

Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury

Edited by

Hui Lu, Shizhang Ling and Lei Xu

Coordinated by

Shihui Gu

Published in

Frontiers in Neurology

Frontiers in Bioengineering and Biotechnology



FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-8325-3369-7
DOI 10.3389/978-2-8325-3369-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury

Topic editors

Hui Lu — Zhejiang University, China

Shizhang Ling — Department of Neurosurgery, The First Affiliated Hospital of Wannan Medical College, China

Lei Xu — Fudan University, China

Topic Coordinator

Shihui Gu — Fudan University, China

Citation

Lu, H., Ling, S., Xu, L., Gu, S., eds. (2023). *Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3369-7

Table of contents

- 06 **Editorial: Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury**
Yanzhao Dong and Hui Lu
- 08 **An experimental and numerical study of the microstructural and biomechanical properties of human peripheral nerve endoneurium for the design of tissue scaffolds**
Liwei Yan, Ali Entezari, Zhongpu Zhang, Jingxiao Zhong, Jing Liang, Qing Li and Jian Qi
- 22 **Automatic detection of abnormal hand gestures in patients with radial, ulnar, or median nerve injury using hand pose estimation**
Fanbin Gu, Jingyuan Fan, Chengfeng Cai, Zhaoyang Wang, Xiaolin Liu, Jiantao Yang and Qingtang Zhu
- 35 **Clinical characteristics and management experience of schwannoma in extremities: Lessons learned from a 10-year retrospective study**
Haiying Zhou, Chengjun Yao, Yanzhao Dong, Ahmad Alhaskawi, Zewei Wang, Jingtian Lai, Sohaib Hasan Abdullah Ezzi, Vishnu Goutham Kota, Mohamed Hasan Abdulla Hasan Abdulla and Hui Lu
- 46 **Ultrasound and elastography role in pre- and post-operative evaluation of median neuropathy in patients with carpal tunnel syndrome**
Han Wu, Hong-Juan Zhao, Wei-Li Xue, Yi-Chun Wang, Wu-Yue Zhang and Xiao-Lei Wang
- 54 ***MTHFR* polymorphism's influence on the clinical features and therapeutic effects in patients with migraine: An observational study**
Jianhao Guo, Xing Hao, Rongrong Wang, Ke Lian, Jun Jiang, Na Chen, Zhiying Feng and Yuefeng Rao
- 62 **Diagnostic performance of preoperative ultrasound for traumatic brachial plexus root injury: A comparison study with an electrophysiology study**
Ailin Liu, Xiaotian Jia, Li Zhang, Xiaoyun Huang, Weimin Chen and Lin Chen
- 72 **The potential roles of dental pulp stem cells in peripheral nerve regeneration**
Jing Fu, Xigong Li, Feilu Jin, Yanzhao Dong, Haiying Zhou, Ahmad Alhaskawi, Zewei Wang, Jingtian Lai, Chengjun Yao, Sohaib Hasan Abdullah Ezzi, Vishnu Goutham Kota, Mohamed Hasan Abdulla Hasan Abdulla, Bin Chen and Hui Lu
- 80 **Traumatic neuromas of peripheral nerves: Diagnosis, management and future perspectives**
Hu Yang, Yanzhao Dong, Zewei Wang, Jingtian Lai, Chenjun Yao, Haiying Zhou, Ahmad Alhaskawi, Sohaib Hasan Abdullah Ezzi, Vishnu Goutham Kota, Mohamed Hasan Abdulla Hasan Abdulla and Hui Lu

- 94 **Case report: Trigeminal neuralgia misdiagnosed as glossopharyngeal neuralgia**
Liangzhe Wu, Jinbiao Xiong, Ying Huang, Kunning Han, Kunhao Cai and Xuejun Fu
- 99 **Case report: A rare case of massive peripheral nerve melanotic schwannoma and review of the literature**
Han Wang, Lin Shi, Tong Tian, Hang Xian, Zhao Song, Rui Cong and Rui Zhao
- 105 **Posterior division of ipsilateral C7 transfer to C5 for shoulder abduction limitation**
Xinying Huang, Zongqi You, Yaoxian Xiang, Junxi Dai and Junjian Jiang
- 112 **Brachial plexus avulsion induced changes in gut microbiota promotes pain related anxiety-like behavior in mice**
Jian-lei Zhang, Hang Xian, Rui Zhao, Ceng Luo, Rou-gang Xie, Tong Tian and Rui Cong
- 124 **Electrical stimulation therapy for peripheral nerve injury**
Lingmei Ni, Zhao Yao, Yifan Zhao, Tianfang Zhang, Jie Wang, Siyue Li and Zuobing Chen
- 137 **The effects of melatonin in the treatment of acute brachial plexus compression injury in rats**
Xigong Li, Jing Fu, Haiying Zhou, Yanzhao Dong, Ahmad Alhaskawi, Zewei Wang, Jingtian Lai, Chengjun Yao, Sohaib Hasan Abdullah Ezzi, Vishnu Goutham Kota, Mohamed Hasan Abdulla Hasan Abdulla, Ming Guan, Xianfeng Lou and Hui Lu
- 143 **Case report: A case of injury to the infrapatellar branch of the saphenous nerve caused by medial approach in knee arthroscopy**
Jiyang Tan, Xunhao Wang, Fei Xiong, Jun Qian, Qiuwen Ying and Jingyi Mi
- 148 **Advances in biotechnology and clinical therapy in the field of peripheral nerve regeneration based on magnetism**
Zheyuan Fan, Xinggui Wen, Xiangdong Ding, Qianqian Wang, Shoushuai Wang and Wei Yu
- 157 **Comparison efficacy of ultrasound-guided needle release plus corticosteroid injection and mini-open surgery in patients with carpal tunnel syndrome**
Zeng Zeng, Nan Lin and Cong-Xian Chen
- 165 **Sensory innervation of the lumbar 5/6 intervertebral disk in mice**
Lunhao Chen, Xuan Lu, Qianjun Jin, Zhihua Gao and Yue Wang
- 173 **Research advances and trends in the surgical treatment of carpal tunnel syndrome from 2003 to 2022: A CiteSpace-based bibliometric analysis**
Daqiang Zheng, Zhiming Wu, Lu Li, Sichao Chen and Jianjun Chang

- 183 **Unilateral biportal endoscopic foraminotomy and discectomy combined with piezosurgery for treating cervical spondylotic radiculopathy with neuropathic radicular pain**
Peng Zhang, Yanghui Jin, Bo Zhu, Mingfeng Zheng, Xiaozhang Ying and Qi Zheng
- 189 **Review of rehabilitation protocols for brachial plexus injury**
Haijun Li, Jinxiu Chen, Juehan Wang, Tianfang Zhang and Zuobing Chen
- 201 **The significance of wrist immobilization for endoscopic carpal tunnel release**
Fei Zhang, Hong Jiang, Zhenfeng Lu, Haoyu Yang, Qian Zhang, Jingyi Mi, Yongjun Rui and Gang Zhao
- 208 **The efficacy and safety of gabapentin vs. carbamazepine in patients with primary trigeminal neuralgia: A systematic review and meta-analysis**
Xin Zhao and Shuyu Ge
- 218 **Referred pain: characteristics, possible mechanisms, and clinical management**
Qianjun Jin, Yuxin Chang, Chenmiao Lu, Lunhao Chen and Yue Wang



OPEN ACCESS

EDITED AND REVIEWED BY
Gila Moalem-Taylor,
University of New South Wales, Australia

*CORRESPONDENCE
Hui Lu
✉ huilu@zju.edu.cn

RECEIVED 25 July 2023
ACCEPTED 07 August 2023
PUBLISHED 15 August 2023

CITATION

Dong Y and Lu H (2023) Editorial: Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury. *Front. Neurol.* 14:1266638. doi: 10.3389/fneur.2023.1266638

COPYRIGHT

© 2023 Dong and Lu. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](#). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury

Yanzhao Dong and Hui Lu*

Department of Orthopedics, The First Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang, China

KEYWORDS

peripheral nerve injuries, nerve regeneration, neurotrophic factor, multidisciplinary collaboration, neuropathic pain

Editorial on the Research Topic

[Surgical treatment of peripheral neuropathic pain, peripheral nerve tumors, and peripheral nerve injury](#)

Peripheral nerve injuries (PNIs) and peripheral nerve tumors can be devastating, leading to impaired motor and sensory function, causing neuropathic pain, and impacting the overall quality of life for affected individuals. As we face an increasing number of PNI cases, there is an urgent need to focus on innovative approaches to promote nerve regeneration and functional recovery.

In this editorial, we explore the latest breakthroughs in the field of peripheral nerve injury regeneration. From tissue engineering and biomaterials to neural tissue transplantation and nerve guidance conduits, researchers are exploring diverse avenues to bridge the gap and stimulate nerve regrowth (Zhang P. et al.; Yang et al.; Fu et al.).

One of the key themes of this editorial is the role of neurotrophic factors and growth-promoting molecules in stimulating nerve regeneration from PNIs and peripheral nerve tumors. Understanding the intricate signaling pathways involved in nerve repair and harnessing the power of stem cells hold promise in revolutionizing PNI treatment (Li et al.).

Moreover, we delve into the importance of precise and personalized therapeutic strategies for peripheral nerve injury regeneration and neuropathic pain. Tailoring treatments to individual patients' needs can significantly enhance the success rates and optimize functional recovery outcomes.

The editorial also highlights the significance of multidisciplinary collaboration in advancing PNI and peripheral nerve tumor research. Neurologists, neurosurgeons, bioengineers, and molecular biologists must unite to share knowledge and insights, fostering a synergistic approach to tackle the complex challenges of nerve regeneration (Zhou et al.).

We shed light on the role of advanced imaging techniques in assessing nerve regeneration progress, providing valuable insights for clinicians and researchers alike. Additionally, rehabilitation protocols play a vital role in maximizing functional recovery post-injury, and we underscore the importance of comprehensive rehabilitation in the editorial (Fan et al.).

While there have been remarkable strides in the field of peripheral nerve regeneration post PNIs or peripheral nerve tumor, we recognize that there are still hurdles to overcome. Ethical considerations, safety, and long-term efficacy remain paramount in the development of novel therapies.

In conclusion, this editorial calls for continued dedication and investment in peripheral nerve regeneration research. Embracing a holistic and interdisciplinary approach, we can forge a brighter future for individuals facing PNI and peripheral nerve tumor, enabling them to regain function and independence.

Author contributions

YD: Conceptualization, Investigation, Methodology, Software, Writing—original draft. HL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing—review and editing.

Funding

The study was funded by Zhejiang Provincial Natural Science Foundation Academic Exchange Program (grant number:

LS2S1H11H0H600010). The funding bodies had no role in the design of the study, in the collection, analysis, and interpretation of data and in drafting the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.



OPEN ACCESS

EDITED BY

Seungik Baek,
Michigan State University, United States

REVIEWED BY

Zhi Yao,
Shenzhen Hospital, Peking University,
China
Justin C. Burrell,
University of Pennsylvania, United States
Teeratrorn Kadeethum,
Sandia National Laboratories (DOE),
United States
Papon Muangsantit,
National Science and Technology
Development Agency (NSTDA),
Thailand

*CORRESPONDENCE

Jian Qi,
qjian@mail.sysu.edu.cn
Qing Li,
qing.li@sydney.edu.au

[†]These authors have contributed equally
to this work

SPECIALTY SECTION

This article was submitted to
Biomechanics,
a section of the journal
Frontiers in Bioengineering and
Biotechnology

RECEIVED 07 September 2022

ACCEPTED 14 November 2022

PUBLISHED 05 December 2022

CITATION

Yan L, Entezari A, Zhang Z, Zhong J,
Liang J, Li Q and Qi J (2022), An
experimental and numerical study of the
microstructural and biomechanical
properties of human peripheral nerve
endoneurium for the design of
tissue scaffolds.
Front. Bioeng. Biotechnol. 10:1029416.
doi: 10.3389/fbioe.2022.1029416

COPYRIGHT

© 2022 Yan, Entezari, Zhang, Zhong,
Liang, Li and Qi. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License](#)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

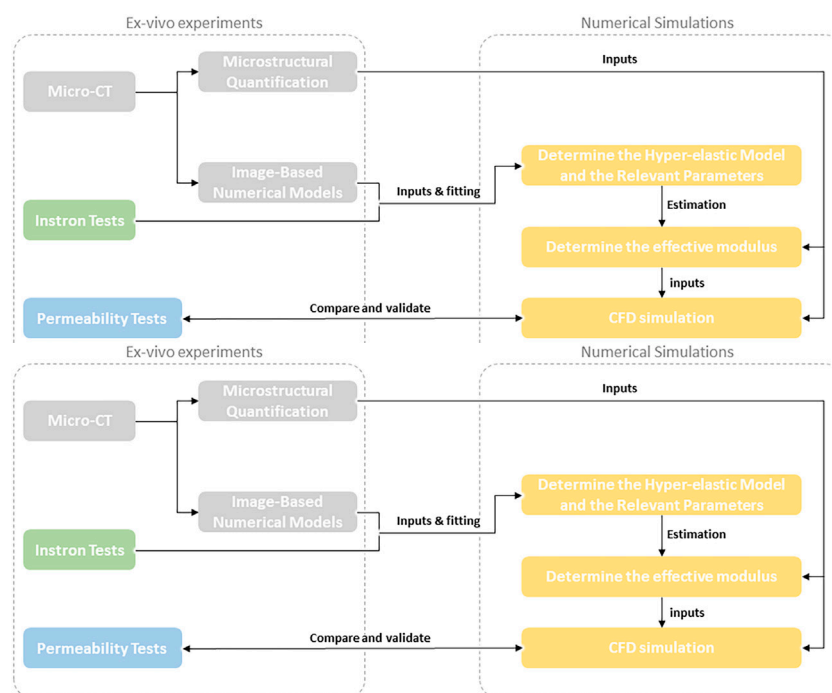
An experimental and numerical study of the microstructural and biomechanical properties of human peripheral nerve endoneurium for the design of tissue scaffolds

Liwei Yan^{1†}, Ali Entezari^{2,3†}, Zhongpu Zhang^{4†}, Jingxiao Zhong³,
Jing Liang¹, Qing Li^{3*} and Jian Qi^{1,5*}

¹Department of Microsurgery, Trauma and Hand Surgery, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, ²School of Biomedical Engineering, University of Technology Sydney, Ultimo, NSW, Australia, ³School of Aerospace, Mechanical and Mechatronic Engineering, University of Sydney, Sydney, NSW, Australia, ⁴School of Computing, Engineering and Mathematics, Western Sydney University, Penrith, NSW, Australia, ⁵Guangdong Provincial Key Laboratory for Orthopedics and Traumatology, Guangzhou, China

Biomimetic design of scaffold architectures represents a promising strategy to enable the repair of tissue defects. Natural endoneurium extracellular matrix (eECM) exhibits a sophisticated microstructure and remarkable microenvironments conducive for guiding neurite regeneration. Therefore, the analysis of eECM is helpful to the design of bionic scaffold. Unfortunately, a fundamental lack of understanding of the microstructural characteristics and biomechanical properties of the human peripheral nerve eECM exists. In this study, we used microscopic computed tomography (micro-CT) to reconstruct a three-dimensional (3D) eECM model sourced from mixed nerves. The tensile strength and effective modulus of human fresh nerve fascicles were characterized experimentally. Permeability was calculated from a computational fluid dynamic (CFD) simulation of the 3D eECM model. Fluid flow of acellular nerve fascicles was tested experimentally to validate the permeability results obtained from CFD simulations. The key microstructural parameters, such as porosity is $35.5 \pm 1.7\%$, tortuosity in endoneurium (X axis is 1.26 ± 0.028 , Y axis is 1.26 ± 0.020 and Z axis is 1.17 ± 0.03 , respectively), tortuosity in pore (X axis is 1.50 ± 0.09 , Y axis is 1.44 ± 0.06 and Z axis is 1.13 ± 0.04 , respectively), surface area-to-volume ratio (SAVR) is $0.165 \pm 0.007 \mu\text{m}^{-1}$ and pore size is $11.8 \pm 2.8 \mu\text{m}$, respectively. These were characterized from the 3D eECM model and may exert different effects on the stiffness and permeability. The 3D microstructure of natural peripheral nerve

Abbreviations: eECM, endoneurium extracellular matrix; micro-CT, microscopic computed tomography; CFD, computational fluid dynamics; SAVR, surface area-to-volume ratio; PNIs, peripheral nerve injuries; ECM, extracellular matrix; NGCs, nerve guidance conduits; IFD, iodine and freeze-dry; ROI, region of interest; RVE, representative volume element; ESD, equivalent spherical diameter; ANA, acellular nerve allograft.



GRAPHICAL ABSTRACT

MicroCT was used to obtain high-resolution/high-contrast images of human peripheral nerve endoneurium extracellular matrix (eECM). A numerical model was constructed to investigate the microstructural and biomechanical properties of eECM.

eECM exhibits relatively lower permeability ($3.10 \text{ m}^2 \times 10^{-12} \text{ m}^2$) than other soft tissues. These key microstructural and biomechanical parameters may play an important role in the design and fabrication of intraluminal guidance scaffolds to replace natural eECM. Our findings can aid the development of regenerative therapies and help improve scaffold design.

KEYWORDS

biomimetic scaffold design, nerve endoneurium, permeability, stiffness, nerve regeneration

Introduction

Peripheral nerve injuries (PNIs) account for 3% of all traumatic patients and could cause partial or complete loss of function in damaged motor and sensory axons (Robinson, 2022). The most effective clinical strategy is graft transplantation, e.g., transplantation of autografts, which is currently used as the gold standard for nerve regeneration (Tiwari et al., 2022). The main advantage of autografts is their ability to largely mimic natural nerve characteristics, such as extracellular matrix (ECM) molecules, chemical and physical cues or topographic support and topological guidance (Wieringa et al., 2018). Nevertheless, the major limitations of autografts are their restrictive availability, donor site morbidity, and immunological rejection, which have prompted the

development of alternative nerve repair strategies, such as nerve guidance conduits (NGCs) (Li et al., 2021).

Recent NGC studies have shown that conduits with proper micro- and nanostructures promote the regeneration of axons across a much longer gap of nerve defects (Li et al., 2021). This approach adopts macroscopic multichannel architectures (Pawelec et al., 2018) loaded with filaments (Yoshii et al., 2003) and unidirectional freeze-dried structures (Pot et al., 2015). Notably, NGC design aims to maximize the degree to which the conduit mimics the natural nerve architecture. According to previous studies, these biomimetic designs help promote nerve regeneration by enhancing the biomechanical behavior and porosity of the luminal wall using different materials (Zhu et al., 2018). However, the efficacy of NGCs is to a considerable extent limited to the defects with a size within a

critical nerve defect, which is species-dependent and can range from 5 to 30 mm in animals (Yoshii et al., 2003). The key issue is usually attributed to inadequate formation of the ECM components and microstructures in the initial stages, which restricts Schwann cell (SC) migration and proliferation, thereby reducing the glial bands of the Büngner formation (Hoffman-Kim et al., 2010; Huang et al., 2022).

To overcome these shortcomings, researchers have sought to design a scaffold with high similarity to the natural microarchitectures of peripheral nerves, especially the microstructure of the endoneurium (Chen et al., 2019; Yang et al., 2021). To realize this task, the first step is to characterize the microstructures and biomechanical properties of the peripheral nerve endoneurium ECM (eECM). The peripheral nerve ECM system is categorized into an external epineurium, an internal epineurium, fascicles and an endoneurium (Schmidt and Leach, 2003; Tiwari et al., 2022). The anatomy of the peripheral nerve ECM is illustrated in Figure 1, which shows that the perineurium surrounds groups of axons to form fascicles, and the epineurium binds individual nerve fascicles into a nerve trunk. The internal epineurium contains a complex microvascular network that plays a vital role in nerve healing and repair after injury/damage.

The biomimetic design of the scaffold architecture is a critical yet complex process requiring the appropriate selection and control of multiple parameters, such as mechanical properties, biodegradation, biocompatibility, pore architecture, porosity, surface properties, and, more importantly, permeability (Singh et al., 2018a; Gotti et al., 2020). Notably, the permeability of porous scaffolds substantially affects cell recruitment, differentiation, and proliferation and tissue regeneration after implantation, which determines the transport of oxygen/nutrients and removal of metabolites (Santos et al., 2020). For example, at a given cell seeding density, high permeability scaffolds will enhance bone regeneration (Mitsak et al., 2011). However, an overly high permeability might be a disadvantage due to an excessive flow rate, which could cause cell washout, thus resulting in a lower tissue growth rate. On the other hand, a permeability lower than the desired value would lead to low nutrient supply or even hypoxia to cells, thus hindering tissue growth. Hence, there is always a range of desired permeabilities as found in different tissues throughout the body (Gomez et al., 2016; Chao et al., 2021). Nevertheless, an optimal permeability of peripheral nerve scaffolds for facilitating nerve regeneration has remained largely unclear.

In a previous study, we proposed to implement an iodine and freeze-drying enhanced microscopic computed tomography (micro-CT) imaging technique that would enable us to obtain the high-resolution microstructure of the human peripheral nerve, which allowed us to construct a baseline model for numerical characterization *in silico* here (Yan et al., 2017a; Yan et al., 2019; Almeida and Bartolo, 2021). The present study aims to investigate the key microstructural characteristics and permeability of the human peripheral

nerve eECM, in which the effects of porosity, tortuosity, surface area-to-volume ratio (SAVR) and pore size on the stiffness and permeability are explored using experimental and numerical approaches. The contributions of this study are as follows (Robinson, 2022): we first applied micro-CT to obtain high-resolution/high-contrast images of human peripheral nerve endoneurium extracellular matrix (eECM) (Tiwari et al., 2022); A numerical model was constructed to investigate the permeability of eECM (Wieringa et al., 2018); We anticipate to provide a fundamental understanding and insights into the design of scaffolds for regenerating peripheral nerves.

Materials and methods

Harvesting of human peripheral nerve specimens

All human experiments were performed using the well-established protocol approved by the institutional review boards of the first Affiliated Hospital of Sun Yat-sen University, China in accordance with the Declaration of Helsinki. In this study, we adopted characteristic human nerves excised from the upper or lower limbs of amputation patients at the first Affiliated Hospital of Sun Yat-sen University. All donors provided specific consent for the use of their donated tissues for teaching and scientific research purposes. We selected three different proximal median nerve as the microCT study. The remaining nerve specimens were used for tensile test and others experiments.

Specimen preparation and protocol for Micro-CT scanning

Micro-CT was used to acquire high-resolution images from the preprocessed samples using the iodine and freeze-dry (IFD) method described previously (Yan et al., 2017a; Yan et al., 2019). Preprocessing steps are briefly summarized here for completeness (Robinson, 2022). Fresh nerves were immersed in 50% Lugol's solution (Sigma-Aldrich, St. Louis, MO, United States, 62,650–100 ML-F; 0.5%–1% potassium iodide and 0.25%–0.5% iodine) for 3 days in a 50-ml centrifuge tube and manually shocked as appropriate. (Tiwari et al., 2022). The samples were placed into liquid nitrogen for 2 min and stored in a drying machine (LABCONCO, FreeZone 6 Plus, United States) for 7 days.

The micro-CT scanning protocol ($\mu 50$, SCANCO Medical AG, Bassersdorf, Zurich, Switzerland) was established as follows: holder, 9 mm; energy/intensity, 55 kVp, 72 μ A, 4 W; filter, 0.1 mm Al; BH, Plexi (PMMA); FOV/diameter, 10.2 mm; integration time, 1,500 ms; average data, 3; voxel size, 3 μ m; and sample pixel, 3,400 $\times$ 3,400. Finally, we obtained at least

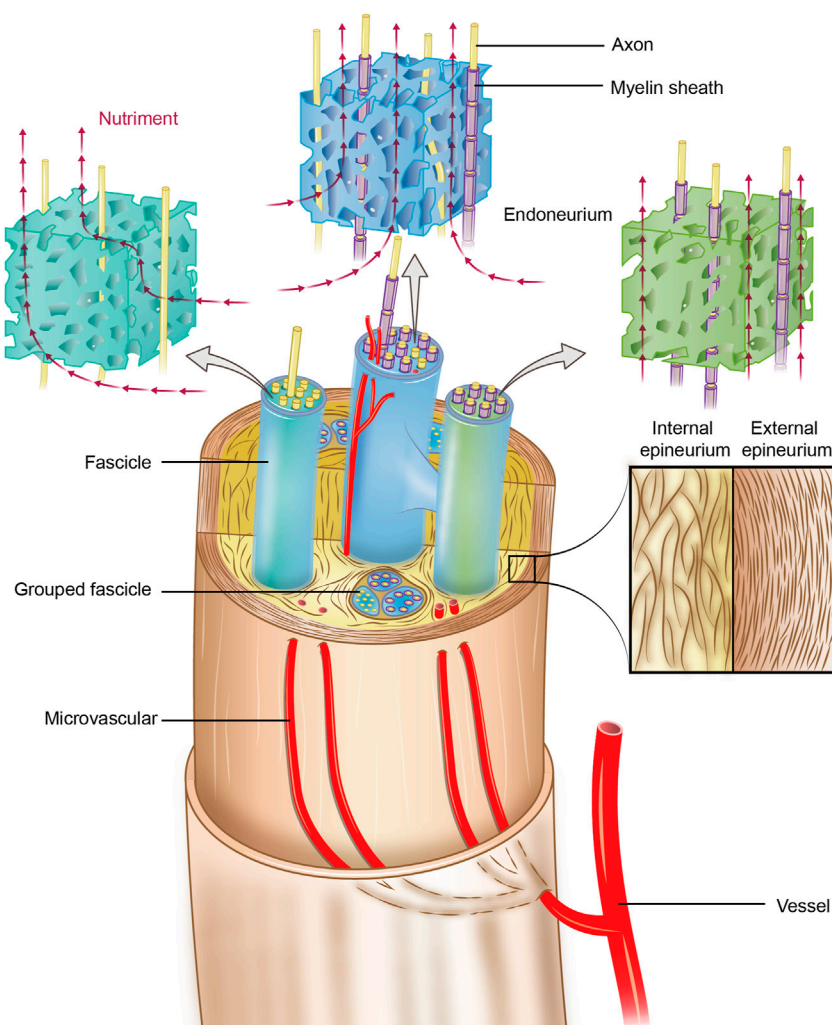


FIGURE 1

Schematic of the anatomy of the peripheral nerve ECM system. The endoneurium is an anisotropic porous structure that provides nutrient diffusivity longitudinally. The perineurium surrounds groups of axons to form fascicles, and the epineurium binds individual nerve fascicles into a nerve trunk. The internal microvasculature runs longitudinally within the internal epineurium and connects with the external vasculature at various points; thus, it plays a particularly vital role in nerve healing and repair after injury/damage.

250 sectional CT images for each sample. After micro-CT scanning, we used the same samples to acquire scanning electron microscopy (SEM) images. The samples were cut transversely into 2-mm-thick sections with a razor blade. After platinum coating, they were examined by SEM (FEI QUANTA 200, Netherlands).

Masson's trichrome staining for histological analysis

The human nerve samples ($n = 3$) were immersed in an optimum cutting temperature compound (O.C.T. compound; Sakura of America, Hayward, California, United States).

Transverse sections were cut using a cryostat (CM3050s; Leica, Wetzlar, Germany) to a thickness of 50 μ m and mounted on microscope slides. They were then subjected to Masson trichrome staining and observed under a light microscope. We collected four photographs from randomly selected fields in each specimen and then measured the cross-sectional area of the pore using Image-Pro Plus software (Media Cybernetics, Bethesda, Maryland, United States).

Micro-CT image segmentation

Voxel-based models were processed using a commercial software package (ScanIP 2017; Simpleware, Exeter,

United Kingdom). A sequence of 250 slices of non-smoothed transverse-plane micro-CT images was imported into the program in a computer [Intel(R) Core (TM) i7-4930K CPU @ 3.40 GB memory]. The threshold for binarizing the images (i.e., segmentation into pores and endoneurium tissue) was set to the minimum value between the two peaks in the grayscale histogram. Two steps are required prior to segmentation: the first is image refinement, in which we used the ScanIP smoothing module plus the mean filter algorithm to remove noise and gradients; and the second is cropping to preserve only the region of the data containing the endoneurium microstructure. Thus, cubic shapes of $200 \times 200 \times 200$ voxels for each sample were generated. As a result, two hundred 2D images were obtained for each region of interest (ROI). In the current study, at least three ROIs at different locations of each individual sample were modeled for characterization.

Reconstruction of the 3D microstructure

The acquired grayscale images were segmented into binary images using a grayscale threshold, where the pixels with grayscale values greater than the preselected threshold (denoted as TV) were labeled as the endoneurium (solid phase), and the pixels with grayscale values less than the TV were classified as background (pore phase). Once this process was performed for each 2D image, the endoneurium region and pore region were interconnected across the third dimension to reconstruct the 3D models.

Selection of the representative volume element

The RVE is commonly used to characterize the effective properties of any porous material, which should be selected appropriately to determine the effective behavior and ensure efficient computational modeling.

Quantification of microstructural parameters

The key microstructural parameters of the endoneurium, such as the porosity, tortuosity, SAVR and pore size, were quantified using the ScanIP 2017 measurement module. Porosity was calculated as the fraction of pore phase volume to the total volume. Tortuosity represents a geometric parameter describing the extent of twisted interconnected paths through the volume. In this study, the tortuosity of both the pore and endoneurium phases was measured using a centerline module based upon a computed path formed by centroids of each interconnected region as the same phase on each image. We used the shortest route module in ScanIP to measure the

tortuosity in different directions. The SAVR measures the relative of the endoneurium phase surface area to the total volume. The pore size was obtained by applying an object separation technique in the microstructures with a watershed algorithm. After the pore/endoneurium phase was separated into a group of individual particles/pores, the volume of each particle/pore was converted to an equivalent spherical diameter (ESD) for plotting particle/pore size distribution histograms (Gentile et al., 2012).

Ex vivo experimental setup

Human fascicle samples ($n = 6$) were carefully isolated within 24 h after harvest from the donors and immersed in a 9% normal saline solution to prevent dehydration during preparation. The diameters of the isolated fascicle samples were measured before the test to calculate the cross-sectional area, in which the average diameter was 2.02 ± 0.78 mm. One end of the sample was wrapped in sandpaper to prevent slippage and mounted on an Instron machine (Instron 3343, INSTRON COMPANY, United States) for the uniaxial tensile test. To precondition the specimens, each specimen was suspended by the top clamp and allowed to hang freely for 2 min. The fascicles were considered under no external loads and fully relaxed, and the length of the fascicles was measured and used as the gauge length. The tensile test was then performed with a 10 mm/min strain rate until complete rupture.

Microstructural finite element modeling

Microstructural finite element (μ -FE) models of endoneurium RVEs ($n = 3$) were constructed and exported using ScanIP N-2018.03 (Simpleware, Exeter, United Kingdom) based on the micro-CT data. The endoneurium was segmented with a proper threshold to ensure that the exported models had the same porosity as the measurements from histological images. The μ -FE models were meshed in linear tetrahedral elements with an average degree of freedom of 1,642,833 and then imported into ABAQUS 6.13 (SIMULIA, Providence, RI, United States), where each μ -FE model of RVEs was placed between the two parallel rigid plates to replicate the tensile tests. One of the rigid plates was fixed to the base, and the other plate was subjected to a load that would generate stress equivalent to that induced by the experimental load on the RVE surface.

Experimental loads beyond the toe region were randomly chosen and converted to corresponding loads on the RVEs as follows:

$$F_{RVE} = \frac{F_{Macro} A_{RVE}}{A_{Macro}} \quad (1)$$

where F_{Macro} and F_{RVE} are the load on the endoneurium (macrolevel) and the corresponding load on the RVE (sublevel), respectively; and A_{Macro} and A_{RVE} are the areas of the endoneurium (macrolevel) and RVE (sublevel), respectively.

Characterization of the mechanical properties of fascicles

A μ -FE analysis was performed to inversely identify the optimal set of material parameters with the best possible match to the characteristics of the human fascicles. The four widely used hyperelastic constitutive models for describing biological soft tissues (Ogden, Yeoh, Marlow, and Mooney-Rivlin) were used here to characterize the hyperelastic properties. To assess the material properties of the μ -FE models that provide the best fit to the experimental data obtained from the uniaxial tensile tests, the minimum mean square error based on the regression curves was calculated. The third-order Yeoh's model best fit the test data and displayed the highest stability. Thus, the third-order Yeoh's hyperelastic model was adopted and carefully adjusted until the resulting effective modulus of the bulk RVE in the z direction (i.e., the longitudinal direction of the nerve) matched the linear phase of the obtained J-shaped stress-strain curve.

Then, the identified material properties were further adopted to determine an effective modulus in the x and y directions (transverse directions of the nerve). The effective modulus was derived using the following equations (Entezari et al., 2019):

$$E_x = \frac{F_x \times l_x}{u_x \times A_{yz}} \quad (2)$$

$$E_y = \frac{F_y \times l_y}{u_y \times A_{xz}} \quad (3)$$

$$E_z = \frac{F_z \times l_z}{u_z \times A_{xy}} \quad (4)$$

where E , F and l denote the effective young's modulus, the applied force and the initial length of the sample in the direction of the corresponding force, respectively; A represents the area of the loaded cross-section; u denotes the displacement; and subscripts x , y , and z indicate the direction of the parameter.

Computational fluid dynamics analyses

The micro-CT-based models of endoneurium RVEs ($n = 3$) with dimensions of $300 \times 300 \times 300 \mu\text{m}$ were created in ScanIP. Notably, the most critical step in creating the micro-CT-based CFD models of fascicle tissue is to estimate a reasonable threshold range for segmentation of the soft tissue. For this purpose, arbitrarily segmented 2D images from each sample were compared with the random images of histology to ensure that the surface area of the soft tissue was the same in both the segmented and histological images.

In the CFD simulation, the fluid was assumed to fully fill the void space in the endoneurium RVEs; therefore, the inverse model of each RVE was first generated in ScanIP to represent the fluidic phase. Then, each RVE model was imported into the Fluid Flow (Fluent) module in ANSYS Workbench 2019 for the permeability analyses. A constant flow rate (Q) and zero static pressure were applied for the inlet and the outlet, respectively. Steady-state Navier–Stokes equations were implemented, and no-slip conditions were considered for the walls of the RVE models. The fluidic phase was considered to be water that was incompressible and homogeneous with a density and dynamic viscosity of $1,000 \text{ kg m}^{-3}$ and $1 \text{ Pa s} \times 10^{-3} \text{ Pa s}$, respectively. The resulting decrease in pressure (ΔP) through the fascicle RVEs was used to calculate the effective permeability from Darcy's law, which is defined by the following equation:

$$k = \frac{\mu L}{A} \frac{Q}{\Delta P} \quad (5)$$

where k denotes the effective permeability (m^2), L is the thickness (m) of RVE, A is the area of the RVE cross-section (m^2), μ is the dynamic fluid viscosity (Pa. s), and ΔP is the pressure gradient (Pa) in the fascicle RVE when fluid flows at a rate of Q ($\text{m}^3 \text{ s}^{-1}$) through the endoneurium model. Since Darcy's law is valid for Reynolds numbers less than one, a small flow rate (Q) was applied as the inlet boundary condition.

Permeability tests

The decellularized human nerve samples were bought from Guangzhou Zhongda Medical Devices Company (Guangzhou, Guangdong Province, China). The nerve fascicles were separated under the microscope due to the experiment in this part. The permeability of the fascicle samples was measured using the falling head method (Zhang et al., 2019a). Using Darcy's law, the permeability of a sample can be derived. The initial heights $L1$ at $t0$ and as the final height $L2$ at $t1$ were recorded in the tests. The values of $L1$ and $L2$ remained the same in each test to simplify the calculation. The hydraulic conductivity K of the sample was calculated from the equation of Darcy's law:

$$K = \frac{aH}{A(t1 - t0)} \ln\left(\frac{L1}{L2}\right) \quad (6)$$

where a is the cross-sectional area of the standpipe, A is the cross-sectional area of the sample, and H is the height of the sample.

The permeability k of the tested sample was calculated using the following equation:

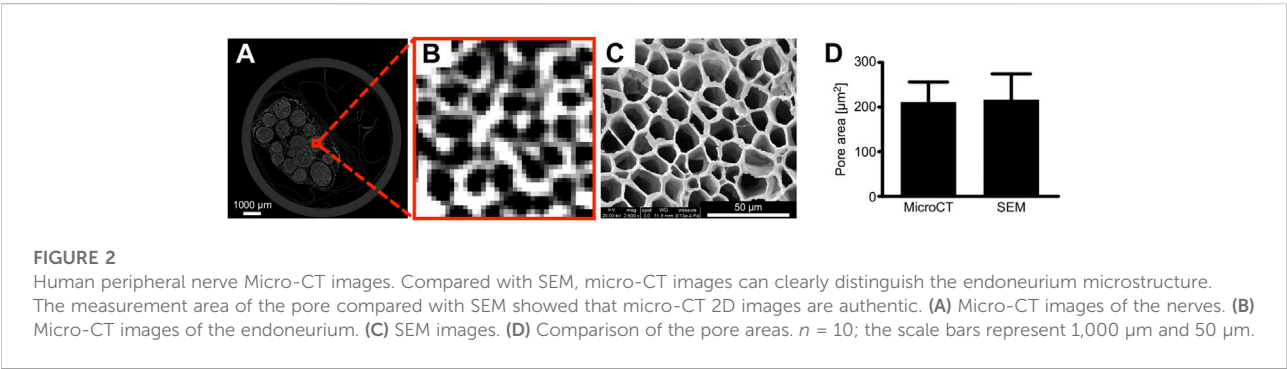
$$k = \frac{K\lambda}{\rho g} \quad (7)$$

where λ is the dynamic viscosity coefficient of water, ρ is the density of water, and g is the acceleration of water due to gravity. The parameters used in the permeability tests are summarized in Table 1.

TABLE 1 Parameters and their values used in the permeability tests.

Parameters	Sample 1	Sample 2	Sample 3
Average height of the sample (H)	$1.00\text{ m} \times 10^{-1}\text{ m}$	$1.00\text{ m} \times 10^{-1}\text{ m}$	$1.00\text{ m} \times 10^{-1}\text{ m}$
Cross-section of the standpipe (a)	$2.16\text{ m}^2 \times 10^{-4}\text{ m}^2$	$2.16\text{ m}^2 \times 10^{-4}\text{ m}^2$	$2.16\text{ m}^2 \times 10^{-4}\text{ m}^2$
Cross-section of the sample (A)	$2.74\text{ m}^2 \times 10^{-6}\text{ m}^2$	$2.52\text{ m}^2 \times 10^{-6}\text{ m}^2$	$2.37\text{ m}^2 \times 10^{-6}\text{ m}^2$
Dynamic viscosity coefficient of water (μ)	$1.00\text{ Pa}\cdot\text{s} \times 10^{-3}\text{ Pa}\cdot\text{s}$	$1.00\text{ Pa}\cdot\text{s} \times 10^{-3}\text{ Pa}\cdot\text{s}$	$1.00\text{ Pa}\cdot\text{s} \times 10^{-3}\text{ Pa}\cdot\text{s}$
Density of water (ρ)	$1.00\text{ kg/m}^3 \times 10^3\text{ kg/m}^3$	$1.00\text{ kg/m}^3 \times 10^3\text{ kg/m}^3$	$1.00\text{ kg/m}^3 \times 10^3\text{ kg/m}^3$
Gravity acceleration (g)	9.8 m/s	9.8 m/s	9.8 m/s
L1	1.46 m	1.46 m	1.46 m
L2	1.44 m	1.44 m	1.44 m
t	350 s	1,196	532
K	3.11×10^{-5}	9.89×10^{-6}	2.36×10^{-5}
$k\text{ [m}^2\text{]}$	3.17×10^{-12}	1.01×10^{-12}	2.41×10^{-12}

K, hydraulic conductivity; k , permeability.



Statistical analysis

All the experiments were performed in triplicate, with a minimum sample size of $n = 3$. The results of the quantitative experiments were expressed as the mean $\pm$ standard deviation. All analyses were carried out using GraphPad Prism 8 with 2-tailed Student's t -tests and one-way ANOVAs. A P value less than 0.05 was considered statistically significant.

Results

Micro-CT imaging and 3D reconstruction of the human peripheral nerve endoneurium

We employed an iodine and freeze-drying method to preprocess human peripheral nerve samples and obtain high-resolution micro-CT images (Figure 2A); and the SEM images were also acquired for comparison with the same sample (Figure 2C). We measured the

area of the endoneurium by comparing the micro-CT scan with the SEM image to confirm that the micro-CT scan generates a sufficiently accurate image (Figure 2D). After the micro-CT scan, some pixels were not captured in the images properly, thereby highlighting the importance of the image threshold for the subsequent segmentation step.

One of the most critical steps in creating micro-CT-based models of nerve tissue for microscopic finite element analysis (μ -FEA) and microscopic computational fluid dynamics (μ -CFD) modeling is to estimate a reasonable threshold range for segmentation of the eECM. For this purpose, arbitrarily segmented 2D images from each sample were compared with some random images of histology to ensure that the surface area of the eECM was the same in both segmented and histological images (Chen et al., 2015a). Here, we adopted the surface area rate obtained from the peripheral nerve 2D histological images to correct the segmentation of micro-CT images (Chen et al., 2015b).

We obtained a 2D histological image (Figure 3A) with a surface area rate of $51.86 \pm 0.25\%$ (Figure 3I). The binarized

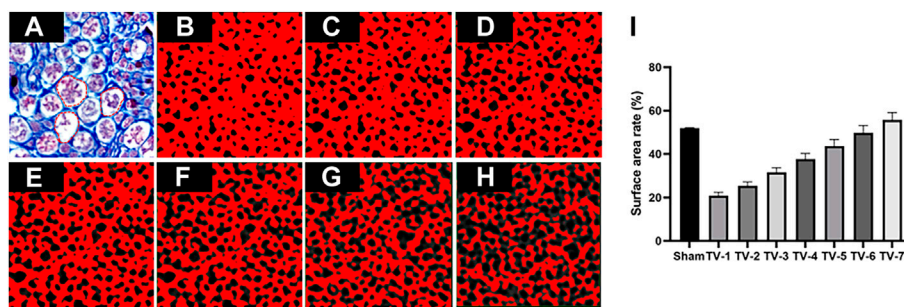


FIGURE 3

Representative TVs used to segment eECM micro-CT images. (A) Masson's trichrome staining and binary image obtained by applying a TV of (B) 25, (C) 26, (D) 27, (E) 28, (F) 29, (G) 30 and (H) 31. (I) Surface rate results. The red dotted line represents the pore of endoneurium. $n = 3$.

endoneurium images are shown in Figures 3B–H. Using the threshold value (TV) to segment endoneurium micro-CT images, 200×200 -pixel eECM micro-CT images were used to obtain a preliminarily defined range of TVs. A number of grayscale values within a limited range close to 30 were selected for segmentation, specifically, $TV = 25, 26, 27, 28, 29$, and 31 , to evaluate the sensitivity of the surface area rate with respect to variation in the TV. The 2D micro-CT images of the surface area rate after applying the aforementioned TVs are illustrated in Figure 3I, which shows a grayscale image from the stack and the resulting binary images generated when different TVs were applied for segmentation. The results indicate that the surface area rate was closest to that of the histological image when the TV was 30. To reduce the computational load, we used 3D porosity to select the RVE of eECM samples.

TABLE 2 3D microstructural parameters of endoneurium ECM.

3D microstructural parameters	Values
Pore size	$11.8 \pm 2.8 \mu\text{m}$
Endoneurium thickness	$11.4 \pm 3.1 \mu\text{m}$
Porosity	$35.5 \pm 1.7\%$
Tortuosity	
Endoneurium-X	1.26 ± 0.028
Endoneurium-Y	1.26 ± 0.020
Endoneurium-Z	1.17 ± 0.03
Pore-X	1.50 ± 0.09
Pore-Y	1.44 ± 0.06
Pore-Z	1.13 ± 0.04
SAVR	$0.165 \pm 0.007 \mu\text{m}^{-1}$

3D, three-dimensional; ECM, extracellular matrix; SAVR, surface area-to-volume ratio.

Key parameters of the 3D endoneurium extracellular matrix microstructure

The key 3D microstructural parameters, such as pore size, eECM thickness, porosity, tortuosity and SAVR, were calculated ($n = 3$) and are summarized in Table 2. For microstructural complexity, the tortuosity of the eECM and the pore phase may not be the same. Tortuosity cannot be less than 1, and microstructures with more twisted and complex features result in a higher tortuosity value. In the current study, the tortuosities of both the endoneurium tissue and the pore space were quantified, and the results are presented in Table 2; both the tortuosities for endoneurium and pores are smaller in the z direction than those in the other directions. The tri-directional tortuosity of each group indicates that the endoneurium microstructure has an anisotropic porous structure. Therefore, tortuosity can also be determined based on the transport equation that governs the permeability in the entire media of the structural phase (Chen et al., 2015a).

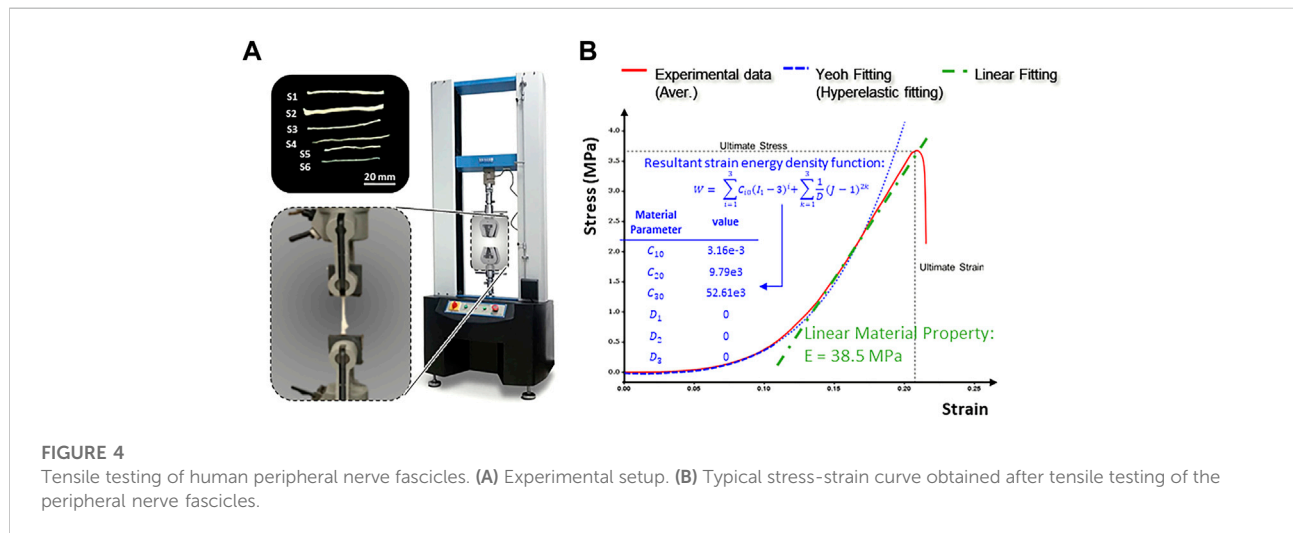
Tensile test

In the present experimental study, we investigated the stress-strain characteristics of the human peripheral nerve fascicles ($n = 6$). The tensile tests showed that the peripheral nerve was easily extensible in the initial stage followed by stiffening, which exhibited a typical J-shaped force-displacement curve similar to those of other soft tissues (El-Bakary et al., 2019). The ultimate loads were recorded from 3.39 to 35.93 N, depending on the diameter of the samples, as shown in Table 3.

Figure 4 shows the stress-strain curve based on the average data for the 6 samples tested, which all exhibited characteristic J-shaped nonlinear behavior. The curve has a large initial toe region, reaching a strain of approximately 13% under minimal stress. Much of the strain within the toe region may be due to relaxation of the slack fibrils within the fascicles. Notably, the nerve strain induced by physiological motion varies with the anatomical position of the nerves and the angle of joint flexion but is generally within the toe region of the J curve (Kwan et al.,

TABLE 3 Sample sizes and ultimate loads.

Sample ID	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Diameter [mm]	3.08	1.72	2.35	3.10	1.22	1.00
Ultimate Load (N)	28.97	7.44	29.59	35.93	6.85	3.39



1992). A linear trend was identified in the stress-strain curve after the toe region, where the slope steadily increased in the initial stage and then remained constant with a young's modulus of 38.5 MPa until the sample ruptured.

A linear regression analysis of the numerical simulation and experimental test results showed an R^2 value greater than 0.83. However, other scientific studies have reported that the elastic modulus of intact human nerves is 8–16 MPa (Chiono and Tonda-Turo, 2015). Our results differed from those of the previous studies mainly because the area of the measured sample was different.

FE simulation of Young's modulus

The μ -FE simulation was performed in the linear phase of the J curve because this region is where pathological stress/strain and thresholds of damage and/or rupture of the nerve are commonly considered. The resulting longitudinal (z direction) effective young's modulus (E) was 37.46 ± 1.20 MPa, which was very close to the experimental effective value of E in the linear phase (38.5 MPa). The simulated effective E of the lateral directions (x and y directions) were 21.69 ± 1.22 MPa and 22.19 ± 0.71 MPa, respectively. According to the present results, human nerve fascicles are transversely isotropic materials; and their

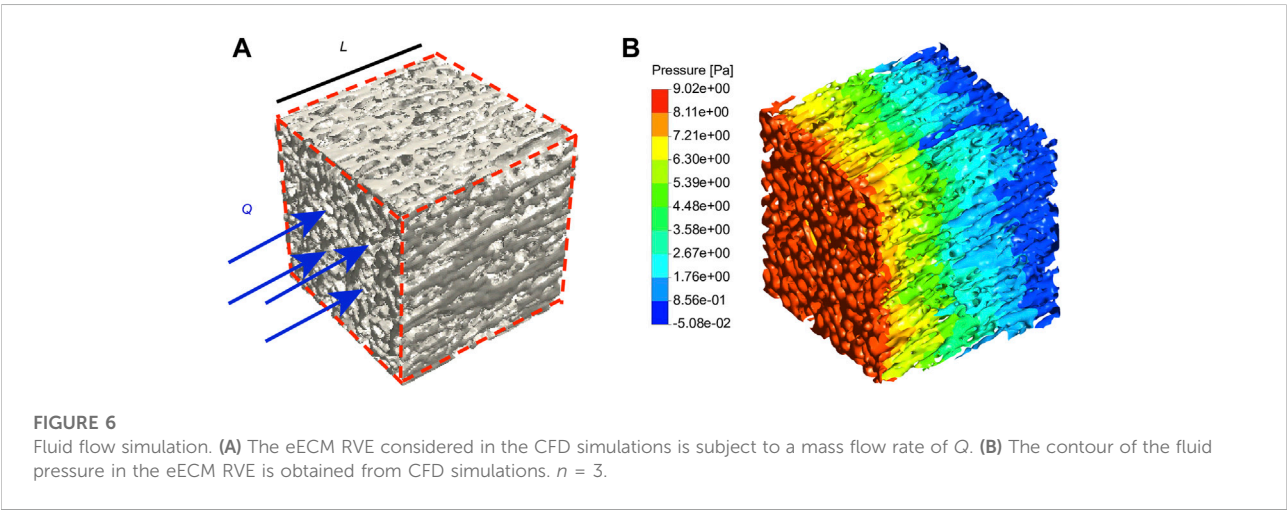
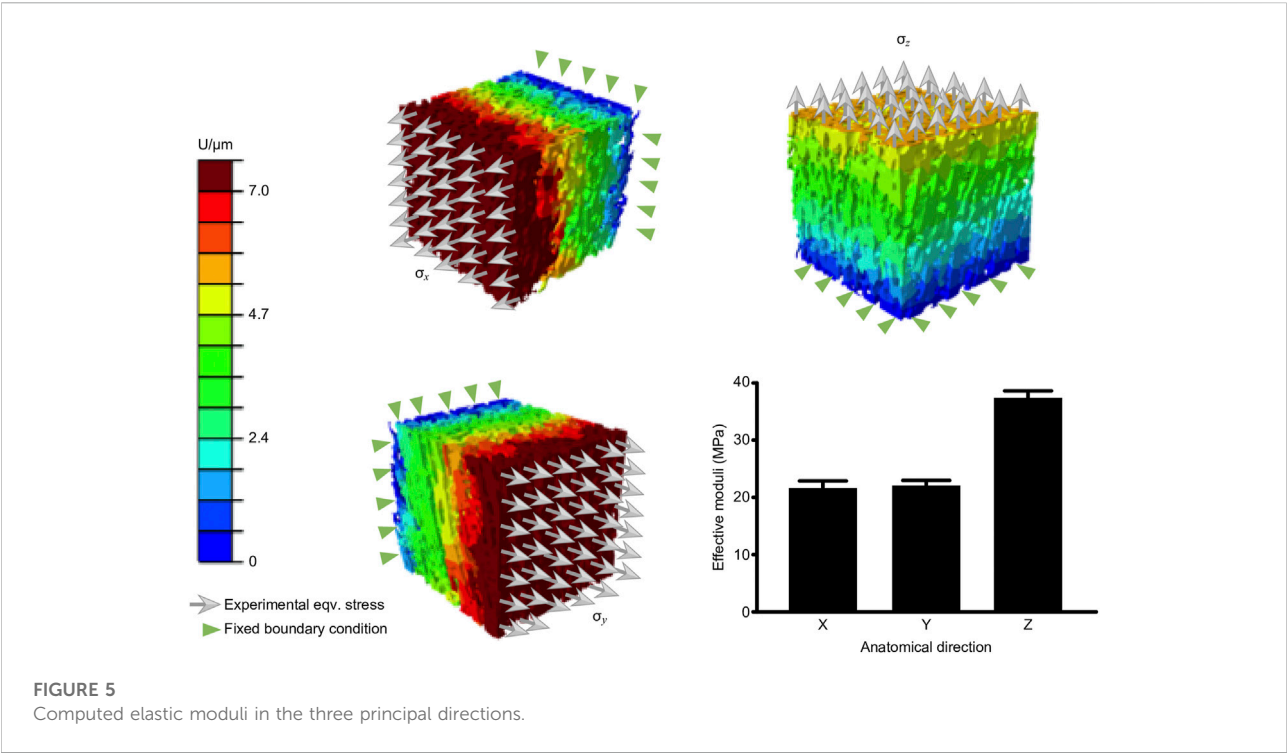
principal direction is along the longitudinal direction, which has higher mechanical properties than those in the transverse directions. Figure 5 summarizes the simulation results.

Computational fluid dynamic simulation of permeability

Figures 6A–B shows the contour of fluidic pressure in an eECM RVE subject to the mass flow rate of 8.75×10^{-9} (kg/s). The decreases in the pressure of each RVE ($n = 3$) were used to calculate the permeability of the eECM according to Darcy's law. Table 4 summarizes the permeability of these three reconstructed eECM specimens (RVE) calculated in the μ -CFD analyses, with a value of $3.10 \pm 1.91 \times 10^{-12}$ m².

Permeability tests *ex vitro*

Permeability, a quantitative measure of cell and nutrient migration through pore channels, has been considered a key criterion for the design of porous scaffolds. Three decellularized human nerve samples were used for the experimental tests, and the results are summarized in Table 5. The average endoneurium permeability value was $2.19 \pm 1.09 \times 10^{-12}$ m².



Discussion

When provided with a suitable ECM microenvironment, the peripheral nerve has an intrinsic capacity for functional recovery over time (Du et al., 2018; Philips et al., 2018). ECM functions both as a material for supporting the microstructure and as a regulator of cell differentiation, signal transduction, proliferation, morphogenesis, and remodeling in the host (Gilbert et al., 2006). Autografts provide a suitable ECM microenvironment, which

TABLE 4 The permeability of the endoneurium ECM RVEs computed using CFD simulations.

Permeability, k [m^2]				
Sample 1	Sample 2	Sample 3	Average	SD
3.29×10^{-12}	2.84×10^{-12}	3.18×10^{-12}	3.10×10^{-12}	1.91×10^{-13}

ECM, extracellular matrix; RVEs, Representative Volume Elements; CFD, computational fluid dynamic; k , permeability.

TABLE 5 Parameters and their values used in the permeability tests of the three groups.

Parameter	Sample 1	Sample 2	Sample 3
Average height of the sample (H)	$1.00 \text{ m} \times 10^{-2} \text{ m}$	$8.00 \text{ m} \times 10^{-3} \text{ m}$	$1.00 \text{ m} \times 10^{-2} \text{ m}$
Cross-section of the standpipe (a)	$3.58 \text{ m}^2 \times 10^{-3} \text{ m}^2$	$3.58 \text{ m}^2 \times 10^{-3} \text{ m}^2$	$3.58 \text{ m}^2 \times 10^{-3} \text{ m}^2$
Cross-section of the sample (A)	$1.42 \text{ m}^2 \times 10^{-5} \text{ m}^2$	$1.24 \text{ m}^2 \times 10^{-5} \text{ m}^2$	$5.20 \text{ m}^2 \times 10^{-5} \text{ m}^2$
Dynamic viscosity coefficient of water (μ)	$1.00 \text{ Pa}\cdot\text{s} \times 10^{-3} \text{ Pa}\cdot\text{s}$	$1.00 \text{ Pa}\cdot\text{s} \times 10^{-3} \text{ Pa}\cdot\text{s}$	$1.00 \text{ Pa}\cdot\text{s} \times 10^{-3} \text{ Pa}\cdot\text{s}$
Density of water (ρ)	$1.00 \text{ kg/m}^3 \times 10^3 \text{ kg/m}^3$	$1.00 \text{ kg/m}^3 \times 10^3 \text{ kg/m}^3$	$1.00 \text{ kg/m}^3 \times 10^3 \text{ kg/m}^3$
Gravity acceleration (g)	9.8 m/s	9.8 m/s	9.8 m/s
L1	0.45 m	0.45 m	0.45 m
L2	0.425 m	0.425 m	0.425 m
t			
#1	950 S	1440 S	5400 S
#2	890 S	1540 S	4460 S
#3	520 S	2130 S	5480 S
K			
#1	1.51×10^{-4}	9.17×10^{-5}	7.29×10^{-6}
#2	1.61×10^{-4}	8.57×10^{-5}	8.23×10^{-6}
#3	2.77×10^{-4}	6.20×10^{-5}	7.18×10^{-6}
k [m^2]			
#1	$1.55 \text{ m}^2 \times 10^{-11} \text{ m}^2$	$9.35 \text{ m}^2 \times 10^{-12} \text{ m}^2$	$7.44 \text{ m}^2 \times 10^{-13} \text{ m}^2$
#2	$1.65 \text{ m}^2 \times 10^{-11} \text{ m}^2$	$8.75 \text{ m}^2 \times 10^{-12} \text{ m}^2$	$9.09 \text{ m}^2 \times 10^{-13} \text{ m}^2$
#3	$2.83 \text{ m}^2 \times 10^{-11} \text{ m}^2$	$6.32 \text{ m}^2 \times 10^{-12} \text{ m}^2$	$7.33 \text{ m}^2 \times 10^{-12} \text{ m}^2$

K, hydraulic conductivity; k, permeability.

currently remain the clinical gold standard for repairing nerve defects (He et al., 2015). Their success has been attributed to the presence of an intact ECM microarchitecture and viable SCs, which concomitantly provide ideal support for orientated axon regeneration (Singh et al., 2018b). However, nerve autografting has inherent flaws: specifically, a secondary surgery at the donor site is required, dysfunction of distal organ may occur in the donor nerve, and the length and/or diameter of the autologous nerve may be insufficient for effective reconstruction (Kehoe et al., 2012). Another major shortcoming of autografts is the microstructural mismatch of nerves from different anatomic sites, which may lead to inappropriate and incomplete reinnervation and subsequently poor functional recovery (Bain et al., 1989). In previous animal experiments, we varied the fascicle spatial position to explore the effect of precise fascicle matching on the effectiveness of nerve function recovery and found that a microstructural mismatch causes poor functional recovery (Yan et al., 2017b).

Compared with autografts, decellularized allografts well confirm the role of ECM microarchitecture in nerve regeneration (Zaminy et al., 2021). In this approach, cells are removed from the decellularized allograft but the 3D microstructure and composition of the ECM are preserved (Sondell et al., 1998). The preserved ECM components create a milieu similar to peripheral nerve tissue for schwann cell adhesion and proliferation (Su et al., 2022). Some studies

show that removing the factors inhibiting axonal growth from ECM will enhance the effect of acellular nerve allograft (ANA) (Li et al., 2022). Our team developed a ANA product approved by the Chinese Food and Drug Administration (CFDA) (Guangzhou Zhongda Medical Devices Company, Guangzhou, Guangdong Province, China; Approval No. (2012) 3460641), and it has been used in clinical repair over the years. The treatments show that ANA facilitate safe and effective reconstruction of distal nerve defects with a length of 10–60 mm in human patients (Zhu et al., 2017). In the literature, another study used allografts to repair nerve defects with gaps of 30–70 mm in the clinic (Safa and Buncke, 2016). Despite these clinical successes, the reason for the limited effectiveness of ANAs in repairing proximal mixed nerve defects remains largely unknown (Thomson et al., 2022).

ANA's materials are mainly from cadavers, which limits its widespread application (Jiang et al., 2022). When ANA is used to repair long or large nerve defects, neural cell necrosis may occur due to insufficient early vascularization, especially when the graft is too thick and revascularization does not reach the center of the graft (Ray and Mackinnon, 2010). Consistent with the results of clinical and fundamental experimental studies, we speculate that ANAs do not form an optimal microenvironment for regeneration (Thomson et al., 2022). The bionic scaffold not only requires sufficient ECM components, but also provide topological guidance by mimicry of microstructure of natural eECM (Rao et al., 2021; Tiwari et al., 2022). The biomechanical

properties, such as stiffness and permeability, can be modulated during the design and fabrication of NGCs to replicate the physical cues and structural characteristics of natural ECM (Wegst et al., 2015; Yang et al., 2021).

Importantly, natural biomaterials are commonly arranged in rather sophisticated hierarchical architectures, and their components exhibit extraordinary biomechanical characteristics. In the literature, three factors were found to be critical for the development of new scaffolds: chemical composition, nano/microstructure and biomechanical properties (Sun et al., 2020). As exemplified by the development of peripheral nerve repair grafts, the first step is to analyze the microstructural characteristics of the eECM. Studies show that stiffness mediates peripheral nerve regeneration through YAP/TAZ regulation of SCs phenotype (Xu et al., 2021). The permeability of scaffold affects the nutrient mass transport, cellular infiltration (such as, macrophage, Schwann cell, fibroblast, endothelial cells), and axonal outgrowth (Varley et al., 2016; Zhang et al., 2019b). Therefore, the stiffness and permeability of the scaffold is a crucial factor for nerve regeneration in early stages.

In the current study, we used micro-CT to acquire high-resolution and high-contrast images of the microstructures of the peripheral nerve eECM (Figure 2). The micro-CT measured area of the endoneurium was larger than that measured from the SEM image, possibly because the micro-CT images lost part of the endoneurium edge as a result of the threshold setting. To the best of the authors' knowledge, we are the first to obtain detailed images of the human nerve eECM structure. We also obtained the young's modulus of human peripheral nerve, which is about 38.5 MPa. Compared with other studies, the nerve tissue obtained is relatively soft, which may be due to the relatively long time *in vitro* (Figure 5) (Zhang et al., 2019b; Bartlett et al., 2020). Permeability is widely used to characterize scaffolds for Tissue Engineering (TE) applications as it gives information about the structure porosity, pore size, tortuosity and pore interconnectivity which have an important impact in cell seeding and proliferation (Santos et al., 2020). In this study, we also quantified the porosity, pore size, tortuosity of eECM (Table 2). After reconstruction, the permeability of the three-dimensional microstructure of the nerve eECM analyzed by μ -CFD was $3.10 \pm 1.91 \times 10^{-12} \text{ m}^2$. The permeability of the fascicles was $2.19 \pm 1.09 \times 10^{-12} \text{ m}^2$, reflecting the reasonable accuracy of the image-based μ -CFD model.

The limitations of this study mainly include (Robinson, 2022): this study only analyzed the mixed nerves, lacking the analysis of sensory nerves and motor nerves, and the control group (Tiware et al., 2022); Peripheral nerve has viscoelasticity, and only tensile test cannot accurately reflect its biomechanical characteristics. It is better to use dynamic mechanical analysis for measurement (Wieringa et al., 2018); This study did not verify that bionic permeability is conducive to nerve regeneration *in vivo* and *in vitro* (Li et al., 2021); The data obtained in this experiment are difficult to construct biomimetic scaffolds on the current biological manufacturing technology, and only stay in the theoretical design.

In summary, to the best of the authors' knowledge, this study is the first of its kind to use micro-CT to obtain high-resolution and high-contrast images of the peripheral nerve eECM, thus enabling the reconstruction of realistic 3D μ -FEA and μ -CFD models for characterizing young's modulus and permeability through correlations with the experimental results from natural human nerve eECM structures. Analysis of the microstructure and biomechanical characteristics of peripheral nerve eECM would extend the current biomechanical knowledge of nerves. This study is anticipated to provide a fundamental basis for the optimal design of peripheral nerve grafts and/or scaffolds and potentially impacting the regenerative medicine.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the medical ethical committee for Clinical Research and Animal Trials of The First Affiliated Hospital of Sun Yat-sen University. The ethics committee waived the requirement of written informed consent for participation.

Author contributions

LY, QL, and JQ conceived and designed the study and wrote the manuscript. JL conducted microCT scanning and obtained 2D images. LY analyzed the data and reconstructed the endoneurium ECM of peripheral nerves. JZ, AE, QL, and ZZ analyzed the FEA model. All authors discussed the conceptual and practical implications of the methods and approved the final version of the paper.

Funding

This study was supported by the grants from the Guangdong Basic and Applied Basic Research Foundation, 2022A1515012072 (to LY) and 2020A1515010545 (to JQ); and the Science and Technology Project of Guangzhou of China, 201704030041 (to JQ).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

References

- Almeida, H. A., and Bartolo, P. J. (2021). Biomimetic boundary-based scaffold design for tissue engineering applications. *Methods Mol. Biol.* 2147, 3–18. doi:10.1007/978-1-0716-0611-7_1
- Bain, J. R., Mackinnon, S. E., and Hunter, D. A. (1989). Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plastic Reconstr. Surg.* 83 (1), 129–136. doi:10.1097/00006534-198901000-00024
- Bartlett, R. D., Eleftheriadou, D., Evans, R., Choi, D., and Phillips, J. B. (2020). Mechanical properties of the spinal cord and brain: Comparison with clinical-grade biomaterials for tissue engineering and regenerative medicine. *Biomaterials* 258, 120303. doi:10.1016/j.biomaterials.2020.120303
- Chao, L., Jiao, C., Liang, H., Xie, D., Shen, L., and Liu, Z. (2021). Analysis of mechanical properties and permeability of trabecular-like porous scaffold by additive manufacturing. *Front. Bioeng. Biotechnol.* 9, 779854. doi:10.3389/fbioe.2021.779854
- Chen, S., Du, Z., Zou, J., Qiu, S., Rao, Z., Liu, S., et al. (2019). Promoting neurite growth and schwann cell migration by the harnessing decellularized nerve matrix onto nanofibrous guidance. *ACS Appl. Mat. Interfaces* 11 (19), 17167–17176. doi:10.1021/acsami.9b01066
- Chen, Z., Wang, X., Giuliani, F., and Atkinson, A. (2015). Analyses of microstructural and elastic properties of porous SOFC cathodes based on focused ion beam tomography. *J. Power Sources* 273, 486–494. doi:10.1016/j.jpowsour.2014.09.131
- Chen, Z., Wang, X., Giuliani, F., and Atkinson, A. (2015). Microstructural characteristics and elastic modulus of porous solids. *Acta Mat.* 89, 268–277. doi:10.1016/j.actamat.2015.02.014
- Chiono, V., and Tonda-Turo, C. (2015). Trends in the design of nerve guidance channels in peripheral nerve tissue engineering. *Prog. Neurobiol.* 131, 87–104. doi:10.1016/j.pneurobio.2015.06.001
- Du, J., Chen, H., Qing, L., Yang, X., and Jia, X. (2018). Biomimetic neural scaffolds: A crucial step towards optimal peripheral nerve regeneration. *Biomater. Sci.* 6 (6), 1299–1311. doi:10.1039/c8bm00260f
- El-Bakary, M. A., El-Farahaty, K. A., and El-Sayed, N. M. (2019). Investigating the mechanical behavior of PGA/PCL copolymer surgical suture material using multiple-beam interference microscopy. *Fibers Polym.* 20 (6), 1116–1124. doi:10.1007/s12221-019-1060-9
- Entezari, A., Roohani, I., Li, G., Dunstan, C. R., Rognon, P., Li, Q., et al. (2019). Architectural design of 3D printed scaffolds controls the volume and functionality of newly formed bone. *Adv. Healthc. Mat.* 8 (1), e1801353. doi:10.1002/adhm.201801353
- Gentile, P., Mattioli-Belmonte, M., Chiono, V., Ferretti, C., Baino, F., Tonda-Turo, C., et al. (2012). Bioactive glass/polymer composite scaffolds mimicking bone tissue. *J. Biomed. Mat. Res. A* 100 (10), 2654–2667. doi:10.1002/jbm.a.34205
- Gilbert, T. W., Sellar, T. L., and Badyrak, S. F. (2006). Decellularization of tissues and organs. *Biomaterials* 27 (19), 3675–3683. doi:10.1016/j.biomaterials.2006.02.014
- Gomez, S., Vlad, M. D., Lopez, J., and Fernandez, E. (2016). Design and properties of 3D scaffolds for bone tissue engineering. *Acta Biomater.* 42, 341–350. doi:10.1016/j.actbio.2016.06.032
- Gotti, C., Sensini, A., Fornai, G., Gualandi, C., Zucchelli, A., and Focarete, M. L. (2020). Biomimetic hierarchically arranged nanofibrous structures resembling the architecture and the passive mechanical properties of skeletal muscles: A step forward toward artificial muscle. *Front. Bioeng. Biotechnol.* 8, 767. doi:10.3389/fbioe.2020.00767
- He, B., Zhu, Q. T., Chai, Y. M., Ding, X. H., Tang, J. Y., Gu, L. Q., et al. (2015). Safety and efficacy evaluation of a human acellular nerve graft as a digital nerve scaffold: A prospective, multicentre controlled clinical trial. *J. Tissue Eng. Regen. Med.* 9 (3), 286–295. doi:10.1002/term.1707
- Hoffman-Kim, D., Mitchel, J. A., and Bellamkonda, R. V. (2010). Topography, cell response, and nerve regeneration. *Annu. Rev. Biomed. Eng.* 12, 203–231. doi:10.1146/annurev-bioeng-070909-105351
- Huang, W. C., Lin, C. C., Chiu, T. W., and Chen, S. Y. (2022). 3D gradient and linearly aligned magnetic microcapsules in nerve guidance conduits with remotely spatiotemporally controlled release to enhance peripheral nerve repair. *ACS Appl. Mat. Interfaces* 14 (41), 46188–46200. doi:10.1021/acsami.2c11362
- Jiang, Y., Tang, X., Li, T., Ling, J., and Yang, Y. (2022). The success of biomaterial-based tissue engineering strategies for peripheral nerve regeneration. *Front. Bioeng. Biotechnol.* 10, 1039777. doi:10.3389/fbioe.2022.1039777
- Kehoe, S., Zhang, X. F., and Boyd, D. (2012). FDA approved guidance conduits and wraps for peripheral nerve injury: A review of materials and efficacy. *Injury* 43 (5), 553–572. doi:10.1016/j.injury.2010.12.030
- Kwan, M. K., Wall, E. J., Massie, J., and Garfin, S. R. (1992). Strain, stress and stretch of peripheral nerve. Rabbit experiments *in vitro* and *in vivo*. *Acta Orthop. Scand.* 63 (3), 267–272. doi:10.3109/17453679209154780
- Li, G., Zheng, T., Wu, L., Han, Q., Lei, Y., Xue, L., et al. (2021). Bionic microenvironment-inspired synergistic effect of anisotropic micro-nanocomposite topology and biology cues on peripheral nerve regeneration. *Sci. Adv.* 7 (28), eabi5812. doi:10.1126/sciadv.abi5812
- Li, W., Li, Z. G., Fu, X. M., Wang, X. Y., Lv, Z. X., Sun, P., et al. (2022). Transgenic Schwann cells overexpressing POU6F1 promote sciatic nerve regeneration within acellular nerve allografts. *J. Neural Eng.* 19, 066006. doi:10.1088/1741-2552/ac9e1e
- Mitsak, A. G., Kempainen, J. M., Harris, M. T., and Hollister, S. J. (2011). Effect of polycaprolactone scaffold permeability on bone regeneration *in vivo*. *Tissue Eng. Part A* 17 (13–14), 1831–1839. doi:10.1089/ten.TEA.2010.0560
- Pawelec, K. M., Koffler, J., Shahriari, D., Galvan, A., Tuszyński, M. H., and Sakamoto, J. (2018). Microstructure and *in vivo* characterization of multi-channel nerve guidance scaffolds. *Biomed. Mat.* 13 (4), 044104. doi:10.1088/1748-605X/aaad85
- Philips, C., Cornelissen, M., and Carriel, V. (2018). Evaluation methods as quality control in the generation of decellularized peripheral nerve allografts. *J. Neural Eng.* 15 (2), 021003. doi:10.1088/1741-2552/aa21a
- Pot, M. W., Faraj, K. A., Adawy, A., van Enckevort, W. J., van Moerkerk, H. T., Vlieg, E., et al. (2015). Versatile wedge-based system for the construction of unidirectional collagen scaffolds by directional freezing: Practical and theoretical considerations. *ACS Appl. Mat. Interfaces* 7 (16), 8495–8505. doi:10.1021/acsami.5b00169
- Rao, Z., Lin, T., Qiu, S., Zhou, J., Liu, S., Chen, S., et al. (2021). Decellularized nerve matrix hydrogel scaffolds with longitudinally oriented and size-tunable microchannels for peripheral nerve regeneration. *Mater. Sci. Eng. C* 120, 111791. doi:10.1016/j.msec.2020.111791
- Ray, W. Z., and Mackinnon, S. E. (2010). Management of nerve gaps: Autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. *Exp. Neurol.* 223 (1), 77–85. doi:10.1016/j.expneurol.2009.03.031
- Robinson, L. R. (2022). Traumatic injury to peripheral nerves. *Muscle Nerve* 23, 863–873. doi:10.1002/(sici)1097-4598(200006)23:6<863:aid-mus4>3.0.co;2-0
- Safa, B., and Buncke, G. (2016). Autograft substitutes: Conduits and processed nerve allografts. *Hand Clin.* 32 (2), 127–140. doi:10.1016/j.hcl.2015.12.012
- Santos, J., Pires, T., Gouveia, B. P., Castro, A. P. G., and Fernandes, P. R. (2020). On the permeability of TPMS scaffolds. *J. Mech. Behav. Biomed. Mat.* 110, 103932. doi:10.1016/j.jmbbm.2020.103932
- Schmidt, C. E., and Leach, J. B. (2003). Neural tissue engineering: Strategies for repair and regeneration. *Annu. Rev. Biomed. Eng.* 5, 293–347. doi:10.1146/annurev-bioeng.5.011303.120731
- Singh, A., Asikainen, S., Teotia, A. K., Shiekh, P. A., Huutilainen, E., Qayoom, I., et al. (2018). Biomimetic photocurable three-dimensional printed nerve guidance channels with aligned cryomatrix lumen for peripheral nerve regeneration. *ACS Appl. Mat. Interfaces* 10 (50), 43327–43342. doi:10.1021/acsami.8b11677
- Singh, S. P., Shukla, M., and Srivastava, R. K. (2018). Lattice modeling and CFD simulation for prediction of permeability in porous scaffolds. *Mater. Today Proc.* 5 (9), 18879–18886. doi:10.1016/j.matpr.2018.06.236
- Sondell, M., Lundborg, G., and Kanje, M. (1998). Regeneration of the rat sciatic nerve into allografts made acellular through chemical extraction. *Brain Res.* 795 (1–2), 44–54. doi:10.1016/s0006-8993(98)00251-0

- Su, Q., Nasser, M. I., He, J., Deng, G., Ouyang, Q., Zhuang, D., et al. (2022). Engineered schwann cell-based therapies for injury peripheral nerve reconstruction. *Front. Cell. Neurosci.* 16, 865266. doi:10.3389/fncel.2022.865266
- Sun, J. H., Li, G., Wu, T. T., Lin, Z. J., Zou, J. L., Huang, L. J., et al. (2020). Decellularization optimizes the inhibitory microenvironment of the optic nerve to support neurite growth. *Biomaterials* 258, 120289. doi:10.1016/j.biomaterials.2020.120289
- Thomson, C., Schneider, J. M., Pohl, U., and Power, D. M. (2022). Failed acellular nerve allografts: A critical review. *Ann. Plast. Surg.* 89 (1), 63–71. doi:10.1097/SAP.0000000000003055
- Tiwari, A. P., Lokai, T., Albin, B., and Yang, I. H. (2022). A review on the technological advances and future perspectives of axon guidance and regeneration in peripheral nerve repair. *Bioeng. (Basel)* 9 (10), 562. doi:10.3390/bioengineering9100562
- Varley, M. C., Neelakantan, S., Clyne, T. W., Dean, J., Brooks, R. A., and Markaki, A. E. (2016). Cell structure, stiffness and permeability of freeze-dried collagen scaffolds in dry and hydrated states. *Acta Biomater.* 33, 166–175. doi:10.1016/j.actbio.2016.01.041
- Wegst, U. G., Bai, H., Saiz, E., Tomsia, A. P., and Ritchie, R. O. (2015). Bioinspired structural materials. *Nat. Mat.* 14 (1), 23–36. doi:10.1038/nmat4089
- Wieringa, P. A., Goncalves de Pinho, A. R., Micera, S., van Wezel, R. J. A., and Moroni, L. (2018). Biomimetic architectures for peripheral nerve repair: A review of biofabrication strategies. *Adv. Healthc. Mat.* 7 (8), e1701164. doi:10.1002/adhm.201701164
- Xu, Z., Orkwis, J. A., and Harris, G. M. (2021). Cell shape and matrix stiffness impact schwann cell plasticity via YAP/TAZ and rho GTPases. *Int. J. Mol. Sci.* 22 (9), 4821. doi:10.3390/ijms22094821
- Yan, L., Guo, Y., Qi, J., Zhu, Q., Gu, L., Zheng, C., et al. (2017). Iodine and freeze-drying enhanced high-resolution MicroCT imaging for reconstructing 3D intraneural topography of human peripheral nerve fascicles. *J. Neurosci. Methods* 287, 58–67. doi:10.1016/j.jneumeth.2017.06.009
- Yan, L., Liu, S., Qi, J., Zhang, Z., Zhong, J., Li, Q., et al. (2019). Three-dimensional reconstruction of internal fascicles and microvascular structures of human peripheral nerves. *Int. J. Numer. Method. Biomed. Eng.* 35 (10), e3245. doi:10.1002/cnm.3245
- Yan, L., Yao, Z., Lin, T., Zhu, Q., Qi, J., Gu, L., et al. (2017). The role of precisely matching fascicles in the quick recovery of nerve function in long peripheral nerve defects. *Neuroreport* 28 (15), 1008–1015. doi:10.1097/WNR.0000000000000873
- Yang, C. Y., Huang, W. Y., Chen, L. H., Liang, N. W., Wang, H. C., Lu, J., et al. (2021). Neural tissue engineering: The influence of scaffold surface topography and extracellular matrix microenvironment. *J. Mat. Chem. B* 9 (3), 567–584. doi:10.1039/d0tb01605e
- Yoshii, S., Oka, M., Shima, M., Taniguchi, A., and Akagi, M. (2003). Bridging a 30-mm nerve defect using collagen filaments. *J. Biomed. Mat. Res.* 67a (2), 467–474. doi:10.1002/jbm.a.10103
- Zaminy, A., Sayad-Fathi, S., Kasmaie, F. M., Jahromi, Z., and Zendedel, A. (2021). Decellularized peripheral nerve grafts by a modified protocol for repair of rat sciatic nerve injury. *Neural Regen. Res.* 16 (6), 1086–1092. doi:10.4103/1673-5374.300449
- Zhang, S., Sanjairaj, V., Chong, G. L., Fuh, Y. H. J., and Lu, W. F. (2019). Computational design and optimization of nerve guidance conduits for improved mechanical properties and permeability. *J. Biomech. Eng.* 141. doi:10.1115/1.4043036
- Zhang, X. Y., Fang, G., Leeftang, S., Zadpoor, A. A., and Zhou, J. (2019). Topological design, permeability and mechanical behavior of additively manufactured functionally graded porous metallic biomaterials. *Acta Biomater.* 84, 437–452. doi:10.1016/j.actbio.2018.12.013
- Zhu, S., Liu, J., Zheng, C., Gu, L., Zhu, Q., Xiang, J., et al. (2017). Analysis of human acellular nerve allograft reconstruction of 64 injured nerves in the hand and upper extremity: A 3 year follow-up study. *J. Tissue Eng. Regen. Med.* 11 (8), 2314–2322. doi:10.1002/term.2130
- Zhu, W., Tringale, K. R., Woller, S. A., You, S., Johnson, S., Shen, H., et al. (2018). Rapid continuous 3D printing of customizable peripheral nerve guidance conduits. *Mat. Today (Kidlington)* 21 (9), 951–959. doi:10.1016/j.matod.2018.04.001



OPEN ACCESS

EDITED BY

Lei Xu,
Fudan University, China

REVIEWED BY

Adriano De Oliveira Andrade,
Federal University of Uberlândia, Brazil
Ivan Stojkovic,
Temple University, United States

*CORRESPONDENCE

Jiantao Yang
yangjt5@mail.sysu.edu.cn
Qingtang Zhu
zhuqingt@mail.sysu.edu.cn

†These authors have contributed
equally to this work and share first
authorship

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 24 September 2022

ACCEPTED 14 November 2022

PUBLISHED 07 December 2022

CITATION

Gu F, Fan J, Cai C, Wang Z, Liu X,
Yang J and Zhu Q (2022) Automatic
detection of abnormal hand gestures
in patients with radial, ulnar, or median
nerve injury using hand pose
estimation. *Front. Neurol.* 13:1052505.
doi: 10.3389/fneur.2022.1052505

COPYRIGHT

© 2022 Gu, Fan, Cai, Wang, Liu, Yang
and Zhu. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License](#)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Automatic detection of abnormal hand gestures in patients with radial, ulnar, or median nerve injury using hand pose estimation

Fanbin Gu^{1†}, Jingyuan Fan^{1†}, Chengfeng Cai^{2†},
Zhaoyang Wang¹, Xiaolin Liu^{1,3,4}, Jiantao Yang^{1,3,4*} and
Qingtang Zhu^{1,3,4*}

¹Department of Microsurgery, Orthopedic Trauma and Hand Surgery, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, ²Department of Hand and Foot Rehabilitation, Guangdong Provincial Work Injury Rehabilitation Hospital, Guangzhou, China, ³Guangdong Provincial Engineering Laboratory for Soft Tissue Biofabrication, Guangzhou, China, ⁴Guangdong Provincial Key Laboratory for Orthopedics and Traumatology, Guangzhou, China

Background: Radial, ulnar, or median nerve injuries are common peripheral nerve injuries. They usually present specific abnormal signs on the hands as evidence for hand surgeons to diagnose. However, without specialized knowledge, it is difficult for primary healthcare providers to recognize the clinical meaning and the potential nerve injuries through the abnormalities, often leading to misdiagnosis. Developing technologies for automatically detecting abnormal hand gestures would assist general medical service practitioners with an early diagnosis and treatment.

Methods: Based on expert experience, we selected three hand gestures with predetermined features and rules as three independent binary classification tasks for abnormal gesture detection. Images from patients with unilateral radial, ulnar, or median nerve injuries and healthy volunteers were obtained using a smartphone. The landmark coordinates were extracted using Google MediaPipe Hands to calculate the features. The receiver operating characteristic curve was employed for feature selection. We compared the performance of rule-based models with logistic regression, support vector machine and of random forest machine learning models by evaluating the accuracy, sensitivity, and specificity.

Results: The study included 1,344 images, twenty-two patients, and thirty-four volunteers. In rule-based models, eight features were finally selected. The accuracy, sensitivity, and specificity were (1) 98.2, 91.7, and 99.0% for radial nerve injury detection; (2) 97.3, 83.3, and 99.0% for ulnar nerve injury detection; and (3) 96.4, 87.5, and 97.1% for median nerve injury detection, respectively. All machine learning models had accuracy above 95% and sensitivity ranging from 37.5 to 100%.

Conclusion: Our study provides a helpful tool for detecting abnormal gestures in radial, ulnar, or median nerve injuries with satisfying accuracy, sensitivity, and specificity. It confirms that hand pose estimation could automatically analyze

and detect the abnormalities from images of these patients. It has the potential to be a simple and convenient screening method for primary healthcare and telemedicine application.

KEYWORDS

peripheral nerve injury, hand pose estimation, hand gesture, abnormal gesture detection, expert system, machine learning

Introduction

Peripheral nerve injury (PNI) is devastating and frequently results in life-long disability, severely decreasing patients' quality of life. It affects more than one million people worldwide, with an incidence of ~13–23 per 100,000 persons per year (1, 2). Among PNIs of the upper limb, radial, ulnar, and median nerve injuries are common, causing sensory and motor dysfunction in the control area of the damaged nerves. In medical practice, early detection of nerve injury may prevent patients from delayed diagnoses, delayed repairs, and potentially worse outcomes. However, the anatomical complexity of the hand may bring difficulties in examination and diagnosis (3). According to a large German database of hand and forearm injuries, nerve injuries were among the most commonly missed injuries, accounting for 24.8% of missed hand injuries (4). The misdiagnosis rate would be considerably higher in areas with insufficient specialized services. In China, for example, there have been reports that most treated patients with missed nerve injuries were from primary healthcare hospitals (5, 6).

The clinical diagnosis of nerve injury depends on clinical history, clinical symptoms, and physical and neurological examinations (7, 8). Among the examinations, electromyography (EMG), nerve conduction studies (NCS), MRI, and high-resolution ultrasonography have been successfully used as diagnostic methods for PNI (9–11). However, these examinations are expensive, invasive, and rely on specialized equipment and professional operators, restricting their application in primary assessment. Given these problems, there is a need for an affordable, non-invasive, and accessible method to detect possible nerve injuries for initial evaluation of the hand, significantly helping clinicians who are not specialized in hand surgery and benefiting patients in remote or rural areas. Some abnormal signs or deformities of the hand may indicate the nerve injuries, such as the limited extension of the wrist and digits for radial nerve injury, the claw hand deformity and the Wartenberg sign for ulnar nerve injury, and the ape hand deformity for median nerve injury. These clinical experiences have been well-acknowledged in hand surgery, where nerve injuries can lead to various morphological changes based on the different innervation of the hand (12, 13). So far, deformity of digit(s)' joint(s) has been used to describe specific nerve injury as supporting evidence (14–16). This

unique connection between abnormalities and nerve injury was determined by the independent anatomical nerve innervation to the muscle. Additionally, it is possible to observe these abnormalities through several gestures. Therefore, detecting abnormal gestures can be a simple method for predicting hand nerve injuries.

With the advancement in computer vision, hand pose estimation is possible using automatic analysis techniques for detecting the hand and predicting the articulated joint locations from images or videos (17, 18). Hand pose estimation is crucial and popular in achieving several tasks, such as gesture recognition (19), action recognition (20), and sign language recognition (21), in general populations. There has been an increasing interest in hand pose estimation techniques for medical research and application, such as investigating automatic assessment of hand rehabilitation (22) and using 3D cameras for the classification of Parkinson's disease (23). These techniques seem to help extract the features of the hand. Among them, MediaPipe Hands is a deep-learning-based hand-tracking solution provided by Google. It does not require specialized hardware and is adequately light to run in real-time on mobile devices; it can also predict 21 landmarks of a hand from a single RGB camera with high prediction quality (24). MediaPipe Hands are popular in human-computer interaction systems and applications of virtual and augmented reality (21, 24, 25). However, it remains unknown whether MediaPipe Hands could detect the abnormalities in patients with nerve injuries, distinguish normal and abnormal gestures in the medical application, and further predict possible injuries based on rule-based methods.

The purpose of the present study was to propose an automatic method for abnormal hand gesture detection caused by radial, ulnar, or median nerve injury. We hypothesize that hand pose estimation could provide specific features to classify the presence of nerve injury and predict the exact type of injury.

Materials and methods

Participants recruitment

The experimental procedure of this study is illustrated in Figure 1. From June 2021 to October 2022, we recruited

preoperative patients with unilateral radial, ulnar, or median nerve injuries who were scheduled to undergo surgery in our department. The diagnosis was made after a comprehensive evaluation of the related history, the observation of typical abnormal gestures, and EMG or ultrasound examination, which was further confirmed in the surgical exploration. Patients with any musculoskeletal disease (such as tendon rupture or arthritis) and neurological diseases (such as stroke and traumatic brain injury) that would influence the movement of the hands (not including the wrist joint) and those who refused to participate were excluded. A group of healthy volunteers were also invited during this period. The inclusion criteria for the volunteers were the absence of symptoms such as clumsiness or numbness of hands and no abnormal findings in the physical examination that indicated nerve injuries. All participants understood the study's protocol and cooperated in taking the images. Verbal consent was obtained for the usage of their images for research purposes. This study was approved by the institutional review board of the First Affiliated Hospital of Sun Yat-sen University (ID: [2021]387).

Design of gestures

Based on the expert experience mentioned above (12, 13), we chose three gestures (Figure 2) to detect abnormalities caused by radial, ulnar, or median nerve injuries as three binary classification tasks. The normal and the expected abnormal gestures were demonstrated as follows:

- Gesture 1 was used to detect radial nerve injuries. Participants were requested to make a maximum abduction of digits to the medial and lateral sides with full extension. Patients with radial nerve injury would have impaired functions of the musculus abductor pollicis longus, the musculus extensor pollicis longus, the musculus extensor pollicis brevis, and the musculus extensor digitorum. The patients were expected to have difficulties extending all metacarpophalangeal (MCP) joints.
- Gesture 2 was used to detect ulnar nerve injuries. Participants were requested to make adduction of digits in full extension toward the middle finger. Patients with ulnar nerve injury would have impaired the function of the musculus lumbricales 3 and 4 and the musculus interosseous palmaris. This would lead to flexed proximal interphalangeal (PIP) joints and distal interphalangeal (DIP) joints of the ring and little fingers (the claw hand deformity) and limited adduction of the ring and particularly the little fingers (the Wartenberg sign).
- Gesture 3 was used to detect median nerve injuries. Participants were requested to perform a tip-to-tip pinch between the thumb and the index finger, i.e., to form an "OK" gesture. Patients with median nerve injury would have impaired functions of the musculus opponens pollicis,

the musculus flexor pollicis brevis, and the musculus lumbricales 1 and 2. When performing this gesture, the decreased range of palmar abduction of the thumb would occur, and it could have trouble flexing the thumb and index finger, even a failure to make contact with the tips of the two digits.

Experimental setup

Before taking images, all participants washed and cleaned their hands. Jewelry, watches, and clothing on the wrist or hand were removed. Then, the hand was placed on the test table with the forearm kept in a supination position. Participants would first perform Gesture 1 for radial nerve classification. Injured hands with radial nerve injury were put in the target group; injured hands with ulnar or median nerve injury and hands without nerve injury were put in the non-target group. Then, stratified random sampling was used between the target and non-target groups to form the training and testing sets. The ratio of training to testing sets was $\sim 3:1$. Next, Gesture 2 and Gesture 3 were performed in the same way to separate respective training and testing sets.

Each gesture was requested to be performed four times with a rest interval of ~ 10 s to obtain more available data. For every gesture of a hand, four images were obtained. The images were taken 40–50 cm above the hand between a slightly radial view and a slightly ulnar view using a smartphone (iPhone XS Max, Apple Inc., image resolution: 1,980*1,080 p) to make them more different. If the forearm had limited supination, images would be taken from the exact distance parallel to the palm.

Data processing

All images were analyzed using MediaPipe Hands solution (Version 0.8.9) with Python (Version 3.8). MediaPipe Hands would first detect and locate the hand through a palm detector algorithm. If the hand was successfully located in the image, then the coordinates of the landmarks could be obtained by its landmark model (24) (Figure 3). If the algorithm did not detect the hand, the image could not be recognized and was excluded from further analysis. After receiving the coordinates, features could be automatically calculated using the following equations:

$$\theta = \cos^{-1} \left(\frac{\vec{S}_1 \cdot \vec{S}_2}{|\vec{S}_1| \cdot |\vec{S}_2|} \right) \quad (1)$$

$$d = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2} \quad (2)$$

$$d' = \frac{d_{\text{original}}}{d_{\text{standard}}} \quad (3)$$

where

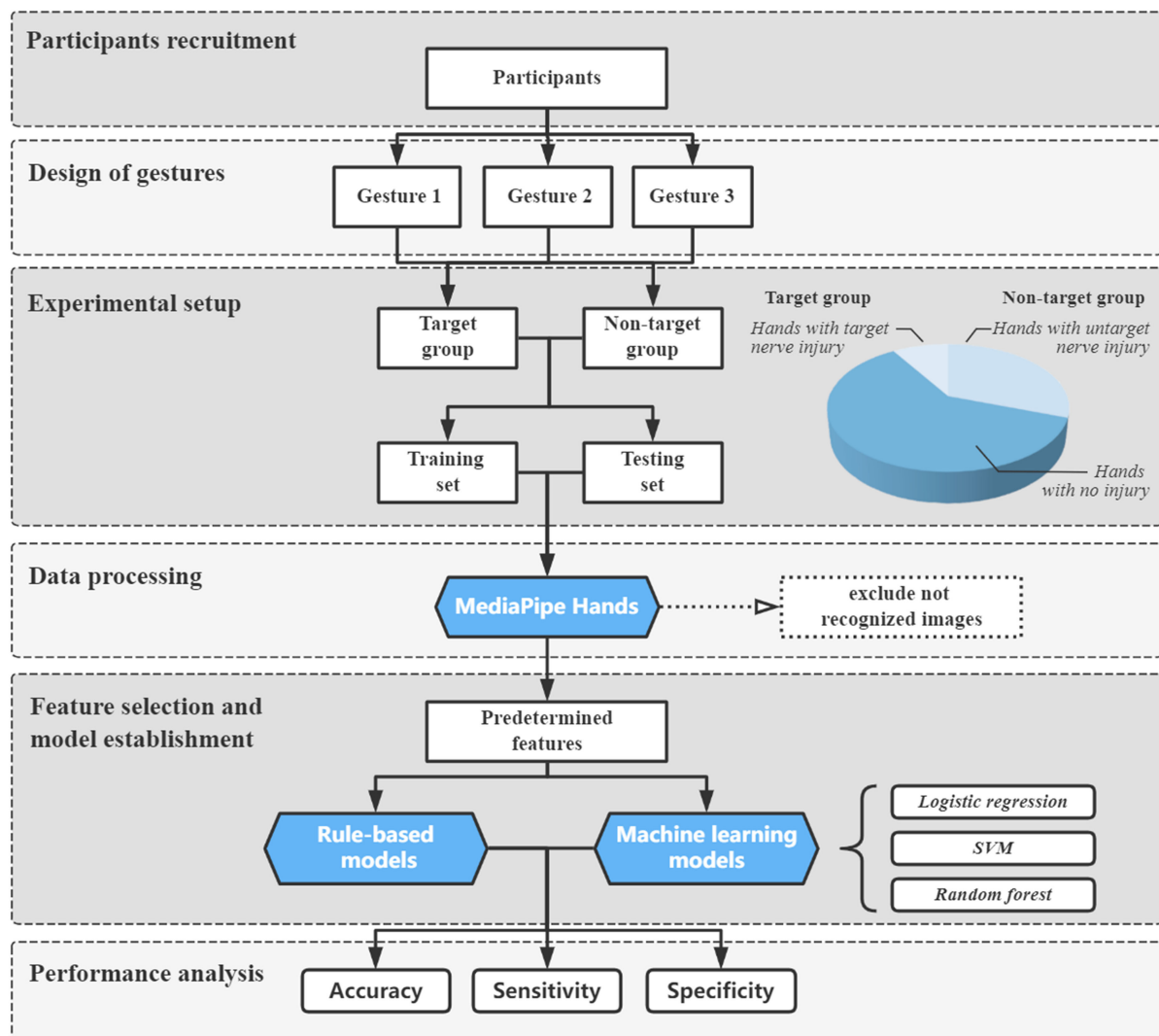


FIGURE 1
Diagram of the experimental procedure.

Equation (1) calculates the targeted angle θ , where $\vec{S}_1$ and $\vec{S}_2$ represent the vectors of the related phalanges (26);

Equation (2) calculates the targeted distance d , where x_1, y_1, z_1 and x_2, y_2, z_2 represent the coordinates of the related landmarks;

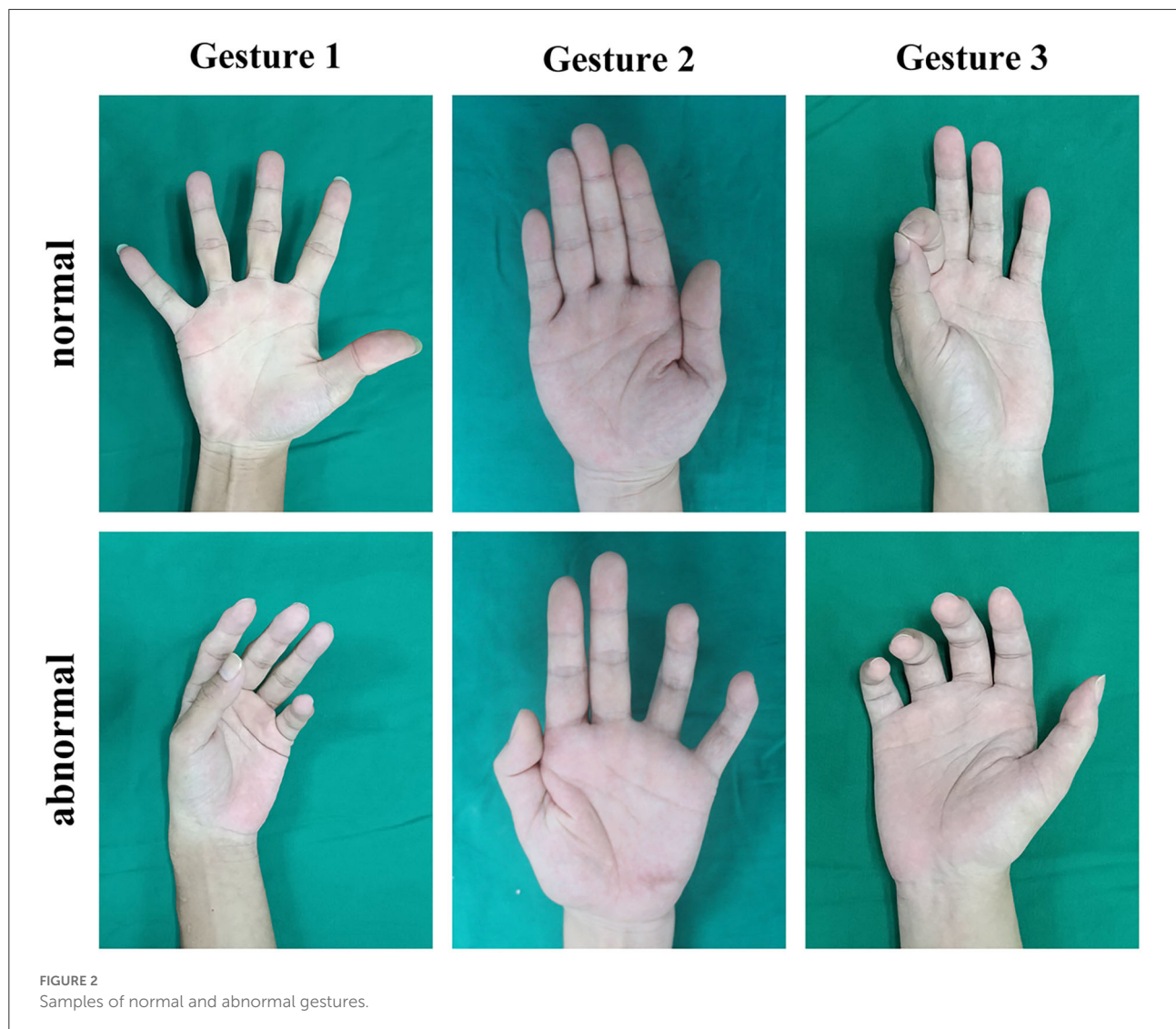
Equation (3) standardizes any distance values d' by dividing its original distance $d_{original}$ by the distance between the landmarks of the tip and IP joints of the thumb $d_{standard}$.

Feature selection and model establishment

Since the radial, ulnar, and median nerves have independent innervations to the muscle (mentioned in the "Design of

gestures" section), medical knowledge and expert experience can provide a series of primary and anatomical-based features. For example, the ulnar nerve is related to the movement of the ring and little fingers, so the features of these two fingers are much more important than the other digits, and irrelevant features could be ignored. In this consideration, we predetermined 23 features with our experience, including joints' angles, key point's distance, and their combinations (Table 1), for feature selection and model establishment.

In the study, two different methods for model establishment were compared. One was the rule-based method, which means that the model was built under a manually designed feature selection and decision-making processes based on knowledge and experience. The other was the machine learning (ML) method, which means that the whole process was learned from



the data. The logistic regression (LR) model, the support vector machine (SVM) model, and the random forest (RF) model were chosen for the ML method. For each gesture, one rule-based model and three ML models were established.

In the rule-based method, feature selection for the predetermined features was analyzed using the receiver operating characteristic (ROC) curve, a standard method to assess the performance of binary classification models (27, 28). The classification efficacy of features could be assessed by calculating the area under the curve (AUC), which was thought to be poor (<0.6), fair ($0.6\text{--}0.7$), good ($0.7\text{--}0.8$), very good ($0.8\text{--}0.9$), or excellent (>0.90) (29). We calculated the AUC of predetermined features using the training sets, filtered features with an AUC below 0.8, and selected at least two features in each gesture classification model as the classifiers. Next, we determined the threshold value of each classifier according to

ROC curves. Finally, all classifiers were ensembled according to the rules of the model: if every classifier was below the threshold, the gesture was predicted as normal and regarded as uninjured; otherwise, the gesture was predicted as abnormal and regarded as injured.

In the ML models, all predetermined features were taken, standardized, and trained in the training sets using scikit-learn python application program interface (API) (<https://scikit-learn.org.cn/>). Grid search with 5-fold cross-validation was performed to find the best hyperparameters, enhancing the efficacy of the ML models. The feature selection of ML models was “embedded” and completed in the training process. We used “coef_” API in the LR and SVM models and “feature_importances_” API in the RF model to evaluate the importance of features and compare the results of feature selection between rule-based and machine learning methods.

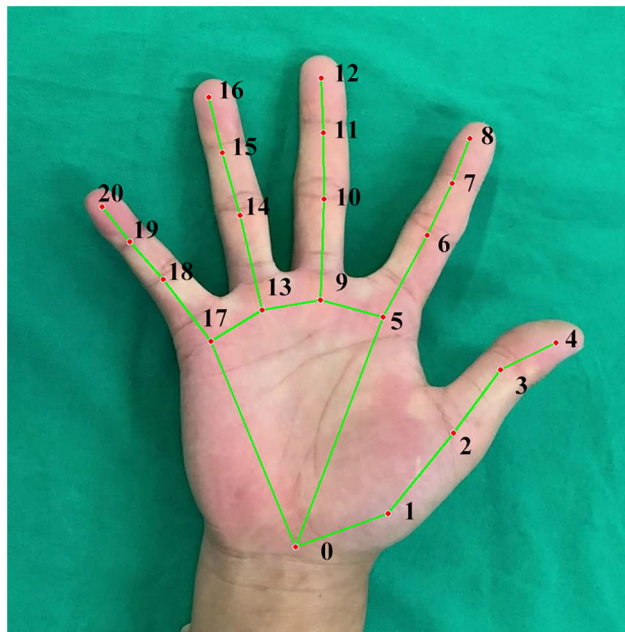


FIGURE 3
The 21 landmarks of the skeleton model of MediaPipe Hands.

0	Wrist	11	Middle_DIP
01	Thumb_CMC	12	Middle_TIP
02	Thumb_MCP	13	Ring_MCP
03	Thumb_IP	14	Ring_PIP
04	Thumb_TIP	15	Ring_DIP
05	Index_MCP	16	Ring_TIP
06	Index_PIP	17	Little_MCP
07	Index_DIP	18	Little_PIP
08	Index_TIP	19	Little_DIP
09	Middle_MCP	20	Little_TIP
10	Middle_PIP		

Performance analysis

The performance of the rule-based model and the LR, SVM, and RF models was evaluated using the testing sets for accuracy, sensitivity, and specificity. The confusion matrixes were used to visualize the agreement between the prediction and the actual label. The indices used in this study were calculated as follows:

$$accuracy = \frac{TP + TN}{TP + FP + TN + FN} \times 100\%$$

$$sensitivity = \frac{TP}{TP + FN} \times 100\%$$

$$specificity = \frac{TN}{FP + TN} \times 100\%$$

where

TP represents the number of abnormal gestures correctly predicted as abnormal,

FP represents the number of normal gestures wrongly predicted as abnormal,

FN represents the number of abnormal gestures wrongly predicted as normal,

TN represents the number of normal gestures correctly predicted as normal.

Statistical analysis

For predetermined features, the mean values in all target and non-target groups were presented as mean $\pm$ standard deviation (SD) with a 95% confidence interval (CI). An independent *t*-test was used to analyze the difference between the target and non-target groups. Subgroup analysis was also performed for features in each rule-based model with an independent *t*-test. A *P*-value of <0.05 was considered statistically significant. The statistical analysis was performed using IBM SPSS version 25 (IBM, Armonk, NY, USA).

Results

The study included twenty-two patients and thirty-four healthy volunteers who met the inclusion and exclusion criteria. The demographic of the participants is summarized in Table 2, where the mean ages of patients and volunteers were 33.04 ± 12.00 and 34.67 ± 13.84 , respectively. Detailed information on patients is shown in Table 3. Among the patients, ten were diagnosed with radial nerve injury, five with ulnar nerve injury, one with median nerve injury, and six with combined ulnar and median nerve injuries. We obtained 1,344 images in total, with 448 images in each gesture classification task. MediaPipe Hands failed to recognize four images, and the overall recognition rate was 99.7%. All non-recognized images belonged to the training

TABLE 1 Description and statistical analysis of the predetermined features.

No.	Feature name	Description	Group	Mean	SD	95% CI	P	AUC
Gesture 1								
01	ag_thumb_MCP	Angle of thumb MCP joint	Non-target	5.49	3.59	(5.083, 5.889)	0.00	0.95
			Target	21.36	7.27	(18.540, 24.181)		
02	ag_index_MCP	Angle of index MCP joint	Non-target	8.46	3.30	(8.090, 8.831)	0.00	0.85
			Target	19.02	9.95	(15.163, 22.876)		
03	ag_middle_MCP	Angle of middle MCP joint	Non-target	4.87	2.39	(4.606, 5.142)	0.00	0.89
			Target	17.44	11.74	(12.884, 21.987)		
04	ag_ring_MCP	Angle of ring MCP joint	Non-target	6.99	3.14	(6.639, 7.343)	0.00	0.85
			Target	19.80	11.87	(15.203, 24.407)		
05	ag_little_MCP	Angle of little MCP joint	Non-target	13.75	6.37	(13.038, 14.466)	0.00	0.68
			Target	24.73	16.13	(18.479, 30.989)		
Gesture 2								
06	ag_ring_PIP	Angle of ring PIP joint	Non-target	6.75	7.08	(5.950, 7.548)	0.00	0.68
			Target	13.81	12.95	(9.145, 18.482)		
07	ag_ring_DIP	Angle of ring DIP joint	Non-target	4.74	6.28	(4.030, 5.447)	0.00	0.68
			Target	13.05	15.13	(7.596, 18.507)		
08	ag_ring	Sum of feature 06 and 07	Non-target	11.49	12.51	(10.076, 12.899)	0.00	0.67
			Target	26.87	27.13	(17.084, 36.646)		
09	ag_little_PIP	Angle of little PIP joint	Non-target	7.02	4.48	(6.511, 7.523)	0.00	0.66
			Target	12.46	9.35	(9.086, 15.828)		
10	ag_little_DIP	Angle of little DIP joint	Non-target	5.85	5.72	(5.208, 6.498)	0.00	0.71
			Target	13.70	12.43	(9.218, 18.184)		
11	ag_little	Sum of feature 09 and 10	Non-target	12.87	9.57	(11.790, 13.950)	0.00	0.66
			Target	26.16	21.39	(18.445, 33.871)		
12	web3	Angle of the third webspace	Non-Target	1.58	1.00	(1.462, 1.688)	0.00	0.99
			Target	6.50	1.83	(5.837, 7.158)		
13	web4	Angle of the fourth webspace	Non-target	1.75	1.51	(1.583, 1.924)	0.00	0.89
			Target	8.33	5.27	(6.433, 10.234)		
Gesture 3								
14	ag_thumb_CMC*	Angle of thumb CMC joint	Non-target	−28.16	6.62	(−28.902, −27.427)	0.19	0.59
			Target	−30.16	5.61	(−32.785, −27.534)		
15	ag_thumb_MCP*	Angle of thumb MCP joint	Non-target	−24.10	9.88	(−25.198, −22.997)	0.01	0.65
			Target	−30.61	12.01	(−36.229, −24.991)		
16	ag_thumb_IP*	Angle of thumb IP joint	Non-target	−42.20	19.62	(−44.39, −40.019)	0.72	0.49
			Target	−39.89	28.04	(−53.009, −26.765)		
17	ag_thumb*	Sum of feature 14, 15, and 16	Non-target	−94.47	19.51	(−96.64, −92.293)	0.38	0.61
			Target	−100.66	30.51	(−114.934, −86.378)		
18	ag_index_MCP*	Angle of index MCP joint	Non-target	−32.72	5.41	(−33.327, −32.122)	0.03	0.39
			Target	−29.91	6.87	(−33.129, −26.694)		
19	ag_index_PIP*	Angle of index PIP joint	Non-target	−70.28	18.33	(−72.319, −68.237)	0.02	0.33
			Target	−55.08	25.78	(−67.146, −43.018)		
20	ag_index_DIP*	Angle of thumb DIP joint	Non-target	−36.99	13.85	(−38.531, −35.445)	0.62	0.54
			Target	−40.21	28.77	(−53.675, −26.748)		
21	ag_index*	Sum of feature 18, 19, and 20	Non-target	−139.99	19.53	(−142.166, −137.815)	0.20	0.60
			Target	−125.21	49.30	(−148.279, −102.131)		
22	ag_palmab*	Angle of thumb palmar abduction	Non-target	−19.95	8.34	(−20.881, −19.023)	0.00	0.87
			Target	−9.30	4.43	(−11.379, −7.231)		
23	dis_tip	Distance of thumb tip to index tip	Non-target	0.72	0.28	(0.693, 0.756)	0.02	0.66
			Target	1.19	0.81	(0.813, 1.575)		

SD, standard deviation; CI, confidence interval; AUC, area under the curve. The unit for all angle features was degree. Feature 23 was a standardized value with no unit. Features after selection in the rule-based models were presented in bold.

*Represents a negative value that was taken for this feature to adapt the classification rules.

set of Gesture 3. In total, three rule-based models and nine ML models were established.

The statistical analysis of the predetermined features is shown in Table 1. In the independent *t*-test, all 23 predetermined features significantly differed between target and non-target groups, except 5 in Gesture 3. In the rule-based methods, eight features were selected after feature selection. In the subgroup analysis (shown in Figure 4), most selected features showed no significant difference in the non-target groups, except the angle of the third webspace and the tip distance between the thumb and the index finger. When compared with the ML method, selected features in the rule-based method also showed higher weight or importance in the ML models. For example, in Gesture 2, the angles of the third and fourth webspaces were selected in the rule-based model and were the only features in the logistic regression model. The hyperparameters, coefficients,

and feature importance of all ML models are summarized in Supplemental material 1.

The performance of all models is shown in Table 4, Figure 5. The accuracy, sensitivity, and specificity of rule-based models were (1) 98.2, 91.7, and 99.0% for radial nerve injury detection; (2) 97.3, 83.3% and 99.0% for ulnar nerve injury detection; and (3) 96.4, 87.5, and 97.1% for median nerve injury detection, respectively. In the ML models, radial nerve injury classification achieved the best performance with 100% accuracy. In all classification tasks, the rule-based method maintained sensitivity above 80%. In comparison, lower sensitivity appears in median nerve injury classification, with 62.5% in the SVM and RF models and only 37.5% in the RL model.

Discussion

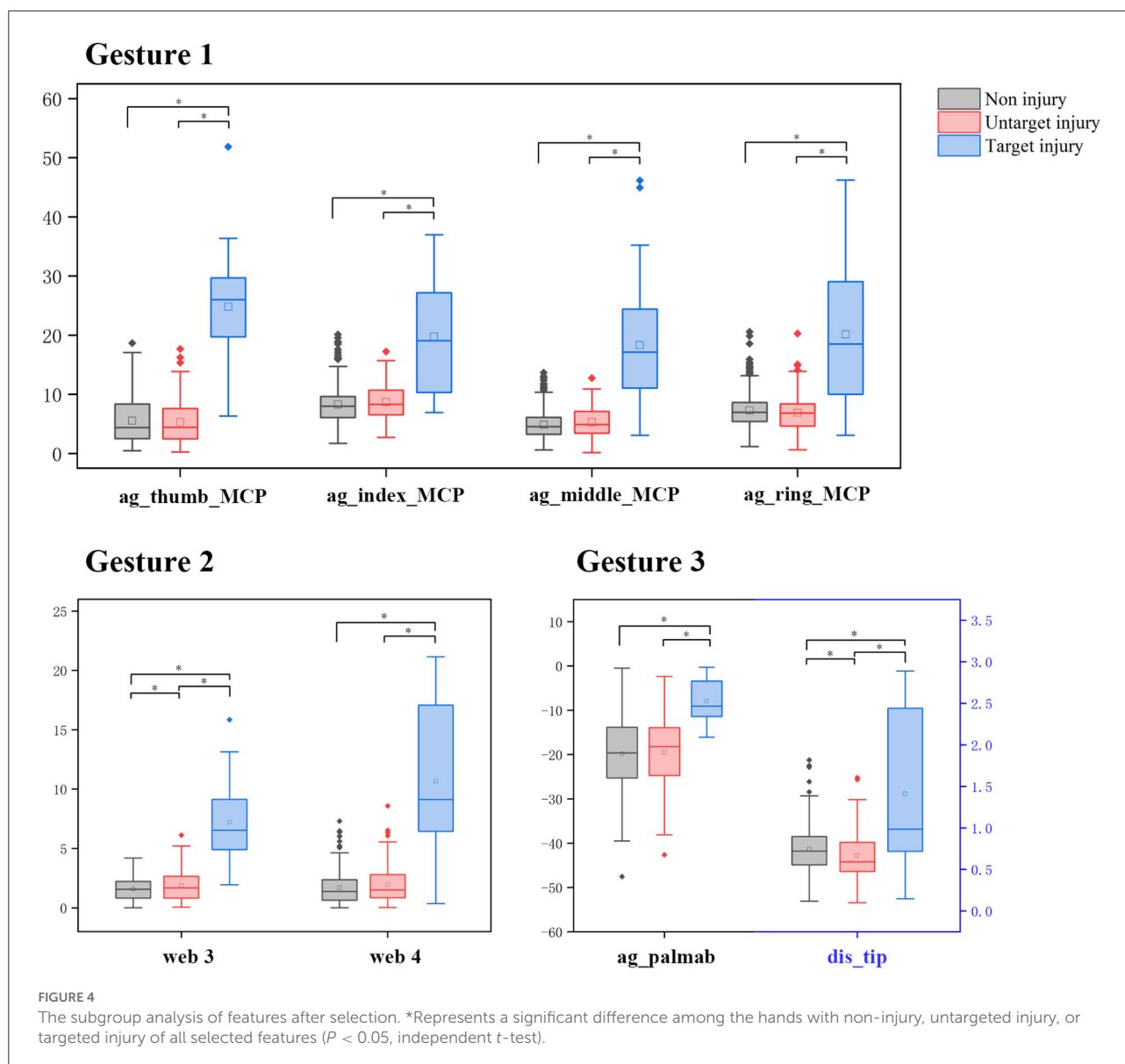
An abnormal gesture can be recognized by well-trained hand surgeons and indicates the presence of radial, ulnar, or median nerve injury before the diagnostic examination. In line with this diagnosis, we designed an automatic detection procedure for abnormal gestures enabled by an advanced hand pose estimation algorithm and rule-based models. According to the result, several features help detect the abnormalities

TABLE 2 Demographics of the participants.

	Patients (N = 22)	Volunteers (N = 34)
Age (year)	33.04 ± 12.00	34.67 ± 13.84
Gender (male)	21 (95.5%)	17 (50%)
Dominate hand (right)	22 (100%)	34 (100%)

TABLE 3 Characteristics of the patients.

No.	Gender	Age	Injured side	Radial nerve injury	Ulnar nerve injury	Median nerve injury
P1	Men	67	Right		✓	✓
P2	Men	27	Left		✓	✓
P3	Men	36	Left	✓		
P4	Women	27	Left	✓		
P5	Men	36	Right	✓		
P6	Men	37	Right		✓	
P7	Men	31	Left		✓	
P8	Men	29	Right		✓	
P9	Men	58	Right	✓		
P10	Men	26	Left	✓		
P11	Men	19	Right		✓	✓
P12	Men	21	Right		✓	
P13	Men	26	Right			✓
P14	Men	21	Left	✓		
P15	Men	32	Left	✓		
P16	Men	35	Left	✓		
P17	Men	36	Right	✓		
P18	Men	27	Left		✓	✓
P19	Men	17	Right		✓	✓
P20	Men	32	Left	✓		
P21	Men	46	Left		✓	✓
P22	Men	41	Right		✓	



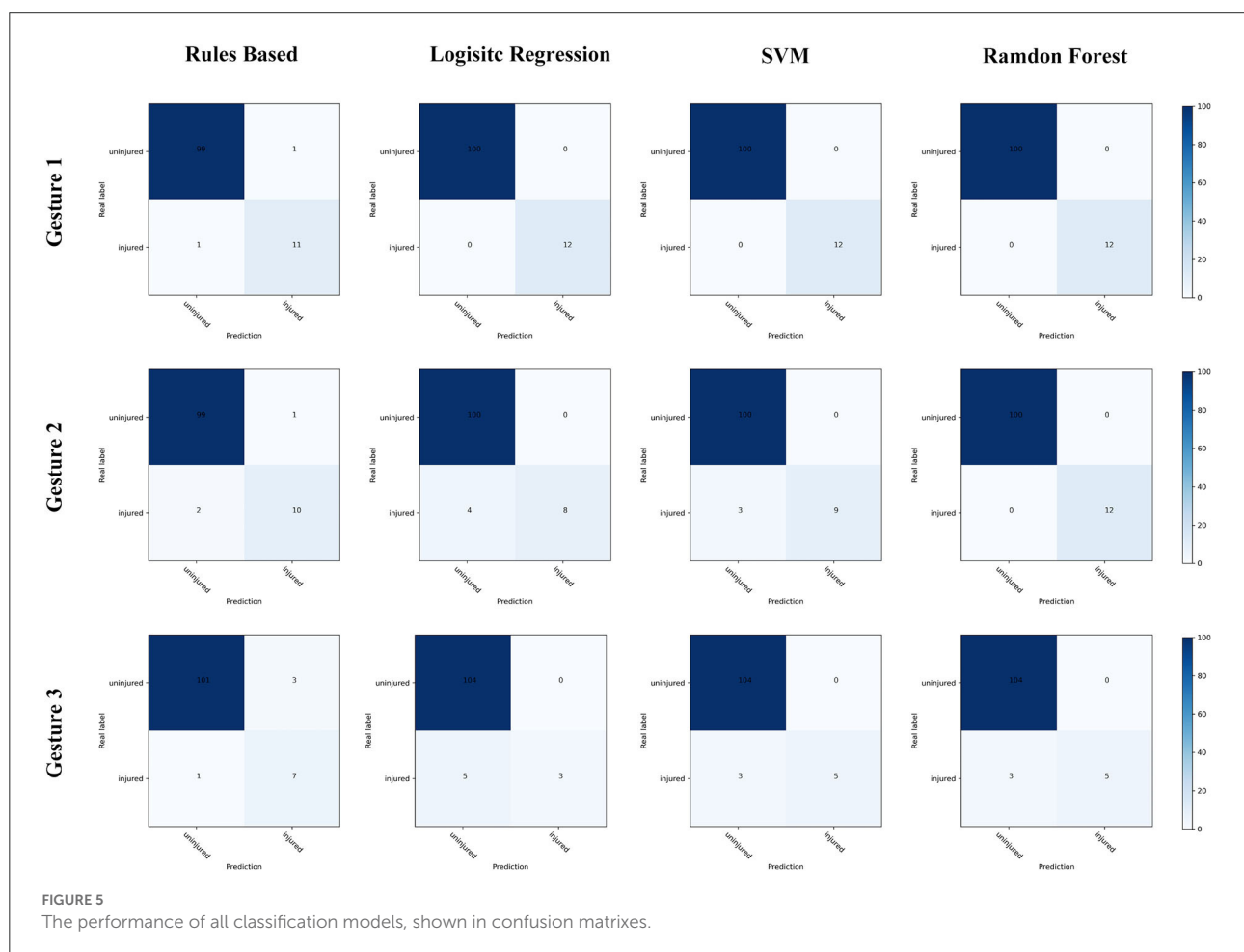
from images to predict the type of nerve injuries. Nevertheless, our study is not intended to replace the role of clinicians or create a diagnostic method. We aimed to provide a simple, effective, and convenient way to alert the possible nerve injuries for unspecialized healthcare providers based on anatomical knowledge and clinical experience.

In this study, MediaPipe Hands, one of the novel pose estimation techniques, has been used as an automatic feature extraction method for classification tasks. We observed that the calculated angles from the landmark coordinates were not precisely in agreement with the manual assessment. For example, in the non-target groups of Gesture 1 and Gesture 2, all MCP joints and the PIP and DIP joints of the ring and the little fingers were higher than 5° . The mean value

of the little MCP joint even reached 13.75° . When the digits are completely extended, these values are expected to be near 0° for people without nerve injury. There are several possible reasons to explain the differences. First, the pose estimation predicts the spatial coordinates of the landmark, yet manual goniometry usually measures the landmark on the surface of the hand. Second, the landmarks are not precisely the same, for there are no landmarks at the base of the metacarpals but only a single wrist landmark in MediaPipe skeleton models (24, 30), which may lead to noticeable differences in measuring the angles of MCP joints. In similar studies, a portable infrared camera called Leap Motion has been reported in functional assessments of hand rehabilitation (22). Researchers also found that the measurement result of

TABLE 4 Performance of all classification models.

	Rules based	Logistic regression	SVM	Random forest
Gesture 1				
Accuracy	98.21%	100.00%	100.00%	100.00%
Sensitivity	91.67%	100.00%	100.00%	100.00%
Specificity	99.00%	100.00%	100.00%	100.00%
Gesture 2				
Accuracy	97.32%	96.43%	97.32%	100.00%
Sensitivity	83.33%	66.67%	75.00%	100.00%
Specificity	99.00%	100.00%	100.00%	100.00%
Gesture 3				
Accuracy	96.43%	95.54%	97.32%	97.32%
Sensitivity	87.50%	37.50%	62.50%	62.50%
Specificity	97.12%	100.00%	100.00%	100.00%

FIGURE 5
The performance of all classification models, shown in confusion matrixes.

Leap Motion was not favorable with manual measurement (31–34).

Under such circumstances, the threshold value of features could not be directly determined by clinical experience.

Therefore, the training sets were used for feature selection and finding the cutoff. In the rule-based method, we used a filter strategy with a ROC curve because absolute distinctions exist between the injured and uninjured hands both in

images and from the clinical practice experience. According to the independent *t*-test, all selected features could effectively categorize normal and abnormal gestures. Besides, the subgroup analysis further proved that, in each gesture, features between targeted and untargeted injuries were completely different, while features between untargeted injuries and non-injury were similar. In other words, our method is consistent with anatomical knowledge. Our study shows that MediaPipe Hands is competent at providing features for qualitative analysis. However, four unrecognized images in Gesture 3, one from the uninjured hand of a patient and three from the hand with radial nerve injury, also suggest that there is still room to propose estimation improvement. More annotated data and datasets are needed (35), especially for the medical population and the medical setting.

The rule-based models have been compared with three machine-learning models commonly used for binary classification in medical research (36–38). In the feature selection process, similar results are obtained for rule-based and ML methods, for the selected features in rule-based models have higher coefficients in the LR models and higher importance in the RF models. In the performance analysis, high specificity in all models shows the prediction for uninjured people is easy and correct. The sensitivity of ML models fluctuates from 37.5 to 100%. In comparison, the sensitivity of our models is stable from 83.3 to 91.7%, mainly because our rules seek the most essential and valuable features but might miss some synergistic effects among them. The overall performance of our methods is satisfactory. We believe that medical knowledge and clinical experience were the keys to maximizing the classification performance, even using minimal features and simple rules. They work as a shortcut to find the right features and make the detection performance in our tasks comparable to machine learning models.

The rule-based method is also interpretable (39, 40), which means the process is easier to modify and understand in human terms. It can provide expertise more conveniently and spread expert knowledge to primary healthcare providers. Tang et al. (11) reported a quantitative assessment method for upper-limb traumatic PNI. In their study, the presence of radial, ulnar, or median nerve injury was identified under an expert system using the data of surface EMG with 81.82% sensitivity and 98.90% specificity. Compared with Tang et al., our study possesses a little higher sensitivity and more straightforward implementation. Unlike surface EMG, taking images is more convenient and comfortable for patients. We are confident that our method has reliable results and is much easier to be applied by primary healthcare providers. As smartphones have become available and acceptable tools for telemedicine (41) and during the COVID-19 pandemic, performing virtual hand examinations through images and videos raises excellent interests in the medical field (42, 43). The proposed method has the potential to be a

convenient screening tool for online health services and remote areas.

The relatively small sample size should be the main limitation of the present study. Since this was a prospective study and there was no available dataset of hand images with PNIs, continually updating our dataset is necessary to provide better automatic healthcare solutions. The smallest sample size in our study is patients with median nerve injury, which might weaken the generalizability of the proposed method. Although the differences between normal and abnormal gestures are noticeable, minor feature changes are highly possible when the sample size becomes more extensive. At present, our study only focuses on detecting nerve injuries and cannot detect abnormal gestures caused by other injuries, such as tendon rupture. In addition, the detection accuracy relies on the compliance of the users. Further study should invite more users in remote or rural areas to verify whether the rightful gestures can also be performed without the supervision of clinicians. Finally, our method functions as decision support to prevent missing nerve injuries in the primary assessment. After the initial discovery of the nerve injury, comprehensive examinations, differential diagnoses, and medical treatments are necessary.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of The First Affiliated Hospital of Sun Yat-sen University (ID: [2021]387). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XL, JY, and QZ contributed to the conception and design of the study. FG, JF, and CC performed the investigation and wrote the original draft. ZW organized the database and performed the statistical analysis. All authors contributed to the manuscript revision, read, and approved the submitted version.

Acknowledgments

The authors thank Mr. Bai Leng, Mr. Zhenguo Lao, and Ms. Jianwen Zheng for technical help, writing assistance, and general support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.1052505/full#supplementary-material>

References

1. Yu TH, Xu YX, Ahmad MA, Javed R, Hagiwara H, Tian XH. Exosomes as a promising therapeutic strategy for peripheral nerve injury. *Curr Neuropharmacol*. (2021) 19:2141–51. doi: 10.2174/1570159X19666210203161559
2. Sullivan R, Dailey T, Duncan K, Abel N, Borlongan CV. Peripheral nerve injury: stem cell therapy and peripheral nerve transfer. *Int J Mol Sci*. (2016) 17:1608–17. doi: 10.3390/ijms17122101
3. Sanderson M, Mohr B, Abraham MK. The emergent evaluation and treatment of hand and wrist injuries: an update. *Emerg Med Clin North Am*. (2020) 38:61–79. doi: 10.1016/j.emc.2019.09.004
4. Fitschen-Oestern S, Lippross S, Lefering R, Kluter T, Behrendt P, Weuster M, et al. Missed hand and forearm injuries in multiple trauma patients: an analysis from the TraumaRegister DGU(R). *Injury*. (2020) 51:1608–17. doi: 10.1016/j.injury.2020.04.022
5. Che Y, Xu L. Analysis on misdiagnosis of ulnar nerve injury: a report of 18 cases. *China J Orthop Trauma*. (2008) 21:769–70.
6. Liang XJ, Liu YL, Yan XF. Analysis of misdiagnosis of 49 cases of peripheral nerve injury. *Chin J Mod Med*. (1998) 8:36–7.
7. Edshage S. Peripheral nerve injuries—diagnosis and treatment. *N Engl J Med*. (1968) 278:1431–6. doi: 10.1056/NEJM196806272782606
8. Dahlin LB, Wiberg M. Nerve injuries of the upper extremity and hand. *EFORT Open Rev*. (2017) 2:158–70. doi: 10.1302/2058-5241.2.160071
9. Pridmore MD, Glassman GE, Pollins AC, Manzanera Esteve IV, Drolet BC, Weikert DR, et al. Initial findings in traumatic peripheral nerve injury and repair with diffusion tensor imaging. *Ann Clin Transl Neurol*. (2021) 8:332–47. doi: 10.1002/acn3.51270
10. Zeidenberg J, Burks SS, Jose J, Subhawong TK, Levi AD. The utility of ultrasound in the assessment of traumatic peripheral nerve lesions: report of 4 cases. *Neurosurg Focus*. (2015) 39:E3. doi: 10.3171/2015.6.FOCUS15214
11. Tang W, Zhang X, Sun Y, Yao B, Chen X, Chen X, et al. Quantitative assessment of traumatic upper-limb peripheral nerve injuries using surface electromyography. *Front Bioeng Biotechnol*. (2020) 8:795. doi: 10.3389/fbioe.2020.00795
12. Gu YD, Wang SD, Shi D. *Gu Yudong and Wang Shuhuan's Hand Surgery*. Shanghai: Shanghai Scientific & Technical Publishers Press (2002).
13. Kim DH, Kline DG, Hudson AR. *Kline and Hudson's Nerve Injuries: Operative Results for Major Nerve Injuries, Entrapments and Tumors*. Singapore: Saunders Elsevier Press (2008).
14. Larson EL, Santosa KB, Mackinnon SE, Snyder-Warwick AK. Median to radial nerve transfer after traumatic radial nerve avulsion in a pediatric patient. *J Neurosurg Pediatr*. (2019) 24:209–14. doi: 10.3171/2019.3.PEDS18550
15. Bauer B, Chaise F. Correction of ulnar claw hand and Wartenberg's sign. *Hand Surg Rehabil*. (2022) 41S:S118–27. doi: 10.1016/j.hansur.2020.11.012
16. Soldado F, Bertelli JA, Ghizoni MF. High median nerve injury: motor and sensory nerve transfers to restore function. *Hand Clin*. (2016) 32:209–17. doi: 10.1016/j.hcl.2015.12.008
17. Erol A, Bebis G, Nicolescu M, Boyle RD, Twombly X. Vision-based hand pose estimation: a review. *Comp Vis Image Understand*. (2007) 108:52–73. doi: 10.1016/j.cviu.2006.10.012
18. Pham T, Pathirana PN, Trinh H, Fay P. A non-contact measurement system for the range of motion of the hand. *Sensors*. (2015) 15:18315–33. doi: 10.3390/s150818315
19. Zhou YM, Jiang GL, Lin YR. A novel finger and hand pose estimation technique for real-time hand gesture recognition. *Pattern Recognit*. (2016) 49:102–14. doi: 10.1016/j.patcog.2015.07.014
20. Hong S, Kim Y. Dynamic pose estimation using multiple RGB-D cameras. *Sensors*. (2018) 18:3865. doi: 10.3390/s18113865
21. Adhikary S, Talukdar AK, Sarma KK. *A Vision-Based System for Recognition of Words used in Indian Sign Language Using MediaPipe*. Guwahati: Gauhati University (2021).
22. Li C, Cheng L, Yang H, Zou Y, Huang F. An automatic rehabilitation assessment system for hand function based on leap motion and ensemble learning. *Cybern Syst*. (2021) 52:3–25. doi: 10.1080/01969722.2020.1827798
23. Butt AH, Rovini E, Dolciotti C, De Petris G, Bongioanni P, Carboncini MC, et al. Objective and automatic classification of Parkinson disease with Leap Motion controller. *Biomed Eng Online*. (2018) 17:168. doi: 10.1186/s12938-018-0600-7
24. Zhang F, Bazarevsky V, Vakunov A, Tkachenka A, Sung G, Chang CL, et al. MediaPipe hands: On-device real-time hand tracking. *arXiv [Preprint]*. (2020). arXiv: 2006.10214. Available online at: <https://arxiv.org/abs/2006.10214>
25. Chunduru V, Roy M, Chittawadigi RG. *Hand Tracking in 3D Space Using MediaPipe and PnP Method for Intuitive Control of Virtual Globe*. Bengaluru: Amrita Vishwa Vidyapeetham, Amrita School of Engineering (2021).
26. Zhu Y, Lu W, Gan W, Hou W. A contactless method to measure real-time finger motion using depth-based pose estimation. *Comput Biol Med*. (2021) 131:104282. doi: 10.1016/j.combiomed.2021.104282
27. Mushari NA, Soultanidis G, Duff L, Trivieri MG, Fayad ZA, Robson P, et al. Exploring the utility of radiomic feature extraction to improve the diagnostic accuracy of cardiac sarcoidosis using FDG PET. *Front Med*. (2022) 9:840261. doi: 10.3389/fmed.2022.840261
28. Koyama T, Fujita K, Watanabe M, Kato K, Sasaki T, Yoshii T, et al. Cervical myelopathy screening with machine learning algorithm focusing on finger motion using noncontact sensor. *Spine*. (2022) 47:163–71. doi: 10.1097/BRS.0000000000004243
29. Jorgensen HS, Behets G, Viaene L, Bammens B, Claes K, Meijers B, et al. Diagnostic accuracy of noninvasive bone turnover markers in renal osteodystrophy. *Am J Kidney Dis*. (2022) 79:667–76 e1. doi: 10.1053/j.ajkd.2021.07.027
30. Ganguly A, Rashidi G, Mombaur K. Comparison of the performance of the leap motion controller (TM) with a standard marker-based motion capture system. *Sensors*. (2021) 21:1750. doi: 10.3390/s21051750
31. Gamboa E, Serrato A, Castro J, Toro D, Trujillo M. Advantages and limitations of leap motion from a developers', physical therapists', and patients' perspective. *Methods Inf Med*. (2020) 59:110–6. doi: 10.1055/s-0040-1715127
32. Chopchuk P, Chumpen S, Tungjitkusolmun S, Phasukkitt P. Hand postures for evaluating trigger finger using leap motion controller. In: *2015 8th Biomedical Engineering International Conference (BMEiCON)*. Pattaya (2015).
33. Nizamis K, Rijken NHM, Mendes A, Janssen M, Bergsma A, Koopman B. A novel setup and protocol to measure the range of motion of the wrist and the hand. *Sensors*. (2018) 18:3230. doi: 10.3390/s18103230

34. Trejo RL, Vazquez JPG, Ramirez MLG, Corral LEV, Marquez IR. Hand goniometric measurements using leap motion. In: *2017 14th IEEE Annual Consumer Communications & Networking Conference (CCNC)*. Las Vegas, NV (2017). p. 137–41.
35. Chatzis T, Stergioulas A, Konstantinidis D, Dimitropoulos K, Daras P. A comprehensive study on deep learning-based 3D hand pose estimation methods. *Appl Sci*. (2020) 10:6850. doi: 10.3390/app10196850
36. Yokoyama M, Yanagisawa M. Logistic regression analysis of multiple interosseous hand-muscle activities using surface electromyography during finger-oriented tasks. *J Electromyogr Kinesiol*. (2019) 44:117–23. doi: 10.1016/j.jelekin.2018.12.006
37. Mebarkia K, Refad A. Multi optimized SVM classifiers for motor imagery left and right hand movement identification. *Australas Phys Eng Sci Med*. (2019) 42:949–58. doi: 10.1007/s13246-019-00793-y
38. Bouacheria M, Cherfa Y, Cherfa A, Belkhamisa N. Automatic glaucoma screening using optic nerve head measurements and random forest classifier on fundus images. *Phys Eng Sci Med*. (2020) 43:1265–77. doi: 10.1007/s13246-020-00930-y
39. Ribeiro MT, Singh S, Guestrin C. “Why should i trust you?” Explaining the predictions of any classifier. In: *Kdd’16: Proceedings of the 22nd Acm Sigkdd International Conference on Knowledge Discovery and Data Mining*. California (2016). p. 1135–44.
40. Doshi-Velez F, Kim B. Towards a rigorous science of interpretable machine learning. *arXiv [Preprint]*. (2017). arXiv: 1702.08608. Available online at: <https://arxiv.org/abs/1702.08608>
41. Hsieh C, Yun D, Bhatia AC, Hsu JT, Ruiz De Luzuriaga AM. Patient perception on the usage of smartphones for medical photography and for reference in dermatology. *Dermatol Surg*. (2015) 41:149–54. doi: 10.1097/DSS.0000000000000213
42. Wainberg MC, Jurisson ML, Johnson SE, Brault JS. The telemedicine hand examination. *Am J Phys Med Rehabil*. (2020) 99:883. doi: 10.1097/PHM.00000000000001555
43. Welle K, Tager S, Hackenberg RK, Markowetz A, Schildberg FA, Burger C, et al. Examining the hand in the video consultation. *Z Orthop Unfall*. (2021) 159:202–8. doi: 10.1055/a-1304-3593



OPEN ACCESS

EDITED BY

Jian-Quan Shi,
Nanjing Medical University, China

REVIEWED BY

S. Ottavio Tomasi,
Paracelsus Medical University, Austria
Gang Zhao,
Soochow University, China

*CORRESPONDENCE

Hui Lu
huilu@zju.edu.cn

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 29 October 2022

ACCEPTED 16 November 2022

PUBLISHED 15 December 2022

CITATION

Zhou H, Yao C, Dong Y, Alhaskawi A,
Wang Z, Lai J, Ezzi SHA, Kota VG,
Abdulla MHAH and Lu H (2022) Clinical
characteristics and management
experience of schwannoma in
extremities: Lessons learned from a
10-year retrospective study.
Front. Neurol. 13:1083896.
doi: 10.3389/fneur.2022.1083896

COPYRIGHT

© 2022 Zhou, Yao, Dong, Alhaskawi,
Wang, Lai, Ezzi, Kota, Abdulla and Lu.
This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Clinical characteristics and management experience of schwannoma in extremities: Lessons learned from a 10-year retrospective study

Haiying Zhou¹, Chengjun Yao², Yanzhao Dong¹,
Ahmad Alhaskawi¹, Zewei Wang², Jingtian Lai²,
Sohaib Hasan Abdullah Ezzi³, Vishnu Goutham Kota²,
Mohamed Hasan Abdulla Hasan Abdulla² and Hui Lu^{1,4*}

¹Department of Orthopedics, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, ²Department of Clinical Medicine, Zhejiang University School of Medicine, Hangzhou, China, ³Department of Orthopaedics of the Third Xiangya Hospital, Central South University, Changsha, China, ⁴Alibaba-Zhejiang University Joint Research Center of Future Digital Healthcare, Zhejiang University, Hangzhou, China

Introduction: Schwannomas are the most common neoplastic lesions of the peripheral nerves when growing on the extremities, they usually have adverse effects on patients due to the exposed and functional nature of the region.

Methods: In the present single-center retrospective study, we included all patients with pathologically confirmed schwannoma located in extremities between 2011 and 2021 totaling 183 patients. Data on gender, age, duration history, clinical presentation, occurrence region, nerve affiliation, imaging data, modus operation, mass volume, immunohistochemistry, postoperative neurological function, and recurrence were collected.

Results: As in previous studies, patients were predominantly middle-aged with a mean age of 49.5, without gender preference and a male-to-female ratio of 1.2:1. Most patients are first seen for this disease, and only five of them are recurrent. The majority presented with an isolated (91.26%), asymptomatic (37.7%) mass, with tenderness (34.97%) being the second frequent complaint. 60% of lesions occurred in the upper extremity, more commonly on the left side (55.26%) than the right. The average duration of onset was 47.50 months. MRI is more sensitive for neurogenic tumors than ultrasound, as it owns 78.93% correct. In immunohistochemistry, the top three markers for positive labeling schwannoma are S-100 (98.95%), Ki67 (98.68%) and β -Catenin. 98.36% of patients underwent complete resection of the lesion, of which 14.44% required partial sacrifice of the nerve fibers. Thanks to the application of intraoperative peripheral nerve microscopic operation, only 6 patients showed symptoms of postoperative nerve injury, and 3 of them received second surgery. Intraoperative microscopic manipulation, preservation of the main nerve, and the need for reconstruction of the affected nerve fibers are some of the points worth noting.

Discussion: In summary, the possibility of schwannoma should not be overlooked in the identification of masses that occur in the upper extremities of the middle-aged population. Preoperative ultrasound and MR are useful for

determining the nature of the mass, and S100, Ki67, and β -Catenin are sensitive to it. Surgical resection can achieve satisfying functional results and a low risk of nerve injury.

KEYWORDS

schwannoma, extremity, retrospective study, diagnosis, microsurgery, prognosis

Introduction

Schwannoma, also known as neurilemmoma, is a rare disease but is also the most common type of peripheral nerve sheath tumor (1). It consists solely of benign tumorigenic Schwann cells, therefore, often has an intact capsule. Schwannoma can occur anywhere in the body where nerve fibers are located, with the extremities being one of the most common sites, accounting for 32.6% of occurrences (2, 3). Although they often appear as isolated, painless, slow-growing masses, the abundant nerve peripheral occupation region and variable size of the masses can sometimes present with physical symptoms such as nerve deficits or pain. Complete surgical resection is often considered sequelae-free, but in some research, a poor prognosis of neurological deficits has been reported (4–7).

Because of its rarity and rich clinical presentation, patients with schwannoma consult diverse departments, resulting in delays and missed diagnoses, as well as different treatment options. Meanwhile, only a small portion of the literature has meticulously investigated schwannoma in extremities, and data on masses originating from the terminal branches are often neglected, resulting in rare relevant diagnoses and treatment experiences (8–10). Therefore, this study collected clinical data from 183 patients with schwannoma at the extremity treated at a large general hospital in the last decade, and to our knowledge, this is the largest retrospective study of schwannomas of the extremities. In this study, we summarize the clinical features of schwannoma and explore the key points of clinical diagnosis and treatment of this disease.

Materials and methods

We retrospectively reviewed all medical records of the patients who were hospitalized in our hospital from 2011 to 2021, received surgical treatment, and were finally confirmed as schwannoma by pathology. The tumors must be located at the extremities, not the trunk or head and neck. Patients with indefinite pathological types such as spindle cell soft tissue tumors, traumatic neuroma, and neurogenic tumors were excluded. Data included clinical, surgical, and radiological findings. All patients were followed up for 1–10 years. Most

patients were discharged after 1 year of follow-up due to no discomfort.

The preoperative characteristics containing gender, age, time of the first detection of the mass, location, size of the mass, major complaints, previous medical experience, and physical examination discovery were collected. It is important to note that the physical examination is often incomplete as the judgment of such disease may vary between different medical history collectors. Patient imaging data, including ultrasound, CT, X-rays, and MRI, were also recorded in detail. However, as X-rays and CT were used less frequently on such patients and the data were so sketchy, we removed them from the analysis. The results of ultrasound and MRI, on the other hand, were categorized into three types based on the diagnostic information they ultimately provided, namely negative, where the mass could not be detected; positive, where the mass was detected but the provisional diagnosis did not consider a neurogenic tumor; and correct, where the diagnosis considered the mass to be a neurogenic tumor.

All masses were surgically excised, and the surgical approach and intraoperative relationship between the mass and surrounding tissues, the size and number of mass, and the involved nerves were tracked. During the surgery, we found that the peeled masses were mostly ellipsoidal or fusiform, so the volume of the mass was calculated using the formula of an ellipsoid: $\text{Volume (cm}^3\text{)} = (V = 4/3 \times \pi \times a \times b \times c; \text{ where } a, b, \text{ and } c \text{ represent the three semi-axes})$. The results of postoperative pathology and immunohistochemical markers were also collected as the gold standard for diagnosis. Clinical follow-up started at 2 weeks postoperatively and then continued for 1, 3 months, or even longer; subsequently, every patient should be followed over 1 year. At each follow-up visit, clinical outcomes, such as the healing of the patient's incision, improvement in chief complaints, and limb sensation, and motor function were assessed and recorded.

Quantitative variables were described using means and standard deviations (SD) and compared using *t*-tests or non-parametric tests, when the data are not a normal distribution, such as the tumor volume and medical history time. Qualitative data were described using percentages or numbers and used one-way ANOVA or Chi-square test to analyze the difference. The level of significance was set to $p < 0.05$. All analyses were done in SPSS 23.0.

TABLE 1 Summary of clinical characteristics of patients and masses.

Clinical characteristics		No. of patients of masses
Age (years)	≤20	2
49.5 ± 14.5	21–30	34
	31–40	34
	41–50	35
	51–60	49
	61–70	22
	>70	7
Gender	Male	100
	Female	83
Duration time (months)	<12	47
47.97 ± 57.64	12–60	102
	>60	41
Location	Upper / Lower	114/76
	Flexor / Extensor	115/75
	Left / Right	105/85
Nerve	Main trunk	72
	Branches	118
Volume (cm ³)	≤64.45 (d = 5 cm)	136
9.44 ± 28.62	>64.45	4
Radiology	Ultrasound	68/13
(right/wrong)	MRI	95/20

Results

General characteristics of patients and tumors

Data were collected from a total of 330 patients with a pathological diagnosis of schwannomas; of which, 147 patients were screened out due to their inadequate medical records, insufficient follow-up time, and incorrect tumor site. Eventually, 183 patients and 190 sites of masses were included in the study, with five patients having two different sites of schwannoma and one patient having three different sites of masses. The mean age of the patients was 49.5 ± 14.5 years, ranging from 7 to 80 years, and male patients were slightly more than female patients (100:83) (Table 1). Five of the patients had a history of schwannoma resection at other hospitals before this hospitalization, ranging from 1 to more than 10 years.

In total, 115 masses were found on the flexor side of the body, while 75 masses were found on the extensor side. The majority of masses occurred in the upper extremity (114 cases in total), at the same time, more schwannomas were found in the left side of the extremity (105 cases). Further, according to its soft tissue occupancy, the extremity sites were subdivided into the digit/toe area (digit); the metacarpal region of the hand

and foot (palmoplantar); the articular area (joint); and the long bone area of the extremity (limb), with the limb area having the most tumors (93 cases) and the digital area the least (25 cases) (Table 1).

The duration of the disease varies from 7 days to 20 years, and the mean time span between the discovery of the tumor and hospitalization was 47.50 ± 57.40 months. Schwannomas located on the extensor side seeking medical help are at an average of 42.44 months after discovery and the limb mass required an average of 42.57 months. There were no significant differences in the time to seek medical help between sites. Based on the patient's history and a brief physical examination, we classified the patient's complaints into five major categories, namely, no discomfort, pain, tenderness, numbness, and other discomforts (including impaired mobility, decreased muscle strength, pruritus, and interference with nail growth). The majority of patients seek medical treatment solely for masses without any discomfort, accounting for about 37.7%, and their average duration time was as long as 39.08 months. In contrast, patients with numbness had the shortest time to seek medical help with a mean of 15.54 months, significantly shorter than those without complaints. Over 1 year after the discovery of an asymptomatic mass, 87.5% of patients from the discomfort group presented with complaints of discomfort (Figure 1).

Information of tumors

A total of 159 patients provided preoperative localized imaging of the mass, either at our hospital or the outside, of whom 102 underwent ultrasound and 119 performed MRI, including enhanced MRI. Ultrasound had a sensitivity of 66.67% for neurogenic masses, while 79.83% for MRI is significantly higher (Table 1).

Combining the intraoperative findings with the physical examination and preoperative image, we clarified the nerve to which the patient's schwannomas belonged. Still, 25 sites of masses could not be accurately localized. According to the results, we classified the affected nerves as digital nerve (31 cases), radial nerve (9 cases), ulnar nerve (22 cases), median nerve (24 cases), sciatic nerve (7 cases), common peroneal nerve (18 cases), tibial nerve (18 cases), and other nerves (62 cases) (Table 2). Incidentally, one of the patients had multiple masses in the wrist, one in the ulnar nerve and one in the median nerve. On the other hand, based on whether they originated from the nerve trunk, masses were divided into the main trunks group (72 cases) and the branches group (118 cases). The branches were mostly seen with no complaints, accounting for 33.56%, which was significantly higher than that of the main trunk. In contrast, the nerve trunk was more often accompanied by a numbness (27.55%) and pressure pain (28.57%), and the incidence of numbness was significantly higher than that of the branches (Figure 2). The digital nerve, radial nerve, and sciatic nerve were

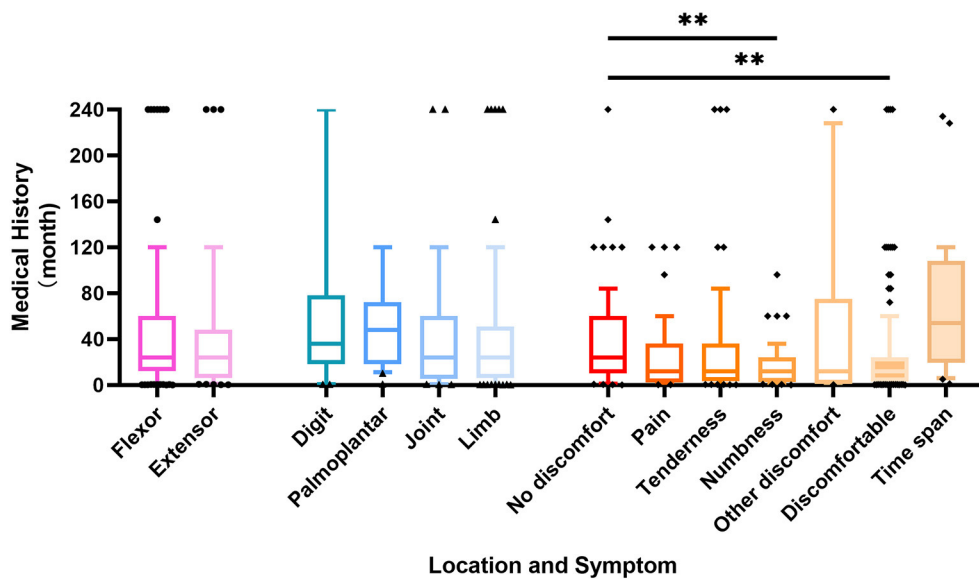


FIGURE 1

Box plot representing the correlation between patient history time and schwannomas location and symptoms. p -value ** <0.01.

most often seen without significant discomfort, while the ulnar and common peroneal nerves were often seen with tenderness, the median nerve was frequently seen with pain, and the tibial nerve was seen with more variable complaints (Table 2).

The volume data of the schwannomas are best obtained from the pathologist's report, followed by the surgical records and imaging reports, and the data obtained from the physical examination is generally not considered. However, due to the different labeling habits of physicians, some masses lacked data or only possessed two-dimensional data, which made the volume of the masses difficult to estimate. Consequently, a total of 140 masses obtained accurate volume, which varies from 0.025 to 274.89 cm³. The mean volume of the schwannomas was 9.44 ± 28.62 cm³. The masses on the extensor side were relatively small at 5.94 cm³ and, not surprisingly, the masses on the digital region were the smallest at 0.78 cm³, significantly smaller than the masses on the joint and limb. There was no significant difference in the size of schwannomas located in the main trunk or the branches of nerves. While talking about the exact origin nerve, the masses from the tibial nerve were significantly larger than the rest of the groups, with a mean volume of 29.36 cm³. Meanwhile, there was also no significant difference in the volume of the masses among symptom groups (Figure 3).

Intraoperative findings and prognosis

Intraoperatively, multiple masses were found in a single site in 10 patients, and in three cases, the nerve showed bead-like

masses, and in most cases, 12 small masses were stripped from a single nerve (Figure 4; Supplementary Figure 1). A total of 187 sites of masses were completely excised, and among them, 50 masses affected their surrounding tissues, of which 26 masses sacrificed the encroached nerve fibers or the distal nerves and 24 masses underwent neurolysis and tenolysis.

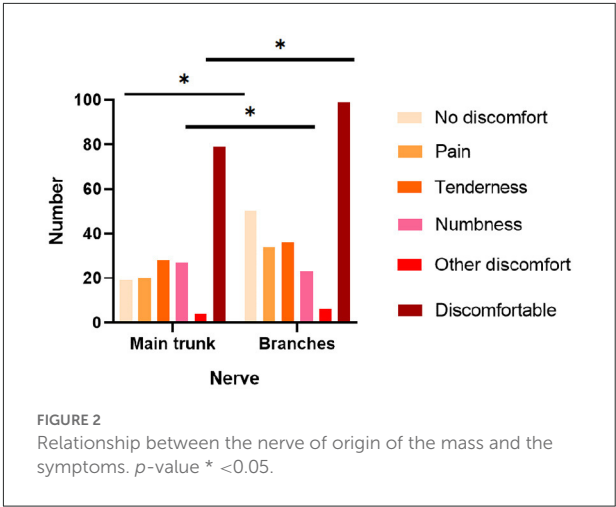
During the postoperative follow-up, we did not consider the patient's short-term, about 14 days, postoperative symptoms as sequelae of the surgery, given that this could be a transient manifestation of postoperative tissue edema and not due to the nerve injury; also, studies have confirmed an extremely high rate of spontaneous remission of short-term postoperative sensory loss (9). In this definition, 96.72% of patients achieved a complete functional improvement of the extremities without any sequelae after the sophisticated microsurgery. Of note, one patient had a recurrence of the mass 2 years after surgery and underwent surgical resection again. A total of six patients had discomforts such as pain and swelling in the operated area or even limitation of limb movement during the postoperative follow-up period and three of them received further surgical treatment (Supplementary Figure 2; Table 2).

Immunohistochemical characteristics

During the data collecting process, we found that many patients had an initial pathological diagnosis of spindle cell soft tissue tumor, which was later confirmed by immunohistochemistry as schwannomas, while a part of patients, who did not undergo follow-up to reimbursement for

TABLE 2 Detailed information of the single site Schwannomas (n = 190).

Location and affiliated nerve		Symptom				Surgical treatment			Postoperative	
		No discomfort	Pain	Tenderness	Numbness	Other discomfort	Partial resection	Complete resection (Sacrifice nerve / neurolysis)	Discomfort	Reoperation
Upper extremity	Digital nerve	14	5	9	3	1	0	30 (3/4)	0	0
	Radial nerve	2	4	3	2	2	0	9 (2/1)	1	1
	Ulnar nerve	5	7	9	7	0	1	21 (4/3)	1	1
	Median nerve	10	4	6	8	0	1	23 (2/4)	2	1 (recurrence)
	Other nerves	12	10	11	10	1	0	30 (3/4)	1	0
Lower extremity	Digital nerve	1	0	0	0	0	0	1 (0/1)	0	0
	Sciatic nerve	4	1	1	2	1	0	7 (0/2)	0	0
	Common peroneal nerve	4	7	11	6	2	2	16 (6/0)	0	0
	Tibial nerve	3	8	8	8	1	0	18 (3/3)	2	1
	Other nerves	14	10	8	5	2	0	32 (1/3)	0	0



immunohistochemistry due to good postoperative functional recovery, did not have further clarification of the tumor type. A total of 95 masses underwent further immunohistochemical analysis, with the highest positive rate of 98.95% for S-100, followed by 98.68% for Ki-67, and 89.66% for β -Catenin (Table 3).

Discussion

Schwannomas are benign tumors of the peripheral nerves commonly found in the percutaneous nerves and the head, neck, or extremities. Due to their rarity and the diversity of symptoms, only a few studies have been conducted to analyze and summarize the clinical features and treatment points of schwannoma in detail. Furthermore, as far as the author knows, this study investigated the largest number of patients with schwannomas in the extremities (8, 11).

Epidemiological information of schwannomas

In line with the currently accepted concept, isolation and singularity are the characteristics of the schwannomas in this study, with the onset peak age ranging from 20 to 60 years and no significant gender bias in the patient population (12). Similarly, the tumors often present as asymptomatic masses, and the flexor surfaces are the frequent site of schwannoma occurrence (13). However, the distribution preference of schwannomas in the upper or lower parts of the extremity is controversial. In studies discussing schwannomas of the extremity, the lower extremity tends to be more frequent (8, 9), but in retrospective studies discussing schwannomas in humans, the upper extremity tends to be more frequent (2, 14, 15). In our study, upper extremity masses were more frequent, which may result from the

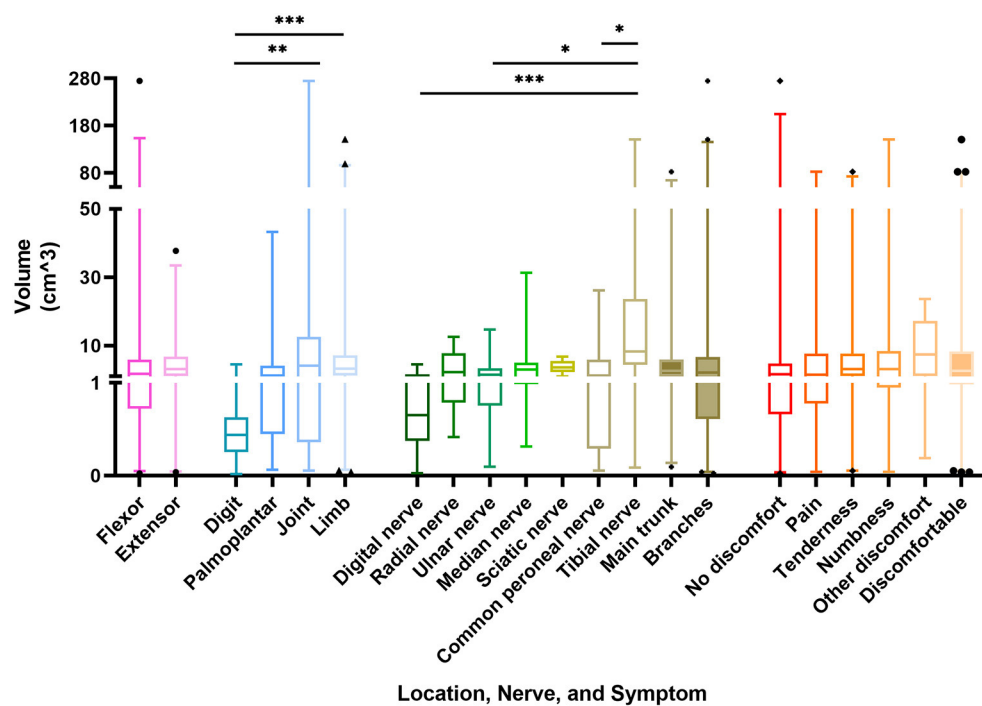


FIGURE 3

Box plot representing the correlation between the volume of schwannomas and its location, nerve of origin, and symptoms. p -value * <0.05 , ** <0.01 , *** <0.005 .

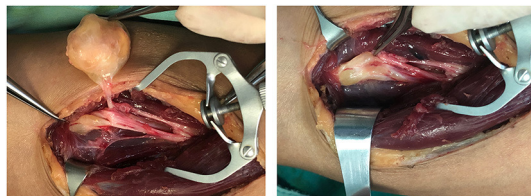


FIGURE 4

Multiple schwannomas on the median nerve.

different definitions for the extremities. The upper extremity was defined by the shoulder, while the lower extremity was defined by the groin, and the buttock, as an intermediate region, was not included. Certainly, the variability in the characteristics of patients attending different medical centers may also contribute to the difference. Furthermore, there is a higher incidence of masses in the left extremity (55.26%), while the long bone region had the highest incidence (Figure 5).

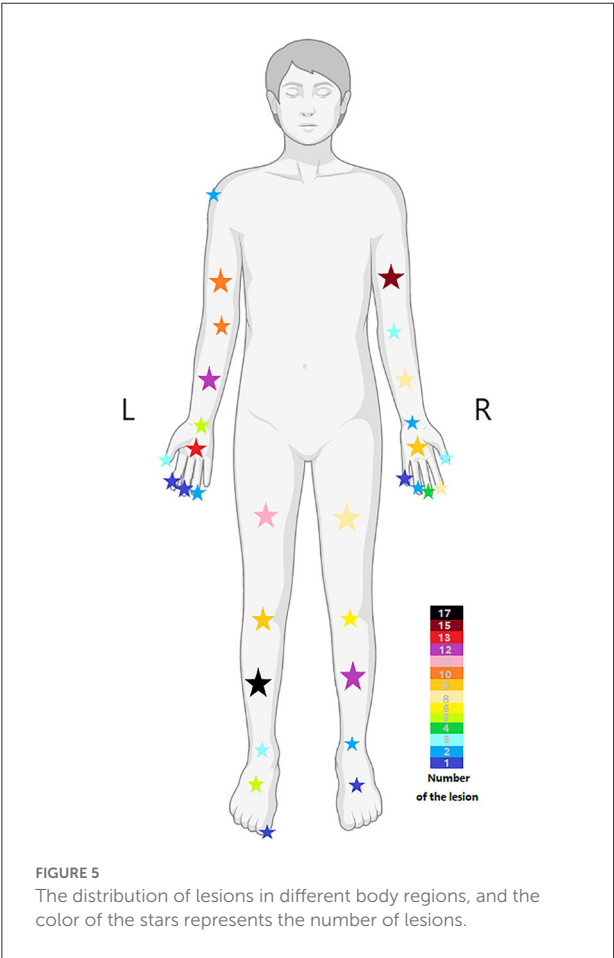
It is generally accepted that the clinical presentation of a mass often influences whether a patient seeks medical help and that the length of medical history may likewise contribute to the manifestation of discomfort. In this study, the patients had an

average medical history of about 2 years, with the longest being more than 20 years, and the large degree of dispersion led to an interest in the factors influencing the length of the disease course. The results showed that patients were more likely to notice masses in the extensor surfaces and long bone regions, and then seek early medical treatment. Besides, nearly one-third of the 114 patients with complaints of discomfort started with asymptomatic masses, and as time went by, patients gradually developed pain, numbness, tenderness, and even impaired movement, most of which appeared 1 year or more after the discovery of the masses. Therefore, early surgical removal of suspected neurogenic masses can prevent the occurrence of concomitant symptoms and even postoperative complications, thus improving the quality of patients' life (Figure 1).

Schwannomas are mostly disseminated and their most common nerve of origin is inconclusive, but the main trunk nerves, such as the median, brachial plexus, and tibial nerves, tend to have the highest incidence of masses in previous studies (6, 8, 11, 16, 17). However, we found that the digital nerve was the most commonly involved, followed by the median nerve. This reminds clinicians to consider the possibility of schwannomas in the differential diagnosis of finger masses. In addition, masses of nerve trunk origin have a higher incidence of complaints, especially numbness and tenderness, than branch nerves, and therefore surgeons need to plan better and perform

TABLE 3 Number of positive (+) and negative (-) staining cases of different immunohistochemical markers.

	(+)	(+) / (-)	(-)
S-100	94	0	1
Ki-67	75	0	1
SMA	5	0	66
CD34	25	0	46
Desmin	0	1	65
CD117	2	1	47
CK(pan)	32	0	32
β-Catenin	26	0	6
DOG-1	0	0	26
EMA	0	0	15
HMB45	1	0	13
SOX10	12	0	0
Staining cases < 10	Nestin (4,0,1); SDHB (4,0,0); Vimentin (3,0,0); GFAP (1,0,2); H3K27Me3 (1,0,0); Fli-1 (1,0,0)		
		/ STAT6 (0,0,6); ALk (0,0,4); Melan A (0,0,2); NF (0,0,2); CD68 (0,0,1); Calponin (CP) (0,0,1); Syn (0,0,1); CgA (0,0,1); ERG (0,0,1); MUC-4 (0,0,1); Myogenin (0,0,1); MyoD1 (0,0,1)	



Careful tissue separation in patients with such complaints to minimize the possible impact on the nerve trunk. Also, unlike the findings of Abe et al. (18), there was no significant correlation between clinical symptoms and the volume of the mass in our patients. Perhaps, the volume of the tumor occurrence site may have a greater impact on the volume of the tumor (Figure 3).

Diagnosis of schwannomas

Correct clinical diagnosis helps to better plan surgical procedures, thus reducing the possibility of intraoperative nerve injury, and the first evidence of which comes from the patient's complaints of discomfort and physical examination. However, the majority of patients presented with asymptomatic masses. Meanwhile, the knowledge of the Tinel sign varied between history-takers, and some clinicians may not even consider the Tinel sign, resulting in very limited information provided by such evidence. In this survey, only one-third of 24 patients who had not undergone imaging examination considered the possibility of a neurogenic tumor in initial diagnosis, with hemangiomas, lipomas, subcutaneous nodules, and ganglions being common considerations in the residual patients. Therefore, preoperative imaging is critical to provide abundant information about the mass, especially ultrasound and MRI. Ultrasound, with the development and application of higher resolution techniques, the characteristic mouse tail sign of schwannomas and the relationship between the mass and nerve can be better demonstrated. Although, in small

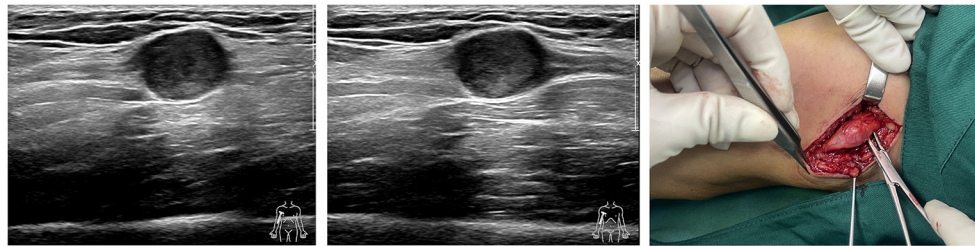


FIGURE 6
Ultrasound images and intraoperative images of schwannomas of ulnar nerve origin in the upper arm.

peripheral nerves, such as the fingertips, the detection rate of neurogenic tumors still needs to be improved (Figure 6) (19, 20). Similar to previous studies, the characteristic target sign of MRI has a sensitivity of nearly 80%, and its eccentric position of the lesion imaging helps in the differential diagnosis of neurofibroma (8, 20). MRI also helps the surgeon to better locate, avoid possible intraoperative nerve injury, and achieve complete lesion resection (Figure 7). However, from another perspective, the fineness and complexity of MRI also limit its clinical application, due to the requirements of the higher ability of radiologists and longer review time. In our previous study, we tried to draw artificial intelligence into medical image reading and achieved a good correct rate (21). We believe that in the future, with the continuous progress of information technology, medical imaging diagnosis will enter the era of intelligence, and we are also looking forward to the feasibility of multimodal diagnosis in the field of artificial intelligence medical diagnosis, such as combining the results of multiple types of radiology, combining imaging and medical history, and combining imaging and pathology.

The definitive diagnosis of schwannomas still relies on pathology, and in the classic form, the alternating presence of Antoni A and B zones is the key to diagnosis (22). However, there are exceptions to everything. Atypical schwannomas require a combination of immunohistochemical findings to differentiate them from tumors such as neurofibromas and solitary circumscribed neuroma (23). S-100 and SOX10 are recognized positive markers, but the ancillary role of other markers, such as EMA, INI-1, and TTF-1, in the diagnosis is rarely discussed and demonstrated (24). In this study, we verified the high positive rate of S-100 and SOX in schwannomas and identified several immunohistochemical markers that could be used as potential adjunctive diagnostic markers, such as Ki-67 and β -Catenin, which had high positive rates, and SDH8, Vimentin, H3K27Me3, and Fli-1, which stained a small number of cases but achieved 100% positive rate, and perhaps they can be tested more in future immunohistochemistry to obtain more data. Interestingly, EMA markers were 100% negative in this study, which may be related to the intracapsular resection of the tumor.

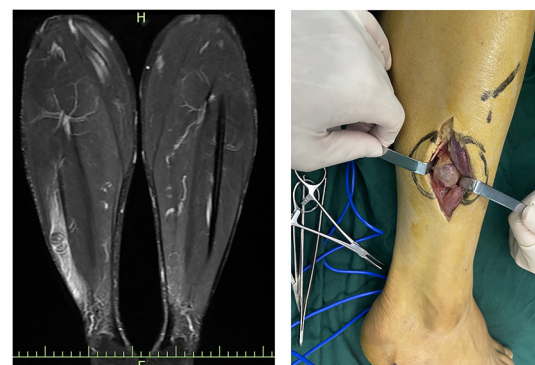


FIGURE 7
MRI shows that the tumor is located between the peroneus longus muscle.

Surgical treatment and prognosis

The treatment options for schwannomas are still controversial, given the risk of medically induced nerve injury from surgery and anesthesia and incisional problems. However, with the continuous development and application of microsurgical techniques, more investigators are recommending schwannomas undergo surgical resection (25–28). In the literature, we observed two main types of complete tumor resection, intracapsular resection and extracapsular resection. Extracapsular resection ensures maximum complete removal of the tumor, and studies have reported good clinical outcomes (8, 29). However, some investigators pointed out that extracapsular resection carries the possibility of damaging the functional nerve fascicles in the capsular layer, while intracapsular resection minimizes the risk of nerve injury without a high recurrence rate (26, 30). In this study, four patients with extracapsular resection reported varying degrees of nerve injury symptoms, including simple numbness and pain, severe clawed hands, and walking discomfort, and three required reoperations. However, patients with intracapsular resection were not completely free from the risk of nerve

injury, and two patients also presented with varying degrees of numbness and sensory abnormalities. All six patients had a solitary mass, and five of them were from the nerve trunk, which may be one of the risk factors for postoperative complications. Also, five patients had a medical history of more than 1 year, ranging from 20 years to 7 days. The patient with a 20-year medical history also had the largest schwannoma, about 23.56 cm³, but postoperatively only complained about abnormal skin sensations and tingling, which gradually vanished after 3 months. The patient with a mass history of only 7 days had the second largest schwannoma, 7.84 cm³, with postoperative symptoms of radial nerve entrapment and surgical release. Unlike previous studies (31, 32), tumor size, nerve origin, and the age of the patient did not show significant correlations with the occurrence of postoperative complications in the study. More data need to be collected to clarify the impact of multiple factors on postoperative complications.

Based on the above, we suggest that (1) meticulous microsurgical dissection should be performed for schwannomas to gain maximum preservation of nerve function; (2) sacrificing some of the nerve fiber bundles, such as the nerve fascicles that have entered the tumor, and the distal nerve fasciculus will not lead to postoperative neurological dysfunction, and to some extent, can reduce the intraoperative stimulation of the nerve trunk; (3) intracapsular resection of schwannomas located on the nerve trunk is recommended to reduce or mitigate the sequelae of nerve injury; (4) after revealing the mass and before intracapsular resection, the mass needs to be 360° exposed and searched for the presence of normal nerve fiber bundles passing through the mass, and if so, the mass is rotated vertically in the direction of the nerve trunk to find the best nerve-free zone for the incision, thus ensures complete resection of the mass and maximum preservation of the surrounding fascicles (Figure 8); (5) After shelling out the tumor, the preserved nerve should be meticulously hemostatic by electrocoagulation, or, in case of rich blood supply, local drainage and negative pressure suction are feasible to avoid nerve entrapment due to hematoma (Figure 9).

Another technical point of the operation process worth discussing is intraoperative monitoring, including nerve action potentials, compound muscle action potentials, and somatosensory evoked potentials (33). Intraoperative electrophysiological monitoring helps to clarify the anatomy and nerve orientation and to distinguish the involved nerves to maximize the preservation of healthy nerves and achieve complete tumor resection (6, 33, 34). However, there are several limitations in its clinical application. Most basically, most surgeons lack experience with the technique, and the instruments required for its implementation are absent from the operating rooms of most hospitals, such as ours (35). Second, the technique limits the choice of anesthesia; general anesthesia is required, as well as larger surgical incisions and longer operative time (33, 35, 36). This results in greater anesthetic risk, longer awakening time, and more postoperative

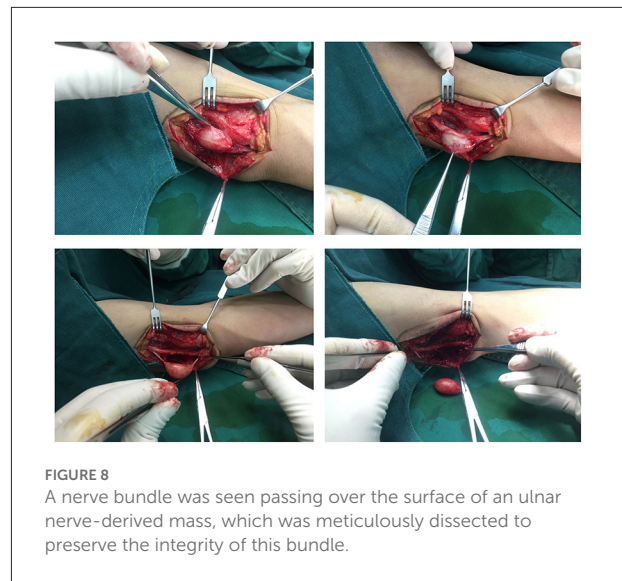


FIGURE 8

A nerve bundle was seen passing over the surface of an ulnar nerve-derived mass, which was meticulously dissected to preserve the integrity of this bundle.

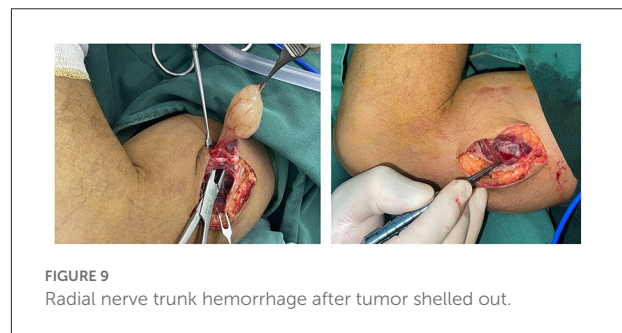


FIGURE 9

Radial nerve trunk hemorrhage after tumor shelled out.

discomfort than with routine nerve-blocking anesthesia. The larger surgical incision also increases the risk of infection and scar growth, and somewhat affects esthetics. These may explain the relatively limited number of studies exploring this technique, and more common reports of brachial plexus schwannomas and major nerve schwannomas (6, 33, 36–38), given their deeper location and greater potential of nerve invasion and complications occurrence. In addition, the lesser neural association and better prognosis of schwannomas compared with neurofibromas and malignant peripheral nerve sheath tumors may also limit its use to some extent (37). Therefore, for intraoperative electrophysiological monitoring, we recommend it for schwannomas with significant preoperative symptoms, involvement of the nerve trunk, deep sites, or large size.

Recurrence of schwannomas after surgical resection is extremely rare (6, 8, 31, 32). In this study, five patients were found to have postoperative recurrences, ranging from 1 to 20 years, and four of them had undergone tumor resection at an outside hospital before attending our hospital, thus detailed medical records are not available. It is interesting to note that in three cases, the site of recurrence was different from the primary

site and recurred in the ipsilateral upper extremity or even the contralateral lower extremity.

Limitations

The limitations of our study are inherent to its retrospective nature. Given the rarity of schwannomas, clinicians have varying degrees of awareness of such a disease, leading to possible omissions in their history-taking and examination of signs, as well as different choices of examination tools. Second, patients with schwannomatosis were not carefully screened in our study, and some patients with multiple masses may be classified as having this disease. Additionally, the description of the surgical procedure by the surgeon varies from person to person, which may have an impact on the classification of the surgical treatment. Also, the duration of follow-up varies from patient to patient, with most patients having relatively short follow-ups (Supplementary Figure 2). For the analysis of the factors influencing postoperative complications, more patients' data need to be collected to achieve this.

Conclusion

We explored the clinical features and management of 183 cases of schwannomas in a single center, which is the largest clinical retrospective study of schwannomas in extremities. The study showed that schwannomas tend to be isolated, asymptomatic masses, occurring mostly in the left limb, flexor surface, upper extremity, and long bone regions, and are more common in middle-aged adults, without gender preference. Patients are more likely to find a mass on the extensor side and in the long bone region and have the lowest tolerance for numbness. Symptoms of the schwannomas appear progressively with time. Digital nerves are a more common origin. The discomfort of nerve trunk-originated tumor is more common. MRI has a high sensitivity for the preoperative detection of schwannomas. Early microsurgical resection of the schwannomas with meticulous hemostasis and dissection is recommended. Intracapsular resection is recommended for tumors located in the nerve trunk, and the application of intraoperative electrophysiological monitoring depends on the characteristics of the schwannomas. Surgical resection of schwannomas has few complications and rare recurrences postoperatively.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of the College of Medicine, Zhejiang University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HL designed the study. HZ, CY, and JL performed data collection. AA and YD analyzed the results. ZW, VK, MA, and SE drafted the manuscript. All authors have read and approved the final manuscript.

Funding

The study was funded by the Zhejiang Provincial Continuing Education Program (Grant Number 2022-04-07-001). This work was supported by Alibaba Cloud. The funding bodies had no role in the design of the study, in the collection, analysis and interpretation of data, and in drafting the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.1083896/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

The number of surgeons and their operation type.

SUPPLEMENTARY FIGURE 2

The time span of patients' follow-up.

References

1. Institute NC. *Schwannoma Was Originally Published by the National Cancer Institute 2020* (accessed July 30, 2020).
2. Das Gupta TK, Brasfield RD, Strong EW, Hajdu SI. Benign solitary Schwannomas (neurilemmomas). *Cancer*. (1969) 24:355–66. doi: 10.1002/1097-0142(196908)24:2<355::aid-cnrcr2820240218>3.0.co;2-2
3. Zhou H, Jiang S, Ma F, Lu H. Peripheral nerve tumors of the hand: clinical features, diagnosis, and treatment. *World J Clin Cases*. (2020) 8:5086–98. doi: 10.12998/wjcc.v8.i21.5086
4. Tang CY, Fung B, Fok M, Zhu J. Schwannoma in the upper limbs. *Biomed Res Int*. (2013) 2013:167196. doi: 10.1155/2013/167196
5. Sawada T, Sano M, Ogihara H, Omura T, Miura K, Nagano A. The relationship between pre-operative symptoms, operative findings and postoperative complications in schwannomas. *J Hand Surg*. (2006) 31:629–34. doi: 10.1016/j.jhsb.2006.06.008
6. Desai KI. The surgical management of symptomatic benign peripheral nerve sheath tumors of the neck and extremities: an experience of 442 cases. *Neurosurgery*. (2017) 81:568–80. doi: 10.1093/neuros/nyx076
7. Jin Q, Lu H. Angioleiomyoma of the hand with nerve compression. *J Int Med Res*. (2020) 48:300060520928683. doi: 10.1177/0300060520928683
8. El Sayed L, Masmejean EH, Lavollé A, Biau D, Peyre M. Clinical results after surgical resection of benign solitary schwannomas: a review of 150 cases. *Orthop Traumatol Surg Res*. (2022) 108:103281. doi: 10.1016/j.otsr.2022.103281
9. Granlund AS, Sørensen MS, Jensen CL, Bech BH, Petersen MM. Clinical outcome after surgery on schwannomas in the extremities. *World J Orthop*. (2021) 12:760–7. doi: 10.5312/wjo.v12.i10.760
10. Suzuki A, Kanda T, Yoshimizu T, Mukoda M, Inoue Y. Investigation of schwannomas in the forearms, hands, and digits: a retrospective study. *J Hand Surg Asian Pac Vol*. (2021) 26:17–23. doi: 10.1142/S2424835521500028
11. Hirai T, Kobayashi H, Akiyama T, Okuma T, Oka H, Shinoda Y, et al. Predictive factors for complications after surgical treatment for schwannomas of the extremities. *BMC Musculoskelet Disord*. (2019) 20:166. doi: 10.1186/s12891-019-2538-8
12. Kokkalis ZT, Stavropoulos NA, Mavrogenis AF, Panagopoulos A, Soucacos PN. Benign peripheral nerve tumors. *Injury*. (2019) 50 Suppl 5:S77–83. doi: 10.1016/j.injury.2019.10.054
13. Bhattacharyya AK, Perrin R, Guha A. Peripheral nerve tumors: management strategies and molecular insights. *J Neurooncol*. (2004) 69:335–49. doi: 10.1023/B:NEON.0000041891.39474.cb
14. Knight DM, Birch R, Pringle J. Benign solitary schwannomas: a review of 234 cases. *J Bone Joint Surg Br*. (2007) 89:382–7. doi: 10.1302/0301-620X.89B3.18123
15. Lee SH, Jung HG, Park YC, Kim HS. Results of neurilemmoma treatment: a review of 78 cases. *Orthopedics*. (2001) 24:977–80. doi: 10.3928/0147-7447-20011001-18
16. Guha D, Davidson B, Nadi M, Alotaibi NM, Fehlings MG, Gentili F, et al. Management of peripheral nerve sheath tumors: 17 years of experience at Toronto Western Hospital. *J Neurosurg*. (2018) 128:1226–34. doi: 10.3171/2017.1.JNS162292
17. Wang Y, Lu H. Multiple intraneural glomus tumors in different digital nerve fascicles. *BMC Cancer*. (2019) 19:888. doi: 10.1186/s12885-019-6098-y
18. Abe K, Takeuchi A, Yamamoto N, Hayashi K, Tada K, Miwa S, et al. Symptomatic small schwannoma is a risk factor for surgical complications and correlates with difficulty of enucleation. *Springerplus*. (2015) 4:751. doi: 10.1186/s40064-015-1547-9
19. Lee SJ, Yoon ST. Ultrasonographic and clinical characteristics of schwannoma of the hand. *Clin Orthop Surg*. (2017) 9:91–5. doi: 10.4055/cios.2017.9.1.91
20. Yang F, Chen XX, Wu HL, Zhu JF, Chen Y, Yu LF, et al. Sonographic features and diagnosis of peripheral schwannomas. *J Clinical Ultrasound*. (2017) 45:127–33. doi: 10.1002/jcu.22438
21. Hu X, Liu Z, Zhou H, Fang J, Lu H. Deep HT: A deep neural network for diagnosis on MR images of tumors of the hand. *PLoS ONE*. (2020) 15:e0237606. doi: 10.1371/journal.pone.0237606
22. Belakhova SM, Rodriguez FJ. Diagnostic pathology of tumors of peripheral nerve. *Neurosurgery*. (2021) 88:443–56. doi: 10.1093/neuros/nyab021
23. Meyer A, Billings SD. What's new in nerve sheath tumors. *Virchows Archiv*. (2020) 476:65–80. doi: 10.1007/s00428-019-02671-0
24. Magro G, Broggi G, Angelico G, Puzzo L, Vecchio GM, Virzi V, et al. Practical approach to histological diagnosis of peripheral nerve sheath tumors: an update. *Diagnostics*. (2022) 12:1463. doi: 10.3390/diagnostics12061463
25. Tiel R, Kline D. Peripheral nerve tumors: surgical principles, approaches, and techniques. *Neurosurg Clin N Am*. (2004) 15:167–75. vi. doi: 10.1016/j.nec.2004.02.003
26. Park MJ, Seo KN, Kang HJ. Neurological deficit after surgical enucleation of schwannomas of the upper limb. *J Bone Joint Surg Br*. (2009) 91:1482–6. doi: 10.1302/0301-620X.91B11.22519
27. Siqueira MG, Martins RS, Teixeira MJ. Management of brachial plexus region tumours and tumour-like conditions: relevant diagnostic and surgical features in a consecutive series of eighteen patients. *Acta Neurochir*. (2009) 151:1089–98. doi: 10.1007/s00701-009-0380-8
28. Zhou H, Lu H. A solitary giant neurofibroma of inguinal region: a case report. *Sci Prog*. (2021) 104:368504211004269. doi: 10.1177/00368504211004269
29. Artico M, Cervoni L, Wierzbicki V, D'Andrea V, Nucci F. Benign neural sheath tumours of major nerves: characteristics in 119 surgical cases. *Acta Neurochir*. (1997) 139:1108–16. doi: 10.1007/BF01410969
30. Kim SM, Seo SW, Lee JY, Sung KS. Surgical outcome of schwannomas arising from major peripheral nerves in the lower limb. *Int Orthop*. (2012) 36:1721–5. doi: 10.1007/s00701-012-1560-3
31. Montano N, D'Alessandris QG, D'Ercole M, Lauretti L, Pallini R, Di Bonaventura R, et al. Tumors of the peripheral nervous system: analysis of prognostic factors in a series with long-term follow-up and review of the literature. *J Neurosurg*. (2016) 125:363–71. doi: 10.3171/2015.6.JNS15596
32. Siqueira MG, Socolovsky M, Martins RS, Robla-Costales J, Di Masi G, Heise CO, et al. Surgical treatment of typical peripheral schwannomas: the risk of new postoperative deficits. *Acta Neurochir*. (2013) 155:1745–9. doi: 10.1007/s00701-013-1818-6
33. Huang JH, Zaghoul K, Zager EL. Surgical management of brachial plexus region tumors. *Surg Neurol*. (2004) 61:372–8. doi: 10.1016/j.surneu.2003.08.006
34. Kline DG. Nerve surgery as it is now and as it may be. *Neurosurgery*. (2000) 46:1285–93. doi: 10.1097/00006123-200006000-00001
35. Tiel RL, Happel LT Jr, Kline DG. Nerve action potential recording method and equipment. *Neurosurgery*. (1996) 39:103–8. doi: 10.1097/00006123-199607000-00019
36. Huang JH, Samadani U, Zager EL. Brachial plexus region tumors: a review of their history, classification, surgical management, and outcomes. *Neurosurg Q*. (2003) 13:151–61. doi: 10.1097/00013414-200309000-00001
37. Donner TR, Voorhies RM, Kline DG. Neural sheath tumors of major nerves. *J Neurosurg*. (1994) 81:362–73. doi: 10.3171/jns.1994.81.3.0362
38. Desai KI. Primary benign brachial plexus tumors: an experience of 115 operated cases. *Neurosurgery*. (2012) 70:220–33. doi: 10.1227/NEU.0b013e31822d276a



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University, China

REVIEWED BY

Francesco Agostini,
Sapienza University of Rome, Italy
Luca Leonardi,
Sapienza University of Rome, Italy

*CORRESPONDENCE

Xiao-Lei Wang
wxlghb@126.com

†These authors have contributed
equally to this work

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 25 October 2022

ACCEPTED 23 November 2022

PUBLISHED 16 December 2022

CITATION

Wu H, Zhao H-J, Xue W-L, Wang Y-C,
Zhang W-Y and Wang X-L (2022)
Ultrasound and elastography role in
pre- and post-operative evaluation of
median neuropathy in patients with
carpal tunnel syndrome.
Front. Neurol. 13:1079737.
doi: 10.3389/fneur.2022.1079737

COPYRIGHT

© 2022 Wu, Zhao, Xue, Wang, Zhang
and Wang. This is an open-access
article distributed under the terms of
the [Creative Commons Attribution
License \(CC BY\)](#). The use, distribution
or reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Ultrasound and elastography role in pre- and post-operative evaluation of median neuropathy in patients with carpal tunnel syndrome

Han Wu[†], Hong-Juan Zhao[†], Wei-Li Xue, Yi-Chun Wang,
Wu-Yue Zhang and Xiao-Lei Wang*

In-Patient Ultrasound Department, Second Affiliated Hospital of Harbin Medical University, Harbin,
China

Introduction: Carpal tunnel syndrome (CTS) is a common compression neuropathy of the median nerve in the wrist. Early diagnosis of CTS is essential for selecting treatment options and assessing prognosis. The current diagnosis of CTS is based on the patient's clinical symptoms, signs, and an electromyography (EMG) test. However, they have some limitations. Recently, ultrasound has been adopted as an adjunct diagnostic tool for electromyography (EMG). Ultrasound is a non-invasive and cost-effective technique. It provides a dynamic display of morphological changes in the median nerve and an assessment of CTS etiology such as tenosynovitis, mass compression, and tendon disease. This study aimed to investigate the value of conventional ultrasound and real-time shear wave elastography (SWE) in evaluation of median neuropathy in patients with carpal tunnel syndrome (CTS) before and after surgery.

Methods: First, the Boston Carpal Tunnel Questionnaire (BCTQ) was administered to patients with CTS. All subjects were measured at three levels: the distal 1/3 of the forearm, the carpal tunnel inlet, and the distal carpal tunnel using conventional ultrasound and SWE. Median nerve parameters were examined in patients with CTS 1 week after surgery.

Results: The cross-sectional area (CSA) and stiffness of the median nerve at the carpal tunnel inlet and distal carpal tunnel were significantly higher in patients with CTS than in healthy controls ($p < 0.001$). The CSA and stiffness of the median nerve at the carpal tunnel inlet were statistically significantly significantly between pre- and postoperative patients with CTS ($p < 0.001$). The CSA and stiffness of the nerve in patients with CTS had a positive correlation with electrophysiology severity.

Conclusions and discussion: Conventional ultrasound and elastography are valuable in the diagnosis of CTS and are useful in the clinical assessment of patient's nerve recovery after operation.

KEYWORDS

carpal tunnel syndrome, acoustic radiation force impulse, shear wave elastography, Boston Carpal Tunnel Questionnaire, cross-sectional area

1. Introduction

Carpal tunnel syndrome (CTS) is a common compression neuropathy of the median nerve in the wrist. It accounts for 90% of all entrapment neuropathies, with a prevalence rate of approximately 3.8% in the population (1). Nerve damage can be reversed with early treatment and diagnosis to prevent permanent damage. Early diagnosis of CTS is essential for selecting treatment options and assessing prognosis (2, 3). The current diagnosis of CTS is based on the patient's clinical symptoms, signs, and an electromyography (EMG) test. However, symptoms and signs in patients with mild disease are unclear and lack objective judgment criteria (4). Electromyography is the gold standard for CTS diagnosis and yields 98% specificity and 75% sensitivity. This examination has some limitations, including being time-consuming, invasive, and has a 10–25% false-negative rate. In addition, it cannot directly assess the anatomical relationship between the median nerve and surrounding tissues (4–6). Certain imaging examinations are significant in CTS diagnosis. According to Park et al. (7), magnetic resonance imaging (MRI) can be a diagnostic tool for median neuropathy. This technology can measure the median nerve cross-sectional area (CSA), despite the procedure being expensive and time-consuming. Also, computed tomography (CT) can diagnose CTS by showing changes in the density of the compressed median nerve and occupying lesions (8, 9).

Recently, ultrasound has been adopted as an adjunct diagnostic tool for electromyography (EMG). Ultrasound is a non-invasive and cost-effective technique. It provides a dynamic display of morphological changes in the median nerve and an assessment of CTS etiology such as tenosynovitis, mass compression, and tendon disease (10–12). Previous studies have focused on the CSA of the median nerve at the carpal tunnel entrance in CTS patients with sensitivity and specificity comparable to electromyography examinations (13–16). On the contrary, shear-wave elastography (SWE) is a new technique for the quantitative measurement of tissue stiffness (17). It is a complement to conventional ultrasound in the assessment of neuropathy (18–21). Studies have reported the significance of elastography in the assessment of CTS (22–25). However, to the extent of our knowledge, there are few reports on the evaluation of pre- and post-operative changes in nerve stiffness in patients with CTS. Therefore, the purpose of this study was to investigate the value of conventional ultrasound and SWE on the median nerve in patients with CTS before and after surgery.

2. Materials and methods

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University. All

examinations were conducted after written informed consent was obtained from all subjects.

2.1. Patients

In total, 50 wrists of 25 healthy volunteers and 54 wrists of 30 patients who underwent carpal tunnel release surgery were studied from October 2020 to June 2021. Inclusion criteria were as follows: patients who reported abnormal sensation or pain in their extremities and had a positive electromyography examination (26). Exclusion criteria were as follows: age < 18 years, history of previous hand surgery, and other conditions affecting the measurement of the median nerve. The scale used to measure the severity of CTS was as follows: negative (grade 0), mild (grade 1), moderate (grade 2), severe (grade 3), and extremely severe (grade 4) according to the results of electromyography. Questionnaires were administered to patients with CTS, and basic information was recorded for all participants.

2.2. Questionnaires

The Boston Carpal Tunnel Questionnaire (BCTQ) consists of two self-rating scales: the Symptom Severity Scale (SSS) and the Functional Status Scale (FSS) (27). High scores indicated more severe disease conditions. It was used to assess all patients with CTS.

2.3. Ultrasound examination

All examinations were performed using a 4C15 MHz linear array transducer (Aixplorer; Supersonic Imagine, Aix en Provence, France). All patients were placed in a seated position, facing the examiner, with the forearm fully exposed, the elbow bent at 90°, and the hand kept in a resting position. The median nerve CSA and nerve flattening rate (NFR) were measured at three sites: the distal 1/3 of the forearm, the carpal tunnel inlet (at the level of the doughy bone), and the distal carpal tunnel (at the level of the hook bone). NFR is the ratio of the transverse diameter of the nerve to the anterior-posterior diameter when the median nerve is compressed by entrapment. It is an ultrasound parameter to determine if the nerve is compressed. The mean value was calculated by taking the average of the replicate measurements. The transducer was rotated 90° to obtain a longitudinal image, and the image was frozen after obtaining the ideal image. SWE was activated at the same three loci as the 2D ultrasound. Three regions of interest (ROI) with a diameter of 1 mm were selected to calculate the average value. Each locus was measured in triplicate. During the measurement, a sufficient coupling agent was applied, and the

probe was placed gently on the skin to avoid pressure. A week after the surgery, the conventional ultrasound and SWE were repeated on the same patients. The above operations and image analysis were performed by a qualified ultrasound physician with over 10 years of research in musculoskeletal aspects.

2.4. Statistical analyses

Data were analyzed using the SPSS version 23.0 (SPSS Inc., Chicago, USA) and MedCalc 20.0 software (Ostend, West-Vlaanderen, Belgium). Continuous variables were expressed as mean $\pm$ standard deviation (SD). The Kolmogorov-Smirnov test was used to analyze the normal distribution. Mann-Whitney U test was used to analyze the non-normally distributed measures. The correlation was expressed by Spearman's correlation coefficient. Receiver operating characteristic (ROC) curves for CSA and elasticity were obtained to calculate sensitivity, specificity, accuracy, the area under the curve (AUC), and optimal cutoff values. Z-test was used for AUC. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Study demographics

The demographic data for the patients are summarized in Table 1. There was no statistical difference in gender, age, and body mass index (BMI) between patients with CTS and healthy controls at $p > 0.05$. BCTQ scores (SSS and FSS) between pre- and postoperative groups with CTS did not show any significant statistical difference ($p > 0.05$). In total, six wrists (11.1%) reported negative electromyography results. Grading CTS by severity showed mild in 15 (27.8%), moderate in 18 (33.3%), severe in 13 (24.1%), and extremely severe in 2 (3.7%) cases with a positive neurophysiological result.

3.2. Conventional ultrasound and SWE parameters in healthy controls and preoperative groups

The distal 1/3 of the forearm in the preoperative group was not statistically different at $p > 0.05$ in CSA, flattening rate, and E_{mean} of the median nerve compared to the healthy control group. However, the CSA, flattening rate, and E_{mean} of the median nerve at the carpal tunnel inlet and the distal carpal tunnel in the preoperative group were significantly higher than in healthy controls at $p < 0.001$ (Table 2).

TABLE 1 Characteristics of patients.

	Control group (<i>n</i> = 50)	CTS group (<i>n</i> = 54)	<i>p</i> -value
Age (y) [#]	55.84 $\pm$ 7.59	53.57 $\pm$ 8.72	0.400
Sex			0.299
Female	42 (84)	48 (89)	
Male	8 (16)	6 (11)	
BMI (kg/m ²) [#]	22.22 $\pm$ 1.75	22.31 $\pm$ 1.98	0.670
BCTQ score [#]		Preoperative group Postoperative group	
SSS	/	4.87 $\pm$ 3.30 4.81 $\pm$ 3.22	0.257
FSS	/	2.20 $\pm$ 1.91 2.11 $\pm$ 1.75	0.132

CTS, carpal tunnel syndrome; BMI, body mass index; BCTQ, The Boston Carpal Tunnel Questionnaire; SSS, Symptom Severity Scale; FSS, Functional Status Scale. [#]Data are means $\pm$ standard deviations.

TABLE 2 Conventional ultrasound and shear wave elastography parameters of the control group and the preoperative group.

	Control group (<i>n</i> = 50)	Preoperative group (<i>n</i> = 54)	<i>p</i> -value
Distal 1/3 of the forearm			
CSA (mm ²) [#]	5.95 $\pm$ 0.75	5.96 $\pm$ 0.83	0.661
NFR [#]	1.78 $\pm$ 0.34	1.73 $\pm$ 0.29	0.470
E_{mean} (kPa) [#]	25.38 $\pm$ 3.99	25.87 $\pm$ 4.81	0.271
Carpal tunnel inlet			
CSA (mm ²) [#]	5.91 $\pm$ 0.90	16.00 $\pm$ 5.63	0.000*
NFR [#]	2.51 $\pm$ 0.48	3.11 $\pm$ 0.53	0.000*
E_{mean} (kPa) [#]	35.25 $\pm$ 23.38	188.21 $\pm$ 53.09	0.000*
Distal carpal tunnel			
CSA (mm ²) [#]	4.86 $\pm$ 0.88	8.70 $\pm$ 2.48	0.000*
NFR [#]	2.05 $\pm$ 0.44	2.67 $\pm$ 0.78	0.000*
E_{mean} (kPa) [#]	27.83 $\pm$ 9.75	113.48 $\pm$ 51.74	0.000*

CSA, cross-sectional area; NFR, neural flattening rate; E_{mean} , the mean shear wave velocity of the nerve. [#]Data are means $\pm$ standard deviations. *Indicates statistically significant difference.

3.3. Conventional ultrasound and SWE parameters between pre- and postoperative CTS groups

There was no significant difference in CSA, flattening rate, and E_{mean} of the median nerve at the distal 1/3 of the forearm ($p > 0.05$). The CSA, flattening rate, and E_{mean} of the median nerve at the carpal tunnel inlet and distal carpal tunnel were significantly lower in the postoperative group than in the preoperative group ($p < 0.05$) (Table 3).

TABLE 3 Conventional ultrasound and shear wave elastography parameters of the preoperative group and the postoperative group.

	Preoperative group (<i>n</i> = 19)	Postoperative group (<i>n</i> = 19)	<i>p</i> -value
Distal 1/3 of the forearm			
CSA (mm ²) [#]	6.51 ± 1.59	6.23 ± 0.84	0.954
NFR [#]	1.76 ± 0.25	1.71 ± 0.21	0.327
E _{mean} (kPa) [#]	25.45 ± 4.99	23.54 ± 3.67	0.133
Carpal tunnel inlet			
CSA (mm ²) [#]	14.89 ± 4.25	11.63 ± 3.69	0.000*
NFR [#]	3.06 ± 0.51	2.44 ± 0.46	0.001*
E _{mean} (kPa) [#]	185.20 ± 34.97	67.46 ± 21.19	0.000*
Distal carpal tunnel			
CSA (mm ²) [#]	8.44 ± 2.75	8.02 ± 2.90	0.389
NFR [#]	2.68 ± 0.56	2.34 ± 0.71	0.049*
E _{mean} (kPa) [#]	102.32 ± 37.61	34.88 ± 15.78	0.000*

CSA, cross-sectional area; NFR, neural flattening rate; E_{mean}, the mean shear wave velocity of the nerve. [#]Data are means ± standard deviations. *Indicates statistically significant difference.

TABLE 4 Conventional ultrasound and shear wave elastography parameters of the control group and the postoperative group.

	Control group (<i>n</i> = 50)	Postoperative group (<i>n</i> = 19)	<i>p</i> -value
Distal 1/3 of the forearm			
CSA(mm ²) [#]	5.95 ± 0.75	6.23 ± 0.84	0.597
NFR [#]	1.78 ± 0.34	1.71 ± 0.21	0.498
E _{mean} (kPa) [#]	25.38 ± 3.99	23.54 ± 3.67	0.712
Carpal tunnel inlet			
CSA(mm ²) [#]	5.91 ± 0.90	11.63 ± 3.69	0.000*
NFR [#]	2.51 ± 0.48	2.44 ± 0.46	0.577
E _{mean} (kPa) [#]	35.25 ± 23.38	67.46 ± 21.19	0.000*
Distal carpal tunnel			
CSA(mm ²) [#]	4.86 ± 0.88	8.02 ± 2.90	0.000*
NFR [#]	2.05 ± 0.44	2.34 ± 0.71	0.127
E _{mean} (kPa) [#]	27.83 ± 9.75	34.88 ± 15.78	0.041*

CSA, cross-sectional area; NFR, neural flattening rate; E_{mean}, the mean shear wave velocity of the nerve. [#]Data are means ± standard deviations. *Indicates statistically significant difference.

3.4. Conventional ultrasound and SWE parameters of healthy controls and postoperative CTS groups

The postoperative group had a significantly lower CSA and E_{mean} of the median nerve at the carpal tunnel inlet and the distal carpal tunnel than the healthy controls ($p < 0.05$) (Table 4).

TABLE 5 AUC, sensitivity, specificity, and optimal cut-off values of nerve parameters for the diagnosis of CTS.

	Sensitivity (%)	Specificity (%)	AUC
Carpal tunnel inlet CSA ≥ 8.2 mm ²	96.2	100	0.999
Inlet/ distal 1/3 of the forearm CSA ratio ≥ 1.68	86.8	100	0.999
Carpal tunnel inlet E _{mean} ≥ 102.9 kPa	96.3	98	0.993
Inlet/ distal 1/3 of the Forearm E _{mean} ratio ≥ 3.72	96	96	0.992

CSA, cross-sectional area; E_{mean}, the mean shear wave velocity of the nerve. Data were tested with the area under the receiver operating characteristic (ROC) curve.

3.5. Diagnostic performance of conventional ultrasound and SWE parameters

The CSA and E_{mean} at the carpal tunnel inlet and the distal 1/3 of the forearm showed good diagnostic performance (0.999, 0.993, 0.999, 0.992, $p < 0.001$). In this study, the corresponding cutoff value of CSA (8.2 mm²) was used to diagnose CTS, with a sensitivity of 96.2% and a specificity of 100% at the carpal tunnel inlet. We chose the optimal cutoff value of E_{mean} (102.9 Kpa) to diagnose CTS, with a sensitivity of 96.3% and a specificity of 98%. The difference in AUC between CSA and E_{mean} for CTS diagnosis was not statistically significant ($Z = 1.15$, $p = 0.25$). The CSA cutoff value (1.68 mm²) of the carpal tunnel inlet and the distal 1/3 of the forearm produced sensitivity and specificity of 86.6 and 100%, respectively. However, the cutoff value (3.72 mm²) of the E_{mean} ratio of the carpal tunnel inlet and the distal 1/3 of the forearm produced a sensitivity of 96% and a specificity of 96% (Table 5).

3.6. Correlation of electrophysiology severity with CSA and E_{mean}

Electrophysiological severity had a positive correlation with CSA ($r^2 = 0.93$, $p < 0.001$), whereas electrophysiological severity had a moderate correlation with E_{mean} ($r^2 = 0.60$, $p < 0.001$).

4. Discussion

Studies suggest high pressure in the carpal tunnel to be the cause of CTS (28). The increased pressure affects the microcirculation in the median nerve, leading to edema and demyelinating lesions. Hence, there is a thickening of the nerve bundle and vessel walls (29, 30). Nerve entrapment injury causes intraneural ischemia, which leads to abnormal vascular

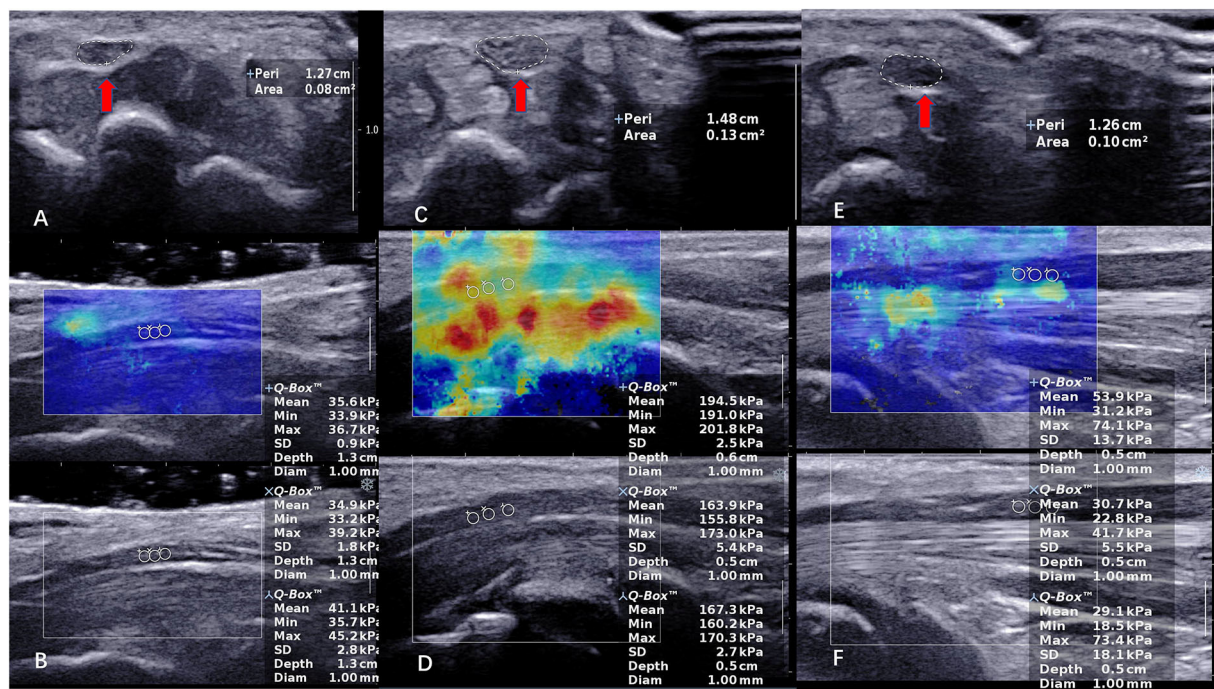


FIGURE 1

The CSA and SWE of the median nerve (MN) in a 40-year-old female control subject at carpal tunnel inlet. (A,B) (CSA = 0.08 cm², E_{mean} = 37.2 kPa). The CSA and SWE of the median nerve (MN) in a 51-year-old female patient with severe CTS at carpal tunnel inlet in preoperative. (C,D) (CSA = 0.13 cm², E_{mean} = 175.3 kPa). The CSA and SWE of the median nerve (MN) in a 51-year-old female patient with severe CTS at carpal tunnel inlet in postoperative. (E,F) (CSA = 0.10 cm², E_{mean} = 37.9 kPa). The red arrow indicates the CSA of the nerve.

endothelial permeability and manifests as intra-nerve edema and increased CSA measurement (29, 31). High-frequency ultrasound can detect changes in the median nerve, such as hypoechoic nerve, blurred nerve bundle, and increased cross-sectional area.

Byra et al. (32) reported variability in the echogenicity and shape of the median nerve in CTS and healthy patients. Also, the CSA of patients with CTS was significantly higher than healthy individuals (33). Notably, these findings were consistent with our study.

Furthermore, we found that the lack of differences between CSA at the distal carpal tunnel pre- and postoperatively could be attributed to delayed decompression.

We also found that the injury to the nerve did not involve the distal 1/3 of the forearm, and because of this, we could use the CSA at the distal 1/3 of the forearm as a reference value in this experiment to obtain a truncated value with less individual differentiation compared with the CSA at other locations.

Compression of microcirculation in the median nerve leads to focal demyelination and axonal degeneration with a fibrotic response (14, 30). Wang et al. (34) documented a relationship between carpal tunnel pressure and shear wave velocity. In our study, the cutoff values of the median nerve at the carpal tunnel inlet were higher than in other studies. We concede that participants had moderate and severe

CTS with a higher degree of neuropathy, thus leading to a higher E_{mean} .

In our study, we found that the variance of E_{mean} at the carpal tunnel inlet and outlet in all populations is very high compared to 1/3 of the distal forearm (Figure 1). We hypothesized that there are more bones in the carpal tunnel inlet and outlet, which cause the “bone-proximity” hardening artifacts. These artifacts occur when the nerve travels near a bone plane that prevents the shear wave from homogeneously propagating at depth, thus causing local stress inhomogeneity. As Bortolotto reported, the stiffness of the median nerve progressively increases in its distal portions, where the nerve approaches the bone surface (35).

The correlation of electrophysiology severity with CSA and E_{mean} is consistent with the results of Choi et al. (36) and Drakopoulos et al. (37). Therefore, we suggest that it is feasible to determine the severity of the disease in patients with CTS based on conventional ultrasound and SWE. Conversely, another study showed that nerve stiffness was a better predictor of disease severity than CSA, due to substantial axonal loss in extremely severe cases, which produces less swelling of the compressed nerve than in moderately severe cases (38).

There were two cases of extreme severity in our study that had no significant difference in assessing CTS severity. The quantitative evaluation of the median nerve injury in patients

by conventional ultrasound and SWE had high diagnostic values (Table 5). Although there were no significant differences between the two techniques, the application of the SWE technique did not improve the diagnostic efficacy of CTS. Kantarci et al. (28) and Arslanet et al. (39) documented similar findings.

Currently, the best treatment for CTS is carpal tunnel release. Approximately, 70–90% of patients undergo carpal tunnel dissection to release the median nerve and achieve satisfactory results (40, 41). The questionnaires can be used as a method to assess postoperative recovery (42). Compared to the BCTQ score, ultrasound better reflects the recovery of neuropathy before the symptoms improve.

The reduction in CSA and elasticity is due to the successful severance of the transverse carpal ligament, which releases nerve compression and reduces nerve edema. In our study, the differences in CSA and E_{mean} between postoperative patients with CTS and volunteers were statistically significant (Table 4). It can be attributed to the presence of severe permanent structural changes in the median nerve in some patients, as reported by Inui et al. (40). In addition, neurological symptoms were not completely relieved because of the short recovery time.

The differences in CSA of the median nerve measured at the carpal tunnel and proximal levels provided higher diagnostic efficacy than a single CSA measure (43). Scholars have suggested the use of different elasticity ratios to reduce individual variability and stiffness (13, 33, 44) due to the variability of CSA and elasticity in patients of different ages, weights, and genders.

In this experiment, we used CSA and E_{mean} of the nerve at the distal 1/3 of the forearm as internal control parameters. We analyzed the diagnostic performance of the CSA ratio and E_{mean} of the nerve at the carpal tunnel inlet and the distal 1/3 of the forearm. The ratio had a high diagnostic efficacy; this strategy increased the assessment efficiency and sensitivity of median nerve injury (45) but was not optimized by applying CSA or E_{mean} alone due to the low number of patients.

This study has some limitations: (a) A single-center study with a small sample size, (b) a preliminary study of postoperative nerve recovery in the short term; in future studies, we will refine the records of longer follow-ups. (c) We detected microvascular changes within the median nerve in some moderate to severe patients, which we believe is also a sign of pathological changes. However, we did not conduct further analysis. (d) The pressure applied to the surface with the probe cannot be controlled completely at the carpal tunnel inlet and outlet. Therefore, the recommendations for future research are to (a) increase the number of patients with mild and extremely severe CTS, (b) increase the patient follow-up information to assess long-term nerve recovery and explore the potential correlations between SWE parameters and clinical score in a later stage after surgery, (c) examine the microvascular anatomy of the median nerve in all patients in future studies, and (d) add an ultrasound gel pad

to reduce excessive pressure when measuring the elasticity of the superficial nerve.

5. Conclusion

Conventional ultrasound and shear wave elastography have a strong correlation with clinical symptom scores and electromyography severity grading. This allowed the assessment of changes in postoperative nerve stiffness, which assisted in the clinical judgment of postoperative nerve recovery in patients with CTS.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design of the study: X-LW. Ultrasound data acquisition: HW. Analysis of data: W-LX, Y-CW, and W-YZ. Drafting the manuscript: HW and H-JZ. Revising and final approval of the version to be published: X-LW. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the scientific research project of the Heilongjiang Provincial Health Commission (Grant No. 202109020156).

Acknowledgments

We sincerely thank all participants in the study for their cooperation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Yao Y, Grandy E, Jenkins L, Hou J, Evans PJ, Seitz Jr WH, et al. Changes of median nerve conduction, cross-sectional area and mobility by radioulnar wrist compression intervention in patients with carpal tunnel syndrome. *J Ortho Translat*. (2019) 18:13–9. doi: 10.1016/j.jot.2019.01.002
- Padua L, Coraci D, Erra C, Pazzaglia C, Paolasso I, Loreti C, et al. Webb Carpal tunnel syndrome: clinical features, diagnosis, and management. *Lancet Neurol*. (2016) 15:1273–84. doi: 10.1016/S1474-4422(16)30231-9
- Wipperfman J, Goerl K. Carpal tunnel syndrome: diagnosis and management. *Am Fam Phys*. (2016) 94:993–999. doi: 10.1016/j.wneu.2022.11.115
- Chang YW, Hsieh TC, Tzeng I, Chiu V, Huang PJ, Horng YS. Ratio and difference of the cross-sectional area of median nerve to ulnar nerve in diagnosing carpal tunnel syndrome: a case control study. *BMC Med Imag*. (2019) 19:52. doi: 10.1186/s12880-019-0351-3
- Cartwright MS, Hobson-Webb LD, Boon AJ, Alter KE, Hunt CH, Flores VH, et al. Evidence-based guideline: neuromuscular ultrasound for the diagnosis of carpal tunnel syndrome. *Mus Nerve*. (2012) 46:287–93. doi: 10.1002/mus.23389
- T Wee TC, Simon NG. Ultrasound elastography for the evaluation of peripheral nerves: A systematic review. *Mus Nerve*. (2019) 60:501–512. doi: 10.1002/mus.26624
- Park JS, Won HC, Oh JY, Kim DH, Hwang SC, Yoo JI. Value of cross-sectional area of median nerve by MRI in carpal tunnel syndrome. *As J Surg*. (2020) 43:654–9. doi: 10.1016/j.asjsur.2019.08.001
- Deniz EO, Sarikaya S, Kurt U, Erkorkmaz H, Ulusoy, Arslan S. Comparison of the diagnostic utility of electromyography, ultrasonography, computed tomography, and magnetic resonance imaging in idiopathic carpal tunnel syndrome determined by clinical findings. *Neurosurgery*. (2012) 70:610–6. doi: 10.1227/NEU.0b013e318233868f
- Lee S, Cho HR, Yoo JS, Kim YU. The prognostic value of median nerve thickness in diagnosing carpal tunnel syndrome using magnetic resonance imaging: a pilot study. *Kor J Pain*. (2020) 33:54–9. doi: 10.3344/kjp.2020.33.1.54
- Jiang W, Huang S, Teng H, Wang P, Wu M, Zhou X, et al. Diagnostic performance of two-dimensional shear wave elastography for evaluating tibial nerve stiffness in patients with diabetic peripheral neuropathy. *Eur Radiol*. (2019) 29:2167–74. doi: 10.1007/s00330-018-5858-4
- Faeghi F, Ardakani AA, Acharya UR, Mirza-Aghazadeh-Attari M, Abolghasemi J, Ejtehadifar S, et al. Accurate automated diagnosis of carpal tunnel syndrome using radiomics features with ultrasound images: a comparison with radiologists' assessment. *Eur J Radiol*. (2021) 136:109518. doi: 10.1016/j.ejrad.2020.109518
- Gonzalez-Suarez CB, Fidel BC, Cabrera JT, Dela Cruz FC, Gesmundo MV, et al. Diagnostic accuracy of ultrasound parameters in carpal tunnel syndrome: additional criteria for diagnosis. *J Am Inst Ultrasound Med*. (2019) 38:3043–52. doi: 10.1002/jum.15012
- Moran L, Royuela A, de Vargas AP, Lopez A, Cepeda Y, Martinelli G. Carpal tunnel syndrome: diagnostic usefulness of ultrasound measurement of the median nerve area and quantitative elastographic measurement of the median nerve stiffness. *J Am Inst Ultrasound Med*. (2020) 39:331–9. doi: 10.1002/jum.15111
- Miyamoto H, Halpern EJ, Kastlunger M, Gabl M, Arora R, Bellmann-Weiler R, et al. Carpal tunnel syndrome: diagnosis by means of median nerve elasticity—improved diagnostic accuracy of US with sonoelastography. *Radiology*. (2014) 270:481–6. doi: 10.1148/radiol.13122901
- Smerilli G, Di Matteo A, Cipolletta E, Carloni S, Incorvaia A, Di Carlo M, et al. Ultrasound assessment of carpal tunnel in rheumatoid arthritis and idiopathic carpal tunnel syndrome. *Clin Rheumatol*. (2021) 40:1085–92. doi: 10.1007/s10067-020-05293-z
- Fowler JR, Munsch M, Tosti R, Hagberg WC, Imbriglia JE. Comparison of ultrasound and electrodiagnostic testing for diagnosis of carpal tunnel syndrome: study using a validated clinical tool as the reference standard. *J Bone Joint Surg*. (2014) 96:e148. doi: 10.2106/JBJS.M.01250
- Schillizzi G, Alviti F, D'Ercole C, Elia D, Agostini F, Mangone M, et al. Evaluation of plantar fasciopathy shear wave elastography: a comparison between patients and healthy subjects. *J Ultrasound*. (2021) 24:417–22. doi: 10.1007/s40477-020-00474-7
- Harmon B, Wells M, Park D, Gao J. Ultrasound elastography in neuromuscular and movement disorders. *Clin Imag*. (2019) 53:35–42. doi: 10.1016/j.clinimag.2018.10.008
- Liao YY, Lee WN, Lee MR, Chen WS, Chiou HJ, Kuo TT, Yeh CK. Carpal tunnel syndrome: US strain imaging for diagnosis. *Radiology*. (2015) 275:205–14. doi: 10.1148/radiol.14140017
- Paluch Ł, Noszczyk B, Nitek Z, Walecki J, Osiak K, Pietruski P. Shear-wave elastography: a new potential method to diagnose ulnar neuropathy at the elbow. *Eur Radiol*. (2018) 28:4932–9. doi: 10.1007/s00330-018-5517-9
- Zhu B, Yan F, He Y, Wang L, Xiang X, Tang Y, et al. Evaluation of the healthy median nerve elasticity: feasibility and reliability of shear wave elastography. *Medicine*. (2018) 97:e12956. doi: 10.1097/MD.00000000000012956
- Zhang C, Li M, Jiang J, Zhou Q, Xiang L, Huang Y, et al. Diagnostic value of virtual touch tissue imaging quantification for evaluating median nerve stiffness in carpal tunnel syndrome. *J Am Inst Ultrasound Med*. (2017) 36:1783–91. doi: 10.1002/jum.14213
- Lai ZH, Yang SP, Shen HL, Luo Y, Cai XH, Jiang WT, et al. Combination of high-frequency ultrasound and virtual touch tissue imaging and quantification improve the diagnostic efficiency for mild carpal tunnel syndrome. *BMC Musculoske Disord*. (2021) 22:112. doi: 10.1186/s12891-021-03982-7
- Cingoz M, Kandemirli SG, Alis DC, Samanci C, Kandemirli GC, Adatepe NU. Evaluation of median nerve by shear wave elastography and diffusion tensor imaging in carpal tunnel syndrome. *Eur J Radiol*. (2018) 101:59–64. doi: 10.1016/j.ejrad.2018.02.005
- Lin CP, Chen J, Chang KV, Wu WT, Özçakar L. Utility of ultrasound elastography in evaluation of carpal tunnel syndrome: a systematic review and meta-analysis. *Ultrasound Med Biol*. (2019) 45:2855–65. doi: 10.1016/j.ultrasmedbio.2019.07.409
- Shen YP, Li TY, Chou YC, Ho TY, Ke MJ, Chen LC, et al. Comparison of perineural platelet-rich plasma and dextrose injections for moderate carpal tunnel syndrome: a prospective randomized, single-blind, head-to-head comparative trial. *J Tissue Eng Regen Med*. (2019) 13:2009–2017. doi: 10.1002/term.2950
- Fischer J, Thompson NW, Harrison JW. A self-administered questionnaire for the assessment of severity of symptoms and functional status in carpal tunnel syndrome. *J Bone Joint Surg*. (1993) 75:1585–92. doi: 10.2106/00004623-199311000-00002
- Kantarci F, Ustabasioglu FE, Delil S, Olgun DC, Korkmazer B, Dikici AS, et al. Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. *Eur Radiol*. (2014) 24:434–40. doi: 10.1007/s00330-013-3023-7
- Yayama T, Kobayashi S, Nakanishi Y, Uchida K, Kokubo Y, Miyazaki T, et al. Effects of graded mechanical compression of rabbit sciatic nerve on nerve blood flow and electrophysiological properties. *J Clin Neurosci*. (2010) 17:501–5. doi: 10.1016/j.jocn.2009.07.110
- Pindrik J, Belzberg AJ. Peripheral nerve surgery: primer for the imagers. *Neuroimag Clin North Am*. (2014) 24:193–210. doi: 10.1016/j.nic.2013.03.034
- Xu C, Zhou Y, He Z, Liu W, Zou M, Sun Y, et al. Difference and ratio of the cross-sectional area of median nerve at the carpal tunnel and the pronator quadratus muscle in diagnosing carpal tunnel syndrome: a cross-sectional study. *Annals Translat Med*. (2022) 10:340. doi: 10.21037/atm-22-1128
- Byra M, Hentzen E, Du J, Andre M, Chang EY, Shah S. Assessing the performance of morphologic and echogenic features in median nerve ultrasound for carpal tunnel syndrome diagnosis. *J Ultrasound Med*. (2020) 39:1165–74. doi: 10.1002/jum.15201
- Paluch Ł, Pietruski P, Walecki J, Noszczyk BH. Wrist to forearm ratio as a median nerve shear wave elastography test in carpal tunnel syndrome diagnosis. *J Plastic Reconst Aesth Surg JPRAS*. (2018) 71:1146–52. doi: 10.1016/j.bjps.2018.03.022

34. Wang Y, Qiang B, Zhang X, Greenleaf JF, An KN, Amadio PC, et al. A non-invasive technique for estimating carpal tunnel pressure by measuring shear wave speed in tendon: a feasibility study. *J Biomech.* (2012) 45:2927–30. doi: 10.1016/j.jbiomech.2012.09.002
35. Bhatia KS, Cho C, Tong CS, Lee YY, Yuen EH, Ahuja AT. Shear wave elastography of focal salivary gland lesions: preliminary experience in a routine head and neck US clinic. *Eur Radiol.* (2012) 22:957–65. doi: 10.1007/s00330-011-2364-3
36. Choi J, Kim SU, Kim DG, Park KS. Characteristics of forearm mixed nerve conduction study in carpal tunnel syndrome: comparison with ultrasound assessments. *J Neurosurg Soc Au.* (2020) 73:155–8. doi: 10.1016/j.jocn.2019.12.053
37. Drakopoulos D, Mitsiokapa E, Karamanis E, Kontogeorgakos V, Mavrogenis AF. ultrasonography provides a diagnosis similar to that of nerve conduction studies for carpal tunnel. *Synd Orthoped.* (2019) 42:e460–4. doi: 10.3928/01477447-20190604-02
38. Shen. Neuromuscular ultrasound in the assessment of polyneuropathies and motor neuron disease. *J Clin Neurophysiol.* (2016) 33:86–93. doi: 10.1097/WNP.0000000000000241
39. Arslan H, Yavuz A, Ilgen F, Aycan A, Ozgokce M, Akdeniz H, et al. The efficiency of acoustic radiation force impulse (ARFI) elastography in the diagnosis and staging of carpal tunnel syndrome. *J Med Ultrasonics.* (2001) 45:453–9. doi: 10.1007/s10396-017-0857-7
40. Inui A, Nishimoto H, Mifune Y, Kokubu T, Sakata R, Kurosaka M. Ultrasound measurement of median nerve cross-sectional area at the inlet and outlet of carpal tunnel after carpal tunnel release compared to electrodiagnostic findings. *Arc Ortho Trauma Surg.* (2016) 136:1325–30. doi: 10.1007/s00402-016-2514-9
41. Peters BR, Martin AM, Memauri BF, Bock HW, Turner RB, Murray KA, et al. Morphologic analysis of the carpal tunnel and median nerve following open and endoscopic carpal tunnel. *Rel Hand.* (2021) 16:310–5. doi: 10.1177/1558944719861711
42. Schrier VJ, Evers S, Geske JR, Kremers WK, Villarraga HR, Kakar S, et al. Median nerve transverse mobility and outcome after carpal tunnel release. *Ultra Med Biol.* (2019) 45:2887–97. doi: 10.1016/j.ultrasmedbio.2019.06.422
43. Klauser AS, Halpern EJ, De Zordo T, Feuchtner GM, Arora R, Gruber J, et al. Carpal tunnel syndrome assessment with US: value of additional cross-sectional area measurements of the median nerve in patients versus healthy volunteers. *Radiology.* (2009) 250:171–7. doi: 10.1148/radiol.2501080397
44. Bedewi MA, Coraci D, Ruggeri F, Giovannini S, Gentile L, Padua L. Shear wave elastography of median nerve at wrist and forearm Heterogeneity of normative values. *J Plast Reconst Aesth Surgery JPRAS.* (2019) 72:137–71. doi: 10.1016/j.bjps.2018.09.015
45. Paliwal PR, Therimadasamy AK, Chan YC, Wilder-Smith EP. Does measuring the median nerve at the carpal tunnel outlet improve ultrasound CTS diagnosis? *J Neurol Sci.* (2014) 339:47–51. doi: 10.1016/j.jns.2014.01.018



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan
Medical College, China

REVIEWED BY

Ali Sazci,
Okan University, Turkey
Harpreet Kaur,
Cleveland Clinic, United States

*CORRESPONDENCE

Yuefeng Rao
✉ raoyf@zju.edu.cn

[†]These authors have contributed
equally to this work

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 20 October 2022

ACCEPTED 08 December 2022

PUBLISHED 23 December 2022

CITATION

Guo J, Hao X, Wang R, Lian K, Jiang J,
Chen N, Feng Z and Rao Y (2022)
MTHFR polymorphism's influence on
the clinical features and therapeutic
effects in patients with migraine: An
observational study.
Front. Neurol. 13:1074857.
doi: 10.3389/fneur.2022.1074857

COPYRIGHT

© 2022 Guo, Hao, Wang, Lian, Jiang,
Chen, Feng and Rao. This is an
open-access article distributed under
the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other
forums is permitted, provided the
original author(s) and the copyright
owner(s) are credited and that the
original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution
or reproduction is permitted which
does not comply with these terms.

MTHFR polymorphism's influence on the clinical features and therapeutic effects in patients with migraine: An observational study

Jianhao Guo^{1†}, Xing Hao^{1†}, Rongrong Wang¹, Ke Lian¹,
Jun Jiang², Na Chen¹, Zhiying Feng¹ and Yuefeng Rao^{1*}

¹The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, ²Hangzhou
Dunen Medical Laboratory Co., Ltd., Hangzhou, China

Objective: Our study aimed to evaluate the influence of
methylenetetrahydrofolate reductase (*MTHFR*) polymorphism on the clinical
features and therapeutic effects in patients with migraine.

Methods: The data of 135 patients with migraine were collected from
January 2021 to December 2021. The *MTHFR* C677T polymorphism was
analyzed. The pain intensity was evaluated using a numerical rating scale (NRS)
during treatment. The levels of folic acid, homocysteine (Hcy), vitamin B12,
interleukin-2 (IL-2), IL-4, and ferritin, and changes of NRS were compared
between folic acid and conventional treatment groups stratified by different
genotypes of *MTHFR* in migraine patients.

Results: The levels of Hcy and ferritin in male patients were higher than that
in female patients ($P < 0.05$); Compared with CC and CT genotype groups,
the TT genotype group showed significantly higher Hcy levels ($P < 0.05$)
and lower folic acid levels ($P < 0.05$); In both folic acid and conventional
treatment groups, a significant decrease in NRS score was observed in different
genotypes post-treatment ($P < 0.05$). Patients with TT genotype in the folic
acid treatment group showed better therapeutic efficacy than conventional
treatment group ($P < 0.05$). There is no significant difference in the therapeutic
efficacy in other genotypes between the two groups ($P > 0.05$).

Conclusion: The *MTHFR* C677T genotyping may provide a new method to
guide and optimize individualized medication for migraine patients.

KEYWORDS

migraine, folic acid, individualized medicine, *MTHFR* polymorphism, therapeutic
efficacy

Introduction

Migraine is a chronic, multifactorial neurovascular disease that is typically
characterized by recurring and disabling headache attacks, as well as an impaired
autonomic nervous system (1). Symptoms associated with migraine include paroxysmal
unilateral throbbing pains, nausea, vomiting, photophobia and phonophobia (2). The

incidence of migraine is about 15% worldwide, the prevalence rate is 9.3% in China, and the ratio of males to females is about 1:3 (3, 4).

The pathogenesis of migraine is complex and remains unclear. Migraine is a multifactorial disorder with pathogenesis influenced by dietary, psychological, environmental, and genetic factors. Except for symptomatic treatments, no special drugs are available for migraine. The first-line therapeutic drugs for migraine include triptans, non-steroidal anti-inflammatory drugs, antiemetics, and so on. A combination strategy is commonly used to reduce the degree of the headache and control other symptoms; however, it is related to medication-overuse headaches, adverse reactions, and substantial financial burdens (5, 6), so the treatment discontinuation rate is high. Previous studies reported that 66.7% of patients discontinued their medications (7). Therefore, a significant need exists for other effective, safe, and affordable treatments at present.

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme for homocysteine (Hcy) metabolism and regulates Hcy level *in vivo*, mainly by affecting folic acid absorption and metabolism (8, 9). MTHFR catalyzes the 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate (CH₃-THF) to provide the carbon in the metabolism process of Hcy. The *MTHFR* 677C→T polymorphism reduces MTHFR enzyme activity, resulting in hyperhomocysteinemia (HHcy). HHcy has become a well-established healthy risk factor associated with neurological disorders, cognitive decline, and cardiovascular disease. HHcy may increase neuronal excitability and release many headache mediators and other inflammatory factors; finally, it induces migraine (10–12). Supplementation of folic acid can reduce the level of Hcy, which has been used to prevent and treat other diseases related to Hcy (13, 14). Several clinical studies preliminarily suggested that targeted folic acid supplementation for migraine patients can help prevent the onset and treatment of migraine (10, 15). Some people may respond better to folic acid supplementation, but the conclusions are inconsistent (16). The *MTHFR* 677C→T variant can decrease the activity of MTHFR, reduce the tetrahydrofolate levels intracellularly, increase the folic acid demand, and counteract the folic acid supplement (9). However, limited studies about the influence of *MTHFR* C677T polymorphism on migraine's clinical features and effects from different treatments have been carried out, such as folic acid. Thus, this article is aimed to determine the role of *MTHFR* polymorphism in the treatment of migraine and the optimization of individualized medication for migraine patients.

Materials and methods

Study design

This is a retrospective study using the detection of the *MTHFR* polymorphism to evaluate the efficacy of patients who

received treatments for migraine between January 2021 and December 2021 in the First Affiliated Hospital of Zhejiang University (FAHZJ), a hospital located in the Zhejiang province, China. A total of 135 patients diagnosed with migraine by physicians were enrolled in our hospital. Patients received folic acid (5 mg), or conventional drugs for migraine treatment were eligible. Ethical approval was obtained from the authorizing ethics committee of FAHZJ (IIT20220564A). The data were anonymous, and the requirement for informed consent was waived for our retrospective study.

MTHFR genotyping and laboratory testing

Genomic DNA was prepared from peripheral blood samples by cell lysis assay. For each patient, 2 mL of peripheral venous blood was collected with EDTA as an anticoagulant for testing *MTHFR* C677T genotypes. A test kit (PCR and microarray method, Shanghai Bio Science and Technology Co., Ltd., China) was used to identify the *MTHFR* C677T genotypes as follows: CC genotype (heterozygous, fast metabolizer), CT genotype (mutant, intermediate metabolizer) and TT genotype (homozygous mutant, slow metabolizer). The serum analytes measured were folic acid, Hcy, ferritin, vitamin B12, interleukin-2 (IL-2), IL-4, IL-6, IL-10, TNF- α , IFN- γ , and IL-17A.

Efficacy assessment

The treatment duration was 1 month. The pain intensity was evaluated using a numerical rating scale (NRS) ranging from 0 to 10. Higher scores indicated greater pain: mild pain (NRS 1–3), moderate pain (NRS 4–6), and severe pain (NRS 7–10). Furthermore, changes in NRS scores were determined by comparing the NRS scores before and after treatment.

Statistical analysis

Data were statistically processed and analyzed by SPSS version 26.0. Measurement data were presented as mean $\pm$ standard deviation (SD) and were compared with *t*-test or One-Way ANOVA. Enumeration data were presented as percentages. A two-tailed *P* < 0.05 demonstrated statistically significant differences.

Results

Clinical characteristics

Clinical characteristics are shown in Table 1 and Supplemental Table 1. Among the 135 migraine patients,

TABLE 1 Clinical characteristics of migraine patients at baseline.

Variables		N (%) or (Mean ± SD)
Age (years)		35.20 ± 12.64
Gender	Male	43 (31.9)
	Female	92 (68.1)
NRS	Mild (0~3)	52 (38.5)
	Moderate(4~6)	70 (51.9)
	Severe (7~10)	13 (9.62)
Pain sensation	Persistent	21 (15.6)
	Paroxysmal	6 (4.4)
	Pulsatile	18 (13.3)
	Splitting	4 (3.0)
	Stabbing	3 (2.2)
Pain location	Bilateral	54 (40.0)
	Unilateral	81 (60.0)
	Temporal	16 (11.9)
	Occiput	59 (43.7)
	Parietal	55 (40.7)
	Frontal	47 (34.8)
	Neck-Shoulder	30 (22.2)
Tenderness	Temple	61 (45.2)
	Periocular	35 (25.9)
	Occiput	23 (17.0)
	Temporal	23 (17.0)
	Occipital nerve	21 (15.6)
	Parapophyses of cervical	27 (20)
	Trapezius	29 (21.5)
	Sternocleidomastoid	1 (0.7)
	Neck-Shoulder	15 (11.1)
Pain duration (years)	0~5	50 (37.0)
	6~10	40 (29.6)
	11~15	12 (8.9)
	16~20	20 (14.8)
	20~	13 (9.6)
Duration of increased pain (months)	0~1	11 (8.1)
	2~5	13 (9.6)
	6~12	16 (11.9)
	12~	10 (7.4)

(Continued)

TABLE 1 (Continued)

Variables		N (%) or (Mean ± SD)
Pain frequency	Once a month	23 (17.0)
	Twice a month	57 (42.2)
	Thrice a month	44 (32.6)
	More than thrice a month	11 (8.1)
Family history for migraine		9 (6.7)
Analgesic drugs	Tizanidine	105 (77.8)
	Methylcobalamin	60 (44.4)
	Folic acid	45 (33.3)
	Qing Peng Ruan Gao	38 (28.1)
	Du Liang Ruan Jiao Nang	37 (27.4)
	Pregabalin	35 (25.9)
	Acetaminophen	34 (25.2)
	Eperisone	34 (25.2)
	Calcitriol	30 (22.2)
	Imrecoxib	26 (19.3)
	Gu Tong Tie Gao	25 (18.5)
	Gabapentin	24 (17.8)
	Topiramate	19 (14.1)
	Promethazine	16 (11.9)
	Flunarizine	15 (11.1)
	Lidocaine	14 (10.4)
	Loxoprofen	13 (9.6)
	Ropivacaine	12 (8.9)
	Botulinum toxin	12 (8.9)
	Vitamin B6	12 (8.9)
	Bai Mai Ruan Gao	9 (6.7)
	Tandospirone	8 (5.9)
	Propacetamol	8 (5.9)
	Celecoxib	7 (5.2)
	Diclofenac diethylamine	7 (5.2)
	Adenosylcobalamin	7 (5.2)

43 were males, and 92 were females, ranged from 13 to 69 years old (mean and median age, 35.2 and 34 years old, respectively). Based on gene detection, 35 patients (25.92%)

TABLE 2 Comparison of serum indexes in different genders (mean $\pm$ SD).

	Male (<i>n</i> = 43)	Female (<i>n</i> = 92)	<i>t</i>	<i>P</i> -value
Folic acid	7.106 (6.09)	9.704 (12.80)	1.350	0.18
Vitamin B12	468.9 (273.0)	739.2 (972.2)	1.809	0.072
Ferritin	224.4 (192.5)	68.23 (61.5)	7.375	<0.0001
Hcy	13.94 (9.671)	7.764 (3.074)	5.691	<0.0001
IL-2	0.686 (1.420)	1.091 (1.676)	0.412	0.681
IL-4	1.232 (1.481)	1.853 (5.790)	0.684	0.4952
IL-6	2.464 (1.803)	2.818 (2.812)	0.747	0.456
IL-10	1.133 (0.962)	1.245 (1.573)	0.431	0.667
TNF- α	3.243 (5.040)	3.863 (6.750)	0.530	0.596
IFN- γ	3.733 (4.526)	6.240 (19.400)	0.826	0.41
IL-17A	7.954 (20.470)	7.657 (19.970)	0.060	0.947

were fast metabolizer (CC genotype), 72 patients (53.33%) were intermediate metabolizer (CT genotype), and 28 patients were (20.74%) slow metabolizer (TT genotype). The conventional drugs used were listed in Table 1 and mainly included non-steroidal anti-inflammatory drugs (acetaminophen, imrecoxib, celecoxib, diclofenac diethylamine), muscle relaxant (tizanidine, eperisone), methylcobalamin, pregabalin, gabapentin, and topiramate.

Serum indexes in different genders

Serum indexes are shown in Table 2. Serum levels of Hcy and ferritin were significantly higher in males than those in females ($P < 0.05$). There was no significant difference between genders on the levels of vitamin B12, IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , and IL-17A ($P > 0.05$).

Serum indexes in different genotypes of *MTHFR* C677T

Summary data are shown in Figure 1 and Table 3. Data analysis showed lower folic acid levels and higher Hcy levels in the TT genotype group ($P < 0.05$). There was a significant difference in vitamin B12 levels between the CC and CT genotype groups ($P < 0.05$, Figure 1). No significant difference was found on the levels of ferritin, IL-2, IL-4, IL-6, IL-10, TNF- α , IFN- γ , and IL-17A among the three genotype groups ($P > 0.05$).

TABLE 3 Comparison of serological indicators in migraine patients with different *MTHFR* genotypes (Mean $\pm$ SD).

Serological indicators	C/C (<i>n</i> = 35)	C/T (<i>n</i> = 72)	T/T (<i>n</i> = 28)
Ferritin	100.9 $\pm$ 99.37	117.2 $\pm$ 128.8	85.79 $\pm$ 73.83
IL-2	0.90 $\pm$ 0.86	0.92 $\pm$ 0.91	0.83 $\pm$ 0.79
IL-4	1.33 $\pm$ 1.49	1.22 $\pm$ 1.2	1.23 $\pm$ 1.55
IL-6	2.91 $\pm$ 2.64	2.70 $\pm$ 2.76	2.40 $\pm$ 1.70
IL-10	1.48 $\pm$ 2.10	1.08 $\pm$ 0.91	1.10 $\pm$ 0.87
TNF- α	3.65 $\pm$ 5.11	3.90 $\pm$ 7.11	4.68 $\pm$ 7.46
TNF- γ	4.39 $\pm$ 6.36	2.91 $\pm$ 3.29	3.86 $\pm$ 4.57
IL-17A	9.35 $\pm$ 25.27	7.20 $\pm$ 18.58	6.62 $\pm$ 14.28

CC genotype: homozygous; CT genotype: heterozygous; TT genotype: homozygous mutation.

Treatment response by *MTHFR* C677T genotype

The NRS score variations during treatment are shown in Figure 2 and Table 4. A significant decrease in NRS score was observed post-treatment ($P < 0.05$). Treatment with folic acid was effective in three genotype groups ($P < 0.05$). Patients with TT genotype in the folic acid treatment group showed better therapeutic efficacy than conventional treatment group ($P < 0.05$). There is no significant difference in the therapeutic efficacy in other genotypes between the two groups ($P > 0.05$).

Discussion

Migraine is a neurovascular disorder characterized by recurrent moderate to severe headache, usually near the hemicranial head and often accompanied by vomiting, diarrhea, photophobia, and fear of sound. If one parent has migraine, the children are about 40 percent more likely to have migraine (17). Moreover, while both parents have migraines, the risk of migraine (17) for their children is up to 75%.

The *MTHFR* enzyme encoded by the *MTHFR* gene is critical in metabolizing methionine folate. This enzyme reduces 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate (5-MTHF) (8). As a methyl donor, 5-MTHF can maintain the balance of Hcy through the methylation of Hcy in the blood (18). Inadequate consumption of folic acid, or abnormality of the enzymes involved in folic acid metabolism, can lead to decreased levels of folic acid. Thus, the methionine production can be inhibited, resulting in an accumulation of Hcy in the body and HHcy. At the same time, the increased Hcy level contributes to the development of migraine through a series of complex events. A high level of Hcy may impair methylation reactions, which disrupts the normal functions of blood vessels and nerves, inhibit the activity of the cerebral cortex, and

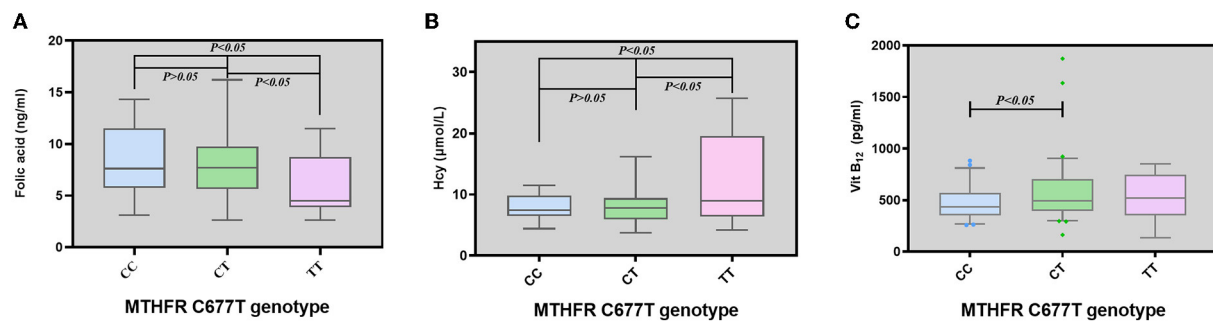


FIGURE 1

Levels of folic acid, homocysteine, and vitamin B12 among different genotypes of *MTHFR* in migraine patients. (A) Levels of folic acid among migraine patients with different genotypes of *MTHFR*. (B) Levels of Hcy among migraine patients with different genotypes of *MTHFR*. (C) Levels of vitamin B12 among migraine patients with different genotypes of *MTHFR*. Horizontal lines represent median values, box represent the quartiles, and whiskers represent the range of data.

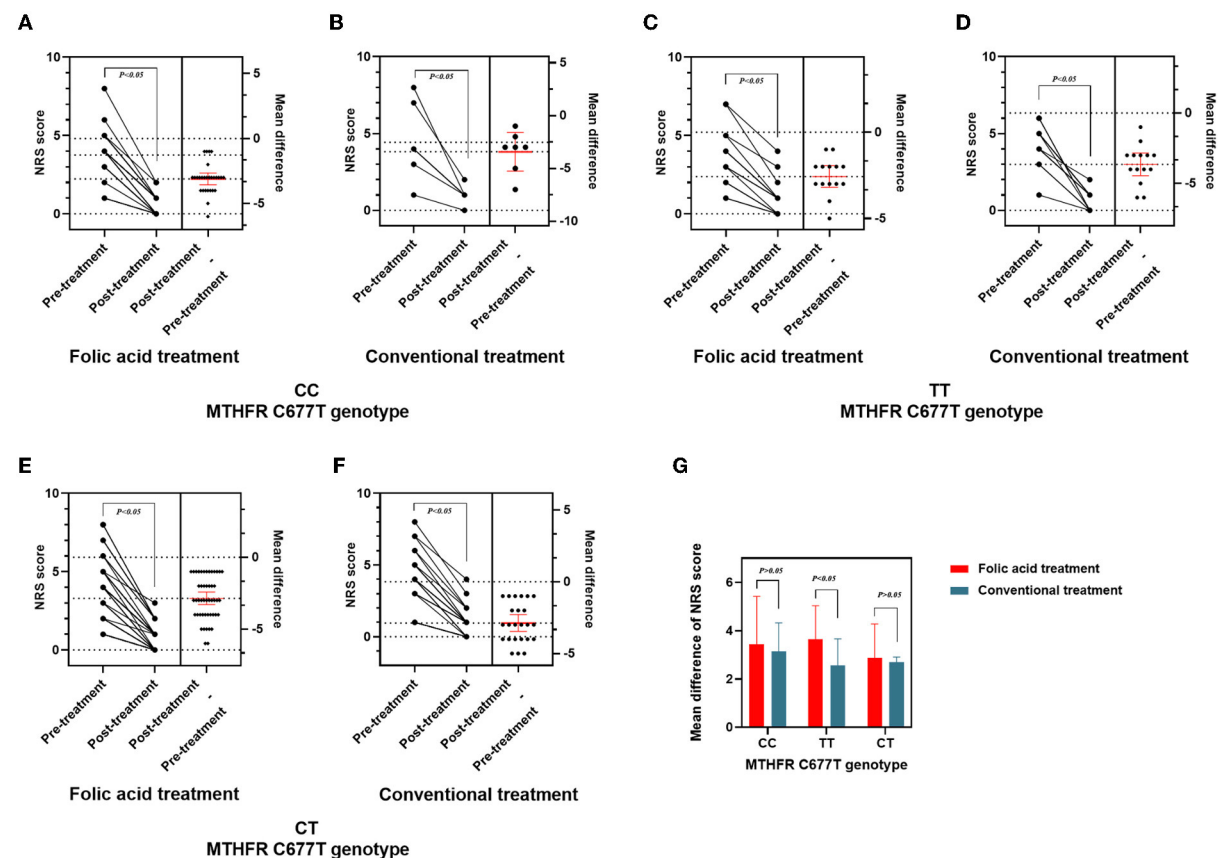


FIGURE 2

Therapeutic effects for migraine patients with different *MTHFR* genotypes before and after treatment. (A–F) NRS score and changes of NRS in patients with different genotypes of *MTHFR* before and after treatment. (G) Mean difference of NRS score between folic acid and conventional treatment groups stratified by different genotypes of *MTHFR* in migraine patients.

decrease local blood flow (19). These reactions can further increase the inflammatory cytokines (such as 5-HT, IL-6, and TNF- α) in cerebral microvascular endothelial cells (20), and finally lead to migraine.

Recently, numerous studies have demonstrated that *MTHFR* polymorphism is associated with migraine and serum Hcy concentrations (21). The genetic polymorphism of the *MTHFR* can cause a decrease in *MTHFR* enzyme activity. Compared

TABLE 4 Comparison of treatment response by *MTHFR* C677T genotype.

<i>MTHFR</i> C677T genotype	NRS	Folic acid treatment group	Conventional treatment group	<i>t</i>	<i>P</i> ¹
TT	NRS pre-treatment	4.29 ± 1.33	3.64 ± 1.91	1.036	0.309
	NRS post-treatment	0.64 ± 0.63	1.07 ± 1.21	1.177	0.25
	Variation of NRS pre-and post-treatment	3.64 ± 1.39	2.57 ± 1.09	2.267	0.0319
	<i>n</i>	14	14		
CT	NRS pre-treatment	3.83 ± 2.12	3.33 ± 2.19	0.932	0.354
	NRS post-treatment	0.96 ± 1.04	0.65 ± 0.80	1.428	0.156
	Variation of NRS pre-and post-treatment	2.88 ± 1.40	2.69 ± 0.22	0.623	0.6225
	<i>n</i>	24	48		
CC	NRS pre-treatment	4.429 ± 2.37	3.75 ± 1.58	0.918	0.365
	NRS post-treatment	1.00 ± 0.57	0.64 ± 0.62	1.377	0.177
	Variation of NRS pre-and post-treatment	3.43 ± 1.99	3.14 ± 1.18	0.497	0.623
	<i>n</i>	7	28		

¹ Comparisons were performed between folic acid treatment group and conventional treatment group among different *MTHFR* C677T genotypes at different time point.

with the CC genotype, the CT and the TT genotypes show 65% and 30% of *MTHFR* enzyme activities, respectively (22). Decreased folic acid levels were observed in patients with the slow metabolism TT genotype. Wang et al. studied the genetic susceptibility of C677T polymorphism to migraine in Asians (23). They suggested that the increase of Hcy may be involved in the pathogenesis of migraine and folic acid, Hcy serum level, and *MTHFR* polymorphism are related to the occurrence of migraine (23). Some studies showed that the Hcy level in patients with migraine is significantly higher than that of the control group, especially during migraine attacks (24). Our study compared the serum folate and Hcy levels among migraine patients with different genotypes. The result showed that the serum folate levels of patients with slow and intermediate metabolic types were significantly lower than those with fast metabolic types. This result is consistent with the data reported in previous literature about the mechanism of *MTHFR* enzymes.

The study of Pan et al. showed that (25) the vitamin B12, folic acid, and Hcy levels in the migraine group were negatively correlated, indicating that the elevation of Hcy level may be related to the inducement of migraine and that supplementation of folic acid and vitamin B12 may prevent migraine. Lea et al. (26) suggested that vitamin supplements (2 mg folic acid, 25 mg vitamin B6, and 400 micrograms of vitamin B12 every day) and lower Hcy levels can effectively reduce headache frequency and pain severity in 52 migraine patients with aura. Menon et al. (15) treated 206 migraine patients with vitamin supplements. The results showed that vitamin supplements effectively reduced Hcy levels and headache severity in migraine patients. The C allele of the *MTHFR* C677T variant has more reductions in Hcy levels and migraine pain severity compared with the TT genotype. Stratified analysis by the genotype of the vitamin treatment

group, *MTHFR* genotype variation affects the therapeutic effect of migraine patients. In our study, migraine patients with CT and TT genotype were divided into a folic acid treatment group and a conventional treatment group. Our results showed that NRS decreased after treatment, with significant differences in numerical values of NRS before and after treatment. The pain and symptoms of migraine patients with the TT genotype were significantly relieved after treatment with folic acid supplementation. Besides, the curative effect of the TT genotype group treated with folic acid supplementation was better than that of conventional treatment group ($P < 0.05$). Fourteen migraine patients with high Hcy had a noticeable curative effect after taking medicine for 1 month. The doctors used folic acid and methylcobalamin to treat migraine the first time and then adjusted the treatment regimen until the above indexes returned to normal range according to the levels of folic acid, vitamin B12, and Hcy in serum. Meanwhile, the patients should pay attention to eating more foods rich in folic acid in their diet. After treatment, the patients' headache was significantly relieved, suggesting that the *MTHFR* C677T genotyping has a particular clinical value in guiding the individualized treatment of migraine. Apart from this, it affirms the curative effect of folic acid supplementation on migraine patients with high Hcy.

Some limitations still exist. In our study, 35 patients (25.92%) were fast metabolizer (CC genotype), 72 patients (53.95%) were intermediate metabolizer (CT genotype), and 28 patients were (20.74%) slow metabolizer (TT genotype). The result seems different from the data reported in public databases (27). However, in Chinese populations, geographical and ethnic variations were observed in *MTHFR* C677T genotypes. In a large sample that pooled the results of epidemiological studies on the distributions of *MTHFR* C677T genotypes in healthy

populations living in Mainland China, the mean frequency of 677TT genotype was 20% (95% confidence interval: 18–23%) (28). The frequency of the 677T allele ranged from 24.0% in Hainan province and 63.1% in Shangdong province. Thus, the polymorphic distribution of *MTHFR* C677T in our study is within the reported range in previous studies among the Chinese population (28). In addition, this sample size of our study is relatively small, so potential confounders to migraine (such as age distribution, sex percentage, and pain duration) were not further checked *via* multivariate logistic regression. Therefore, the results of this study also need to be further verified with a well-designed and large sample study. In summary, this study has provided valuable information from real-world clinical practices about individualized medication for migraine treatments.

Conclusion

In conclusion, *MTHFR* polymorphism may be beneficial to guide and optimize individualized medication for migraine treatments. In the future, the relationship between *MTHFR* polymorphism and migraine treatment can be further studied in larger sample populations with a well-controlled experimental design, especially for vitamin B supplementation.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the First Affiliated Hospital of Zhejiang University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

References

1. Xuejun S, Bifa F, You W, Daying Z, Yan L, Jisheng H, et al. revision of IASP definition of pain. *Chin J Pain Med.* (2020) 26:641–4. doi: 10.3969/j.issn.1006-9852.2020.09.001
2. De Felice M, Ossipov MH, Wang R, Dussor G, Lai J, Meng ID, et al. Triptan-induced enhancement of neuronal nitric oxide synthase in trigeminal ganglion dural afferents underlies increased responsiveness to potential migraine triggers. *Brain.* (2010) 133:2475–88. doi: 10.1093/brain/awq159
3. Shunwei L, Yansheng L, ruozhuo L, Xiangyang Q, Qi W, Xiaosu Y, et al. The Chinese guidelines for the diagnosis and treatment of migraine. *Chin J Pain Med.* (2011) 17:65–86. doi: 10.3969/j.issn.1006-9852.2011.02.001
4. Vos T, Lim SS, Bisignano C, Cruz J, Mirzaei-Alavijeh M. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019—a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9
5. Archibald N, Lipscomb J, McCrory DC, AHRQ. *Technical Reviews. Resource Utilization and Costs of Care for Treatment of Chronic Headache.* Rockville, MD: Agency for Health Care Policy and Research (US). (1999).
6. Wells RE, O'Connell N, Pierce CR, Estave P, Penzien DB, Loder E, et al. Effectiveness of mindfulness meditation vs headache education for adults with migraine: a randomized clinical trial. *JAMA Intern Med.* (2021) 181:317–28. doi: 10.1001/jamainternmed.2020.7090

Author contributions

Conception and design: JG, YR, and XH. Provision of study materials or patients: XH, NC, and RW. Collection and assembly of data: ZF, KL, and JJ. Data analysis and interpretation: KL. All authors contributed to the writing and final approval of manuscript.

Funding

This study was supported by the project of the Zhejiang Pharmaceutical Association (2019ZYYG02).

Conflict of interest

JJ was employed by Hangzhou Dunen Medical Laboratory Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.1074857/full#supplementary-material>

7. Hepp Z, Dodick DW, Varon SF, Chia J, Matthew N, Gillard P, et al. Persistence and switching patterns of oral migraine prophylactic medications among patients with chronic migraine: a retrospective claims analysis. *Cephalalgia*. (2017) 37:470–85. doi: 10.1177/0333102416678382
8. van der Pol KH, Nijenhuis M, Soree B, de Boer-Veger NJ, Buunk AM, Guchelaar HJ, et al. Dutch pharmacogenetics working group guideline for the gene-drug interaction of ABCG2, HLA-B and Allopurinol, and MTHFR, folic acid and methotrexate. *Eur J Hum Genet*. (2022) 1–8. doi: 10.1038/s41431-022-01180-0. [Epub ahead of print].
9. Shaik MM, Gan SH. Vitamin supplementation as possible prophylactic treatment against migraine with aura and menstrual migraine. *Biomed Res Int*. (2015) 469529. doi: 10.1155/2015/469529
10. Askari G, Nasiri M, Mozaffari-Khosravi H, Rezaie M, Bagheri-Bidakhavidi M, Sadeghi O. The effects of folic acid and pyridoxine supplementation on characteristics of migraine attacks in migraine patients with aura: a double-blind, randomized placebo-controlled, clinical trial. *Nutrition*. (2017) 38:74–9. doi: 10.1016/j.nut.2017.01.007
11. Liu L, Yu Y, He J, Guo L, Li H, Teng J. Effects of MTHFR C677T and A1298C polymorphisms on migraine susceptibility: a meta-analysis of 26 studies. *Headache*. (2019) 59:891–905. doi: 10.1111/head.13540
12. Tietjen GE, Collins SA. Hypercoagulability and migraine. *Headache*. (2018) 58:173–83. doi: 10.1111/head.13044
13. Chen H, Liu S, Ge B, Zhou D, Li M, Li W, et al. Effects of folic acid and vitamin b12 supplementation on cognitive impairment and inflammation in patients with alzheimer's disease: a randomized, single-blinded, placebo-controlled trial. *J Prev Alzheimers Dis*. (2021) 8:249–56. doi: 10.14283/jpad.2021.22
14. Chen H, Liu S, Ji L, Wu T, Ji Y, Zhou Y, et al. Folic acid supplementation mitigates alzheimer's disease by reducing inflammation: a randomized controlled trial. *Mediators Inflamm*. (2016) 5912146. doi: 10.1155/2016/5912146
15. Menon S, Lea RA, Roy B, Hanna M, Wee S, Haupt LM, et al. Genotypes of the MTHFR C677T and MTRR A66G genes act independently to reduce migraine disability in response to vitamin supplementation. *Pharmacogenet Genom*. (2012) 22:741–9. doi: 10.1097/FPC.0b013e3283576b6b
16. Di Rosa G, Attinà S, Spanò M, Ingegneri G, Sgrò DL, Pustorino G, et al. Efficacy of folic acid in children with migraine, hyperhomocysteinemia and MTHFR polymorphisms. *Headache*. (2007) 47:1342–4. doi: 10.1111/j.1526-4610.2007.00932.x
17. Yang Y, Ligthart L, Terwindt GM, Boomsma DI, Rodriguez-Acevedo AJ, Nyholt DR. Genetic epidemiology of migraine and depression. *Cephalalgia Int J Headache*. (2016) 36:1097–25. doi: 10.1177/0333102416638520
18. Zhuan Z, Ying O. Inference of MTHFR polymorphisms in migraine. *J Clin Neurol*. (2015) 28:3.
19. Shiyin Z. Change of plasma homocysteine in migraine patients. *Clin J Med Officers*. (2002) 30:3. doi: 10.3969/j.issn.1671-3826.2002.01.007
20. Feng G, Zhanxiu R, Qiu H, Shanshan S, Meixi J. The contents and correlation studies of HCY and CRP in plasma of migraine in attack stage. *J Apoplexy Nervous Dis*. (2015) 32:3.
21. Shuowen W. Prevention of folate deficiency with 5-methyl-tetrahydrofolate. *Int J Pediatr*. (2020) 47:4. doi: 10.3760/cma.j.issn.1673-4408.2020.10.011
22. van der Put NMJ, Gabreels F, Stevens EMB, Smeitink JAM, Trijbels FJM, Eskes T, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural-tube defects? *Am J Hum Genet*. (1998) 62:1044–51. doi: 10.1086/301825
23. Min W, Xiaobin C, Jing L, Gang L. MTHFR gene C677T polymorphism and migraine susceptibility among Asian populations: a meta analysis. *Chongqing Med*. (2020) 49:6. doi: 10.3969/j.issn.1671-8348.2020.12.024
24. Lippi G, Mattiuzzi C, Meschi T, Cervellin G, Borghi L. Homocysteine and migraine. A narrative review. *Clin Chim Acta*. (2014) 433:5–11. doi: 10.1016/j.cca.2014.02.028
25. Yan P. The clinical significance of change of serum Hcy in patients with migraine. *Chin J Tissue Eng Res*. (2004) 8:92–3. doi: 10.3321/j.issn:1673-8225.2004.01.047
26. Lea R, Colson N, Quinlan S, Macmillan J, Griffiths L. The effects of vitamin supplementation and MTHFR (C677T) genotype on homocysteine-lowering and migraine disability. *Pharmacogenet Genom*. (2009) 19:422. doi: 10.1097/FPC.0b013e32832af5a3
27. Sherry ST, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. (2001) 29:308–11. doi: 10.1093/nar/29.1.308
28. Wang X, Fu J, Li Q, Zeng D. Geographical and ethnic distributions of the MTHFR C677T, A1298C and MTRR A66G gene polymorphisms in chinese populations: a meta-analysis. *PLoS ONE*. (2016) 11:e0152414. doi: 10.1371/journal.pone.0152414



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University, China

REVIEWED BY

Vincenzo Ricci,
Luigi Sacco Hospital, Italy
Marcello Romano,
Azienda Ospedaliera Ospedali Riuniti
Villa Sofia Cervello, Italy

*CORRESPONDENCE

Weimin Chen
✉ baion2301@foxmail.com
Lin Chen
✉ peng_f@163.com

[†]These authors have contributed
equally to this work and share first
authorship

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 23 October 2022

ACCEPTED 28 November 2022

PUBLISHED 06 January 2023

CITATION

Liu A, Jia X, Zhang L, Huang X, Chen W
and Chen L (2023) Diagnostic
performance of preoperative
ultrasound for traumatic brachial
plexus root injury: A comparison study
with an electrophysiology study.
Front. Neurol. 13:1077830.
doi: 10.3389/fneur.2022.1077830

COPYRIGHT

© 2023 Liu, Jia, Zhang, Huang, Chen
and Chen. This is an open-access
article distributed under the terms of
the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution
or reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Diagnostic performance of preoperative ultrasound for traumatic brachial plexus root injury: A comparison study with an electrophysiology study

Ailin Liu^{1†}, Xiaotian Jia^{2†}, Li Zhang², Xiaoyun Huang²,
Weimin Chen^{1*} and Lin Chen^{2*}

¹Department of Medical Ultrasound, Huashan Hospital, Fudan University, Shanghai, China,

²Department of Hand Surgery, Huashan Hospital, Fudan University, Shanghai, China

Purpose: Accurate preoperative assessment for traumatic brachial plexus injury (BPI) is critical for clinicians to establish a treatment plan. The objective of this study was to investigate the diagnostic performance of preoperative ultrasound (US) through comparison with an electrophysiology study (EPS) in the assessment of traumatic brachial plexus (BP) root injury.

Materials and methods: We performed a retrospective study in patients with traumatic BPI who had preoperative US and EPS, excluding obstetric palsy and other nontraumatic neuropathies. US examination was performed on an EPIQ 5 color Doppler equipment. EPS was performed on a Keypoint 9033A07 Electromyograph/Evoked Potentials Equipment, testing electromyography (EMG), nerve conduction studies (NCS), and somatosensory evoked potentials (SEP). Each BP root of all patients was assessed by US and EPS as completely injured or incompletely injured, respectively. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated based on the correlation with intraoperative findings. The accuracy of US and EPS were compared using the McNemar test. The added benefit of US was evaluated by comparing the sensitivity and specificity between the combined tests with EPS using the McNemar test.

Results: This study included 49 patients with traumatic BPI who underwent BP surgeries from October 2018 to September 2022. Surgical exploration confirmed 89 completely injured BP roots in 28 patients. US correctly detected 80 completely injured BP roots (sensitivity, 0.899; specificity, 0.981; PPV, 0.964; NPV, 0.944; accuracy, 0.951). EPS correctly detected 75 completely injured BP roots (sensitivity, 0.843; specificity, 0.929; PPV, 0.872; NPV, 0.912; accuracy, 0.898). US showed significantly higher accuracy than EPS ($p = 0.03$). When combining US and EPS for completely injured BP root detection, the sensitivity of the inclusive combination (0.966) was significantly higher than EPS alone ($p = 0.000977$), and the specificity of the exclusive combination (1.000) was significantly higher than EPS alone ($p = 0.000977$).

Conclusion: Preoperative US is an effective diagnostic tool in the assessment of traumatic BP root injury. US had higher accuracy than EPS in this study. Sensitivity and specificity were significantly higher than EPS when US was combined with EPS.

KEYWORDS

ultrasound, brachial plexus, trauma, root injury, electrophysiology, comparison

1. Introduction

Traumatic brachial plexus injury (BPI) commonly affects younger people and causes significant disability of the upper extremity. Early exploration and nerve repair or nerve grafting are recommended in open sharp injuries, while the management of closed injuries requires patience and careful monitoring (1–5). As nonsurgical treatment is likely to have a good prognosis, surgery is not recommended for some patients, such as those with neuropraxia (4–6). Therefore, accurate preoperative assessment to stratify patients who require surgical intervention by identifying a complete injury is critical for establishing a treatment plan.

Apart from clinical symptoms and physical examinations, an electrophysiology study (EPS) is one of the most commonly used methods for preoperative assessments of traumatic BPI (3, 7–10). However, in complete injuries, EPS cannot provide reliable results within 3 to 4 weeks (1, 3, 7–9, 11), which might decrease the possibility of early surgical intervention and better functional outcomes (12, 13). Currently, ultrasound (US) has gained its role as a noninvasive and cost-effective imaging modality through recent successful attempts to assess brachial plexus (BP) neuropathy, showing that US might be a valuable alternative method (14–24).

To the best of our knowledge, although there have been a series of publications that reported the sensitivities of US for BPI diagnosis, ranging from 0.75 to 0.80 (16–18, 20, 24–26), fewer studies reported the specificity, and the sample sizes were limited (18, 20, 24). Furthermore, there is a shortage of published studies comparing US and EPS in a head-to-head fashion in the same set of patients with BPI (27, 28). Therefore, the aim of this study was to investigate the diagnostic performance of US through comparison with EPS in the detection of completely injured BP roots.

2. Materials and methods

This retrospective study was approved by the ethical institutional review board of Huashan Hospital, and all procedures were performed according to the Helsinki Declaration. Patients with traumatic BPI who underwent BP surgeries between October 2018 and September 2022 at Huashan Hospital, Fudan University (Shanghai, China), were

included in this study. We searched our database of patients who (1) had a clear history of trauma; (2) had suspected BP root injury based on physical examination; and (3) underwent US and EPS examination before BP surgery. The exclusion criteria were as follows: (1) patients with obstetric plexus palsy, (2) patients who underwent previous BP surgery, (3) patients who showed obvious functional recovery after nonsurgical treatment before surgery, and (4) patients with other nontraumatic neuropathies. Medical records of each patient, such as age, sex, etiology, treatment history, US, and EPS results, were reviewed.

Preoperative US examination, including image collection and diagnosis, was performed by a certified radiologist with 9 years of experience in peripheral nerve imaging who was blinded to EPS and other imaging results. An EPIQ 5 color Doppler equipment (Philips Medical Systems, Bothell, WA, USA) was used for the standardized examination protocol. High-frequency linear-array transducers, namely, L18-5 MHz and L12-5 MHz, were used for deep structures. The patient was in a supine position with the neck extended to the noninjured side. As traditionally advised, US scanning started from the cranial part of the BP, e.g., C5 root, after identifying its corresponding transverse processes in a horizontal plane on the supraclavicular fossa. The C7 transverse process was the most commonly used bony landmark to locate the C7 root. Thus, the C5 to C8 roots could be depicted in the interscalene groove, arranged in a line as a series of hypoechoic nodules between the anterior and middle scalene muscles (29). The deepest T1 root could be visualized caudal to the C8 root coursing on top of the first rib. Each BP root was scanned along its inferior and lateral courses and tracked proximally in the transverse section and possible longitudinal section. In cases of variations, there might be BP roots coursing anterior to the anterior scalene muscle or piercing the muscle belly instead of traveling inside the interscalene groove; a rudimentary anterior tubercle might be visualized at the C7 transverse process; and a cervical rib might also appear (30). As such, following the caudal-to-cranial tracking technique by shifting the probe back and forth could effectively clarify the C5 to T1 BP roots and recognize variations (14, 30). Gentle tilting of the probe during the US scanning helped better delineate the edges of the nerve epineurium (31). The continuity of the BP root epineurium and fascicles and the existence of any accumulated cerebrospinal fluid or neuroma-like enlargement were recorded as observed (Supplementary Table 1). In US examination, a completely injured BP root was recorded as a

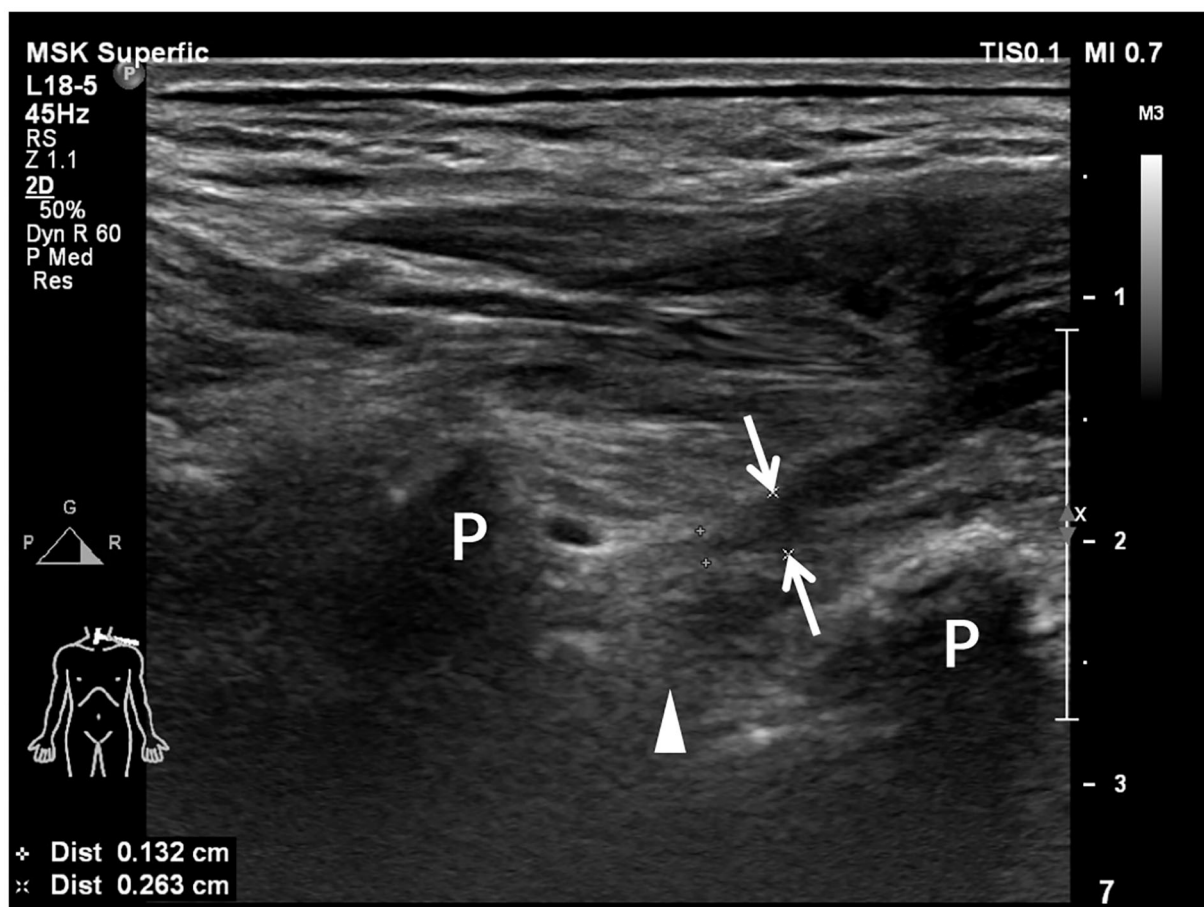


FIGURE 1

Longitudinal sonogram shows a hypoechoic irregular neural stump (arrows) with a blind-ending of the hypoechoic brachial plexus root. An empty neural foramen (arrowhead) is visualized, indicating preganglionic avulsion of the nerve root. Transverse processes (P) of cervical vertebrae are shown as hyperechoic bone prominences with posterior acoustic shadowing.

positive result, which should have at least one of the following appearances: (1) neural stump (Figure 1), (2) neural gap (Figure 2), (3) cerebrospinal fluid adjacent to the intervertebral foramen (Figure 3), and (4) neuroma-like enlargement adjacent to the transverse process (Figure 4).

Preoperative EPS examination was performed by a certified neurophysiologist blinded to the US and other imaging results, following the information collection forms reflecting the content of examination standards (Supplementary Tables 2–5). A Keypoint 9033A07 Electromyograph/Evoked Potentials Equipment (Alpine bioMedApS, Skovlunde, DK-2740 Denmark) was used. The patients were asked to wait for at least 30 min before the examination, and room temperature (25°C) was required during the examination. Needle electromyography (EMG), nerve conduction studies (NCS), and somatosensory-evoked potential (SEP) were tested for each patient. Sensory NCS included sensory nerve conduction velocity (SNCV) and sensory nerve action potential (SNAP). Motor NCS included motor nerve conduction velocity (MNCV) and compound

muscle action potential (CMAP). EMG was performed on relevant muscle sets using disposable concentric needle electrodes. Motor unit action potential (MUP) recruitment pattern characteristics were used to determine injury presence, severity, and recovery. Diagnosis of BPI was finally determined by the results of these EPS tests according to the anatomy of the brachial plexus. In EPS, a completely injured BP root was recorded as a positive result, which should meet all these characteristics: (1) abnormal spontaneous activities; (2) no motor units; and (3) no CMAP or MNCV.

The intraoperative findings were used as the reference standard. Exploratory BP surgeries were made *via* supraclavicular incisions. Observation and evaluation of the continuity of each BP root were performed up to the intervertebral foramen under a microscope. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of US and EPS for detecting completely injured roots were calculated using a 2×2 table. The statistical difference in diagnostic accuracy

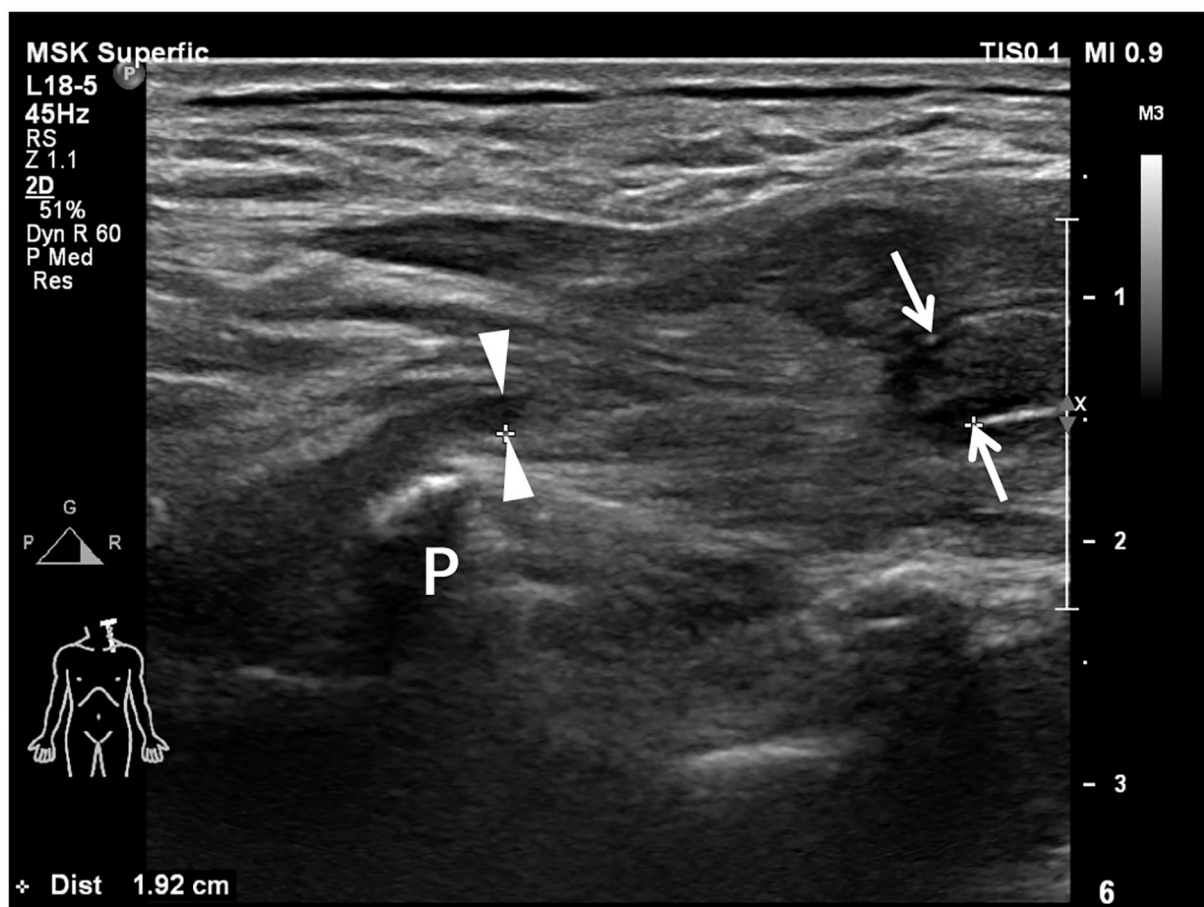


FIGURE 2

Longitudinal sonogram shows a completely transected brachial plexus root with a 1.92-cm-long neural gap between the proximal end (arrowheads) and the distal end (arrows). The proximal root end is adjacent to the transverse process (P). The caliber of the distal root end is enlarged in comparison with the proximal root end.

between US and EPS was examined by the exact, two-sided McNemar test using VassarStats.net (<http://vassarstats.net/>). To evaluate the added benefit of US when combined with EPS, sensitivity and specificity were calculated based on two models, namely, inclusive combination (if the result of either US or EPS was positive, it was considered positive) and exclusive combination (if both the results of US and EPS were positive, it was considered positive). Then, the sensitivity and specificity of these two models were compared with those of EPS alone by the exact, two-sided McNemar test. A *p*-value of 0.05 was considered the limit for a statistically significant difference.

3. Results

3.1. Patient characteristics

The clinical characteristics of the 49 patients are summarized in Table 1. Among them, 40 of the patients were men and

nine were women. The majority of patients had a trauma of collision (41). The other patients suffered from stretch (4), crush (1), incision (1), and iatrogenic injuries (2). The age of the patients ranged from 5 to 78 years, with an average age of 42.6 years. The median time interval between injury and surgery was 87 days. The median time intervals between injury and preoperative examination were 75 days and 68 days for US and EPS, respectively.

3.2. Diagnostic performance of US and EPS

Surgical exploration confirmed 89 completely injured roots in 28 patients. US correctly detected 80 completely injured BP roots, with 80 true-positive results, 153 true-negative results, three false-positive results, and nine false-negative results (Table 2). EPS correctly detected 75 completely injured BP roots, with 75 true-positive results, 145 true-negative results, 11

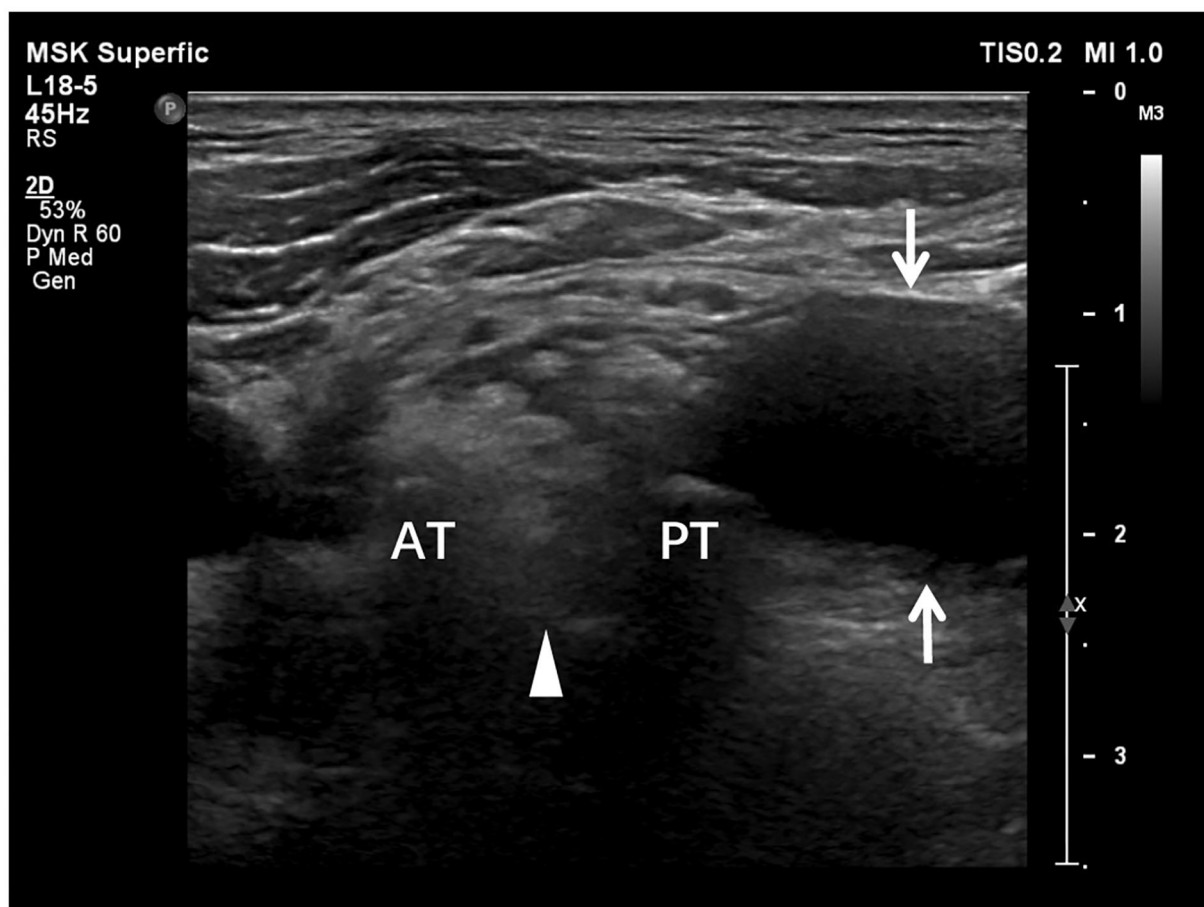


FIGURE 3

Transverse sonogram shows the accumulated cerebrospinal fluid, i.e., pseudomeningocele, adjacent to the intervertebral foramen. The cerebrospinal fluid (arrows) appears anechoic. The absence of brachial plexus root (arrowhead) between anterior (AT) and posterior tubercles (PT) represents preganglionic root avulsion.

false-positive results, and 14 false-negative results (Table 2). The number of positive and negative cases based on preoperative US and EPS examination is shown by root level in Table 2. In our study, US had fewer false-positive and false-negative results than EPS (Table 2). The overall sensitivity, specificity, PPV, NPV, and accuracy of detecting completely injured BP roots by US were 0.899, 0.981, 0.964, 0.944, and 0.951, respectively (Table 3). The overall sensitivity, specificity, PPV, NPV, and accuracy of detecting completely injured BP roots by EPS were 0.843, 0.929, 0.872, 0.912, and 0.898, respectively (Table 3). There was a significant difference in diagnostic accuracy between US and EPS ($p = 0.03$, Table 4).

3.3. Combination of US and EPS

The sensitivity of the inclusive combination of US and EPS was significantly higher than EPS alone ($p = 0.000977$), while the

difference in specificity was not statistically significant ($p = 0.25$, Table 5). The specificity of the exclusive combination of US and EPS was significantly higher ($p = 0.000977$), while the sensitivity was significantly lower ($p = 0.03125$) compared to EPS alone (Table 6).

4. Discussion

A series of publications reported that both US and EPS are valuable tools for evaluating BPI (8, 25, 26, 32–35). However, to the best of our knowledge, published literature comparing the diagnostic performance of US and EPS on BPI is limited (27, 28). To investigate their diagnostic performance, US was compared head-to-head with EPS in 245 BP roots from 49 patients in our study by using intraoperative findings as the reference standard. Furthermore, the added benefit of US was shown when we combined it with EPS.

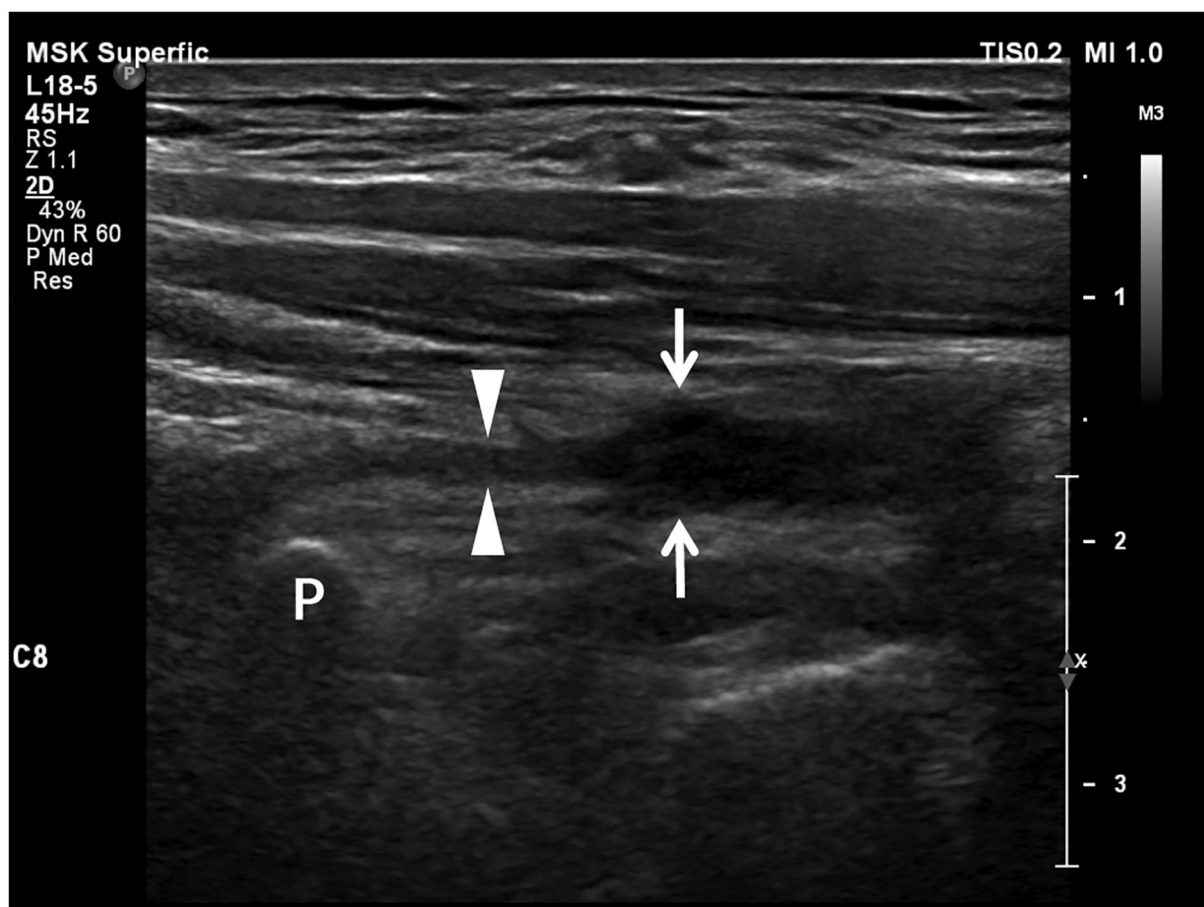


FIGURE 4

Longitudinal sonogram shows a neuroma-like enlargement of a brachial plexus root. The proximal part (arrowheads) of the nerve root adjacent to the transverse process (P) appears as a thin stripe. The distal part (arrows) of the root is hypoechoic and significantly enlarged as a neuroma losing smooth contour and regularly arranged fascicles due to retraction.

Our study showed a higher sensitivity (0.899) of US than previous reports (0.75–0.80) (16–18, 20, 24–26) and a higher accuracy of US (0.951) than EPS (0.898). One possible factor was that the radiologist in our study had extensive experience in peripheral nerve imaging. Another possible explanation was that the result of EPS might change during the spontaneous nerve recovery process, which might cause false diagnoses (3, 7–9, 11, 36).

The results obtained by US were consistent with EPS in 69 true-positive BP roots, 142 true-negative BP roots, and three false-negative BP roots. In this study, when US and EPS were both diagnosed positive, the BP roots were all completely injured. When US and EPS were both diagnosed negative, most (142/145) of the BP roots were not completely injured.

Despite the high concordance rate between US and EPS, 22 BP roots were correctly diagnosed by US but not by EPS, and 9 BP roots were correctly diagnosed by EPS but not by US. Notably, the inclusive combination of US and EPS could

correct 11 out of 22 roots falsely diagnosed by EPS and 6 out of 9 roots falsely diagnosed by US. The exclusive combination of US and EPS could correct the other 11 out of 22 roots falsely diagnosed by EPS. Therefore, we suggest the inclusive and exclusive combination might be taken into consideration for assessing BP root injuries.

As a nerve conduction test, EPS could verify the continuity of a neural pathway to distinguish partial rupture from complete rupture when the neuroma-like enlargement is ambiguous for US. The advantages of Doppler US include high spatial resolution capabilities, portability, easy access, low cost, comparison with the contralateral side, and vascular information. Besides, this noninvasive and radiation-free method can generate real-time images, which allows extremity movement and muscle contraction during the scanning (37). A series of publications showed that US may provide useful morphological information (14–21, 23, 24, 36, 38) to help surgeons choose the appropriate surgical time and surgery type

TABLE 1 Demographic characteristics of 49 patients.

Variables	Value
Sex	
Men	40 (82%)
Women	9 (18%)
Injury-involved side	
Left	27 (55%)
Right	22 (45%)
Etiology	
Collision	41 (84%)
Stretch	4 (8%)
Crush	1 (2%)
Incision	1 (2%)
Iatrogenic injury	2 (4%)
Age (years)	
	Mean ± SD (range)
	42.6 ± 16.8 (5–78)
Time interval (days)	
	Median (Q1, Q3; Min–Max)
Between injury and surgery	87 (42, 137; 4–1,193)
Between injury and US examination	75 (32, 123; 0–1,189)
Between injury and EPS	68 (29, 123; 0–1,188)
Between US examination and surgery	3 (2, 6; 0–94)
Between EPS and surgery	6 (3, 12; 1–94)

as well as refine surgical decisions in advance. Although the time for nerve exploration in closed injuries remains controversial, it is accepted that early nerve transfer for preganglionic root avulsion and early nerve repair or nerve grafts for postganglionic root rupture correlate with better outcomes (1, 4–6). Since the denervated muscle of the preganglionic avulsed root can only be reactivated by nerve transfer, it is vital to diagnose which roots were avulsed in preoperative preparation. If a preganglionic root avulsion is diagnosed, clinicians can better plan the nerve transfer surgery by choosing the suitable donor nerve and testing its function earlier. In addition to clarifying which roots are postganglionic ruptured, US can measure the distance between the neural stumps. An accurate measurement can help inform clinicians in advance to determine whether a neuroanastomosis can be performed or a nerve grafting surgery is necessary. US can visualize the internal texture and surrounding structures of the nerve (25, 31), which is a suitable method for noninvasive monitoring of lesions in continuity at the same anatomical site during spontaneous recovery (4–6). In addition, US can help in making a clinical decision about whether adopting neurolysis for treating continuous lesions with postganglionic swelling or compression when nonsurgical treatment does not lead to functional recovery after a period of time, usually at

TABLE 2 Preoperative US and EPS examination of completely Injured BP roots in correlation with intraoperative results.

BP roots	Number of examined BP roots	Intraoperative results		US				EPS			
		Positive	Negative	True-positive	True-negative	False-positive	False-negative	True-positive	True-negative	False-positive	False-negative
C5-T1	245	89	156	80	153	3	9	75	145	11	14
C5	49	19	30	17	29	1	2	17	28	2	2
C6	49	18	31	18	31	0	0	17	27	4	1
C7	49	21	28	19	28	0	2	16	26	2	5
C8	49	15	34	13	32	2	2	12	32	2	3
T1	49	16	33	13	33	0	3	13	32	1	3

TABLE 3 The sensitivity, specificity, PPV, NPV, and accuracy of preoperative US and EPS examination of completely injured BP roots.

BP Roots	Number of examined BP roots	Number of completely-injured BP roots confirmed in surgery	Sensitivity of preoperative examination		Specificity of preoperative examination		Positive predictive value of preoperative examination		Negative predictive value of preoperative examination		Accuracy of preoperative examination	
			US	EPS	US	EPS	US	EPS	US	EPS	US	EPS
C5-T1	245	89	0.899	0.843	0.981	0.929	0.964	0.872	0.944	0.912	0.951	0.898
C5	49	19	0.895	0.895	0.997	0.933	0.944	0.895	0.935	0.933	0.939	0.918
C6	49	18	1.000	0.944	1.000	0.871	1.000	0.910	1.000	0.964	1.000	0.898
C7	49	21	0.905	0.762	1.000	0.929	1.000	0.889	0.933	0.839	0.959	0.857
C8	49	15	0.867	0.941	0.941	0.941	0.867	0.857	0.941	0.914	0.918	0.898
T1	49	16	0.813	0.813	1.000	0.970	1.000	0.929	0.917	0.914	0.939	0.918

TABLE 4 Paired contingency table of diagnostic accuracy of preoperative US and EPS examination of completely injured BP roots.

	EPS correct	EPS error	Total
US correct	211	22	233
US error	9	3	12
Total	220	25	245

TABLE 5 Comparison of sensitivity and specificity between EPS and the inclusive combination of US and EPS.

	Inclusive combination (US or EPS)	EPS	p-value*
Sensitivity	0.966	0.843	0.000977
Specificity	0.910	0.929	0.25

*Two-sided McNemar test.

TABLE 6 Comparison of sensitivity and specificity between EPS and the exclusive combination of US and EPS.

	Exclusive combination (US and EPS)	EPS	p-value*
Sensitivity	0.775	0.843	0.03125
Specificity	1.000	0.929	0.000977

*Two-sided McNemar test.

least 3–6 months based on the opinion of the majority of clinicians, is necessary (1, 4–6, 11–13). Moreover, the additional morphological information provided by US could improve the preoperational preparation of the surgery and, hence, reduce the time of surgery and the risk of infection.

There were three falsely diagnosed BP roots by both US and EPS. US images of these completely injured roots did not show any positive signs defined in this study, e.g., neural stump, neural gap, cerebrospinal fluid, or neuroma-like enlargement. In this study, two of these three roots (C8 and T1) were from one patient who was injured around 3 years before the examinations. Despite the operator-dependent nature (17–20, 23, 24), the prominent deficiency of US is its poor visualization of deep roots due to the anatomic location, edema, or obscuration by the bone (16–20, 23, 24, 36), which might be the reason for the relatively lower sensitivity of deeper roots in our study. Besides the features investigated in our study, ultrasonographic researchers also studied other parameters of histological structure that might support the diagnosis of peripheral nerve injuries. For example, in a recent publication, Ricci et al. recommended (semi)-quantitative measurement of the peripheral nerve internal structures and the nerve microcirculation by using high-frequency probes and high-sensitive color/power Doppler. The sonographic parameters of interest included (1) thickness of epifascicular epineurium; (2) texture of interfascicular epineurium; (3) number, size, and echogenicity of fascicles; and (4) intrafascicular/extrafascicular

vascular signals. By combining these parameters, grayscale and vascular US allowed clear visualization of two pathological sonographic patterns. The first one was the hypoechoic nerve due to fascicular edema, which is usually observed in the acute phase of pathology. The second one was the hyperechoic nerve due to fibrotic involution of the interfascicular tissue and reduced number and size of fascicles, which correlated with the chronic phase of pathology. These abovementioned sonographic features could be considered potential additional sonographic features to augment the diagnostic accuracy of US in future studies, especially for neural stumps and neuroma-like enlargement (31).

The main bias of this study resulted from the observer of the reference standard, who also made the decision to operate and was not blinded to preoperative US or EPS results. Second, all the patients included in this retrospective study were surgical cases, which indicates that this study was enriched with severe patients with BPI who needed surgeries. This did not reflect the proportion of severe BPI in the real-world BPI population, so the performance of US or the combination of US and EPS needed to be further investigated in a medium or minor level of BPI.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Institutional Review Board of Huashan Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

AL and XJ collected and analyzed the data and wrote the manuscript. AL, XJ, LZ, XH, WC, and LC collected the data. WC

and LC supervised the analyzing and writing work. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Shanghai's Rising Stars of Medical Talent Youth Development Program (Youth Medical Talents—Medical Imaging Practitioner Program).

Acknowledgments

The authors would like to thank Dr. Minzhen Wang for her assistance in statistical analysis and Dr. Nira Beck-Razi for her editorial assistance provided during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2022.1077830/full#supplementary-material>

References

- Shin AY, Spinner RJ, Steinmann SP, Bishop AT. Adult traumatic brachial plexus injuries. *J Am Acad Orthop Surg.* (2005) 13:382–96. doi: 10.5435/00124635-200510000-00003
- Kato N, Htut M, Taggart M, Carlstedt T, Birch R. The effects of operative delay on the relief of neuropathic pain after injury to the brachial plexus: a review of 148 cases. *J Bone Joint Surg.* (2006) 88B:756–9. doi: 10.1302/0301-620X.88B6.16995
- Ferrante MA, Wilbourn AJ. Electrodiagnostic approach to the patient with suspected brachial plexopathy. *Neurol Clin.* (2002) 20:423–50. doi: 10.1016/s0733-8619(01)00007-x
- Fox IK, Mackinnon SE. Adult peripheral nerve disorders: nerve entrapment, repair, transfer, and brachial plexus disorders. *Plast Reconstr Surg.* (2011) 127:105e–18e. doi: 10.1097/PRS.0b013e31820cf556
- Limthongthang R, Bachoura A, Songcharoen P, Osterman AL. Adult brachial plexus injury: evaluation and management. *Orthop Clin North Am.* (2013) 44:591–603. doi: 10.1016/j.jocl.2013.06.011
- Arzillo S, Gishen K, Askari M. Brachial plexus injury: treatment options and outcomes. *J Craniofac Surg.* (2014) 25:1200–6. doi: 10.1097/SCS.0000000000000841

7. Caporrio FA, Moreira L, Moraes VY, Bellotti JC, Gomes dos Santos JB, Faloppa F. Brachial plexus injuries: diagnosis performance and reliability of everyday tools. *Hand Surg.* (2014) 19:7–11. doi: 10.1142/S0218810414500026
8. Mansukhani KA. Electrodiagnosis in traumatic brachial plexus injury. *Ann Indian Acad Neurol.* (2013) 16:19–25. doi: 10.4103/0972-2327.107682
9. O'Shea K, Feinberg J, Wolfe SW. Imaging and electrodiagnostic work-up of acute adult brachial plexus injuries. *J Hand Surg Eur Vol.* (2011) 36:747–59. doi: 10.1177/1753193411422313
10. Levin KH, Wilbourn AJ, Maggiano HJ. Cervical rib and median sternotomy-related brachial plexopathies: a reassessment. *Neurology.* (1998) 50:1407–13. doi: 10.1212/wnl.50.5.1407
11. Lim SH, Lee JS, Kim YH, Kim TW, Kwon KM. Spontaneous recovery of non-operated traumatic brachial plexus injury. *Eur J Trauma Emerg Surg.* (2018) 44:443–49. doi: 10.1007/s00068-017-0810-x
12. Giuffrè JL, Kakar S, Bishop AT, Spinner RJ, Shin AY. Current concepts of the treatment of adult brachial plexus injuries. *J Hand Surg Am.* (2010) 35:678–88. doi: 10.1016/j.jhsa.2010.01.021
13. Martin E, Senders JT, DiRisio AC, Smith TR, Broekman MLD. Timing of surgery in traumatic brachial plexus injury: a systematic review. *J Neurosurg.* (2018) 1:1–13. doi: 10.3171/2018.1.JNS172068
14. Martinoli C, Bianchi S, Santacrocce E, Pugliese F, Graif M, Derchi LE. Brachial plexus sonography: a technique for assessing the root level. *AJR Am J Roentgenol.* (2002) 179:699–702. doi: 10.2214/ajr.179.3.1790699
15. Shafiqhi M, Gurunluoglu R, Ninkovic M, Mallouhi A, Bodner G. Ultrasonography for depiction of brachial plexus injury. *J Ultrasound Med.* (2003) 22:631–4. doi: 10.7863/jum.2003.22.6.631
16. Graif M, Martinoli C, Rochkind S, Blank A, Trejo L, Weiss J, et al. Sonographic evaluation of brachial plexus pathology. *Eur Radiol.* (2004) 14:193–200. doi: 10.1007/s00330-003-1997-2
17. Haber HP, Sinis N, Haerle M, Schaller HE. Sonography of brachial plexus traction injuries. *AJR Am J Roentgenol.* (2006) 186:1787–91. doi: 10.2214/AJR.04.1861
18. Gruber H, Glodny B, Galiano K, Kamelger F, Bodner G, Hussl H, et al. High-resolution ultrasound of the supraclavicular brachial plexus – can it improve therapeutic decisions in patients with plexus trauma. *Eur Radiol.* (2007) 17:1611–20. doi: 10.1007/s00330-006-0464-2
19. Chen DZ, Cong R, Zheng MJ, Zhu T, Coles G, Feng H, et al. Differential diagnosis between pre- and postganglionic adult traumatic brachial plexus lesions by ultrasonography. *Ultrasound Med Biol.* (2011) 37:1196–203. doi: 10.1016/j.ultrasmedbio.2011.04.015
20. Tagliafico A, Succio G, Serafini G, Martinoli C. Diagnostic performance of ultrasound in patients with suspected brachial plexus lesions in adults: a multicenter retrospective study with MRI, surgical findings and clinical follow-up as reference standard. *Skeletal Radiol.* (2013) 42:371–6. doi: 10.1007/s00256-012-1471-9
21. Zheng M, Zhu Y, Zhou X, Chen S, Cong R, Chen D. Diagnosis of closed injury and neoplasm of the brachial plexus by ultrasonography. *J Clin Ultrasound.* (2014) 42:417–22. doi: 10.1002/jcu.22155
22. Zhu YS, Mu NN, Zheng MJ, Zhang YC, Feng H, Cong R, et al. High-resolution ultrasonography for the diagnosis of brachial plexus root lesions. *Ultrasound Med Biol.* (2014) 4:1420–6. doi: 10.1016/j.ultrasmedbio.2014.02.012
23. Nwawka OK, Casaletto E, Wolfe SW, Feinberg JH. Ultrasound imaging of brachial plexus trauma in gunshot injury. *Muscle Nerve.* (2019) 59:707–11. doi: 10.1002/mus.26461
24. Gu S, Zhao Q, Yao J, Zhang L, Xu L, Chen W, et al. Diagnostic ability of ultrasonography in brachial plexus root injury at different stages post-trauma. *Ultrasound Med Biol.* (2022) 48:1122–30. doi: 10.1016/j.ultrasmedbio.2022.02.013
25. Chin B, Ramji M, Farrokhhyar F, Bain JR. Efficient imaging: examining the value of ultrasound in the diagnosis of traumatic adult brachial plexus injuries, a systematic review. *Neurosurgery.* (2018) 83:323–32. doi: 10.1093/neuros/nyx483
26. Padua L, Martinoli C. From square to cube: ultrasound as a natural complement of neurophysiology. *Clin Neurophysiol.* (2008) 119:1217–8. doi: 10.1016/j.clinph.2008.02.005
27. Toia F, Gagliardo A, D'Arpa S, Gagliardo C, Gagliardo G, Cordova A. Preoperative evaluation of peripheral nerve injuries: What is the place for ultrasound? *J Neurosurg.* (2016) 125:603–14. doi: 10.3171/2015.6.JNS151001
28. Jakobsen RL, Frederiksen AF, Hellfritsch MB, Qerama EA. prospective study of high resolution ultrasound in brachial plexopathies - correlation with electrophysiological measurements. *Clin Neurophysiol.* (2019) 130:1144–50. doi: 10.1016/j.clinph.2019.03.034
29. Perlas A, Chan VW, Simons M. Brachial plexus examination and localization using ultrasound and electrical stimulation: a volunteer study. *Anesthesiology.* (2003) 99:429–35. doi: 10.1097/0000542-200308000-00025
30. Hsu PC, Chang KV, Mezian K, Nanka O, Wu WT, Yang YC, et al. Sonographic pearls for imaging the Brachial Plexus and its pathologies. *Diagnostics (Basel).* (2020) 20:324. doi: 10.3390/diagnostics10050324
31. Ricci V, Ricci C, Cocco G, Gervasoni F, Donati D, Fari G, et al. Histopathology and high-resolution ultrasound imaging for peripheral nerve (injuries). *J Neurol.* (2022) 269:3663–75. doi: 10.1007/s00415-022-10988-1
32. Sinha S, Pemmaiah D, Midha R. Management of brachial plexus injuries in adults: Clinical evaluation and diagnosis. *Neurol India.* (2015) 63:918–25. doi: 10.4103/0028-3886.170114
33. Singer AD, Meals C, Kesner V, Boulis N, Gonzalez FM, Umpierrez M, et al. The Multidisciplinary approach to the diagnosis and management of nonobstetric traumatic brachial plexus injuries. *AJR Am J Roentgenol.* (2018) 211:1319–31. doi: 10.2214/AJR.18.19887
34. Krawczuk AW, Huber J. Standard neurophysiological studies and motor evoked potentials in evaluation of traumatic brachial plexus injuries – a brief review of the literature. *Neurol Neurochir Pol.* (2018) 52:549–54. doi: 10.1016/j.pjnns.2018.05.004
35. Griffith JF. Ultrasound of the brachial plexus. *Semin Musculoskelet Radiol.* (2018) 22:323–33. doi: 10.1055/s-0038-1645862
36. Padua L, Pasquale AD, Liotta G, Granata G, Pazzaglia C, Erra C, et al. Ultrasound as a useful tool in the diagnosis and management of traumatic nerve lesions. *Clin Neurophysiol.* (2013) 124:1236–43. doi: 10.1016/j.clinph.2012.10.024
37. Jacobson JA, Middleton WD, Allison SJ, Dahiya N, Lee KS, Levine BD, et al. Ultrasonography of superficial soft-tissue masses: society of radiologists in ultrasound consensus conference statement. *Radiology.* (2022) 304:18–30. doi: 10.1148/radiol.211101
38. Kumar AH, Kim J, Sadeghi N, Leversedge FJ, Moorman CT, Grant SA. The use of ultrasound imaging for brachial plexus injury assessment following operative clavicle repair. *Can J Anaesth.* (2018) 65:739–41. doi: 10.1007/s12630-018-1076-4



OPEN ACCESS

EDITED BY

Bruce Douglas Carter,
Vanderbilt University, United States

REVIEWED BY

Nareshwaran Gnanasegaran,
Singapore-MIT Alliance for Research
and Technology (SMART), Singapore
Luca Testarelli,
Sapienza University of Rome, Italy

*CORRESPONDENCE

Hui Lu
✉ huilu@zju.edu.cn

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 15 November 2022

ACCEPTED 28 December 2022

PUBLISHED 11 January 2023

CITATION

Fu J, Li X, Jin F, Dong Y, Zhou H,
Alhaskawi A, Wang Z, Lai J, Yao C,
Ezzi SHA, Kota VG, Hasan Abdulla
Hasan Abdulla M, Chen B and Lu H
(2023) The potential roles of dental
pulp stem cells in peripheral nerve
regeneration.
Front. Neurol. 13:1098857.
doi: 10.3389/fneur.2022.1098857

COPYRIGHT

© 2023 Fu, Li, Jin, Dong, Zhou,
Alhaskawi, Wang, Lai, Yao, Ezzi, Kota,
Hasan Abdulla Hasan Abdulla, Chen
and Lu. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

The potential roles of dental pulp stem cells in peripheral nerve regeneration

Jing Fu¹, Xigong Li², Feilu Jin³, Yanzhao Dong², Haiying Zhou²,
Ahmad Alhaskawi², Zewei Wang⁴, Jingtian Lai⁴, Chengjun Yao⁴,
Sohaib Hasan Abdullah Ezzi⁵, Vishnu Goutham Kota²,
Mohamed Hasan Abdulla Hasan Abdulla², Bin Chen² and
Hui Lu^{2,6*}

¹Department of Stomatology, Affiliated Hangzhou Xixi Hospital, Zhejiang University School of Medicine, Hangzhou, China, ²Department of Orthopedics, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China, ³Oral and Maxillofacial Surgery Department, The Second Affiliated Hospital of Zhejiang University, Hangzhou, Zhejiang, China, ⁴Zhejiang University School of Medicine, Hangzhou, Zhejiang, China, ⁵Department of Orthopedics, Third Xiangya Hospital, Central South University, Changsha, Hunan, China, ⁶Alibaba-Zhejiang University Joint Research Center of Future Digital Healthcare, Zhejiang University, Hangzhou, Zhejiang, China

Peripheral nerve diseases are significantly correlated with severe fractures or trauma and surgeries, leading to poor life quality and impairment of physical and mental health. Human dental pulp stem cells (DPSCs) are neural crest stem cells with a strong multi-directional differentiation potential and proliferation capacity that provide a novel cell source for nerve regeneration. DPSCs are easily extracted from dental pulp tissue of human permanent or deciduous teeth. DPSCs can express neurotrophic and immunomodulatory factors and, subsequently, induce blood vessel formation and nerve regeneration. Therefore, DPSCs yield valuable therapeutic potential in the management of peripheral neuropathies. With the purpose of summarizing the advances in DPSCs and their potential applications in peripheral neuropathies, this article reviews the biological characteristics of DPSCs in association with the mechanisms of peripheral nerve regeneration.

KEYWORDS

dental pulp stem cells, neuron, Schwann cells, peripheral nerve diseases, neurotrophic factors

1. Introduction

Peripheral neuron degeneration, inflammation, and necroptosis caused by trauma, diabetes, and neurodegenerative disorders may cause motor-sensory dysfunctions (1–3). Therefore, the current therapeutic regimen mainly focuses on neuron regeneration and function restoration in post-traumatic events.

Conventional therapies have limited efficacy in restoring nerve function since the regeneration of neurons, and glial cells require sufficient neuronal precursor cells, which are absent or lacking in the mature nervous system (4). Stem cell-based therapies bring new insight into the biotherapy of peripheral neuropathies, providing adequate cell

sources capable of self-renewal and multi-directional differentiation (4–8). However, challenges remain in the mass production of autografts or autologous cells for sufficient nerve regeneration (5–8).

Derived from the neural crest, DPSCs yield great potential in differentiation into neurons, expression of various neurotrophic factors for axonal regeneration, and functions of immunomodulation, indicating that DPSCs are an ideal cell source for peripheral nerve regeneration (5–7). In this review, we will summarize the biological characteristics of DPSCs and their respective application in animal models of peripheral neuropathies, with a focus on their regenerative mechanisms for future application.

1.1. Biological characteristics of DPSCs

Dental pulp, identified as a typical soft tissue, is rich in blood vessels, nerves, and mesenchymal tissue. Dental pulp has a central role in the development of primary and secondary teeth and further maintenance throughout life (7, 9, 10). Gronthos et al. (11) first described that DPSCs were initially discovered from the third molar dental pulp, which is later found in other dental pulps including deciduous teeth, permanent teeth, and supernumerary teeth. DPSCs display fibroblast-like morphology with higher proliferation capacity but lack specific surface biomarkers (Table 1) (12–20). While highly express MSC-like phenotypic biomarkers including CD29, CD90, and CD73 (12, 13), DPSCs are also found to express stemness-related markers such as Oct-4, Nanog, and Sox-2 (14, 15), and cytoskeleton-related markers such as Nestin and Vimentin (15). Moreover, several studies have demonstrated the expression of cranial neural crest cell-related neural markers by DPSCs, including glial fibrillary acidic protein (GFAP), β -III tubulin, and microtubule-associated protein-2 (MAP-2) (15, 16, 21). Recently, several special markers are proposed to distinguish DPSCs from gingiva-derived mesenchymal stem cells (GMSCs) including Calreticulin, Annexin A5, and Rho GDP dissociation inhibitor alpha (17). Furthermore, a recent study by Lei et al. (18) demonstrated that the CD271 is the most effective stem

cell surface marker for dental mesenchymal stem cells (DMSCs), which display high odontogenic potential.

2. The potential mechanisms of DPSCs in neural regeneration

DPSCs have shown the potential of multiple differentiations, with promising therapeutic value as bioengineered autografts for different tissue repair (Figure 1). Therefore, DPSCs can be applied in the biotherapy for a variety of peripheral neuropathies (5–7, 19, 20, 22–26). Studies show that DPSCs could induce restoration in peripheral nerves *via* three mechanisms, which include neuronal differentiation, paracrine and immunomodulatory effects (Figure 2) (5–7, 19, 20, 22–26).

2.1. Neuronal differentiation

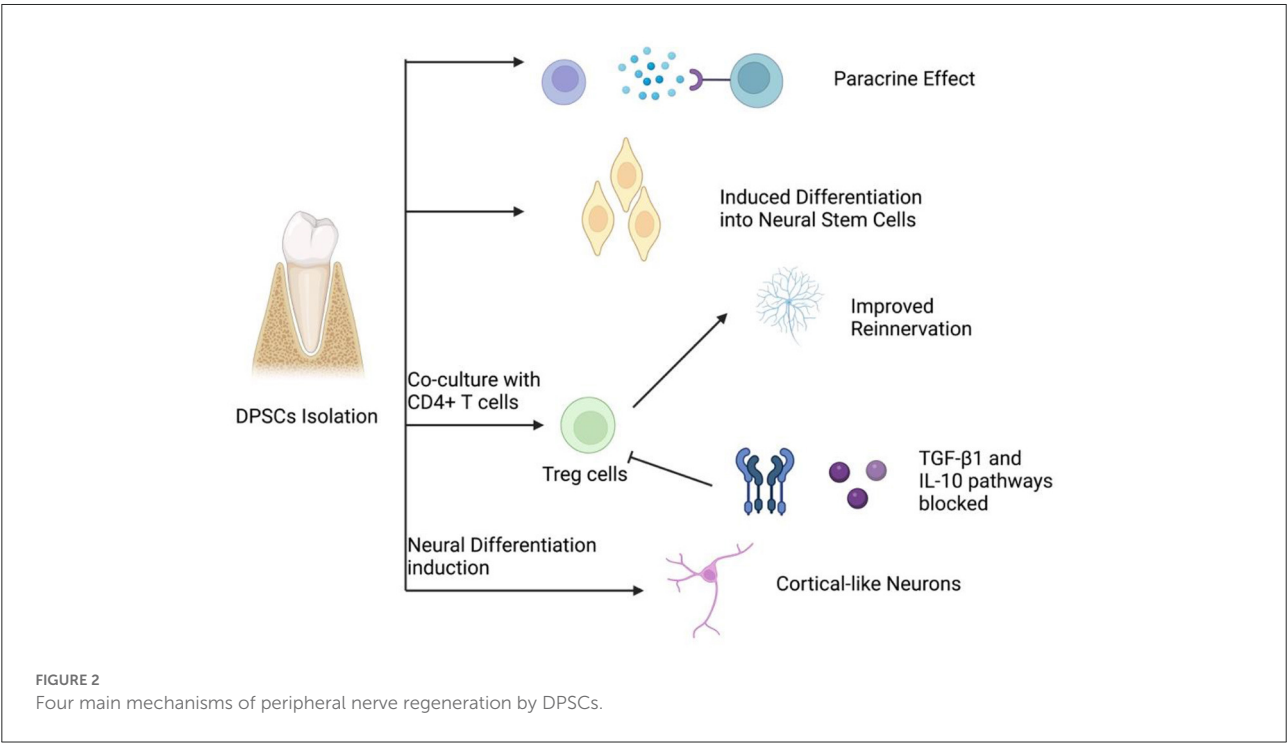
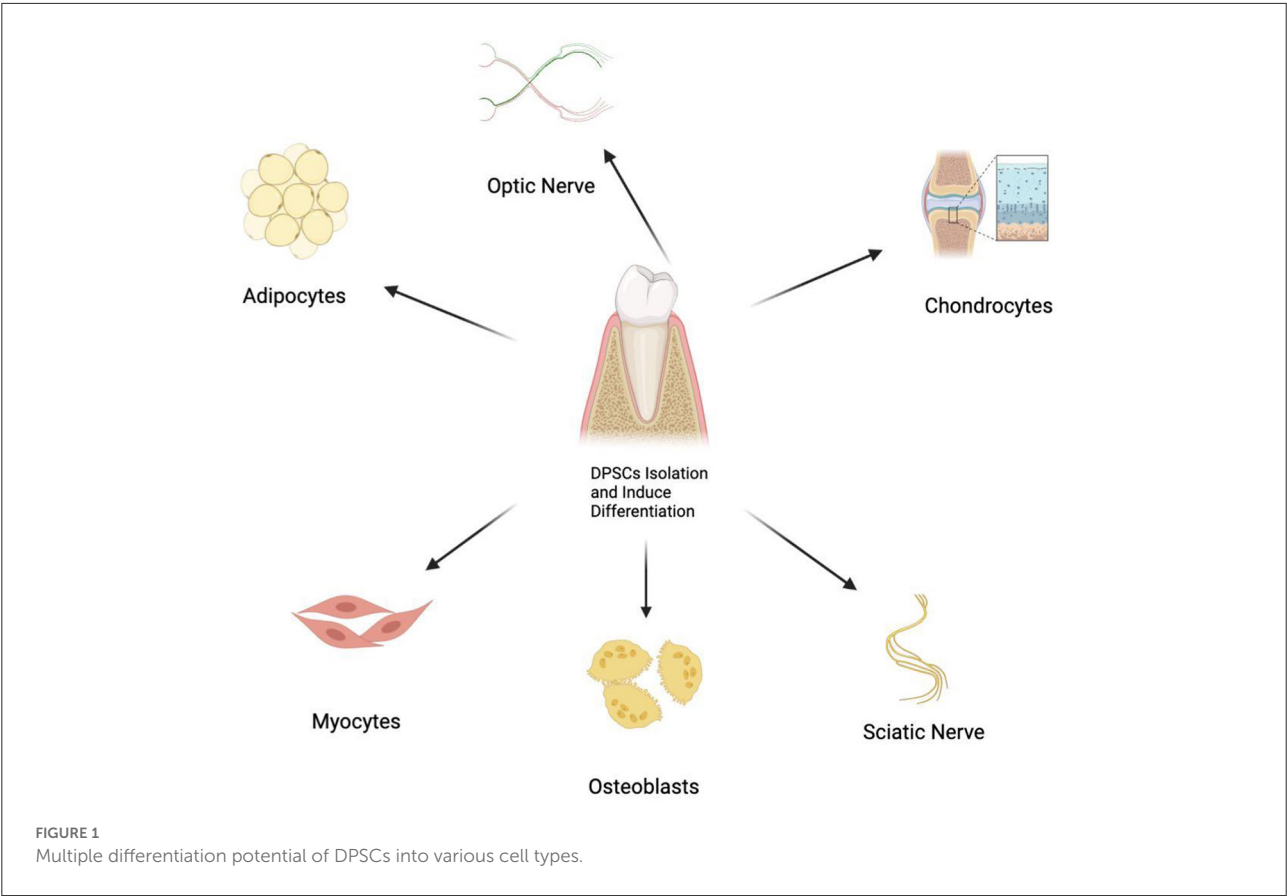
DPSCs can directly differentiate into neuron-like cells and express early neural markers (such as Nestin) (15). These neuron-like cells could migrate to the lesion sites and subsequently participate in the nerve regeneration process (5–7). Some studies have also shown that during migration, the transplanted DPSCs can recruit endogenous neural stem cells for tissue reconstruction (20). In addition, Kiraly et al. (15) revealed that the induction and differentiation of DPSCs are promoted by activating the intracellular cyclic adenosine phosphate signaling pathway. Chen et al. (27) proposed that the elevated intracellular cyclic adenosine phosphate can activate protein kinase A (PKA), which then up-regulate regeneration-related genes, such as arginase I, and promotes peptide synthesis. On the other hand, PKA also inhibits the Rho protein activation induced by myelin and induce nerve regeneration. Heng et al. (28) suggest that EphrinB2 signaling can modulate the neural differentiation of DPSCs, while EphB4 -inhibition in DPSCs could significantly up-regulate expression of the neural markers microtubule-associated protein 2, Musashi1, NGN2, and neuron-specific enolase. In 2018 Urraca et al. (29) described DPSC-derived neurons expressing GABAA and MAP2 genes, which is previously absent in undifferentiated DPSC and may provide future neurogenetic research with a useful tool.

TABLE 1 The features of DPSCs.

Type	Surface markers expression
MSC-like phenotypic markers	CD27, CD29, CD44, CD73, CD90, CD105, CD146, CD166, CD271, and STRO-1
Stemness-related markers	Oct-4, Nanog, and Sox-2
Cytoskeleton-related markers	Nestin and Vimentin
Neural crest cell-related markers	GFAP, β -III tubulin, and MAP-2
DPSC Specific markers	Calreticulin and CD271

2.2. Paracrine effects

Several studies have indicated that DPSCs could participate in the process of nerve repair in a paracrine manner (10, 20, 30). DPSCs can express brain-derived neurotrophic factor (BDNF), glia cell-line derived neurotrophic factor (GDNF), NGF, and neurotrophin-3 (NT3) at a substantial level, and exert neuroprotective functions in the process of peripheral nerve regeneration (10). Some researchers propose that the effects



of these neurotrophic factors on neural cells are achieved *via* the intervention of PI3K/AKT signaling pathway. Even in the presence of neurotrophic factors such as NGF and GDNF, inhibition of PI3K or AKT activation can result in neuron apoptosis or necroptosis (31). The Erk signaling pathway plays a essential role in axonal growth stimulation by modulating neurotrophic factors (32). Additionally, the neurotrophin expression in DPSCs is enhanced in certain neural inductive conditions (33). The conditioned medium of DPSCs has been shown to increase Schwann cell proliferation rate while inducing neurite growth *in vitro* (31, 32). Previous studies revealed that the DPSC-derived secretome or DPSC conditional medium, which includes soluble factors and extracellular vesicles, proved to be therapeutically relevant in the management of neurodegenerative disorders and nerve injuries *via* the regulation of several processes, including neuroprotection, anti-inflammation, anti-apoptosis, and angiogenesis (34).

2.3. Immunomodulatory properties

The immunomodulatory properties of DPSCs may also exert crucial functions during the neural repair mechanism (35–38). Several studies have shown not only CD4⁺T cells co-cultured with DPSCs can highly express regulatory T cells (Treg), but DPSCs implanted *in vivo* could reverse the decrease in Treg expression induced by transforming growth factor β 1 (TGF β 1) and interleukin-10 signaling pathways inhibition, suggesting that DPSCs could interfere with immunoregulation during nerve regeneration (35, 36). On the other hand, it has been discovered that DPSCs may inhibit TNF- α , and thus up-regulate anti-inflammation cytokines and promote nerve regeneration (37). Furthermore, DPSCs can induce inhibition in cytotoxic T cell proliferation and activation *via* CD73, a central enzyme in the crosstalk of immunosuppressive adenosine and extracellular pro-inflammatory ATP, which is highly expressed in DPSCs (38).

3. The treatment of transplanted DPSCs in peripheral nerve disease

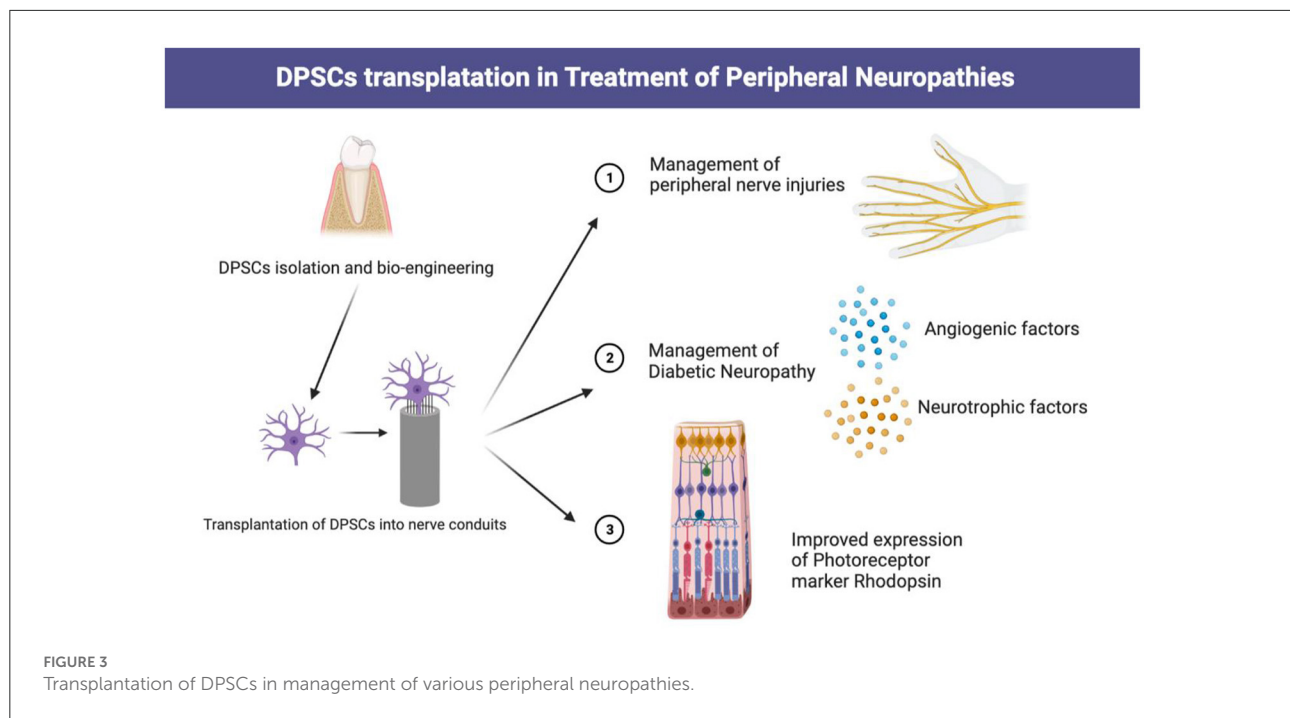
3.1. Peripheral nerve injuries

Peripheral nerve injuries lead to notable functional impairments and decreased quality of life (39). Despite advances in microscopic techniques for neurosurgery, clinicians always desire to improve postoperative nerve regeneration and rehabilitation for better functional restoration *via* various methods including biotherapy (40, 41). Currently, the transplanted DPSCs alone or in combination with several novel nerve conduits are promising therapy for patients suffering from peripheral nerve injury (42, 43). Takaoka et al.

(44) transplanted DPSCs into a rat model with a 10-mm sciatic nerve defect and found improvement in axon growth, remyelination, electrophysiological activities, and alleviated muscle atrophy at 12 weeks post-transplantation. DPSC-embedded polymeric biomaterial based on ethyl acrylate and hydroxy ethyl acrylate copolymer shows sufficient bioactivity to promote regeneration of the injured sciatic nerve (45). A similar study has shown that preloading collagen conduits with Schwann cell-like cells (SCLCs) induced from DPSCs could enhance sciatic nerve repair (46). In addition, collagen scaffolds preloaded with DPSCs post-differentiation could exhibit certain traits of SCLC that promote the outgrowth of axons and myelination in 2-dimensional or 3-dimensional culture conditions (47). These results demonstrate that DPSCs are excellent stem cell sources for peripheral nerve regeneration (Figure 3).

3.2. Diabetic neuropathy

Diabetic neuropathy, the most common complication of type 1 and type 2 Diabetes Mellitus (DM), and has become a substantial health concern worldwide, especially for the elderly. It has been estimated that over 50% of long-term DM patients will eventually develop neuropathy, which could lead to diabetic foot ulcers associated with serious disabilities (48). The COVID milieu since 2019 has driven healthcare professionals to emphasize the matter of management and nursing of patients suffering from diabetic neuropathy since DM patients are prone to have compromised immunity and disturbed microenvironment (49). Typically, patients suffering from diabetic neuropathy show decreased peripheral nerve vascularity and a deficiency of angiogenic and neurotrophic factors, which may account for the pathogenesis of neuropathies (50, 51). Recently, the therapeutic effects of DPSCs in diabetic neuropathy have become recognized by many researchers, which raised controversies regarding the optimal application method of DPSCs. Makino et al. suggested that DPSCs transplantation can significantly improve the blood flow, nerve conduction velocity, capillary density, and intra-epidermal nerve fiber density of the damaged nerves while up-regulating the expression levels of angiogenic and neurotrophic factor genes (52). Another study proved that transplanted-DPSCs can significantly reduce the number of macrophages in the diabetic peripheral nerve microenvironment and specifically inhibit M1 macrophage expression while up-regulating M2 macrophage expression, eventually decreasing the M1/M2 macrophage ratio (19). Apart from regulating macrophage expression, DPSCs could also exert anti-inflammatory effects *via* inhibiting tumor necrosis factor α (TNF α) and interleukin-6 (IL-6) expression while up-regulating TGF- β expression. These findings provide some perspectives on possible future applications of DPSCs in diabetic neuropathy management.



3.3. Retina injury

Neurons in the retina and optic nerve share a mutual origin from the embryonic diencephalon. After neuroepithelium formation of the retina, neurons lose the ability to divide, rendering neuronal renewal in the retina impossible (53). Therefore, blindness caused by retinal injuries remains a major cause of disability worldwide. Retinal ganglion cells express a large number of neurotrophic factor receptors, which may enhance retinal ganglion cell survival and axonal regeneration (54). Mead et al. found that DPSCs secreted a large number of neurofibrillary tangles (NTF), which enhanced neural β III-tubulin+ retinal cell proliferation and lengthened the neuritis (55). In addition, transplantation of DPSCs into the vitreous humor of mice after optic nerve injury promoted Brn-3a+ retinal ganglion cell survival and axonal regeneration (55). It has been reported that 44% of DPSCs expressed a photoreceptor marker rhodopsin in a conditioned medium from the damaged retina (56). This promising novel mechanism should be further explored for clinical applications.

4. Conclusions

4.1. Current research and challenges

Dental pulp tissue yields great reproductive ability and is rich in varying categories of stem cells with unique differentiation potentials. It has been concluded that DPSCs may be isolated from both postnatal teeth and extremely rare natal teeth (11). Immortalized DPSCs are also an excellent source of pluripotent

stem cells with high molecular, morphological and genetic resemblance with non-immortalized DPSCs, which introduces the possibility of building a reservoir with immortalized DPSCs from patients suffering from a wide spectrum of neurogenetic disorders (57). On the other hand, Wilson et al. (58) have assessed the tumorigenic potential of immortalized DPSC *in vitro* and in mice and observed no tumor formation, indicating the probable safety of immortalized DPSC in future clinical applications.

As presented in Table 2, current research on the application of DPSC in the management of central and peripheral neuropathies is majorly concentrated on differentiation induced *in vitro* and implantation either *via* nerve conduit scaffold or direct injections. However, for such a therapeutic regimen to be carried out clinically, further clinical and lab research are required to achieve large-scale DPSC manufacture, storage, and transportation with minimum possibility of contamination. Concerns have been raised about the compromised quality of DPSC culture possibly in association with poor oral hygiene and long-distance transportation (57).

4.2. Future prospects

Stem cell-based therapies shed light on the biotherapies of peripheral nerve disease. DPSCs may enhance peripheral nerve regeneration *via* the induction of neuronal differentiation and the up-regulation of various neurotrophic factors. DPSCs in combination with biomaterials could be the prospect of neural tissue repair. Furthermore, DPSCs have a wide

TABLE 2 Several current studies involving the application of DPSCs in nerve regeneration.

No.	Method/treatment of DPSCs	Study type (<i>in vitro</i> versus <i>in vivo</i>)	Biomarkers expressed by DPSCs	Outcome	Reference
1	Nerve conduit preloaded with DPSCs	<i>In vivo</i> rat sciatic nerve	GFAP and β -tubulin III	Promote functional repair of peripheral nerve injuries	(59)
2.	Hypoxia-treated DPSCs transplantation into damaged rat spinal cord	<i>In vivo</i> rat spinal cord	CD13+, N-cadherin and bFGF	Increase vascularization and oxygenation of the injured spinal cord	(60)
3.	5-Aza pre-condition to induce myogenic commitment; injection into rat urethral sphincter post pudendal nerve transection	<i>In vitro</i> and <i>in vivo</i> rat urethral sphincter	HLA-ABC	<i>In vitro</i> : DPSCs committed toward myogenic lineage; <i>In vivo</i> : promoted vascularization, recovered sphincter thickness and detected within the nerve	(61)
4.	DPSC auto-transplantation in unilateral hindlimb of diabetic rats	<i>In vivo</i> in diabetic rat sciatic nerve	CD29, CD34, CD49d, CD45 and CD90	Improved blood flow, nerve conduction velocity, capillary number, and intra-dermal nerve fiber density	(62)
5.	Exposure of DPSCs to midbrain cues	<i>In vitro</i>	CD73, CD90, CD105, CD34, and HLADR	Midbrain cues could dictate DPSCs to dopaminergic cell-type	(63)
6.	DPSCs pre-labeled with PKH 26 is cultured and injected intravenously in PD rat model	<i>In vivo</i> in PD rat model	CD34, CD45, CD73, CD90, CD166, and HLA-DR	Amelioration of degenerated neurons, and enhancement to impaired behavioral performances	(64)

GFAP, glial fibrillary acidic protein; bFGF, basic fibroblast growth factors; HLA-ABC, Human Leukocyte Antigen–A, B, and C isotype; HLA-DR, Human Leukocyte Antigen –DR isotype; PD, Parkinson's disease.

range of application prospects in peripheral nerve diseases, such as peripheral nerve injury, diabetic neuropathy, and retina injury. While DPSCs transplantation shows promising therapeutic potential in the management of peripheral nerve diseases, further research is required to establish a therapeutic approach and a regimen of dosage, efficacy, and safety. In conclusion, DPSCs yield great potential in peripheral neural tissue regeneration and repair, yet various issues remain to be solved through further assessment and experimentation.

Author contributions

HL and JF designed the study. FJ, XL, and YD performed data collection. HZ, AA, ZW, and JL analyzed the results. CY, SE, VK, BC, and MH drafted the manuscript. All authors have read and approved the final manuscript.

Funding

This study was funded by Zhejiang Provincial Natural Science Foundation Academic Exchange Program (Grant Numbers: LY20H060007 and LY20H090018). The funding

bodies had no role in the design of the study; in the collection, analysis, and interpretation of data; in drafting the manuscript.

Acknowledgments

We thank Zhenyu Sun for comments on the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Donzelli R, Capone C, Sgulò FG, Mariniello G, Donzelli O, Maiuri F. Microsurgical repair by autografting in traumatic injuries of peripheral nerves. *J Neurosurg Sci.* (2022) 66:208–14. doi: 10.23736/S0390-5616.19.04572-7
2. Baumard J, Lesourd M, Remigereau C, Jarry C, Etcharry-Bouyx F, Chauviré V, et al. Tool use in neurodegenerative diseases: planning or technical reasoning? *J Neuropsychol.* (2018) 12:409–26. doi: 10.1111/jnp.12121

3. Farrell MC, Weiss SJ, Goodrich C, Lehmann MP, Delarato N. Food aversion leading to nutritional optic neuropathy in a child with severe vitamin A deficiency. *J Neuroophthalmol.* (2021) 41:e718–9. doi: 10.1097/WNO.0000000000001142
4. Klimovich P, Rubina K, Syssoeva V, Semina E. New Frontiers in Peripheral Nerve Regeneration: Concerns and Remedies. *Int J Mol Sci.* (2021) 22(24). doi: 10.3390/ijms222413380
5. Zainal Ariffin SH, Kermani S, Zainol Abidin IZ, Megat Abdul Wahab R, Yamamoto Z, et al. Differentiation of dental pulp stem cells into neuron-like cells in serum-free medium. *Stem Cells Int.* (2013) 2013:250740. doi: 10.1155/2013/250740
6. Luo L, He Y, Wang X, Key B, Lee BH, Li H, et al. Potential roles of dental pulp stem cells in neural regeneration and repair. *Stem Cells Int.* (2018) 2018:1731289. doi: 10.1155/2018/1731289
7. Young F, Sloan A, Song B. Dental pulp stem cells and their potential roles in central nervous system regeneration and repair. *J Neurosci Res.* (2013) 91:1383–93. doi: 10.1002/jntr.23250
8. de Freria CM, Van Niekerk E, Blesch A, Lu P. Neural stem cells: promoting axonal regeneration and spinal cord connectivity. *Cells.* (2021) 10:3296. doi: 10.3390/cells10123296
9. Karaöz E, Demircan PC, Saglam O, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. *Histochem Cell Biol.* (2011) 136:455–73. doi: 10.1007/s00418-011-0858-3
10. Martens W, Bronckaers A, Politis C, Jacobs R, Lambrechts I. Dental stem cells and their promising role in neural regeneration: an update. *Clin Oral Investig.* (2013) 17:1969–83. doi: 10.1007/s00784-013-1030-3
11. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A.* (2000) 97:13625–30. doi: 10.1073/pnas.240309797
12. Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. *J Dent Res.* (2002) 81:531–5. doi: 10.1177/154405910208100806
13. Kawashima N. Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? *Arch Oral Biol.* (2012) 57:1439–58. doi: 10.1016/j.archoralbio.2012.08.010
14. Cheng PH, Snyder B, Fillos D, Ibegbu CC, Huang AHC, Chan AWS. Postnatal stem/progenitor cells derived from the dental pulp of adult chimpanzee. *BMC Cell Biol.* (2008) 9:20. doi: 10.1186/1471-2121-9-20
15. Király M, Porcsalmy B, Pataki A, Kádár K, Jelítai M, Molnár B, et al. Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. *Neurochem Int.* (2009) 55:323–32. doi: 10.1016/j.neuint.2009.03.017
16. Feng X, Xing J, Feng G, Sang A, Shen B, Xu Y, et al. Age-dependent impaired neurogenic differentiation capacity of dental stem cell is associated with Wnt/beta-catenin signaling. *Cell Mol Neurobiol.* (2013) 33:1023–31. doi: 10.1007/s10571-013-9965-0
17. Alvarez R, Lee HL, Hong C, Wang CY. Single CD271 marker isolates mesenchymal stem cells from human dental pulp. *Int J Oral Sci.* (2015) 7:205–12. doi: 10.1038/ijos.2015.29
18. Lei T, Wang J, Liu Y, Chen P, Zhang Z, Zhang X, et al. Calreticulin as a special marker to distinguish dental pulp stem cells from gingival mesenchymal stem cells. *Int J Biol Macromol.* (2021) 178:229–39. doi: 10.1016/j.ijbiomac.2021.02.126
19. Omi M, Hata M, Nakamura N, Miyabe M, Ozawa S, Nukada H, et al. Transplantation of dental pulp stem cells improves long-term diabetic polyneuropathy together with improvement of nerve morphometrical evaluation. *Stem Cell Res Ther.* (2017) 8:279. doi: 10.1186/s13287-017-0729-5
20. Sugimura-Wakayama Y, Katagiri W, Osugi M, Kawai T, Ogata K, Sakaguchi K, et al. Peripheral nerve regeneration by secretomes of stem cells from human exfoliated deciduous teeth. *Stem Cells Dev.* (2015) 24:2687–99. doi: 10.1089/scd.2015.0104
21. Bhandi S, Alkahtani A, Reda R, Mashyaky M, Boreak N, Maganur PC, et al. Parathyroid hormone secretion and receptor expression determine the age-related degree of osteogenic differentiation in dental pulp stem cells. *J Pers Med.* (2021) 11:349. doi: 10.3390/jpm11050349
22. Sharma. Changes in neurotrophic factors of adult rat laryngeal muscles during nerve regeneration. *Neuroscience.* (2016) 333:44–53. doi: 10.1016/j.neuroscience.2016.07.004
23. Zheng K, Feng G, Zhang J, Xing J, Huang D, Lian M, et al. Basic fibroblast growth factor promotes human dental pulp stem cells cultured in 3D porous chitosan scaffolds to neural differentiation. *Int J Neurosci.* (2021) 131:625–33. doi: 10.1080/00207454.2020.1744592
24. Tsutsui TW. Dental pulp stem cells: advances to applications. *Stem Cells Cloning.* (2020) 13:33–42. doi: 10.2147/SCCAA.S166759
25. Li Z, Li K, Zhu L, Kan Q, Yan Y, Kumar P, et al. Inhibitory effect of IL-17 on neural stem cell proliferation and neural cell differentiation. *BMC Immunol.* (2013) 14:20. doi: 10.1186/1471-2172-14-20
26. Kumar A, Kumar V, Rattan V, Jha V, Bhattacharyya S. Secretome cues modulate the neurogenic potential of bone marrow and dental stem cells. *Mol Neurobiol.* (2017) 54:4672–82. doi: 10.1007/s12035-016-0011-3
27. Chen ZL, Yu WM, Strickland S. Peripheral regeneration. *Annu Rev Neurosci.* (2007) 30:209–33. doi: 10.1146/annurev.neuro.30.051606.094337
28. Heng BC, Gong T, Xu J, Lim LW, Zhang C. EphrinB2 signalling modulates the neural differentiation of human dental pulp stem cells. *Biomed Rep.* (2018) 9:161–8. doi: 10.3892/br.2018.1108
29. Urraca N, Hope K, Victor AK, Belgard TG, Memon R, Goorha S, et al. Significant transcriptional changes in 15q duplication but not Angelman syndrome deletion stem cell-derived neurons. *Mol Autism.* (2018) 9:6. doi: 10.1186/s13229-018-0191-y
30. Kolar MK, Itte VN, Kingham PJ, Novikov LN, Wiberg M, Kelk P. The neurotrophic effects of different human dental mesenchymal stem cells. *Sci Rep.* (2017) 7:12605. doi: 10.1038/s41598-017-12969-1
31. Yao R, Cooper GM. Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science.* (1995) 267:2003–6. doi: 10.1126/science.7701324
32. Traverse S, Gomez N, Paterson H, Marshall C, Cohen P. Sustained activation of the mitogen-activated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor. *Biochem J.* (1992) 288(Pt 2):351–5. doi: 10.1042/bj2880351
33. Sun ZG, Ma JT, Liu HW, Hu M, Huang HT. ERK/MAPK and PI3K/AKT signal channels simultaneously activated in nerve cell and axon after facial nerve injury. *Saudi J Biol Sci.* (2017) 24:1853–8. doi: 10.1016/j.sjbs.2017.11.027
34. Kichenbrand C, Velot E, Menu P, Moby V. Dental pulp stem cell-derived conditioned medium: an attractive alternative for regenerative therapy. *Tissue Eng Part B Rev.* (2019) 25:78–88. doi: 10.1089/ten.teb.2018.0168
35. Raza SS, Wagner AP, Hussain YS, Khan MA. Mechanisms underlying dental-derived stem cell-mediated neurorestoration in neurodegenerative disorders. *Stem Cell Res Ther.* (2018) 9:245. doi: 10.1186/s13287-018-1005-z
36. Hong JW, Lim JH, Chung CJ, Kang TJ, Kim TY, Kim YS, et al. Immune tolerance of human dental pulp-derived mesenchymal stem cells mediated by CD4(+)CD25(+)FoxP3(+) regulatory T-cells and induced by TGF-beta1 and IL-10. *Yonsei Med J.* (2017) 58:1031–9. doi: 10.3349/ymj.2017.58.5.1031
37. Yang C, Li X, Sun L, Guo W, Tian W. Potential of human dental stem cells in repairing the complete transection of rat spinal cord. *J Neural Eng.* (2017) 14:026005. doi: 10.1088/1741-2552/aa596b
38. Ahmadi P, Yan M, Bauche A, Smeets R, Müller CE, Koch-Nolte F, et al. Human dental pulp cells modulate CD8(+) T cell proliferation and efficiently degrade extracellular ATP to adenosine *in vitro*. *Cell Immunol.* (2022) 380:104589. doi: 10.1016/j.cellimm.2022.104589
39. Lopes B, Sousa P, Alvites R, Branquinho M, Sousa AC, Mendonça C, et al. Peripheral nerve injury treatments and advances: one health perspective. *Int J Mol Sci.* (2022) 23:918. doi: 10.3390/ijms23020918
40. Zhou HY, Jiang S, Ma FX, Lu H. Peripheral nerve tumors of the hand: clinical features diagnosis and treatment. *World J Clin Cases.* (2020) 8:5086–98. doi: 10.12998/wjcc.v8.i21.5086
41. Lu H, Chen L, Jiang S, Shen H. A rapidly progressive foot drop caused by the posttraumatic intraneural ganglion cyst of the deep peroneal nerve. *BMC Musculoskelet Disord.* (2018) 19:298. doi: 10.1186/s12891-018-2229-x
42. Pfister BJ, Gordon T, Loverde JR, Kocher AS, Mackinnon SE, Cullen DK. Biomedical engineering strategies for peripheral nerve repair: surgical applications state of the art and future challenges. *Crit Rev Biomed Eng.* (2011) 39:81–124. doi: 10.1615/CritRevBiomedEng.v39.i2.20
43. Luzuriaga J, Polo Y, Pastor-Alonso O, Pardo-Rodríguez B, Larrañaga A, Unda F, et al. Advances and perspectives in dental pulp stem cell based neuroregeneration therapies. *Int J Mol Sci.* (2021) 22:3546. doi: 10.3390/ijms22073546
44. Takaoka S, Uchida F, Ishikawa H, Toyomura J, Ohyama A, Watanabe M, et al. Transplanted neural lineage cells derived from dental pulp stem cells promote peripheral nerve regeneration. *Hum Cell.* (2022) 35:462–71. doi: 10.1007/s13577-021-00634-9
45. Soria JM, Sancho-Tello M, Esparza MA, Mirabet V, Bagan JV, Monleón M, et al. Biomaterials coated by dental pulp cells as substrate for neural stem cell differentiation. *J Biomed Mater Res A.* (2011) 97:85–92. doi: 10.1002/jbm.a.33032

46. Sanen K, Martens W, Georgiou M, Ameloot M, Lambrichts I, Phillips J. Engineered neural tissue with Schwann cell differentiated human dental pulp stem cells: potential for peripheral nerve repair? *J Tissue Eng Regen Med.* (2017) 11:3362–72. doi: 10.1002/term.2249
47. Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, et al. Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. *FASEB J.* (2014) 28:1634–43. doi: 10.1096/fj.13-243980
48. Reardon R, Simring D, Kim B, Mortensen J, Williams D, Leslie A. The diabetic foot ulcer. *Aust J Gen Pract.* (2020) 49:250–5. doi: 10.31128/AJGP-11-19-5161
49. Zhou H, Jin Q, Lu H. Exposure risk of patients with chronic infectious wounds during the COVID-19 outbreak and its countermeasures. *J Orthop Surg Res.* (2020) 15:452. doi: 10.1186/s13018-020-01976-0
50. Feldman EL, Nave KA, Jensen TS, Bennett DLH. New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. *Neuron.* (2017) 93:1296–313. doi: 10.1016/j.neuron.2017.02.005
51. Zhang. Mesenchymal stem cells to treat diabetic neuropathy: a long and strenuous way from bench to the clinic. *Cell Death Discov.* (2016) 2:16055. doi: 10.1038/cddiscovery.2016.55
52. Makino E, Nakamura N, Miyabe M, Ito M, Kanada S, Hata M, et al. Conditioned media from dental pulp stem cells improved diabetic polyneuropathy through anti-inflammatory neuroprotective and angiogenic actions: Cell-free regenerative medicine for diabetic polyneuropathy. *J Diabetes Investig.* (2019) 10:1199–208. doi: 10.1111/jdi.13045
53. Stafford. Regenerating optic pathways from the eye to the brain. *Science.* (2017) 356:1031–4. doi: 10.1126/science.aal5060
54. Berry M, Ahmed Z, Lorber B, Douglas M, Logan A. Regeneration of axons in the visual system. *Restor Neurol Neurosci.* (2008) 26:147–74. doi: 10.2174/1570159X14666161227150250
55. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci.* (2013) 54:7544–56. doi: 10.1167/iovs.13-13045
56. Bray AF, Cevallos RR, Gazarian K, Lamas M. Human dental pulp stem cells respond to cues from the rat retina and differentiate to express the retinal neuronal marker rhodopsin. *Neuroscience.* (2014) 280:142–55. doi: 10.1016/j.neuroscience.2014.09.023
57. Urraca N, Memon R, El-Iyachi I, Goorha S, Valdez C, Tran QT, et al. Characterization of neurons from immortalized dental pulp stem cells for the study of neurogenetic disorders. *Stem Cell Res.* (2015) 15:722–30. doi: 10.1016/j.scr.2015.11.004
58. Wilson R, Urraca N, Skobowiat C, Hope KA, Miravalle L, Chamberlin R, et al. Assessment of the tumorigenic potential of spontaneously immortalized and hTERT-immortalized cultured dental pulp stem cells. *Stem Cells Transl Med.* (2015) 4:905–12. doi: 10.5966/sctm.2014-0196
59. Luo L, He Y, Jin L, Zhang Y, Guastaldi FP, Albashari AA, et al. Application of bioactive hydrogels combined with dental pulp stem cells for the repair of large gap peripheral nerve injuries. *Bioact Mater.* (2021) 6:638–54. doi: 10.1016/j.bioactmat.2020.08.028
60. Zhu S, Ying Y, He Y, Zhong X, Ye J, Huang Z, et al. Hypoxia response element-directed expression of bFGF in dental pulp stem cells improve the hypoxic environment by targeting pericytes in SCI rats. *Bioact Mater.* (2021) 6:2452–66. doi: 10.1016/j.bioactmat.2021.01.024
61. Zordani A, Pisciotto A, Bertoni L, Bertani G, Vallarola A, Giuliani D, et al. Regenerative potential of human dental pulp stem cells in the treatment of stress urinary incontinence: *in vitro* and *in vivo* study. *Cell Prolif.* (2019) 52(6):e12675. doi: 10.1111/cpr.12675
62. Hata M, Omi M, Kobayashi Y, Nakamura N, Tosaki T, Miyabe M, et al. Transplantation of cultured dental pulp stem cells into the skeletal muscles ameliorated diabetic polyneuropathy: therapeutic plausibility of freshly isolated and cryopreserved dental pulp stem cells. *Stem Cell Res Ther.* (2015) 6:162. doi: 10.1186/s13287-015-0156-4
63. Kanafi M, Majumdar D, Bhonde R, Gupta P, Datta I. Midbrain cues dictate differentiation of human dental pulp stem cells towards functional dopaminergic neurons. *J Cell Physiol.* (2014) 229:1369–77. doi: 10.1002/jcp.24570
64. Simon C, Gan QF, Kathivaloo P, Mohamad NA, Dhamodharan J, Krishnan A, et al. Deciduous DPSCs ameliorate MPTP-mediated neurotoxicity sensorimotor coordination and olfactory function in parkinsonian mice. *Int J Mol Sci.* (2019) 20:30568 doi: 10.3390/ijms20030568



OPEN ACCESS

EDITED BY

Federica Ginanneschi,
University of Siena, Italy

REVIEWED BY

Luca Padua,
Agostino Gemelli University Polyclinic
(IRCCS), Italy
Hede Yan,
The Second Affiliated Hospital and
Yuying Children's Hospital of Wenzhou
Medical University, China

*CORRESPONDENCE

Hui Lu
✉ huilu@zju.edu.cn

[†]These authors have contributed
equally to this work

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and
Peripheral Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 08 September 2022

ACCEPTED 19 December 2022

PUBLISHED 11 January 2023

CITATION

Yang H, Dong Y, Wang Z, Lai J, Yao C,
Zhou H, Alhaskawi A, Hasan Abdullah
Ezzi S, Kota VG, Hasan Abdulla Hasan
Abdulla M and Lu H (2023) Traumatic
neuromas of peripheral nerves:
Diagnosis, management and future
perspectives.
Front. Neurol. 13:1039529.
doi: 10.3389/fneur.2022.1039529

COPYRIGHT

© 2023 Yang, Dong, Wang, Lai, Yao,
Zhou, Alhaskawi, Hasan Abdullah Ezzi,
Kota, Hasan Abdulla Hasan Abdulla
and Lu. This is an open-access article
distributed under the terms of the
[Creative Commons Attribution License
\(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s)
are credited and that the original
publication in this journal is cited, in
accordance with accepted academic
practice. No use, distribution or
reproduction is permitted which does
not comply with these terms.

Traumatic neuromas of peripheral nerves: Diagnosis, management and future perspectives

Hu Yang^{1†}, Yanzhao Dong^{1†}, Zewei Wang², Jingtian Lai²,
Chenjun Yao², Haiying Zhou¹, Ahmad Alhaskawi¹,
Sohaib Hasan Abdullah Ezzi², Vishnu Goutham Kota²,
Mohamed Hasan Abdulla Hasan Abdulla² and Hui Lu^{1,3*}

¹Department of Orthopedics, The First Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang, China, ²Zhejiang University School of Medicine, Hangzhou, Zhejiang, China, ³Alibaba-Zhejiang University Joint Research Center of Future Digital Healthcare, Zhejiang University, Hangzhou, Zhejiang, China

Traumatic neuromas are infrequent in clinical settings but are prevalent following trauma or surgery. A traumatic neuroma is not a true malignancy, rather, it is a hyperplastic, reparative nerve reaction after injury and typically manifests as a nodular mass. The most common clinical manifestations include painful hypersensitivity and the presence of a trigger point that causes neuralgic pain, which could seriously decrease the living standards of patients. While various studies are conducted aiming to improve current diagnosis and management strategies via the induction of emerging imaging tools and surgical or conservative treatment. However, researchers and clinicians have yet to reach a consensus regarding traumatic neuromas. In this review, we aim to start with the possible underlying mechanisms of traumatic neuromas, elaborate on the diagnosis, treatment, and prevention schemes, and discuss the current experiment models and advances in research for the future management of traumatic neuromas.

KEYWORDS

traumatic neuroma, peripheral nerve, hand surgery, clinical management, diagnosis

1. Introduction

The history of neuromas dates back to 1634 when Ambroise Pare first described the painful neuroma and treated neuroma with massage and oil. In 1811, Odier (1) discovered that the bulbous stump tissue in the proximal ending of a transected nerve could be extremely sensitive. It was not until 1828 that the term “neuroma” was coined by Wood (2), who conducted the first pathological research on such nerve injuries. From then on, the understanding of traumatic neuromas grew steadily, including the introduction of Morton's neuroma by Morton (3), the elucidation of the actual mechanism forming these neuromas by Huber and Lewis (4), and the classification system put forward by Swanson (5), who also defined neuromas as cutaneous and post-traumatic.

A neuroma is not a conventional neoplasm that arises from malignant cells, but a local non-neoplastic proliferation of the injured nerve. Neuromas mostly occur when normal nerve conduction is damaged by injury, inadequate surgical repair, or in some cases, chronic fibro-inflammatory irritation. In this case, the reinnervation process is deterred when nerve reconstruction is interrupted by the interpolation of scar tissue. Depending on the nature of the trauma, traumatic neuromas can be largely categorized into terminal neuroma and neuroma-in-continuity (6). Terminal neuromas are generally observed in limb amputation, lower extremities in particular, where the nerve is completely transected without a distal end, rendering it impossible to reestablish innervation by the injured nerve. On the other hand, neuroma-in-continuity results from a fusiform swelling of the injured site following the trauma and is further divided into two subtypes: (1) total or partial transection of the nerve; (2) repeated blunt trauma to the nerve. The former type of trauma could cause fusiform neuromas, which are commonly seen in the digital nerve and median nerve, while the latter form of trauma causes thickening of the fibrous tissue surrounding the nerve, which is involved in cases of Morton neuroma, Bowler's thumb (7), and lateral femoral cutaneous neuropathy (8). It can be concluded that given the mechanism of traumatic neuromas, it is highly related to traumatic limb injury and occurs mostly in orthopedic patients.

The most common main complaint in patients suffering from peripheral traumatic neuromas remains paresthesia in the innervated area and a painful nodule at the site of injury. Pain, as mentioned before, is among the most common clinical symptoms, including painful hypersensitivity and the presence of a trigger point that causes neuralgic pain. Patients may feel burning, stabbing, raw, gnawing, or sickening sensations. These symptoms could lead to psychological distress and a severe decrease in the quality of life (9). In this review, we aimed to provide a comprehensive understanding of traumatic peripheral neuroma and its related research progress.

2. Epidemiology

Traumatic neuromas, as infrequent as it is, are prevalent following trauma or surgery. As previously described, a traumatic neuroma is not a true malignancy. Instead, it is a hyperplastic, reparative nerve reaction after injury and typically manifests as a nodular mass. It is secondary to the abnormal growth of nerves and connective tissue attempting to reinnervate the region following an entire or partial nerve segment due to an accidental or surgical trauma (10, 11). As nerve lesions, traumatic neuromas are characterized as neuroma-in-continuity (NIC) following partial nerve transection or end-bulb neuromas (EBN) following the total disruption (12). Traumatic neuromas exhibit proximal continuity with the parent nerve, similar to the "tail sign"

shown in peripheral nerve sheath tumors (PNSTs), indicating a neurogenic origin. On the other hand, EBNs do not present distal continuity with the parent nerve, whereas NICs are continuous both proximally and distally (13).

3. Risk factors and pathophysiology

Many studies have found that inhibiting nerve growth factor (NGF) following nerve damage lowers neuroma growth and neuropathic pain in rat models (14). Furthermore, localized deactivation of the brain-derived nerve factor (BDNF) has been found to greatly reduce neuropathic pain and influence the regeneration of sensory fibers. In contrast, excessive concentrations of BDNF promote the growth of neuromas and neuropathic pain (15).

Traumatic damage to a peripheral nerve leads to multidimensional cell proliferation, regeneration failure, and deformed architecture of the nerve. Due to post-traumatic obstruction of axonal flow and subsequent Wallerian degeneration, the nerve segment distant to the site of damage has unique and complicated physiology (16).

Nevertheless, the ability of axons to regenerate and the growing support of Schwann cells (SCs) decrease with time and distance from a trauma (17). On condition that two severed nerve segments are distant respectively, or the proximal end is missing (amputations), axon regeneration occurs in an unstructured manner (18, 19). In some instances, the simultaneous proliferation of wound-healing cells and signaling molecules might result in collagen remodeling and scar formation and eventually forms a neuroma (9). Several studies and case reports have defined traumatic neuroma as having a tangled shape consisting of connective tissue, Schwann cells, and regenerated axons (20–23).

In painful neuromas, inflammatory signaling factors (22, 23) and myofibroblasts (24) have been reported. While the microscopic characteristics of a fully formed neuroma have been thoroughly recorded, little is known about the cellular structure of neuromas in their early stages of development, from nerve damage to initial neuroma formation. Further studies in this field is helpful to generate new and improved therapies that target the earliest stages of neuroma development and prevent the accompanying discomfort (25).

4. Diagnosis and clinical evaluation of traumatic neuromas

As a result of injuries or surgical procedures, traumatic neuromas generally present as a firm, oval, slow-growing, palpable nodule with a painful sensation, not larger than 2 cm in diameter. Common symptoms include pain, stiffness, pain hypersensitivity to light tactile stimuli, or neuralgic pain with

a trigger point (26). It is vitally important that clinicians pay close attention to the previous medical history of patients with the above symptoms for an accurate initial diagnosis.

Regarding histopathology, Seddon's initial classification of peripheral nerve injuries is based on a three-tiered severity scale: neurapraxia, axonotmesis, and neurotmesis (27). In terms of Electrodiagnosis, axonotmesis and neurotmesis have the same characteristics. The symptoms of axonotmesis are reversible, but those of neurotmesis are irreversible due to disordered axon regeneration. In such instances, complete realignment of the sectioned fascicles and optimum neural tube repair necessitates surgery (28). Following Sudden, the traumatic damage to peripheral nerves was further classified into five classes by Sunderland (29). However, these categories are determined using a presumed prognosis without objective data on the anatomical damage. From this perspective, imaging may be useful for distinguishing between reversible axonotmesis and irreversible neurotmesis.

As trauma majorly affects working-age individuals, delayed management also causes economic and social harm (30, 31). While the classification systems mentioned proved effective in clinical practice, more often than not clinicians are faced with the dilemma that the injured nerve may branch into more regional nerves, even along the length of a nerve the degree of injury may vary. The localization of traumatic neuromas primarily depends on physical examination and medical history, and the application of medical imaging tools. Ultrasound (US) and magnetic resonance imaging (MRI) can be utilized to examine the anatomy and topography of peripheral nerves in order to determine the location, extent, and type of damage (32–37). In examining a limb trauma, it is crucial to examine the probability of nerve structure involvement. A thorough clinical history, physical examination, and electrodiagnostic testing (electromyography—EMG and nerve conduction velocity studies—NCVs) are sufficient to diagnose a nerve injury; nevertheless, a complete qualitative and quantitative assessment of the structural damage is not possible. Determining the kind of anatomical damage and the injury's severity is crucial in deciding whether surgical therapy is necessary since time is crucial for a successful prognosis (38). In addition, a comprehensive morphological diagnosis of a traumatic lesion is crucial in determining optimal care (conservative or surgical treatment) (32, 39). Therefore, the clinical examination strongly recommends morphological imaging using US or MRI (Table 1). The US is a useful utility not only to access the continuity of nerve but also in post-operative follow-up and detection of complications (39). MRI T2-weighted imaging provides high-resolution imaging of peripheral nerve anatomy in combination with fat and flow suppression (34, 41). Many researchers recommend MRI for examining anatomical nerve damage, considering it appropriate for a high-quality assessment (42–45). On the other hand, other researchers believe the two procedures are complementary,

favoring the US as an initial examination and MRI for evaluating anatomical regions where US access is difficult or impossible or where US details seem insufficient or do not correspond with clinical suspicion and/or EMG results (33, 36).

Currently, few researchers have compared the diagnostic accuracy of the two techniques (36, 46), indicating that both US and MRI can detect the damaged region with a high level of anatomical details and pathological results that correspond with EMG testing. In research by Zaidman et al. (36), it was determined that the US had a greater sensitivity than MRI (93 vs. 67%, respectively), assuming the same level of specificity (86%). In contrast, Aggarwal et al. (46) observed that MRI is more sensitive than US (95 vs. 81%), attributing this to the deployment of a high-field (3 Tesla) MRI scanner; nevertheless, these scanners are not yet accessible for all clinicians. The advantages and limitations of US and MRI are listed in Table 2. The US offers a more affordable alternative, and while well-tolerated by patients, the US shows results in real time. Obtaining dynamic information is also achievable if the patient is requested to undertake certain actions while the physician performs the exam (Figure 1) (60). This dynamic examination provides essential objective data for an overall evaluation of nerve and surrounding tissue damage. On the other hand, high-resolution ultrasound (HRU) is now been identified as a useful tool in the diagnosis of lesions of traumatic neuromas (39, 61–63). HRU can achieve imaging of all main nerves running the limbs including the medial nerve, ulnar nerve, radial nerves, sciatic nerves, common peroneal nerves, and posterior tibial nerves, while still demonstrating the transections, lacerations, hematoma or neuroma formation clearly and accurately (35).

Early diagnosis of the location and type of a lesion is crucial for prognosis and treatment since there is a limited clear opportunity for efficient reinnervation and, if fails, the commencement of alternative treatment measures, such as nerve surgery. Nerve recovery begins immediately upon axonal transection when the denervated muscle fibers and skin regions begin producing neurotrophic signals that attract any adjacent surviving axons and induce sprouting of these axons into the denervated tissue (64). The rapid process of reinnervation is known as collateral reinnervation, where the surviving axons in the fascicle innervate the motor units and skin regions of their injured counterparts. In severe nerve damage, when over 75% of the axons in a fascicle is destroyed, the remaining axons in the bundle will be unable to reinnervate every motor unit and skin region, which calls for proximal ingrowth of new axons. The rate of proximal reinnervation is around 1 mm each day, which can be deterred if the length between the nerve lesion and target muscle is too great, as in the case of distal muscles of the lower leg and sciatic nerve injury, since over time there are irreversible alterations in the muscle and neural axon tubes that inhibit further abnormal growth and reinnervation (65). Moreover, injury to the connective tissue constructs of the nerves may result in perineural fibrosis and scarring that

TABLE 1 Nerve injury classification following Seddon and Sunderland theories.

Seddon	Sunderland	Description	MRI*	Ultrasound*
Neuropraxia	I	Conduction block	T2 hyperintensity	The decreased echogenicity of the nerve (hypoechoic)
Axonotmesis	II	Discontinuity of axon with Wallerian degeneration	T2 hyperintensity with increased size Hyperintensity in muscles due to denervation	Decreased echogenicity and increased caliber of the nerve
	III	Scarring of the endoneurium	Endoneurium can-not is delineated with the current MR technique T2 hyperintensity with increased size Hyperintensity in muscles due to denervation	Focal echogenicity decreases with the increased caliber with a change in echotexture of the affected muscles
	IV	A neuroma in continuity with the formation of scar which blocks nerve regeneration	T1 hypointense, T2 hyperintense focal enlargement with loss of fascicular pattern. Hyperintensity in muscles due to denervation	The hypoechoic fusiform lesion is in continuity with the nerve with loss of fascicular architecture and altered echogenicity of denervated muscles
Neurotmesis	V	Rupture of the nerve	End neuroma formation at the proximal end with denervation changes in muscle	Hypoechoic neuroma at the proximal end with local soft tissue edema and denervation changes in muscle
	Mackinnon and Dellon type VI	Mixed injury	Variable findings with nerve heterogeneity and muscle denervation changes	Hypoechoic enlarged with mixed findings of scarring, discontinuity, or neuroma formation

*The data was obtained from Agarwal et al. (40).

TABLE 2 Advantages and disadvantages of US and MRI in the traumatic damage to peripheral nerves.

Comparison items	Ultrasonography	Magnetic resonance imaging
Spatial resolution	Excellent	Good
Contrast resolution	Good	Excellent
canning planes	Multiple planes (the transducer can be oriented in multiple ways, referring to nerve anatomy)	Multiple planes (2D and 3D acquisitions)
Scan times	Fast	Long
Nerve assessment	Dynamic	Static
Extension of nerve tract examined	Wide, based on need	Limited by the dimensions of the FOV
Denervation changes in muscles	Chronic (atrophy, fatty infiltration)	Early and chronic (edema, atrophy, fatty infiltration)
Deep structures	Limitation	No limitations
Less accessible anatomical areas	Limitation	No limitations
Contraindications	No	Yes
Patient compliance	Good	Poor
Cost	Low	High
Operator dependence	High	Less

The data was obtained and modified after Visalli et al. (47).

can inhibit future axon development. The therapeutic frame for nerve surgery focuses on an effective evaluation of the extent of the injury, which is typically performed clinically and may be enhanced by electromyography (EMG) *via* localizing the exact site of the lesion and the distance between it and the influenced muscle and skin, and identifying morphologic changes of the damaged nerve that indicate transaction or intraneural scarring. By analyzing the regeneration potential, this data will influence surgical decision-making (66).

5. Treatment

The process of nerve reconstruction is accompanied by scar formation in the injured site, which eventually results in traumatic neuroma formation (67, 68). Treatment of traumatic neuromas is based on the removal of disturbed nervous tissue or neurolysis to improve the microenvironment surrounding axons and achieving partial or complete remission of painful symptoms (Table 3). Once the neuroma is formed, the priority

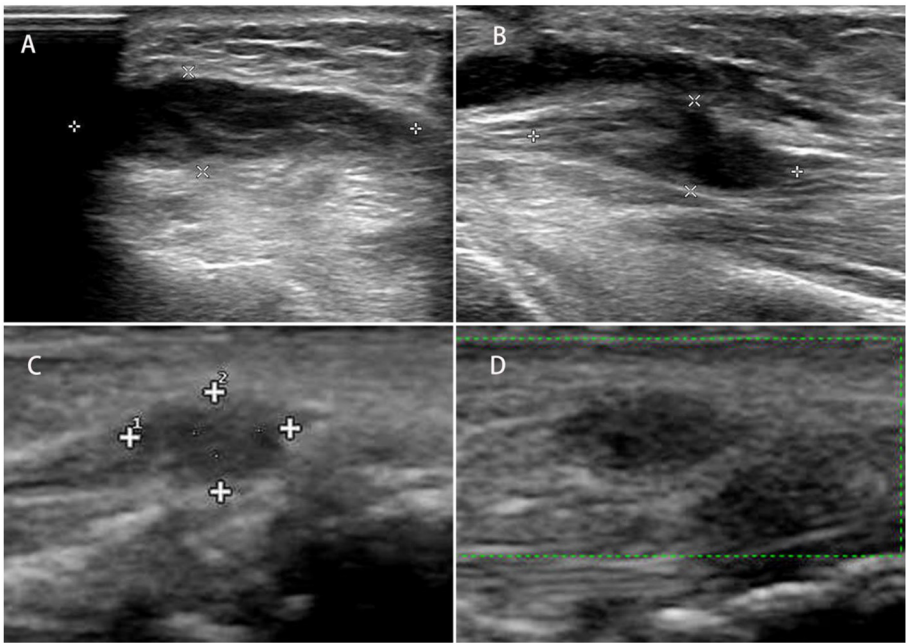


FIGURE 1
(A) Longitudinal and transverse ultrasound images of the left traumatic neuroma of the superficial branch of the radial nerve; (B) longitudinal and transverse images of the neuroma; (C, D) traumatic neuroma of the superficial branch of the radial nerve of the left wrist.

TABLE 3 Possible methods during or post-operation for prevention of traumatic neuromas formation.

Prevention categories	Prevention methods	Effectiveness
Surgical skills	Tight ligation, scissors cut, CO ₂ laser, and oblique nerve cutting (48–50)	Good (regular connection between fibers formation)
No nerve gap or free tension injury	Direct nerve repair with or without supporting conduits or gel (51, 52)	Good (depends on surgeon skills)
Nerve gap	Veins autologous, autologous nerve graft (53)	Various (depending on the size of the nerve gap)
Recurrent traumatic neuroma Amputation	Targeted nerve implantation, Terminated nerve stump (49, 54, 55)	Excellent (axons arborize into the intramuscular motor nerve branches)
Nerve support	Nerve conduits, fibrin glue, tacrolimus (FK506) (56, 57)	Various (depending on the severity of the injury)
Others	NGF inhibitor, stem cells, and 3D conduits (58, 59)	Under observation, promising result

for clinicians is to relieve symptoms and limit further disease development (54).

5.1. Surgical treatment

Surgical treatment is so far the most effective therapeutic method to manage peripheral traumatic neuromas (Figure 2). Various operative techniques have been invented for the management of peripheral traumatic neuroma occurring at different sites (69, 70), including nerve repair, distal nerve ending transposition to muscle, vein, or bone (71, 72), capping of distal nerve-ending with soft tissues or conduits (73–76). In the

surgical management of traumatic neuroma, one predominant factor for the selection of operative technique is the continuity of the severed nerve, that is whether there is an adjacent distal nerve stump for future nerve reconstruction and reinnervation. As the described condition is vitally important and greatly affects the selection of operation method, conventional surgical treatment in this section is categorized following this criterion before the proposition of several emerging surgical approaches (Figure 3).

For terminal peripheral neuroma where the distal nerve ending is not available, surgical neurectomy is considered where the target nerve is first identified *via* neurolysis. At this point, palpation of the target nerve should reproduce neuropathic

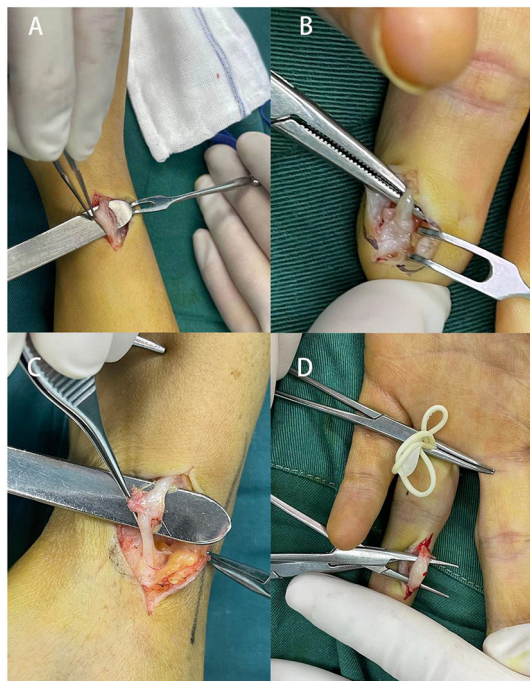


FIGURE 2
Cases of surgical management in four patients suffering from traumatic neuromas. (A) A 32-year-old female patient with a 2-year history of painful mass on the radial side of the right wrist; (B) a traumatic neuroma of the middle finger in a 34-year-old female patient as a result of previous finger tope amputation, (C) a 24-year-old patient with a traumatic neuroma on the wrist, (D) 33-year-old female presented a left index finger traumatic neuroma with local radiation pain for half a month, the patient had a history of left index finger injury by scissors several years ago.

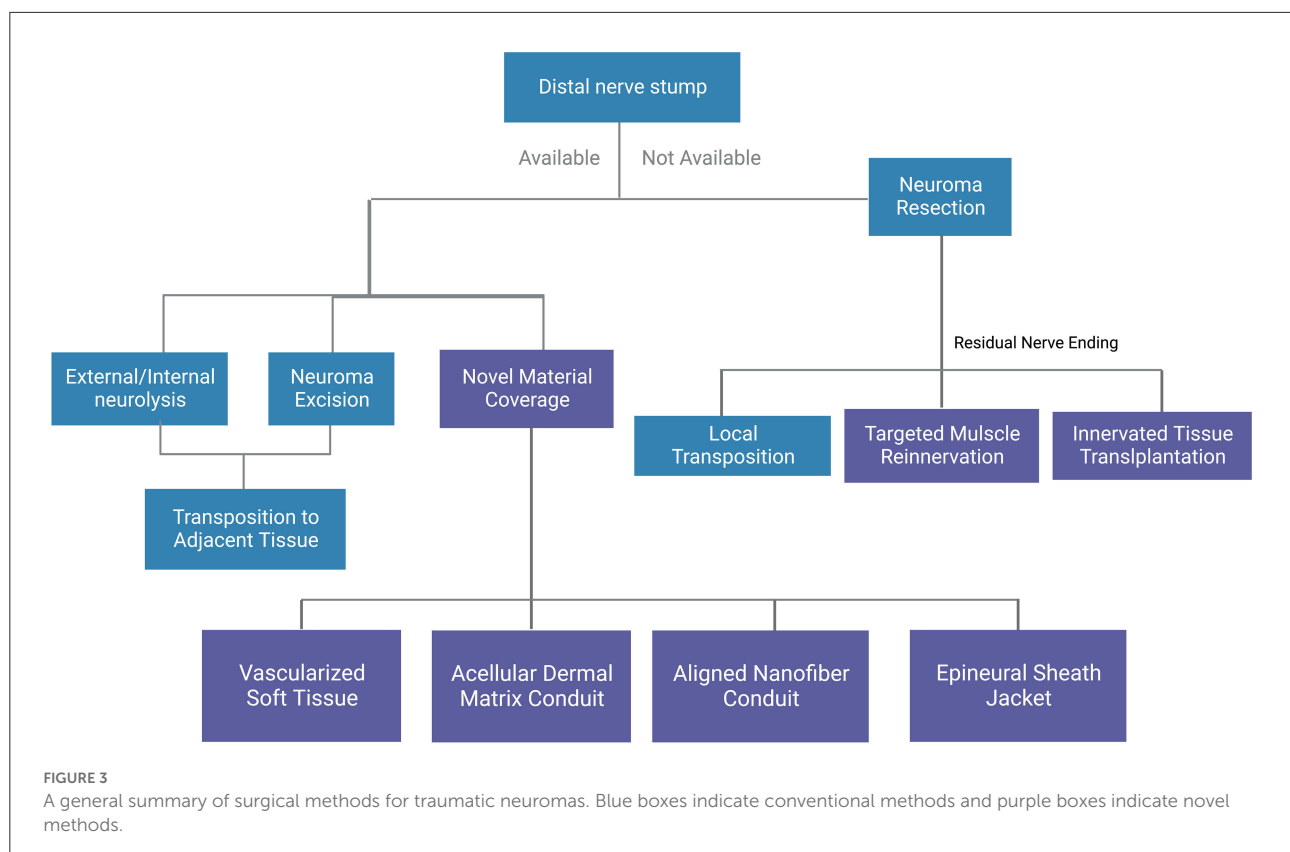
pain in the patient, and resection proximal to the neuroma is performed, followed by transposition of the proximal nerve ending to nearby muscles, bones, and veins, thus reducing connective tissue formation around the nerve post-surgery to obtain a better pain relief effect. This surgical method has been tested out by various researchers and clinicians. In 1984, Mass et al. (77) transposed the hand neuroma into the bone, and 18 of 20 neuromas showed acceptable results. Laborde et al. (72) combined excision of the neuroma with transposition of the proximal palmar cutaneous branch of the median nerve to the pronator quadratus muscle, which freed the nerve from wrist motion and environmental stimulation. Transposition between the superficial and deep flexor muscles is also applicable. Koch et al. (78) studied the resection of the neuroma and transposition of the nerve stump into an adjacent vein, where 22 of 23 patients reported positive long-term results. While the above surgical methods are mainly aimed at nerve trunks such as radial nerve and ulnar nerve, smaller, unknown sensory nerve injuries require a more specific mapping technique. Conventionally, this

is achieved by marking the painful area while the patients are awake and applying local anesthetic while incising progressively to identify discrete painful areas (79).

For traumatic peripheral neuroma-in-continuity where the distal ending is available, traditional treatments include external neurolysis, internal neurolysis, excision of the neuroma and transposition of the nerve, etc. External neurolysis or internal neurolysis is used to free the nerve from surrounding scar tissue while maintaining its integrity. Patients with an unsuccessful neurectomy as the primary treatment often suffer from a true neuroma. When the nerve is dispensable, the neuroma-in-continuity can be excised and transposed elsewhere.

Novel treatments targeting neuromas without distal ends mainly include transplantation of innervated tissue to cover the neuroma and targeted muscle reinnervation. In the former type, a muscle flap is always used, Reisman and Dellon (80) adopted a local transfer of the abductor *digiti minimi* muscle flap to treat palmar wrist pain. In the latter type, the cut, distal end of a peripheral nerve is transferred to a nearby muscle target or nerve target, for example, the deep peroneal nerve is transferred to the tibialis anterior muscle and the superficial peroneal nerve is transferred to the peroneus longus muscle (81), interdigital neurorrhaphy for treatment of digital neuromas. In addition to the techniques mentioned above, repeated intervention and higher amputations are rarely advised since it adds to the anxiety and pain of the patient (82). On the other hand, novel treatments for neuroma-in-continuity include the application of vascularized soft tissue or nerve conduits to cover the neuromas. A study by Krishnan et al. (83) showed that vascularized soft tissue coverage of painful peripheral nerve neuromas could be an effective method. Peterson and Adham (84) achieved satisfactory outcomes using an acellular dermal matrix conduit in the treatment of traumatic neuropathic pain at the wrist. Yan et al. (85) found that an aligned nanofiber conduit can significantly facilitate nerve regeneration, inhibit neuroma growth, and reduce traumatic neuropathic pain after neurectomy in a rat sciatic nerve model. Siemionow et al. (86) investigated the efficacy of the epineurial sheath jacket as a novel technique for neuroma prevention in the rat sciatic nerve model and gained outcomes comparable to the muscle burying technique. Synthetic conduits may be a promising field in treating traumatic neuroma in the future. In general, traditional techniques combined with novel techniques are more effective in treating traumatic peripheral neuroma. Recently, treatment of traumatic peripheral neuroma has shifted from hiding injured nerves to attempting nerve healing (81). Some literature reviews support this conceptual shift (87, 88). However, more research is required in the future to match suitable operative methods with specific clinical settings.

The numerous techniques that have been attempted suggest that no one technique is completely effective or superior to other techniques, instead, each technique fits the certain situation of nerve injuries. Poppler et al. (89) and Ives et al.



(90) compared various surgical treatments for neuroma of the extremities, including excision alone, excision and cap, excision and transposition, excision and repair, neurolysis and coverage, and found no difference in efficacy.

5.2. Conservative treatment

Treatment of traumatic peripheral neuroma usually starts with medication. Anti-neuropathic drugs include membrane-stabilizing agents (gabapentin, pregabalin, etc), anticonvulsants [carbamazepine (91), topiramate, phenytoin, lamotrigine, etc], antidepressants (amitriptyline, doxepin, etc), opioid analgesics (pethidine hydrochloride, etc) and muscle relaxants (baclofen, etc). Gabapentin and pregabalin (92) are considered the first-line effective medicine to inhibit central sensitization by affecting the calcium channels and reducing excessive neurotransmitter release. Although anti-neuropathic drugs were used as initial treatment, they often failed to obtain effective results while exposing the patients to side effects.

Other nonoperative management of traumatic peripheral neuroma includes physiotherapy, local injections (anesthetic, steroid, and alcohol), cryotherapy, radiofrequency ablation, shockwave therapy, and electrical stimulation. According to a systematic review from Samaila et al. (93), corticosteroid

injections appear to be a safe treatment allowing good results with a very low complication rate. Alcohol injections in a study by Gurdezi et al. (94) showed that although the short-term results are encouraging, alcohol injection does not offer a permanent resolution of symptoms for most patients and can be associated with considerable morbidity. Ultrasound-guided radiofrequency ablation is a novel treatment modality, where a probe is inserted at the site of the neuroma, and the temperature is raised at 85°C for 90 s in an attempt to sever adjacent nerve endings and shows short-term success rates of 80%–85% (95–97).

Friedman et al. (98) performed a retrospective case series review in patients who had undergone sonographically guided cryotherapy for Morton's neuromas, postsurgical and posttraumatic neuromas, and idiopathic neuralgia, where 15 of 20 patients had a positive response to cryotherapy. On the other hand, Friedman et al. (99) did a study of extracorporeal shockwave therapy for an interdigital neuroma in 13 patients and obtained positive therapeutic results.

Continuous high-frequency electrical stimulation can be used to control the hypersensitive state of the injured nerve according to the gate control theory proposed by Wall et al. (100). A study by Stevanato et al. (101) showed that all patients experienced pain relief within a few minutes of treatment (>75 and >95% in most), with long-lasting pain relief with

a reduction in mean Numerical Rating Scale of 76.2% after 6 months and of 71.5% after 12 months. No significant adverse events were observed.

6. Prevention

Traumatic neuroma patients generally suffer from low life quality caused by function abnormalities, chronic pain syndrome (traumatic neuropathic pain) which may continue for weeks or years, and psychological issues (26, 102, 103). As prevention is better than cure, the first defense line against traumatic neuroma is the prevention of such events. Reconnecting the two ends of the injured nerve after nerve transection is important to obtain a better outcome and decrease the incidence of traumatic neuroma (104).

Several techniques have emerged to prevent traumatic neuroma based on better surgical treatment of peripheral nerve injuries. The incidence of developing traumatic neuroma increases along with the application of electrocoagulation or cryo-neurolysis, compared with other methods such as tight ligation or scissors cut and CO₂ laser, indicating that applying the right neurectomy method helps to prevent traumatic neuroma (48, 49). Furthermore, some studies have investigated how the direction of nerve cutting can prevent traumatic neuroma formation, and they found that oblique nerve cutting displayed a better outcome compared with perpendicular or transverse cutting. This phenomenon occurred majorly because oblique nerve cutting leaves long and short nerve fibers, where a growth pathway will be formed between them (50). Amputated or untreated peripheral nerve injuries have a higher incidence of traumatic neuroma formation than neurorrhaphy (51). Direct nerve repair, on the other hand, can be divided into epineural repair and grouped fascicular repair and requires a tension-free environment and microscope magnification to obtain better recovery and prevention of traumatic neuromas (52).

NGF was experimentally proved to inhibit NGF decreasing neuroma formation, and minimizing neuropathic pain in traumatic neuroma formation after peripheral nerve injury (14), by applying an antibody against a specific receptor (trkA), or antibody against brain-derived neurotrophic factor (BDNF) which is usually upregulated after nerve injury by NGF (56). Fibrin glue proves its effectiveness in peripheral nerve repair by reducing the inflammatory response and improving axon regeneration. Also, Davis et al. showed that the continuous release of local tacrolimus (FK506) to repair site could actively inhibits neuroma formation (57). They used 3D-printing technology to create a polyethylene Y-shape conduit loop, where the nerve will be connected instead of direct connection to autografts. Other studies pointed out that the use of stem cells could improve nerve regeneration and possibly reduce neuroma

formation, which could be a potential advanced traumatic neuroma prevention method (58, 59).

7. Experimental neuroma model

Experimental research attaches great importance to the treatment and management of traumatic neuromas. Over the years, with an enormous amount of neuroma models proposed in different animals, there is no model that could be of reference, for each model has its own strengths and weaknesses (105).

Rats are the most common species studied in animal models of traumatic neuroma, for the anatomy of rat nerves is well-established and similar to human anatomy (58, 86, 106–109). Besides, the model has a large number of standardized functional tests, making the experimental results easy to evaluate (110, 111). However, one of the major drawbacks of the rat models is that peripheral nerve regeneration is much faster in rodents than in humans, and made worse by the fact that only relative short nerve gaps can be obtained in rodents, making it difficult to compare this *in vivo* model with humans and to further apply treatment into clinical trials. Compared to rats, limiting further use of these mice models.

Rabbits used to be common traumatic neuroma models (112–115). Compared with mice models, rabbits yield longer regeneration time, worse nerve regeneration, and longer gaps which resembles humans more (116). However, the major disadvantage of the rabbit neuroma models is that rabbit nerve anatomy and limb muscle function differ significantly from human beings (117). Although similar to human beings, Primates are rarely used in research because of ethical concerns (118).

According to the research, the sciatic nerve, saphenous nerve (48), sural nerve (119), and tibial nerve (120, 121) can be used for modeling. Among these nerves, the sciatic nerve has gradually become the most common site for the animal model of a traumatic neuroma given the fact that the sciatic nerve is easy to expose and observe, while others are rarely used now (58, 86, 106, 107). After exposure of the sciatic nerve and its trifurcation under the microscope, the nerve is sharply dissected 3–10 mm distal from the trifurcation (108, 113, 114). To prevent spontaneous regeneration of the distal nerve stump, at least 10 mm of the distal nerve stumps should be removed and discarded (106–108). Finally, the presence or absence of a traumatic neuroma was determined by gross observation, ultrasound, hematoxylin-eosin staining, and immunofluorescence (106, 122). After reaching the predetermined time of the experiment or the growth and formation of the neuroma, the animals were sacrificed, and the nerve stump on the severed side and the contralateral normal nerve were harvested for neuroma adhesion evaluation, weight ratios, western blot analysis, and histological analysis.

8. Advances in research

In order to cover and cope with the recent advances and findings of traumatic neuromas of peripheral nerves, research has been conducted to improve and decrease the scientific gaps in this issue. Agarwal et al. (40) assessed the role of using imaging tools (high-resolution ultrasonography and MR neurography) and precise anatomical localization in diagnosing peripheral mononeuropathy. With IRB consent, a hospital-based prospective analytical investigation was conducted on 180 peripheral nerves in 131 individuals with symptoms of peripheral mononeuropathy in a context with limited resources. Generally, the proton density fat-saturated MR sequence had the best diagnosis accuracy (93.89%), followed by high-resolution ultrasonography (80%). The proton density fat-saturated sequence showed the maximum sensitivity, whereas the T1 MR sequence had the highest specificity. It was determined that the cumulative diagnostic accuracy of both modalities was 93.33%, with an 80% negative predictive value. Ultrasound and MRI revealed nerve interruption cases, but MRI was more effective at identifying neuromas. With the development of devices with a higher frequency and enhanced MR field strength, imaging of peripheral nerves is more accurate. Nerve imaging permits anatomical delineation and identification of the precise location of involvement. This comparative analysis highlights the significance of imaging in detecting peripheral nerve diseases, with an accuracy of 93.89% for MRI, which might act as the imaging gold standard. High-resolution ultrasonography can serve as a viable screening method since it is faster, more cost-effective, and has an accuracy of 80%, which is equivalent to other diagnostic techniques. The authors concluded that these two imaging techniques are not mutually exclusive. Instead, they complement one another and can be utilized in tandem to diagnose peripheral neuropathies using imaging.

Around 2.8% of hospitalized trauma patients suffer from acute peripheral nerve damage (123). Consequent significant disorder and socioeconomic effects have prompted continued research efforts on this subject (124). If a tension-free direct approximation of the nerve stumps is achievable, the epineural nerve suture is the treatment option. However, if tension-free coaptation is impossible, the current gold standard is autologous nerve transplantation (ANT) (125). Nevertheless, given the restricted accessibility of donor nerves and the morbidity associated with donor sites, new procedures are required to assist in peripheral nerve surgery. Nowadays, it is generally accepted that the material utilized to assist peripheral nerve regeneration should ideally consist of a totally biodegradable matrix that does not negatively impact regeneration during biodegradation (126). Despite significant advances in tissue engineering, no substance or bio-mimicking idea has yet demonstrated improved peripheral nerve regeneration results compared to the ANT, the current gold standard for bridging

peripheral nerve deficits (125). In addition to the well-existing substances, chitosan is a relatively potential new substance in peripheral nerve regeneration. Due to its global availability, low cost, complete biodegradability, safe byproducts, and potentially compromising on the regeneration process (127). According to the literature, chitosan was shown to promote axonal regeneration [Kanazawa et al. (128), Stenberg et al. (129)], minimize severe scarring and enhance functional recovery (130), and inhibit further neuroma development peripheral nerve damage (131).

Relying on the processing of chitosan, the degree of acetylation (DOA) might vary, influencing both the molecular weights and solvent properties (132). In addition, the DOA has been found to be a factor that influences the survival, proliferation, and cellular activity of regeneration-supporting cells such as SC (133). However, the accurate adjustments of chitosan matrices remain problematic, as the mechanical rigidity, the biodegradation period, the spatial architecture, and the sterilization process all have the potential to influence the axonal regeneration process and must be considered across the manufacturing system (134). Furthermore, chitoooligosaccharide (COS), a byproduct of chitosan, has been discovered to stimulate cell proliferation and inhibit apoptosis in SC, the essential cell for adequate axonal regeneration (135, 136). Furthermore, Wang et al. (137) contributed stimulating effects of COS to an expedited cell cycle leading to enhanced SC proliferation. Additionally, COS boosts the CCL2 production by down-regulating the miR-327 of the SC, resulting in improved migration to the damaged area (138). He et al. (139) examined the anti-apoptotic impacts of carboxymethylated chitosan (CMC) on SC by lowering caspase-3, -9, and Bax activities and enhancing Bcl-2 activities in CMC-treated SC. To protect the SC from oxidative stress, COS led to a decline in malondialdehyde activity and an increase in superoxide dismutase (SOD) activity. Subsequent *in vivo* tests on a rabbit model of axotomy revealed that daily intravenous injections of COS for 6 weeks dramatically enhanced peripheral nerve regeneration. Interestingly, the amount of regenerated myelinated nerve fibers, the thickness of the myelin sheath, and the compound muscle action potential (CMAP) as a measure of electrophysiological recovery were considerably greater in COS-treated individuals.

Traumatic neuroma-caused traumatic neuropathic pain has long bothered doctors and patients, scientists widely debate the reasons for traumatic neuropathic pain, and the therapy is difficult. Uncertain is the clinical therapy of painful neuroma. Specialists have developed numerous therapeutic methods in this discipline. However, there is currently no accepted standard treatment (26). Treatment strategies have been explored in animals and people, but pharmacotherapies (antidepressants, antiepileptics) continue to be the core of the care of traumatic neuropathic pain. Nerve stump transpositions into a muscle, vein, or bone are regarded as effective, traditional surgical

treatments for persistent conditions, especially using soft tissue (83, 131, 140), and the usage of conduits (24, 131, 141, 142); which reflected an effective potential in traumatic neuropathic pain treatment. In recent decades, novel surgical procedures, including tube-guided nerve capping, electrical stimulation, and adipose autograft, have significantly increased the variety of treatments for traumatic neuropathic pain (143). Balcin et al. (144) hypothesized that nerve transplantation into a vein might limit the growth of painful neuromas. Contrasted the transposition of the nerve stump into a nearby vein or muscle as a surgical therapy for a painful neuroma. According to their pre-operative proportions in the muscle group 3 and 12 months after surgery, translocation into a vein resulted in decreased intensity and evaluating pain levels and enhanced sensory, as measured by the visual analog scale and the McGill pain score. This was connected with greater activity levels and enhanced function. In addition, the transposition of the nerve stump into an adjacent vein is favored over its relocation into a muscle. Myofibroblasts are strongly expressed in neuromas, and it is believed that they contribute to pain by compressing the collagen matrix around the sensitive non-myelinated fibers that proliferate to produce a neuromatous protuberance (83). Krishnan et al. (83) found that covering painful peripheral nerve neuromas with vascularized soft tissue might be an effective but difficult therapeutic strategy. For years, all types of nerve conduits have been used to repair nerve abnormalities (145), and have also been launched to treat painful neuromas (141).

9. Conclusion and future directions

Traumatic neuromas have long been a clinical challenge for doctors and researchers. While the standard classification system has been set based on Seddon's and Sunderland's theories, the imaging technology remains in dispute as opinions are divided in selecting the optimal tools among US, MRI, HRUS, EMG, and ect. The diagnosis of traumatic neuromas, with the utility of imaging tools, is based most generally on the previous medical history of nerve injury or operation and symptoms including pain hypersensitivity and the presence of trigger points that causes neuralgic pain. Concerning the management of traumatic neuromas, while surgical procedures are still the most effective treatment method, researchers are eager to develop more non-surgical methods including medication and

physical therapy for treatment and prevention of traumatic neuromas. Although clinicians have yet to reach a consensus on a standardized management strategy of traumatic neuromas, it is clear that proper surgical procedures are vital for the prevention of traumatic neuromas. The future perspectives for management of traumatic neuromas, therefore, is most likely the prevention strategy during and post-operation, including an improved surgical approach, or the application of implants with a sustained release of medication that guides proper nerve regeneration.

Author contributions

HL and HY designed the study. YD, ZW, and JL performed data collection. HZ, AA, and SH analyzed the results. VK, MH, CY, and YD drafted the manuscript. The authors have read and approved the final manuscript.

Funding

The study was funded by the Zhejiang Provincial Natural Science Foundation (Grant number LS21H060001) and Alibaba Youth Studio Project (Grant number ZJU-032). The funding bodies had no role in the design of the study; in collection, analysis, and interpretation of data; and in drafting the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Odier L. *Manual de Medecine Pratique*. Geneva: J.J. Pachoud (1811), p. 362.
2. Wood W. Observations on neuroma, with cases and histories of the disease. *Trans Med Chir Soc Edinb*. (1828) 3(Pt 2):367.
3. Morton TG. A peculiar and painful affection of the fourth metatarsophalangeal articulation. *Am J Med Sci*. (1876) 71:9. doi: 10.1097/00000441-187601000-00002
4. Huber GC, Lewis D. Amputation neuromas: their development and prevention. *Arch Surg*. (1920) 1:85–113. doi: 10.1001/archsurg.1920.01110010098007
5. Swanson HH. Traumatic neuromas. A review of the literature. *Oral Surg Oral Med Oral Pathol*. (1961) 14:317–26. doi: 10.1016/0030-4220(61)90297-3

6. Burchiel KJ, Ochoa JL. Surgical management of post-traumatic neuropathic pain. *Neurosurg Clin N Am.* (1991) 2:117–26. doi: 10.1016/S1042-3680(18)30761-7
7. Howell AE, Leach RE. Bowler's thumb. Perineural fibrosis of the digital nerve. *J Bone Joint Surg Am.* (1970) 52:379–81. doi: 10.2106/00004623-197052020-00024
8. Mukherjee K, Bassett IB. Meralgia paraesthetica. *Br Med J.* (1969) 2:55. doi: 10.1136/bmj.2.5648.55
9. Foltán R, Klíma K, Spacková J, Sedý J. Mechanism of traumatic neuroma development. *Med Hypotheses.* (2008) 71:572–6. doi: 10.1016/j.mehy.2008.05.010
10. Rainsbury JW, Whiteside OJH, Bottrill ID. Traumatic facial nerve neuroma following mastoid surgery: a case report and literature review. *J Laryngol Otol.* (2007) 121:601–5. doi: 10.1017/S0022215106004993
11. Li Q, Gao E-L, Yang Y-L, Hu H-Y, Hu X-Q. Traumatic neuroma in a patient with breast cancer after mastectomy: a case report and review of the literature. *World J Surg Oncol.* (2012) 10:35. doi: 10.1186/1477-7819-10-35
12. Chhabra A, Williams EH, Wang KC, Dellon AL, Carrino JA. MR neurography of neuromas related to nerve injury and entrapment with surgical correlation. *AJNR Am J Neuroradiol.* (2010) 31:1363–8. doi: 10.3174/ajnr.A2002
13. Ahlawat S, Belzberg AJ, Montgomery EA, Fayad LM. MRI features of peripheral traumatic neuromas. *Eur Radiol.* (2016) 26:1204–12. doi: 10.1007/s00330-015-3907-9
14. Kryger GS, Kryger Z, Zhang F, Shelton DL, Lineaweaver WC, Buncke HJ. Nerve growth factor inhibition prevents traumatic neuroma formation in the rat. *J Hand Surg Am.* (2001) 26:635–44. doi: 10.1053/jhsu.2001.26035
15. Marcol W, Kotulska K, Larysz-Brysz M, Kowalik JL. BDNF contributes to animal model neuropathic pain after peripheral nerve transection. *Neurosurg Rev.* (2007) 30:235–43. discussion 243. doi: 10.1007/s10143-007-0085-5
16. Carroll SL WS. Wallerian degeneration? In: *Reference Module in Neuroscience and Biobehavioral Psychology.* Netherlands: Elsevier. (2017), p. 1–8. doi: 10.1016/B978-0-12-809324-5.02077-0
17. Chan KM, Gordon T, Zochodne DW, Power HA. Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. *Exp Neurol.* (2014) 261:826–35. doi: 10.1016/j.expneurol.2014.09.006
18. Wood, M.D. and S.E. Mackinnon. Pathways regulating modality-specific axonal regeneration in peripheral nerve. *Exp Neurol.* (2015) 265:171–5. doi: 10.1016/j.expneurol.2015.02.001
19. Faroni A, Mobasser SA, Kingham PJ, Reid AJ. Peripheral nerve regeneration: experimental strategies and future perspectives. *Adv Drug Deliv Rev.* (2015) 82:83:160–7. doi: 10.1016/j.addr.2014.11.010
20. Hirose T, Tani T, Shimada T, Ishizawa K, Shimada S, Sano T. Immunohistochemical demonstration of EMA/Glut1-positive perineurial cells and CD34-positive fibroblastic cells in peripheral nerve sheath tumors. *Mod Pathol.* (2003) 16:293–8. doi: 10.1097/01.MP.0000062654.83617.B7
21. Vora AR, Bodell SM, Loescher AR, Smith KG, Robinson PP, Boissonade FM. Inflammatory cell accumulation in traumatic neuromas of the human lingual nerve. *Arch Oral Biol.* (2007) 52:74–82. doi: 10.1016/j.archoralbio.2006.08.015
22. Egami S, Tanese K, Honda H, Kasai H, Yokoyama T, Sugiura M. Traumatic neuroma on the digital tip: immunohistochemical analysis of inflammatory signaling pathways. *J Dermatol.* (2016) 43:836–7. doi: 10.1111/1346-8138.13297
23. Khan J, Noboru N, Young A, Thomas D. Pro and anti-inflammatory cytokine levels (TNF- α , IL-1 β , IL-6 and IL-10) in rat model of neuroma. *Pathophysiology.* (2017) 24:155–9. doi: 10.1016/j.pathophys.2017.04.001
24. Yan H, Zhang F, Kolkin J, Wang C, Xia Z, Fan C. Mechanisms of nerve capping technique in prevention of painful neuroma formation. *PLoS ONE.* (2014) 9:e93973. doi: 10.1371/journal.pone.0093973
25. Oliveira KMC, Pindur L, Han Z, Bhavsar MB, Barker JH, Leppik L. Time course of traumatic neuroma development. *PLoS ONE.* (2018) 13:e0200548. doi: 10.1371/journal.pone.0200548
26. Yao C, Zhou X, Zhao B, Sun C, Poonit K, Yan H. Treatments of traumatic neuropathic pain: a systematic review. *Oncotarget.* (2017) 8:57670–9. doi: 10.18632/oncotarget.16917
27. Seddon HJ. A classification of nerve injuries. *Br Med J.* (1942) 2:237–9. doi: 10.1136/bmj.2.4260.237
28. Griffin MF, Malahias M, Hindocha S, Khan WS. Peripheral nerve injury: principles for repair and regeneration. *Open Orthop J.* (2014) 8:199–203. doi: 10.2174/1874325001408010199
29. Sunderland S. A classification of peripheral nerve injuries producing loss of function. *Brain.* (1951) 74:491–516. doi: 10.1093/brain/74.4.491
30. Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, et al. Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. *J Peripher Nerv Syst.* (2010) 15:120–7. doi: 10.1111/j.1529-8027.2010.00260.x
31. Dahlin LB, Wiberg M. Nerve injuries of the upper extremity and hand. *EFORT Open Rev.* (2017) 2:158–70. doi: 10.1302/2058-5241.2.160071
32. Koenig RW, Pedro MT, Heinen CPG, Schmidt T, Richter H-P, Antoniadis G, et al. High-resolution ultrasonography in evaluating peripheral nerve entrapment and trauma. *Neurosurg Focus.* (2009) 26:E13. doi: 10.3171/FOC.2009.26.2.E13
33. Kermarrec E, Demondion X, Khalil C, Thuc VL, Boutry N, Cotten A. Ultrasound and magnetic resonance imaging of the peripheral nerves: current techniques, promising directions, and open issues. *Semin Musculoskelet Radiol.* (2010) 14:463–72. doi: 10.1055/s-0030-1268067
34. Lee FC, Singh H, Nazarian LN, Ratliff JK. High-resolution ultrasonography in the diagnosis and intraoperative management of peripheral nerve lesions. *J Neurosurg.* (2011) 114:206–11. doi: 10.3171/2010.2.JNS091324
35. Zeidenberg J, Burks SS, Jose J, Subhawong TK, Levi AD. The utility of ultrasound in the assessment of traumatic peripheral nerve lesions: report of 4 cases. *Neurosurg Focus.* (2015) 39:E3. doi: 10.3171/2015.6.FOCUS15214
36. Zaidman CM, Seelig MJ, Baker JC, Mackinnon SE, Pestronk A. Detection of peripheral nerve pathology: comparison of ultrasound and MRI. *Neurology.* (2013) 80:1634–40. doi: 10.1212/WNL.0b013e3182904f3f
37. Ng ES, Vijayan J, Therimadasamy AK, Tan TC, Chan YC, Lim A, et al. High resolution ultrasonography in the diagnosis of ulnar nerve lesions with particular reference to post-traumatic lesions and sites outside the elbow. *Clin Neurophysiol.* (2011) 122:188–93. doi: 10.1016/j.clinph.2010.04.035
38. Karabay N, Toros T, Ademoglu Y, Ada S. Ultrasonographic evaluation of the iatrogenic peripheral nerve injuries in upper extremity. *Eur J Radiol.* (2010) 73:234–40. doi: 10.1016/j.ejrad.2008.10.038
39. Padua L, Di Pasquale A, Liotta G, Granata G, Pazzaglia C, Erra C, et al. Ultrasound as a useful tool in the diagnosis and management of traumatic nerve lesions. *Clin Neurophysiol.* (2013) 124:1237–43. doi: 10.1016/j.clinph.2012.10.024
40. Agarwal A, Chandra A, Jaipal U, Bagarhatta M, Mendiratta K, Goyal A, et al. Can imaging be the new yardstick for diagnosing peripheral neuropathy?—a comparison between high resolution ultrasound and MR neurography with an approach to diagnosis. *Insights Imaging.* (2019) 10:104. doi: 10.1186/s13244-019-0787-6
41. Chhabra A, Carrino J. Current MR neurography techniques and whole-body MR neurography. *Semin Musculoskelet Radiol.* (2015) 19:79–85. doi: 10.1055/s-0035-1545074
42. Koltzenburg M, Bendszus M. Imaging of peripheral nerve lesions. *Curr Opin Neurol.* (2004) 17:621–6. doi: 10.1097/00019052-200410000-00013
43. Gallagher TA, Simon NG, Klot M. Diffusion tensor imaging to visualize axons in the setting of nerve injury and recovery. *Neurosurg Focus.* (2015) 39:E10. doi: 10.3171/2015.6.FOCUS15211
44. Chhabra A, Ahlawat S, Belzberg A, Andreiseik G. Peripheral nerve injury grading simplified on MR neurography: as referenced to Seddon and Sunderland classifications. *Indian J Radiol Imaging.* (2014) 24:217–24. doi: 10.4103/0971-3026.137025
45. Hassanien OA, Younes RL, Dawoud RM, Younis LM, Hamoda IM. Reliable MRI and MRN signs of nerve and muscle injury following trauma to the shoulder with EMG and Clinical correlation. *Egypt J Radiol Nucl Med.* (2016) 47:929–36. doi: 10.1016/j.ejrm.2016.03.015
46. Aggarwal A, Srivastava DN, Jana M, Sharma R, Gamanagatti S, Kumar A, et al. Comparison of different sequences of magnetic resonance imaging and ultrasonography with nerve conduction studies in peripheral neuropathies. *World Neurosurg.* (2017) 108:185–200. doi: 10.1016/j.wneu.2017.08.054
47. Visalli C, Cavallaro M, Concerto A, Torre DL, Di Salvo R, Mazzioti S, et al. Ultrasonography of traumatic injuries to limb peripheral nerves: technical aspects and spectrum of features. *Jpn J Radiol.* (2018) 36:592–602. doi: 10.1007/s11604-018-0765-9
48. Zeltser R, Beilin B-Z, Zaslansky R, Seltzer Z. Comparison of autotomy behavior induced in rats by various clinically-used neurectomy methods. *Pain.* (2000) 89:19–24. doi: 10.1016/S0304-3959(00)00342-0
49. Lewin-Kowalik J, Marcol W, Kotulska K, Mander M, Klimczak A. Prevention and management of painful neuroma. *Neuro Med Chir.* (2006) 46:62–7. discussion 67–8. doi: 10.2176/nmc.46.62
50. Marcol W, Kotulska K, Larysz-Brysz M, Bierzynska-Macyszyn G, Wlasczuk P, Lewin-Kowalik J. Prevention of painful neuromas by oblique transection of peripheral nerves. *J Neurosurg.* (2006) 104:285–9. doi: 10.3171/jns.2006.104.2.285
51. van der Avoort DJJC, Hovius SER, Selles RW, van Neck JW, Coert JH. The incidence of symptomatic neuroma in amputation and neurotaphy patients. *J Plast Reconstr Aesthet Surg.* (2013) 66:1330–4. doi: 10.1016/j.bjps.2013.06.019

52. Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomed Res Int.* (2014) 2014:698256. doi: 10.1155/2014/698256
53. Colen KL, Choi M, Chiu DTW. Nerve grafts and conduits. *Plast Reconstr Surg.* (2009) 124(6 Suppl):e386–94. doi: 10.1097/PRS.0b013e3181bf8430
54. Vernadakis AJ, Koch H, Mackinnon SE. Management of neuromas. *Clin Plast Surg.* (2003) 30:247–68, vii. doi: 10.1016/S0094-1298(02)00104-9
55. Pet MA, Ko JH, Friedly JL, Mourad PD, Smith DG. Does targeted nerve implantation reduce neuroma pain in amputees? *Clin Orthop Relat Res.* (2014) 472:2991–3001. doi: 10.1007/s11999-014-3602-1
56. Apfel SC, Wright DE, Wiideman AM, Dormia C, Snider WD, Kessler JA. Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system. *Mol Cell Neurosci.* (1996) 7:134–42. doi: 10.1006/mcne.1996.0010
57. Davis B, Hilgart D, Erickson S, Labroo P, Burton J, Sant H, et al. Local FK506 delivery at the direct nerve repair site improves nerve regeneration. *Muscle Nerve.* (2019) 60:613–20. doi: 10.1002/mus.26656
58. Bolleboom A, de Ruiter GCW, Coert JH, Tuk B, Holstege JC, van Neck JW. Novel experimental surgical strategy to prevent traumatic neuroma formation by combining a 3D-printed Y-tube with an autograft. *J Neurosurg.* (2018) 130:184–96. doi: 10.3171/2017.8.JNS17276
59. Sullivan R, Dailey T, Duncan K, Abel N, Borlongan CV. Peripheral nerve injury: stem cell therapy and peripheral nerve transfer. *Int J Mol Sci.* (2016) 17:2101. doi: 10.3390/ijms17122101
60. Löfstedt T, Ahnlund O, Peolsson M, Trygg J. Dynamic ultrasound imaging—a multivariate approach for the analysis and comparison of time-dependent musculoskeletal movements. *BMC Med Imaging.* (2012) 12:29. doi: 10.1186/1471-2342-12-29
61. Lee TH, Hsieh ST, Chiang HY. Fibronectin inhibitor pUR4 attenuates tumor necrosis factor alpha-induced endothelial hyperpermeability by modulating beta1 integrin activation. *J Biomed Sci.* (2019) 26:37. doi: 10.1186/s12929-019-0529-6
62. Alaqeel A, Alshomer F. High resolution ultrasound in the evaluation and management of traumatic peripheral nerve injuries: review of the literature. *Oman Med J.* (2014) 29:314–9. doi: 10.5001/omj.2014.86
63. Beekman R, Visser LH. High-resolution sonography of the peripheral nervous system – a review of the literature. *Eur J Neurol.* (2004) 11:305–14. doi: 10.1111/j.1468-1331.2004.00773.x
64. Wijntjes J, Borchert A, van Alfen N. Nerve ultrasound in traumatic and iatrogenic peripheral nerve injury. *Diagnostics.* (2020) 11:30. doi: 10.3390/diagnostics11010030
65. Sulaiman W, Gordon T. Neurobiology of peripheral nerve injury, regeneration, and functional recovery: from bench top research to bedside application. *Ochsner J.* (2013) 13:100–8. doi: 10.1186/1749-7221-5-6
66. Antoniadis G, Kretschmer T, Pedro MT, König RW, Heinen CPG, Richter H-P. Iatrogenic nerve injuries: prevalence, diagnosis and treatment. *Dtsch Arztebl Int.* (2014) 111:273–9. doi: 10.3238/arztebl.2014.0273
67. Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Alteration of neuroma formation by manipulation of its microenvironment. *Plast Reconstr Surg.* (1985) 76:345–53. doi: 10.1097/00006534-198509000-00002
68. Dellon AL, Mackinnon SE, Pestronk A. Implantation of sensory nerve into muscle: preliminary clinical and experimental observations on neuroma formation. *Ann Plast Surg.* (1984) 12:30–40. doi: 10.1097/00006537-198401000-00006
69. Burchiel KJ, Johans TJ, Ochoa J. The surgical treatment of painful traumatic neuromas. *J Neurosurg.* (1993) 78:714–9. doi: 10.3171/jns.1993.78.5.0714
70. Wu J, Chiu DT. Painful neuromas: a review of treatment modalities. *Ann Plast Surg.* (1999) 43:661–7. doi: 10.1097/00006537-199912000-00016
71. Herndon JH, Eaton RG, Littler JW. Management of painful neuromas in the hand. *J Bone Joint Surg Am.* (1976) 58:369–73. doi: 10.2106/00004623-197658030-00013
72. Laborde KJ, Kalisman M, Tsai TM. Results of surgical treatment of painful neuromas of the hand. *J Hand Surg Am.* (1982) 7:190–3. doi: 10.1016/S0363-5023(82)80086-5
73. Robbins TH. Nerve capping in the treatment of troublesome terminal neuromata. *Br J Plast Surg.* (1986) 39:239–40. doi: 10.1016/0007-1226(86)90089-5
74. Battista AF, Cravioto HM, Budzilovich GN. Painful neuroma: changes produced in peripheral nerve after fascicle ligation. *Neurosurgery.* (1981) 9:589–600. doi: 10.1097/00006123-198111000-00019
75. Swanson AB, Boeve NR, Lumsden RM. The prevention and treatment of amputation neuromata by silicone capping. *J Hand Surg Am.* (1977) 2:70–8. doi: 10.1016/S0363-5023(77)80013-0
76. Yüksel F, Kışlaoglu E, Durak N, Uçar C, Karacaoglu E. Prevention of painful neuromas by epineural ligatures, flaps and grafts. *Br J Plast Surg.* (1997) 50:182–5. doi: 10.1016/S0007-1226(97)91367-9
77. Mass DP, Ciano MC, Tortosa R, Newmeyer WL, Kilgore ES Jr. Treatment of painful hand neuromas by their transfer into bone. *Plast Reconstr Surg.* (1984) 74:182–5. doi: 10.1097/00006534-198408000-00002
78. Koch H, Haas F, Hubmer M, Rappl T, Scharnagl E. Treatment of painful neuroma by resection and nerve stump transplantation into a vein. *Ann Plast Surg.* (2003) 51:45–50. doi: 10.1097/01.SAP.0000054187.72439.57
79. Youmans & Winn *Neurological Surgery*, 8th ed. Philadelphia, PA: Elsevier(2022).
80. Reisman NR, Dellon AL. The abductor digiti minimi muscle flap: a salvage technique for palmar wrist pain. *Plast Reconstr Surg.* (1983) 72:859–65. doi: 10.1097/00006534-198312000-00025
81. Lanier ST, Jordan SW, Ko JH, Dumanian GA. Targeted muscle reinnervation as a solution for nerve pain. *Plast Reconstr Surg.* (2020) 146:651e–63e. doi: 10.1097/PRS.0000000000007235
82. Grant GH. Methods of treatment of neuromata of the hand. *J Bone Joint Surg Am.* (1951) 33-A:841–8. doi: 10.2106/00004623-195133040-00003
83. Krishnan KG, Pinzer T, Schackert G. Coverage of painful peripheral nerve neuromas with vascularized soft tissue: method and results. *Neurosurgery.* (2005) 56(2 Suppl):369–78. discussion 369–78. doi: 10.1227/01.NEU.0000156881.10388.D8
84. Peterson SL, Adham MN. Acellular dermal matrix as an adjunct in treatment of neuropathic pain at the wrist. *J Trauma.* (2006) 61:392–5. doi: 10.1097/01.ta.0000224139.74143.f9
85. Yan H, Zhang F, Wang C, Xia Z, Mo X, Fan C. The role of an aligned nanofiber conduit in the management of painful neuromas in rat sciatic nerves. *Ann Plast Surg.* (2015) 74:454–61. doi: 10.1097/SAP.0000000000000266
86. Siemionow M, Bobkiewicz A, Cwykiel J, Uygur S, Francuzik W. Epineural sheath jacket as a new surgical technique for neuroma prevention in the rat sciatic nerve model. *Ann Plast Surg.* (2017) 79:377–84. doi: 10.1097/SAP.0000000000001117
87. Wolvetang NHA, Lans J, Verhulst SHWL, Notermans BJW, Chen NC, Eberlin KR. Surgery for symptomatic neuroma: anatomic distribution and predictors of secondary surgery. *Plast Reconstr Surg.* (2019) 143:1762–71. doi: 10.1097/PRS.0000000000005664
88. Guse DM, Moran SL. Outcomes of the surgical treatment of peripheral neuromas of the hand and forearm: a 25-year comparative outcome study. *Ann Plast Surg.* (2013) 71:654–8. doi: 10.1097/SAP.0b013e3182583cf9
89. Poppler LH, Parikh RP, Bichanich MJ, Rebehn K, Bettlach CR, Mackinnon SE, et al. Surgical interventions for the treatment of painful neuroma: a comparative meta-analysis. *Pain.* (2018) 159:214–23. doi: 10.1097/j.pain.0000000000001101
90. Ives GC, Kung TA, Nghiem BT, Ursu DC, Brown DL, Cederna PS, et al. Current state of the surgical treatment of terminal neuromas. *Neurosurgery.* (2018) 83:354–64. doi: 10.1093/neuros/nyx500
91. Rizzo MA. Successful treatment of painful traumatic mononeuropathy with carbamazepine: insights into a possible molecular pain mechanism. *J Neurol Sci.* (1997) 152:103–6. doi: 10.1016/S0022-510X(97)00143-3
92. Alles SRA, Cain SM, Snutch TP. Pregabalin as a pain therapeutic: beyond calcium channels. *Front Cell Neurosci.* (2020) 14:83. doi: 10.3389/fncel.2020.00083
93. Samaila E, Colò G, Rava A, Negri S, Valentini R, Felli L, et al. Effectiveness of corticosteroid injections in Civinini-Morton's syndrome: a systematic review. *Foot Ankle Surg.* (2021) 27:357–65. doi: 10.1016/j.fas.2020.05.001
94. Gurdezi S, White T, Ramesh P. Alcohol injection for Morton's neuroma: a five-year follow-up. *Foot Ankle Int.* (2013) 34:1064–7. doi: 10.1177/1071100713489555
95. Cione JA, Cozzarelli J, Mullin CJ. A retrospective study of radiofrequency thermal lesioning for the treatment of neuritis of the medial calcaneal nerve and its terminal branches in chronic heel pain. *J Foot Ankle Surg.* (2009) 48:142–7. doi: 10.1053/j.fas.2008.11.007
96. Moore JL, Rosen R, Cohen J, Rosen B. Radiofrequency thermoneurolysis for the treatment of Morton's neuroma. *J Foot Ankle Surg.* (2012) 51:20–2. doi: 10.1053/j.fas.2011.10.007
97. Chuter GSJ, Chua YP, Connell DA, Blackney MC. Ultrasound-guided radiofrequency ablation in the management of interdigital (Morton's) neuroma. *Skeletal Radiol.* (2013) 42:107–11. doi: 10.1007/s00256-012-1527-x

98. Friedman T, Richman D, Adler R. Sonographically guided cryoneurolysis: preliminary experience and clinical outcomes. *J Ultrasound Med.* (2012) 31:2025–34. doi: 10.7863/jum.2012.31.12.2025
99. Friedman R, Cain JD, Weill L Jr. Extracorporeal shockwave therapy for interdigital neuroma: a randomized, placebo-controlled, double-blind trial. *J Am Podiatr Med Assoc.* (2009) 99:191–3. doi: 10.7547/0980191
100. Wall PD, Melzack R, Bonica JJ. *Textbook of Pain.* Philadelphia, PA: Elsevier (1999).
101. Stevanato G, Devigili G, Eleopra R, Fontana P, Lettieri C, Baracco C, et al. Chronic post-traumatic neuropathic pain of brachial plexus and upper limb: a new technique of peripheral nerve stimulation. *Neurosurg Rev.* (2014) 37:473–79. discussion 479–80. doi: 10.1007/s10143-014-0523-0
102. Hanna SA, Catapano J, Borschel GH. Painful pediatric traumatic neuroma: surgical management and clinical outcomes. *Childs Nerv Syst.* (2016) 32:1191–4. doi: 10.1007/s00381-016-3109-z
103. Humberto C, Arthur B. Clinical and ultrastructural study of painful neuroma. *Neurosurgery.* (1981) 2:181–90.
104. Wang ML, Rivlin M, Graham JG, Beredjikian PK. Peripheral nerve injury, scarring, and recovery. *Connect Tissue Res.* (2019) 60:3–9. doi: 10.1080/0308207.2018.1489381
105. Toia F, Giesen T, Giovanoli P, Calcagni M. A systematic review of animal models for experimental neuroma. *J Plast Reconstr Aesthet Surg.* (2015) 68:1447–63. doi: 10.1016/j.bjps.2015.05.013
106. He F-L, Qiu S, Zou J-L, Gu F-B, Yao Z, Tu Z-H, et al. Covering the proximal nerve stump with chondroitin sulfate proteoglycans prevents traumatic painful neuroma formation by blocking axon regeneration after neurotomy in Sprague Dawley rats. *J Neurosurg.* (2020) 134:1599–609. doi: 10.3171/2020.3.JNS193202
107. Weng W, Zhao B, Lin D, Gao W, Li Z, Yan H. Significance of alpha smooth muscle actin expression in traumatic painful neuromas: a pilot study in rats. *Sci Rep.* (2016) 6:23828. doi: 10.1038/srep23828
108. Yao C, Zhou X, Weng W, Poonit K, Sun C, Yan H. Aligned nanofiber nerve conduits inhibit alpha smooth muscle actin expression and collagen proliferation by suppressing TGF- β 1/SMAD signaling in traumatic neuromas. *Exp Ther Med.* (2021) 22:1414. doi: 10.3892/etm.2021.10850
109. Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, et al. Chapter 4 Methods and protocols in peripheral nerve regeneration experimental research: part I—Experimental models. *Int Rev Neurobiol.* (2009) 87:47–79. doi: 10.1016/S0074-7742(09)87004-9
110. Bozkurt A, Tholl S, Wehner S, Tank J, Cortese M, O'Dey Dm, et al. Evaluation of functional nerve recovery with visual-SSI—a novel computerized approach for the assessment of the static sciatic index (SSI). *J Neurosci Methods.* (2008) 170:117–22. doi: 10.1016/j.jneumeth.2008.01.006
111. Nichols CM, Myckatyn TM, Rickman SR, Fox IK, Hadlock T, Mackinnon SE. Choosing the correct functional assay: a comprehensive assessment of functional tests in the rat. *Behav Brain Res.* (2005) 163:143–58. doi: 10.1016/j.bbr.2005.05.003
112. Song C, Zhang F, Zhang J, Mustain WC, Chen MB, Chen T, et al. Neuroma-in-continuity model in rabbits. *Ann Plast Surg.* (2006) 57:317–22. doi: 10.1097/01.sap.0000221512.06129.d3
113. Kim PS, Ko J, O'Shaughnessy KK, Kuiken TA, Dumanian GA. Novel model for end-neuroma formation in the amputated rabbit forelimb. *J Brachial Plex Peripher Nerve Inj.* (2010) 5:6.
114. Kim PS, Ko JH, O'Shaughnessy KK, Kuiken TA, Pohlmeier EA, Dumanian GA. The effects of targeted muscle reinnervation on neuromas in a rabbit rectus abdominis flap model. *J Hand Surg Am.* (2012) 37:1609–16. doi: 10.1016/j.jhnsa.2012.04.044
115. Ko JH, Kim PS, O'Shaughnessy KD, Ding X, Kuiken TA, Dumanian GA, et al. Quantitative evaluation of gross versus histologic neuroma formation in a rabbit forelimb amputation model: potential implications for the operative treatment and study of neuromas. *J Brachial Plex Peripher Nerve Inj.* (2011) 6:e23–32.
116. Kerns JM, Sladek EH, Malushte TS, Bach H, Elhassan B, Kitidumrongsook P, et al. End-to-side nerve grafting of the tibial nerve to bridge a neuroma-in-continuity. *Microsurgery.* (2005) 25:155–64. discussion 164–6. doi: 10.1002/micr.20096
117. Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ, et al. Systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials.* (2012) 33:8034–9. doi: 10.1016/j.biomaterials.2012.07.056
118. Arnason G. The emergence and development of animal research ethics: a review with a focus on nonhuman primates. *Sci Eng Ethics.* (2020) 26:2277–93. doi: 10.1007/s11948-020-00219-z
119. Chim H, Miller E, Gliniak C, Cohen ML, Guyuron B. The role of different methods of nerve ablation in prevention of neuroma. *Plast Reconstr Surg.* (2013) 131:1004–12. doi: 10.1097/PRS.0b013e3182879ec2
120. Adelson PD, Bonaroti EA, Thompson TP, Tran M, Nystrom NA. End-to-side neurorrhaphies in a rodent model of peripheral nerve injury: a preliminary report of a novel technique. *J Neurosurg.* (2004) 101:78–84. doi: 10.3171/ped.2004.101.2.0078
121. Huang H-L, Cendan C-M, Roza C, Okuse K, Cramer R, Timms JF, et al. Proteomic profiling of neuromas reveals alterations in protein composition and local protein synthesis in hyper-excitable nerves. *Mol Pain.* (2008) 4:33. doi: 10.1186/1744-8069-4-33
122. Jing W, Changfeng L, Jiang P, Chen Z, Wenjing X, Xiaoqing C, et al. Establishment and evaluation of traumatic neuroma model. *Chin J Tissue Eng Res.* (2020) 24:716–9. doi: 10.3969/j.issn.2095-4344.2431
123. Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma.* (1998) 45:116–22. doi: 10.1097/00005373-199807000-00025
124. Nicholson B, Verma S. Comorbidities in chronic neuropathic pain. *Pain Med.* (2004) 5(Suppl 1):S9–27. doi: 10.1111/j.1526-4637.2004.04019.x
125. Deumens R, Bozkurt A, Meek MF, Marcus MAE, Joosten EAJ, Weis J, et al. Repairing injured peripheral nerves: bridging the gap. *Prog Neurobiol.* (2010) 92:245–76. doi: 10.1016/j.pneurobio.2010.10.002
126. Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng.* (2003) 5:293–347. doi: 10.1146/annurev.bioeng.5.011303.120731
127. Freier T, Montenegro R, Koh HS, Shoichet MS. Chitin-based tubes for tissue engineering in the nervous system. *Biomaterials.* (2005) 26:4624–32. doi: 10.1016/j.biomaterials.2004.11.040
128. Kanazawa T, Akiyama F, Kakizaki S, Takashima Y, Seta Y. Delivery of siRNA to the brain using a combination of nose-to-brain delivery and cell-penetrating peptide-modified nano-micelles. *Biomaterials.* (2013) 34:9220–6. doi: 10.1016/j.biomaterials.2013.08.036
129. Stenberg L, Kodama A, Lindwall-Blom C, Dahlin LB. Nerve regeneration in chitosan conduits and in autologous nerve grafts in healthy and in type 2 diabetic Goto-Kakizaki rats. *Eur J Neurosci.* (2016) 43:463–73. doi: 10.1111/ejn.13068
130. Neubrech F, Sauerbier M, Moll W, Seegmüller J, Heider S, Harhaus L, et al. Enhancing the outcome of traumatic sensory nerve lesions of the hand by additional use of a chitosan nerve tube in primary nerve repair: a randomized controlled bicentric trial. *Plast Reconstr Surg.* (2018) 142:415–24. doi: 10.1097/PRS.0000000000004574
131. Marcol W, Larysz-Brysz M, Kucharska M, Niekaszewicz A, Slusarczyk W, Kotulska K, et al. Reduction of post-traumatic neuroma and epineural scar formation in rat sciatic nerve by application of microcrystalline chitosan. *Microsurgery.* (2011) 31:642–9. doi: 10.1002/micr.20945
132. Chatelet C, Damour O, Domard A. Influence of the degree of acetylation on some biological properties of chitosan films. *Biomaterials.* (2001) 22:261–8. doi: 10.1016/S0142-9612(00)00183-6
133. Carvalho CR, López-Cebal R, Silva-Correia J, Silva JM, Mano JF, Silva TH, et al. Investigation of cell adhesion in chitosan membranes for peripheral nerve regeneration. *Mater Sci Eng C Mater Biol Appl.* (2017) 71:1122–34. doi: 10.1016/j.msec.2016.11.100
134. Stöfel M, Wildhagen VM, Helmecke O, Metzen J, Pfund CB, Freier T, et al. Comparative evaluation of chitosan nerve guides with regular or increased bendability for acute and delayed peripheral nerve repair: a comprehensive comparison with autologous nerve grafts and muscle-in-vein grafts. *Anat Rec.* (2018) 301:1697–713. doi: 10.1002/ar.23847
135. Zhou S, Yang Y, Gu X, Ding F. Chitooligosaccharides protect cultured hippocampal neurons against glutamate-induced neurotoxicity. *Neurosci Lett.* (2008) 444:270–4. doi: 10.1016/j.neulet.2008.08.040
136. Huang H-C, Hong L, Chang P, Zhang J, Lu S-Y, Zheng B-W, et al. Chitooligosaccharides attenuate Cu²⁺-induced cellular oxidative damage and cell apoptosis involving Nrf2 activation. *Neurotox Res.* (2015) 27:411–20. doi: 10.1007/s12640-014-9512-x
137. Wang Y, Zhao Y, Sun C, Hu W, Zhao J, Li G, et al. Chitosan degradation products promote nerve regeneration by stimulating schwann cell proliferation via miR-27a/FOXO1 axis. *Mol Neurobiol.* (2016) 53:28–39. doi: 10.1007/s12035-014-8968-2
138. Zhao Y, Wang Y, Gong J, Yang L, Niu C, Ni X, et al. Chitosan degradation products facilitate peripheral nerve regeneration by improving macrophage-constructed microenvironments. *Biomaterials.* (2017) 134:64–77. doi: 10.1016/j.biomaterials.2017.02.026

139. He B, Tao HY, Liu SQ. Neuroprotective effects of carboxymethylated chitosan on hydrogen peroxide induced apoptosis in Schwann cells. *Eur J Pharmacol.* (2014) 740:127–34. doi: 10.1016/j.ejphar.2014.07.008
140. Ulrich D, van Doorn L, Hovius S. Fat injection for treatment of painful neuroma after episiotomy. *Int J Gynaecol Obstet.* (2011) 115:290–1. doi: 10.1016/j.ijgo.2011.07.023
141. Thomsen L, Bellemere P, Loubersac T, Gaisne E, Poirier P, Chaise F. Treatment by collagen conduit of painful post-traumatic neuromas of the sensitive digital nerve: a retrospective study of 10 cases. *Chir Main.* (2010) 29:255–62. doi: 10.1016/j.main.2010.07.004
142. Economides JM, DeFazio MV, Attinger CE, Barbour JR. Prevention of painful neuroma and phantom limb pain after transfemoral amputations through concomitant nerve coaptation and collagen nerve wrapping. *Neurosurgery.* (2016) 79:508–13. doi: 10.1227/NEU.0000000000001313
143. Koch H, Hubmer M, Welkerling H, Sandner-Kiesling A, Scharnagl E. The treatment of painful neuroma on the lower extremity by resection and nerve stump transplantation into a vein. *Foot Ankle Int.* (2004) 25:476–81. doi: 10.1177/107110070402500706
144. Balcin H, Erba P, Wettstein R, Schaefer DJ, Pierer G, Kalbermatten DF, et al. A comparative study of two methods of surgical treatment for painful neuroma. *J Bone Joint Surg Br.* (2009) 91:803–8. doi: 10.1302/0301-620X.91B6.22145
145. Gaudin R, Knipfer C, Henningsen A, Smeets R, Heiland M, Hadlock T. Approaches to peripheral nerve repair: generations of biomaterial conduits yielding to replacing autologous nerve grafts in craniomaxillofacial surgery. *Biomed Res Int.* (2016) 2016:3856262. doi: 10.1155/2016/3856262



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan Medical
College, China

REVIEWED BY

J. Geraldo R. Vaz,
Cliniques Universitaires Saint-Luc, Belgium
Christian Scheiwe,
University Hospital Freiburg, Germany

*CORRESPONDENCE

Kunhao Cai
✉ 2259584745@qq.com
Xuejun Fu
✉ fuxuejun999@sina.com

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 25 October 2022

ACCEPTED 02 January 2023

PUBLISHED 19 January 2023

CITATION

Wu L, Xiong J, Huang Y, Han K, Cai K and Fu X
(2023) Case report: Trigeminal neuralgia
misdiagnosed as glossopharyngeal neuralgia.
Front. Neurol. 14:1079914.
doi: 10.3389/fneur.2023.1079914

COPYRIGHT

© 2023 Wu, Xiong, Huang, Han, Cai and Fu.
This is an open-access article distributed under
the terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Case report: Trigeminal neuralgia misdiagnosed as glossopharyngeal neuralgia

Liangzhe Wu¹, Jinbiao Xiong², Ying Huang³, Kunning Han¹,
Kunhao Cai^{2*} and Xuejun Fu^{3*}

¹The Second Clinical Medical College, Jinan University, Shenzhen, Guangdong, China, ²Department of Neurosurgery, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University, The First Affiliated Hospital, Southern University of Science and Technology), Shenzhen, Guangdong, China, ³Department of Neurology, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University, The First Affiliated Hospital, Southern University of Science and Technology), Shenzhen, Guangdong, China

Background: Trigeminal neuralgia (TN) and glossopharyngeal neuralgia (GPN) are cranial nerve neuralgias with the same clinical manifestations, pathological features, and trigger factors; their affected sites are adjacent. Performing a magnetic resonance imaging (MRI) examination alone can easily lead to a misdiagnosis.

Case presentation: A 72-year-old man had visited another hospital with severe left-sided tongue pain. On MRI, vascular compression of the glossopharyngeal nerve had been visible, with unclear evidence of trigeminal nerve involvement. He had been diagnosed with left-sided GPN and underwent microvascular decompression (MVD) of the left glossopharyngeal nerve. However, no improvement was observed after surgery. During a second surgery at our hospital, MVD of the trigeminal nerve was performed, and the trigeminal nerve was fully explored and separated. The patient's pain resolved after surgery. Ultimately, the patient was definitively diagnosed with left-sided TN.

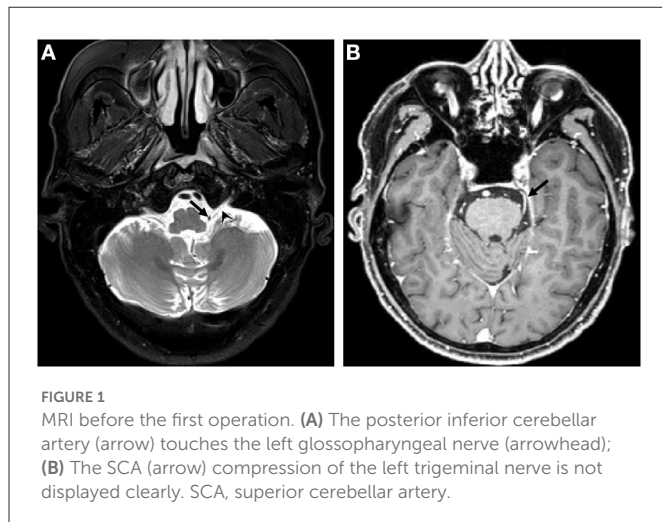
Discussion and conclusion: MVD is currently the most efficacious surgical option for treating cranial nerve neuralgia. To select patients for MVD, having an MRI criteria for identifying true neurovascular compression will be helpful. However, clinicians should focus more on a patient's clinical symptoms and not rely solely on MRI findings. This patient's case can help clinicians distinguish between TN and GPN, improve the understanding of these diseases, avoid misdiagnosis, and reduce the possibility of secondary damage.

KEYWORDS

trigeminal neuralgia, glossopharyngeal neuralgia, microvascular decompression, magnetic resonance imaging, misdiagnosed

1. Introduction

Trigeminal neuralgia (TN) is a chronic neuropathic pain disorder that occurs mainly in the face, especially in the cheeks and corners of the mouth, and rarely in the tongue, mouth, and jaw. Glossopharyngeal neuralgia (GPN) is a rare facial syndrome characterized by pain in the throat, tonsils, and tongue, with occasional pain in the mandibular angle and ear. Both TN and GPN can occur spontaneously or can have specific precipitating factors. The most common etiology involves demyelinating lesions induced by vascular compression of nerves in the brainstem; this is known as the root entry zone (REZ). The antiepileptic drugs carbamazepine and oxcarbazepine are the first-line treatment options; if drug therapy is ineffective or the patient cannot tolerate the side effects, microvascular decompression (MVD) is the preferred surgical option. When a patient presents with tongue pain and magnetic resonance imaging (MRI) shows



intracranial vascular compression of the glossopharyngeal nerve, it is easy to consider a diagnosis of GPN but not TN. A misdiagnosis potentially results in multiple surgeries; therefore, the ability to distinguish between the two diseases is essential. To the best of our knowledge, the present case is the first case of TN misdiagnosed as a GPN.

2. Case description

A 72-year-old man was admitted to the hospital with intermittent severe pain in the left middle one-third of the tongue for 8 months, triggered by tongue movements and chewing of food, lasting from a few to dozens of minutes at a time, and was treated effectively with carbamazepine. Swallowing did not cause any pain. There was no pain at the face, base of the tongue, throat, or tonsils, and no other neurological symptoms. Four months prior, the patient underwent an MRI exam (Figure 1) and was diagnosed with left-sided GPN; he was treated with glossopharyngeal nerve MVD at another hospital. The patient's symptoms did not improve after surgery. The day after the surgery, the pain recurred to the preoperative condition and gradually aggravated. On the third post-operative day, there was clear fluid outflow from the nose, suggesting cerebrospinal fluid rhinorrhea. In addition, the patient developed reduced hearing in the left ear. The symptoms of the clear fluid outflow from the nose disappeared 10 days after the surgery.

Following admission to our hospital, the patient had a second MRI exam (Figure 2). The patient underwent a second surgery for cerebellopontine angle exploration (CPA). The previous craniotomy was reopened in the lateral position, and the dura was cut open. While exploring the trigeminal nerve area, we observed a small blood vessel close to the REZ with a Teflon sponge adhering to it (placed during the first operation) (Figure 3). The vessel was displaced without compressing the trigeminal nerve. The blood vessel was small; therefore, it was not considered to be the responsible vessel. The superior pole of the trigeminal nerve was then examined. After

cutting arachnoid adhesions, the superior cerebellar artery (SCA) was found to be significantly suppressing the REZ area of the trigeminal nerve. Following the placement of a Teflon sponge between the vessel and nerve to make a total separation, we continued to explore the trigeminal nerve near Meckel's cave. We saw that the SCA was turned forward and downward, oppressing the trigeminal nerve (Figure 4), which was separated completely by the Teflon sponge. Finally, the space between the glossopharyngeal nerve and the facial acoustic nerve was explored; the glossopharyngeal nerve anatomy was unclear, and there was no apparent vascular compression. Therefore, no further dissections were performed. The dura mater was sutured in a watertight pattern, and the wound was closed in layers. Postoperatively, the patient's pain resolved completely. No new neurological deficits or surgery-related complications were observed.

3. Diagnostic assessment

In the sensory innervation of the tongue, the mandibular branch of the trigeminal nerve innervates the anterior two-thirds of the tongue, and the glossopharyngeal nerve innervates the posterior one-third of the tongue. The pain in the middle one-third of this patient's tongue was located in the demarcation zone of the trigeminal and glossopharyngeal nerves. Anatomically, this area is involved in the innervation of the trigeminal nerve, but symptoms did not improve after the first surgery. When drug therapy is ineffective, MVD is currently the best surgical plan to treat cranial nerve neuralgia caused by intracranial vascular compression of nerves. Studies report that the success rate of MVD is 83.4% for TN and 97.4% for GPN (1, 2). When postoperative ineffectiveness occurs, we must consider that certain areas were missed during exploration or the diagnosis may be incorrect. Although the trigeminal nerve was not displayed clearly on the patient's MRI, the MRI's negative-predictive value was only 33.3%; thus, TN was still considered. We fully explored and separated the responsible vessels of the trigeminal nerve during surgery, and the tongue pain disappeared after surgery. The trigeminal nerve is a large sensory rootlet that exits the lateral aspect of the midpons medial to the middle cerebellar peduncle; the ophthalmic division is the most inferior, the maxillary division is in the middle, and the mandibular division is in the superior position (3). The trigeminal nerve was compressed from above to below by the SCA during surgery and was therefore diagnosed more definitively as left-sided TN.

4. Discussion

The patient had simple pain in the left middle third of the tongue, without pain in the root of the tongue or throat, and without swallowing dysfunction or other neurological abnormalities. In addition, he had previously undergone MVD of the left GPN.

The etiology of TN can be divided into three categories: classic, secondary, and idiopathic. The classical type is intracranial vascular compression upon the REZ of the trigeminal nerve. Secondary TN, combined with other neurological symptoms, is commonly associated with multiple sclerosis and tumors in the pontocerebellar region. Idiopathic TN indicates that no neurological cause can be found (4). The incidence of GPN is approximately 1/100th that of TN (5). GPN is divided into idiopathic and secondary types. Idiopathic neuralgia has no clear etiology. Secondary causes include intracranial

Abbreviations: TN, trigeminal neuralgia; GPN, glossopharyngeal neuralgia; MRI, magnetic resonance imaging; REZ, root entry zone; MVD, microvascular decompression; SCA, superior cerebellar artery.

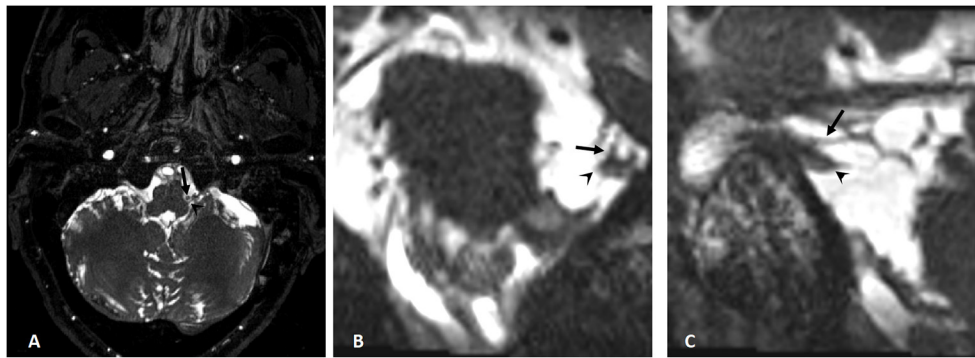


FIGURE 2

MRI before the second operation. (A) The structure around the left glossopharyngeal nerve is disordered, and the original posterior inferior cerebellar artery (arrow) seems to be moved with no compression to the left glossopharyngeal nerve (arrowhead); (B, C) The left trigeminal nerve (arrowhead) touches the superior cerebellar artery (arrow).

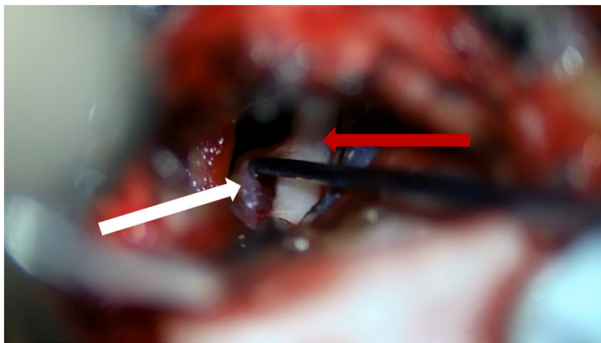


FIGURE 3

Teflon sponges (white arrow) separate small blood vessels away from the trigeminal nerve (red arrow).

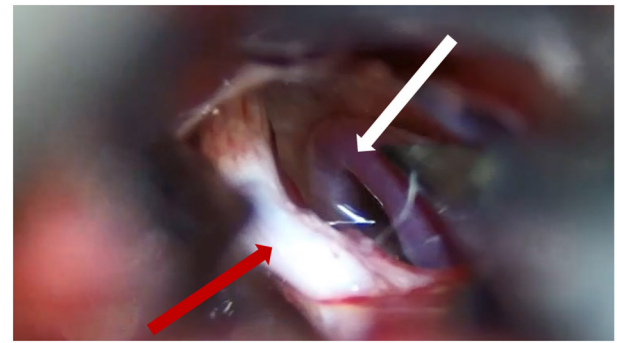


FIGURE 4

The SCA (white arrow) compresses the trigeminal nerve (red arrow). SCA, superior cerebellar artery.

vascular compression, tumors, multiple sclerosis, trauma, and Eagle's syndrome. The differential diagnosis of TN and GPN depends mainly on different clinical manifestations, with MRI performed as an auxiliary diagnosis. The pain in TN occurs in the area controlled by the trigeminal nerve, including the face, tongue, and jaw; trigger points are also present in this area. In GPN, the pain ranges among the auricular and pharyngeal branches of the glossopharyngeal and vagus cranial nerves, including the ear, pharynx, root of the tongue, and mandibular angle; the pain can be spontaneous or induced by swallowing, chewing, coughing, talking, and yawning (6). A cranial MRI exam is the first choice for evaluating the contact between blood vessels and nerves. Preoperative MRI sensitivity is 75.8%, with a specificity of 65.8%, and positive- and negative-predictive values of 92.4 and 33.3%, respectively, for MVD (7).

GPN is often misdiagnosed as TN, nervus intermedius neuralgia, myofascial pain dysfunction syndrome, pharyngitis, or tonsillitis. The main reasons for misdiagnosing the glossopharyngeal nerve as TN include that (1) GPN is much rarer and less recognized than TN, accounting for 1/100th of TN cases; (2) diagnosis of cranial nerve neuralgia is dependent mainly on symptoms and signs; (3) when the patient history description is not accurate, a lack of a specific diagnostic basis may exist;

and (4) being misled is easy. For example, some patients with GPN subjectively describe experiencing pain when chewing and eating. However, this symptom does not differentiate GPN from TN. In addition, if clinical experience is lacking when the medical history is unknown, a misdiagnosis can easily occur.

Having many branches, the glossopharyngeal nerve is widely distributed. The glossopharyngeal nerve, vagus nerve, accessory nerve, and hypoglossal nerve all originate from the medulla oblongata, with some branches of the glossopharyngeal nerve also entering the spinal nucleus of the trigeminal nerve. Therefore, the pain range may extend to the distribution area of the trigeminal nerve or be complicated by TN. When the glossopharyngeal nerve is damaged, the symptoms of damage to the vagus and hypoglossal nerve can coincide; the location of the pain can also change. Therefore, making a diagnosis is difficult. This phenomenon occurs in nearly 20% of GPN cases. MRI has a high predictive value for vascular-nerve contact, but its false-positive and false-negative rates may lead to a misdiagnosis or missed diagnosis.

Interestingly, the opposite occurred in this case: TN was misdiagnosed as GPN. The main cause of the misdiagnosis

was that the patient showed simple pain in the middle one-third of the left tongue without other concomitant symptoms. The pain was located in the adjacent innervation areas of the trigeminal and glossopharyngeal nerves; this does not occur in the classically described TN and GPN. The cranial MRI exam indicated that the glossopharyngeal nerve was compressed, but the trigeminal nerve was not displayed clearly. These MRI results, combined with the patient's medical history, facilitated a misdiagnosis of GPN; the positive-predictive rate of MRI is 92.4%, which is not optimal for diagnosis. Because cranial nerve neuralgia is diagnosed mainly by symptoms and signs, inadequacies in the medical history and physical examination can easily lead to misdiagnosis. If the preoperative diagnosis is unclear, and the vessels responsible for the suspected nerve damage have not been determined, the nerves should be fully explored and separated.

TN and GPN are the types of cranial nerve neuralgia with the same clinical and pathological features and trigger factors, with the pain locations overlapping the two groups of nerves. Because of this, misdiagnosis often occurs. Therefore, it is essential to identify differences between TN and GPN for proper diagnosis and treatment.

In this case, although the trigeminal nerve was separated in the first surgery, it was not carefully explored and separated, so the main responsible vessels were not identified. As a result, the pain persisted after the first surgery. In the second surgery, the trigeminal nerve was explored and decompressed fully, and the postoperative outcomes were good. Therefore, it is necessary to fully explore the trigeminal nerve and separate all potentially responsible vessels during surgery. Although preoperative MRI has a high positive-predictive value and sensitivity, a relatively low negative-predictive value reduces its usefulness in patients without vascular compression of nerves from MVD. Therefore, performing MVD should be coordinated with clinical manifestations and imaging; many MRI patients with negative compression may still benefit from MVD (7).

The diagnosis of cranial nerve disease is based primarily on clinical manifestations. Therefore, a patient's medical history should be reviewed thoroughly before surgery, and the target nerve should be determined. MRI should be performed only as a clinical diagnostic support and not as a determinant. If it is difficult to determine where the cranial nerve pain originates, a full exploration and complete separation of suspicious nerves should be performed simultaneously.

5. Conclusion

Misdiagnosis can easily occur in the area adjacent to the innervation areas of the trigeminal and glossopharyngeal nerves. For neuralgia with vascular compression, the patient's medical history should be investigated carefully, the target nerve should be determined, and full exploration and separation of the target nerves should be performed to avoid the risk of failure and recurrence. Although MRI is the primary method used to identify neurovascular compression, providing operators with a preoperative

evaluation and choice of operation methods, it is only an auxiliary means. Therefore, a patient's clinical symptoms should be identified first, and basic clinical and physical examinations should not be ignored. When a diagnosis is highly suspicious and the MRI exam yields unclear results, MVD should be performed using full exploration.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LW wrote the manuscript and was involved in the diagnostic and therapeutic clinical progress. JX was involved in the patient's care. KH supported the case interpretation. KC revised the manuscript for intellectual content and was responsible for patient diagnosis and treatment. XF contributed to the figures, helped with the diagnostic process, and critically revised the manuscript. All authors contributed to the manuscript and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2023.1079914/full#supplementary-material>

References

1. Xia L, Li YS, Liu MX, Zhong J, Dou NN, Li B, et al. Microvascular decompression for glossopharyngeal neuralgia: a retrospective analysis of 228 cases. *Acta Neurochirurg.* (2018) 160:117–23. doi: 10.1007/s00701-017-3347-1
2. Amaya Pascasio L, De La Casa-Fages B, Esteban de Antonio E, Grandas F, García-Leal R, Ruiz Juretschke F. Microvascular decompression for trigeminal neuralgia: a retrospective analysis of long-term outcomes and prognostic factors. *Neurologia.* (2021) 26:712. doi: 10.1016/j.nrl.2021.03.009
3. Joo W, Yoshioka F, Funaki T, Mizokami K, Rhoton AL. Microsurgical anatomy of the trigeminal nerve. *Clin Anat.* (2014) 27:61–88. doi: 10.1002/ca.22330
4. Cruccu G, Di Stefano G, Truini A. Trigeminal neuralgia. *N Engl J Med.* (2020) 383:754–62. doi: 10.1056/NEJMr1914484
5. Palanisamy D, Kyosuke M, Yasuhiro Y, Tsukasa K, Kato Y. Management of recurrent glossopharyngeal neuralgia following microvascular decompression surgery. *World Neurosurg.* (2018) 117:339–43. doi: 10.1016/j.wneu.2018.06.136
6. Blumenfeld A, Nikolskaya G. Glossopharyngeal neuralgia. *Curr Pain Headache Rep.* (2013) 17:343. doi: 10.1007/s11916-013-0343-x
7. Xu R, Nair SK, Raj D, Materi J, So RJ, Gujar SK, et al. The role of preoperative MRI imaging in assessing neurovascular compression prior to microvascular decompression in trigeminal neuralgia. *World Neurosurg.* (2022) 168:e216–22. doi: 10.1016/j.wneu.2022.09.092



OPEN ACCESS

EDITED BY
Hui Lu,
Zhejiang University, China

REVIEWED BY
Gang Zhao,
Soochow University, China
Hui Lu,
Zhejiang University, China

*CORRESPONDENCE
Rui Cong
✉ congriui@fmmu.edu.cn
Rui Zhao
✉ xjzhaorui@126.com

SPECIALTY SECTION
This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 20 November 2022
ACCEPTED 02 January 2023
PUBLISHED 26 January 2023

CITATION
Wang H, Shi L, Tian T, Xian H, Song Z, Cong R
and Zhao R (2023) Case report: A rare case of
massive peripheral nerve melanotic
schwannoma and review of the literature.
Front. Neurol. 14:1103604.
doi: 10.3389/fneur.2023.1103604

COPYRIGHT
© 2023 Wang, Shi, Tian, Xian, Song, Cong and
Zhao. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Case report: A rare case of massive peripheral nerve melanotic schwannoma and review of the literature

Han Wang¹, Lin Shi², Tong Tian², Hang Xian², Zhao Song²,
Rui Cong^{2*} and Rui Zhao^{2*}

¹Department of Orthopedics, Air Force Medical Center, Beijing, China, ²Department of Orthopedics, Xijing Hospital, Air Force Military Medical University, Xi'an, China

Melanotic schwannoma is a rare tumor with indeterminate biologic behavior and varying treatment recommendations. Just about 200 cases have been reported worldwide, in which occurred in peripheral nerves has even less reported. Due to the lack of cognition of melanotic schwannoma, it is easy to be misdiagnosed and mistreatment in primary hospitals. Herein, we presented a case of massive melanotic schwannoma growing in the brachial plexus of an elderly male patient. First, the patient underwent a left forearm tumor resection in the local primary hospital because a painless lump was found there in 2017, of which details remain unclear. After this operation, the patient developed the symptoms of left median nerve injury. Thus, he came to our hospital and underwent a second operation. During this operation, we found that a part of the median nerve was absent at the left forearm, and the remanent median nerve, from the broken end to the elbow, was totally turned black, which was accompanied by petroleum-like exudate. Losing the opportunity for nerve repair, the black nerve was removed extensively and thoroughly. Postoperative pathological diagnosis revealed that the tumor was melanotic schwannoma. Then 4 years later, the tumor recurrence again, which led to the paralysis of the whole left arm and severe nerve pain, and the pulmonary metastasis of the tumor was detected at the same time. The black nerve was resected again in our hospital, and the nerve pain was partially relieved after the operation. To the best of our knowledge, it is the first time to report a melanotic schwannoma case that happened in the peripheral nerve trunk and then spread to the whole brachial plexus. There were many questions that worthy of discussion could be invited from this case, and we analyzed and discussed them based on the relevant literature. In conclusion, we reported a rare case of melanotic schwannoma that happened in the brachial plexus and illustrated the problems of the diagnosis and treatment of it based on the analysis of the relevant literature, which is helpful for the cognition of this rare nerve tumor.

KEYWORDS

melanotic schwannoma, peripheral nerves, brachial plexus, pathological diagnosis, neuropathic pain

Introduction

Melanotic schwannoma (MS) is a rare tumor of the nervous system, which accounts for <1% of nervous system tumors. However, its specific incidence has not been reported yet. Since it was first reported by Hodson and J. J. in 1961, only about 200 cases have been reported worldwide (1). With the improvement in detection methods, the reports on MS have gradually increased in recent years. It has been reported that MS mainly occurs in the skin, organs, and spinal cord, but rarely in the peripheral nerve trunk. Many studies have pointed out that MS can be divided

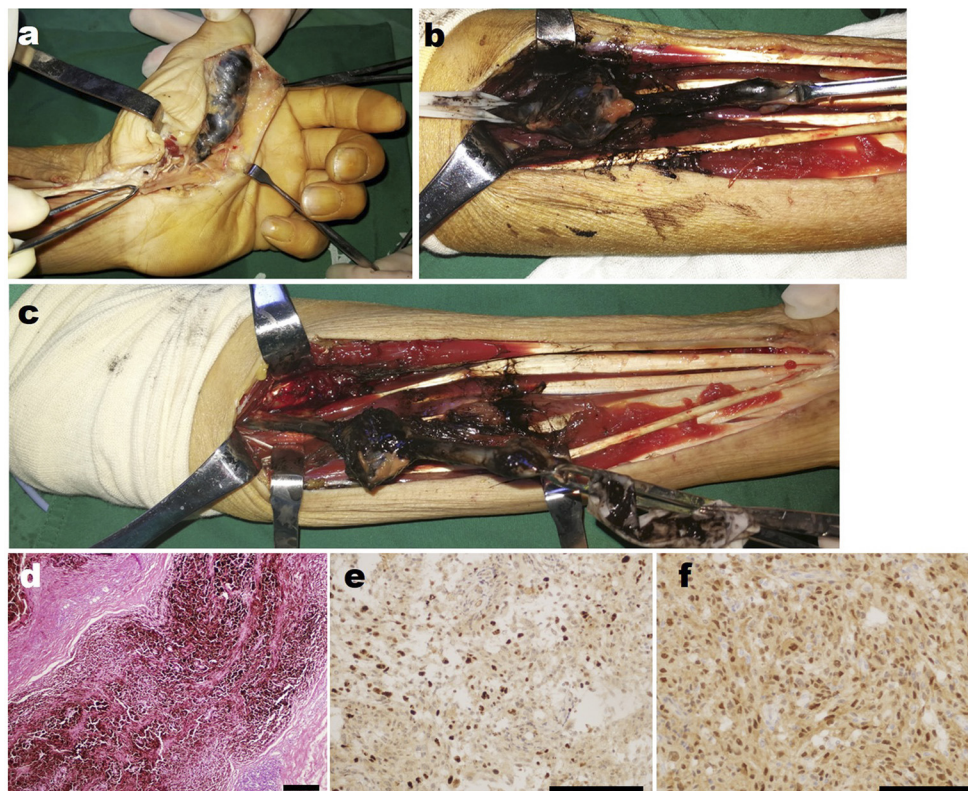


FIGURE 1

Intra-operative findings of the patient's first operation in our hospital. (a) The intraoperative photograph of the mass in the left hand of the patient. (b) The close-up detailed photograph of the mass in the left forearm shows the petroleum-like exudate. (c) The overall photographs of the mass in the left forearm of the patient. (d) The representative HE staining image of the resected tumor. (e) The representative Ki67 immunohistochemical staining image of the resected tumor. (f) The representative S100 immunohistochemical staining image of the resected tumor. Scar bar, 100 μ m.

into the psammomatous type and the non-psammomatous type. Psammomatous melanotic schwannoma (PMS) is considered to have a potential malignant tendency, which can cause distant metastasis (2). Due to the lack of awareness of this disease, it is prone to misdiagnosis and mistreatment during diagnosis and treatment, especially in grass-roots hospitals. Therefore, we reported a rare case of recurrent giant MS in the peripheral nerve and discussed the diagnosis and treatment methods of this rare disease based on the relevant literature.

Case report

A 62-year-old elderly Chinese man visited our hospital because of left hand numbness for 1 year after resection of a tumor in the forearm, accompanied by a tumor of the left hand for half a year. In 2016, the patient found a painless tumor at the left distal forearm of no obvious cause, without left hand numbness, movement disorders, or muscular atrophy. The tumor of the left forearm was resected in a local grass-roots hospital, with an unknown specific surgical condition and no definite postoperative diagnosis. After the surgery, the patient developed numbness of three and a half fingers on the radial side of the left hand, accompanied by the inflexibility of the left thumb. About half a year later, the patient found another painless tumor in the hypothenar region of the left hand, accompanied by progressive tumor enlargement, weakness of the left thumb, and

progressive aggravation of dyskinesia. The patient visited our hospital for further treatment. Considering the recurrent tumor adjacent to the site of tumor resection, accompanied by iatrogenic injury of the median nerve, the patient was admitted to our department with "a tumor of the left palm, and left median nerve injury". After admission, the preoperative examination was completed, and magnetic resonance imaging (MRI) of the left hand suggested a space-occupying lesion in the hypothenar region of the left hand, with a size of about $5 \times 3 \times 2$ cm, and low T1 and high T2 signals.

After preoperative preparation, tumor resection of the left hand and biopsy and median nerve exploration and repair in the left forearm were performed. However, the intraoperative findings were unexpected. A spindle-shaped enlarged mass was found in the thenar region of the left hand, which was completely black and connected with the digital nerve (Figure 1a). When the mass was cut open, melanotic nerve fibers and petroleum-like exudate were observed. A similar phenomenon also occurred in the left forearm. Intraoperatively, findings showed a defect of the left median nerve of about 10 cm in the distal forearm, and melanosis and thickening at about 13 cm from the proximal end of the nerve defect, in the center of which there was a quasi-circular mass, with a diameter of about 4 cm. After cutting open the mass, melanotic nerve fibers and black tumor-like hyperplasia were found, accompanied by black petroleum-like exudate (Figures 1b, c). According to the morphological features, MS was diagnosed intraoperatively, and the diseased nerves that were explored were resected completely and

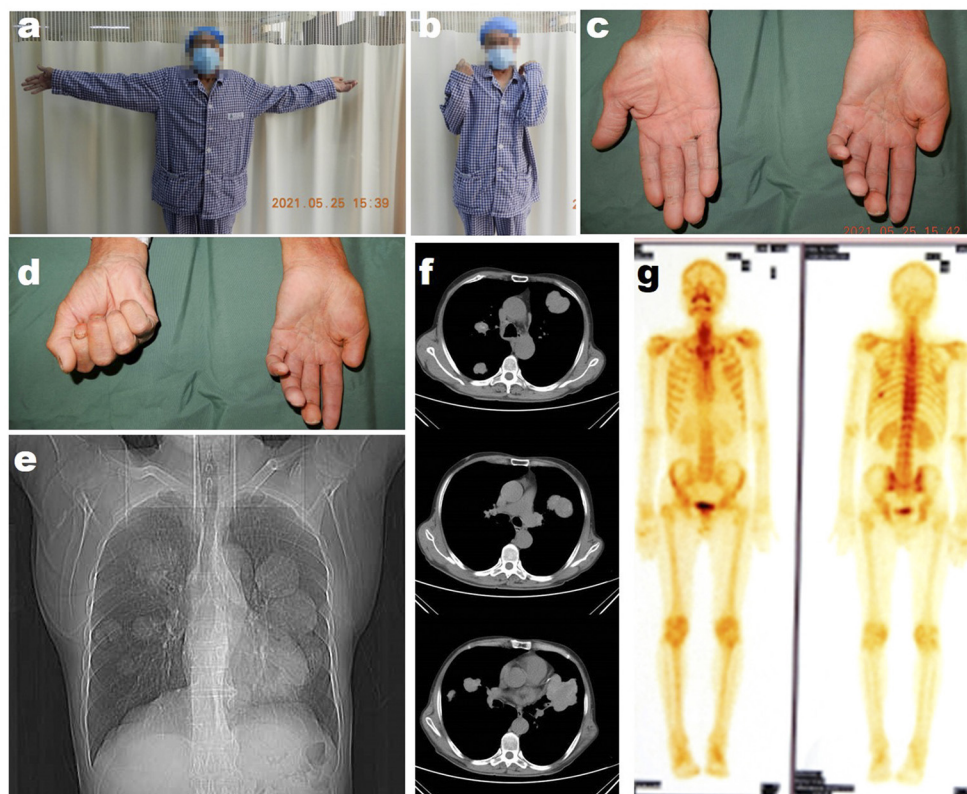


FIGURE 2

The preoperative examination of the patient's second operation in our hospital. (a) The photograph of the patient trying to abduct his bilateral shoulders. (b) The photograph of the patient trying to flex his bilateral elbows. (c) The photograph of the patient trying to extend his bilateral fingers. (d) The photograph of the patient trying to flex his bilateral fingers. (e) The radiological image of the patient's chest. (f) The representative CT images of the patient's chest. (g) The bone scan images of the patient.

extensively. The tumor was extendedly resected at 2 cm from the tumor boundary trying to ensure no melanotic tumor cells at the incisional margin. Because of the lack of nerve repair condition, median nerve repair was abandoned in this surgery. After explaining and communicating with the patient about the condition, the patient and his family members expressed understanding and returned home for resting after the wound healed. Postoperative pathological confirmed MS and it was pathologically manifested as massive melanin deposition in tumor cells, and immunohistochemical staining suggested S100 and Ki67-positive, indicating that this tumor has a potential malignant tendency (Figures 1d–f).

Then 4 years later, the patient returned to our hospital for further treatment due to left upper limb paralysis, accompanied by severe neuropathic pain. The examination after admission showed that the VAS score of the patient was eight. The patient's muscle strength of left wrist flexion, forearm pronation, finger extension, and intrinsic hand muscles was grade 0, and that of left finger flexion, wrist extension, forearm supination, elbow flexion, elbow extension, shoulder abduction, and adduction was grade 3 (Figures 2a–d). Chest X-ray and CT showed multiple quasi-circular negative lesions in both lungs, which were considered metastatic lesions (Figures 2e, f), while the patient did not complain of dry cough or hemoptysis. Bone scanning suggested metastatic lesions in the left humeral head and left eighth rib (Figure 2g). In combination with the results of various examinations, the patient was diagnosed with recurrent MS after surgery. Considering that the severe neuropathic pain of the patient might be related to a nerve tumor, the tumor could be

resected surgically to reduce his pain. Due to distant metastasis, this surgery aimed at tumor reduction but not complete resection. During the surgery, it was found that from the clavicle to the elbow, the median nerve was completely melanotic, and the ulnar, radial, and musculocutaneous nerves were partially melanotic (Figures 3a, c). Exploration of the roots and trunks of the brachial plexus revealed that the upper, middle, and lower trunks of the brachial plexus were completely melanotic, but the suprascapular nerve was not melanotic (Figure 3b). When the melanotic epineurium was cut open, the melanotic nerve fiber bundles could be observed. Among them, there were also not completely melanotic nerve fiber bundles. A nerve fiber with melanotic two ends, but a normal middle part, was also found. All these phenomena suggest the growth pattern of the tumor spreading along the nerve fibers (Figure 3d). Intraoperatively, most of the completely melanotic nerves that were visible were palliatively resected, and the cut ends of these nerves were protected. After surgery, the patient's pain was significantly relieved, and the VAS score was reduced to three. The postoperative pathological analysis showed that the melanin-enriched tumor cells were extensively proliferated and accompanied by focal necrosis. The Ki67 immunohistochemical staining was positive, which was consistent with the previous pathological results (Figures 3e, f). It was recommended that the patient should be transferred to the Department of Oncology for further treatment, but he returned to a local hospital for subsequent treatment due to personal reasons. At the 3-month and half-a-year follow-up, the patient's neuropathic pain could be controlled to a tolerable level by taking pregabalin

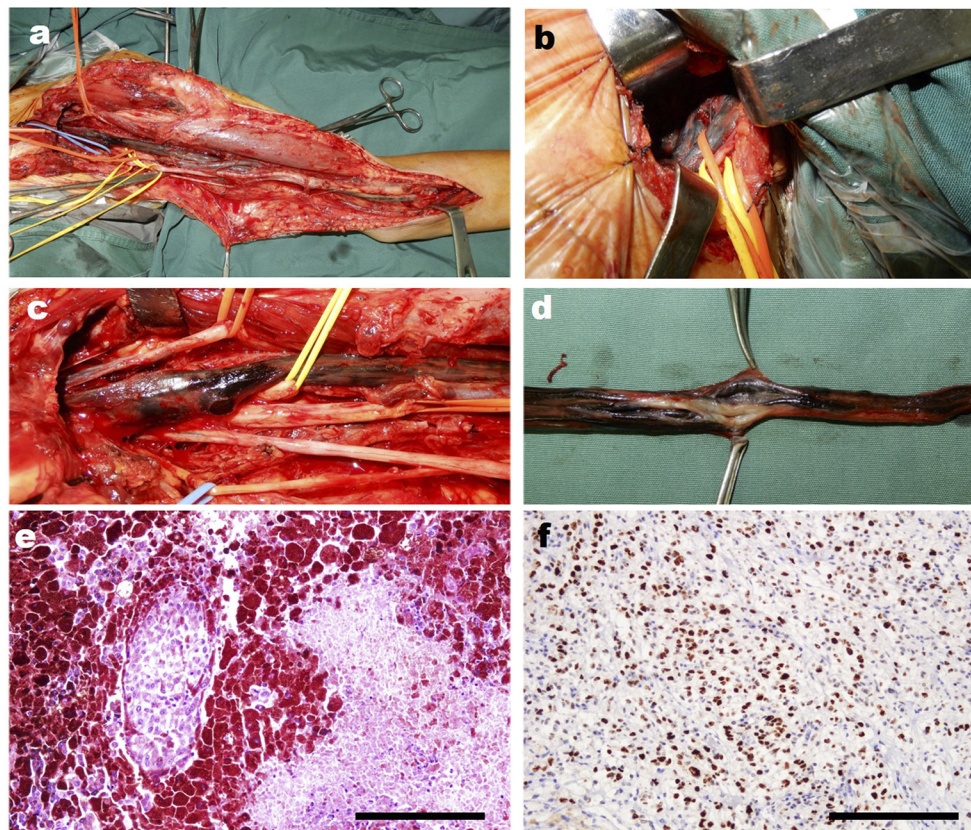


FIGURE 3

The intra-operative photographs of the patient's second operation in our hospital. (a) The overall photograph of the mass in the patient's left arm. (b) The intraoperative photograph of the mass in trunks of the brachial plexus. (c) The close-up detail photograph of the black changed the nerves of the patient. (d) The close-up detail photograph of the black changed nerve shows the change of the never fibers intra epineurium. (e) The representative HE staining image of the resected tumor. (f) The representative Ki67 immunohistochemical staining image of the resected tumor. Scar bar, 100 μ m.

150–225 mg two times daily. Finally, the patient passed away 1 year after surgery.

Discussion

Due to the small number of cases, there is no recognized treatment guideline or clinical experience for MS. Consequently, in the reported cases, the long-term therapeutic effect may not be ideal, but it still accumulates valuable experience for better treatment of this tumor in the future. It has been reported that PMS is one of the manifestations of Carney syndrome and has a malignant tendency (3). In the recently reported cases, recurrence and metastasis have also been found in patients with MS of non-psammomatous type and without Carney syndrome. To better analyze our case, we summarized the treatment and prognosis of MS cases similar to this case, which occurred in the peripheral nerve trunk and did not present Carney syndrome, so as to provide a basis for analyzing the treatment of this disease (Table 1). It was found that although only a few of these cases had tumor recurrence, most of the cases were followed up for a relatively short time, which may lead to many recurrences not being observed. Therefore, because of its uncertain biological features and potential recurrence risk, the treatment of MS should be carefully selected.

Although most literature reports that 90% of MS are benign, and only about 10% are recurred and metastasized, this does not mean

that we can take such cases lightly. It is precisely this uncertainty that brings difficulties in the selection of treatment for such tumors (18). Among them, MS occurring in the peripheral nerve is different from those in other locations in terms of treatment. If the MS of the peripheral nerve trunk is benign, the treatment principle should be similar to that of common schwannomas. Common schwannomas usually do not lead to the paralysis of neurological function. Thus, the treatment principle for schwannomas with (or without) neurostimulation symptoms is to protect the nerve fiber bundles during tumor resection, and not damage nerve fibers, causing iatrogenic neurological impairment after surgery. In the resection of common schwannomas, the tumor tissue can usually be completely dissected from the nerve bundles, so as not to damage nerve fibers. However, benign MS occurring in the peripheral nerve trunk that can be dissected has not been reported.

In our case, because of the invasion of MS cells into the nerve fiber bundles, the nerve fiber bundles would be damaged during tumor resection, resulting in the loss of neurological function, which is a problem that must be fully considered when making a treatment plan for MS. If it is a benign MS, the first principle of treatment is to resect the tumor as thoroughly as possible without damaging the neurological function. When the tumor invades the nerve fibers and it has to remove partial nerve bundles, it is necessary to ensure the primary nerve repair or reconstruction as much as possible. The treatment of malignant MS should be completely different. The first principle of its treatment should be to resect the tumor completely

TABLE 1 Summary of the treatment of melanotic schwannoma cases without Carney complex.

References	Location	Gender	Age	Nationality	Symptom	Duration	Treatment	Follow-up	Recurrence
Hall et al. (4)	S1	Female	18	USA	Radicular pain	Several years	Resection + radiotherapy	2.5 years	No
Yeom et al. (5)	T11	Male	72	South Korea	Lower back pain, paresthesia	6 months	Resection	1 year	No
Georgiev et al. (6)	L3	Male	61	Bulgaria	Lower back pain	3 months	Resection	14 months	No
Soyland et al. (7)	T8	Male	53	USA	Acute chest pain	2 days	Resection	6 months	No
Morgan et al. (8)	C8	Female	50	UK	Tinel's sign	Several years	Resection	3 months	No
Solomou et al. (9)	C6	Female	45	UK	Neck pain, paresthesia	1 year	Resection + immunotherapy	15 months	Yes (death)
Shen et al. (10)	L3	Female	29	China	Lower back pain	1 day	Chemotherapy	1 year	No
Nagashima et al. (11)	S2	Male	48	Japan	Left sciatic pain	6 months	Resection	6 months	No
Kim et al. (12)	Facial nerve	Female	14	USA	Right facial weakness	No mention	Resection + chemotherapy	9 years	Yes
Azarpira et al. (13)	L2	Male	37	Iran	Lower back pain	8 months	Resection	4 months	No
Carrasco et al. (14)	Trigeminal nerve	Female	34	Chile	Odontalgia, hypoesthesia	2 months	Resection	3 months	Yes
Sengoz et al. (15)	S1	Male	41	Turkey	Radicular pain	14 months	Resection	6 months	No
Kuchelmeister et al. (16)	C6	Female	53	Germany	Brachialgia	2 years	Resection	1 year	No
Cummings et al. (17)	S2	Male	51	USA	Lower back pain	8 months	Needle biopsies	No mention	No mention

and extensively and to dissect the surrounding lymph nodes and adjacent tissues, so as to prolong the survival of patients as much as possible. As for the loss of neurological function, it should not be repaired in the primary phase, but in the secondary phase after the tumor was completely resected, there was no recurrence. Based on the above principles, another problem puzzling the treatment of MS is how to determine whether it is benign or malignant. The first difficulty lies in the diagnosis of MS before cutting open the skin and exposing the tumor during surgery. Although some reports have suggested the imaging features of MS, these features are still not specific enough to enable us to distinguish MS from common schwannomas before surgery. Moreover, due to the low incidence, it is unreasonable to conduct a needle biopsy before the resection of all schwannomas for excluding the possibility of MS before surgery. Therefore, the diagnosis of MS is often an emergency during surgery. The second difficulty is that even if MS is found during surgery, we cannot determine whether it is benign or malignant. It is difficult to accurately determine the benign and malignant nature of tumor cells only by histological staining and cell morphology during the frozen pathological examination. The accurate diagnosis still depends on the immunohistochemical analysis. A series of molecular markers of the malignant tumor could be used to decide whether the tumor was malignant, such as Ki67, C-erbB, PCNA, NSE, Vim, and NESTIN. In this case, immunohistochemical analysis showed that the tumor cells were positive with Vim, H3K27m, Pan-mel, S-100, and Ki67, with indicated malignancy.

Based on this, we have reasons to make the following therapeutic decisions, that is, when we detect MS during surgery, the tumor should be completely resected, and the adjacent tissues and lymph nodes should be dissected according to the tumor invasion. Further treatment should be decided according to the pathological condition after surgery. If it is benign, nerve repair should be performed as soon as possible. In case of malignancy, radiotherapy or molecular targeted therapy should be continued, and nerve repair or functional reconstruction should be carried out after the tumor is completely eradicated.

In addition to prolongating the survival period, relieving the patient's severe neuropathic pain is also one of the important purposes of treatment. During the operation of this patient, we found that the invasion of melanin schwannoma resulted in swelling and stiffening of brachial plexus trunks and increased intra-nerve pressure, which may be one of the causes of neuropathic pain. Therefore, the epineural release of the diseased nerves, reducing the pressure within the nerves, can partially relieve the nerve pain.

Conclusion

This article reports a rare case of malignant MS in the peripheral nerve trunk. Combined with the retrospective analysis of similar cases, a new idea on the treatment of MS in the peripheral nerve trunk is discussed and proposed, which will help to better understand the treatment of this rare nerve tumor in the future.

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions.

Requests to access the datasets should be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Xijing Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HW wrote the manuscript. LS and TT summarized the literature. ZS took the photographs. RC and RZ revised the

manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Alexiev BA, Chou PM, Jennings LJ. Pathology of melanotic schwannoma. *Arch Pathol Lab Med.* (2018) 142:1517–23. doi: 10.5858/arpa.2017-0162-RA
- Belakhova SM, Rodriguez FJ. Diagnostic pathology of tumors of peripheral nerve. *Neurosurgery.* (2021) 88:443–56. doi: 10.1093/neuros/nyab021
- Gupta A, Dwivedi T. A simplified overview of World Health Organization classification update of central nervous system tumors 2016. *J Neurosci Rural Pract.* (2017) 8:629–41. doi: 10.4103/jnpr.jnpr_168_17
- Hall JC, Chang SD, Wilson TJ, Ganjoo KN, Toland A, Vogel H, et al. Post-operative stereotactic radiosurgery of malignant melanotic schwannoma. *Cureus.* (2022) 14:e22849. doi: 10.7759/cureus.22849
- Yeom JA, Song YS, Lee IS, Han IH, Choi KU. Malignant melanotic nerve sheath tumors in the spinal canal of psammomatous and non-psammomatous type: two case reports. *World J Clin Cases.* (2022) 10:8735–41. doi: 10.12998/wjcc.v10.i24.8735
- Georgiev GK, Todorov IV, Shamov TP, Krushovenski IV, Fakirova AD, Ilcheva BN, et al. L3 rootlet recurrent melanocytic schwannoma - case report and literature review. *Folia Med.* (2021) 63:448–56. doi: 10.3897/folmed.63.e55295
- Soyland DJ, Goehner DR, Hoerschgen KM, Gust TD, Vuong SM. Hemorrhagic spinal melanotic schwannoma presenting as acute chest pain: a case report and literature review. *Surg Neurol Int.* (2021) 12:164. doi: 10.25259/SNI_786_2020
- Morgan RV, Simpson A, Vaghela KR, Quick T. Psammomatous melanotic schwannoma - a rare neck lump. *Ann R Coll Surg Engl.* (2021) 103:e124–6. doi: 10.1308/rcsann.2020.7083
- Solomou G, Dulanka Silva AH, Wong A, Pohl U, Tzerakis N. Extramedullary malignant schwannoma of the spine: Case report and an up to date systematic review of the literature. *Ann Med Surg.* (2020) 59:217–23. doi: 10.1016/j.amsu.2020.10.003
- Shen XZ, Wang W, Luo ZY. 18F-FDG PET/CT imaging for aggressive melanotic schwannoma of the L3 spinal root: a case report. *Medicine.* (2021) 100:e24803. doi: 10.1097/MD.00000000000024803
- Nagashima Y, Nishimura Y, Eguchi K, Awaya T, Yoshikawa S, Haimoto S, et al. Intraosseous melanotic schwannoma in the sacrum mimicking primary bone tumor. *NMC Case Rep J.* (2020) 7:107–11. doi: 10.2176/nmcrcr.2019-0238
- Kim S, Federman N, Gordon EM, Hall FL, Chawla SP. Rexin-G((R)), a tumor-targeted retrovector for malignant peripheral nerve sheath tumor: a case report. *Mol Clin Oncol.* (2017) 6:861–5. doi: 10.3892/mco.2017.1231
- Azarpira N, Torabineghad S, Sepidbakht S, Rakei M, Bagheri MH. Cytologic findings in pigmented melanotic schwannoma: a case report. *Acta Cytol.* (2009) 53:113–5. doi: 10.1159/000325096
- Carrasco CA, Rojas-Salazar D, Chiorino R, Venega JC, Wohllk N. Melanotic nonpsammomatous trigeminal schwannoma as the first manifestation of Carney complex: case report. *Neurosurgery.* (2006) 59:E1334–5; discussion E1335. doi: 10.1227/01.NEU.0000245608.07570.D2
- Sengoz A, Tasdemiroglu E, Togay H. Is clear cell sarcoma a malignant form of psammomatous melanotic schwannoma? Case report. *Neurosurg Focus.* (2006) 21:E11. doi: 10.3171/foc.2006.21.6.3
- Kuchelmeister K, Lotz C, Schonmayr R, Schachenmayr W. April 2004: woman in her early fifties with a cervical intraspinal and extraspinal mass lesion. *Brain Pathol.* (2004) 14:453–4, 458–9. doi: 10.1111/j.1750-3639.2004.tb00090.x
- Cummings TJ, Liu K, Jordan LK 3rd, Dodd LG. Fine-needle aspiration diagnosis of psammomatous melanotic schwannoma. *Diagn Cytopathol.* (2000) 23:55–8. doi: 10.1002/1097-0339(200007)23:1<55::AID-DC13>3.0.CO;2-B
- Rodriguez FJ, Folpe AL, Giannini C, Perry A. Pathology of peripheral nerve sheath tumors: diagnostic overview and update on selected diagnostic problems. *Acta Neuropathol.* (2012) 123:295–319. doi: 10.1007/s00401-012-0954-z



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan Medical
College, China

REVIEWED BY

Hiroyuki Nodera,
Tenri Hospital, Japan
Hui Lu,
Zhejiang University, China

*CORRESPONDENCE

Junjian Jiang
✉ jjjdoctor@126.com

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 06 August 2022

ACCEPTED 02 January 2023

PUBLISHED 02 February 2023

CITATION

Huang X, You Z, Xiang Y, Dai J and Jiang J
(2023) Posterior division of ipsilateral C7
transfer to C5 for shoulder abduction limitation.
Front. Neurol. 14:1012977.
doi: 10.3389/fneur.2023.1012977

COPYRIGHT

© 2023 Huang, You, Xiang, Dai and Jiang. This
is an open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](#). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Posterior division of ipsilateral C7 transfer to C5 for shoulder abduction limitation

Xinying Huang ^{1,2,3,4}, Zongqi You ^{1,2,3}, Yaoxian Xiang ^{1,2,3}, Junxi Dai ^{1,2,3}
and Junjian Jiang ^{1,2,3*}

¹Department of Hand Surgery, Huashan Hospital, Fudan University, Shanghai, China, ²Key Laboratory of Hand Reconstruction, Ministry of Health, Shanghai, China, ³Shanghai Key Laboratory of Peripheral Nerve and Microsurgery, Shanghai, China, ⁴Shanghai Medical College, Fudan University, Shanghai, China

Background: Reparation of C5 by proximal selective ipsilateral C7 transfer has been reported for the treatment of neurogenic shoulder abduction limitation as an alternative to the reparation of the suprascapular nerve (SSN) and the axillary nerve (AXN) by distal nerve transfers. However, there is a lack of evidence to support either strategy leading to better outcomes based on long-term follow-up.

Objective: The purpose of the study was to investigate the safety and long-term outcomes of the posterior division of ipsilateral C7 (PDIC7) transfer to C5 in treating neurogenic shoulder abduction limitation.

Methods: A total of 27 cases with limited shoulder abduction caused by C5 injury (24 cases of trauma, 2 cases of neuritis, and 1 case of iatrogenic injury) underwent PDIC7 transfer to the C5 root. A total of 12 cases (11 cases of trauma and 1 case of neuritis) of C5 injury underwent spinal accessory nerve (SAN) transfer to SSN plus the triceps muscular branch of the radial nerve (TMBRN) transfer to AXN. The patients were followed up for at least 12 months for muscle strength and shoulder abduction range of motion (ROM).

Results: In cases that underwent PDIC7 transfer, the average shoulder abduction was 105.9° at the 12-month follow-up. In total, 26 of 27 patients recovered at least M3 (13 reached M4) (Medical Research Council Grading) of the deltoid. In cases that underwent SAN transfer to SSN plus TMBRN to AXN, the average shoulder abduction was 84.6° at the 12-month follow-up. In total, 11 of 12 patients recovered at least M3 (4 reached M4) of the deltoid.

Conclusion: Posterior division of ipsilateral C7 transfer is a one-stage, safe, and effective surgical procedure for patients with neurogenic shoulder abduction limitation.

KEYWORDS

nerve transfer, ipsilateral C7 transfer, brachial plexus injuries, neurogenic shoulder abduction limitation, C5 injury

Introduction

Neurogenic shoulder abduction limitation, a pathological change of the nerves innervating shoulder abductors, can be caused by C5 disorder. C5 contributes to the axillary nerve (AXN), which dominates the deltoid, and the suprascapular nerve (SSN), which dominates the musculus supraspinatus and the musculus infraspinatus (1). Based on our clinical experiences and previous studies, trauma, especially traffic accidents, is the most common cause leading to the direct injury of C5. However, the direct injury of C5 can also be caused by other neurological reasons, such as neuritis, peripheral neuropathy, and iatrogenic injury, in some patients. Those patients were treated with conservative therapies such as drugs or physical therapies, but they received

poor results. In a retrospective study reported by Thompson et al. (2), among 59 patients with postoperative C5 disorder who received conservative therapies, 15 patients had symptoms resolved with residual effects (25.4%) and 10 patients did not recover (17.0%).

Nerve transfer strategies have been reported to mainly focus on the distal nerve transfer as repairing SSN and AXN separately since they are the main branches of the C5 nerve root and their targeting muscles are involved in shoulder movement. Surgeons are used to choosing the donor nerves outside the brachial plexus such as accessory nerve (3, 4) and phrenic nerve (5). However, several issues with previous strategies need to be addressed. First, muscle function recovery requires adequate motive power from the donor nerve, matching donor nerve, and the recipient's nerve. It is a dilemma that choosing a donor nerve with sufficient motive power may lead to a severe loss of their original target muscle function such as diaphragm disorder caused by phrenic nerve transfer. Another drawback of previous strategies focused on distal nerve transfer is that C5 injury cannot be directly diagnosed without an additional incision to explore the brachial plexus. For intraoperative diagnosis, a specific incision for brachial exploration is required, resulting in more surgical injury and even a two-stage surgical strategy. Furthermore, another problem is that if the cerebral cortex regions of the donor's nerve and recipient's nerve are distant, it could be more difficult for patients to voluntarily move after surgery and the recovery process places high demands on brain plasticity (6).

Since distal nerve transfer may lead to the problems mentioned earlier, Gu et al. (7) developed ipsilateral C7 nerve root transfer to treat C5 rupture and Xu et al. (8, 9) further developed selective ipsilateral C7 nerve root transfer with less donor nerve function loss. To investigate further, here, we studied the clinical outcomes of 27 patients with the posterior division of ipsilateral C7 (PDIC7) transfer, a more selective proximal C5 repair strategy compared to distal SSN and AXN repair strategies for the treatment of neurogenic shoulder abduction limitation.

Methods

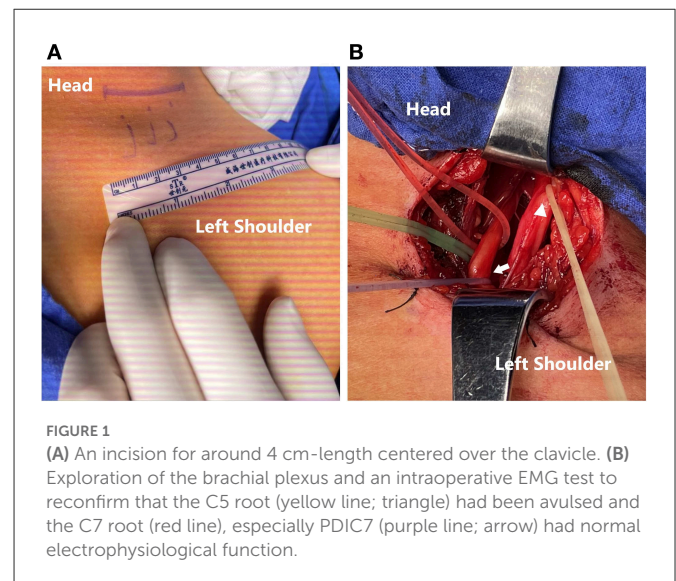
Patients

This is a retrospective study carried out in accordance with the Declaration of Helsinki. Each participant provided informed written consent. IRB approval was granted by our institution. Based on the following criteria, we compared 27 patients who underwent PDIC7 transfer to C5 with 12 patients who underwent SAN transfer to SSN plus TMBRN transfer to AXN.

The inclusion criteria are as follows:

1. Patients with shoulder abduction limitation whose muscle strength of the musculus supraspinatus and deltoid was M0.
2. C5 injury which was diagnosed by neurophysiological investigations and ultrasound examination and then confirmed by subsequent intraoperative exploration and neurophysiological investigation.

Abbreviations: PDIC7, posterior division of ipsilateral C7; SSN, suprascapular nerve; AXN, axillary nerve; SAN, spinal accessory nerve; TMBRN, triceps muscle branch of radial nerve; EMG, electromyogram; SEP, sensory-evoked potential; CMAP, compound muscle action potential; CSA, cross-sectional area.



3. Reserved C7 function was diagnosed by the neurophysiological investigations and confirmed by subsequent intraoperative exploration and neurophysiological investigation.

The exclusion criteria are as follows:

1. History of diabetic mellitus and smoking history.
2. Fractures of the affected limb, neuromuscular disorders, and musculoskeletal disorders such as tendon or ligament injury were excluded.
3. Cases with an interval time between injury and surgery of less than 1 month or more than 12 months.

Surgical procedures

PDIC7 transfer to C5

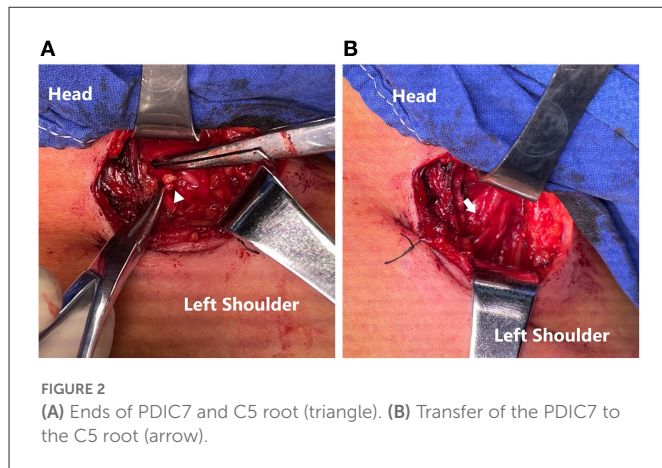
The operation was carried out under general anesthesia without using muscle relaxants. The arm, shoulder, neck, and chest were prepared with the patient supine.

The brachial plexus was explored through an incision of around 4 cm in length centered over the clavicle (Figure 1A), followed by an exploration of the anatomical structure of the 5 roots (Figure 1B). The continuity of the upper, middle, and lower trunks of the brachial plexus was then confirmed. C5 injury was identified by the absence of sensory-evoked potential (SEP) and compound muscle action potential (CMAP) of the deltoid while C6-T1 roots were unaffected. CMAP of the triceps and latissimus dorsi identified PDIC7. Using 8–0 sutures under a magnification of 2.5x, the PDIC7 was transferred to the C5 root (Figure 2).

SAN transfer to SSN plus TMBRN transfer to AXN

The operation was carried out as a two-stage procedure performed under general anesthesia without using muscle relaxants.

In the first stage, the brachial plexus was explored through an incision centered over the clavicle. The continuity of the upper, middle, and lower trunks of the brachial plexus was then confirmed. C5 injury was identified by the absence of sensory-evoked potential



(SEP) and compound muscle action potential (CMAP) of targeted muscles. The SSN was identified from the injured C5 root and the SAN was also separated in the same incision. The SAN was transferred to SSN. Additionally, in the second stage, the TMBRN was separated and transferred to AXN through a longitudinal incision on the posterior aspect of the arm. Details of the surgical procedures have been described before (10).

Postoperative management

Patients were asked to use a custom-made neck splint postoperatively for 3 weeks. Physiotherapy began 4 weeks after surgery to maintain the ROM in all joints, such as shoulder abduction, external rotation, and elbow flexion. However, passive shoulder abduction beyond 90° was avoided within 4 weeks after nerve repair.

Postoperative evaluation

Postoperative evaluations were performed at 14 days and then at approximate intervals of 3 months, with at least 12 months until no improvement can be observed.

A physical assessment and an electromyographic test were conducted in the clinic. The motor function was classified according to the Medical Research Council grading. The active and passive ranges of external rotation were evaluated beginning with the arm in the neutral position. The sagittal plane was defined as 0°. The range of movement was defined as the angle between the 0 and 180° position and the forearm position upon an external abduction (9).

Electromyography was conducted 3 months after surgery. CMAP of the deltoid was recorded as the sign of successful C5 regeneration and CMAP of the latissimus dorsi was recorded for evaluating the function loss of PDIC7.

Statistical analysis

Outcomes comprising continuous variables such as ROM were presented as mean $\pm$ std and analyzed using the *t*-test. The *p*-values were two-tailed and *p*-values of <0.05 were considered significant. All statistical analyses were conducted using SPSS for Windows.

Results

The characteristics of patients

From 2018 to 2020, 27 patients, 23 men, and 4 women, aged 16 to 63 years (average, 38.7 years), had brachial plexus C5 injury, and the average interval between injury and surgery was 3.9 months. A total of 12 patients were investigated in the group of PDIC7 transfer to C5. A total of 11 men and 1 woman, aged 36–67 years (average, 45.6 years), were investigated in the group of SAN transfer to SSN plus TMBRN transfer to AXN, and the average interval between injury and surgery was 4.1 months.

Motor and sensory function of the donor site

During the follow-up period, no patient in PDIC7 to C5 was observed with obvious elbow extension disorder or upper extreme adduction and pronation disorder. For PDIC7, we focused on the function of the triceps and latissimus dorsi with manual muscle testing, and reversible muscle strength decrease within 1 level (more than M3 postoperation) was thought to be acceptable. The follow-up EMG did not show the severe loss of function of the triceps and the latissimus dorsi, which showed that the motor function of the PDIC7 can be compensated. In terms of sensory function, 2 patients complained of transient middle finger numbness, 1 patient disappeared spontaneously during the follow-up period, and 1 patient with persistent numbness could adjust the discomfort by himself, and it was considered that it did not affect the quality of life.

For patients who underwent SAN transfer to SSN plus TMBRN transfer to AXN, we also tested the trapezius muscle and triceps in SAN transfer to SSN plus TMBRN transfer to AXN with the same standard as PDIC7. There were no evident motor or sensory function disorders in the dominated area of the donor's nerve, and the EMG test showed acceptable nerve and muscle function of the radial nerve and triceps.

Recovery of shoulder function

In all 27 patients who underwent PDIC7 to C5, the average maximal range of shoulder abduction was 105.9° from the worst case 65° to the best case 170° (Figure 3). A total of 26 of 27 patients achieved at least M3 muscle strength within 12 months and 50% of cases achieved M4 level. Among those cases of M3, 7 of 13 patients' injured side was not the dominant side. In contrast, in 12 patients who underwent SAN transfer to SSN plus TMBRN transfer to AXN, the average maximal range of shoulder abduction was 84.6° from the worst case 50° to the best case 100° and 11 of 12 patients achieved at least M3 muscle strength within 12 months and 36.4% cases achieved M4 level. The maximal range of shoulder abduction in patients who underwent PDIC7 to C5 was significantly greater than those who underwent SAN transfer to SSN plus TMBRN transfer to AXN ($p < 0.05$, Table 1).

All the detailed information of patients in our study is listed in Table 2.

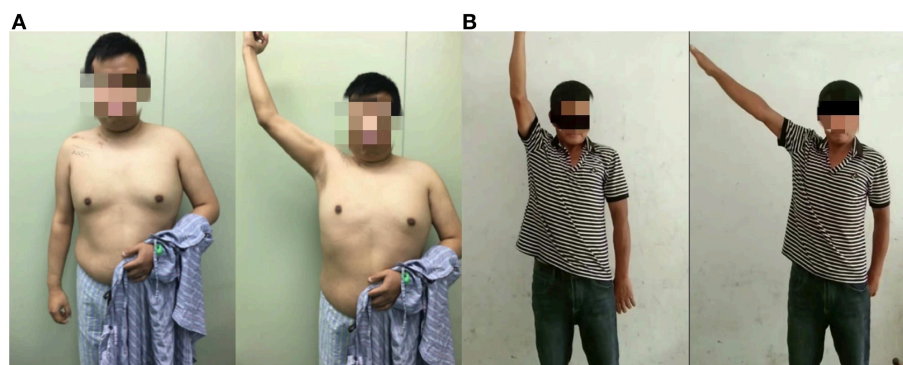


FIGURE 3
(A) Patient's functional recovery at 1 month. (B) Patient's functional recovery at 2 years.

TABLE 1 *t*-test result of the maximal range of shoulder abduction in two groups.

Method (Mean $\pm$ s)	ROM
PDIC7 ($n = 27$)	105.93 $\pm$ 27.18
SAN-SSN + TMBRN-AXN ($n = 12$)	84.58 $\pm$ 14.05
<i>t</i>	3.224
<i>p</i>	0.003**

ROM, Range of motion. ** $p < 0.01$.

Discussion

In our study, we demonstrate a novel surgical strategy, posterior division of ipsilateral C7 (PDIC7) transfer, for the treatment of neurogenic shoulder abduction limitation and compared its effect on improving shoulder function with a widely-accepted surgical strategy, the spinal accessory nerve (SAN) transfer to the suprascapular nerve (SSN) plus TMBRN transfer to AXN.

Lu et al. (11) have explored the motor fiber counts of the human C7 roots and their branches. They showed that the posterior division of C7 owned 66.2% of all the motor fibers from the C7 root and these fibers finally joined to the axillary nerve (27.94%), radial nerve (49.75%), and thoracodorsal nerve (52.7%). Lu et al. (12) also observed in a rat model that the functions of C7 dominated muscles, including the triceps brachii and latissimus dorsi, can be compensated in 2 months after ipsilateral C7 transfer procedures. In conclusion, previous studies supported at the anatomic level that PDIC7 transfer could provide sufficient motive power for functional improvement without apparent donor deficits. In this study, we revealed that PDIC7 transfer may be an effective surgical strategy to improve shoulder function in cases of C5 injury. Also, all cases receiving PDIC7 transfer showed acceptable triceps brachii and latissimus dorsi function in EMG during follow-up.

Previous studies reported cross-sectional area (CSA) of the human brachial plexus in ultrasonography. Niu et al. (13) reported that the CSA of the C5 root was $5.3 \pm 1.3 \text{ mm}^2$ and of the C7 root was $8.5 \pm 1.3 \text{ mm}^2$. Similarly, Bedewi et al. (14) reported that the CSA of the C5 root at the interscalene groove was $5.1 \pm 1.8 \text{ mm}^2$ and of the C7 root was $6.3 \pm 3.4 \text{ mm}^2$. Won et al. (15) reported that the CSA of the C5 root was $5.66 \pm 1.02 \text{ mm}^2$ and of the C7 root was 10.43

$\pm 1.86 \text{ mm}^2$. In our study, we observed that the CSA of PDIC7 and C5 roots matched well during operation. Also, as the donor nerve and acceptor nerve are close enough, the tension at the suture site can be small, which is beneficial for nerve regeneration.

Compared with SAN transfer to SSN plus TMBRN transfer to AXN, a widely-accepted surgical strategy, PDIC7 transfer to C5, has several advantages. First, surgeons could use a single supraclavicular incision for both brachial plexus exploration and repair. It is necessary to clarify the diagnosis of C5 injury during operation because C5 neurolysis alone may be sufficient to restore shoulder abduction if the continuity of C5 is reserved, whereas distal nerve transfer may lead to iatrogenic injury of SAN and TMBRN. Supportive evidence from Chuang et al. (16) showed that in their series, 9.5% of cases were identified intraoperatively as ruptured spinal nerves despite the preoperative imaging indicating complete root avulsion. Moreover, SAN transfer to SSN plus TMBRN transfer to AXN can only repair two nerves while PDIC7 transfer is to repair at the root level, which targets a group of muscles and can provide more motive power. Patients in the SAN-to-SSN plus TMBRN-to-AXN group did not experience sensory deficit, which is indeed a benefit of peripheral nerve transfer due to the presence of somatosensory fibers in either SAN or TMBRN. Although the patients in the PDIC7 group experienced sensory deficits, the sensory deficit was transient and not severe, which did not obscure the better motor recovery ability of PDIC7 transfer.

Another vital issue that should be considered for nerve transfer surgery is the matching of the original dominant muscles of the donor's nerve and the recipient nerve. Since cerebral control of the donor nerve differs from that of the recipient nerve, volitional movement of muscles dominated by the injured nerve after nerve transfer could be restricted and prognosis may be limited by cerebral plasticity (17). In our strategy, both PDIC7 and C5 were initially targeted to the deltoid, which avoided the cerebral function transfer phase of voluntary movement.

In addition, there are other factors that contribute to different outcomes of neurogenic shoulder function improvement, such as the interval between injury and surgery (18), age, and BMI (19), thus, future studies should examine these factors in greater detail. In our study, we applied PDIC7 transfer to the C5 root in patients aged 16–63 years and the interval between injury and surgery was 1–9 months. Most of them achieved satisfied prognosis without related side effects observed during the follow-up. We may, therefore,

TABLE 2 Detailed information on patients who underwent one of the two types of surgeries.

Sex	Age	Dominant side	Injured side	Cause	Surgery	Interval between injury and surgery	Duration of follow-up /months	Active ROM of shoulder abduction improvement	Shoulder abduction strength improvement	Preoperative EMG (deltoid)
Male	27	Right	Left	surgical injury	PDIC7—C5	2	12	0–90	M1-M3	MUP(–), CMAP(–)
Male	35	Left	Left	traffic accident	PDIC7—C5	2	12	0–100	M1-M4	MUP(–), CMAP(–)
Male	51	Right	Right	traffic accident	PDIC7—C5	3	24	0–100	M1-M4	MUP(–), CMAP(–)
Male	31	Right	Right	traffic accident	PDIC7—C5	3	12	0–90	M1-M4	MUP(–), CMAP(–)
Male	23	Right	Right	traffic accident	PDIC7—C5	2	12	0–170	M1-M4	MUP(–), CMAP(–)
Female	46	Right	Left	crush injury	PDIC7—C5	4	12	0–80	M1-M3	MUP(–), CMAP(–)
Male	37	Right	Right	neuritis	PDIC7—C5	6	12	0–95	M1-M3	MUP(–), CMAP(–)
Male	63	Right	Right	traffic accident	PDIC7—C5	3	12	0–80	M1-M3	MUP(–), CMAP(–)
Male	52	Right	Left	traffic accident	PDIC7—C5	3	30	0–80	M1-M3	MUP(–), CMAP(–)
Male	47	Right	Right	traffic accident	PDIC7—C5	4	36	0–120	M1-M4	MUP(–), CMAP(–)
Female	42	Right	Right	traffic accident	PDIC7—C5	4	12	0–130	M1-M4	MUP(–), CMAP(–)
Male	19	Right	Left	traffic accident	PDIC7—C5	3	12	0–120	M1-M4	MUP(–), CMAP(–)
Male	20	Right	Left	traffic accident	PDIC7—C5	5	12	0–90	M1-M3	MUP(–), CMAP(–)
Male	55	Right	Right	traffic accident	PDIC7—C5	8	30	0–90	M1-M3	MUP(–), CMAP(–)
Female	34	Right	Left	traffic accident	PDIC7—C5	2	12	0–90	M1-M3	MUP(–), CMAP(–)
Male	54	Right	Right	falling injury	PDIC7—C5	2	12	0–100	M1-M4	MUP(–), CMAP(–)
Male	31	Right	Right	traffic accident	PDIC7—C5	6	12	0–90	M1-M3	MUP(–), CMAP(–)
Male	47	Left	Right	traffic accident	PDIC7—C5	9	12	0–65	M1-M2	MUP(–), CMAP(–)
Male	52	Right	Left	traffic accident	PDIC7—C5	5	12	0–100	M1-M3	MUP(–), CMAP(–)
Male	16	Right	Left	neuritis	PDIC7—C5	7	12	0–95	M1-M3	MUP(–), CMAP(–)
Male	29	Right	Right	falling injury	PDIC7—C5	3	12	0–155	M1-M4	MUP(–), CMAP(–)
Male	45	Right	Right	strangulation	PDIC7—C5	6	21	0–100	M1-M4	MUP(–), CMAP(–)
Male	54	Right	Right	crush injury	PDIC7—C5	1	27	0–165	M1-M4	MUP(–), CMAP(–)
Male	51	Right	Right	falling injury	PDIC7—C5	1	12	0–100	M1-M3	MUP(–), CMAP(–)
Female	26	Right	Left	incised injury	PDIC7—C5	2	12	0–125	M1-M4	MUP(–), CMAP(–)
Male	63	Right	Right	crush injury	PDIC7—C5	4	24	0–90	M1-M3	MUP(–), CMAP(–)
Male	16	Right	Left	surgical injury	PDIC7—C5	3	12	0–150	M1-M4	MUP(–), CMAP(–)
Male	51	Right	Left	crush injury	SAN-SSN TMBRN-AXN	7	12	0–50	M1-M2	MUP(–), CMAP(–)

(Continued)

TABLE 2 (Continued)

Sex	Age	Dominant side	Injured side	Cause	Surgery	Interval between injury and surgery	Duration of follow-up /months	Active ROM of shoulder abduction improvement	Shoulder abduction strength improvement	Preoperative EMG (deltoid)
Male	36	Right	Right	traffic accident	SAN-SSN TMBRN-AXN	1	12	0-80	M1-M3	MUP(-), CMAP(-)
Male	36	Right	Left	traffic accident	SAN-SSN TMBRN-AXN	8	12	0-95	M1-M3	MUP(-), CMAP(-)
Male	45	Right	Left	neuritis	SAN-SSN TMBRN-AXN	7	12	0-90	M1-M3	MUP(-), CMAP(-)
Male	39	Right	Right	crush injury	SAN-SSN TMBRN-AXN	2	12	0-90	M1-M4	MUP(-), CMAP(-)
Male	32	Right	Right	crush injury	SAN-SSN TMBRN-AXN	5	12	0-80	M1-M3	MUP(-), CMAP(-)
Male	42	Right	Left	crush injury	SAN-SSN TMBRN-AXN	2	12	0-90	M1-M3	MUP(-), CMAP(-)
Male	53	Right	Left	crush injury	SAN-SSN TMBRN-AXN	2	12	0-100	M1-M4	MUP(-), CMAP(-)
Male	56	Right	Right	falling injury	SAN-SSN TMBRN-AXN	3	12	0-70	M1-M3	MUP(-), CMAP(-)
Male	33	Right	Right	traffic accident	SAN-SSN TMBRN-AXN	2	12	0-100	M1-M4	MUP(-), CMAP(-)
Male	57	Right	Right	traffic accident	SAN-SSN TMBRN-AXN	5	12	0-90	M1-M4	MUP(-), CMAP(-)
Female	67	Right	Right	traffic accident	SAN-SSN TMBRN-AXN	5	12	0-80	M1-M3	MUP(-), CMAP(-)

preliminarily demonstrate that PDIC7 transfer is a safe, one-stage, and effective surgical procedure for patients with neurogenic shoulder abduction limitation. Furthermore, based on our clinical experience, patients with C5 injury who received only PDIC7 transfer can achieve satisfactory improvement in shoulder function without combining other additional surgical strategies.

In our study, the causes behind shoulder abduction limitation varied. Among all, traffic accidents were the most common cause (51.3%) and mechanical injuries accounted for 92.3% of all causes. Also, there were two neuritis cases and one postoperative injury case that underwent our strategy and received a good prognosis. As for patients with neuritis or other neurologic problems, our strategy can also be available for those who did not receive satisfying functional improvement after conservative therapies such as waiting, medicines, or physical therapies. It should be noted that the two neuritis cases in this study had received simple neurolysis surgery but had not obtained satisfying recovery more than 12 months after surgery, thus, we performed a PDIC7 transfer for them and they received benign effects.

Furthermore, the patient whose shoulder abduction only achieved 85° was a 47-year-old male patient. There may be two main reasons for his poor recovery. One is that the injured side was not his dominant side and the other is the long interval between injury and surgery. Before his surgery, there had been apparent muscle atrophy on his injured upper extremity. This question is worth discussing and studying if the long interval time and irreversible muscle atrophy may predict a poor prognosis and the probable degeneration of nerve or muscle tissue during the interval time should be studied more, which is of great clinical value to choosing better treatment strategies for patients.

Although in our study, PDIC7 transfer to C5 has shown a better therapeutic effect than traditional surgical strategies, we still hope to explore more for improvement. For further research, we are curious about if PDIC7 could be divided into more selective branches, which requires not only clinical but also more basic and anatomical studies to get a better understanding of the brachial plexus nerves.

For all patients with neurogenic shoulder abduction limitation, PDIC7 transfer is a generally safe, one-stage, and effective strategy for restoring shoulder function.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XH and ZY collected the cases and analyzed the data. ZY and JD reviewed the previous literatures. XH and YX wrote the draft. YX and JD revised the manuscript. JJ conceptualized the main idea and supervised the whole study. XH, ZY, YX, JD, and JJ contributed to review the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was sponsored by the Shanghai Municipal Key Clinical Specialty (shslczdk05601).

Acknowledgments

We would like to thank our colleagues from Huashan Hospital (Fudan University) who provided

insight and expertise that greatly assisted with the research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Hsueh YH, Tu YK. Surgical reconstructions for adult brachial plexus injuries. Part I: Treatments for combined C5 and C6 injuries, with or without C7 injuries. *Injury*. (2020) 51:787–803. doi: 10.1016/j.injury.2020.02.076
2. Thompson SE, Smith ZA, Hsu WK, Nassr A, Mroz TE, Fish DE, et al. C5 Palsy after cervical spine surgery: a multicenter retrospective review of 59 cases. *Global Spine J*. (2017) 7:64s–70s. doi: 10.1177/2192568216688189
3. Suzuki K, Doi K, Hattori Y, Pagsaligan JM. Long-term results of spinal accessory nerve transfer to the suprascapular nerve in upper-type paralysis of brachial plexus injury. *J Reconstr Microsurg*. (2007) 23:295–9. doi: 10.1055/s-2007-985205
4. Leechavengvongs S, Witoonchart K, Uerpaiojkit C, Thuvathakul P, Malungpaishrope K. Combined nerve transfers for C5 and C6 brachial plexus avulsion injury. *J Hand Surg Am*. (2006) 31:183–9. doi: 10.1016/j.jhssa.2005.09.019
5. Dong Z, Zhang CG, Gu YD. Surgical outcome of phrenic nerve transfer to the anterior division of the upper trunk in treating brachial plexus avulsion. *J Neurosurg*. (2010) 112:383–5. doi: 10.3171/2009.4.JNS081064
6. Anastakis DJ, Malessy MJ, Chen R, Davis KD, Mikulis D. Cortical plasticity following nerve transfer in the upper extremity. *Hand Clin*. (2008) 24:425–44, vi–vii. doi: 10.1016/j.hcl.2008.04.005
7. Gu YD, Cai PQ, Xu F, Peng F, Chen L. Clinical application of ipsilateral C7 nerve root transfer for treatment of C5 and C6 avulsion of brachial plexus. *Microsurgery*. (2003) 23:105–8. doi: 10.1002/micr.10113
8. Xu JG, Wang H, Hu SN, Gu YD. Selective transfer of the C7 nerve root: an experimental study. *J Reconstr Microsurg*. (2004) 20:463–70. doi: 10.1055/s-2004-833503
9. Yin HW, Jiang S, Xu WD, Xu L, Xu JG, Gu YD. Partial ipsilateral C7 transfer to the upper trunk for C5–C6 avulsion of the brachial plexus. *Neurosurgery*. (2012) 70:1176–81. doi: 10.1227/NEU.0b013e3182400a91
10. Wolfe SW, Hotchkiss RN, Pederson WC, Kozin SH, Cohen MS. *Green's Operative Hand Surgery*. Seventh ed. Philadelphia, PA: Elsevier (2017).
11. Wei LU, Jiang-Guang XU, Xiao JD. A study of the motor fiber counts of the human 7th cervical nerve root and its branches. *Chin J Clin Anat*. (2004) 2004:518–21. doi: 10.13418/j.issn.1001-165x.2004.05.022
12. Lu W, Xiao J, Xu J, He Q, Li J, Wang D, et al. [Study on the quantity and distribution of motor fiber of rat's C7 nerve root]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi*. (2005) 19:857–9.
13. Niu J, Li Y, Zhang L, Ding Q, Cui L, Liu M. Cross-sectional area reference values for sonography of nerves in the upper extremities. *Muscle Nerve*. (2020) 61:338–46. doi: 10.1002/mus.26781
14. Bedewi MA, Kotb MA. Ultrasound reference values of C5, C6, and C7 brachial plexus roots at the interscalene groove. *Neurol Sci*. (2021) 42:2425–9. doi: 10.1007/s10072-020-04836-1
15. Won SJ, Kim BJ, Park KS, Kim SH, Yoon JS. Measurement of cross-sectional area of cervical roots and brachial plexus trunks. *Muscle Nerve*. (2012) 46:711–6. doi: 10.1002/mus.23503
16. Hu CH, Chang TN, Lu JC, Laurence VG, Chuang DC. Comparison of surgical strategies between proximal nerve graft and/or nerve transfer and distal nerve transfer based on functional restoration of elbow flexion: a retrospective review of 147 patients. *Plast Reconstr Surg*. (2018) 141:68e–79e. doi: 10.1097/PRS.0000000000003935
17. Dahlin LB, Andersson G, Backman C, Svensson H, Björkman A. Rehabilitation, using guided cerebral plasticity, of a brachial plexus injury treated with intercostal and phrenic nerve transfers. *Front Neurol*. (2017) 8:72. doi: 10.3389/fneur.2017.00072
18. Solla DJF, de Oliveira AJM, Riechelmann RS, Martins RS, Siqueira MG. Functional outcome predictors after spinal accessory nerve to suprascapular nerve transfer for restoration of shoulder abduction in traumatic brachial plexus injuries in adults: the effect of time from injury to surgery. *Eur J Trauma Emerg Surg*. (2020). doi: 10.1007/s00068-020-01501-2
19. Xiao C, Lao J, Wang T, Zhao X, Liu J, Gu Y. Intercostal nerve transfer to neurotize the musculocutaneous nerve after traumatic brachial plexus avulsion: a comparison of two, three, and four nerve transfers. *J Reconstr Microsurg*. (2014) 30:297–304. doi: 10.1055/s-0033-1361840



OPEN ACCESS

EDITED BY
Hui Lu,
Zhejiang University, China

REVIEWED BY
Quanjian Dong,
Qingdao University Medical College, China
Marco Rinaudo,
Agostino Gemelli University Polyclinic
(IRCCS), Italy

*CORRESPONDENCE
Rui Cong
✉ congri@fmmu.edu.cn

[†]These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 30 October 2022
ACCEPTED 24 January 2023
PUBLISHED 08 February 2023

CITATION
Zhang J-I, Xian H, Zhao R, Luo C, Xie R-g,
Tian T and Cong R (2023) Brachial plexus
avulsion induced changes in gut microbiota
promotes pain related anxiety-like behavior in
mice. *Front. Neurol.* 14:1084494.
doi: 10.3389/fneur.2023.1084494

COPYRIGHT
© 2023 Zhang, Xian, Zhao, Luo, Xie, Tian and
Cong. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Brachial plexus avulsion induced changes in gut microbiota promotes pain related anxiety-like behavior in mice

Jian-lei Zhang^{1†}, Hang Xian^{1†}, Rui Zhao¹, Ceng Luo², Rou-gang Xie²,
Tong Tian¹ and Rui Cong^{1*}

¹Department of Orthopedics, Xijing Hospital, Air Force Medical University, Xi'an, China, ²Department of Neurobiology, School of Basic Medicine, Air Force Medical University, Xi'an, China

Introduction: Brachial plexus avulsion (BPA) injury develops frequent and intense neuropathic pain, involving in both peripheral and central nervous systems. The incidence of anxiety or depression caused by BPA-induced neuropathic pain is high, but the underlying mechanism remains unclear.

Methods: We established a BPA mice model and assessed its negative emotions through behavioral tests. To further explore the role of the microbiota-gut-brain axis in the unique emotional behavior after BPA, we performed intestinal fecal 16s and metabolomics assays. Psychobiotics (PB) supplementation was administered to BPA mice to check the probiotics effects on BPA-induced anxiety behaviors.

Results: Pain related anxiety-like behavior was observed at the early stage after BPA (7 days), while no depression-like behavior was detected. Intriguingly, gut microbiota diversity was increased in BPA mice, and the most abundant probiotics, *Lactobacillus*, showed obvious changes. *Lactobacillus_reuteri* was significantly decreased in BPA mice. Metabolomics analysis showed that *Lactobacillus_reuteri*-related bile acid pathway and some neurotransmitter amino acids were significantly altered. Further PB (dominated by *Lactobacillus_reuteri*) supplementation could significantly relieve BPA-induced anxiety-like behaviors in mice.

Conclusion: Our study suggests that pathological neuralgia after BPA could alter intestinal microbiota diversity, especially *Lactobacillus*, and the changes in neurotransmitter amino acid metabolites may be the key reason for the onset of anxiety-like behaviors in BPA mice.

KEYWORDS

brachial plexus avulsion, microbiota-gut-brain axis, metabolites, anxiety, psychobiotics

1. Introduction

Brachial plexus injury (BPI) is a common and special type of peripheral nerve injury, which usually occurs in traffic accidents, especially motorcycles (1). BPI is often accompanied by severe pain complications called neuropathic pain (NPP), which is characterized by spontaneous pain, hyperalgesia, and hyperalgesia. The incidence of NPP is high in the patients with BPI. Around 30–80% patients within brachial plexus avulsion (BPA) show symptoms of NPP (2, 3). BPA is a mixed injury involving both peripheral and central nervous mechanisms (4). BPA causes immediate pain which gets rapid onset after trauma; along with the progression of long-term neuropathy, this pain may be observed away from the lesions (5). Nowadays, the research on BPA mainly focused on nerve transplantation procedures, which ignores BPA-induced pain and related comorbidities like emotional and/or cognitive impairments that largely worsen the quality of patients' life (6). Previous reports have revealed that the incidence of negative stress responses, including anxiety and depression, of patients with BPA could reach up to 30–50% (7). A follow-up study of 415 patients with BPA found

that 81.20% of them appeared anxiety or depression (8). Another clinical trial of BPA patients also showed that even with active surgical intervention, there were still 76% of patients suffered by frequent pain and 18.8% of them accompanied with depression or anxiety (9). Therefore, BPA induced neuropathic pain and related negative emotions should get more attention.

The impact of symbiotic microbiota on various host functions has been increasingly recognized over the past decade. It is well-studied that gut microbes influence many biological processes including immunity, metabolism, and central nervous system (CNS) (10–12). The effects of gut microbiota on NPP animal models have also been characterized (13, 14). Extensive studies have shown that the microbiota can directly or indirectly influence brain homeostasis through affecting anxiety and the occurrence of neurological diseases were related to nerve conduction dysfunction in animal models (15). Therapeutic intervention with probiotic can affect the emotional behaviors of depressive mice and alleviate depression in patients (16, 17). In this study, to explore whether gut microbiota also play roles in the negative emotions caused by BPA, we investigated the changes of behaviors, gut microbes, and metabolites in BPA mice through bioinformatics analysis of 16s sequencing and metabolomics, and also provided supporting evidence for the therapeutic intervention with intestinal probiotics.

2. Methods and materials

2.1. Establishment of BPA mice model

Adult female C57BL/6 mice (6–8 weeks old, 20–25 g) were randomly divided into sham and BPA group ($n = 10$ in each). The mice were housed in the specific pathogen-free laboratory of Animal Center of Air Force Medical University. During modeling, the mice were anesthetized with 1% sodium pentobarbital (45 mg/kg) and fixed on the operating table. The upper limbs were stretched, and the left limb was chosen to carry out a transverse incision (0.8–1.2 cm) at 0.3–0.5 cm under the collarbone. Brachial plexus was separated and the C7 nerve root was exposed. The 2–0 silk thread was used to wrap the C7 nerve, then the avulsion injury was performed through the stretch of the thread under a constant speed. The visibility of the corresponding nerve root and dorsal root body are considered as successful avulsion (Figure 1A). After avulsion, the stump was put back to the muscle space between pectoralis major and minor muscles, followed by incision suture using 3–0 silk thread. The mice were reared in cages after waking up. Mice in sham group underwent same operation to expose the brachial plexus without any further injury.

2.2. Behavioral testing

2.2.1. Pain behavior detection

The basal and threshold value of pain behavior on the left forepaw and left hindpaw were measured on 1 day before and 7, 14, and 21 days after BPA modeling. Behavioral parameters of paw mechanical withdrawal threshold (PMWT) and thermal withdrawal latency (TWL) were assessed.

To evaluate PMWT, one black plastic box with a lid was placed on a rack with a metal mesh (0.3 × 0.3 cm) at the bottom, and the test was carried out after 0.5–1 h adaptation. Von Frey filaments of

different grams were used to detect the skin sensation of the mice planta pedis. The planta was stimulated 5 times at an interval of 3 min, and mice showed a positive selection when they lifted or licked their paws. The gram thresholds were recorded and the average of them was calculated.

To evaluate TWL, the black plastic box with a lid was placed on a glass plate (35°C). Environment adaptation was conducted 0.5–1 h before testing. The thermal radiation intensity and the irradiation time were set in advance to prevent skin burns caused by prolonged irradiation to the mice planta pedis. The maximum irradiation time was set as 20 s. The mice planta pedis was irradiated with pre-set thermal radiation intensity. The irradiation was stopped immediately and the time was recorded when the mice retracted, lifted, shook, or licked their paws. After five repetitions, the average time was calculated as the TWL value.

2.2.2. Motor ability detection

The beam walking test (BWT) and Rota-Rod test were conducted to detect the effects of BPA on the murine motor function. For BWT, one side of the balance beam was placed in a black square box, and the mouse was placed on the other side. The time and number of skids for mice entering the black square box through the balance beam were recorded within 60 s. The test included training period and testing period. If the mouse failed to reach the box, then the time was recorded as 60 s. Rota-Rod test also had pre-training and testing periods. During testing, the animal was placed on the rotating rod. Once fell off the rod, the mice were put back immediately. The latency time for falling and rotation speed during falling were recorded. After three repetitions, the average delay time and rotation speed were calculated.

2.2.3. Behavioral testing for anxiety and depression

Open field test (OFT): OFT was performed to detect the animal autonomous behavior, exploratory behavior, and tension in a new environment. The open field box (50 × 50 × 35 cm, L × W × H) was placed with a camera directly above the box connecting to the computer. EthoVision XT behavioral video recording software was used to record the animal behavior during experiments. The mouse was placed in the center of the open field with the back to the researcher. Moving trajectory was recorded for 10 min, and the movement distance, speed, and residence time in the central area were analyzed.

Elevated plus-maze test (EPM): The EPM equipment consisted of two open arms (30 × 7 cm) and two closed arms (30 × 7 × 15 cm) with a height of 60 cm from the ground. During experiments, all mice were placed in the same position at the center of the maze with their head to the open arms. The entry times of open and closed arms and the duration within each arm in 5 min were recorded.

Tail suspension test (TST): The rear 1/3 of the tail of the mouse was hung on a support with the head 15 cm away from the table. Six minutes later, the immobile time of mice was recorded and analyzed using Smart v3.0 animal behavior analysis software.

Forced swimming test (FST): The mouse was put into a transparent cylinder (10 × 18 cm, Diameter × H) for 6 min that had been filled with water at 25 ± 1°C. The animal activity in the water was recorded automatically by Smart v3.0 animal behavior analysis software. The immobile duration of mice was analyzed.

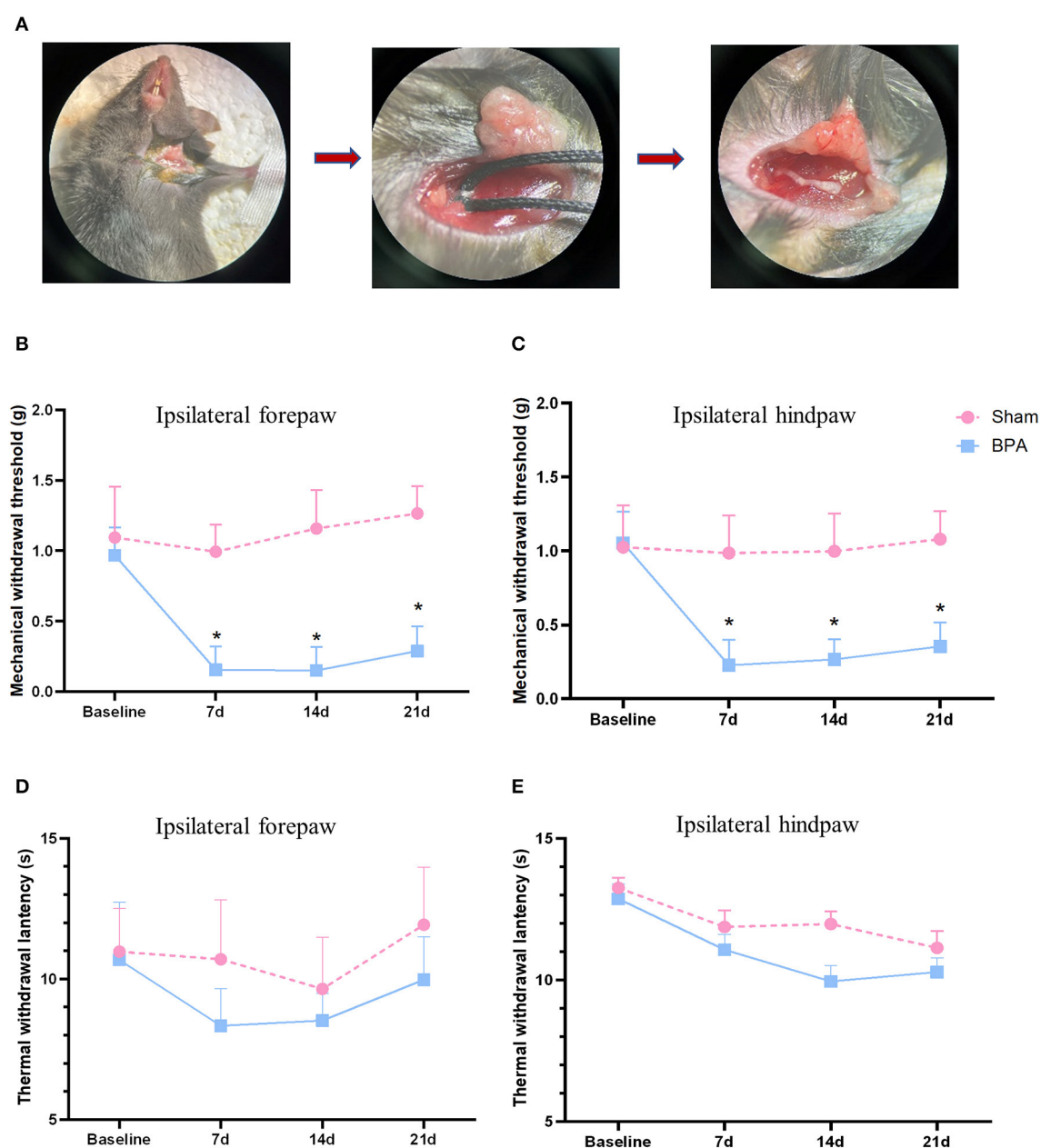


FIGURE 1 BPA modeling and pain behavior detection over time. **(A)** Schematic diagram of BPA operation: from brachial plexus dissociate the C7 nerve separation to nerve avulsion. **(B)** PMWT results of the operative limb 1 day before and 7, 14, and 21 days after modeling. **(C)** PMWT results of the ipsilateral hindlimb of sham and BPA mice. **(D)** No significant difference of TWL in the operative limb between sham and BPA mice at 1 day before and 7, 14, and 21 days after modeling. **(E)** TWL results of the ipsilateral hindlimb of the two group at each time point. $N = 10$ per group. * $P < 0.05$ vs. sham at the same time point. Unpaired T -test was used for data analysis. BPA, brachial plexus avulsion; PMWT, paw mechanical withdrawal threshold; TWL, thermal withdrawal latency.

Each group of 10 mice was independent of the different behavioral tests at each time point.

2.3. Gut microbiota 16s sequencing of BPA mice

Fecal samples (150–200 mg) from ileocecal sections were collected on 14 days after BPA modeling. Each group contained 8 mice at the beginning, and only 5 samples in the sham and 7

samples in the BPA group passed quality detection and go forward following sequencing and analysis. HiPure Stool DNA Kits (#D3141, Megan Biotechnology Co., LTD., Guangzhou, China) was used to extract fecal DNAs, that were then detected by Nanorop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), agarose gel electrophoresis apparatus DYY-6C (BJLiuYi, Beijing, China), and gel imaging system Tanon-2500 (Shanghai Tianeng Technology Co., LTD., China). The second round pf PCR was performed after quality qualified. AMPure XP Beads were used to purify the amplification products, and ABI StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA) was

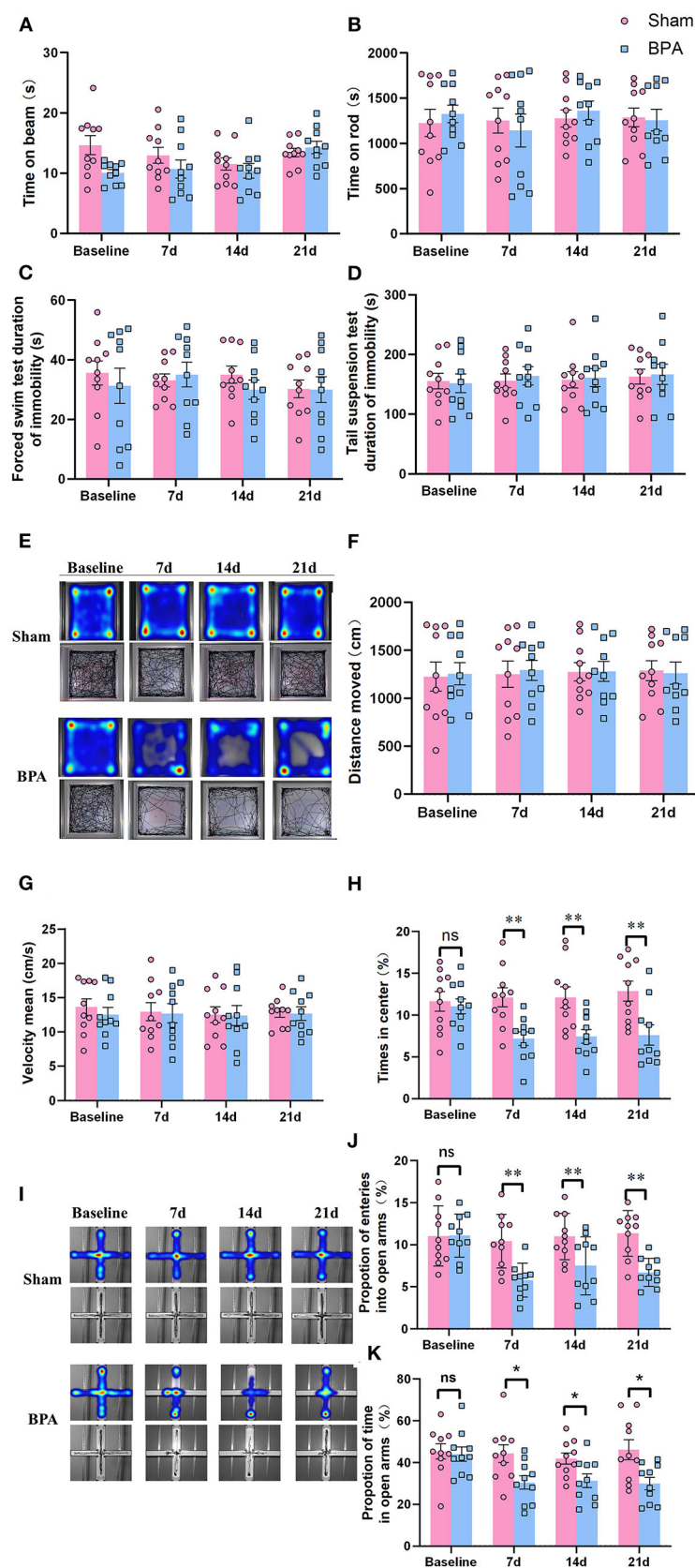


FIGURE 2

Detection of depression and anxiety-like behaviors in BPA mice over time. (A) BWT detection showed no difference of the time on the beam at each time point. (B) Rota-Rod test showed no difference of the time on the rod at each time point. (C) FST showed no difference of the immobility duration at each time point. (D) TST shows no difference of the immobility duration at each time point. (E) OFT trajectory diagram in the two groups over time. (F) The movement distance from OFT test between the two groups at each time point. (G) The movement speed from OFT test between the two groups at each time point. (H) The central area residence time from OFT test between the two groups at each time point. (I) EPM trajectory diagram in the two groups. (Continued)

FIGURE 2 (Continued)

(J) EPM: The entering times of the open arms at each time point. (K) EPM: The open arm residence time at each time point. $N = 10$ per group. Ns = no statistical significance, $*P < 0.05$, $**P < 0.01$ vs. sham. Unpaired T -test was used for data analysis. BPA, brachial plexus avulsion; BWT, beam walking test; FST, forced swimming test; TST, tail suspension test; OFT, open field test; EPM, elevated plus-maze test.

used for quantification. Sequencing was performed according to the PE250 mode pooling of Novaseq 6000. For 16s sequencing data analysis, raw reads were firstly filtered by the rules: reads contained $>10\%$ of unknown nucleotides and/or $<50\%$ of bases with quality >20 . Then, filtered reads were assembled into raw tags through FLASH. After raw tag filtering, operational taxonomic units (OTUs) were obtained *via* clean tag clustering by UPARSE pipeline and chimera removal was conducted to get effective tags for further analysis.

2.4. Sequencing of intestinal metabolites in BPA mice

The cultural medium from the mice fecal samples was removed. LC-MS/MS analyses were performed using an UHPLC system (1,290, Agilent Technologies, Santa Clara, CA, USA) with a UPLC HSS T3 column coupled to a Q Exactive mass spectrometer (Orbitrap MS, Thermo Fisher Scientific). The mobile phase A was 0.1% formic acid in water for positive mode, and 5 mmol/L ammonium acetate in water for negative mode, and the mobile phase B was acetonitrile. The injection volume was 2 μ L. The QE mass spectrometer was used because its ability to acquire MS/MS spectra on an information-dependent basis (IDA) during an LC/MS experiment. In this mode, the acquisition software (Xcalibur 4.0.27, Thermo Fisher Scientific) continuously evaluates the full scan survey MS data as it collects and triggers the acquisition of MS/MS spectra depending on preselected criteria. ESI source conditions were set as follows: sheath gas flow rate was 45 Arb, aux gas flow rate was 15 Arb, capillary temperature was 320°C, full MS resolution was 70,000, MS/MS resolution was 17,500, collision energy was 20/40/60 eV in the NCE model, spray voltage was 3.8 (+) or -3.1 kV (-). Through bioinformatics analysis, potential differentially expressed metabolites were identified according to specific screening conditions ($VIP > 1$ and $P < 0.05$). Metabolomic profiling was performed in collaboration with Guangzhou Genedenovo Biotechnology Co., Ltd. (Guangzhou, China).

2.5. Association analysis of the intestinal microbiota and metabolites bioinformatics in BPA mice

Pearson correlation coefficient of microbiota and metabolomic datasets between every level were calculated in R (version 3.5.1). The correlation heatmap was generated using pheatmap package in R. The network analysis was performed using igraph package in R.

2.6. Effects of psychobiotics (PB) supplementation on BPA mice behavior

The mice were randomly divided into sham, BPA, and BPA + PB groups ($n = 10$ per group). Mice in the BPA + PB group were intragastrically administrated with PB every day after BPA modeling. The PB used was purchased from Jiangnan University containing *Bifidobacterium bifidum* F-35, *Bifidobacterium longum* CCFM729, *Lactobacillus plantarum* CCFM639, *Lactobacillus acidophilus* CCFM137, *Lactobacillus casei* CN1566, *Lactobacillus reuteri* DSM17938, and *Lactobacillus rhamnosus* CCFM10281 (0.02 mg/kg). Mice in sham and BPA groups were intragastrically administrated with galactose oligosaccharide (20.2%). Pain behavior detection, OFT, and EPM tests were carried out 1 day before and 14 days after operation.

2.7. Statistical analysis

A total of 126 mice were used and randomly divided into each group for behavior tests ($n = 10$ per group) and 16s sequencing ($n = 8$ per group) in this study. Analyzed data were expressed as mean $\pm$ standard deviation (SD), and statistically analyzed by unpaired T -test within two groups and ordinary one-way ANOVA within three groups using GraphPad Prism 8.0 software. Besides, p -value < 0.05 was considered statistically significant.

3. Results

3.1. BPA could induce stable mechanical allodynia of the affected forepaw

After successful procedure of avulsion injury, the single C7 nerve root could be observed (Figure 1A). Mechanical threshold of the affected forepaw and ipsilateral hindpaw decreased at 7 days after BPA and lasted until 21 days, which had significant differences compared to sham group (Figures 1B, C). While, no obvious difference was observed in TWL tests between the two groups of the ipsilateral forepaw and ipsilateral hindpaw (Figures 1D, E). These results indicated that this single C7 root avulsion model could mimic the neuropathic pain state like BPA patients which fulfills the needs of the following investigations.

3.2. Comorbidity of pain related anxiety-like behavior occurred after BPA happens

BWT detection showed that there was no significant difference in balance ability, muscle strength, and motor coordination between sham and BPA mice at 1 day before and 7, 14, and 21 days after

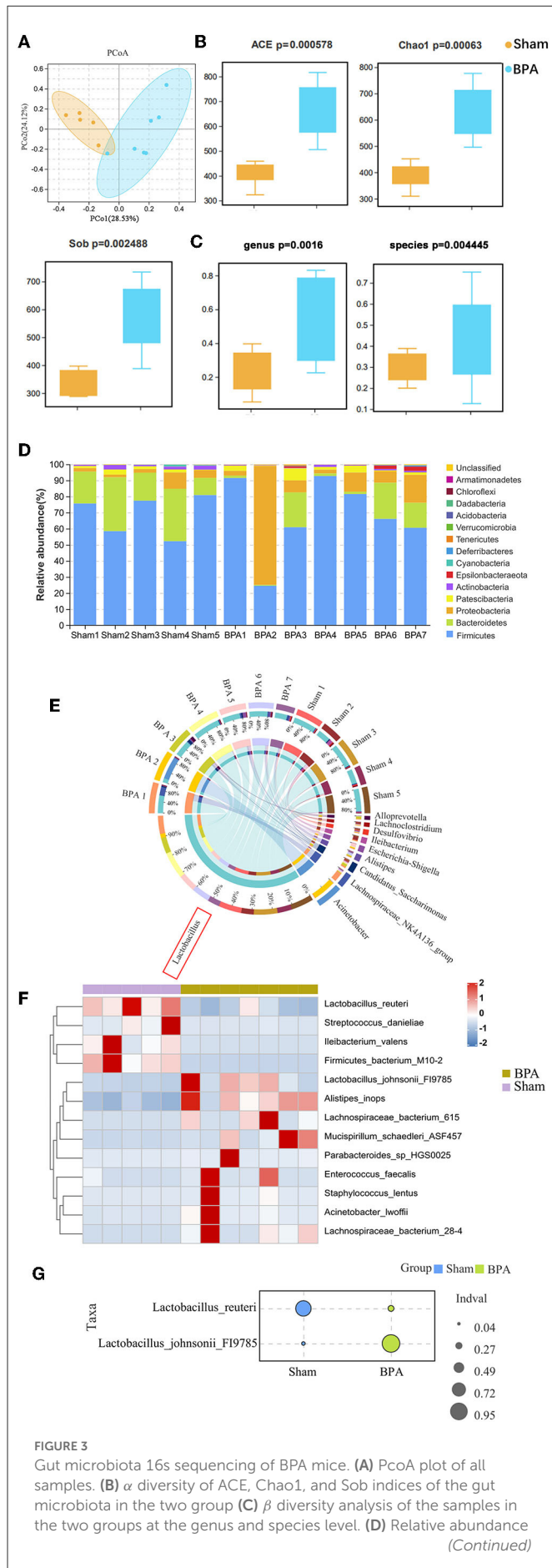


FIGURE 3 (Continued)

of gut microbiota at the phylum levels in the two groups. (E) Distribution circle map of 16s species composition. (F) Heat map showing that indicator species analysis between the groups. (G) Indicator value analysis of 16s indicator species, and *Lactobacillus_reuteri* and *Lactobacillus_johnsonii* with the most significant differences between groups were shown. $N = 5$ in BPA group and $N = 7$ in sham group. BPA, brachial plexus avulsion; PcoA, principal coordinates analysis.

modeling (Figure 2A). Similarly, in Rota-Rod test, no difference of time on rod was observed between sham and BPA mice at each time point (Figure 2B). The immobile duration from FST and TST results indicated that mice in BPA group had no significant depression-like behavior before and after modeling compared to the sham group (Figures 2C, D). In addition, the trajectory diagram of OFT experiments clearly showed a decrease of the residence track in the center area at 7, 14, and 21 days after BPA modeling (Figure 2E). Statistical analysis showed no significant difference in the movement distance and speed of mice between the two groups (Figures 2F, G), but the residence time at the center area was significantly decreased at 7, 14, and 21 days after BPA compared with sham group (Figure 2H). Moreover, EPM trajectory diagram showed that the open arm entering frequency was reduced after BPA modeling (Figure 2I). Meanwhile, the entry times of open arms and the duration were significantly reduced in BPA group compared with the sham (Figures 2J, K). The above results showed single root avulsion won't affect the motor function of the BPA mice, and the pain related anxiety-like behavior occurred after BPA.

3.3. BPA altered gut microbiota composition

To investigate changes in gut microbiota induced by BPA-caused NPP in mice, 16s rDNA amplicons from fecal samples of sham ($n = 5$) and BPA groups ($n = 7$) were sequenced. Principal coordinates analysis (PcoA) of groups indicated that in general, the species community structure was similar within the two groups but distinct between them (Figure 3A). The α -diversity of microbiota between sham and BPA samples was compared by ACE, chao1, and Sob indices. Alpha-diversity mainly reveals species richness by counting the number of species or OTUs. Results from all three α -diversity indices showed that compared to the sham, samples in BPA had significantly increased α -diversity, indicating elevated species richness (Figure 3B). Another β diversity analysis informs the diversity of microbiota. Results of the β diversity of the samples in the BPA groups were also significantly higher than that in the sham, at both genus and species levels (Figure 3C). Hence, BPA could increase the richness and diversity of intestinal flora. Moreover, relative abundance of gut microbiota at the phylum level in the two groups was shown. The three largest bacterial phyla were Firmicutes, Bacteroidetes, and Proteobacteria (Figure 3D). Moreover, species composition distribution showed that *Lactobacillus* genera had the most obvious change (Figure 3E). We further analyzed the significant flora changes at the species level, and 13 significantly-altered species were shown (Figure 3F). Among them, *Lactobacillus_reuteri* and *Lactobacillus_johnsonii* were the two species with the most significantly different indicator

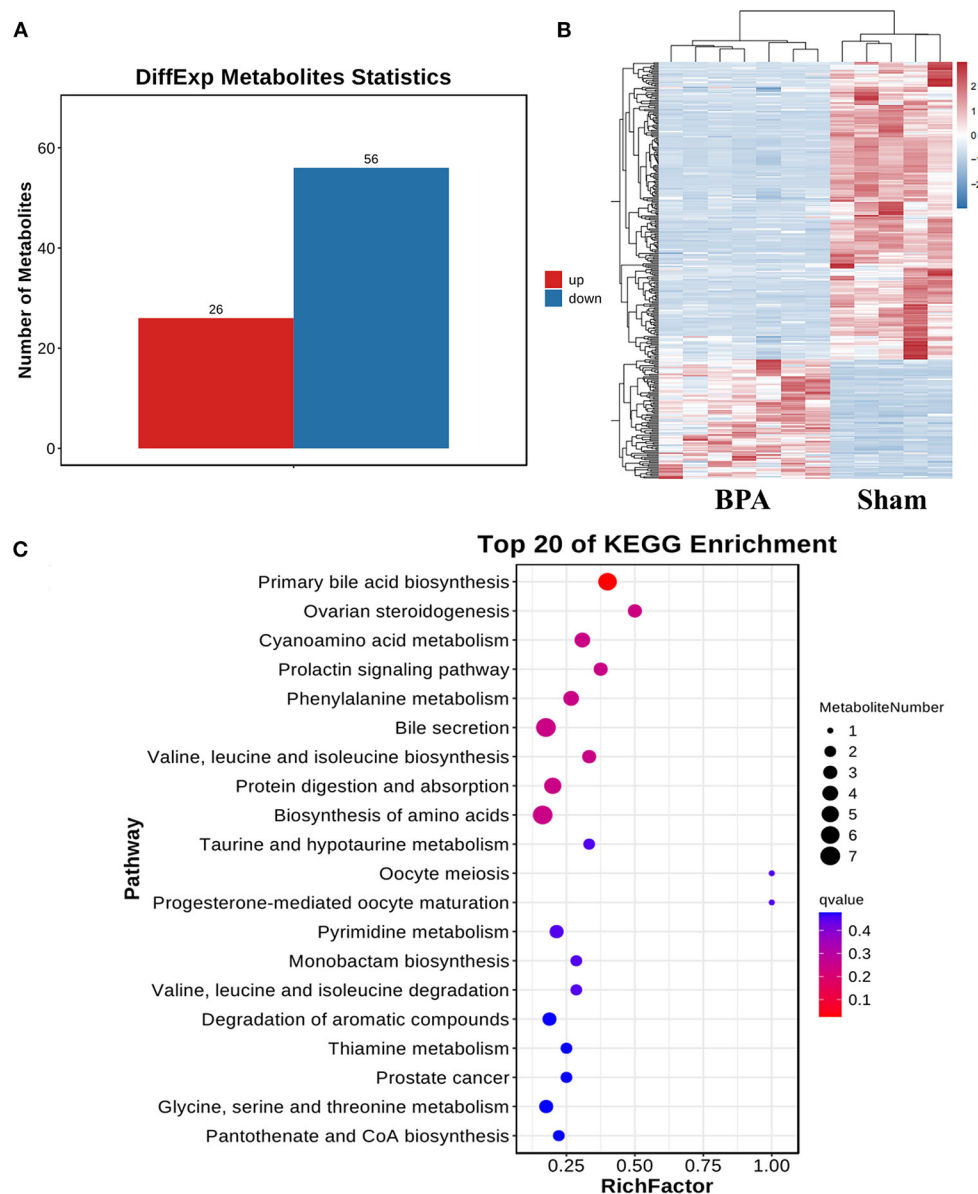


FIGURE 4

Gut bacteria metabolites sequencing analysis of sham and BPA mice. (A) Differentially expressed metabolites in BPA groups compared to the sham group. (B) Cluster heat map of the differentially expressed metabolites between the two groups. (C) KEGG functional enrichment analysis of the differentially expressed metabolites.

values after BPA through the indicator value analysis *via* R package (Figure 3G).

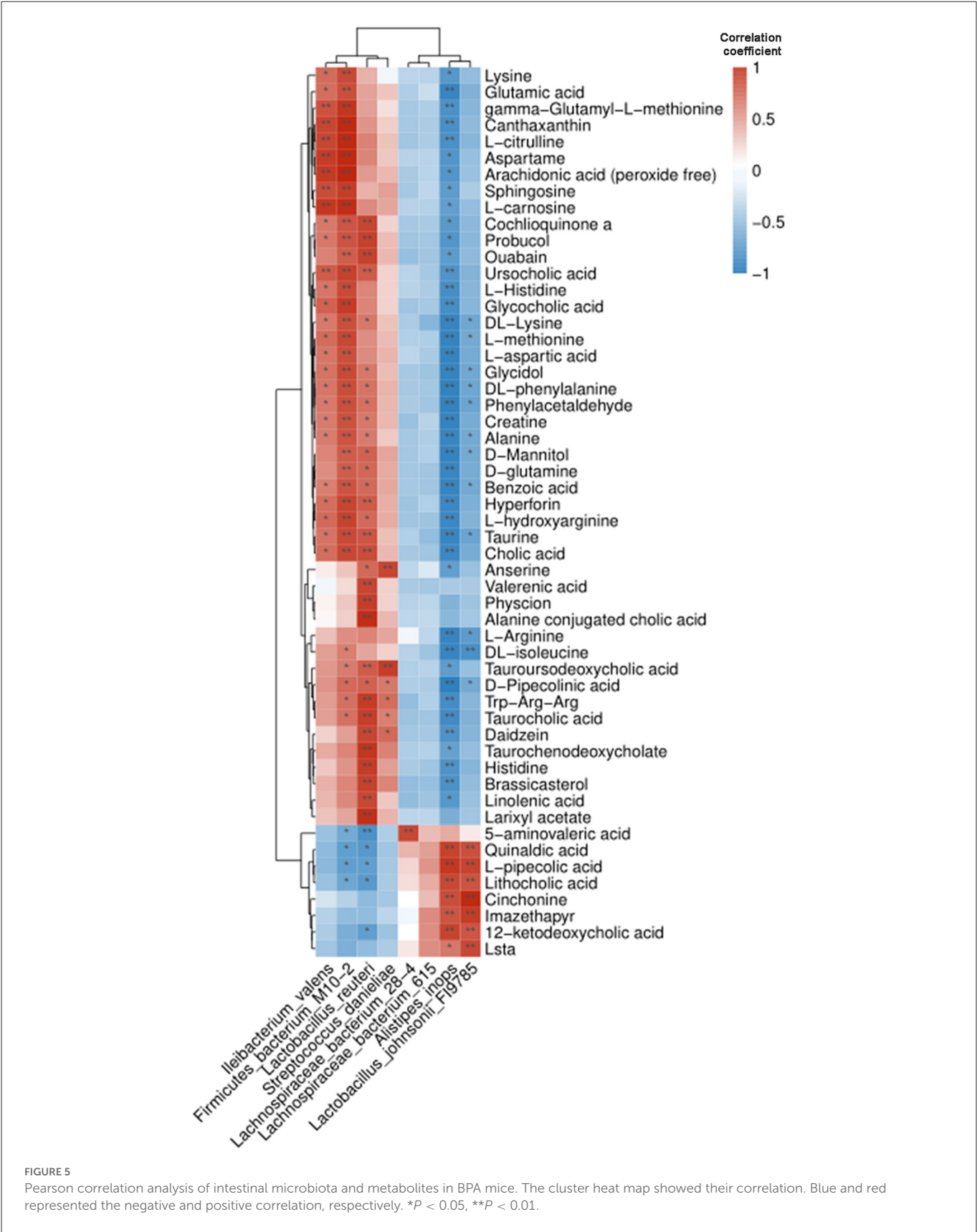
3.4. BPA altered gut metabolites

To identify BPA-induced metabolic disorders, metabolomics analysis was performed between sham ($n = 5$) and BPA ($n = 7$) groups. Compared with sham, 82 differentially expressed metabolites were identified in BPA, including 26 up-regulated and 56 down-regulated metabolites (Figure 4A). Clustering heat maps were used to visualize the significantly differential-expressed metabolites between the two groups

(Figure 4B). Further KEGG functional enrichment analysis of these differentially expressed metabolites showed that differentially expressed metabolites mainly participated in Pantothenate and CoA biosynthesis, Primary bile acid biosynthesis, Bile Hair's-gain, Glycine, serine and threonine metabolism (Figure 4C).

3.5. Correlation analysis between intestinal microbiota and metabolites in BPA mice

To further investigate the functional significance of metabolic dysfunction caused by intestinal microbiota disorder in BPA mice,



Pearson correlation analysis was applied to find the association between 8 abundant intestinal bacteria at the genus level and 54 differentially expressed metabolites. The cluster heat map visualized the Pearson correlation coefficient (Figure 5).

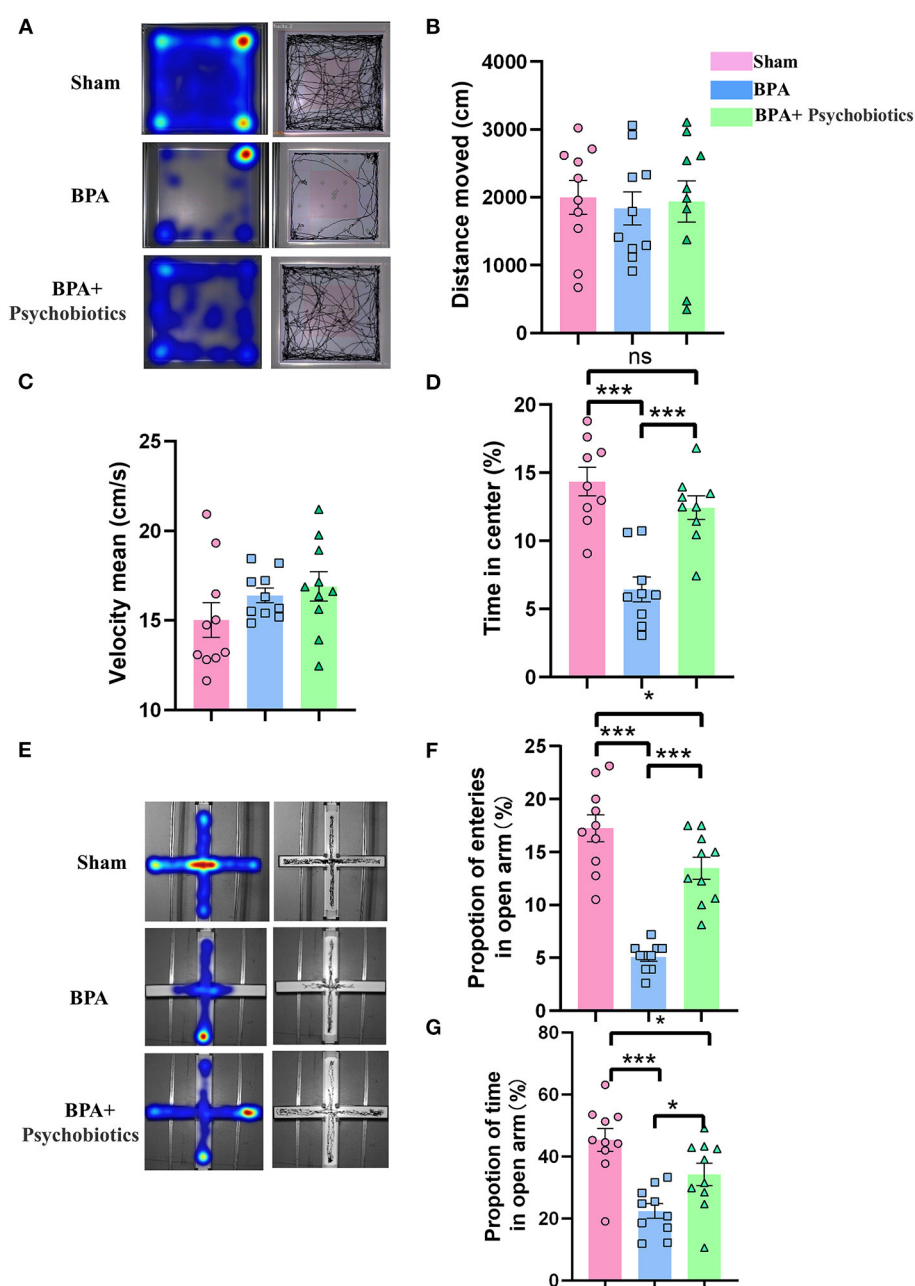


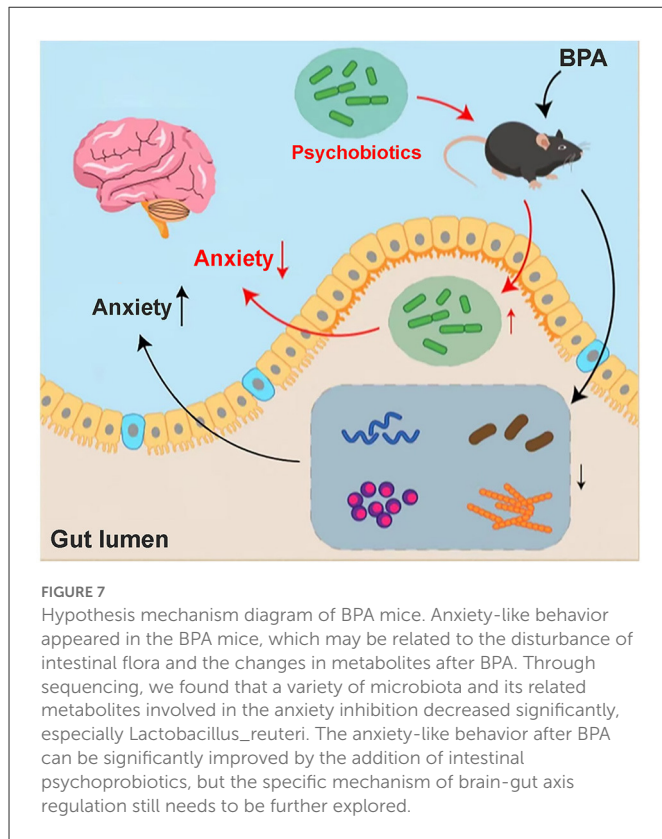
FIGURE 6

Effects of PB supplementation on anxiety-like behaviors in BPA mice. (A) OFT trajectory diagram. (B) The movement distance from OFT tests among the three groups. (C) The movement speed from OFT tests among the three groups. (D) The residence time in the central area from OFT among groups (E) EPM trajectory diagram. (F) EPM: The proportion of open arm entry times in the three groups. (G) EPM: The open arm residence time among groups. $N = 10$ per group. $ns =$ no statistical significance, $*P < 0.05$, $***P < 0.001$. Ordinary one-way ANOVA was used for data analysis. BPA, brachial plexus avulsion; PB, psychobiotics; OFT, open field test; EPM, elevated plus-maze test.

3.6. PB supplementation could relieve anxiety-like behaviors in BPA mice

BPA mice were then intragastrically administrated with PB every day until 14 days after modeling, followed by pain detection, OFT, and EPM testing. Pain detection showed that PB treatment significantly attenuate the mechanical allodynia of BPA mice (Supplementary Figure 1). OFT results showed that there was no significant difference in the movement distance

and speed among the three groups (Figures 6A–C), while the reduced residence time in the center area of the BPA group was significantly increased by PB supplementation (Figure 6D). EPM results showed that BPA decreased the frequency of entering the open arm and the residence time of the open arm compared with the sham, which were significantly reversed after PB supplementation (Figures 6E–G). These results showed that probiotics could significantly relieve pain related anxiety-like behavior after BPA.



4. Discussion

Our study found the evidence of the participation of microbiota-gut-brain axis in the pathological process of BPA induced pain related anxiety-like behavior in mice. 16s rDNA amplification of intestinal microbiota showed that BPA significantly affected the diversity of intestinal microbiota, and a variety of mental probiotics were significantly decreased. The differentially expressed metabolites were involved in the metabolism and synthesis of various amino acids in response to BPA stress in the gut. Some of the metabolites as neurotransmitters or neurotransmitter precursors are significantly different, and most of the intestinal flora related to these metabolites belong to the category of neuroprobiotics. Therefore, we considered that the onset of BPA-induced anxiety-like behaviors may be related to the altered gut microbiota, especially the reduction of psychogenic probiotics. Supplementation with related probiotics significantly attenuated anxiety-like behaviors in BPA mice, which supported that the occurrence of anxiety-like behaviors after BPA is close to the changes of related microbiota and metabolites.

Nerve injury-induced acute pain is a warning mechanism of the human body, which prevents further tissue damage (18). Due to the great changes in peripheral nervous system (PNS) and CNS after nerve injury, nociceptive sensitization happens, which leads to intestinal microbiota disorders, and in turn affects the PNS and CNS through metabolites after adapting to the pain stimuli. This microbiota-gut-brain interaction involves in the regulation of neuroimmunity, host metabolism, neurotransmitter system, and vague nerve (19). Peripheral and central sensitization are crucial issues during the chronicity of pain, and are also the main reasons

for the comorbidities including anxiety, depression, etc. About 30–60% of patients with chronic pain suffered by depression, and the prevalence of suicide consciousness is 32% (20). Even with active surgical treatment, the incidence of anxiety or depression in BPI patients is still higher than 18.8% (9), and in BPA patients is as high as 81.2% (8). In order to explore the underlying mechanisms, we constructed a BPA mouse model according to the operation process described by Xian et al. (21). The results showed that this model could mimic the pain state like BPA patients and single C7 nerve root avulsion had little effect on motor ability of mice. It is the pain related anxiety-like behaviors was observed at 7, 14, and 21 days after BPA but no depression-like behavior. Compared with previously reported results in chronic constriction injury (22) and partial sciatic nerve ligation neuralgia models (23), the onset of anxiety-like behaviors in BPA mice is earlier, the mechanism under this difference still worth deep mining.

In order to explore the effects of BPA on intestinal microbiota, we conducted 16s sequencing of intestinal microbiota of BPA mice to explore the correlation between microbiota and BPA induced pain related anxiety-like behaviors. Sequencing results found that the α and β diversity of the microbiota of BPA mice increased significantly, indicating the changes of species richness and diversity after BPA. The most obvious change was *Lactobacillus*. *Lactobacillus* is the most abundant probiotic bacteria in the gut, which maintains many functions of the body. Studies have found that *Lactobacillus* is an important member of the microorganism of the NPP mouse (24), and it has obvious changes both in acute and chronic neuralgia (25). Among the 13 species with the significant changes, *Lactobacillus_reuteri* showed significantly decreased indicator value after BPA, which may be related to the intestinal stress on pain, because *Lactobacillus_reuteri* can reduce the pain stimulation caused by nerve discharge (26). *Lactobacillus* increases short chain fatty acid (SCFA) metabolites, such as acetate, propionate, and butyrate, which are important regulators in the synthesis and degradation of neurotransmitters. Moreover, the colonization of *Lactobacillus_rhamnosus* is beneficial to reduce anxiety (27). Therefore, we speculate that the reduction of *Lactobacillus* abundance induced by BPA may regulate the microbial-gut-brain axis through altering metabolites.

To investigate the changes of intestinal metabolites, we performed untargeted metabolomics of intestinal feces. The expression of 26 metabolites increased and 56 metabolites decreased in BPA mice. KEGG enrichment pathways of differentially expressed metabolites mainly focused on Pantothenate and CoA biosynthesis, Primary bile acid biosynthesis, Bile acid development, Glycine, serine and threonine metabolism. These pathways are related to the abnormal metabolism of bile acid. Intestinal microbiota synthesizes hundreds of metabolites that affect host physiology, among which the most abundant metabolites are secondary bile acids (28). Primary bile acids produced by liver are further synthesized by intestinal microbiota to about 20 types of bile acids (29). Bile acids regulate the neurotransmitter receptor functions, like muscarinic acetylcholine receptor and γ -aminobutyric acid receptor. Bile acids can also be protective against neurodegeneration (30). Another metabolite that was significantly elevated was glutamate, a key excitatory neurotransmitter for emotion regulation (31). The apparent rise in glutamate expression may be the body's response to painful stimuli. However, further studies are needed to determine whether these metabolites are related to the dysregulation of microbiota.

We further investigated the association between BPA microbiota and corresponding metabolites. We found that *Alistipes_inops*, *Lactobacillus_johnsonii_FI9785*, *Lactobacillus_reuteri*, and *Firmicutes_bacterium* were correlated with the expression of a large number of metabolites, including γ -aminobutyric acid (GABA), anandamide (AEA), and amino acid neurotransmitters. An interesting phenomenon was found that *Lactobacillus_reuteri* was positively correlated with AEA. AEA is closely related to the body's endogenous cannabinoid system (eCBS) (32). eCBS have pleiotropic functions *in vivo*. It plays a key role in the development of energy homeostasis and metabolic disorders and is a mediator of the relationship between the gut microbiota and host metabolism. These neurotransmitter amino acid changes were associated with four microbiotas, two of which were *Lactobacillus*, suggesting that the related metabolite changes caused by *Lactobacillus* microbiota may be a key factor in BPA-induced anxiety-like behavior. Therefore, we supplemented PB in BPA mice to test its effect on anxiety. PB is a probiotic strain to affect gut-brain axis, and has been reported to improve microbiota and alleviate some symptoms of CNS disorders (33). The results showed anxiety-like behaviors of BPA mice were significantly relieved after PB supplementation. Therefore, we confirmed that the decrease of intestinal microbiota diversity caused by BPA, especially the changes of neurotransmitter amino acid metabolites caused by altered *Lactobacillus* diversity, may be the key causes of anxiety-like behavior.

In the current study, we found BPA could induce pain and related anxiety-like behavior in mice, and the participation of microbiota-gut-brain axis may be the critical point during this pathological process (Figure 7). BPA causes significant intestinal microbiota disorder and altered metabolites. Regulation of the microbiota and metabolites maybe the key point to cure this comorbidity induced by BPA. While, this is just a preliminary research, and what specific kind of metabolite affects the brain through microbiota-gut-brain axis remains unclear. As the influence of the microbiota-gut-brain axis is mutual, how the CNS regulates the corresponding neurotransmitters after receiving the signals of peripheral nervous system injury and the mutual feedback mechanism between the CNS and intestinal flora are still worth further explorations

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA899015.

References

1. Kouyoumdjian JA, Graca CR, Ferreira VFM. Peripheral nerve injuries: a retrospective survey of 1124 cases. *Neurol India*. (2017) 65:551–5. doi: 10.4103/neuroindia.NI_987_16
2. Zhou Y, Liu P, Rui J, Zhao X, Lao J. The clinical characteristics of neuropathic pain in patients with total brachial plexus avulsion: a 30-case study. *Injury*. (2016) 47:1719–24. doi: 10.1016/j.injury.2016.05.022
3. Zhou Y, Liu P, Rui J, Zhao X, Lao J. The associated factors and clinical features of neuropathic pain after brachial plexus injuries: a cross-sectional study. *Clin J Pain*. (2017) 33:1030–6. doi: 10.1097/AJP.0000000000000493
4. Teixeira MJ, da Paz MG, Bina MT, Santos SN, Raicher I, Galhardoni R, et al. Neuropathic pain after brachial plexus avulsion: central and peripheral mechanisms. *BMC Neurol*. (2015) 15:73. doi: 10.1186/s12883-015-0329-x
5. Shen J, Huo BB, Zheng MX, Hua XY, Shen H, Lu YC, et al. Evaluation of neuropathic pain in a rat model of total brachial plexus avulsion from behavior to brain metabolism. *Pain Phys*. (2019) 22:E215–24. doi: 10.36076/ppj/2019.22.E215
6. Fonseca-Rodrigues D, Amorim D, Almeida A, Pinto-Ribeiro F. Emotional and cognitive impairments in the peripheral nerve chronic constriction injury model (CCI) of neuropathic pain: a systematic review. *Behav Brain Res*. (2021) 399:113008. doi: 10.1016/j.bbr.2020.113008
7. Franzblau L, Chung KC. Psychosocial outcomes and coping after complete avulsion traumatic brachial plexus injury. *Disabil Rehabil*. (2015) 37:135–43. doi: 10.3109/09638288.2014.911971

Ethics statement

The animal study was reviewed and approved by the Fourth Military Medical University Laboratory Animal Center of Welfare and Ethics Committee.

Author contributions

J-LZ and RC proposed the idea and drafted the manuscript. J-LZ and HX performed animal models, behavioral tests, and data analysis. RZ and CL performed data analysis. R-gX and TT assisted figures editing. RC was responsible for supervising. All authors contributed to the article and approved the submitted version.

Funding

The work was supported by the National Natural Science Foundation of China (82072526).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2023.1084494/full#supplementary-material>

8. Li GY, Xue MQ, Wang JW, Zeng XY, Qin J, Sha K. Traumatic brachial plexus injury: a study of 510 surgical cases from multicenter services in Guangxi, China. *Acta Neurochir.* (2019) 161:899–906. doi: 10.1007/s00701-019-03871-y
9. Rasulic L, Savić A, Zivković B, Vitosevic F, Micović M, Bascarevic V, et al. Outcome after brachial plexus injury surgery and impact on quality of life. *Acta Neurochir.* (2017) 159:1257–64. doi: 10.1007/s00701-017-3205-1
10. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell.* (2014) 157:121–41. doi: 10.1016/j.cell.2014.03.011
11. Cani PD. Metabolism in 2013: the gut microbiota manages host metabolism. *Nat Rev Endocrinol.* (2014) 10:74–6. doi: 10.1038/nrendo.2013.240
12. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The central nervous system and the gut microbiome. *Cell.* (2016) 167:915–32. doi: 10.1016/j.cell.2016.10.027
13. Ramakrishna C, Corleto J, Ruegger PM, Logan GD, Peacock BB, Mendonça S, et al. Dominant role of the gut microbiota in chemotherapy induced neuropathic pain. *Sci Rep.* (2019) 9:20324. doi: 10.1038/s41598-019-56832-x
14. Brandon-Mong GJ, Shaw GT, Chen WH, Chen CC, Wang D, A. network approach to investigating the key microbes and stability of gut microbial communities in a mouse neuropathic pain model. *BMC Microbiol.* (2020) 20:295. doi: 10.1186/s12866-020-01981-7
15. Chevalier G, Siopi E, Guenin-Mace L, Pascal M, Laval T, Rifflet A, et al. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. *Nat Commun.* (2020) 11:6363. doi: 10.1038/s41467-020-19931-2
16. Yang C, Fujita Y, Ren Q, Ma M, Dong C, Hashimoto K. Bifidobacterium in the gut microbiota confer resilience to chronic social defeat stress in mice. *Sci Rep.* (2017) 7:45942. doi: 10.1038/srep45942
17. Ng QX, Peters C, Ho CYX, Lim DY, Yeo WS. A meta-analysis of the use of probiotics to alleviate depressive symptoms. *J Affect Disord.* (2018) 228:13–9. doi: 10.1016/j.jad.2017.11.063
18. Malcangio M. Role of the immune system in neuropathic pain. *Scand J Pain.* (2019) 20:33–7. doi: 10.1515/sjpain-2019-0138
19. Morkl S, Butler MI, Holl A, Cryan JE, Dinan TG. Probiotics and the microbiota-gut-brain axis: focus on psychiatry. *Curr Nutr Rep.* (2020) 9:171–82. doi: 10.1007/s13668-020-00313-5
20. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. *Lancet.* (2021) 397:2082–97. doi: 10.1016/S0140-6736(21)00393-7
21. Xian H, Xie R, Luo C, Cong R. Comparison of different *in vivo* animal models of brachial plexus avulsion and its application in pain study. *Neural Plast.* (2020) 2020:8875915. doi: 10.1155/2020/8875915
22. Bagdas D, Sevdar G, Gul Z, Younis R, Cavun S, Tae HS, et al. (E)-3-furan-2-yl-N-phenylacrylamide (PAM-4) decreases nociception and emotional manifestations of neuropathic pain in mice by $\alpha 7$ nicotinic acetylcholine receptor potentiation. *Neurol Res.* (2021) 43:1056–68. doi: 10.1080/01616412.2021.1949684
23. Gai BM, Bortolatto CF, Bruning CA, Zborowski VA, Stein AL, Zeni G, et al. Depression-related behavior and mechanical allodynia are blocked by 3-(4-fluorophenylselenenyl)-2,5-diphenylselenophene in a mouse model of neuropathic pain induced by partial sciatic nerve ligation. *Neuropharmacology.* (2014) 79:580–9. doi: 10.1016/j.neuropharm.2014.01.020
24. Li R, Wang F, Dang S, Yao M, Zhang W, Wang J. Integrated 16S rRNA gene sequencing and metabolomics analysis to investigate the important role of osteon on gut microbiota and serum metabolites in neuropathic pain mice. *Front Physiol.* (2022) 13:813626. doi: 10.3389/fphys.2022.813626
25. Chen P, Wang C, Ren YN, Ye ZJ, Jiang C, Wu ZB. Alterations in the gut microbiota and metabolite profiles in the context of neuropathic pain. *Mol Brain.* (2021) 14:50. doi: 10.1186/s13041-021-00765-y
26. Perez-Burgos A, Wang L, McVey Neufeld KA, Mao YK, Ahmadzai M, Janssen LJ, et al. The TRPV1 channel in rodents is a major target for antinociceptive effect of the probiotic *Lactobacillus reuteri* DSM 17938. *J Physiol.* (2015) 593:3943–57. doi: 10.1113/JP270229
27. Zhou B, Jin G, Pang X, Mo Q, Bao J, Liu T, et al. *Lactobacillus rhamnosus* GG colonization in early life regulates gut-brain axis and relieves anxiety-like behavior in adulthood. *Pharmacol Res.* (2022) 177:106090. doi: 10.1016/j.phrs.2022.106090
28. Funabashi M, Grove TL, Wang M, Varma Y, McFadden ME, Brown LC, et al. A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature.* (2020) 582:566–70. doi: 10.1038/s41586-020-2396-4
29. Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed hepatic bile acid signaling despite elevated production of primary and secondary bile acids in NAFLD. *Gut.* (2018) 67:1881–91. doi: 10.1136/gutjnl-2017-314307
30. Kiriya Y, Nochi H. The biosynthesis, signaling, and neurological functions of bile acids. *Biomolecules.* (2019) 9:232. doi: 10.3390/biom9060232
31. Henter ID, Park IT, Zarate CA. Novel glutamatergic modulators for the treatment of mood disorders: current status. *CNS Drugs.* (2021) 35:527–43. doi: 10.1007/s40263-021-00816-x
32. Petrie GN, Nastase AS, Aukema RJ, Hill MN. Endocannabinoids, cannabinoids and the regulation of anxiety. *Neuropharmacology.* (2021) 195:108626. doi: 10.1016/j.neuropharm.2021.108626
33. Oroojzadeh P, Bostanabad SY, Lotfi H. Psychobiotics: the influence of gut microbiota on the gut-brain axis in neurological disorders. *J Mol Neurosci.* (2022) 72:1952–64. doi: 10.1007/s12031-022-02053-3



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan Medical
College, China

REVIEWED BY

Gang Zhao,
Soochow University, China
Rui Li,
Sun Yat-sen University, China

*CORRESPONDENCE

Zuobing Chen
✉ czb1971@zju.edu.cn

†These authors share first authorship

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 27 October 2022

ACCEPTED 06 February 2023

PUBLISHED 24 February 2023

CITATION

Ni L, Yao Z, Zhao Y, Zhang T, Wang J, Li S and
Chen Z (2023) Electrical stimulation therapy for
peripheral nerve injury.
Front. Neurol. 14:1081458.
doi: 10.3389/fneur.2023.1081458

COPYRIGHT

© 2023 Ni, Yao, Zhao, Zhang, Wang, Li and
Chen. This is an open-access article distributed
under the terms of the [Creative Commons
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Electrical stimulation therapy for peripheral nerve injury

Lingmei Ni^{1†}, Zhao Yao^{2†}, Yifan Zhao³, Tianfang Zhang³,
Jie Wang², Siyue Li³ and Zuobing Chen^{2,3*}

¹Infection Prevention and Control Department, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, ²The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University, Wenzhou, China, ³Department of Rehabilitation Medicine, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Peripheral nerve injury is common and frequently occurs in extremity trauma patients. The motor and sensory impairment caused by the injury will affect patients' daily life and social work. Surgical therapeutic approaches don't assure functional recovery, which may lead to neuronal atrophy and hinder accelerated regeneration. Rehabilitation is a necessary stage for patients to recover better. A meaningful role in non-pharmacological intervention is played by rehabilitation, through individualized electrical stimulation therapy. Clinical studies have shown that electrical stimulation enhances axon growth during nerve repair and accelerates sensorimotor recovery. According to different effects and parameters, electrical stimulation can be divided into neuromuscular, transcutaneous, and functional electrical stimulation. The therapeutic mechanism of electrical stimulation may be to reduce muscle atrophy and promote muscle reinnervation by increasing the expression of structural protective proteins and neurotrophic factors. Meanwhile, it can modulate sensory feedback and reduce neuralgia by inhibiting the descending pathway. However, there are not many summary clinical application parameters of electrical stimulation, and the long-term effectiveness and safety also need to be further explored. This article aims to explore application methodologies for effective electrical stimulation in the rehabilitation of peripheral nerve injury, with simultaneous consideration for fundamental principles of electrical stimulation and the latest technology. The highlight of this paper is to identify the most appropriate stimulation parameters (frequency, intensity, duration) to achieve efficacious electrical stimulation in the rehabilitation of peripheral nerve injury.

KEYWORDS

peripheral nerve injury, peripheral nerve regeneration, electrical stimulation (ES), therapy, mechanism

1. Introduction

The peripheral nervous system, including cranial nerves, spinal nerves, and autonomic nerves, is a nerve trunk, nerve plexus, ganglion, and nerve terminal composed of perikarya and nerve fibers, which mainly connect the peripheral sensory apparatus and the central nervous system. Peripheral nerve injury (PNI) is a kind of motor and sensory disorder caused by damage to the structure of peripheral nerves. The incidence of PNI caused by trauma is roughly 5%, including brachial plexus and root injuries (1). After nerve injury, damaged axons are not able to regenerate completely. Therefore, it is important to provide appropriate therapies to reconnect nerves in the injured area and to accelerate the growth rate of nerve (2). At present, the treatment methods for nerve injury are mainly divided into surgical treatment and non-surgical treatment (3–5). Electrical stimulation (ES) is the most

commonly used non-surgical treatment. Most studies used low-frequency ES to promote nerve regeneration, but the method and frequency range of ES need to be standardized because high-frequency ES will aggravate nerve damage (6). The standard ES parameter of 20 Hz for one hour immediately after the repair is well known. However, its utility and efficacy for various nerves have never been defined. This review focuses on a framework to develop a new ES paradigm enabling future clinical translation.

2. Peripheral nerve anatomy

Peripheral nerves refer to all nerves other than the brain and spinal cord, including ganglia, nerve trunks, nerve plexus and nerve ending. The center of the anatomical structure of the peripheral nerve is the nerve fiber. The endoneurium wraps around the nerve fibers to form the nerve bundle that is surrounded by loose connective tissue. The epineurium then wraps the nerve bundle to form a complete peripheral nerve (7). There are many pathogenic factors, such as infection, ischemia, trauma, metabolic disorders, poisoning, nutritional deficiency, and iatrogenic injury (such as chemotherapy, radiotherapy, etc.). Peripheral injury can lead to severe dysfunction, often affecting the ability of patients to perform activities of daily living.

3. Classification of peripheral nerve injury

Common peripheral nerve injuries include brachial plexus nerve injury, axillary nerve injury, cutaneous nerve injury, median nerve injury, radial nerve injury, ulnar nerve injury, femoral nerve injury, sciatic nerve injury, and common peroneal nerve injury.

According to Seddon's classification in 1943, PNI was divided into three types: neurapraxia, axonal disruption, and nerve rupture. According to the 1951 Sunderland classification, the PNI was divided into 5 types. Type I: focal demyelination; Type II: damage to axons but endoneurium, perineurium, and epineurium intact; Type III: damage to axons and endoneurium but perineurium and epineurium intact; Type IV: damage to axons, endoneurium, and perineurium but epineurium intact; Type V: complete loss of continuity (Figure 1). For non-severe injuries (Types I-III), patients only required exercise training and physical therapy as treatment because adult nerves have some intrinsic regenerative capacity. For type IV and V injuries, it is necessary to suture the nerve transection by surgeries, including nerve manipulation and bridging (8).

4. Treatments of electrical stimulation

Currently, the surgical treatment of PNI includes microsurgical end-to-end repair, tension-free nerve epineurium suture, and autologous nerve grafting (9, 10). Less than half of patients recover satisfactory motor and sensory function after nerve repair. A third of patients have little or no recovery despite proper surgery.

Although great advances have been made in surgical strategies to treat PNI, most patients did not undergo systematic rehabilitation after surgery, leaving them with lifelong sensorimotor disorders and chronic neuropathic pain. There is a need for a treatment to overcome the limitations of peripheral nerve recovery and improve patient physical function. ES is a promising method to accelerate peripheral nerve regeneration. After PNI, ES has been shown to promote early stages of nerve regeneration, including neuronal survival and axon bud formation (11).

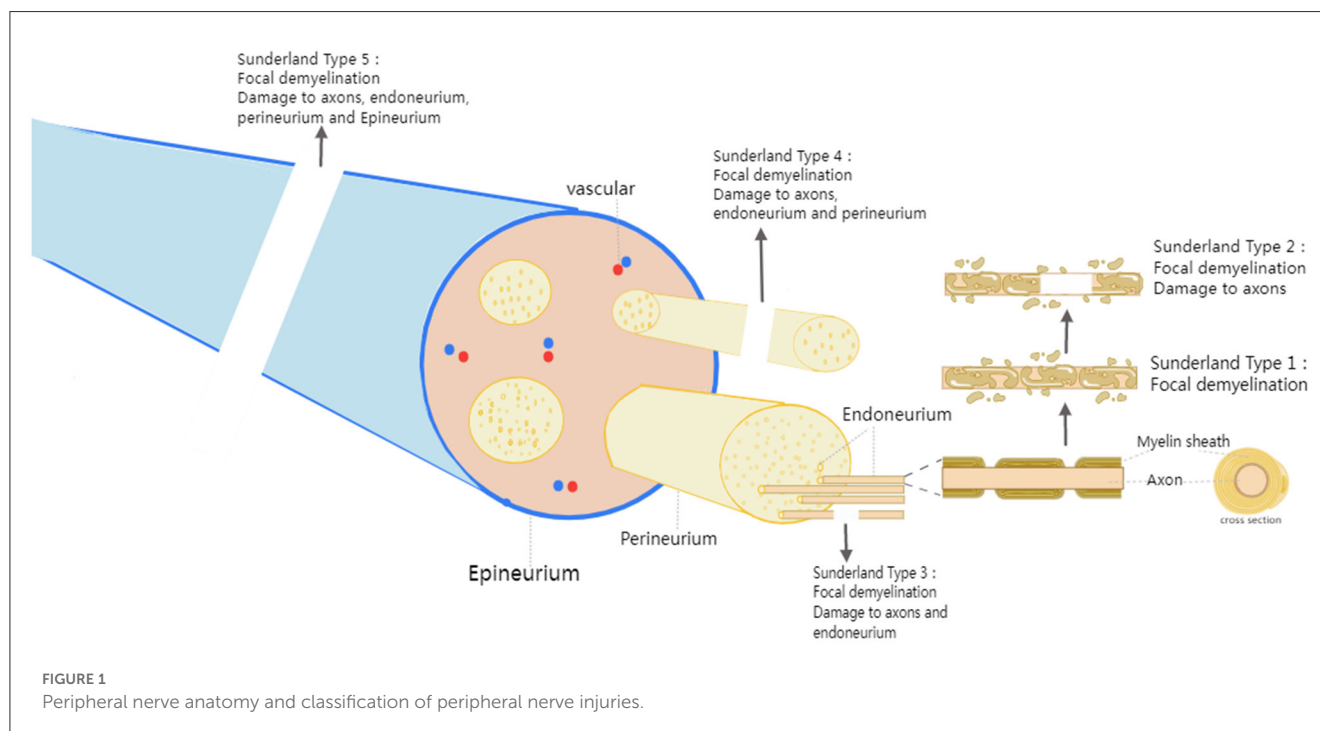




FIGURE 2
TENS, NMES (Empi 300PV unit).



FIGURE 3
Demonstration of Empi 300PV unit.

ES can not only treat PNI but also show the changes in the process of injury. The latest research has shown that the use of pulsed ES to act on the injured muscle and record the changes in muscle fibers, called the muscle velocity recovery cycle (MVRC), can provide a detailed understanding of the *in vivo* evidence of depolarized resting potential after PNI. To provide the reason for neurogenic muscle weakness caused by reduced muscle excitability (12).

Since the 1980s multiple, animal studies have been conducted and have shown a positive effect of ES on peripheral nerve recovery. In a rat femoral nerve model, the use of 20 Hz continuous ES at

the proximal end of the nerve reduced the axonal growth period from 10 to 3 weeks (13). ES that alters neuromuscular activity by electrical currents mainly includes neuromuscular electrical stimulation (NMES), transcutaneous electrical nerve stimulation (TENS), and functional electrical stimulation (FES). NMES usually produces muscle contraction at a frequency of 20–50 Hz and is used to improve patient function. TENS is usually used to relieve pain at a low frequency of 2–10 Hz or ultra-high frequency. Low-frequency TENS generally targets sensory nerves and does not produce visible muscle contraction. FES is a functional task, in which the target muscle is initially stimulated to generate movement, and the next step is to achieve the upper limb grasping the object or the lower limb walking. The current ES instrument is mostly small portable devices, such as 300 PV Empi, Bioness® L300 Go, and Bioness® H200 Wireless (Figures 2–5).

4.1. Neuromuscular electrical stimulation (NMES)

4.1.1. Stimulation mode and system composition

When ES induces muscle contraction, H-reflex and M-wave waves are recorded by electromyography. (H-reflex is an action potential triggered by the current to afferent axons of LA fibers, which recruits low-threshold motor units; When the current intensity gradually increases, the motor axon is activated to produce a motor response called M wave.) This method of muscle activation, when the strength of the current applied by electrodes placed on the muscle or nerve causes muscle contraction, is referred to as NMES. NMES is commonly used to restore skeletal muscle mass and function in patients of PNI and is also applied to activate the nervous system in healthy individuals (14).

4.1.2. Clinical application (nerve ES, direct muscle ES)

Some studies have investigated the effect of ES on nerve repair by directly connecting the two ends of the injured nerve to electrodes for brief ES when the skin is not sutured after nerve surgery. Gordon et al. (15) carried out a randomized controlled clinical trial (RCT) that assessed the post-surgical outcomes of acute brief low-frequency (20 Hz) ES. The researchers used ES (1 h; 20 Hz; 4–6 V; 0.1–0.8 ms) to improve neurological function in patients with severe carpal tunnel syndrome who had undergone surgery. It was shown that ES could promote axonal regeneration and accelerate muscle nerve regeneration (16). ES at 20 Hz for 1 h was also effective in cubital tunnel compression. One study involved Patients with severe cubital tunnel syndrome (CuTS), in which compression of the ulnar nerve in the cubital tunnel resulted in decreased neuromuscular function, and patients who underwent cubital tunnel release surgery were randomized in a 1:2 ratio into the control or PES groups. Patients in ES groups underwent ES (1 h; 20 Hz; balanced biphasic pulses; 30 V, 0.1 ms). Through three years of follow-up, using EMG signals to record motor unit number estimation (MUNE), grip, and key pinch strength, the study showed that, Brief-ES immediately after decompression surgery accelerated axon regeneration. Compared with the control



FIGURE 4
FES of foot (Bioness L300 Go).



FIGURE 5
FES of hand (Bioness H200 wireless).

group, postoperative MUNE function was better in the ES group, and grip and key pinch strength were also significantly improved. Patients who underwent surgery alone did not show any significant improvement in the number of MUNE, grip strength, or pinch strength. In one RCT (17), patients with complete digital nerve rupture received nerve repair and postoperative brief-ES (1 h; 20 HZ; balanced biphasic pulses; 30 V, 0.1–0.4 ms). At 6-month follow-up, patients in the ES group showed significant improvements in temperature discrimination, pressure detection, and spatial discrimination compared with controls.

Some researchers vary the duration of the stimulation cycle, the type of target muscle fiber, the width of the ES pulse, and the individualized setting of the ES intensity to explore the influence of different parameters on the effect of ES. In the studies of this paragraph, electrodes were attached to the proximal and distal ends of the muscle for direct muscle ES. Acaroz Candan S et al. (18) proved that NMES (100 Hz; symmetrical biphasic squared waveform; 400 μ s) both a short stimulation period (SNMES 5 min $\times$ 4 sets) and a long stimulation period (LNMES 10 min $\times$ 2 sets) could improve quadriceps femoris function in the elderly, but there was no significant difference between the two groups. Toth et al. (19) used NMES (1 h; 50 Hz; symmetrical biphasic pulses; 400 μ s) to intervention patients after anterior cruciate ligament injuries surgery, and the result showed that NMES could reduce the atrophy of slow-twitch fibers and fast-twitch fibers, and could maintain the muscle contraction strength and output power of slow-twitch fibers. The study by Stevens-Lapsley JE, et al. (20) demonstrated the ability to increase muscle strength and function by setting different personalized parameters according to the patient's maximum tolerance. Pinto Damo NL, et al. (21) used four different parameters of NMES, respectively narrow pulses (PC 200 μ s), wide pulses (PC 500 μ s), 500 μ s phase duration, and low carrier frequency (KFAC 500: 1 kHz/Aussie current), and 200 μ s phase duration and high carrier frequency (KFAC 200: 2.5 kHz/Russian current). The results concluded that KFAC and PC currents produced similar effects with the same phase duration. Currents with 500 μ s induced higher muscle torque and efficiency, but patients felt more uncomfortable. Mani et al. (22) used both narrow pulses (0.26 ms) at 50 Hz and wide pulses (1 ms) at 100 Hz

to improve mobility function in the elderly. The result showed both pulse methods improved lower limb strength and functional performance, but there were no significant differences between each other.

Moreover, excessive ES induces muscle fatigue and weakens the effect of improving nerve recovery. Vanderthommen et al. (23) suggested that brief intermittent ES was the best choice and high-frequency currents caused premature muscle fatigue. To avoid muscle fatigue, some scholars have done comparative studies between muscle ES and nerve ES. The result suggested that electrical nerve stimulation might be more comfortable and less to cause muscle fatigue than electrical muscle stimulation (24).

However, not all clinical applications of NMES are effective. Hyer et al. (25) used NMES to treat the calf muscles of patients after achilles tendon surgery. Neither muscle mass nor function was improved in the NMES group or the sham-stimulation group. Also in a double-blind randomized clinical trial, after traumatic peripheral nerve injuries with axonal damage and clinical impairment of two muscles. There was no significant difference between the ES group and the control group (26). Therefore, further studies are needed to determine the optimal parameters of ES. More clinical trials are expected to prove the effect of ES in the treatment of PNI.

4.1.3. Animal models

In animal experiments, most scholars used the rat sciatic nerve transection and repair model to verify the effect and mechanism of NMES on nerve regeneration and prevention of muscle atrophy.

Compared with delayed ES after surgery, immediate ES is more conducive to neuromuscular recovery. It was reported that after sciatic nerve transection in rats, direct ES of the proximal sciatic nerve, immediate motor cortex stimulation (MCS) (60 Hz, 3–10 V, 200 μ s) for 15 min and treatment for 2 weeks. The results showed that MCS was more effective than direct nerve stimulation in nerve regeneration and muscle nerve reinnervation, especially in the immediate postoperative period (27).

A similar effect was seen with 10 min of ES compared with 60 min. A study of sciatic nerve transection in mice was conducted and compared 10 min and 60 min ESs with pulsed current (28). The experimental results have shown that both settings facilitated motor neuron regeneration with increased axonal excitability, axonal myelin, and improved motor function. This finding has also been validated in tibial nerve transection repair, where as little as 10 min of ES could increase early axonal regeneration and produce similar benefits to 60 min stimulation (29).

ES may have different effects on different peripheral nerves. Researchers used ES (20 Hz, 3–4 V, 0.1 ms, 60 min) on the proximal femoral and facial nerves of rats after nerve cutting. The results showed that transient ES of the femoral nerve could promote nerve regeneration, but did not improve the facial nerve repair (30).

In the implantable ES experiment in rats, the contact area between the coil of the electrode and the nerve also had different effects on nerve regeneration. Some studies have compared three different nerve contact modes, which were point contact, 1/4 contact, and full coil contact. The results showed that the electrodes

with point contact and 1/4 contact were more effective in promoting nerve regeneration and functional recovery (31).

4.1.4. Mechanism

Peripheral nerve ES can be divided into direct muscle ES and nerve ES according to the electrode location. The mechanisms of the two kinds of ES have similarities and differences, which are summarized separately in terms of mechanism.

4.1.4.1. Direct muscle ES

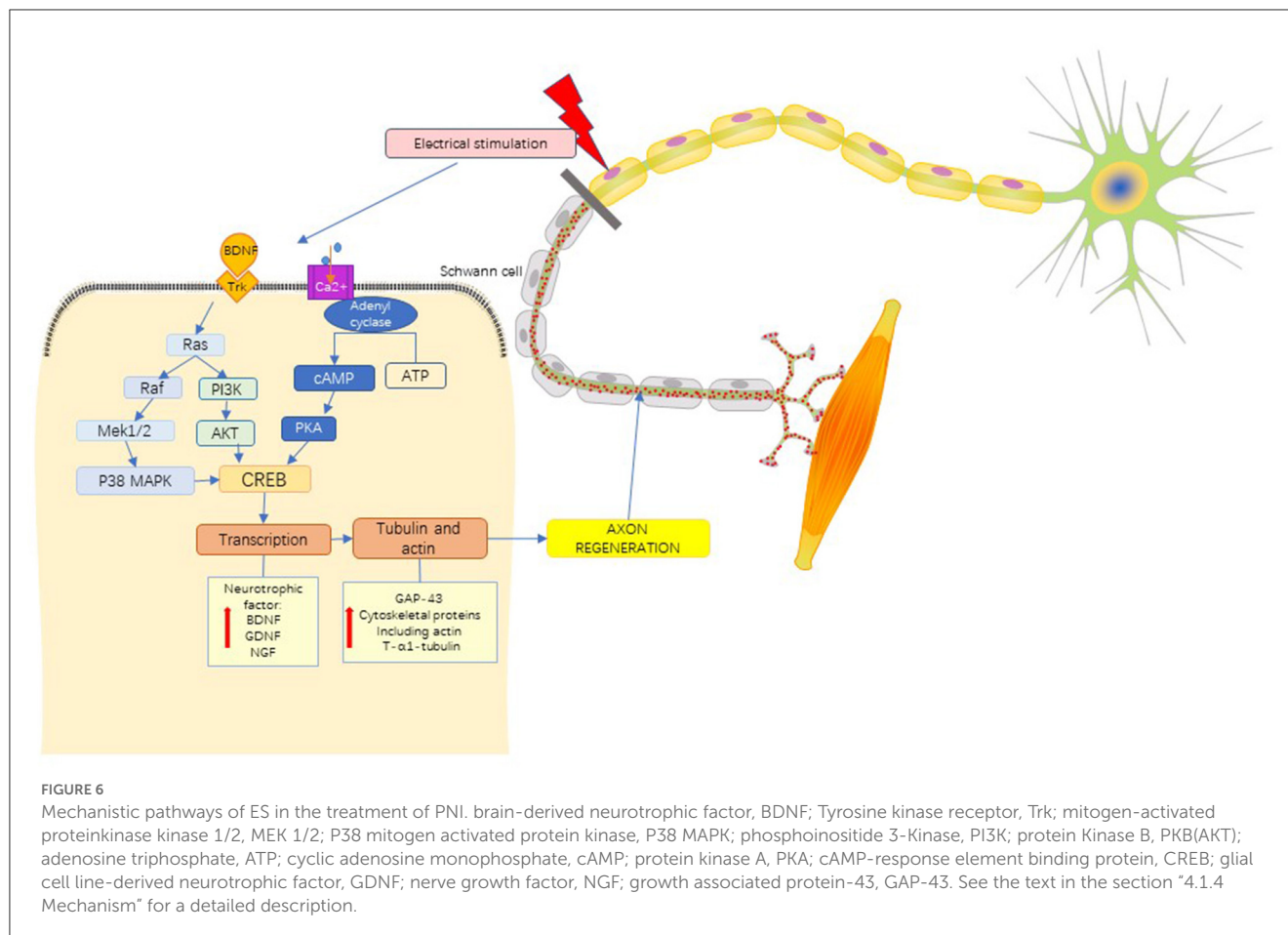
From animal studies, the mechanism of direct muscle ES on PNI may be high expression of myosin heavy chain (MHC) gene (32, 33). In experiments, surface electrodes were fixed to the skin of rats with nerve damage. The results showed that MHC expression in muscle was increased after ES, which promoted muscle strength recovery compared with the control group. A clinical trial has also verified that ES may achieve its effect by increasing MHC gene expression (34).

In addition, ES can also lead to an increase in light chain 3B-II (LC3-II) autophagy level. In one research, the sciatic nerve of rats was transected, and the proximal and distal parts of the nerve were repaired. The rats received ES (100 Hz, 200 μ s, stimulation 5 s and intermittent 10 s, 30 min/day) for 2 weeks. The outcome measurements had the sciatic function index, the structure of muscle fiber, and the growth of nerve axons. The results have shown that the rehabilitation plus ES group was better than the control group. Among them, studies have observed that ES could increase LC3-II whose level represented the degree of autophagy. After adding an autophagy inhibitor, the effect of ES was attenuated. More powerful evidence was provided (35).

Moreover, ES can increase beneficial M2 macrophage, allowing faster nerve repair (36). In a clinical trial, ES combined with protein intake increased pro-inflammatory-like macrophages, which could activate the early step of muscle regeneration and accelerate collagen synthesis. ES might cause muscle damage, thereby accumulating inflammatory macrophages. Pro-inflammatory-like macrophages were decreased with aging, and the data from this study suggested that the increase of macrophages might be a positive adaptive response, and muscle loading alleviated muscle atrophy during the cessation of ES (36).

4.1.4.2. Nerve ES

Previous studies have suggested that the effect of nerve ES is achieved by the up-regulation expression of brain-derived neurotrophic factor (BDNF) (37–39), glial cell line-derived neurotrophic factor (GDNF) (40), Tyrosine Kinase receptor B (TrkB) (41, 42) and Increasing of adenosine monophosphate (cAMP) (43). ES can cause Ca^{2+} influx, and the increase of Ca^{2+} in nerve cells induces the up-regulation of BDNF and TrkB expression (44). Overexpression of BDNF can inhibit phosphodiesterase activity, resulting in a sustained increase in cAMP levels (38, 45). Cytoskeleton formation is accelerated by activation of cAMP-response element binding protein (CREB), upregulation of RAGs such as T- α -1 tubulin and growth-associated protein-43 (GAP-43) expression (46), and inhibition of Rho (47). In addition, trkB-stimulated Ras activated CREB through P38 mitogen-activated protein kinase (P38 MAPK) pathway (18) and activation of



kinases phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway (48) to enhance the effect of ES on peripheral nerve regeneration (Figure 6).

The repair of nerve injury was delayed for different time durations (2, 4, 12, and 24 weeks), brief depolarizing ES (20 Hz, 3 V, 20 min) was applied to rats that were bridged with a hollow nerve conduit after sciatic nerve transection. The results have shown that the diameter and number of regenerated axons, myelin sheath thickness, and the number of the motor and sensory neurons significantly increased in delayed nerve injury repair. The brief ES also increased the expression of BDNF, which accelerated axon regeneration by BDNF-mediated neurotrophin signaling (37).

ES to promote regeneration of PNI may be through the increase of neurotrophic factors and cAMP. One study involved patients with severe carpal tunnel syndrome, the results proved that ES affected nerve recovery. To further elucidate the underlying mechanisms, they also injected agonists of cAMP into rats after femoral nerve transection surgery. Similar effects were achieved, which demonstrated the mechanism by which ES accelerated axonal regeneration (49).

Formation of synapses and the sustaining of motor neuron's cell body including vesicle glutamate transporters 1 (VGLUT 1) (50, 51). Park et al. (50) used mice with sciatic nerve transection to show that both single and repeated ES increased the direct muscle responses. Mice that received a single ES showed a progressive

increase in muscle contraction amplitude during recovery. Only in repeated treatment mice, cell bodies of excitatory and inhibitory synaptic contacts were sustained, including VGLUT 1. The maintenance of VGLUT1+ inputs onto motoneurons might be related to the expression of BDNF and its trkB receptor. Moreover, the H-reflex became twice its pre-injury level after repeated ES, which indicated that repeated ES could preserve muscle reflex by sustaining of VGLUT 1 (50).

In addition to nerve cells, Schwann cells (SC) are also affected by ES. In the animal model of tibial nerve transection, 1-time brief ES during surgery was used to improve the axonal regeneration of the transplanted nerve. The results observed that ES could convert M1 macrophages, which cause inflammation, into M2 macrophages, which are beneficial for repair, thereby rapidly removing myelin debris and improving neurological function (29, 52).

Some scholars believed that ES could redistribute blood flow to active muscles and meet muscle metabolic demands, which encouraged muscle contraction (53, 54). In animal experiments, nerve ES can increase the structural changes of capillaries in rats with nerve injury, thereby increasing blood flow, providing sufficient oxygen, and accelerating peripheral nerve regeneration (53). Especially at the current intensity of 10 mA and above, the effect of ES on accelerating the recovery of PNI was more significant (54).

4.2. Transcutaneous electrical nerve stimulation (TENS)

4.2.1. Stimulation mode and system composition

ES that uses a small current to activate sensory axons without triggering muscle contraction is called TENS. It is often used to treat pain, spasticity, and urinary incontinence (55). The commonly used frequency in clinical practice is 1–150 Hz (56). In rehabilitation, TENS are used to improve sensory feedback and adjust neural network pathways. Sensory feedback plays an important role in completing an action. When the motor nerve is damaged and unable to conduct, the sensory nerve will temporarily innervate the muscle to prevent muscle atrophy until the motor nerve recovers. A concept is termed chipmaker protection or chipmaker “babysitting” (57). TENS can improve sensory feedback which is important for the movement of the body. Pain is one of the sensory disorders that patients complain about most obviously and affect their daily life most, and it is also the most subjective sensory disorder (56). Therefore, there is more corresponding research in this area.

4.2.2. Clinical application (ultra-high, high, and low-frequency; implantable)

TENS has high and low-frequency modes. Low-frequency TENS is defined as the delivery of pulsed currents of 10 Hz or less (burst mode of TENS). High-frequency TENS is used to describe frequencies more than 10 Hz to the maximum setting on TENS devices, typically 150–200 Hz (58).

Bergeron-Vézina et al. (59) have compared the improvement effect of low-frequency TENS (110 Hz; 200 ms) and high-frequency TENS (unmodulated 5-kHz; 200 ms) on neurological function. Outcomes were no differences in mechanical pain threshold (MPT), heat pain threshold (HPT), tactile threshold (TT), and peripheral nerve conduction (PNC). However, patients felt more comfortable with Unmodulated 5-kHz currents. In an RCT (60), both high-frequency (100 Hz, 60 μ s) and low-frequency (3 Hz, 400 μ s) TENS improved pain in young adults, but there was no effect on the pain perception threshold of 15 elderly people (67 years old), reflecting that the elderly are less sensitive to TENS than young people.

One paper has shown that TENS with the patient's tolerance intensity can also improve pain. A meta-analysis of RCT has shown that non-invasive TENS could reduce pain by 30%, with the recommended intensity being “strong but comfortable” as optimal, and “mild”, “faint” or “barely perceptible” as suboptimal (58). In one study, TENS could improve 72 patients of non-cancer pain including peripheral nerve pain, which had affected life severely lasting more than 3 months. The ES parameter was the optimal frequency provided by the machine, and the intensity was standardized on patient tolerance. With pain improvement in 40% of patients at 6 months of treatment (61).

Some researchers have used ultra-high-frequency TENS to treat pain. The frequency was higher than 10 kHz and the treatment had a significant effect (62, 63). In a trial, 20 kHz of percutaneous high-frequency alternating current (HFAC) was applied to the ulnar and median nerves of volunteers for 20 min.

The current intensity from the beginning to the end of the stimulation ranged from 44.2 mA to an average final intensity of 85.0 mA. Outcome measurements had maximal handgrip strength, mechanical threshold, maximal handgrip strength (MHS), mechanical detection threshold (MDT), and pressure pain threshold (PPT). The results have shown that 1. The 20 kHz stimulation showed the lower MHS during the stimulation at the 15 min and 20 min when compared to the sham-stimulation group 2. The 20 kHz stimulation resulted in a slight increase in MDT at 15 min when compared to the sham-stimulation group 3. No effects were shown for PPT. The conclusion was that HFAC caused a partial block of nerve activity, which might be a therapeutic approach to the hyperactivity of the nervous system. Chronic pain can also be alleviated by nerve conduction block, but the optimal parameters are unknown (62). A double-blind trial used invasive ES at 10 kHz for chronic pain in 58 spinal cord (SCS) and 11 peripheral nerve (PNS) patients. Both groups showed a reduction of pain and disability after 3–6 months of stimulation, but PNS relieved pain to a greater extent than SCS. In patients with PNS, the effect of pain relief after stimulation was maintained for 12 h. There was no change in pain 2 h after stimulation in PNS patients, but the pain was significantly reduced after 4 h. In summary, 10 kHz ES performed for at least 4 h could reduce pain in patients with PNS (63).

For TENS and HFAC in improving pain which one is more effective, one study has shown that compared with the sham-stimulation group, 40 min of TENS can significantly inhibit the amplitude and lengthen the latency of the soleus H-reflex. TENS and HFAC at a frequency of 10 kHz had the effect of regulating the soleus H-reflex, but there was no meaningful difference between the two groups (62).

At the same time, a lot of clinical trials have shown that implanted TENS had a significant improvement in pain after PNI. Implantable TENS is an effective method for peripheral nerve pain caused by upper limb trauma. This technology in the armpit implantation of a nerve stimulator and four electrodes were placed on the affected sensory nerve branches (mainly brachial plexus, median nerve, and radial nerve). According to the characteristics of each patient set the stimulation parameters. Stimulation was an action for 24 h after nerve repair surgery. The results have shown that implantable TENS could significantly relieve and prevent intractable pain caused by PNI. Implantable TENS preserved the structure of neuroanatomy, which was an effective and feasible treatment method (64). Similarly, implanted TENS (12 Hz, 20 mA, 20–200 μ s) was used to treat refractory pain caused by subacromial impingement syndrome (SIS). The electrodes of a small ES wearable device were implanted into the end of the axillary nerve and the deltoid muscle, and the surface electrode was attached to the skin. Longitudinal analysis has shown a significant reduction in pain, which was most pronounced at 5, 8, and 16 weeks after treatment (65). In an RCT, electrodes were implanted at the affected shoulder axillary nerve in patients with shoulder impingement syndrome (12 Hz, 0.2–30 mA, 10–200 μ s). The results have shown that axillary nerve ES had a good effect on chronic shoulder pain (66).

Some studies have developed an intermediate frequency alternating current (10 kHz, 0.3 s), and surface electrodes were

applied to the median nerve. The results have shown that this intermediate frequency alternating current could be used to inhibit undesired sensory and motor activities and accelerate nerve repair (67).

However, there are conflicting results compared to previous studies. In a study to treat decreased shoulder strength after exercise-induced acute muscle pain, TENS (85 Hz, 0–80 mA, 75 μ s) was compared with the sham-stimulation group, and electrodes were placed around the rotator cuff. The results have shown that the two groups had no significant effect on pain relief, but had a Nocebo effect on shoulder muscle strength. This study suggested that the application of TENS was complex, and it was necessary to separate the psychological effects and sensory mechanisms of TENS to determine the outcomes of patients with pain (68).

One study has shown that the use of TENS during exercise in elderly patients with chronic pain could not improve the prognosis of patients, but it had a transient analgesic effect during exercise and was well tolerated by the elderly (69).

4.2.3. Animal model

A previous study used rats with sciatic nerve crush to verify the effect of ES, low-frequency ES (LFES 5 Hz), and high-frequency ES (HFES 100 Hz) in the early stage (at that time after injury) and late stage (7 days after injury). The results of the experiment were evaluated by motor function recovery score, thermal hyperalgesia test, gait analysis, and somatosensory cortex evoked potential. The results showed that immediate HFES significantly improved motor function but increased the susceptibility to neuropathic pain. Compared with LFES, HFES can increase the growth of nerve myelin sheath in both early and late stages. Late stage of HFES increased nerve regeneration without aggravating neuropathic pain in nerve crush injury. Moreover, cell experiments were also performed, in which the dorsal root ganglion cells of rats received ES at 5 Hz and 100 Hz for 30 min at 50 mA. The results of cell experiments, ES could activate dorsal root ganglion cells to express inflammatory cytokines, such as TNF- α , synapsin, and NGF (70).

In an animal experiment, 60 rats were divided into a sham operation control group (SHAM), a sciatic nerve denervation group (DN), and sciatic nerve denervation plus ES (DN-SM). Surface ES (2 Hz, 25 V, 1 mA, 300 ms, 10 min/day) was performed for 28 days with one electrode attached to the achilles tendon and the other to the popliteal fossa. Proteomics, transcriptomics, bioinformatics, and skeletal muscle function analysis were used to observe the molecular expression changes induced by ES. The results showed that the DN-SM group was better than the DN group in terms of muscle mass, muscle fiber diameter, and contractile properties. At the molecular cellular level, the FoxO and p53 signaling pathways are important in structural protection by bioinformatics analysis. Anti-apoptosis proteins (KCN A7, KCNJ11) were down-regulated after denervation but up-regulated after ES. Muscle fiber type-related proteins (TNNI1, TNNT1, ACTN2), myosin light chain kinase 2 and myomesin 2, fibrosis-associated proteins (POSTN, COL1A1, COL1A2, COL6A1, COL6A2, COL6A3, FN1, and LUM) were all increased after denervation but decreased by ES. All these results explained the mechanism of ES in promoting nerve regeneration (71).

4.2.4. Mechanism

The mechanism of TENS to relieve pain and promote nerve regeneration may be that (1) TENS can stimulate low-threshold skin afferents to inhibit the positive transmission of nociceptive information in the central nervous system, thereby relieving pain (also known as, segmental modulation). In addition, TENS can stimulate the small-diameter afferent pathway to activate the descending pain inhibition pathway or block the afferent activity of peripheral neurons, forming a “busy line” effect (72). (2) It has been confirmed in the human body that ES can block nerve conduction and reduce H-reflex excitability, thereby improving the situation of abnormal increase in H-reflex caused by nerve overactivities, such as spasticity and pain (73). (3) Powerful evidence confirmed that from the aspects of hemodynamics, increasing blood flow to the ES can restore nourishment. In a study comparing TENS and NMES influence on the hemodynamics of the gastrocnemius muscle, the results showed that ES can increase muscle hemodynamics. Compared to the NMES, TENS can increase blood flow even more (74). (4) Animal experiments have shown that ES can activate nerve cells to express inflammatory cytokines. For example, the FoxO and p53 signaling pathways were activated, and muscle-related structural proteins were increased (71).

4.3. Functional electrical stimulation (FES)

4.3.1. Stimulation mode

FES is the ES of muscles or nerves to provide functional improvement. Applications of FES include restoration of upper limb functions, such as stretching and grasping and lower limb functions, such as standing, balance, posture, and gait. There are three types of stimulation methods called fully implanted, percutaneous stimulation, and surface stimulation (75). For treatment, surface stimulation is preferable because it does not invade the body. Current surface electrodes use biocompatible gels that provide stability on the skin and uniform current distribution on the electrode surface. Conventional surface electrodes are suitable for innervating large muscles close to the skin (76).

4.3.2. Clinical application (activity function, muscle fatigue, latest technology)

The FES mainly focuses on the walking function of the lower limbs and the finger-grasping function of the upper limbs. A surface FES of the tibial nerve in healthy participants showed that FES activated both thigh and calf muscle contraction. During walking, contraction of the thigh and calf muscles plays an important role in gait. In the experiment, the anode (50 Hz, 300 ms) was fixed on the calf gastrocnemius muscle, and the medial gastrocnemius muscle, tibialis anterior, semitendinosus, and rectus femoris were recorded by the electromyography (EMG). This study has shown that FES had a significant effect on the contraction and activation patterns of muscles (77).

Using FES during walking, 30 min of stimulation could increase the half-maximum peak-to-peak motor evoked potential (MEPh) of the tibial nerve, and the effect lasted for at least 30 min. In addition, the results showed that only the combination of FES

and exercise could lead to an increase in corticospinal excitability without cortical inhibition and further promote the afferent of the central nervous system (78).

It has been performed that FES on the ulnar nerve and median nerve was effective for upper limb motor function. A non-transcutaneous FES was performed on the proximal ulnar and median nerve bundle. Individual finger and joint grasping movements were observed using 24 fingers movement to quantify hand grasping patterns. The results have shown that this stimulation technique was able to stimulate individual and coordinated movements. The grasping pattern was different depending on the location of the stimulation. In the future, it can be used for the treatment of grasping with weak fingers (79). A similar trial used 30 Hz ES with three anode electrodes placed in the proximal segment of the inferior radial nerve to activate and control different finger and wrist extension movements (80).

ES of the radial and median nerves in eight tetraplegic patients was able to provide useful grasping movements. A multi-contact cuff electrode (25 Hz, 250 μ s) was implanted around the median or radial nerve 5 cm above the elbow of the subjects. Through evaluating the flexion and extension of the thumb, finger, wrist, and functional movements, the results have shown that this minimally invasive ES could effectively restore the patient's grip function. Future studies using two microelectrodes to activate more muscle activity are expected (81).

In addition to the coordination of various parameters to achieve the purpose of stimulating muscle and nerve recovery, excessive muscle fatigue caused by FES will reduce the effect. To alleviate muscle fatigue, some studies have further improved the stimulation parameters. Some scholars have used ultrasonic echogenicity as an evaluation index of FES-induced muscle fatigue, and there was a strong linear relationship between ultrasonic echogenicity and muscle fatigue level. Muscle-in-the-loop FES controllers considering muscle fatigue are helpful to produce better stimulation effects (82).

Researchers have used multiple electrodes to activate a larger volume of muscle to reduce muscle fatigue. The results have proved that ES with four intramuscular electrodes was more conducive to muscle contraction and endurance than ES with a single intramuscular electrode. In addition, it was further demonstrated that muscle fatigue might be caused by single FES not activating the intact motor synapses of the muscle (83). For muscle fatigue or damage caused by prolonged stimulation, a cross-sectional study used ES with different parameters to treat 24 cases of denervated extensor digitorum communis muscle and 24 cases of denervated tibialis anterior muscle. Different combinations of pulse duration and polarity were evaluated using triangular pulses, with current increasing from 0.1 mA and a frequency of 1 Hz. The results have shown that the triangular current with a duration of 200 ms and cathode polarity had a better effect on the denervated tibialis anterior muscle, which was statistically significant (84).

One study used FES at 10, 35, and 50 Hz on two muscles (vastus lateralis, VL; abductor pollicis brevis, APB) with different proportions of fast fibers and slow fibers. The results have shown that, In high-frequency stimulation, VL with more fast muscle fibers was tired faster than APB with more slow muscle fibers, and the treatment time should not exceed 14–16 min (85).

Jaramillo Cienfuegos et al. (86) have used a proportional integral (PI) controller to achieve classical and adaptive control of FES for isolated skeletal muscle contraction, which provided the best closed-loop performance for contraction speed and anti-interference. Future research should use algorithms to control FES muscle contraction, focusing on different stimulation sites. On the premise of avoiding muscle fatigue caused by stimulation as much as possible, the best stimulation effect was evaluated by the combination of each stimulus parameter. We summarized the various ES parameters applied to PNI (Supplementary Table 1).

4.3.3. Animal models

Animal experiments have shown that ES could significantly accelerate the regeneration of injured peripheral nerves. Some scholars have conducted animal experiments to prove that ES could promote the recovery of animal nerve function and increase the strength of muscle contraction (87). In the experiment, FES was used to activate the maximum muscle contraction force in the anterior deltoid muscle of monkeys, showing that the distributed dual electrode could generate an additional 50% contraction force compared with the single electrode, which improved the effect of FES.

In the experiment of horses with recurrent laryngeal nerve injury, FES was implanted into the ipsilateral posterior cricoarytenoid muscle (PCA) after 20 weeks of ES, which improved muscle strength and laryngeal function (88).

An implantable microelectrode array (MEA) belongs to the scope of FES, which is used to prevent muscle atrophy and acetylcholine receptor degradation during nerve regeneration after PNI. In one study (89), the tibial nerves of rats were cut, and MEA was placed on the surface of the biceps femoris muscle. The results have shown that the atrophy degree of muscle fiber cross-section in MEA-mediated FES rats was less than that in control rats, and the area of acetylcholine receptor was significantly increased.

4.3.4. Mechanism

At present, the mechanism hypothesis of FES promoting nerve regeneration and muscle contraction after PNI is as follows: (1) It increases the blood flow of the stimulated muscle capillaries and the flux of red blood cells (90). (2) FES is mainly applied at the motor point (motor point stimulation, MPS). Some studies have explored the neural pathways activated by MPS, and the results have shown that MPS did not induce H reflex or LA sensory nerve activation, but it could induce H reflex inhibition of stimulated muscles and skin inhibition (91).

5. Combined application of ES and other rehabilitation methods (exercise, phototherapy, etc.)

ES plays a role in promoting the recovery of PNI. Some studies have combined ES with other rehabilitation methods to achieve better therapeutic effects. The main combined rehabilitation methods include exercise training (40, 50, 92), phototherapy, magnetic stimulation, cryotherapy, etc.

ES combined with exercise can more strongly promote peripheral axon regeneration and relieve pain after PNI (40, 50). An RCT has shown that physical therapy exercise training combined with TENS was more effective in treating nerve pain in men (92). In a controlled study, low-level laser (continuous wave: 15 mW, 632.8 nm; Pulsed: 9.4 W, 904 nm) plus microamperes TENS had a significant effect compared with the control group in the treatment of carpal tunnel syndrome pain. And the improvement effect could be maintained for 1–3 years (93). The combination of low-frequency transcutaneous magnetic stimulation and ES in the treatment of PNI could counteract the slowing effect of TENS on the fast conduction fibers, and regulate the slow conduction of pain afferent fibers, resulting in better analgesic effect (94). Some scholars have combined cryotherapy with burst TENS to effectively improve the pain threshold of participants, and the effect of the combination was better, but the combination of cryotherapy and ordinary TENS had no improvement (95). Some researchers have also obtained negative results. Compared with the use of NMES or motor images alone, the combination of NMES and motor images did not achieve better results (96).

Similar studies in animal experiments have shown that ES could achieve better results when combined with exercise training, magnetic stimulation, and stem cell therapy. After sciatic nerve transection repair in adult rats, the rats were divided into 4 groups, the immediate ES after injury (ESA), delayed ES, ES+ exercise, and exercise groups. The results have shown that compared with the control group and the rats receiving delayed ES, ESA with or without exercise group improved muscle reinnervation and increased the number of regenerated myelin axons. ESA combined with exercise significantly improved muscle reinnervation at an early stage (97). In an animal experiment, electrical muscle stimulation prevented muscle mass loss. Combined with exercise, twitch characteristics, fatigue index, mechanical sensitivity, and mechanosensitivity could be further recovered (98). PNI would enhance the transmission of pain fibers and lead to chronic post-traumatic pain. Animal experiments have shown that magnetic stimulation and ES could promote the recovery of PNI and allow a tolerance of high-intensity output (99). The combined effect of ES and stem cell therapy could better promote nerve regeneration and improve functional recovery after sciatic nerve transection in rats. ES was able to up-regulate the expression of neurotrophic factors (BDNF, NTF-3) and increase the expression of neurotrophic factor receptor (Trk) in human neural progenitor cells (hNPC). Thus, it was capable of promoting angiogenesis, axon dendrite growth, myelin sheath thickening, and accelerate peripheral nerve regeneration and functional recovery (39).

6. Limitations of clinical application

In conclusion, ES can accelerate the recovery of body function after PNI, but ES still has the following problems to be solved, (1) What are the changes in brain activity induced by ES. (2) Optimal ES parameters are expected. (3) Further clinical applications provide more authority evidence to verify the effectiveness of ES. (4) Solving the side effects of ES.

Most importantly, the changes in the central nervous system after PNI are not well understood, which is the basis for determining the appropriate method of ES and finding new

methods. Although there are many positive results of ES, there are also some doubts about perceptions. For example, some studies have shown that ES could aggravate muscle fiber atrophy, reduce muscle excitability, and inhibit peripheral nerve regeneration (100).

7. Conclusion

The advantage of NMES can activate type II fibers that are most affected by aging and responsible for the decline in functional activity (18). The disadvantage of high-frequency continuous NMES is easy to causes muscle fatigue. PNS patients have higher comfort than NMES patients.

TENS has a good effect on the treatment of stubborn pain caused by PNI, and non-invasive or minimally invasive TENS is easy to be accepted by patients. The disadvantages of TENS are that the effect is short, and the elderly are less effective than the young because of their decreased sensitivity.

The advantage of FES is that targeted stimulation of a nerve or muscle can produce stable effects for different motor functions. However, it is uncomfortable to wear an ES instrument for a long time. Prolonged FES may cause muscle fatigue and decrease responsiveness.

With the number of patients with PNI increasing, more attention should be paid to the repair effect of peripheral nerves. This paper believes that ES can accelerate neurological recovery of PNI, effectively relieve pain and increase muscle mass and strength in patients, and it is necessary to select appropriate ES methods and parameters according to the actual situation of patients.

Author contributions

LN: conceptualization, methodology, and validation. ZY: searching literatures and writing—original draft preparation. YZ: visualization and formal analysis. TZ: supervision and data curation. JW: software and validation. SL: writing—reviewing and editing. ZC: funding acquisition and supervision. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Zhejiang Key Research and Development Program of China (No. 2021C03050) and the Zhejiang Provincial Chinese Medicine Science and Technology Program of China (No. 2023ZL489).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of

their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Robinson LR. Traumatic injury to peripheral nerves. *Muscle Nerve*. (2022). doi: 10.1002/mus.27706
- Jensen PH, Li JY, Dahlström A, Dotti CG. Axonal transport of synucleins is mediated by all rate components. *Eur J Neurosci*. (1999) 11:3369–76. doi: 10.1046/j.1460-9568.1999.00754.x
- Rabia J, Qiang A. Nanoparticles in peripheral nerve regeneration: a mini-review. *J Neurorestoratology*. (2022) 10:1–12. doi: 10.26599/JNR.2022.9040001
- Sharma HS, Chopp M, Chen L, Sarnowska A, Xue M, Ao Q, et al. The 2021 yearbook of Neurorestoratology. *J Neurorestoratology*. (2022) 10:100008. doi: 10.1016/j.jnrt.2022.100008
- Huang H, Chen L, Chopp M, Young W, Robert Bach J, He X, et al. The 2020 yearbook of Neurorestoratology. *J Neurorestoratology*. (2021) 9:1–12. doi: 10.26599/JNR.2021.9040002
- Haastert-Talini K, Schmitte R, Korte N, Klode D, Ratzka A, Grothe C. Electrical stimulation accelerates axonal and functional peripheral nerve regeneration across long gaps. *J Neurotrauma*. (2011) 28:661–74. doi: 10.1089/neu.2010.1637
- Sunderland S. A classification of peripheral nerve injuries producing loss of function. *Brain*. (1951) 74:491–516. doi: 10.1093/brain/74.4.491
- Raza C, Riaz HA, Anjum R, Shakeel NUA. Repair strategies for injured peripheral nerve: Review. *Life Sci*. (2020) 243:117308. doi: 10.1016/j.lfs.2020.117308
- Lal D, Hetzler LT, Sharma N, Wurster RD, Marzo SJ, Jones KJ, et al. Electrical stimulation facilitates rat facial nerve recovery from a crush injury. *Otolaryngol Head Neck Surg*. (2008) 139:68–73. doi: 10.1016/j.otohns.2008.04.030
- Al-Majed AA, Neumann CM, Brushart TM, Gordon T. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci*. (2000) 20:2602–8. doi: 10.1523/JNEUROSCI.20-07-02602.2000
- Mestriner RG, Oliveira CBD, Melo DAS, Menezes MFd, Filho IG, Silva JB, et al. Chordata method combined with electrotherapy in functional recovery after brachial plexus injury: report of three clinical cases. *Social Sci Med*. (2016) 26:22425. doi: 10.15448/1980-6108.2016.2.22425
- Witt A, Kristensen RS, Fuglsang-Frederiksen A, Pedersen TH, Finnerup NB, Kasch H, et al. Muscle velocity recovery cycles in neurogenic muscles. *Clin Neurophysiol*. (2019) 130:1520–7. doi: 10.1016/j.clinph.2019.05.030
- ElAbd R, Alabdulkarim A, AlSabah S, Hazan J, Alhalabi B, Thibaudeau S. Role of electrical stimulation in peripheral nerve regeneration: a systematic review. *Plast Reconstr Surg Glob Open*. (2022) 10:e4115. doi: 10.1097/GOX.0000000000004115
- Truong AD, Kho ME, Brower RG, Feldman DR, Colantuoni E, Needham DM. Effects of neuromuscular electrical stimulation on cytokines in peripheral blood for healthy participants: a prospective, single-blinded Study. *Clin Physiol Funct Imaging*. (2017) 37:255–62. doi: 10.1111/cpf.12290
- Gordon T, Amirjani N, Edwards DC, Chan KM. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol*. (2010) 223:192–202. doi: 10.1016/j.expneurol.2009.09.020
- Power HA, Morhart MJ, Olson JL, Chan KM. Postsurgical electrical stimulation enhances recovery following surgery for severe cubital tunnel syndrome: a double-blind randomized controlled trial. *Neurosurgery*. (2020) 86:769–77. doi: 10.1093/neuros/nyz322
- Wong JN, Olson JL, Morhart MJ, Chan KM. Electrical stimulation enhances sensory recovery: a randomized controlled trial. *Ann Neurol*. (2015) 77:996–1006. doi: 10.1002/ana.24397
- Acaröz Candan S, Akoglu AS, Büyükan S, Yüksel F. Effects of neuromuscular electrical stimulation of quadriceps on the quadriceps strength and functional performance in nursing home residents: A comparison of short and long stimulation periods. *Geriatr Gerontol Int*. (2019) 19:409–13. doi: 10.1111/ggi.13633
- Toth MJ, Tourville TW, Voigt TB, Choquette RH, Anair BM, Falcone MJ, et al. Utility of neuromuscular electrical stimulation to preserve quadriceps muscle fiber size and contractility after anterior cruciate ligament injuries and reconstruction: a randomized, sham-controlled, blinded trial. *Am J Sports Med*. (2020) 48:2429–37. doi: 10.1177/0363546520933622
- Stevens-Lapsley JE, Balter JE, Wolfe P, Eckhoff DG, Kohrt WM. Early neuromuscular electrical stimulation to improve quadriceps muscle strength after total knee arthroplasty: a randomized controlled trial. *Phys Ther*. (2012) 92:210–26. doi: 10.2522/ptj.20110124
- Pinto Damo NL, Modesto KA, Neto IVS, Bottaro M, Babault N, Durigan JLQ. Effects of different electrical stimulation currents and phase durations on submaximal and maximum torque, efficiency, and discomfort: a randomized crossover trial. *Braz J Phys Ther*. (2021) 25:593–600. doi: 10.1016/j.bjpt.2021.03.001
- Mani D, Almklass AM, Amiridis IG, Enoka RM. Neuromuscular electrical stimulation can improve mobility in older adults but the time course varies across tasks: Double-blind, randomized trial. *Exp Gerontol*. (2018) 108:269–75. doi: 10.1016/j.exger.2018.04.018
- Vanderthommen M, Duchateau J. Electrical stimulation as a modality to improve performance of the neuromuscular system. *Exerc Sport Sci Rev*. (2007) 35:180–5. doi: 10.1097/jes.0b013e318156e785
- Inns TB, McCormick D, Greig CA, Atherton PJ, Phillips BE, Piasecki M. Factors associated with electrical stimulation-induced performance fatigability are dependent upon stimulation location. *Exp Physiol*. (2021) 106:828–36. doi: 10.1111/EP089204
- Hyer CF, Berlet G, Philbin T, Bull P, Brandão R, Prissel M, et al. Does functional neuromuscular electrical stimulation (NMES) influence calf atrophy following achilles tendon surgery? Prospective double-blind randomized controlled trial on the use of immediate postoperative electrical muscle stimulation to preserve muscle function and volume. *J Foot Ankle Surg*. (2021) 60:683–8. doi: 10.1053/j.jfas.2020.12.005
- Piccinini G, Cuccagna C, Caliendo P, Coraci D, Germanotta M, Pecchioli C, et al. Efficacy of electrical stimulation of denervated muscle: a multicenter, double-blind, randomized clinical trial. *Muscle Nerve*. (2020) 61:773–8. doi: 10.1002/mus.26880
- Nicolas N, Kobaiter-Maarrawi S, Georges S, Abadjian G, Maarrawi J. Motor cortex stimulation regenerative effects in peripheral nerve injury: an experimental rat model. *World Neurosurg*. (2018) 114:e800–e8. doi: 10.1016/j.wneu.2018.03.090
- Sayanagi J, Acevedo-Cintrón JA, Pan D, Schellhardt L, Hunter DA, Snyder-Warwick AK, et al. Brief electrical stimulation accelerates axon regeneration and promotes recovery following nerve transection and repair in mice. *J Bone Joint Surg Am*. (2021) 103:e80. doi: 10.2106/JBJS.20.01965
- Keane GC, Pan D, Roh J, Larson EL, Schellhardt L, Hunter DA, et al. The effects of intraoperative electrical stimulation on regeneration and recovery after nerve isograft repair in a rat model. *Hand (N Y)*. (2022) 17:540–8. doi: 10.1177/1558944720939200
- Raslan A, Salem MAM, Al-Hussaini A, Guntinas-Lichius O, Irintchev A. Brief electrical stimulation improves functional recovery after femoral but not after facial nerve injury in rats. *Anat Rec (Hoboken)*. (2019) 302:1304–13. doi: 10.1002/ar.24127
- Yu AP, Shen YJ, Qiu YQ, Li J, Shen YD, Wang XM, et al. Comparative effects of implanted electrodes with differing contact patterns on peripheral nerve regeneration and functional recovery. *Neurosci Res*. (2019) 145:22–9. doi: 10.1016/j.neures.2018.08.007
- Xing H, Zhou M, Assinck P, Liu N. Electrical stimulation influences satellite cell differentiation after sciatic nerve crush injury in rats. *Muscle Nerve*. (2015) 51:400–11. doi: 10.1002/mus.24322
- Koh ES, Kim HC, Lim JY. The effects of electromyostimulation application timing on denervated skeletal muscle atrophy. *Muscle Nerve*. (2017) 56:E154–e61. doi: 10.1002/mus.25656
- Segers J, Vanhorebeek I, Langer D, Charusisin N, Wei W, Frickx B, et al. Early neuromuscular electrical stimulation reduces the loss of muscle mass in critically ill patients—a within subject randomized controlled trial. *J Crit Care*. (2021) 62:65–71. doi: 10.1016/j.jcrc.2020.11.018
- Fu T, Jiang L, Peng Y, Li Z, Liu S, Lu J, et al. Electrical muscle stimulation accelerates functional recovery after nerve injury. *Neuroscience*. (2020) 426:179–88. doi: 10.1016/j.neuroscience.2019.10.052
- Reidy PT, Edvalson LT, McKenzie AI, Petrocelli JJ, Mahmassani ZS, Drummond MJ. Neuromuscular electrical stimulation and protein during bed rest increases CD11b(+) skeletal muscle macrophages but does not correspond to muscle size or insulin sensitivity. *Appl Physiol Nutr Metab*. (2020) 45:1261–9. doi: 10.1139/apnm-2020-0064
- Huang J, Zhang Y, Lu L, Hu X, Luo Z. Electrical stimulation accelerates nerve regeneration and functional recovery in delayed peripheral nerve injury in rats. *Eur J Neurosci*. (2013) 38:3691–701. doi: 10.1111/ejn.12370

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2023.1081458/full#supplementary-material>

38. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. *Eur J Neurosci.* (2000) 12:4381–90. doi: 10.1046/j.1460-9568.2000.01341.x
39. Song S, McConnell KW, Amores D, Levinson A, Vogel H, Quarta M, et al. Electrical stimulation of human neural stem cells via conductive polymer nerve guides enhances peripheral nerve recovery. *Biomaterials.* (2021) 275:120982. doi: 10.1016/j.biomaterials.2021.120982
40. Cobianchi S, Casals-Diaz L, Jaramillo J, Navarro X. Differential effects of activity dependent treatments on axonal regeneration and neuropathic pain after peripheral nerve injury. *Exp Neurol.* (2013) 240:157–67. doi: 10.1016/j.expneurol.2012.11.023
41. Al-Majed AA, Tam SL, Gordon T. Electrical stimulation accelerates and enhances expression of regeneration-associated genes in regenerating rat femoral motoneurons. *Cell Mol Neurobiol.* (2004) 24:379–402. doi: 10.1023/B:CEMN.0000022770.66463.f7
42. Eberhardt KA, Irintchev A, Al-Majed AA, Simova O, Brushart TM, Gordon T, et al. BDNF/TrkB signaling regulates HNK-1 carbohydrate expression in regenerating motor nerves and promotes functional recovery after peripheral nerve repair. *Exp Neurol.* (2006) 198:500–10. doi: 10.1016/j.expneurol.2005.12.018
43. Batty NJ, Torres-Espin A, Vavrek R, Raposo P, Fouad K. Single-session cortical electrical stimulation enhances the efficacy of rehabilitative motor training after spinal cord injury in rats. *Exp Neurol.* (2020) 324:113136. doi: 10.1016/j.expneurol.2019.113136
44. Wenjin W, Wenchao L, Hao Z, Feng L, Yan W, Wodong S, et al. Electrical stimulation promotes BDNF expression in spinal cord neurons through Ca(2+)- and Erk-dependent signaling pathways. *Cell Mol Neurobiol.* (2011) 31:459–67. doi: 10.1007/s10571-010-9639-0
45. Udina E, Furey M, Busch S, Silver J, Gordon T, Fouad K. Electrical stimulation of intact peripheral sensory axons in rats promotes outgrowth of their central projections. *Exp Neurol.* (2008) 210:238–47. doi: 10.1016/j.expneurol.2007.11.007
46. Geremia NM, Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. *Exp Neurol.* (2007) 205:347–59. doi: 10.1016/j.expneurol.2007.01.040
47. Han PJ, Shukla S, Subramanian PS, Hoffman PN. Cyclic AMP elevates tubulin expression without increasing intrinsic axon growth capacity. *Exp Neurol.* (2004) 189:293–302. doi: 10.1016/j.expneurol.2004.03.010
48. Osorio-Fuentealba C, Contreras-Ferrat AE, Altamirano F, Espinosa A, Li Q, Niu W, et al. Electrical stimuli release ATP to increase GLUT4 translocation and glucose uptake via PI3K-Akt-AS160 in skeletal muscle cells. *Diabetes.* (2013) 62:1519–26. doi: 10.2337/db12-1066
49. Gordon T, Chan KM, Sulaiman OA, Udina E, Amirjani N, Brushart TM. Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury. *Neurosurgery.* (2009) 65:A132–44. doi: 10.1227/01.NEU.0000335650.09473.D3
50. Park S, Liu CY, Ward PJ, Jaiswal PB, English AW. Effects of repeated 20-Hz electrical stimulation on functional recovery following peripheral nerve injury. *Neurorehabil Neural Repair.* (2019) 33:775–84. doi: 10.1177/1545968319862563
51. Cirillo G, Colangelo AM, Berbeni M, Ippolito VM, De Luca C, Verdesca F, et al. Purinergic modulation of spinal neuroglial maladaptive plasticity following peripheral nerve injury. *Mol Neurobiol.* (2015) 52:1440–57. doi: 10.1007/s12035-014-8943-y
52. McLean NA, Verge VM. Dynamic impact of brief electrical nerve stimulation on the neural immune axis-polarization of macrophages toward a pro-repair phenotype in demyelinated peripheral nerve. *Glia.* (2016) 64:1546–61. doi: 10.1002/glia.23021
53. Egginton S, Hudlická O. Early changes in performance, blood flow and capillary fine structure in rat fast muscles induced by electrical stimulation. *J Physiol.* (1999) 515:265–75. doi: 10.1111/j.1469-7793.1999.265ad.x
54. Lin YC, Kao CH, Cheng YK, Chen JJ, Yao CH, Chen YS. Current-modulated electrical stimulation as a treatment for peripheral nerve regeneration in diabetic rats. *Restor Neurol Neurosci.* (2014) 32:437–46. doi: 10.3233/RNN-130381
55. Johnson MI, Paley CA, Jones G, Mulvey MR, Wittkopf PG. Efficacy and safety of transcutaneous electrical nerve stimulation (TENS) for acute and chronic pain in adults: a systematic review and meta-analysis of 381 studies (the meta-TENS study). *BMJ Open.* (2022) 12:e051073. doi: 10.1136/bmjopen-2021-051073
56. Vance CG, Dailey DL, Rakel BA, Sluka KA. Using TENS for pain control: the state of the evidence. *Pain Manag.* (2014) 4:197–209. doi: 10.2217/pmt.14.13
57. Adidharma W, Khouri AN, Lee JC, Vanderboll K, Kung TA, Cederna PS, et al. Sensory nerve regeneration and reinnervation in muscle following peripheral nerve injury. *Muscle Nerve.* (2022) 66:384–96. doi: 10.1002/mus.27661
58. Johnson MI, Claydon LS, Herbison GP, Jones G, Paley CA. Transcutaneous electrical nerve stimulation (TENS) for fibromyalgia in adults. *Cochrane Database Syst Rev.* (2017) 10:Cd012172. doi: 10.1002/14651858.Cd012172.pub2
59. Avendaño-Coy J, Gómez-Soriano J, Goicoechea-García C, Basco-López JA, Taylor J. Effect of unmodulated 5-kHz alternating currents versus transcutaneous electrical nerve stimulation on mechanical and thermal pain, tactile threshold, and peripheral nerve conduction: a double-blind, placebo-controlled crossover trial. *Arch Phys Med Rehabil.* (2017) 98:888–95. doi: 10.1016/j.apmr.2016.11.020
60. Bergeron-Vézina K, Corriveau H, Martel M, Harvey MP, Léonard G. High- and low-frequency transcutaneous electrical nerve stimulation does not reduce experimental pain in elderly individuals. *Pain.* (2015) 156:2093–9. doi: 10.1097/j.pain.0000000000000276
61. Tonye-Geoffroy L, Mauboussin Carlos S, Tuffet S, Fromentin H, Berard L, Leblanc J, et al. Efficacy of a combination of hypnosis and transcutaneous electrical nerve stimulation for chronic non-cancer pain: a randomized controlled trial. *J Adv Nurs.* (2021) 77:2875–86. doi: 10.1111/jan.14833
62. Serrano-Muñoz D, Avendaño-Coy J, Simón-Martínez C, Taylor J, Gómez-Soriano J. 20-kHz alternating current stimulation: effects on motor and somatosensory thresholds. *J Neuroeng Rehabil.* (2020) 17:22. doi: 10.1186/s12984-020-00661-x
63. Finch P, Price L, Drummond P. High-frequency (10 kHz) electrical stimulation of peripheral nerves for treating chronic pain: a double-blind trial of presence vs absence of stimulation. *Neuromodulation.* (2019) 22:529–36. doi: 10.1111/ner.12877
64. Stevanato G, Devigili G, Eleopra R, Fontana P, Lettieri C, Baracco C, et al. Chronic post-traumatic neuropathic pain of brachial plexus and upper limb: a new technique of peripheral nerve stimulation. *Neurosurg Rev.* (2014) 37:473–79. doi: 10.1007/s10143-014-0523-0
65. Wilson RD, Harris MA, Gunzler DD, Bennett ME, Chae J. Percutaneous peripheral nerve stimulation for chronic pain in subacromial impingement syndrome: a case series. *Neuromodulation.* (2014) 17:771–6. doi: 10.1111/ner.12152
66. Cleland T, Jain NB, Chae J, Hansen KM, Hisel TZ, Gunzler DD, et al. The protocol for a multisite, double blind, randomized, placebo-controlled trial of axillary nerve stimulation for chronic shoulder pain. *Trials.* (2020) 21:248. doi: 10.1186/s13063-020-4174-x
67. Kim Y, Cho HJ, Park HS. Technical development of transcutaneous electrical nerve inhibition using medium-frequency alternating current. *J Neuroeng Rehabil.* (2018) 15:80. doi: 10.1186/s12984-018-0421-8
68. Butera KA, George SZ, Borsa PA, Dover GC. Prolonged reduction in shoulder strength after transcutaneous electrical nerve stimulation treatment of exercise-induced acute muscle pain. *Pain Pract.* (2018) 18:954–68. doi: 10.1111/papr.12690
69. Vaillancourt S, Coulombe-Lévêque A, Fradette J, Martel S, Naour W, da Silva RA, et al. Combining transcutaneous electrical nerve stimulation with therapeutic exercise to reduce pain in an elderly population: a pilot study. *Disabil Rehabil.* (2021) 43:2141–48. doi: 10.1080/09638288.2019.1693639
70. Su HL, Chiang CY, Lu ZH, Cheng FC, Chen CJ, Sheu ML, et al. Late administration of high-frequency electrical stimulation increases nerve regeneration without aggravating neuropathic pain in a nerve crush injury. *BMC Neurosci.* (2018) 19:37. doi: 10.1186/s12868-018-0437-9
71. La G, Zhou M, Lim JY, Oh S, Xing H, Liu N, et al. Proteomics and transcriptomics analysis reveals clues into the mechanism of the beneficial effect of electrical stimulation on rat denervated gastrocnemius muscle. *Cell Physiol Biochem.* (2019) 52:769–86. doi: 10.33594/0000000054
72. Johnson MI, Jones G, Paley CA, Wittkopf PG. The clinical efficacy of transcutaneous electrical nerve stimulation (TENS) for acute and chronic pain: a protocol for a meta-analysis of randomised controlled trials (RCTs). *BMJ Open.* (2019) 9:e029999. doi: 10.1136/bmjopen-2019-029999
73. Serrano-Muñoz D, Gómez-Soriano J, Bravo-Esteban E, Ávila-Martín G, Galán-Arriero I, Taylor J, et al. Soleus H-reflex modulation following transcutaneous high- and low-frequency spinal stimulation in healthy volunteers. *J Electromyogr Kinesiol.* (2019) 46:1–7. doi: 10.1016/j.jelekin.2019.03.004
74. Senin-Camargo F, Martínez-Rodríguez A, Chouza-Insua M, Raposo-Vidal I, Jácome MA. Effects on venous flow of transcutaneous electrical stimulation, neuromuscular stimulation, and sham stimulation on soleus muscle: A randomized crossover study in healthy subjects. *Medicine (Baltimore).* (2022) 101:e30121. doi: 10.1097/MD.00000000000030121
75. Popovic MR, Popovic DB, Keller T. Neuroprostheses for grasping. *Neurol Res.* (2002) 24:443–52. doi: 10.1179/016164102101200311
76. Miller L, McFadyen A, Lord AC, Hunter R, Paul L, Rafferty D, et al. Functional electrical stimulation for foot drop in multiple sclerosis: a systematic review and meta-analysis of the effect on gait speed. *Arch Phys Med Rehabil.* (2017) 98:1435–52. doi: 10.1016/j.apmr.2016.12.007
77. Monaghan CC, Hermens HJ, Nene AV, Tenniglo MJ, Veltink PH. The effect of FES of the tibial nerve on physiological activation of leg muscles during gait. *Med Eng Phys.* (2010) 32:332–8. doi: 10.1016/j.medengphy.2010.01.003
78. Kido Thompson A, Stein RB. Short-term effects of functional electrical stimulation on motor-evoked potentials in ankle flexor and extensor muscles. *Exp Brain Res.* (2004) 159:491–500. doi: 10.1007/s00221-004-1972-4
79. Shin H, Watkins Z, Hu X. Exploration of hand grasp patterns elicitable through non-invasive proximal nerve stimulation. *Sci Rep.* (2017) 7:16595. doi: 10.1038/s41598-017-16824-1
80. Zheng Y, Hu X. Elicited finger and wrist extension through transcutaneous radial nerve stimulation. *IEEE Trans Neural Syst Rehabil Eng.* (2019) 27:1875–82. doi: 10.1109/TNSRE.2019.2930669
81. Tigra W, Dali M, William L, Fattal C, Gélis A, Divoux JL, et al. Selective neural electrical stimulation restores hand and forearm movements

- in individuals with complete tetraplegia. *J Neuroeng Rehabil.* (2020) 17:66. doi: 10.1186/s12984-020-00676-4
82. Zhang Q, Iyer A, Lambeth K, Kim K, Sharma N. Ultrasound echogenicity-based assessment of muscle fatigue during functional electrical stimulation. *Annu Int Conf IEEE Eng Med Biol Soc.* (2021) 2021:5948–52. doi: 10.1109/EMBC46164.2021.9630325
83. Buckmire AJ, Arakeri TJ, Reinhard JP, Fuglevand AJ. Mitigation of excessive fatigue associated with functional electrical stimulation. *J Neural Eng.* (2018) 15:066004. doi: 10.1088/1741-2552/aade1c
84. Pieber K, Hecceg M, Paternostro-Sluga T, Schuhfried O. Optimizing stimulation parameters in functional electrical stimulation of denervated muscles: a cross-sectional study. *J Neuroeng Rehabil.* (2015) 12:51. doi: 10.1186/s12984-015-0046-0
85. Vromans M, Faghri PD. Functional electrical stimulation-induced muscular fatigue: effect of fiber composition and stimulation frequency on rate of fatigue development. *J Electromyogr Kinesiol.* (2018) 38:67–72. doi: 10.1016/j.jelekin.2017.11.006
86. Jaramillo Cienfuegos P, Shoemaker A, Grange RW, Abaid N, Leonessa A. Classical and adaptive control of *ex vivo* skeletal muscle contractions using Functional Electrical Stimulation (FES). *PLoS ONE.* (2017) 12:e0172761. doi: 10.1371/journal.pone.0172761
87. Buckmire AJ, Lockwood DR, Doane CJ, Fuglevand AJ. Distributed stimulation increases force elicited with functional electrical stimulation. *J Neural Eng.* (2018) 15:026001. doi: 10.1088/1741-2552/aa9820
88. Cercone M, Jarvis JC, Ducharme NG, Perkins J, Piercy RJ, Willand MP, et al. Functional electrical stimulation following nerve injury in a large animal model. *Muscle Nerve.* (2019) 59:717–25. doi: 10.1002/mus.26460
89. McAvoy M, Tsosie JK, Vyas KN, Khan OF, Sadtler K, Langer R, et al. Flexible multielectrode array for skeletal muscle conditioning, acetylcholine receptor stabilization and epimysial recording after critical peripheral nerve injury. *Theranostics.* (2019) 9:7099–107. doi: 10.7150/thno.35436
90. Puttaswamy SV, Bhattacharya G, Raj S, Bhalla N, Lee C, McLaughlin J. Effect of functional electrical stimulation on capillary blood flow to muscle. *Annu Int Conf IEEE Eng Med Biol Soc.* (2022) 2022:1573–6. doi: 10.1109/EMBC48229.2022.9871395
91. Nakagawa K, Bergquist AJ, Yamashita T, Yoshida T, Masani K. Motor point stimulation primarily activates motor nerve. *Neurosci Lett.* (2020) 736:135246. doi: 10.1016/j.neulet.2020.135246
92. Eid MM, Rawash MF, Sharaf MA, Eladl HM. Effectiveness of transcutaneous electrical nerve stimulation as an adjunct to selected physical therapy exercise program on male patients with pudendal neuralgia: a randomized controlled trial. *Clin Rehabil.* (2021) 35:1142–50. doi: 10.1177/0269215521995338
93. Naeser MA, Hahn KA, Lieberman BE, Branco KF. Carpal tunnel syndrome pain treated with low-level laser and microamperes transcutaneous electric nerve stimulation: a controlled study. *Arch Phys Med Rehabil.* (2002) 83:978–88. doi: 10.1053/apmr.2002.33096
94. Leung A, Shukla S, Lee J, Metzger-Smith V, He Y, Chen J, et al. Effect of low frequency transcutaneous magnetic stimulation on sensory and motor transmission. *Bioelectromagnetics.* (2015) 36:410–9. doi: 10.1002/bem.21921
95. Macedo LB, Josué AM, Maia PH, Câmara AE, Brasileiro JS. Effect of burst TENS and conventional TENS combined with cryotherapy on pressure pain threshold: randomised, controlled, clinical trial. *Physiotherapy.* (2015) 101:155–60. doi: 10.1016/j.physio.2014.07.004
96. Bouguetoch A, Martin A, Grosprêtre S. Insights into the combination of neuromuscular electrical stimulation and motor imagery in a training-based approach. *Eur J Appl Physiol.* (2021) 121:941–55. doi: 10.1007/s00421-020-04582-4
97. Asensio-Pinilla E, Udina E, Jaramillo J, Navarro X. Electrical stimulation combined with exercise increase axonal regeneration after peripheral nerve injury. *Exp Neurol.* (2009) 219:258–65. doi: 10.1016/j.expneurol.2009.05.034
98. Marqueste T, Alliez JR, Alluin O, Jammes Y, Decherchi P. Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. *J Appl Physiol.* (2004) 96:1988–95. doi: 10.1152/japplphysiol.00775.2003
99. Bannaga A, Guo T, Ouyang X, Hu D, Lin C, Cao F, et al. A comparative study of the effects of magnetic stimulation and electric stimulation on peripheral nerve injury in rat. *J Tongji Med Univ.* (2001) 21:159–62. doi: 10.1007/BF02888085
100. Gigo-Benato D, Russo TL, Geuna S, Domingues NR, Salvini TF, Parizotto NA. Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. *Muscle Nerve.* (2010) 41:685–93. doi: 10.1002/mus.21549



OPEN ACCESS

EDITED BY

Jian-Quan Shi,
Nanjing Medical University, China

REVIEWED BY

A. Emre Öge,
Istanbul University, Türkiye
Adem Kara,
Erzurum Technical University, Türkiye

*CORRESPONDENCE

Hui Lu
✉ huilu@zju.edu.cn

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 29 November 2022

ACCEPTED 30 January 2023

PUBLISHED 01 March 2023

CITATION

Li X, Fu J, Zhou H, Dong Y, Alhaskawi A,
Wang Z, Lai J, Yao C, Ezzi SHA, Kota VG,
Abdulla MHAH, Guan M, Lou X and Lu H (2023)
The effects of melatonin in the treatment of
acute brachial plexus compression injury in
rats. *Front. Neurol.* 14:1111101.
doi: 10.3389/fneur.2023.1111101

COPYRIGHT

© 2023 Li, Fu, Zhou, Dong, Alhaskawi, Wang,
Lai, Yao, Ezzi, Kota, Abdulla, Guan, Lou and Lu.
This is an open-access article distributed under
the terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

The effects of melatonin in the treatment of acute brachial plexus compression injury in rats

Xigong Li¹, Jing Fu², Haiying Zhou¹, Yanzhao Dong¹,
Ahmad Alhaskawi¹, Zewei Wang³, Jingtian Lai³, Chengjun Yao³,
Sohaib Hasan Abdullah Ezzi⁴, Vishnu Goutham Kota³,
Mohamed Hasan Abdulla Hasan Abdulla³, Ming Guan¹,
Xianfeng Lou¹ and Hui Lu^{1,5*}

¹Department of Orthopedics, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China, ²Department of Stomatology, Affiliated Hangzhou Xixi Hospital, Zhejiang University School of Medicine, Hangzhou, China, ³Department of Clinical Medicine, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China, ⁴Department of Orthopaedics of the 3rd Xiangya Hospital, Central South University, Changsha, Hunan, China, ⁵Alibaba-Zhejiang University Joint Research Center of Future Digital Healthcare, Zhejiang University, Hangzhou, Zhejiang, China

Introduction: Brachial plexus injury (BPI) is one of the most destructive peripheral nerve injuries and there is still a lack of effective treatment.

Methods: This study was conducted to evaluate the effects of melatonin in the treatment of acute brachial plexus compression injury in rats using histopathological, histomorphometric, immunohistochemical and electrophysiological methods. Forty-eight adult male Sprague Dawley rats were randomly allocated into three groups: sham, melatonin and vehicle groups. The brachial plexus compression injury model was performed by a vascular clamp. Melatonin group received intraperitoneal injection of melatonin at doses of 10 mg/kg for 21 days after crush injury. The conduction velocity and amplitude of compound muscle action potential (CAMP) in the regenerated nerve, and nerve histomorphometry, as well as levels of myelin protein zero (P0) protein of the crush region were assessed.

Results: Compared with the vehicle group, the melatonin group which reported significant increased CMAP conduction velocity and amplitude also showed thicker myelin sheath and lower levels of P0 protein.

Discussion: Our results suggest that melatonin effectively promotes nerve regeneration and improves the function of damaged nerves. Melatonin treatment is a promising strategy for the treatment of acute brachial plexus compression injury.

KEYWORDS

acute brachial plexus compression injury, melatonin, rat model, myelin protein zero, nerve regeneration

1. Introduction

Brachial plexus injury (BPI) is one of the most destructive peripheral nerve injuries, which can result in notable motorsensory deficits (1–3). Despite neurosurgical techniques are available to repair the lesion, function restoration of BPI remains unsatisfactory in clinical practice (2). The outcomes after nerve reconstruction are adversely affected not only by mechanical injury, also by several secondary factors such as inflammatory responses, oxidative stress and regenerative misdirection (4). Several recent studies have focused on the promising approaches involving therapeutic management of the secondary cascade resulting from BPI (4, 5).

Melatonin, the main hormone of the pineal gland, daily regulates circadian rhythms (6, 7). Various formulations of melatonin have been patented and used in the treatment of sleep dysfunction. Some previous studies show that melatonin can regulate various physiological functions including anti-inflammation, free radical scavenging and anti-oxidative properties (7). Due to these biological properties, melatonin has been used as potential pharmacotherapy for traumatic events in the central nerve system. Recently, it has been demonstrated that melatonin has have beneficial effects on peripheral nerve repair in models of sciatic nerve injury (8–13).

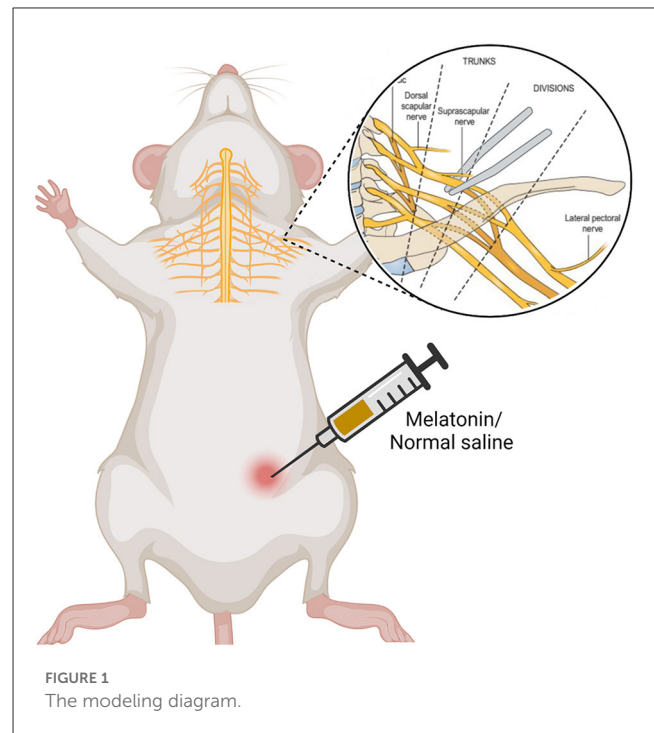
In this study, we evaluated the potential neuroprotective roles of melatonin in brachial plexus compression injury models of rats.

2. Materials and methods

A total of 48 adult male Sprague Dawley (SD) rats, weighing about 250 g, were obtained from experimental animal center of Zhejiang University. The animal experiment was approved by the Zhejiang University's Animal Experimentation Ethics Committee. All rats were housed in a room with constant and appropriate temperature and humidity and are subjected to a 12-h diurnal cycle. Drinking water and standard laboratory feed were available in sufficient quantities. They were allowed to acclimatize for 1 week prior to the experiments. Efforts were made to minimize animal suffering and to reduce the number of animals used during the experiments.

The animals were randomly divided into 3 groups ($n = 16$ each group): sham, vehicle and melatonin groups. Sham group had no operation on the brachial plexus nerve, instead, they just underwent skin incision and suturing. In melatonin and vehicle groups, all surgical procedures were performed after anesthesia with an intraperitoneal injection of xylazine and ketamine (10 and 80 mg/kg). The left brachial plexus was identified and exposed using an operating microscope. Distal to the suprascapular nerve branch, the upper trunk of the brachial plexus were crushed by a mosquito vascular clamp (Shanghai Medical Instruments Corp. Ltd.) that was fixed to the maximum extent for 30 s. Afterwards, the incision was closed by using 4.0 silk sutures. Melatonin group received intraperitoneal injection of melatonin at doses of 10 mg/kg for 21 days after injury. Normal saline was injected intraperitoneally in vehicle group (Figure 1).

By 7 and 14 days after surgery, 6 rats were anesthetized and perfused transcardially with 0.5% glutaraldehyde in 0.1 M phosphate buffer. Short 5 mm segments of the brachial plexus were collected. The specimens were fixed in 2.5% phosphate-buffered glutaraldehyde solution (PBS) for 1 h at room temperature and then at 4°C, until processed. Tissue paraffin blocks were fabricated by post-fixing specimens in 1% OsO₄ in PBS, dehydrating in a graded series of alcohol and propylene oxide, embedding in resin, and then polymerizing at 60°C. Transverse semi-thin sections (1 mm) were obtained using an ultramicrotome, and were respectively stained with Fast blue (Abcam, UK), silver (Amresco, USA) and H&E staining (Abcam, UK). By 7 days after surgery, ultrathin sections (70 nm) of nerve samples were stained with uranyl acetate and citrate and were evaluated by JEM1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan).



Three weeks after surgery, the injured sites of the brachial plexus were excised and lysed, and resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then transferred to a polyvinylidene difluoride membrane, blocked with 5% non-fat dry milk. Subsequently, the membrane was incubated with mouse polyclonal antibody to P0 (1:1,000; Santa Cruz Biotechnology, USA) and mouse monoclonal antibody to GAPDH (1:5,000; Santa Cruz Biotechnology). After washing with 0.1 M Tris buffered saline (Ph 7.2) containing 0.1% Tween-20 (TBST) for three times (10 min for each), the membranes were incubated with rabbit anti-mouse IgG (1:5,000; Santa Cruz Biotechnology), for 2 h at room temperature. Band optical density values were determined using Gel-Pro Analyzer Software, version 4.0 (Media Cybernetics, USA).

Three weeks after surgery, 4 rats were anesthetized and the left brachial plexus was isolated. Bipolar stimulating electrodes were placed near the injury site while the bipolar recording electrode was placed in the upper limb muscle. The amplitude and latency of compound muscle action potential (CMAP) were recorded with an electromyogram instrument. The bipolar stimulating electrodes were used to stimulate the regenerated nerves and the bipolar recording electrodes inserted into the muscle were to record electrical activity through a digital MYTO electromyograph machine (Esaote, Genoa, Italy).

All pathological manifestations, Fast blue staining, HE staining, silver staining or transmission electron microscopic images, were evaluated by a pathologist who was blinded to this study. Quantitative variables were described using means and standard deviations (SD), and compared using *t*-tests. The level of significance was set to $p < 0.05$. All analyses were done in SPSS 23.0.

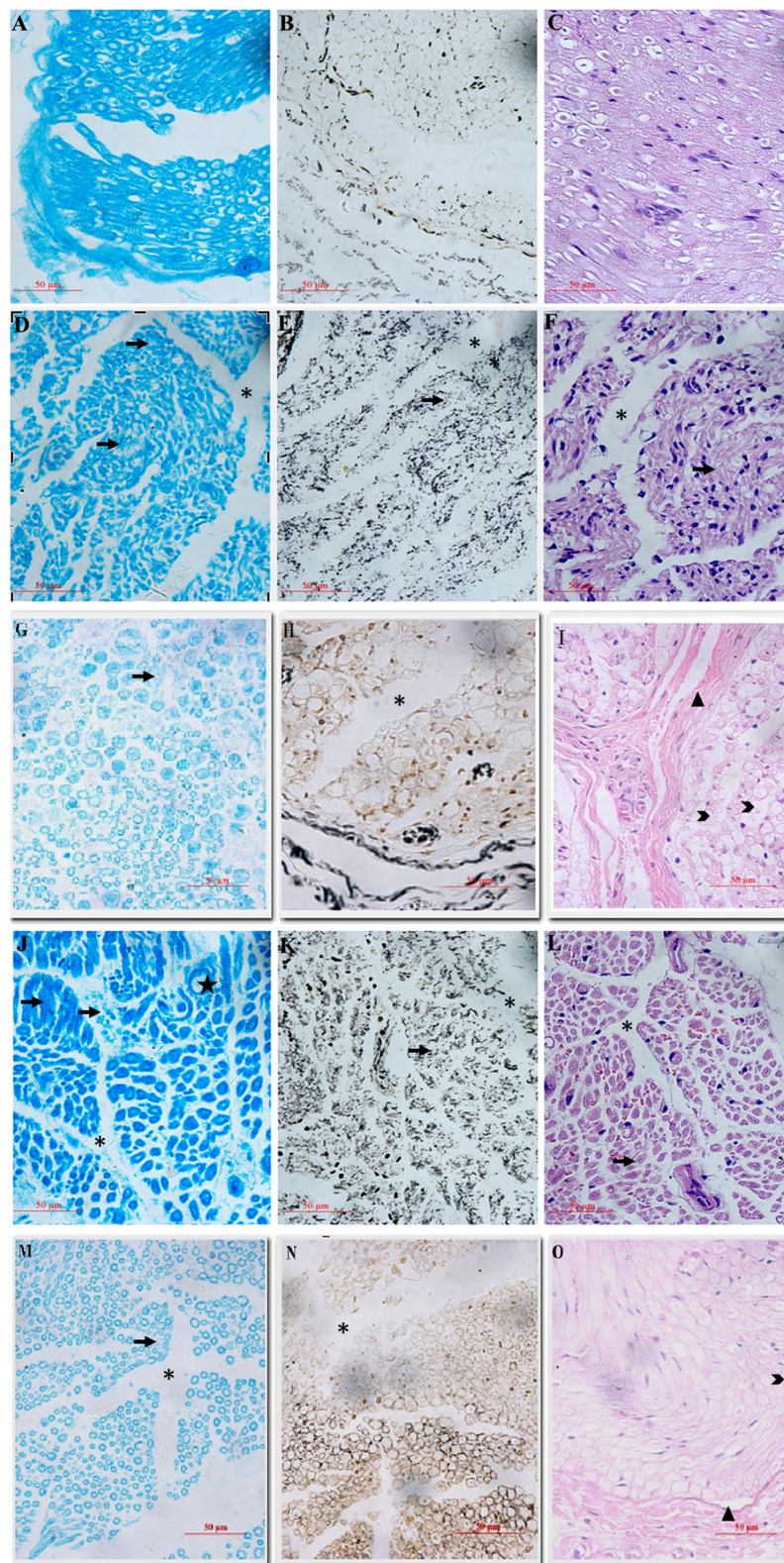


FIGURE 2

The general views of histological sections stained by Fast blue, silver and H&E. (A–C) Sham group: well-arranged and distributed nerve fibers were observed; (D–I) vehicle groups at 7 and 14 days: typical features of peripheral nerve injury after 7 days could be seen in (D–F), including myelin loss with Wallerian degeneration (arrow), and endoneurial edema (*). (G–I) A further decrease in nerve fiber density, disruption of fiber arrangement, and a marked increase in fibrosis (triangle) and axonal vacuolization (arrowhead) at day 14. (J–O) Melatonin groups at 7 and 14 days: compared with vehicle group, less axonal degeneration and vacuolization, as well as fibrosis were observed, with more regenerating axon clusters (star) and the nerve fibers were neatly aligned.

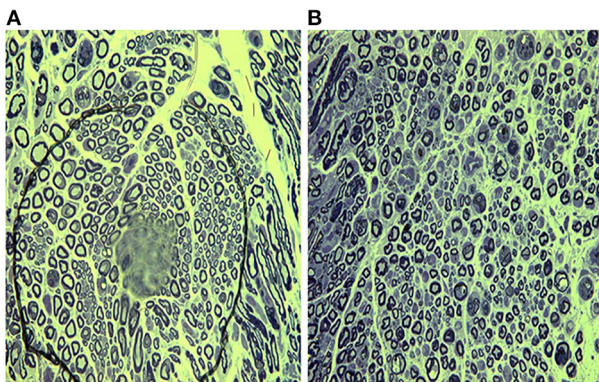


FIGURE 3
Transmission electron micrographs showing the ultrastructure of brachial plexus day 7 after injury. (A) Vehicle group; (B) melatonin group. Nerves in the melatonin group showed thicker myelin sheaths and narrower endoneurial gaps compared to the vehicle group. Scale bar = 5 mm.

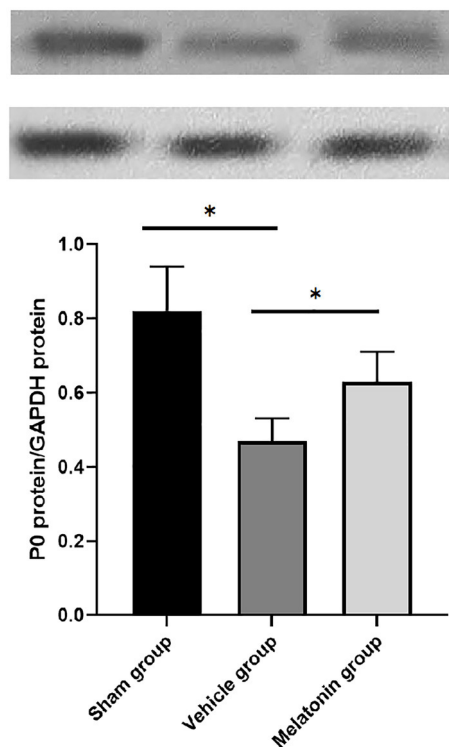


FIGURE 4
P0 protein/GAPDH protein levels were assessed using western blotting in the injured sites of rats in all 3 groups, 3 weeks after injury. p -value * <0.05 .

3. Results

Well-arranged and distributed nerve fibers were observed in sham group (Figures 2A–C). On the contrary, the typical characteristics of peripheral nerve injury were present in

vehicle group on day 7, including axonal degeneration, myelin abnormalities, and endoneurial edema (Figures 2D–F). By day 14, the density of nerve fibers was further reduced, and the axonal degeneration was increased markedly. More axons had collapsed and the number of Schwann's cells increased (Figures 2G–I). Compared with vehicle group, less axonal degeneration and vacuolization were observed in melatonin group (Figures 2J–O). And the vehicle group showed slightly more increase in endoneurial space than the melatonin group. Similarly, the transmission electron microscope analysis showed there was a significant difference in myelin sheath thickness and endoneurial space between the vehicle and melatonin group (Figures 3A, B).

The levels of P0 protein at 3 weeks after surgery in all three groups were shown in Figure 4. The levels of P0 protein were significantly decreased in vehicle group compared with the sham group ($P < 0.05$). Whereas, the P0 protein levels in the melatonin group, although slightly lower than the sham group, were significantly higher compared with vehicle group ($P < 0.05$).

Table 1 showed the results of electrophysiological assessment at 3 weeks after surgery in all three groups. The CMAP amplitude was reduced markedly ($p < 0.05$), while the CMAP latency was prolonged significantly ($p < 0.05$) in vehicle group compared with that in Sham group. Moreover, melatonin-treated group displayed distinctly higher CMAP amplitude ($p < 0.05$) and markedly shorter CMAP latency ($p < 0.05$) compared with vehicle group.

4. Discussion

Peripheral nerve injury of the upper limb is a common and extremely inconvenient clinical disease. The main mechanisms of its occurrence are compression, trauma, peripheral nerve tumor, inflammation, neuronal degeneration, and radiation exposure (14–16). The brachial plexus is not only the most complicated structure in the peripheral nervous system, but highly susceptible to trauma, or may be damaged secondary to lesions of adjacent structures (17). The present study demonstrated continuous treatment with melatonin can significantly increase CMAP conduction velocity, enhance myelin sheath thickness and at the same time, increase P0 protein levels in the regenerated brachial plexus nerve after injury compared with vehicle treatment. These results suggest that melatonin can effectively promotes nerve regeneration and improves the function of damaged nerves. Melatonin treatment is a promising strategy for the treatment of acute brachial plexus compression injury.

There are some basic pathological changes in the early stage of peripheral nerve injuries, including axonal degeneration, myelin abnormalities, and endoneurial edema (4). And severe peripheral nerve injuries can finally lead to the Wallerian degeneration of the distal segment of the nerve, which means rupture of the axonal membrane, degradation of cytoskeletal components and lysis of myelin sheaths (4, 18). Therefore, several pharmacological agents have been investigated to

TABLE 1 The results of electrophysiological evaluation in all three groups.

Group	Sham	Vehicle	Melatonin
CMAP latency (ms)	2.21 ± 0.08	3.57 ± 0.16*	3.08 ± 0.06 [#]
CMAP amplitude (Mv)	11.68 ± 0.8	1.92 ± 0.24*	5.34 ± 0.14 [#]

* $P < 0.05$ for the sham compared with vehicle group; [#] $P < 0.05$ for the melatonin compared with vehicle group.

facilitate myelination and functional recovery after peripheral nerve injury.

In the process of peripheral nerve repair, Schwann cells proliferate and construct the myelin sheath to enhance axonal regeneration from proximal to distal (18). P0, the major peripheral nervous system myelin protein, belongs to the immunoglobulin supergene family of membrane proteins and can mediate homotypic adhesion (19, 20). P0 is considered as a fundamental structural component of peripheral nervous system myelin, and is directly or indirectly involved in the regulation of myelin gene expression and myelin morphogenesis (20). And the levels of P0 expression are related to Schwann cell myelination in peripheral nerve repair (21). The experimental results of the present study showed that P0 protein levels in the myelin sheath were increased after melatonin treatment, from which it can be inferred that melatonin treatment triggered Schwann cell proliferation and myelination, thus promoting the repair and regeneration of damaged nerves. Other histomorphological findings corroborate this supposition. The electrophysiological assessment showed that injured nerve after melatonin treatment can provides lower latency recordings and higher amplitude measures, which indicated a higher number of normally functioning axons in the melatonin treatment group. And histological observation manifested the presence but not nimity of Wallerian degeneration, more regenerating axon clusters, higher myelin thickness and nerve fiber density, more orderly nerve fiber arrangement in the melatonin group, all of which support the promotion of melatonin for nerve regeneration.

Melatonin is a hormone secreted by the pineal gland of brain and produce a marked effect on the regulation of sleep and circadian rhythm (1, 2). Previous studies demonstrated melatonin has neuroprotective effects in the treatment of peripheral nerve injury (8–13, 22). Melatonin treatment can markedly increase the number of axons and thickness of myelin sheath in rat models of sciatic nerve injury (8–13, 22). And structural protection of the myelin lamellae can be preserved following repeated low dosage of melatonin administration (13). Even a single injection of high-dose melatonin can act to protect myelin sheath, prevent axonal loss, and accelerate functional recovery of the injured sciatic nerve (12). Pan et al. (11) illustrated that melatonin might promote peripheral nerve regeneration by improving the proliferation and migration of Schwann cells through the Shh signaling pathway after sciatic nerve injury. Moreover, recently simultaneous use of melatonin and chondroitin sulfate ABC synergistically promoted nerve regeneration in the brachial plexus nerve-root avulsion model (23). All these experimental results were consistent with our findings.

Overall, this study strengthens the idea that melatonin promoted axonal regeneration after brachial plexus compression injury, which may provide a promising therapeutic strategy for peripheral nerve injury. However, our study had some limitations. Firstly, histomorphometric changes with experimental injury and healing response were assessed only by histology and immunohistochemistry. Secondly, we did not conduct functional evaluation. In addition, further experiments should be conducted on the proper dosage and possible side effects.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the First Affiliated Hospital, College of Medicine, Zhejiang University.

Author contributions

HL and XL designed the study. XL, HZ, and MG performed data collection. AA, JF, YD, and CY analyzed the results. JL, ZW, VK, MA, and SE drafted the manuscript. All authors have read and approved the final manuscript.

Funding

This study was funded by the Zhejiang Provincial Continuing Education Program (grant number 2022-04-07-001). The funding bodies had no role in the design of the study, in collection, analysis, and interpretation of data, and in drafting the manuscript.

Acknowledgments

This work was supported by Alibaba Cloud.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Noland SS, Bishop AT, Spinner RJ, Shin AY. Adult traumatic brachial plexus injuries. *J Am Acad Orthop Surg.* (2019) 27:705–16. doi: 10.5435/JAAOS-D-18-00433
- Limthongthang R, Bachoura A, Songcharoen P, Osterman AL. Adult brachial plexus injury: evaluation and management. *Orthop Clin N Am.* (2013) 44:591–603. doi: 10.1016/j.ocl.2013.06.011
- Jiang S, Shen H, Tan WQ, Lu H. Arterial thoracic outlet syndrome caused by cervical ribs—an unusual case report. *Medicine.* (2019) 98:e14778. doi: 10.1097/MD.00000000000014778
- Modrak M, Talukder MAH, Gurgenshvili K, Noble M, Elfar JC. Peripheral nerve injury and myelination: potential therapeutic strategies. *J Neurosci Res.* (2020) 98:780–95. doi: 10.1002/jnr.24538
- Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *BioMed Res Int.* (2014) 2014:698256. doi: 10.1155/2014/698256
- Cipolla-Neto J, Amaral FGD. Melatonin as a hormone: new physiological and clinical insights. *Endocrine Rev.* (2018) 39:990–1028. doi: 10.1210/er.2018-00084
- Chitimus DM, Popescu MR, Voiculescu SE, Panaitescu AM, Pavel B, Zagrean L, et al. Melatonin's impact on antioxidative and anti-inflammatory reprogramming in homeostasis and disease. *Biomolecules.* (2020) 10:1211. doi: 10.3390/biom10091211
- Sayan H, Ozacmak VH, Ozen OA, Coskun O, Arslan SO, Sezen SC, et al. Beneficial effects of melatonin on reperfusion injury in rat sciatic nerve. *J Pineal Res.* (2004) 37:143–8. doi: 10.1111/j.1600-079X.2004.00145.x
- Zencirci SG, Bilgin MD, Yaraneri H. Electrophysiological and theoretical analysis of melatonin in peripheral nerve crush injury. *J Neurosci Methods.* (2010) 191:277–82. doi: 10.1016/j.jneumeth.2010.07.008
- Turgut M, Kaplan S. Effects of melatonin on peripheral nerve regeneration. *Recent Patents Endocrine Metabol Immune Drug Discov.* (2011) 5:100–8. doi: 10.2174/187221411799015336
- Pan B, Jing L, Cao M, Hu Y, Gao X, Bu X, et al. Melatonin promotes Schwann cell proliferation and migration via the shh signalling pathway after peripheral nerve injury. *Eur J Neurosci.* (2021) 53:720–31. doi: 10.1111/ejn.14998
- Yazar U, Çakir E, Boz C, Çobanoğlu Ü, Baykal S. Electrophysiological, functional and histopathological assessments of high dose melatonin on regeneration after blunt sciatic nerve injury. *J Clin Neurosci Off J Neurosurg Soc Aust.* (2020) 72:370–7. doi: 10.1016/j.jocn.2020.01.006
- Daglioglu E, Serdar Dike M, Kilinc K, Erdogan D, Take G, Ergungor F, et al. Neuroprotective effect of melatonin on experimental peripheral nerve injury: an electron microscopic and biochemical study. *Central Eur Neurosurg.* (2009) 70:109–14. doi: 10.1055/s-0029-1220712
- Jiang S, Shen H, Lu H. Multiple schwannomas of the digital nerves and common palmar digital nerves: an unusual case report of multiple schwannomas in one hand. *Medicine.* (2019) 98:e14605. doi: 10.1097/MD.00000000000014605
- Li P, Lou D, Lu H. The cubital tunnel syndrome caused by intraneural ganglion cyst of the ulnar nerve at the elbow: a case report. *BMC Neurol.* (2018) 18:217. doi: 10.1186/s12883-018-1229-7
- Zhou H, Bai Q, Hu X, Alhaskawi A, Dong Y, Wang Z, et al. Deep CTS: a deep neural network for identification MRI of carpal tunnel syndrome. *J Dig Imag.* (2022) 4:1–12. doi: 10.1007/s10278-022-00661-4
- Ferrante MA. Brachial plexopathies: classification, causes, and consequences. *Muscle Nerve.* (2004) 30:547–68. doi: 10.1002/mus.20131
- Nocera G, Jacob C. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. *Cell Mol Life Sci CMLS.* (2020) 77:3977–89. doi: 10.1007/s00018-020-03516-9
- Eichberg J. Myelin P0: new knowledge and new roles. *Neurochem Res.* (2002) 27:1331–40. doi: 10.1023/A:1021619631869
- Xu W, Manichella D, Jiang H, Vallat JM, Lilien J, Baron P, et al. Absence of P0 leads to the dysregulation of myelin gene expression and myelin morphogenesis. *J Neurosci Res.* (2000) 60:714–24. doi: 10.1002/1097-4547(20000615)60:6<714::AID-JNR3>3.0.CO;2-1
- Morrison S, Mitchell LS, Ecob-Prince MS, Griffiths IR, Thomson CE, Barrie JA, et al. P0 gene expression in cultured Schwann cells. *J Neurocytol.* (1991) 20:769–80.
- Atik B, Erkuğlu I, Tercan M, Buyukhatipoglu H, Bekerecioglu M, Pence S. The effects of exogenous melatonin on peripheral nerve regeneration and collagen formation in rats. *J Surg Res.* (2011) 166:330–6. doi: 10.1016/j.jss.2009.06.002
- Guo WL, Qi ZP, Yu L, Sun TW, Qu WR, Liu QQ, et al. Melatonin combined with chondroitin sulfate ABC promotes nerve regeneration after root-avulsion brachial plexus injury. *Neural Regen Res.* (2019) 14:328–38. doi: 10.4103/1673-5374.244796



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University, China

REVIEWED BY

Aleksandras Vilionskis,
Vilnius University, Lithuania
Yuneng Li,
Beijing Jishuitan Hospital, China

*CORRESPONDENCE

Jingyi Mi
✉ Jy8586yz@163.com

[†]These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 29 October 2022

ACCEPTED 09 February 2023

PUBLISHED 03 March 2023

CITATION

Tan J, Wang X, Xiong F, Qian J, Ying Q and Mi J
(2023) Case report: A case of injury to the
infrapatellar branch of the saphenous nerve
caused by medial approach in knee
arthroscopy. *Front. Neurol.* 14:1083871.
doi: 10.3389/fneur.2023.1083871

COPYRIGHT

© 2023 Tan, Wang, Xiong, Qian, Ying and Mi.
This is an open-access article distributed under
the terms of the [Creative Commons Attribution
License \(CC BY\)](#). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Case report: A case of injury to the infrapatellar branch of the saphenous nerve caused by medial approach in knee arthroscopy

Jiyang Tan^{1†}, Xunhao Wang^{2†}, Fei Xiong¹, Jun Qian¹, Qiuwen Ying¹ and Jingyi Mi^{1*}

¹Department of Sports Medicine, Wuxi 9th People's Hospital Affiliated to Soochow University, Wuxi, Jiangsu, China, ²Medical College, Soochow University, Suzhou, China

We present the case of a 72-year-old man who was referred to our department for treatment of pain on the anteromedial infrapatellar side of the right knee with sensory disturbance that began 2 years earlier. The patient previously underwent right knee arthroscopy at another hospital for a meniscus injury 2 years earlier, which relieved his knee pain, but pain and discomfort near the incision of the medial portal persisted. Given this situation, various physical treatments, such as ice compress, were administered postoperatively. However, the symptom was only partially relieved before discharge. Subsequently, the patient visited two other hospitals and began taking oral pregabalin and duloxetine for treatment of the pain based on a diagnosis of right common peroneal nerve injury. The pain in the same dermatomal distribution was slightly relieved, but a withdrawal reaction was observed. However, the results of an ultrasound at our hospital indicated that the right medial quadriceps femoris tendon showed a hypoechoic area suggesting inflammatory changes. Physical examination of the right knee detected atrophy of the quadriceps femoris muscle, decreased muscle strength (M4), obvious tenderness in the medial side, radiating pain along the anterior tibia, and sensory disturbance (S3+); the results of a drawer test, McMurray test, pivot shift test, and lateral stress test were negative. Based on the aforementioned evidence, a diagnosis was made of injury to the infrapatellar branch of the saphenous nerve, after which neurolysis of the nerve in question was carried out. An enlarged incision was made along the original medial approach. Scar hyperplasia was observed after careful separation of the subcutaneous tissue. During neurolysis, branches were found wrapped in the scar; their continuity and integrity were confirmed after relief. The released nerve was placed in a physiological position. The patient's pain was clearly relieved, and numbness disappeared on the first postoperative day. At 1-month follow-up, all symptoms were found to have resolved.

KEYWORDS

saphenous nerve, knee arthroscopy, medial approach, pain, numbness

Introduction

Injury to the infrapatellar branch of the saphenous nerve (IBSN) can be triggered by many factors, including arthroscopic portals, incision for drilling of the tibial tunnel or dissection, and graft harvest. Most studies report that IBSN injury leads to sensory comorbidities in most cases. Problems can include loss of sensation, paresthesia, neuralgia, or hypersensitivity in the medial infrapatellar area of the lower extremity (1). Persistent pain after knee arthroscopy is rarely reported. We recently admitted a patient with pain on the anteromedial infrapatellar side of the right knee due to knee arthroscopy.

Case presentation

Chief complaints

A 72-year-old man presented with complaints of pain on the anteromedial infrapatellar side of the right knee with sensory disturbance that began 2 years earlier.

History of present illness

The patient previously underwent right knee arthroscopy at another hospital for a meniscus injury in May 2020, which gave him relief from knee pain, but pain and discomfort near the incision of the medial portal persisted. Given this situation, various physical treatments, such as ice compress, were administered postoperatively. However, the symptom was only partially relieved before discharge. Subsequently, the patient visited two other hospitals and began taking oral pregabalin and duloxetine for treatment of the pain based on a diagnosis of right common peroneal nerve injury. Pain in the same dermatomal distribution was slightly relieved, but a withdrawal reaction was observed. The patient still felt migratory pain at the inner foretibia.

History of past illness

The patient had high blood pressure for 5 years and had no other history of chronic disease or surgery.

Family history

The patient's parents were deceased, cause of death unknown; he had one brother in good health. The patient denied (1) any similar disease in the family; (2) tuberculosis, hepatitis, or other infectious diseases in the family; and (3) hereditary or familial diseases, such as diabetes or hemophilia, in the family.

Physical examination

Physical examination of the right knee detected atrophy of the quadriceps femoris muscle, decreased muscle strength (M4), obvious tenderness in the medial side, radiating pain along the anterior tibia, sensory disturbance (S3+), and positive Tinel's sign. The results of a drawer test, McMurray test, pivot shift test, and lateral stress test were negative.

Imaging examinations

Color ultrasound and magnetic resonance imaging (MRI) examination of the patient's right knee were performed. Color ultrasound showed a hypoechoic area suggesting inflammatory changes in the right medial quadriceps femoris tendon. MRI

showed level II injury in the posterior horn of the medial meniscus of the right knee joint and edema of the infrapatellar fat pad.

Final diagnosis

The final diagnosis was injury to the infrapatellar branch of the saphenous nerve.

Treatment

The patient underwent neurolysis for the infrapatellar branch of the saphenous nerve under epidural anesthesia in July 2022. An enlarged incision was made along the original medial approach. Scar hyperplasia was observed after careful separation of the subcutaneous tissue. During neurolysis, branches were found wrapped in the scar; their continuity and integrity were confirmed after relief. We translocated the nerve and embedded it under the fat (Figure 1). After surgery, the patient took 0.5 mg mecobalamin orally three times per day for 4 weeks.

Outcome and follow-up

The patient's pain was clearly relieved, and numbness disappeared on the first postoperative day. One month after surgery, the pain had disappeared completely and the patient was satisfied with the outcome.

Discussion

The saphenous nerve is the longest cutaneous branch of the femoral nerve. It divides into the IBSN, which serves the proximal tibia inferior medial to the patella (2, 3). The infrapatellar branch of the saphenous nerve splits off from the saphenous nerve in a highly variable way. It penetrates the sartorius muscle, after which it runs on a superficial course and generally forms two branches. Both branches cross the patellar tendon in the transverse direction to form the infrapatellar plexus. The infrapatellar branch of the saphenous nerve innervates the anteromedial aspect of the knee, the anterolateral aspect of the proximal part of the lower leg, and the anteroinferior part of the knee joint capsule (4–6). There are many reports of IBSN injury caused by surgery, especially knee arthroplasty (7), tendon extraction incision for anterior cruciate ligament reconstruction (8), and incisions for anteromedial knee surgery (9). Among these reports, the proportion of IBSN injuries caused by total knee replacement is 50–100% (10). Because the IBSN is highly variable, nerve damage in this area is considered inevitable (11). The most common symptoms associated with IBSN injury are hypoesthesia, numbness, or allergy in the anteromedial knee joint (12). Usually, these symptoms trouble the patient only for a short time, but in this case, the symptoms continued over a long period of time. We believe that the patient's IBSN, which was not completely disconnected, was the reason for the continued presence of sensory conduction; therefore, the patient's symptoms were primarily pain rather than numbness. The presence

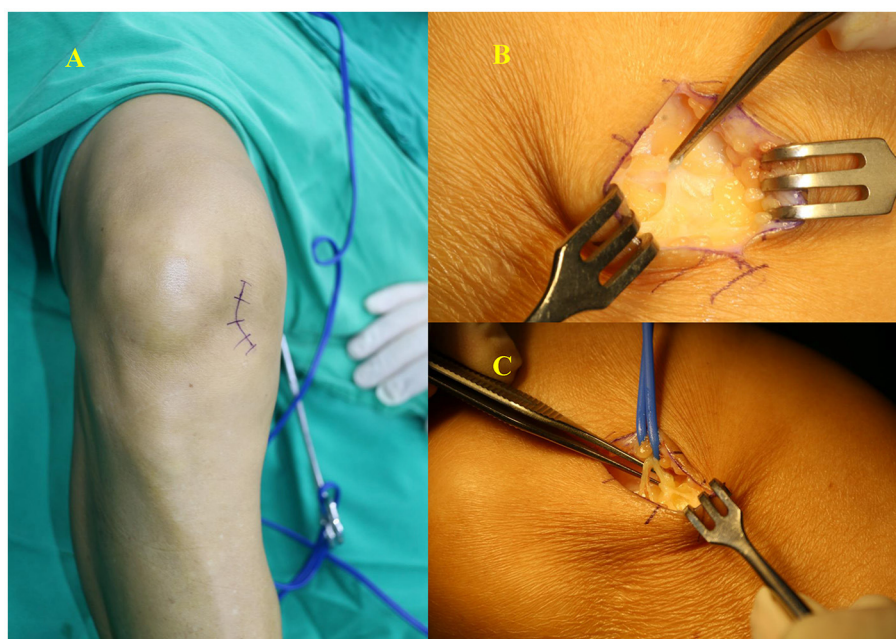


FIGURE 1

(A) An enlarged incision was made along the original medial approach. (B) Branches were found wrapped in the scar. (C) Continuity and integrity of the IBSN were confirmed after relief.

of scarring around the nerve, trapping the nerve, was what caused the symptoms to persist.

Prior to surgery, we had suspected subpatellar fat pad inflammation to be the cause of the patient's pain, but it was not until we observed positive Tinel's sign that the diagnosis was biased toward nerve damage or neuroma formation. However, this patient only underwent arthroscopic meniscus formation, and no large surgical incision was found; this made our diagnosis questionable, because the incision made by arthroscopic portal is small, and IBSN injury due to arthroscopic portal is rarely reported (13). Therefore, we used ultrasound to examine the affected area before the surgery (14). Unfortunately, ultrasound did not detect the formation of neuroma. Eventually, we had to make an enlarged incision along the original incision, and finally discovered the trapped nerve.

In general, symptoms of nerve entrapment do not progress rapidly, and there are few reports of neuroma formation leading to persistent pain 1 week after surgery. However, this patient felt pain 1 day after surgery, with a VAS score of up to 5. We do not believe that direct injury to the nerve was caused by the arthroscopic portal, but rather that the cause was unintended irritation of the nerve by the suture used to close the portal. Over the course of a few weeks, the sutures were absorbed and caused scarring around the nerve, which further perpetuated the symptoms. Therefore, we believe that the arthroscopic approach does not need to pursue aesthetics too strongly, and the epidermis can be directly sutured with less irritating sutures, without the need to apply too much suturing to subcutaneous tissue.

Krijgh et al. dissected 18 cadavers from adult donors and found a total of 23 infrapatellar branches. Based on this, they

argued that the origin of the infrapatellar branch from the saphenous nerve is highly variable, as well as the position at which the infrapatellar branch passes the knee joint (15). This makes IBSN injury difficult to avoid. Gousopoulos et al. dissected the space between the subcutaneous and the capsule; this dissection is performed by grasping and knotting the sutures through the anteromedial portal to avoid iatrogenic saphenous nerve injury (16). Although there are many ways to avoid nerve injury, diagnosis and early treatment after injury are more important.

There are many ways to diagnose IBSN. Riegler et al. have reported that ultrasound successfully pinpoints the variable course of the IBSN from the origin to the most distal point and may therefore enable the correct identification of (iatrogenic) nerve damage in any location (17). We also initially used ultrasound to examine the patient's nerves after suspecting IBSN injury. Unfortunately, no positive results were observed in our examination.

After knee arthroplasty and other procedures that can lead to IBSN injury, denervation is usually the choice of most surgeons if IBSN injury occurs (18). The current conservative treatment for IBSN neuritis is traditionally performed with nerve blocks containing a steroid and a local anesthetic (19). Reports of cryoneuroablation have also been published (20). Existing nonsurgical treatments can provide symptomatic relief but with variable long-term outcomes.

This case report still has several shortcomings. First, our follow-up time for this patient was short, and the long-term outcome remains unclear. Second, we have reported on only one case, so we are unable to present more useful information, such as

Guidance on how to avoid IBSN injury and the incidence of IBSN injury.

Conclusion

In conclusion, we should always be vigilant in relation to the possibility of IBSN injury during surgery, since there is a risk of saphenous nerve injury even if only a 1-cm long incision is made during arthroscopic surgery. The symptoms after an IBSN injury can easily confuse clinicians and make treatment difficult.

Author's note

The authors have read the CARE Checklist (2016) and the manuscript was prepared and revised according to the CARE Checklist (2016).

Data availability statement

The datasets presented in this article are not readily available because of ethical and privacy restrictions. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Wuxi 9th People's Hospital. The patients/participants provided their written informed consent

to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JT and XW reviewed the literature and contributed to drafting of the manuscript. FX and QY followed up the patients. JQ was the patient's first doctor. JM was responsible for revision of the manuscript for important intellectual content. All authors issued final approval for the version to be submitted.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Shi SM, Meister DW, Graner KC, Ninomiya JT. Selective denervation for persistent knee pain after total knee arthroplasty: a report of 50 cases. *J Arthroplasty*. (2017) 32:968–73. doi: 10.1016/j.arth.2016.09.043
- Nagai K, Muratsu H, Matsumoto T, Fujibayashi I, Kuroda R, Kurosaka M. Early-onset severe neuromatous pain of the infrapatellar branch of the saphenous nerve after total knee arthroplasty. *Asia Pac J Sports Med Arthrosc Rehabil Technol*. (2014) 1:102–5. doi: 10.1016/j.asmart.2013.11.002
- McArthur BA, Henry SL, Rothy A, Bashour LE. Peripheral nerve injury in total knee arthroplasty. In: Dy CJ, Brogan DM, Wagner ER, editors. *Peripheral Nerve Issues after Orthopedic Surgery: A Multidisciplinary Approach to Prevention, Evaluation and Treatment*. Cham: Springer International Publishing (2022). p. 297–323.
- Boyle J, Eason A, Hartnett N, Marks P. Infrapatellar branch of the saphenous nerve: a review. *J Med Imaging Radiat Oncol*. (2021) 65:195–200. doi: 10.1111/1754-9485.13141
- Kerver AL, Leliveld MS, den Hartog D, Verhofstad MH, Kleinrensink GJ. The surgical anatomy of the infrapatellar branch of the saphenous nerve in relation to incisions for anteromedial knee surgery. *J Bone Joint Surg Am*. (2013) 95:2119–25. doi: 10.2106/JBJS.L.01297
- McGinnis Dt M, Gonzalez R, Nyland J, Caborn DN. The posteromedial knee arthroscopy portal: a cadaveric study defining a safety zone for portal placement. *Arthroscopy*. (2011) 27:1090–5. doi: 10.1016/j.arthro.2011.02.031
- Lee SR, Dahlgren NJP, Staggers JR, de Cesar Netto C, Agarwal A, Shah A, et al. Cadaveric study of the infrapatellar branch of the saphenous nerve: can damage be prevented in total knee arthroplasty? *J Clin Orthop Trauma*. (2019) 10:274–7. doi: 10.1016/j.jcot.2018.03.005
- Luo J, Li S, Wang C, Li Q, Lin J, Shen Y, et al. Optimization of surgical exposure for harvesting gracilis-semitendinosus tendons. *Int Orthop*. (2023) 47:131–40. doi: 10.1007/s00264-022-05598-5
- Ngbilo C, Ouabo EC, Jovanovic B, Saithna A, Gillain L, Cottet BS. Partial denervation for neuroma pain after knee anterior tibial tuberosity exostectomy: a case report. *J Orthop Case Rep*. (2019) 9:10–3. doi: 10.13107/jocr.2019.v09.i04.1456
- Haraguchi T, Kume S, Jimbo K, Gotoh M, Shiba N, Okawa T. Saphenous nerve entrapment neuropathy after closed tibial fracture: A case report. *JBJS Case Connect*. (2021) 11:e20. doi: 10.2106/JBJS.CC.20.00670
- Le Corroller T, Lagier A, Pirro N, Champsaur P. Anatomical study of the infrapatellar branch of the saphenous nerve using ultrasonography. *Muscle Nerve*. (2011) 44:50–4. doi: 10.1002/mus.22004
- Phruetthiphath OA, Apinyankul R, Chanpoo M, Chotanaphuti T, Kosuwon W, Callaghan JJ. Anterior skin numbness after total knee arthroplasty: a prospective comparison study between diabetic and non-diabetic patients. *J Orthop*. (2020) 22:12–6. doi: 10.1016/j.jor.2020.03.017
- Henry BM, Tomaszewski KA, Pekala PA, Graves MJ, Pekala JR, Sanna B, et al. Oblique incisions in hamstring tendon harvesting reduce iatrogenic injuries to the infrapatellar branch of the saphenous nerve. *Knee Surg Sports Traumatol Arthrosc*. (2018) 26:1197–203. doi: 10.1007/s00167-017-4590-y

14. Shi GG, Schultz DS Jr, Whalen J, Clendenen S, Wilke B. Midterm outcomes of ultrasound-guided local treatment for infrapatellar saphenous neuroma following total knee arthroplasty. *Cureus*. (2020) 12:e6565. doi: 10.7759/cureus.6565
15. Krijgh DD, List EB, Teunis T, Bleys R, Coert JH. The origin and course of the infrapatellar branch of the saphenous nerve: an anatomical study. *JPRAS Open*. (2022) 34:144–51. doi: 10.1016/j.jpra.2022.08.006
16. Gousopoulos L, Grob C, Ahrens P, Levy Y, Vieira TD, Sonnery-Cottet B. How to avoid iatrogenic saphenous nerve injury during outside-in or inside-out medial meniscus sutures. *Arthrosc Tech*. (2022) 11:e127–32. doi: 10.1016/j.eats.2021.09.012
17. Riegler G, Jengoan S, Mayer JA, Pivec C, Platzgummer H, Brugger PC, et al. Ultrasound anatomic demonstration of the infrapatellar nerve branches. *Arthroscopy*. (2018) 34:2874–83. doi: 10.1016/j.arthro.2018.05.043
18. Giannetti A, Valentino L, Giovanni Mazzoleni M, Tarantino A, Calvisi V. Painful total knee arthroplasty: infrapatellar branch of the saphenous nerve selective denervation. A case series. *Knee*. (2022) 39:197–202. doi: 10.1016/j.knee.2022.09.010
19. Kramer DE, Bahk MS, Cascio BM, Cosgarea AJ. Posterior knee arthroscopy: anatomy, technique, application. *JBJS*. (2006) 88:110–21. doi: 10.2106/00004623-200612001-00013
20. Trescot AM, Brown MN, Karl HW. Infrapatellar saphenous neuralgia-diagnosis and treatment. *Pain Phys*. (2013) 16:E315–24. doi: 10.36076/ppj.2013/16/E315



OPEN ACCESS

EDITED BY

Lei Xu,
Fudan University, China

REVIEWED BY

Giovanna Squintani,
Azienda Ospedaliera Universitaria Integrata
Verona, Italy

Bo Cui,
Xuanwu Hospital, Capital Medical
University, China

*CORRESPONDENCE

Wei Yu
✉ yuweimei@jlu.edu.cn

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 25 October 2022

ACCEPTED 07 February 2023

PUBLISHED 10 March 2023

CITATION

Fan Z, Wen X, Ding X, Wang Q, Wang S and
Yu W (2023) Advances in biotechnology and
clinical therapy in the field of peripheral nerve
regeneration based on magnetism.
Front. Neurol. 14:1079757.
doi: 10.3389/fneur.2023.1079757

COPYRIGHT

© 2023 Fan, Wen, Ding, Wang, Wang and Yu.
This is an open-access article distributed under
the terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Advances in biotechnology and clinical therapy in the field of peripheral nerve regeneration based on magnetism

Zheyuan Fan, Xinggui Wen, Xiangdong Ding, Qianqian Wang,
Shoushuai Wang and Wei Yu*

Department of Hand Surgery, China-Japan Union Hospital of Jilin University, Changchun, China

Peripheral nerve injury (PNI) is one of the most common neurological diseases. Recent studies on nerve cells have provided new ideas for the regeneration of peripheral nerves and treatment of physical trauma or degenerative disease-induced loss of sensory and motor neuron functions. Accumulating evidence suggested that magnetic fields might have a significant impact on the growth of nerve cells. Studies have investigated different magnetic field properties (static or pulsed magnetic field) and intensities, various magnetic nanoparticle-encapsulating cytokines based on superparamagnetism, magnetically functionalized nanofibers, and their relevant mechanisms and clinical applications. This review provides an overview of these aspects as well as their future developmental prospects in related fields.

KEYWORDS

magnetic nanoparticles, magnetical nanofibers, peripheral nerve regeneration, magnetic field, repetitive peripheral magnetic stimulation

1. Introduction

Peripheral nerve injury (PNI) is a major clinical concern, which is caused by the loss of structure or function of peripheral nerves. In developed countries, ~ 13–23 per 100,000 persons are affected by PNI every year (1). It is a common complication in trauma. The regeneration of the peripheral nerve should be improved to recover its function. The core difficulties in nerve regeneration are the directional prolongation of neurites and the proliferation of Schwann cells (SCs), which provide well-functional axons and myelin sheaths. Currently, the studies on peripheral nerve regeneration involve growth factors, nerve conduits, tissue engineering, and genetic engineering (2–5). Since the 1980's, researchers have been continuously studying the effects of magnetism on nerve cells. The magnetic field can induce the orientation of cellular growth. In 1965, Murayama et al. reported that sickled erythrocytes were oriented perpendicular to the magnetic field (6). This was the first study on this phenomenon. Since then, erythrocytes, collagen, fibroblasts, osteoblasts, human glioblastoma A172 cells, SCs, smooth muscle cells, and PC12 cells have been reported to be related to magnetic fields (6–13). In addition, nerve cells have a better growth potential in the magnetic field [16]. Based on the theoretical effects of magnetic fields on nerve cells, biomaterial interventions have shown promising results in cell cultures and animal studies, providing contact guidance for extending neurites or a sustained release of various drugs and growth factors. Moreover, researchers further investigated the magnetic stimulation of peripheral nerves to obtain a better sense and function. This review summarizes the recent studies on the effects of magnetism on peripheral nerves and proposes future developmental directions in this field.

2. PNI and regeneration

PNI is a common clinical issue worldwide, and its prognosis varies with the degree of injury. Unlike the central nerve, the peripheral nerve has a limited regenerative capacity after being injured. After PNI, Wallerian degeneration occurs; the cell bodies of neurons swell, dissolving the chromatin and making Nissl's body disappear, which makes the cell body relatively eosinophilic, shifting the nucleus to the periphery. Moreover, SCs and macrophages phagocytize the myelin and axons, thereby degenerating the myelin sheaths at the distal portion of the nerve injury site. In addition to clearing myelin debris, macrophages and SCs also produce cytokines, which enhance axon growth. After clearing debris, regeneration begins at the proximal portion and continues toward the distal stump. New axonal sprouts generate from the Ranvier nodes (14, 15).

Different cells participate in repairing the injured nerves. SCs are arranged longitudinally to form the bands of Büngner, guiding the direction of regeneration and providing a microenvironment, which promotes regeneration. The tips of the regenerated axons, called growth cones, are composed of flat sheets of cellular matrices with finger-like protrusions (filopodia). Actin polypeptides, which can contract to produce axonal elongation, are in filopodia. The growth cones release proteases, which dissolve the matrix on its path, thereby clearing the way for regeneration. The recovery of nerve function depends on the extension of regenerated axons from the injured site to the target organ at the distant portion (14).

3. Theoretical study based on magnetism and peripheral nerve regeneration

3.1. Properties of magnetic fields and superparamagnetism

Magnetic properties are generated by electrons, spinning around the nuclei of atoms and their axis (16). The magnetic field intensity of a magnetic material is related to its atomic structure and temperature. In some atoms, the magnetic dipoles, arising from the spinning of electrons, do not cancel each other out, thereby creating a permanent dipole. The permanent dipole aligns with the external magnetic field. The magnetism of superparamagnetic materials disappears with the disappearance of the external magnetic field. This provides a basis for strengthening the magnetic field intensity and reducing the adverse effect of a magnetic field (17).

3.2. Growth of nerve cells in a magnetic field

Static magnetic fields (SMFs) or pulsed magnetic fields (PMFs) are usually used in the studies of nerve cells. PMFs are safe and efficient to promote nerve regeneration (18–20). SMFs have constant intensity and direction and a frequency of 0 Hz. Recently, studies have been conducted on the effects of SMFs on nerves at the cellular level. Numerous studies showed that the effects of

exposure to SMFs on cellular proliferation varied depending on the cell type (21, 22). Moreover, most studies, investigating the effects of SMFs on cell cycle distribution, showed that there was no statistical difference between the exposed and control group (23). There are two necessary pathways for the regeneration of peripheral nerves: the growth of neurites and myelin sheaths. Mann et al. provided new ideas for the correlations between these two enhanced pathways (24). They reported that a slight nuclear magnetic resonance therapy (NMRT) could induce an increase in SCs proliferation; however, its effect on repair-associated genes was not statistically significant. Furthermore, NMRT could not only enhance the neuronal maturation of individual neurons but also encouraged neurite outgrowth. Interestingly, they also showed that NMRT could induce the secretion of neurotrophic and neuritogenic factors in SCs, leading to the survival of dorsal root ganglia (DRG) neurons and neurite outgrowth.

These studies suggested that the differences in growth direction in response to magnetic field direction depended on the cell type. Macias made two coils of individual copper sheets folded into a square coil to establish a PMF (25). DRG showed a growth tendency parallel to the electric field (perpendicular to the magnetic field). Interestingly, Eguchi showed that SCs and collagens exhibited different growth directions under strong SMFs (8-T). The directions of the SCs and collagen groups were parallel and vertical to that of the magnetic field, respectively. Moreover, SCs oriented in the direction vertical to the magnetic field in a mixture of Schwann cells and collagen under 2 h of magnetic field exposure. DRG showed the same growth tendency as that of collagens, thereby providing theoretical support for the use of a magnetic field to guide peripheral nerve regeneration. Other studies demonstrated that fibrin (26, 27), PC12 cells (12), erythrocytes (28), and osteoblasts (9, 29) aligned parallel to the magnetic field. This property of the magnetic field might be significant in selecting different ways to guide peripheral nerve regeneration.

A strong magnetic field has a destructive effect on cells and can cause genetic mutation and DNA damage (30, 31). Liu et al. reported that the high-intensity magnetic field might be unfavorable for the growth of Schwann cells. When PMF was set to a frequency of 50 Hz, different from 5.0 or 10.0 mT ($T = \text{tesla}$), 0.5, 1.0, or 2.0 mT each was safe for the growth of Schwann cells (32). Interestingly, Eguchi reported that SCs and collagen exhibited normal growth under strong SMFs (8-T) (11). The reasons for these dramatic differences have not been explored yet, and it was speculated that the effects of magnetic field intensity on cellular growth were related to the type of magnetic field. SCs could better withstand SMF as compared to PMF.

3.3. Magnetic nanoparticles

A major challenge in peripheral nerve regeneration is the local and sustained delivery of bioactive factors. Studies on MNPs showed that the intervention of an external magnetic field was a type of conventional means. Due to good biocompatibility, MNPs can also be used alone as drug-loading tools to promote peripheral nerve regeneration (33). Magnetic liposomes could enhance neurite outgrowth with a nerve growth factor (NGF)

through phosphorylated extracellular regulated protein kinases (ERK) 1/2, which simultaneously upregulated the β -tubulin and integrin β 1 (34). Hu et al. showed an increased extension of spiral ganglion neurons (SGNs) in MNPs and MNPs + magnetic field groups as compared to those in the control group (2). Mitrea et al. used oral chitosan-functionalized MNPs to promote neural regeneration in the PNI experimental model and obtained satisfactory results (35). The mechanism for this phenomenon might be the release of Fe ions from iron oxide NPs inside the cells, promoting neurite outgrowth. Numerous approaches used MNPs to assist in drug or gene delivery, including liposome or micelle encapsulation, polymer coatings, and direct surface functionalization (16). Other applications of MNPs include the directional migration of cells induced by external magnetic fields (2, 36–39). In a review, Gilbert divided MNP-mediated cellular manipulations into two categories: magnetic cell guidance and magnetic cell transplantation (16). In essence, both these methods are based on the combination of MNPs and target cells induced by external magnetic fields.

To solve the problem of instability, aggregations, and cellular toxicity of MNPs, Qin et al. synthesized a novel nanomedicine composed of NGF-functionalized Au-coated MNPs. They showed that both the static and rotation magnetic groups could significantly increase the number of differentiated cells and neurites, exhibiting longer average neurite length and clearer directionality, as compared to the no magnet group (36). Liu et al. designed a type of fluorescent–magnetic bifunctional superparamagnetic iron oxide nanoparticles (SPIONs) to control the phenotypic stability of repaired SCs (37). The SMF-induced SPIONs group showed a significantly higher mRNA expression level of brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), oligodendrocyte transcription factor 1 (Olig1), and vascular endothelial growth factor (VEGF) (qRT-PCR and ELISA). Western blot analysis showed that the expression levels of Beclin1 and lc3b in the experimental group were higher than those in the control group, while the expression level of p62 protein was lower, suggesting the activation of autophagy in Schwann cells by the magnetic stimulation SPIONs. Moreover, for their effects on elongation and branching, there was a statistically significant increase in the expression levels of immune-related cytokines and transcription factors associated with repair phenotypes in the SPION + MF groups. *In vitro* experiments also verified the effects of SPIONs on promoting peripheral nerve regeneration. Hu et al. reported that the combination of SGNs with poly-L-lysine-coated SPIONs could promote the extension of neurites and growth along the direction of a magnetic field (2). Huang et al. reported that the number of SCs with ChABC/PEI-SPIONs, which migrated into the astrocyte region, was 11.6- and 4.6-fold higher than that of control groups under the driven effect of a directional magnetic field (40). Raffa et al. observed that PC12 cells also exhibited more neurite extension and orientation under the guidance of MNPs (39).

3.4. Biomaterials based on magnetic fibers

Due to the advantageous features of nanofibers and MNPs, numerous researchers have combined MNPs with biodegradable

nanofibers to produce magnetic nanofiber scaffolds. Nanofibers have the characteristics of a large surface-to-mass ratio, high porosity, and superior mechanical performance (41–43). Based on electrospinning (ES), there are three techniques for obtaining iron oxide-loaded composite fibers: the introduction of pre-synthesized SPIONs in a polymer solution before ES (used in biodegradable polymers mostly), mixing a precursor before a post-ES process to yield the SPIONs within the fibers, and *in situ* synthesis techniques (44, 45). There are two major drawbacks in the classical precipitation of NPs on the surface of biomaterials, including difficulty in controlling the morphology and aggregation of NPs and the incompatibility of SPIONs formation with a wide range of biopolymers (44). Silanization, polymer brush coating, and grafting have been used for ameliorating the dispersibility of NPs in polymeric matrices (44). Sodium citrate, polyacid, or oleic acid has also been applied for the dispersion of iron oxide NPs. Nottelet et al. combined thiolene photoaddition with free ligand exchange to anchor SPIONs on the surface of nanofibers, which maintained the initial fiber morphology and avoided the unwanted aggregation of MNPs.

A variety of materials have been used in the preparation of magnetic nanofibers, such as chitosan/poly (vinyl alcohol) (PVA), poly (ϵ -caprolactone) (PCL), hydroxyapatite (HA), magnetic poly (L-lactic acid) (PLLA), poly (D, L-lactic acid) (PDLLA), and poly (glycolide-co-L-lactide) (PGLA) (46–50). Due to the tunability, porosity, hydrophilicity, capacity for the incorporation of biological factors, and the polymeric nature of hydrogels, they are increasingly used in bone or nerve regeneration, cancer therapy, and tissue engineering (51). Gilbert et al. designed a method to inject the small conduits of aligned fibers within a hydrogel to reduce fiber tangling (52). They observed that SPION could increase the elongation of neurites by 30%. In addition, they also concluded that SPION content in the electrospinning fibers could be set between 2 and 6% by weight to balance the magnetization and fiber diameter, alignment, and/or density. Gilbert et al. also synthesized aligned control PLLA and SPION-grafted PLLA electro-spun fibers to induce DRG growth. They set up three different types of magnetic fields to study the induction of nerve regeneration by magnetic nanofibers under different magnetic fields: SMF, alternating magnetic field, and linearly moving magnetic field. They observed that neurites could extend 24 and 30% farther on SPION-grafted fibers as compared to that of untethered SPIONs on PLLA fibers under the static or alternating magnetic field. Moreover, 40 and 27% longer neurites were observed in the alternating and linearly moving magnetic fields as compared to that in the SMF (53).

In addition to inducing nerve regeneration under a paramagnetic action, the magnetic field can also be used to form aligned columnar structures in biological scaffolds, especially systematically tuning the mechanical properties of hydrogel scaffolds (54–56). Schmidt et al. used polyimide, magnetic alginate microparticles, and glycidyl methacrylate hyaluronic acid to invent a magnetically aligned regenerative tissue-engineered electronic nerve interface (MARTEENI) (57). This new technology combined TEENI technology with magnetically templated scaffolds to overcome the challenges of prosthetic-limb neural-interfacing technology, such as stiffness mismatch, low axonal population

sampling, and long-term signal decay. After 6–8 weeks of exposure, MARTEENI groups showed similar results in the expression levels of extracellular matrix (ECM) proteins and axonal density as compared to the autograft groups. Under the induction of an external magnetic field, magnetic nanofibers can accelerate drug release. Ming-Wei Chang et al. reported that magnetic hollow fibers could induce greater drug release as compared to the non-treated samples under AMF (~ 40 kHz) (58). This might be due to the magnetocaloric effect caused by superparamagnetism. This kind of nanofiber might have a good prospect in drug delivery with precise positioning.

3.5. Mechanism of magnetic effects on nerve cells

Since the 1970's, increasing evidence has confirmed the neuron-promoting effects of SMF and alternating magnetic fields. The mechanism of the magnetic field, including both external or micromagnetic fields based on superparamagnetism, which affects peripheral nerves, is unclear. A comprehensive understanding of the mechanism at the cellular level might help in developing strategies to use magnet-mediated therapy for PNI. This part of the review highlights the mechanism of stimulating the effects of a magnetic field on cells (Figure 1).

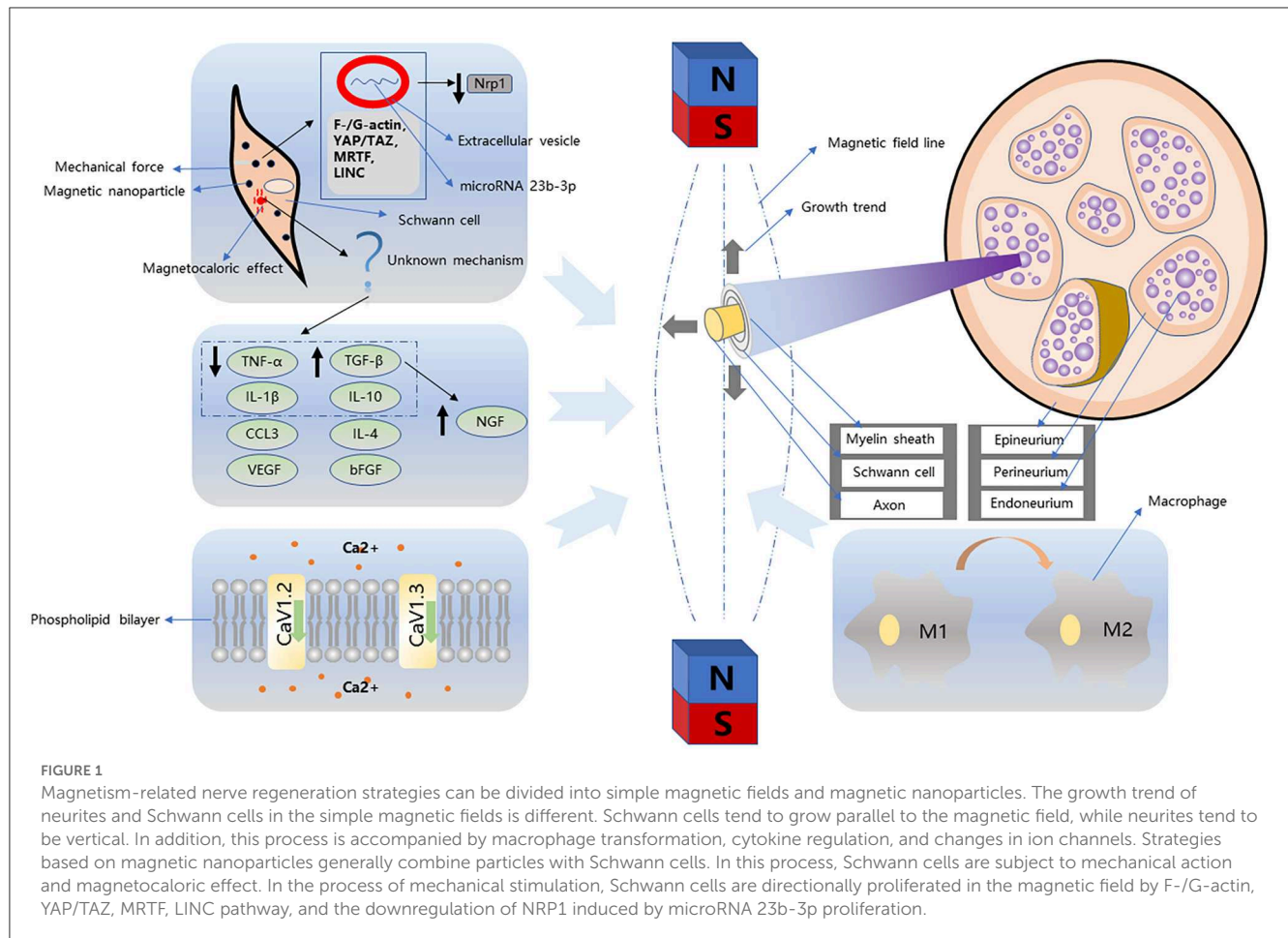
One of the mechanisms of the magnetic field on cells is its effect on the molecular structure of excitable membranes, modifying the function of embedded ion-specific channels (59). Strong magnetic fields can alter the preferred orientation of different diamagnetic anisotropic organic molecules. Low magnetic field intensity relies on the molecular structure of excitable membranes. Researchers have focused on the changes in ion channels under a magnetic field. The voltage-gated channels, including potassium, sodium, and calcium ion channels, are affected by magnetic field exposure, thereby making neurons highly sensitive to magnetic field exposure (60–62). Numerous studies have investigated the internal flow of calcium ions (3, 63). In previous studies, Aldinucci simultaneously exposed human lymphocytes to 4.75 T SMFs and 0.7 mT PMF at 500 MHz for 1 h. The combined SMF and PMF groups could increase the Ca^{2+} influx (64). Balassa et al. reported that the calcium ion channels might play significant roles in synaptic functions under magnetic field stimulation (65). Finberg et al. reported that SMFs could mediate the Ca^{2+} influx through L-type voltage-gated calcium channels (VGCCs), providing a neuroprotective activity related to their anti-apoptotic capacity. They reported that the cellular apoptosis of primary cortical neurons, which were exposed to SMF (50 G), decreased, showing a lower expression level of pro-apoptotic markers, including cleaved caspase-3, cleaved poly ADP ribose polymerase-1, the phospho-histone H2A variant (Ser139), and active caspase-9. Moreover, the expression levels of Cav1.2 and Cav1.3 channels were significantly enhanced (63). Currently, these protective effects on these cells are only reflected in the SMF, while the strong PMF accelerates cellular apoptosis. Prasad et al. reported that the moderate-intensity SMF (0.3 T) could induce the oligodendrocytes precursor cells to increase the intracellular Ca^{2+} influx by increasing the expression levels of L-type channel

subunits—CaV1.2 and CaV1.3 (3). Numerous studies showed that the changes in ion channels were an important part of this process.

Studies on MNPs in various magnetic fields have shown that they have great potential for nerve regeneration. After the binding of MNPs to target cells, the magnetic force acting on the target cells becomes the most intuitive mechanism. Schwann cells, which are the commonly used target cells, are essential for the rapid saltatory propagation of action potential. Studies have focused on the effects of mechanical stimulation on Schwann cells (66). Recent studies showed that Schwann cells could create “stimuli” for themselves even in the absence of external mechanical stimulation (67, 68). They secrete basal lamina, an essential component of the SC's ECM, to enhance mechanical resistance. Mechanical stimulation might have a positive impact on SCs based on mechano-sensors and mechano-transducers (66). The stimulation can activate ECM, cell adhesion molecules, and some mechano-transduction pathways (69) and can enhance the expression levels of $\beta 1$ integrins (70, 71). Moreover, low-level mechanical stimulation can promote the proliferation of SCs accompanied by demyelination and apoptosis (72–74). Xia et al. reported that miR-23b-3p from the extracellular vesicle of SCs might be a key factor of mechanical stimulation, promoting peripheral nerve regeneration (75). Meanwhile, force generation is downstream of many signaling cascades activated by neurotrophic factors, such as netrin-1 and NGF (76, 77). Raffa et al. quantified the correlations between the magnitude of mechanical force and nerve elongation (78, 79). This provided a theoretical basis for the quantitative design of MNPs to promote axonal regeneration.

Another possible mechanism is the magnetocaloric effects of MNPs in an external magnetic field, promoting nerve regeneration. This effect has been applied in bioengineering for a long time. Lin et al. completed and applied the localized heating of magnetic nanofibers to inactivate tumor cells (80). Although there is a lack of studies on the correlation of the magnetocaloric effect with nerve regeneration under a magnetic field, the correlation between temperature and neurite growth has been confirmed. He et al. reported that suitable temperature increased the serum levels of TGF- β and IL-10 and decreased the serum levels of TNF- α and IL-1 β (81). This result is the same as the cytokine changes of nerve cells under the magnetic field. Numerous studies proved that the increase in temperature could positively affect the growth of neurites, and the temperature of 37–42°C might be the most suitable temperature for nerve growth (82).

The effects of the magnetic field on peripheral nerves are also reflected in growth factors and inflammatory factors. Zhang et al. reported that the low-intensity PMF could enhance the expression levels of myelin basic protein (MBP), myelin oligodendrocyte glycoprotein expressions (MOG), and transforming growth factor (TGF) (TGF- $\beta 1$, TGF- $\beta R1$, and TGF- $\beta R2$) in the central nervous system (83). The TGF- β inhibitor could reduce the expression levels of NGF in a mouse model, which suggested that the stimulation of a magnetic field might promote the release of NGF (4). Mert et al. reported that the low-frequency PMF (1, 3, 5, 7 Hz) not only increased compound action potential (CAP) amplitude and sciatic nerve conduction velocity (SNCV) but also reduced the expression levels of chemokines, such as CXCL1 and CCL3, which prevented the infiltration of immune cells and migration of neural progenitors (84). Moreover, PMF treatment could increase the



expression levels of bFGF and decrease that of VEGF in the sciatic nerve. Interestingly, VEGF and bFGF indicated different results in diabetic mouse models, showing decreased expression levels (85). The mRNA expression levels of BDNF, GDNF, and VEGF were higher in the magnetic field-induced Schwann cells than those in the control group, while those of *NT-3* were similar (86). There was no reasonable explanation for this phenomenon. It was speculated that this might be related to the differences in the growth directions of Schwann cells and neurites in the magnetic field.

During peripheral nerve regeneration, macrophages gradually convert from the pro-inflammatory (M1) phenotype to the anti-inflammatory (M2) phenotype (87–90). They are also associated with cellular proliferation and differentiation as well as the release of growth factors (91). Dai et al. reported that the combination of MNPs and alternating magnetic fields could promote macrophage to M2 polarization (92). In addition, they also suggested that this regulation might be directly related to the internalization of MNPs involved in activating the expression of interleukin 10 (IL-10), which might be amplified by the external magnetic field.

Brief exposure to SMF at 100 mT for 15 min led to a marked but transient potentiation of binding of a radiolabeled probe for activator protein-1 (AP1) in immature cultured rat hippocampal neurons. It caused a high expression level of growth-associated protein-43 and increased AP1 DNA binding through the expression of Fra-2, c-jun, and jun-D proteins. A study by

Hirai provided a basis for finding the signaling pathway of the magnetic stimulatory effects on neurons (93). Liu et al. studied the mechanism of remyelination using *in vitro* and *in vivo* experiments on MNPs bound to SCs under SMFs. They suggested that Raf-MEK-ERK1/2, Rac1-MKK7-JNK-c-Jun, and TORC1-c-Jun pathways might be related to peripheral nerve regeneration under superparamagnetism. Notably, although there was a statistically significant increase for regeneration-related protein in the SPION + MF group as compared to the pure magnetic field group, it was not possible to determine whether this effect was due to superparamagnetism or mechanical stimulation (37).

Numerous high-quality studies are still required to establish a complete system for the mechanistic effects of magnetism on peripheral nerves, which should include all the aforementioned experimental phenomena.

4. Clinical applications

With the continuous theoretical studies on peripheral nerve regeneration using a magnetic field, relevant clinical applications are also being developed. Due to its muscle contractions and sensory afferents, repetitive peripheral magnetic stimulation (rPMS) is a non-invasive treatment for the nervous and musculoskeletal system (94) and has been widely used in many

fields, such as reducing spasticity and improving the motor control of paretic limbs (95–97).

There is no conclusion about the underlying mechanism of rPMS. Researchers showed that the cortical plastic effects might be a key factor (98). The activation of the frontoparietal loops and an increase in corticomotor excitability are also important effects after magnetic stimulation (96). Liu et al. reported that the peripheral nerve electrical stimulation for 1 h could increase corticomotor excitability and hand dexterity improvement in patients with motor impairment after stroke (99). Other studies have shown similar results (100, 101).

A randomized controlled trial of 46 samples showed that the repetitive magnetic stimulation of the median nerve with 2,400 pulses (20 Hz over 10 min) could increase the peak motor evoked potential (MEP) amplitudes and RC slope in the contralateral hemisphere (98). Furthermore, the improvement in the Purdue Pegboard Test (PPT) score was significant after 24 h as compared to the baseline ($*P = 0.003$) and immediately after rPMS ($*P = 0.012$). Savulescu et al. reported that rPMS in association with physiotherapy (PKT) groups showed more improved results in the pain score and electromyography (EMG) analysis than in monotherapy groups (102).

rPMS has therapeutic effects on stroke-induced neurological and muscular sequelae. Jiang et al. reported that rPMS groups showed a better arm function and muscle strength for grip and elbow flexion and an extension of patients suffering from an early subacute stroke with severe upper extremity impairment than conventional physiotherapy groups (103). Zschorlich et al. reported that a 5-Hz rPMS could reduce the tendon reflex amplitude, which reduced muscle stiffness and increased mobility (104). Although rPMS involves the direct effect of magnetic field and peripheral nerve, there are few clinical studies on rPMS and peripheral nerve injury. The aforementioned research suggests that rPMS may play a role in promoting peripheral nerve regeneration. We need more high-quality studies to verify the efficacy and specific parameters of rPMS.

Magnetic fields are also applied in chemotherapy-induced peripheral neuropathy (CIPN), which is performed by axonal degeneration (vinca alkaloids) and demyelination (platinum compounds) (105, 106). Rick et al. reported that the 3-month magnetic field treatment could significantly improve NCV and subjectively perceived neurotoxicity (107). Moreover, there was no statistically significant difference in the pain detection end scores.

The International Commission on Non-Ionizing Radiation Protection (lower than 300 Hz for the general public and 400 Hz for occupational exposure) and the Institute of Electrical and Electronics Engineers International Committee on Electromagnetic Safety (lower than 750 Hz) have set limits on electromagnetic field thresholds for protection against stimulation and thermal effects (108–110). Moreover, the threshold of electromagnetic field strength is conservative due to a lack of correlation between internal and external field strengths and nerve activation (108). Hirata et al. used a multi-scale computation to study the threshold of peripheral magnetic stimulation based on a human anatomical model (108). They showed a margin factor of 4–6 and 10–24 times between internal and external protection limits of the international standards.

It is worth noting that the preliminary or explorative studies on the nature of rPMS have reported single cases, case series, or different stimulation protocols (111–115). Sollmann et al. made a checklist, including eight subject-related items, such as age, gender, handedness, or footedness; 16 methodological items, such as coil location, coil type, pulse duration, and shape; and 11 stimulation protocol items, such as stimulation frequency, stimulation intensity, and duty cycle, to guide the research direction of rPMS and contribute to new interventional or exploratory rPMS studies (116).

5. Conclusion and future prospects

This review discusses magnetic field properties and intensities, MNP-encapsulating various cytokines based on superparamagnetism, magnetically functionalized nanofibers, relevant mechanisms, and some clinical applications. This review attempted to completely and systematically analyze the latest studies on these topics.

At the same time, there are numerous problems in this field, which should be solved by researchers in their future studies. First, a suitable magnetic field mode and methods to be used are needed to be formulated. Gleich et al. reported that the cornered FO8 coil was more effective, which could reduce the stimulation voltage, current, and energy of the stimulation device (117). Abe et al. also used a newly developed stimulator to reduce the pain and discomfort of magnetic stimulation (118). Heiland et al. reported that the magnetization transfer ratio in the peripheral nerve tissue altered with age (119). This indicated that the mode setting of peripheral nerve magnetic stimulation therapy should be adjusted based on the age of patients.

In addition, the relevant mechanism requires further exploration. The molecular mechanisms of these problems are still unknown. Using experiments based on MNPs-Schwann cells under SMFs, Liu et al. showed that Raf-MEK-ERK1/2, Rac1-MKK7-JNK-c-Jun, and TORC1-c-Jun pathways might be related to peripheral nerve regeneration; however, there is no evidence determining whether this effect was due to superparamagnetism or mechanical stimulation. Moreover, the direction of cell growth was correlated with the cell type under different magnetic fields. According to previous studies, Schwann cells tended to grow parallel to the direction of the magnetic field, while neurites grew perpendicular to the magnetic field. This difference might limit the potential of axon and myelin sheath growth under a magnetic field. Future studies should focus on finding a more reasonable magnetic field placement mode, strength, and time to obtain the best effects with the smallest damage.

The effects of magnetism on peripheral nerves have always been a research hotspot. A suitable application of magnetic field or magnetic biomaterials can shorten the regeneration time of peripheral nerves, enhance the orientation of nerves, and promote the release of growth factors. In future, more high-quality studies are warranted, which could further clarify the mechanism of magnetic action and formulate guidelines for magnetic therapy.

Author contributions

All authors were involved in the conception and choice of approach for the expert consensus and also contributed findings from their knowledge and experience to the expert consensus, drafted the manuscript, critically revised the manuscript for important intellectual content, and approved the final version of the manuscript.

Funding

This study was supported by the Research Project of the Education Department of Jilin Province (No. JJKH20211187KJ) and the Industrial Technology Research and Development of the Development and Reform Commission of Jilin Province (No. 2021C042-4).

Acknowledgments

The authors would like to acknowledge Xiang Li (College of Chemistry, Jilin University, Changchun, China) for

providing support related to materials science, and Shusen Cui (China-Japan Union Hospital of Jilin University, Changchun, China) for medical writing assistance with the preparation of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Li R, Liu Z, Pan Y, Chen L, Zhang Z, Lu L. Peripheral nerve injuries treatment: a systematic review. *Cell Biochem Biophys.* (2014) 68:449–54. doi: 10.1007/s12013-013-9742-1
- Hu Y, Li D, Wei H, Zhou S, Chen W, Yan X, et al. Neurite extension and orientation of spiral ganglion neurons can be directed by superparamagnetic iron oxide nanoparticles in a magnetic field. *Int J Nanomedicine.* (2021) 16:4515–26. doi: 10.2147/IJN.S313673
- Prasad A, Teh DBL, Blasiak A, Chai C, Wu Y, Gharibani PM, et al. Static magnetic field stimulation enhances oligodendrocyte differentiation and secretion of neurotrophic factors. *Sci Rep.* (2017) 7:6743. doi: 10.1038/s41598-017-06331-8
- Yokozeki Y, Uchida K, Kawakubo A, Nakawaki M, Okubo T, Miyagi M, et al. TGF- β regulates nerve growth factor expression in a mouse intervertebral disc injury model. *BMC Musculoskeletal Disord.* (2021) 22:634. doi: 10.1186/s12891-021-04509-w
- Yang M, Jian L, Fan W, Chen X, Zou H, Huang Y, et al. Axon regeneration after optic nerve injury in rats can be improved via PirB knockdown in the retina. *Cell Biosci.* (2021) 11:158. doi: 10.1186/s13578-021-00670-w
- Murayama M. Orientation of sickled erythrocytes in a magnetic field. *Nature.* (1965) 206:420–2. doi: 10.1038/206420a0
- Emura R, Takeuchi T, Nakaoka Y, Higashi T. Analysis of anisotropic diamagnetic susceptibility of a bull sperm. *Bioelectromagnetics.* (2003) 24:347–55. doi: 10.1002/bem.10109
- Guido S, Tranquillo RT. A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and gel birefringence. *J Cell Sci.* (1993) 105:317–31. doi: 10.1242/jcs.105.2.317
- Kotani, Hiroko, Iwasaka, Masakazu, Ueno, Shoogo, et al. Magnetic orientation of collagen and bone mixture. *J Appl Phys.* (2000) 87:6191. doi: 10.1063/1.372652
- Hirose H, Nakahara T, Miyakoshi J. Orientation of human glioblastoma cells embedded in type I collagen, caused by exposure to a 10 T static magnetic field. *Neurosci Lett.* (2003) 338:88–90. doi: 10.1016/S0304-3940(02)01363-0
- Eguchi Y, Ogiue-Ikeda M, Ueno S. Control of orientation of rat Schwann cells using an 8-T static magnetic field. *Neurosci Lett.* (2003) 351:130–2. doi: 10.1016/S0304-3940(03)00719-5
- Kim S, Im WS, Kang L, Lee ST, Chu K, Kim BI. The application of magnets directs the orientation of neurite outgrowth in cultured human neuronal cells. *J Neurosci Methods.* (2008) 174:91–6. doi: 10.1016/j.jneumeth.2008.07.005
- Torbet J, Ronzière MC. Magnetic alignment of collagen during self-assembly. *Biochem J.* (1984) 219:1057–9. doi: 10.1042/bj2191057
- Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. *Annu Rev Biomed Eng.* (2003) 5:293–347. doi: 10.1146/annurev.bioeng.5.011303.120731
- Stoll G, Griffin JW, Li CY, Trapp BD. Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol.* (1989) 18:671–83. doi: 10.1007/BF01187086
- Funnell JL, Balouch B, Gilbert RJ. Magnetic composite biomaterials for neural regeneration. *Front Bioeng Biotechnol.* (2019) 7:179. doi: 10.3389/fbioe.2019.00179
- D'Agata F, Ruffinatti FA, Boschi S, Stura I, Rainero I, Abollino O, et al. Magnetic nanoparticles in the central nervous system: targeting principles, applications and safety issues. *Molecules.* (2017) 23:9. doi: 10.3390/molecules23010009
- Ito H, Bassett CAL. Effect of weak, pulsing electromagnetic fields on neural regeneration in the rat. *Clin Orthop Relat Res.* (1983) 181:283–90. doi: 10.1097/00003086-198312000-00044
- Kanje M, Rusovan A, Siskin B, Lundborg G. Pretreatment of rats with pulsed electromagnetic fields enhances regeneration of the sciatic nerve. *Bioelectromagnetics.* (1993) 14:353–9. doi: 10.1002/bem.2250140407
- Pedro J, Pérez-Caballer A, Dominguez J, Collía F, Blanco J, Salvado M. Pulsed electromagnetic fields induce peripheral nerve regeneration and endplate enzymatic changes. *Bioelectromagnetics.* (2005) 26:20–7. doi: 10.1002/bem.20049
- Raylman RR, Clavo AC, Wahl RL. Exposure to strong static magnetic field slows the growth of human cancer cells in vitro. *Bioelectromagnetics.* (1996) 17:358–63. doi: 10.1002/(SICI)1521-186X(1996)17:5<358::AID-BEM2>3.0.CO;2-2
- Buemi M, Marino D, Pasquale GD, Floccari F, Senatore M, Aloisi C, et al. Cell proliferation/cell death balance in renal cell cultures after exposure to a static magnetic field. *Nephron.* (2001) 87:269–73. doi: 10.1159/000045925
- Schiffer IB, Schreiber WG, Graf R, Schreiber EM, Jung D, Rose DM, et al. No influence of magnetic fields on cell cycle progression using conditions relevant for patients during MRI. *Bioelectromagnetics.* (2003) 24:241–50. doi: 10.1002/bem.10097
- Mann A, Steinecker-Frohnwieser B, Naghilou A, Milesi F, Supper P, Semmler L, et al. Nuclear magnetic resonance treatment accelerates the regeneration of dorsal root ganglion neurons. *Front Cell Neurosci.* (2022) 16:859545. doi: 10.3389/fncel.2022.859545
- Macias MY, Battocletti JH, Sutton CH, Pintar FA, Maiman DJ. Directed and enhanced neurite growth with pulsed magnetic field stimulation. *Bioelectromagnetics.* (2015) 21:272–86. doi: 10.1002/(SICI)1521-186X(200005)21:4<272::AID-BEM4>3.3.CO;2-X
- Torbet J, Freyssinet JM, Hudry-Clergeon G. Oriented fibrin gels formed by polymerization in strong magnetic fields. *Nature.* (1981) 289:91–3. doi: 10.1038/289091a0
- Heid S, Unterwieser H, Tietze R, Friedrich RP, Weigel B, Cicha I, et al. Synthesis and characterization of tissue plasminogen activator-functionalized

- superparamagnetic iron oxide nanoparticles for targeted fibrin clot dissolution. *Int J Mol Sci.* (2017) 18:1837. doi: 10.3390/ijms18091837
28. Higashi T, Yamagishi A, Takeuchi T, Kawaguchi N, Sagawa S, Onishi S, et al. Orientation of erythrocytes in a strong static magnetic field. *Blood.* (1993) 82:1328–34. doi: 10.1182/blood.V82.4.1328.1328
29. Kotani H, Kawaguchi H, Shimoaka T, Iwasaka M, Ueno S, Ozawa H, et al. Strong static magnetic field stimulates bone formation to a definite orientation *in vitro* and *in vivo*. *J Bone Min Res.* (2002) 17:1814–21. doi: 10.1359/jbmr.2002.17.10.1814
30. Ikehata M, Koana T, Suzuki Y, Shimizu H, Nakagawa M. Mutagenicity and co-mutagenicity of static magnetic fields detected by bacterial mutation assay. *Mutat Res.* (1999) 427:147. doi: 10.1016/S0027-5107(99)00087-1
31. Zmylony M, Palus J, Jajte J, Dziubaltowska E, Rajkowska E. DNA damage in rat lymphocytes treated *in vitro* with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz). *Mutat Res.* (2000) 453:89–96. doi: 10.1016/S0027-5107(00)00094-4
32. Liu L, Liu Z, Huang L, Sun Z, Ma T, Zhu S, et al. Pulsed magnetic field promotes proliferation and neurotrophic genes expression in Schwann cells *in vitro*. *Int J Clin Exp Pathol.* (2015) 8:2343–53.
33. Giannaccini M, Calatayud MP, Poggetti A, Corbianco S, Novelli M, Paoli M, et al. Magnetic nanoparticles for efficient delivery of growth factors: stimulation of peripheral nerve regeneration. *Adv Healthc Mater.* (2017) 6. doi: 10.1002/adhm.201601429
34. Kim JA, Lee N, Kim BH, Rhee WJ, Yoon S, Hyeon T, et al. Enhancement of neurite outgrowth in PC12 cells by iron oxide nanoparticles. *Biomaterials.* (2011) 32:2871–7. doi: 10.1016/j.biomaterials.2011.01.019
35. Pop NL, Nan A, Urda-Cimpean AE, Florea A, Toma VA, Moldovan R, et al. Chitosan functionalized magnetic nanoparticles to provide neural regeneration and recovery after experimental model induced peripheral nerve injury. *Biomolecules.* (2021) 11:676. doi: 10.3390/biom11050676
36. Yuan M, Wang Y, Qin Y-X. Promoting neuroregeneration by applying dynamic magnetic fields to a novel nanomedicine: superparamagnetic iron oxide (SPIO)-gold nanoparticles bounded with nerve growth factor (NGF). *Nanomedicine.* (2018) 14:1337–47. doi: 10.1016/j.nano.2018.03.004
37. Liu T, Wang Y, Lu L, Liu Y. SPIONs mediated magnetic actuation promotes nerve regeneration by inducing and maintaining repair-supportive phenotypes in Schwann cells. *J Nanobiotechnology.* (2022) 20:159. doi: 10.1186/s12951-022-01337-5
38. Wang Y, Du S, Liu T, Ren J, Zhang J, Xu H, et al. Schwann cell migration through magnetic actuation mediated by fluorescent-magnetic bifunctional FeO-rhodamine 6G@polydopamine superparticles. *ACS Chem Neurosci.* (2020) 11:1359–70. doi: 10.1021/acscchemneuro.0c00116
39. Riggio C, Calatayud MP, Giannaccini M, Sanz B, Torres TE, Fernández-Pacheco R, et al. The orientation of the neuronal growth process can be directed via magnetic nanoparticles under an applied magnetic field. *Nanomedicine.* (2014) 10:1549–58. doi: 10.1016/j.nano.2013.12.008
40. Gao J, Xia B, Li S, Huang L, Ma T, Shi X, et al. Magnetic field promotes migration of schwann cells with chondroitinase ABC (ChABC)-loaded superparamagnetic nanoparticles across astrocyte boundary *in vitro*. *Int J Nanomedicine.* (2020) 15:315–32. doi: 10.2147/IJN.S227328
41. Frenot A, Chronakis IS. Polymer nanofibers assembled by electrospinning. *Curr Opin Colloid Interface Sci.* (2003) 8:64–75. doi: 10.1016/S1359-0294(03)00004-9
42. Kim JS. Improved mechanical properties of composites using ultrafine electrospun fibers. *Univ Akron.* (1997) 20:124–31. doi: 10.1002/pc.10340
43. Lee H-J, Lee SJ, Uthaman S, Thomas RG, Hyun H, Jeong YY, et al. Biomedical applications of magnetically functionalized organic/inorganic hybrid nanofibers. *Int J Mol Sci.* (2015) 16:13661–77. doi: 10.3390/ijms160613661
44. Awada H, Al Samad A, Laurencin D, Gilbert R, Dumail X, El Jundi A, et al. Controlled anchoring of iron oxide nanoparticles on polymeric nanofibers: easy access to Core@Shell organic-inorganic nanocomposites for magneto-scaffolds. *ACS Appl Mater Interfaces.* (2019) 11:9519–29. doi: 10.1021/acsami.8b19099
45. Mortimer CJ, Wright CJ. The fabrication of iron oxide nanoparticle-nanofiber composites by electrospinning and their applications in tissue engineering. *Biotechnol J.* (2017) 12. doi: 10.1002/biot.20160069
46. Gao D, Jiang J-J, Gu S-H, Lu J-Z, Xu L. Morphological detection and functional assessment of regenerated nerve after neural prosthesis with a PGLA nerve conduit. *Sci Rep.* (2017) 7:46403. doi: 10.1038/srep46403
47. Singh RK, Patel KD, Lee JH, Lee E-J, Kim J-H, Kim T-H, et al. Potential of magnetic nanofiber scaffolds with mechanical and biological properties applicable for bone regeneration. *PLoS ONE.* (2014) 9:e91584. doi: 10.1371/journal.pone.0091584
48. Wei Y, Zhang X, Song Y, Han B, Hu X, Wang X, et al. Magnetic biodegradable Fe₃O₄/CS/PVA nanofibrous membranes for bone regeneration. *Biomed Mater.* (2011) 6:055008. doi: 10.1088/1748-6041/6/5/055008
49. Zeng XB, Hu H, Xie LQ, Lan F, Jiang W, Wu Y, et al. Magnetic responsive hydroxyapatite composite scaffolds construction for bone defect reparation. *Int J Nanomedicine.* (2012) 7:3365–78. doi: 10.2147/IJN.S32264
50. Shan D, Shi Y, Duan S, Wei Y, Cai Q, Yang X. Electrospun magnetic poly(L-lactide) (PLLA) nanofibers by incorporating PLLA-stabilized Fe₃O₄ nanoparticles. *Mater Sci Eng C Mater Biol Appl.* (2013) 33:3498–505. doi: 10.1016/j.msec.2013.04.040
51. Annabi N, Tamayol A, Uquillas JA, Akbari M, Bertassoni LE, Cha C, et al. 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. *Adv Mater.* (2014) 26:85–123. doi: 10.1002/adma.201303233
52. Johnson CDL, Ganguly D, Zuidema JM, Cardinal TJ, Ziemba AM, Kearns KR, et al. Injectable, magnetically orienting electrospun fiber conduits for neuron Guidance. *ACS Appl Mater Interfaces.* (2019) 11:356–72. doi: 10.1021/acsami.8b18344
53. Funnell JL, Ziemba AM, Nowak JF, Awada H, Prokopiou N, Samuel J, et al. Assessing the combination of magnetic field stimulation, iron oxide nanoparticles, and aligned electrospun fibers for promoting neurite outgrowth from dorsal root ganglia *in vitro*. *Acta Biomater.* (2021) 131:302–13. doi: 10.1016/j.actbio.2021.06.049
54. Lacko CS, Singh I, Wall MA, Garcia AR, Porvasnik SL, Rinaldi C, et al. Magnetic particle templating of hydrogels: engineering naturally derived hydrogel scaffolds with 3D aligned microarchitecture for nerve repair. *J Neural Eng.* (2020) 17:016057. doi: 10.1088/1741-2552/ab4a22
55. Kasper M, Deister C, Beck F, Schmidt CE. Bench-to-bedside lessons learned: commercialization of an acellular nerve graft. *Adv Healthc Mater.* (2020) 9:e2000174. doi: 10.1002/adhm.202000174
56. Kuliasha CA, Judy JW. *In vitro* reactive-accelerated-aging (RAA) assessment of tissue-engineered electronic nerve interfaces (TEENI). *Annu Int Conf IEEE Eng Med Biol Soc.* (2018) 2018:5061–4. doi: 10.1109/EMBC.2018.8513458
57. Kasper M, Ellenbogen B, Hardy R, Cydis M, Mojica-Santiago J, Afridi A, et al. Development of a magnetically aligned regenerative tissue-engineered electronic nerve interface for peripheral nerve applications. *Biomaterials.* (2021) 279:121212. doi: 10.1016/j.biomaterials.2021.121212
58. Wang B, Zheng H, Chang M-W, Ahmad Z, Li J-S. Hollow polycaprolactone composite fibers for controlled magnetic responsive antifungal drug release. *Colloids Surf B Biointerfaces.* (2016) 145:757–67. doi: 10.1016/j.colsurf.2016.05.092
59. Rosen AD. Mechanism of action of moderate-intensity static magnetic fields on biological systems. *Cell Biochem Biophys.* (2003) 39:163–73. doi: 10.1385/CBB:39:2:163
60. Mathie A, Kennard LE, Veale EL. Neuronal ion channels and their sensitivity to extremely low frequency weak electric field effects. *Radiat Prot Dosimetry.* (2003) 106:311–6. doi: 10.1093/oxfordjournals.rpd.a006365
61. Marchionni I, Paffi A, Pellegrino M, Liberti M, Apollonio F, Abeti R, et al. Comparison between low-level 50 Hz and 900 MHz electromagnetic stimulation on single channel ionic currents and on firing frequency in dorsal root ganglion isolated neurons. *Biochim Biophys Acta.* (2006) 1758:597–605. doi: 10.1016/j.bbame.2006.03.014
62. Saunders RD, Jefferys JGR. A neurobiological basis for ELF guidelines. *Health Phys.* (2007) 92:596–603. doi: 10.1097/01.HP.0000257856.83294.3e
63. Ben Yakir-Blumkin M, Loboda Y, Schächter L, Finberg JPM. Neuroprotective effect of weak static magnetic fields in primary neuronal cultures. *Neuroscience.* (2014) 278:313–26. doi: 10.1016/j.neuroscience.2014.08.029
64. Aldinucci C, Garcia JB, Palmi M, Sgaragli G, Benocci A, Meini A, et al. The effect of exposure to high flux density static and pulsed magnetic fields on lymphocyte function. *Bioelectromagnetics.* (2003) 24:373–9. doi: 10.1002/bem.10111
65. Balassa T, Varró P, Elek S, Drozdovszky R, Szemerszky R, Világi I, et al. Changes in synaptic efficacy in rat brain slices following extremely low-frequency magnetic field exposure at embryonic and early postnatal age. *Int J Dev Neurosci.* (2013) 31:724–30. doi: 10.1016/j.ijdevneu.2013.08.004
66. Belin S, Zuloaga KL, Poitelon Y. Influence of mechanical stimuli on Schwann cell biology. *Front Cell Neurosci.* (2017) 11:347. doi: 10.3389/fncel.2017.00347
67. Feltri ML, Poitelon Y, Previtali SC. How Schwann cells sort axons: new concepts. *Neuroscientist.* (2016) 22:252–65. doi: 10.1177/1073858415572361
68. Monk KR, Feltri ML, Taveggia C. New insights on Schwann cell development. *Glia.* (2015) 63:1376–93. doi: 10.1002/glia.22852
69. Poitelon Y, Bogni S, Matafora V, Della-Flora Nunes G, Hurley E, Ghidini M, et al. Spatial mapping of juxtacrine axo-glial interactions identifies novel molecules in peripheral myelination. *Nat Commun.* (2015) 6:8303. doi: 10.1038/ncomms9303
70. Friedland JC, Lee MH, Boettiger D. Mechanically activated integrin switch controls alpha5beta1 function. *Science.* (2009) 323:642–4. doi: 10.1126/science.1168441
71. Kong F, Garcia AJ, Mould AP, Humphries MJ, Zhu C. Demonstration of catch bonds between an integrin and its ligand. *J Cell Biol.* (2009) 185:1275–84. doi: 10.1083/jcb.200810002
72. Gupta R, Steward O. Chronic nerve compression induces concurrent apoptosis and proliferation of Schwann cells. *J Comp Neurol.* (2003) 461:174–86. doi: 10.1002/cne.10692
73. Gupta R, Truong L, Bear D, Chafik D, Modafferi E, Hung CT. Shear stress alters the expression of myelin-associated glycoprotein (MAG) and myelin basic protein (MBP) in Schwann cells. *J Orthopaed Res.* (2005) 23:1232–9. doi: 10.1016/j.jorthres.2004.12.010
74. Salzer JL, Bunge RP. Studies of Schwann cell proliferation. I An analysis in tissue culture of proliferation during development, Wallerian degeneration, and direct injury. *J Cell Biol.* (1980) 84:739–52. doi: 10.1083/jcb.84.3.739
75. Xia B, Gao J, Li S, Huang L, Zhu L, Ma T, et al. Mechanical stimulation of Schwann cells promote peripheral nerve regeneration via

- extracellular vesicle-mediated transfer of microRNA 23b-3p. *Theranostics*. (2020) 10:8974–95. doi: 10.7150/thno.44912
76. Toriyama M, Kozawa S, Sakumura Y, Inagaki N. Conversion of a signal into forces for axon outgrowth through Pak1-mediated shootin1 phosphorylation. *Curr Biol*. (2013) 23:529–34. doi: 10.1016/j.cub.2013.02.017
77. Turney SG, Ahmed M, Chandrasekar I, Wysolmerski RB, Goeckeler ZM, Rioux RM, et al. Nerve growth factor stimulates axon outgrowth through negative regulation of growth cone actomyosin restraint of microtubule advance. *Mol Biol Cell*. (2016) 27:500–17. doi: 10.1091/mbc.e15-09-0636
78. Raffa V, Falcone F, De Vincentiis S, Falconieri A, Calatayud MP, Goya GF, et al. Piconewton mechanical forces promote neurite growth. *Biophys J*. (2018) 115:2026–33. doi: 10.1016/j.bpj.2018.10.009
79. De Vincentiis S, Falconieri A, Mainardi M, Cappello V, Scribano V, Bizzarri R, et al. Extremely low forces induce extreme axon growth. *J Neurosci*. (2020) 40:4997–5007. doi: 10.1523/JNEUROSCI.3075-19.2020
80. Lin T-C, Lin F-H, Lin J-C. *In vitro* feasibility study of the use of a magnetic electrospun chitosan nanofiber composite for hyperthermia treatment of tumor cells. *Acta Biomater*. (2012) 8:2704–11. doi: 10.1016/j.actbio.2012.03.045
81. He L-P, Liu P-Z, Wen Y-M, Wu J. Effect of temperature maintenance by forced-air warming blankets of different temperatures on changes in inflammatory factors in children undergoing congenital hip dislocation surgery. *Chin Med J*. (2020) 133:1768–73. doi: 10.1097/CM9.0000000000000846
82. Cancalon P. Influence of temperature on various mechanisms associated with neuronal growth and nerve regeneration. *Prog Neurobiol*. (1985) 25:27–92. doi: 10.1016/0301-0082(85)90022-X
83. Wang Z, Baharani A, Wei Z, Truong D, Bi X, Wang F, et al. Low field magnetic stimulation promotes myelin repair and cognitive recovery in chronic cuprizone mouse model. *Clin Exp Pharmacol Physiol*. (2021) 48:1090–102. doi: 10.1111/1440-1681.13490
84. Mert T, Metin TO, Sahin E, Yaman S, Sahin M. Neuroprotective and anti-neuropathic actions of pulsed magnetic fields with low frequencies in rats with chronic peripheral neuropathic pain. *Brain Res Bull*. (2021) 177:273–81. doi: 10.1016/j.brainresbull.2021.10.012
85. Mert T, Sahin E, Yaman S, Sahin M. Pulsed magnetic field treatment ameliorates the progression of peripheral neuropathy by modulating the neuronal oxidative stress, apoptosis and angiogenesis in a rat model of experimental diabetes. *Arch Physiol Biochem*. (2020) 128:1658–65. doi: 10.1080/13813455.2020.1788098
86. Liu L, Liu Z, Huang L, Sun Z, Luo Z. Pulsed magnetic field promotes proliferation and neurotrophic genes expression in Schwann cells *in vitro*. *Int J Clin Exp Pathol*. (2015) 8:2343.
87. Ferrante CJ, Leibovich SJ. Regulation of macrophage polarization and wound healing. *Adv Wound Care*. (2012) 1:10–6. doi: 10.1089/wound.2011.0307
88. Novak ML, Koh TJ. Phenotypic transitions of macrophages orchestrate tissue repair. *Am J Pathol*. (2013) 183:1352–63. doi: 10.1016/j.ajpath.2013.06.034
89. Chen P, Piao X, Bonaldo P. Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. *Acta Neuropathol*. (2015) 130:605–18. doi: 10.1007/s00401-015-1482-4
90. Gensel JC, Zhang B. Macrophage activation and its role in repair and pathology after spinal cord injury. *Brain Res*. (2015) 1619:1–11. doi: 10.1016/j.brainres.2014.12.045
91. Mokarram N, Merchant A, Mukhtyar V, Patel G, Bellamkonda RV. Effect of modulating macrophage phenotype on peripheral nerve repair. *Biomaterials*. (2012) 33:8793–801. doi: 10.1016/j.biomaterials.2012.08.050
92. Guo W, Wu X, Wei W, Wang Y, Dai H. Mesoporous hollow FeO nanoparticles regulate the behavior of neuro-associated cells through induction of macrophage polarization in an alternating magnetic field. *J Mater Chem B*. (2022) 10:5633–43. doi: 10.1039/D2TB00527A
93. Hirai T, Nakamichi N, Yoneda Y. Activator protein-1 complex expressed by magnetism in cultured rat hippocampal neurons. *Biochem Biophys Res Commun*. (2002) 292:200–7. doi: 10.1006/bbrc.2002.6618
94. Beaulieu LD, Schneider C. Repetitive peripheral magnetic stimulation to reduce pain or improve sensorimotor impairments: a literature review on parameters of application and afferents recruitment. *Neurophysiol Clin*. (2015) 45:223–37. doi: 10.1016/j.neucli.2015.08.002
95. Struppler A, Havel P, Müller-Barna P. Facilitation of skilled finger movements by repetitive peripheral magnetic stimulation (RPMS) - a new approach in central paresis. *NeuroRehabilitation*. (2003) 18:69–82. doi: 10.3233/NRE-2003-18108
96. Struppler A, Binkofski F, Angerer B, Bernhardt M, Spiegel S, Drzezga A, et al. A fronto-parietal network is mediating improvement of motor function related to repetitive peripheral magnetic stimulation: a PET-H2O15 study. *Neuroimage*. (2007) 36 Suppl 2:T174–T86. doi: 10.1016/j.neuroimage.2007.03.033
97. Beaulieu L-D, Massé-Alarie H, Brouwer B, Schneider C. Noninvasive neurostimulation in chronic stroke: a double-blind randomized sham-controlled testing of clinical and corticomotor effects. *Top Stroke Rehabil*. (2015) 22:8–17. doi: 10.1179/1074935714Z.00000000032
98. Jia Y, Liu X, Wei J, Li D, Wang C, Wang X, et al. Modulation of the corticomotor excitability by repetitive peripheral magnetic stimulation on the median nerve in healthy subjects. *Front Neural Circuits*. (2021) 15:616084. doi: 10.3389/fncir.2021.616084
99. Liu H, Au-Yeung SSY. Corticomotor excitability effects of peripheral nerve electrical stimulation to the paretic arm in stroke. *Am J Phys Med Rehabil*. (2017) 96:687–93. doi: 10.1097/PHM.0000000000000748
100. Guarda SNFD, Conforto AB. Effects of somatosensory stimulation on corticomotor excitability in patients with unilateral cerebellar infarcts and healthy subjects - preliminary results. *Cerebell Ataxias*. (2014) 1:16. doi: 10.1186/s40673-014-0016-5
101. Chen C-C, Chuang Y-F, Yang H-C, Hsu M-J, Huang Y-Z, Chang Y-J. Neuromuscular electrical stimulation of the median nerve facilitates low motor cortex excitability in patients with spinocerebellar ataxia. *J Electromyogr Kinesiol*. (2015) 25:143–50. doi: 10.1016/j.jelekin.2014.10.009
102. Savulescu SE, Berteau M, Filipescu I, Beiu C, Mihai M-M, Popa LG, et al. Repetitive peripheral magnetic stimulation (rPMS) in subjects with lumbar radiculopathy: an electromyography-guided prospective, randomized study. *In Vivo*. (2021) 35:623–7. doi: 10.21873/invivo.12300
103. Jiang Y-F, Zhang D, Zhang J, Hai H, Zhao Y-Y, Ma Y-W, et al. Randomized controlled trial of repetitive peripheral magnetic stimulation applied in early subacute stroke: effects on severe upper-limb impairment. *Clin Rehabil*. (2022) 36:693–702. doi: 10.1177/02692155211072189
104. Zschorlich VR, Hillebrecht M, Tanjour T, Qi F, Behrendt F, Kirschstein T, et al. Repetitive peripheral magnetic nerve stimulation (rPMS) as adjuvant therapy reduces skeletal muscle reflex activity. *Front Neurol*. (2019) 10:930. doi: 10.3389/fneur.2019.00930
105. Sahenk Z, Brady ST, Mendell JR. Studies on the pathogenesis of vincristine-induced neuropathy. *Muscle Nerve*. (1987) 10:80–4. doi: 10.1002/mus.880100115
106. Shemesh OA, Spira ME. Paclitaxel induces axonal microtubules polar reconfiguration and impaired organelle transport: implications for the pathogenesis of paclitaxel-induced polyneuropathy. *Acta Neuropathol*. (2010) 119:235–48. doi: 10.1007/s00401-009-0586-0
107. Rick O, Hehn UV, Mikus E, Dertinger H, Geiger G. Magnetic field therapy in patients with cytostatics-induced polyneuropathy: a prospective randomized placebo-controlled phase-III study. *Bioelectromagnetics*. (2018) 38:85–94. doi: 10.1002/bem.22005
108. Suzuki Y, Gomez-Tames J, Diao Y, Hirata A. Evaluation of peripheral electrostimulation thresholds in human model for uniform magnetic field exposure. *Int J Environ Res Public Health*. (2021) 19:390. doi: 10.3390/ijerph19010390
109. Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). International commission on non-ionizing radiation protection. *Health Phys*. (1998) 74:494–522.
110. Ibey BL, Roth CC, Ledwig PB, Payne JA, Amato AL, Dalzell DR, et al. Cellular effects of acute exposure to high peak power microwave systems: morphology and toxicology. *Bioelectromagnetics*. (2016) 37:141–51. doi: 10.1002/bem.21962
111. Flamand VH, Schneider C. Noninvasive and painless magnetic stimulation of nerves improved brain motor function and mobility in a cerebral palsy case. *Arch Phys Med Rehabil*. (2014) 95:1984–90. doi: 10.1016/j.apmr.2014.05.014
112. Kinoshita S, Ikeda K, Hama M, Suzuki S, Abo M. Repetitive peripheral magnetic stimulation combined with intensive physical therapy for gait disturbance after hemorrhagic stroke: an open-label case series. *Int J Rehabil Res*. (2020) 43:235–9. doi: 10.1097/MRR.0000000000000416
113. Leung A, Fallah A, Shukla S. Transcutaneous magnetic stimulation (TMS) in alleviating post-traumatic peripheral neuropathic pain States: a case series. *Pain Med*. (2014) 15:1196–9. doi: 10.1111/pme.12426
114. Momosaki R, Abo M, Watanabe S, Kakuda W, Yamada N, Kinoshita S. Repetitive peripheral magnetic stimulation with intensive swallowing rehabilitation for poststroke dysphagia: an open-label case series. *Neuromodulation*. (2015) 18:630–4. doi: 10.1111/ner.12308
115. Elena SS, Lavinia G, Florina P, Luminia D, Mihai AB. Peripheral repetitive magnetic stimulation: a novel approach for hand rehabilitation in carpal tunnel syndrome - a pilot study. *Int J Soc Sci Human*. (2017) 6:604–7. doi: 10.7763/IJSSH.2016.V6.718
116. Schneider C, Zangrandi A, Sollmann N, Bonfert MV, Beaulieu L-D. Checklist on the quality of the repetitive peripheral magnetic stimulation (rPMS) methods in research: an international delphi study. *Front Neurol*. (2022) 13:852848. doi: 10.3389/fneur.2022.852848
117. Braun P, Rapp J, Hemmert W, Gleich B. Coil efficiency for inductive peripheral nerve stimulation. *IEEE Trans Neural Syst Rehabil Eng*. (2022) 30:2137–45. doi: 10.1109/TNSRE.2022.3192761
118. Abe G, Oyama H, Liao Z, Honda K, Yashima K, Asao A, et al. Difference in pain and discomfort of comparable wrist movements induced by magnetic or electrical stimulation for peripheral nerves in the dorsal forearm. *Med Devices*. (2020) 13:439–47. doi: 10.2147/MDER.S271258
119. Kollmer J, Kästel T, Jende JME, Bendszus M, Heiland S. Magnetization transfer ratio in peripheral nerve tissue: does it depend on age or location? *Invest Radiol*. (2018) 53:397–402. doi: 10.1097/RLI.0000000000000455



OPEN ACCESS

EDITED BY

Lei Xu,
Fudan University, China

REVIEWED BY

Mina Raouf,
Minia University, Egypt
Yuanyuan Zheng,
Tongji University School of Medicine, China
Juan Mou,
Shanghai Normal University, China

*CORRESPONDENCE

Cong-Xian Chen
✉ kema320@qq.com

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 04 February 2023

ACCEPTED 08 March 2023

PUBLISHED 29 March 2023

CITATION

Zeng Z, Lin N and Chen C-X (2023) Comparison
efficacy of ultrasound-guided needle release
plus corticosteroid injection and mini-open
surgery in patients with carpal tunnel
syndrome. *Front. Neurol.* 14:1158688.
doi: 10.3389/fneur.2023.1158688

COPYRIGHT

© 2023 Zeng, Lin and Chen. This is an
open-access article distributed under the terms
of the [Creative Commons Attribution License](#)
(CC BY). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted which
does not comply with these terms.

Comparison efficacy of ultrasound-guided needle release plus corticosteroid injection and mini-open surgery in patients with carpal tunnel syndrome

Zeng Zeng^{1,2}, Nan Lin³ and Cong-Xian Chen^{1*}

¹Cancer Center, Department of Ultrasound Medicine, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, Zhejiang, China, ²Zhejiang University School of Medicine, Hangzhou, China, ³Plastic Surgery Center, Department of Hand and Reconstruct Surgery, Zhejiang Provincial People's Hospital, Affiliated People's Hospital, Hangzhou Medical College, Hangzhou, Zhejiang, China

This retrospective study was to compare clinical outcomes of ultrasound-guided needle release with corticosteroid injection vs. mini-open surgery in patients with carpal tunnel syndrome (CTS). From January 2021 to December 2021, 40 patients (40 wrists) with CTS were analyzed in this study. The diagnosis was based on clinical symptoms, electrophysiological imaging, and ultrasound imaging. A total of 20 wrists were treated with ultrasound-guided needle release plus corticosteroid injection (Group A), and the other 20 wrists were treated with mini-open surgery (Group B). We evaluated the Boston carpal tunnel questionnaire, electrophysiological parameters (distal motor latency, sensory conduction velocity, and sensory nerve action potential of the median nerve), and ultrasound parameters (cross-sectional area, flattening ratio, and the thicknesses of transverse carpal ligament) both before and 3 months after treatment. Total treatment cost, duration of treatment, healing time, and complications were also recorded for the two groups. The Boston carpal tunnel questionnaire and electrophysiological and ultrasound outcomes at preoperatively and 3 months postoperatively had a significant difference for each group (each with $P < 0.05$). There were no complications such as infection, hemorrhage, vascular, nerve, or tendon injuries in both groups. Ultrasound-guided needle release and mini-open surgery are both effective measures in treating CTS patients. Ultrasound-guided needle release plus corticosteroid injection provides smaller incision, less cost, less time of treatment, and faster recovery compared with mini-open surgery. Ultrasound-guided needle release plus corticosteroid injection is better for clinical application.

KEYWORDS

ultrasound, mini-open surgery, carpal tunnel syndrome, transverse carpal ligament, needle release

Introduction

Carpal tunnel syndrome (CTS) is the most frequent musculoskeletal disorder of compressive neuropathy (1). It is caused by compression of the median nerve within the carpal tunnel between the carpal bones and the transverse carpal ligament (TCL) (2). CTS usually causes sensory (paresthesia and hypoesthesia), pain, sleep disturbance, and motor

deficit. It affects ~1–2 men and 4–5 women in every 1,000 persons and always occurs between the age of 40 and 60 years (3). In most cases, CTS is idiopathic (4). Sometimes, diabetes, pregnancy, amyloidosis, and rheumatic synovitis can also cause CTS (4). In essence, CTS is a multifactorial condition (5). CTS is diagnosed by clinical symptoms and physical examination. The Boston carpal tunnel questionnaire, electrophysiological testing, and ultrasonography as supplementary tools can confirm it (6–10). The electrophysiological test of the median nerve is the key point in diagnosing CTS (11). However, when electrophysiological testing is normal in clinically suspected CTS patients, ultrasound imaging seems to be effective in diagnosing (8). Recently, ultrasound was often used for musculoskeletal diseases, especially in peripheral nerve pathologies and median nerve abnormality (12, 13).

There are mainly two types of CTS treatments: surgical and conservative. The choice of treatment depends on the severity of CTS. Conservative treatments are usually for those patients who are in minor and modest circumstances. Conservative treatments include wrist splinting, local corticosteroid injections, laser therapy, and therapeutic ultrasound (14). When conservative ways cannot alleviate CTS symptoms, or the patient's case was severe at the beginning, surgical treatment should be the preferred option (15). Surgery is also a treatment of choice for persistent or progressive CTS patients, and it mainly includes dividing TCL in order to relieve median nerve compression (16). There are five kinds of incision sizes for carpal tunnel release (CTR), namely classic (>4 cm), limited (2–4 cm), mini (1.0–2 cm), percutaneous (0.4–0.6 cm), and ultra-minimally invasive (≤ 1 mm) (17–22). Smaller incision size shows faster recovery time and lower pain (17, 19). Although endoscopic CTR has superiority compared with classic open CTR (OCTR), it has damage complications to neurovascular structures and tendons (19). Cause mini carpal tunnel release has good outcomes in clinical set-up, and we use mini-incision open surgery when conservative ways cannot alleviate CTS symptoms.

Nowadays, ultrasound-guided corticosteroid injection combined with needle release of the TCL is a new minimally invasive method to treat CTS. Under ultrasound guidance, it is accurate to identify anomalous anatomy, thus avoiding damaging the surrounding tissues of the median nerve. We can observe the median nerve cross-sectional area reduction to demonstrate the effectiveness of corticosteroid injection into the carpal tunnel (23). The purpose of our study was to evaluate the efficacy of surgery and ultrasound-guided corticosteroid injection combined with needle release.

Materials and methods

Patients

Our study was approved by the ethical and scientific review board of our hospital. Written informed consent was obtained from every patient. Between January 2021 and December 2021, we included 40 patients (5 men and 35 women) who were diagnosed with CTS in this retrospective study. CTS diagnosis standard was made by clinical history, physical examination, ultrasound, and electrophysiological evaluation. Clinical history included tests of sensibility and muscle strength, questioning symptoms of sensory

and thenar atrophy examinations. We checked Phalen's test and Tinel's sign on percussion of the wrist. The criteria of CTS in electrophysiological were median distal motor nerve latency of >4.2 ms (stimulation, 2 cm proximal to the wrist crease) or median sensory nerve conduction velocity of <45 m/s (between the 2 cm proximal to the wrist crease and middle crease of the long finger).

The exclusion criteria were poor general physical condition, pregnancy, carpal fractures, contraindicate for corticosteroid drugs, previous wrist surgery, and previous treatment of CTS. A total of 20 patients (Group A) underwent ultrasound-guided corticosteroid injection combined with needle release, and the other 20 patients (Group B) had mini-open surgery. All patients finished the Boston carpal tunnel questionnaire, ultrasound, and electrophysiological examination before and 3 months after treatment.

Ultrasound-guided treatment procedures

Ultrasound-guided treatment as an outpatient procedure was routinely performed in the ultrasound interventional room. Ultrasound examinations were performed by a senior ultrasound doctor using the Mindray Resona7 with a 5–12 MHz transducer (Mindray, Shenzhen, China). We set the median nerve in the middle of the screen, and a 5-ml disposable sterile syringe (with a needle) was used for the administration of local anesthesia and release of TCL.

The patient was asked to sit on a chair, and the affected hand was positioned to the side with the palm up in an extended position. All treatment procedures were performed by C.C.X with the probe covered with surgical gloves. An acoustic coupling agent was used on the probe inside the surgical gloves. The patient's skin was disinfected with complex iodine three times from the metacarpophalangeal joint to 5 cm above the carpal canal. The probe was placed on the wrist with the longitudinal section, and then, the long axis of the median nerve was clearly seen. The insertion point is 0.5 cm proximal to the compression point of the median nerve. The direction of the needle entry is from the proximal to the distal end of the median nerve. When inserting the needle, care was taken to avoid stabbing the median nerve, radial artery, and ulnar neurovascular bundle. The needle entry point was wiped with complex iodine cotton balls under ultrasound guidance, and a 4-ml mixture solution which contained 2 ml 2% lidocaine and 2 ml 0.9% sodium chloride (in a ratio of 1:1) was injected. Thereafter, local anesthesia was performed layer by layer on the median nerve surface under the continuous guidance of ultrasound, and acupuncture compression of the TCL at the median nerve was continuously performed from proximal to distal and from shallow to deep. The operation was completed until there was no resistance to the acupuncture of TCL. Under real-time ultrasound guidance, a 2-ml mixture solution containing 1 ml of 2% lidocaine and 1 ml of betamethasone (Schering Pharmaceutical Co., Ltd. in Shanghai, China) (in a ratio of 1:1) was injected, and the mixture solution could be seen diffusing in the carpal tunnel (Figure 1). After the needle was pulled out, the needle path was pressed with hand for 5 min and then pasted with an adhesive bandage at the insertion point. To prevent infection, the wound should be kept dry for 48 h. The whole process



FIGURE 1

Ultrasound-guided needle release plus corticosteroid injection procedure of a Group A patient. **(a)** The transverse carpal ligament (TCL) was compressed (white arrow) in the ultrasound image before treatment. **(b)** Acupuncture process **(c)** 2 ml mixture solution (white ellipse) which contained 1 ml of 2% lidocaine and 1 ml of betamethasone were injected into the carpal tunnel. **(d)** TCL was normal (no compression) after 3-month follow-up in the ultrasound image. **(e)** Sonographer held the probe covered with surgical gloves with one hand and acupuncture compression of the TCL at the median nerve continuously under the continuous guidance of ultrasound. **(f)** Postoperative image (no scar).

lasted for approximately 5–10 min. During the operation, all patients had no pain or discomfort. There were no postoperative complications, and the patient was reexamined 3 months after the operation.

Mini-open surgery procedures

The patient was placed in a supine position under general anesthesia. A pneumatic tourniquet was set at 40 kpa pressure, with a total time of 1 h and an interval of 15 min. The size of the incision was ~2 cm, and its shape was longitudinal. The skin and subcutaneous tissues were cut open in turn to expose the carpal canal; the thickened and adherent transverse carpal ligament was cut open; the epineurium and bundle membrane of the nerve were loosened; the nerve bundle decompression was performed; and the wound was

rinsed after complete hemostasis (Figure 2). After checking nerve release, it was reclosed layer by layer until it reached the skin.

Boston carpal tunnel questionnaire, and ultrasonic and electrophysiological evaluations before and after treatment

The Boston carpal tunnel questionnaire is the most widely used to assess symptom severity and functional status. It includes two parts: the Symptom Severity Scale (SSS) and the Functional Status Scale (FSS) (6). SSS has 11 questions, and FSS contains 8 items. According to the mean score, patients are divided into five grades: minimal (0.1–1 point), mild (1.1–2 points), moderate (2.1–3 points), severe (3.1–4 points), and extreme (4.1–5 points).

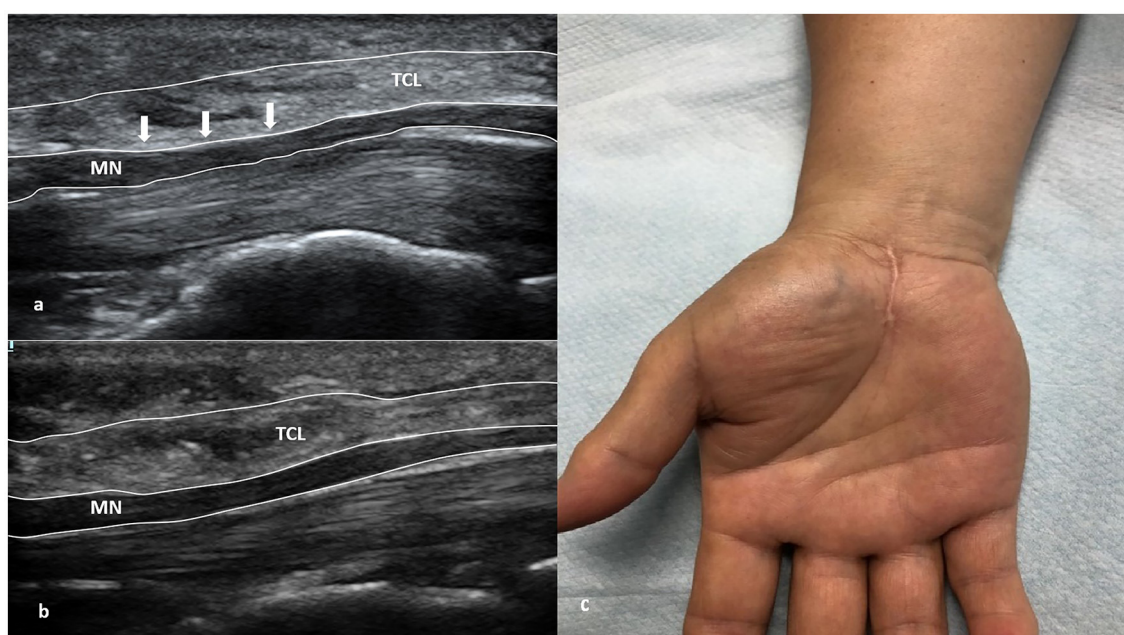


FIGURE 2

Mini-open surgery of a Group B patient. (a) The transverse carpal ligament (TCL) was compressed (white arrow) in the ultrasound image before treatment. (b) TCL was normal (no compression) after 3-month follow-up in the ultrasound image. (c) Postoperative image (with a 2-cm scar).

In ultrasound examinations, the median nerve's flattening ratio (FR) and cross-sectional area (CSA) were measured according to El Miedany and colleagues' grading system (24). Depending on the CSA of the median nerve at the inlet, severity is divided into follows: mild, 10–13 mm²; moderate, >13–15 mm²; and severe, >15 mm² (25). FR was the ratio of the nerve's transverse axis to the anteroposterior axis. It was assessed at the level of the pisiform bone. The transverse carpal ligament (TCL) was measured on the cross-section at the level of the hamate bone.

In electrophysiological examinations, we recorded the median nerve's distal motor latency (DML), sensory conduction velocity (SCV), and sensory nerve action potential (SNAP). According to recommendations of the American Association of Neuromuscular and Electrodiagnostic Medicine (26) and the American Academy of Orthopedic Surgeon (AAOS) work group (27), CTS severity was classified as follows: negative when all tests had normal findings (both comparative and segmental studies); minimal when abnormal on comparative or segmental tests; mild when normal DML with finger-wrist tract SNCV slowed; moderate when increased DML with finger-wrist tract SNCV slowed; severe when increased DML with a finger-wrist tract absence of the sensory response; and extreme when there is a thenar motor absence of the response.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). All quantitative data were expressed as mean $\pm$ standard deviations (SD). All qualitative data were expressed as numbers and percentages. We used Student's *t*-test to

compare quantitative data between groups and Mann–Whitney U-test to compare qualitative data. A *p*-value of < 0.05 was considered to be statistically significant.

Results

In our study, we included 40 eligible patients (40 wrists) with CTS. There were no significant differences between Group A and Group B with respect to average age, gender, side, and duration of disease (each with *P* > 0.05) (Table 1). For the treatment duration, total treatment cost, and healing time, Group A had significance compared with Group B (each with *P* < 0.05). Group A had a shorter treatment duration, costed less, and had a quicker healing time than Group B. There were no complications such as infection, hemorrhage, vascular, nerve, or tendon injuries in both groups. Patients had no recurrent symptoms after treatment in 3 months.

Table 2 shows Boston carpal tunnel questionnaire results before and 3 months after the treatment of the two groups. It showed a significant difference for both groups with respect to the Symptom Severity Scale (SSS) and the Functional Status Scale (FSS) before and 3 months after treatment (each with *P* < 0.05). There was no significance regarding SSS and FSS between the two groups 3 months after treatment (*P* = 0.73).

The results of electrophysiological examinations for two groups before and 3 months after treatment are shown in Table 3. There was no significant difference with respect to DML, SCV, and SNAP before treatment between the two groups (each with *P* > 0.05). Both the groups showed significance in their DML, SCV, and SNAP 3 months after treatment which suggested that both ultrasound-guided needle release plus corticosteroid injection and mini-open

TABLE 1 Clinical data of two groups.

	Group A	Group B	<i>P</i> -value
	(<i>n</i> = 20 wrists)	(<i>n</i> = 20 wrists)	
Number of patients	20	20	NA
Gender			0.633
Male	3	2	
Female	17	18	
Side			0.507
Right	12	14	
Left	8	6	
Age (years)	54.05 ± 9.40	53.70 ± 8.22	0.927
Duration of CTS (months)	19.05 ± 15.74	10.50 ± 7.98	0.526
Duration of treatment (minutes)	5.75 ± 1.37	19.00 ± 1.30	0.000*
Total treatment cost (dollars)	54.30 ± 3.38	644.55 ± 58.49	0.021*
Healing time (months)	1.16 ± 0.14	2.44 ± 0.24	0.000*

**P*-value < 0.05.

TABLE 2 Boston carpal tunnel questionnaire before and 3 months after treatment for two groups.

	Group A			Group B			After treatment Group A vs. Group B
	(n = 20 wrists)			(n = 20 wrists)			
	Before	After	P-value	Before	After	P-value	P-value
Symptom severity scale (SSS)							
Normal	0 (0)	9 (45)	0.00*	0 (0)	7 (35)	0.00*	0.73
Minimal	0 (0)	10 (50)		0 (0)	11 (55)		
Mild	1 (5)	1 (5)		2 (10)	2 (10)		
Moderate	6 (30)	0 (0)		8 (40)	0 (0)		
Severe	10 (50)	0 (0)		8 (40)	0 (0)		
Extreme	3 (15)	0 (0)		2 (10)	0 (0)		
Functional status scale (FSS)							
Normal	0 (0)	9 (45)	0.00*	0 (0)	7 (35)	0.00*	0.73
Minimal	0 (0)	10 (50)		0 (0)	11 (55)		
Mild	1 (5)	1 (5)		2 (10)	2 (10)		
Moderate	6 (30)	0 (0)		8 (40)	0 (0)		
Severe	10 (50)	0 (0)		8 (40)	0 (0)		
Extreme	3 (15)	0 (0)		2 (10)	0 (0)		

**P*-value < 0.05.

TABLE 3 Electrophysiologic results before and 3 months after treatment for two groups.

	Group A	Group B
	(<i>n</i> = 20 wrists)	(<i>n</i> = 20 wrists)
Median nerve	Before	After
Distal motor latency (DML, ms)	4.81 ± 1.85	3.50 ± 1.83 ⁺ #
Sensory conduction velocity (SCV, m/s)	40.65 ± 5.75	51.50 ± 4.51 ⁺ #
Sensory nerve action potential (SNAP, μV)	7.24 ± 3.30	17.42 ± 7.31 ⁺

⁺ vs. Before treatment, *P*-value < 0.05.# vs. Group B after treatment, *P*-value < 0.05.

TABLE 4 Ultrasound results before and 3 months after treatment for two groups.

Median nerve	Group A		Group B	
	(n = 20 wrists)		(n = 20 wrists)	
	Before	After	Before	After
Flattening ratio at the level of hamate (FR)	2.89 ± 0.36	2.31 ± 0.20 [§]	2.88 ± 0.39	2.34 ± 0.18 [§]
Cross-sectional area at the level of pisiform bone (CSA, cm ²)	0.15 ± 0.02	0.09 ± 0.01 [§]	0.14 ± 0.03	0.09 ± 0.02 [§]
Transverse carpal ligament-Thickness on the cross-section at the level of hamate bone (TCL, mm)	4.30 ± 0.34	3.45 ± 0.30 [§]	4.10 ± 0.43	3.48 ± 0.41 [§]

[§]vs. Before, *P*-value < 0.05.

surgery had effectiveness in treating CTS (each with *P* < 0.05). Furthermore, in terms of DML and SCV 3 months after treatment, there was a significant difference between the two groups (each with *P* < 0.05). It suggested that ultrasound-guided needle release plus corticosteroid injection was superior to mini-open surgery in the matter of DML and SCV.

Ultrasound examination results of both groups before and after treatment are shown in Table 4. There was no significant difference with respect to FR, CSA, and TCL before treatment between the two groups (each with *P* > 0.05). There was a significant difference before and 3 months after treatment in the values of FR, CSA, and TCL for both groups (each with *P* < 0.05), which indicated the effectiveness of the two therapeutic approaches. In addition, we found that there was no significant difference between the two groups in the values of FR, CSA, and TCL 3 months after treatment. We performed approximately 1-year follow-up after intervention for our patients. We followed all patients by phone, and the results were that all patients did not develop CTS again.

Discussion

CTS is usually diagnosed by illness history and physical examination (28). Electrophysiological examination is often used to confirm it (29). Ultrasound as a new way to diagnose CTS has now been widely used in clinics because it is easy and non-invasive (30). The principle of treating CTS is the decompression of carpal tunnel by opening the median nerve's flattening ratio. This is usually performed by using conventional open surgery (incision size of ~5 cm), mini-open surgery (incision size of ~2 cm), or endoscopy (incision size of ~1.5 cm) (11). Jugovac et al. found that compared with conventional open surgery, mini-open surgery had smaller incisions and returned to work in a shorter time (i.e., 15 days vs. 30 days) (31). In the 1990's, endoscopy came into effect. It is a safe technique that reduces morbidity, postoperative pain, and recovery time (11). However, endoscopy has its own shortcomings with a potential risk of injury to adjacent neurovascular structures (32). Nowadays, a new procedure that uses a needle to release the nerve based on ultrasound as guidance came into use in clinics. Compared with open surgery and endoscopic treatments, ultrasound-guided needle release allows for a fast recovery and easy postoperative care due to almost no wound.

Our research found that CTS was more common in women; we retrospectively studied 40 CTS patients, and 35 of them were

female, which was consistent with the study of Tang et al. and Baysal et al. where they found that hand-intensive housewives had a higher incidence of CTS (33, 34). The higher incidence of CTS in women may be due to physiological differences, hormonal changes, and intense housework (35). The mean ages of Group A and B patients were 54.05 ± 9.40 and 53.70 ± 8.22, respectively. CTS incidence peaks for individuals were those people in the age bracket of 40 to 60 years (3).

In our study, we compared the clinical outcomes of ultrasound-guided needle release plus corticosteroid injection and mini-open surgery and found that both groups had significant improvement with respect to BCTQ, electrophysiological, and ultrasound results after treatment. Both groups had no recurrent symptoms or pain syndrome 3 months after treatment. These findings indicated that ultrasound-guided needle release plus corticosteroid injection and mini-open surgery had the same effect in treatment. However, in terms of cost, operation time, recovery time, and recurrence, ultrasound-guided needle release plus corticosteroid injection is better than mini-open surgery. Furthermore, ultrasound-guided needle release plus corticosteroid injection has almost no scar and was deemed to be preferable in terms of cosmesis.

In Group A patients, no complications were reported during or after treatment due to the proper visualization of the carpal tunnel structure under ultrasound guidance. Recent studies demonstrated that corticosteroid injection under ultrasound guidance is better than blind injection (17, 31, 36). The advantage of ultrasound guidance can be clearly listed as follows: (1) The structure can be seen so that the doctor knows the location and reaches the carpal tunnel and injects the medicine without damaging the neighbor tissue; (2) offers a great degree of needle control: the doctor can change the distance of the needle and the median nerve in visualization so that the needle can reach close to the median nerve; and (3) ensures uniform distribution of injection solution around the carpal tunnel and peels the median nerve away from adhesions (37, 38). Studies had shown that the injection of corticosteroids was widely used in the field of pain management because it can reduce pain and swelling. In CTS patients, injecting corticosteroids into the flexor pollicis longus tendon sheath and common flexor sheath can relieve pain and improve functional impairment (39, 40).

During ultrasound-guided needle release plus corticosteroid injection, the following points should be noted: (1) The puncture was carried out along the long axis of the median nerve. During the process of releasing the transverse carpal ligament by acupuncture, attention should be paid to protecting the median nerve deep in the transverse carpal ligament. (2) Since the puncture space of the

carpal tunnel is relatively small, appropriate wrist extension can increase the puncture space. The puncture angle should not be too large in the process of puncture, 15°–30° with the skin of the wrist is suitable. (3) Due to the density of the transverse carpal ligament, when the lesion occurs, it will be more tenacious. There will be a sense of resistance during acupuncture, and when the sense of resistance disappears, it indicates that the release is thorough. (4) The puncture should be carried out in the plane so that the needle tip can be observed at all times to avoid injury to the median nerve. (5) Finally, the mixture of lidocaine and betamethasone should not be injected into the transverse carpal ligament during the injection into the carpal canal. When the relationship between the tip and median nerve is not clear, a small amount of normal saline can be injected.

There are many new methods for detecting CTS disease. Shear-wave elastography (SWE) is a new method in the quantitative measurement of tissue stiffness, especially in assessing neuropathy. Several studies have reported the meaning of elastography in diagnosing CTS. Wu et al. investigated the value of both ultrasound and real-time shear wave elastography (SWE) in evaluating the median nerve with CTS and demonstrated that SWE was useful in assessing the median nerve both before and after the operation (41). Recently, some research studies indicated that in CTS patients, blood flow changes of the median nerve can help to confirm. Endo et al. pointed out that using superb microvascular imaging can help clarify CTS patients' blood flow changes (42). They concluded that the diagnostic utility of superb microvascular imaging was superior to conventional color Doppler ultrasound and equivalent to power Doppler ultrasound (42).

There are several limitations to our study. First, the sample size is small, and more data are needed for further study. Second, we only compared ultrasound-guided needle release plus corticosteroid injection with mini-open surgery, and it is still not clear how effective it is compared with endoscopic or other treatment procedures. Furthermore, we only selected 3-month follow-up because some patients returned to work after treatment, and many factors may affect the outcome of follow-up. The long-term effects are still worth watching.

Conclusion

Both ultrasound-guided needle release plus corticosteroid injection and mini-open surgery had great benefits in the treatment of CTS. Ultrasound-guided needle release plus corticosteroid injection had the advantages of a shorter operation time, quicker recovery time, smaller wounds, and less cost compared with mini-open surgery. According to those advantages,

ultrasound-guided needle release plus corticosteroid injection is better for clinical application.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Zhejiang Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZZ, NL, and C-XC contributed to the study concepts and designed the study. C-XC contributed to the data analysis and interpreted the data. ZZ and NL contributed to the statistical analysis. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by the General Scientific Research Project of the Zhejiang Provincial Education Department, China (Grant No. Y202249231).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Alfonso C, Jann S, Massa R, Torreggiani A. Diagnosis, treatment, and follow-up of the carpal tunnel syndrome a review. *Neurol Sci.* (2010) 31:243–52. doi: 10.1007/s10072-009-0213-9
- Katz JN, Simmons BP. Clinical practice. Carpal tunnel syndrome. *N Engl J Med.* (2002) 346:1807–12. doi: 10.1056/NEJMcp013018

3. Ghasemi-Rad M, Nosair E, Vegh A, Mohammadi A, Akkad A, Leshia E, et al. A handy review of carpal tunnel syndrome from anatomy to diagnosis and treatment. *World J Radiol.* (2014) 6:284–300. doi: 10.4329/wjr.v6.i6.284
4. Yoshii Y, Zhao C, Amadio PC. Recent advances in ultrasound diagnosis of carpal tunnel syndrome. *Diagnostics.* (2020) 10:596. doi: 10.3390/diagnostics10080596
5. Genova A, Dix O, Saefan A, Thakur M, Hassan A. Carpal tunnel syndrome a review of literature. *Cureus.* (2020) 12:e7333. doi: 10.7759/cureus.7333
6. Levine DW, Simmons BP, Koris MJ, Daltroy LH, Hohl GG, Fossel AH, et al. Self-administered questionnaire for the assessment of severity of symptoms and functional status in carpal tunnel syndrome. *J Bone Joint Surg Am.* (1993) 75:1585–92. doi: 10.2106/00004623-199311000-00002
7. Padua L, Pazzaglia C, Caliendo P, Granata G, Foschini M, Briani C, et al. Carpal tunnel syndrome ultrasound, neurophysiology, clinical, and patient-oriented assessment. *Clin Neurophysiol.* (2008) 119:2064–9. doi: 10.1016/j.clinph.2008.05.004
8. Rahmani M, Ghasemi Esfe AR, Vaziri-Bozorg SM, Mazloumi M, Khalilzadeh O, Kahnouji H. The ultrasonographic correlates of carpal tunnel syndrome in patients with normal electrodiagnostic tests. *Radiol Med.* (2011) 116:489–96. doi: 10.1007/s11547-011-0632-6
9. Granata G, Caliendo P, Pazzaglia C, Minciotti I, Russo G, Martinoli C, et al. Prevalence of bifid median nerve at wrist assessed through ultrasound. *Neurol Sci.* (2011) 32:615–8. doi: 10.1007/s10072-011-0582-8
10. Chen YT, Williams L, Zak MJ, Fredericson M. Review of ultrasonography in the diagnosis of carpal tunnel syndrome and a proposed scanning protocol. *J Ultrasound Med.* (2016) 35:2311–24. doi: 10.7863/ultra.15.12014
11. Petrover D, Richette P. Treatment of carpal tunnel syndrome from ultrasonography to ultrasound guided carpal tunnel release. *Joint Bone Spine.* (2018) 85:545–52. doi: 10.1016/j.jbspin.2017.11.003
12. Roomzadeh P, Eftekharsadat B, Abedini A, Ranjbar-Kiyakalayeh S, Yousefi N, Ebadi S, et al. Ultrasonographic assessment of carpal tunnel syndrome severity: a systematic review and meta-analysis. *Am J Phys Med Rehabil.* (2019) 98:373–81. doi: 10.1097/PHM.0000000000001104
13. Karahan AY, Arslan S, Ordahan B, Bakdik S, Ekiz T. Superb microvascular imaging of the median nerve in carpal tunnel syndrome: an electrodiagnostic and ultrasonographic study. *J Ultrasound Med.* (2018) 37:2855–61. doi: 10.1002/jum.14645
14. Wiperman J, Goel K. Carpal tunnel syndrome diagnosis and management. *Am Fam Physician.* (2016) 94:993–9.
15. Burton C, Chesterton LS, Davenport G. Diagnosing and managing carpal tunnel syndrome in primary care. *Br J Gen Pract.* (2014) 64:262–3. doi: 10.3399/bjgp14X679903
16. Crnković T, Trkulja V, Bilić R, Gašpar D, Kolundžić R. Carpal tunnel and median nerve volume changes after tunnel release in patients with the carpal tunnel syndrome: a magnetic resonance imaging (MRI) study. *Int Orthop.* (2016) 40:981–7. doi: 10.1007/s00264-015-3052-8
17. Nakamichi K, Tachibana S, Yamamoto S, Ida M. Percutaneous carpal tunnel release compared with mini-open release using ultrasonographic guidance for both techniques. *J Hand Surg Am.* (2010) 35:437–45. doi: 10.1016/j.jhsa.2009.12.016
18. Klein RD, Kotsis SV, Chung KC. Open carpal tunnel release using a 1-centimeter incision technique and outcomes for 104 patients. *Plast Reconstr Surg.* (2003) 111:1616–22. doi: 10.1097/01.PRS.0000057970.87632.7e
19. Nakamichi K, Tachibana S. Ultrasonographically assisted carpal tunnel release. *J Hand Surg Am.* (1997) 22:853–62. doi: 10.1016/S0363-5023(97)80081-0
20. Lecoq B, Hanouz N, Vielpeau C, Marcelli C. Ultrasound-guided percutaneous surgery for carpal tunnel syndrome: a cadaver study. *Joint Bone Spine.* (2011) 78:516–8. doi: 10.1016/j.jbspin.2010.12.006
21. Rowe NM, Michaels J, 5th, Soltanian H, Dobryansky M, Peimer CA, Gurtner GC. Sonographically guided percutaneous carpal tunnel release: an anatomic and cadaveric study. *Ann Plast Surg.* (2005) 3:55–6. doi: 10.1097/01.sap.0000168281.77528.02
22. Rojo-Manaute JM, Capa-Grasa A, Rodríguez-Maruri GE, Moran LM, Martínez MV, Martín JV. Ultra-minimally invasive sonographically guided carpal tunnel release: anatomic study of a new technique. *J Ultrasound Med.* (2013) 32:131–42. doi: 10.7863/jum.2013.32.1.131
23. Cartwright MS, White DL, Demar S, Wiesler ER, Sarlikiotis T, Chloros GD, et al. Median nerve changes following steroid injection for carpal tunnel syndrome. *Muscle Nerve.* (2011) 44:25–9. doi: 10.1002/mus.22067
24. El Miedany YM, Aty SA, Ashour S. Ultrasonography versus nerve conduction study in patients with carpal tunnel syndrome: substantive or complementary tests? *Rheumatology.* (2004) 43:887–95. doi: 10.1093/rheumatology/keh190
25. Giannini F, Cioni R, Mondelli M, Padua R, Gregori B, D'Amico P, et al. New clinical scale of carpal tunnel syndrome: validation of the measurement and clinical-neurophysiological assessment. *Clin Neurophysiol.* (2002) 113:71–7. doi: 10.1016/S1388-2457(01)00704-0
26. England JD, Gronseth GS, Franklin G, Carter GT, Kinsella LJ, Cohen JA, et al. American academy of neurology; American association of neuromuscular and electrodiagnostic medicine; American academy of physical medicine and rehabilitation. Practice parameter: the evaluation of distal symmetric polyneuropathy: the role of laboratory and genetic testing (an evidence-based review) report of the American academy of neurology, the American association of neuromuscular and electrodiagnostic medicine, and the American academy of physical medicine and rehabilitation. *PM R.* (2009) 1:5–13. doi: 10.1016/j.pmrj.2008.11.010
27. Keith MW, Masear V, Chung K, Maupin K, Andary M, Amadio PC, et al. Diagnosis of carpal tunnel syndrome. *J Am Acad Orthop Surg.* (2009) 17:389–96. doi: 10.5435/00124635-200906000-00007
28. Jablęcki CK, Andary MT, Floeter MK, Miller RG, Quartly CA, Vennix MJ, et al. American association of electrodiagnostic medicine; American Academy of neurology; American academy of physical medicine and rehabilitation. Practice parameter: electrodiagnostic studies in carpal tunnel syndrome: report of the American association of electrodiagnostic medicine, American academy of neurology, and the American academy of physical medicine and rehabilitation. *Neurology.* (2002) 58:1589–92. doi: 10.1212/WNL.58.11.1589
29. Kanikannan MA, Boddu DB, Umamahesh, Sarva S, Durga P, Borgohain R. Comparison of high-resolution sonography and electrophysiology in the diagnosis of carpal tunnel syndrome. *Ann Indian Acad Neurol.* (2015) 18:219–25. doi: 10.4103/0972-2327.150590
30. Alemán L, Berná JD, Reus M, Martínez F, Doménech-Ratto G, Campos M. Reproducibility of sonographic measurements of the median nerve. *J Ultrasound Med.* (2008) 27:193–7. doi: 10.7863/jum.2008.27.2.193
31. Jugovac I, Burgić N, Mićović V, Radolović-Prenc L, Uračić M, Golubović V, et al. Carpal tunnel release by limited palmar incision vs traditional open technique: randomized controlled trial. *Croat Med J.* (2002) 43:33–6.
32. Sayegh ET, Strauch RJ. Open versus endoscopic carpal tunnel release: a meta-analysis of randomized controlled trials. *Clin Orthop Relat Res.* (2015) 473:1120–32. doi: 10.1007/s11999-014-3835-z
33. Tang X, Zhuang L, Lu Z. Carpal tunnel syndrome: a retrospective analysis of 262 cases and a one to one matched case-control study of 61 women pairs in relationship between manual housework and carpal tunnel syndrome. *Chin Med J.* (1999) 112:44–8.
34. Baysal O, Altay Z, Özcan C, Ertem K, Yologlu S, Kayhan A. Comparison of three conservative treatment protocols in carpal tunnel syndrome. *Int J Clin Pract.* (2006) 60:820–8. doi: 10.1111/j.1742-1241.2006.00867.x
35. Wilgis EF. Treatment options for carpal tunnel syndrome. *JAMA.* (2002) 288:1281–2. doi: 10.1001/jama.288.10.1281
36. Smith J, Barnes DE, Barnes KJ, Strakowski JA, Lachman N, Kakar S, et al. Sonographic visualization of the motor branch of the median nerve: a cadaveric validation study. *PM R.* (2017) 9:159–69. doi: 10.1016/j.pmrj.2016.05.008
37. Lee JC, Healy JC. Normal sonographic anatomy of the wrist and hand. *Radiographics.* (2005) 25:1577–90. doi: 10.1148/rg.256055028
38. Ustün N, Tok F, Yagz AE, Kizil N, Korkmaz I, Karazincir S, et al. Ultrasound-guided vs. blind steroid injections in carpal tunnel syndrome: A single-blind randomized prospective study. *Am J Phys Med Rehabil.* (2013) 92:999–1004. doi: 10.1097/PHM.0b013e31829b4d72
39. Ly-Pen D, Andréu JL, Millán I, de Blas G, Sánchez-Olaso A. Comparison of surgical decompression and local steroid injection in the treatment of carpal tunnel syndrome: 2-year clinical results from a randomized trial. *Rheumatology.* (2012) 51:1447–54. doi: 10.1093/rheumatology/kes053
40. Huisstede BM, Fridén J, Coert JH, Hooglyet P, European HANDGUIDE. Group. Carpal tunnel syndrome: hand surgeons, hand therapists, and physical medicine and rehabilitation physicians agree on a multidisciplinary treatment guideline—results from the European HANDGUIDE study. *Arch Phys Med Rehabil.* (2014) 95:2253–63. doi: 10.1016/j.apmr.2014.06.022
41. Wu H, Zhao HJ, Xue WL, Wang YC, Zhang WY, Wang XL. Ultrasound and elastography role in pre- and post-operative evaluation of median neuropathy in patients with carpal tunnel syndrome. *Front Neurol.* (2022) 13:1079737. doi: 10.3389/fneur.2022.1079737
42. Endo T, Matsui Y, Kawamura D, Urita A, Momma D, Ota M, et al. Diagnostic utility of superb microvascular imaging and power doppler ultrasonography for visualizing enriched microvascular flow in patients with carpal tunnel syndrome. *Front Neurol.* (2022) 13:832569. doi: 10.3389/fneur.2022.832569



OPEN ACCESS

EDITED BY

Lei Xu,
Fudan University, China

REVIEWED BY

Benjamin Gantenbein,
University of Bern, Switzerland
Ivan A. Stepanov,
Irkutsk State Medical University, Russia

*CORRESPONDENCE

Yue Wang
✉ wangyuespine@zju.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 30 October 2022

ACCEPTED 03 March 2023

PUBLISHED 03 April 2023

CITATION

Chen L, Lu X, Jin Q, Gao Z and Wang Y (2023)
Sensory innervation of the lumbar 5/6
intervertebral disk in mice.
Front. Neurol. 14:1084209.
doi: 10.3389/fneur.2023.1084209

COPYRIGHT

© 2023 Chen, Lu, Jin, Gao and Wang. This is an
open-access article distributed under the terms
of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/)
(CC BY). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted which
does not comply with these terms.

Sensory innervation of the lumbar 5/6 intervertebral disk in mice

Lunhao Chen^{1†}, Xuan Lu^{1†}, Qianjun Jin¹, Zhihua Gao^{2,3,4} and
Yue Wang^{1*}

¹Spine Lab, Department of Orthopedic Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, ²Department of Neurobiology and Department of Neurology of Second Affiliated Hospital, NHC and CAMS Key Laboratory of Medical Neurobiology, Zhejiang University School of Medicine, Hangzhou, China, ³The MOE Frontier Research Center of Brain and Brain-Machine Integration, Zhejiang University School of Brain Science and Brain Medicine, Hangzhou, China, ⁴Liangzhu Laboratory, Zhejiang University Medical Center, Hangzhou, China

Introduction: Over the years, most back pain-related biological studies focused on the pathogenesis of disk degeneration. It is known that nerve distributions at the outer layer of the annulus fibrosus (AF) may be an important contributor to back pain symptoms. However, the types and origins of sensory nerve terminals in the mouse lumbar disks have not been widely studied. Using disk microinjection and nerve retrograde tracing methods, the current study aimed to characterize the nerve types and neuropathway of the lumbar 5/6 (L5/6) disk in mice.

Methods: Using an anterior peritoneal approach, the L5/6 disk of adult C57BL/6 mice (males, 8–12 weeks) disk microinjection was performed. Fluorogold (FG) was injected into the L5/6 disk using the Hamilton syringe with a homemade glass needle driven by a pressure microinjector. The lumbar spine and bilateral thoracic 13 (Th13) to L6 DRGs were harvested at 10 days after injection. The number of FG⁺ neurons among different levels was counted and analyzed. Different nerve markers, including anti-neurofilament 160/200 (NF160/200), anti-calcitonin gene-related peptide (CGRP), anti-parvalbumin (PV), and anti-tyrosine hydroxylase (TH), were used to identify different types of nerve terminals in AF and their origins in DRG neurons.

Results: There were at least three types of nerve terminals at the outer layer of L5/6 AF in mice, including NF160/200⁺ (indicating A β fibers), CGRP⁺ (A δ and C fibers), and PV⁺ (proprioceptive fibers). No TH⁺ fibers (sympathetic nerve fibers and some C-low threshold mechanoreceptors) were noticed in either. Using retrograde tracing methods, we found that nerve terminals in the L5/6 disk were multi-segmentally from Th13–L6 DRGs, with L1 and L5 predominately. An immunofluorescence analysis revealed that FG⁺ neurons in DRGs were co-localized with NF160/200, CGRP, and PV, but not TH.

Conclusion: Intervertebral disks were innervated by multiple types of nerve fibers in mice, including A β , A δ , C, and proprioceptive fibers. No sympathetic nerve fibers were found in AF. The nerve network of the L5/6 disk in mice was multi-segmentally innervated by the Th13–L6 DRGs (mainly L1 and L5 DRGs). Our results may serve as a reference for preclinical studies of discogenic pain in mice.

KEYWORDS

lumbar intervertebral disk, dorsal root ganglia (DRG), neuron, nerve terminal, retrograde tracing, neural pathways

Introduction

Back pain is a common disorder in the lumbar spines of adults, affecting over 50% of people during their lifetimes (1). Although pathologies in a variety of anatomical structures, such as intervertebral disks, facet joints, myofascial tissues, and sacroiliac joints, may lead to back pain (2), intervertebral disk pathology is thought to be a significant contributor to back pain, termed discogenic pain (3). Identifying the innervation patterns of nerve terminals in the disk may serve as an important reference in studying discogenic pain.

The disk is referred to as the largest avascular tissue of the body, which comprises an outer ring of collagen-rich annulus fibrosus (AF) and proteoglycan-rich gelatinous nucleus pulposus (NP) in the center, providing both the mechanical support for compressive forces and the flexibility necessary for the movements (4). Previous studies indicated that the outer one-third layers of the normal AF are innervated by different types of sensory nerve terminals, including both myelinated and unmyelinated fibers (5, 6). Using immunohistochemical staining and structure analysis, peptidergic nerve fibers, containing calcitonin gene-related peptide (CGRP) or substance P (SP), and mechanoreceptors, containing neurofilament heavy polypeptide (NEFH), were found in disks and suggested to sense nociceptive and mechanical stimuli (7, 8). After disk injury and degeneration, those nerve terminals can sprout and extend deeper, even up to the inner two-thirds of AF and NP, which was considered as a possible pathomechanism of discogenic pain (9).

Moreover, during disk degeneration, AF and NP cells upregulate the expression of proinflammatory cytokines, including interleukins, tumor necrosis factors (TNFs), and metalloproteases (MMPs), which interact with corresponding receptors on nerve terminals to promote nerve ingrowth, and therefore facilitate ectopic action potentials and discogenic pain (10–12). Using the mouse model of disk degeneration, disk puncture induced progressive disk degeneration, along with the increased expression of colony-stimulating factor 1 (CSF1) in dorsal root ganglion (DRG) and activated microglia in the lumbar spinal dorsal horn (13), suggesting that neural plasticity was involved in back pain.

Anatomically, the intervertebral disk is assumed to receive afferent fibers principally from DRG neurons of the same segment. Yet, the lower lumbar intervertebral disks are multi-segmentally innervated by DRG neurons, whose nerve terminals

enter the paravertebral sympathetic trunks and ascend to upper-level DRGs (2, 14, 15). Local anesthetic blocks to sympathetic ganglia at the L2 level alleviated discogenic pain in patients (16, 17), and experimental studies in rats demonstrated a raised pain threshold after sympathectomy (18). However, due to the adjacent distance between the disk and corresponding DRG, proinflammatory cytokines are presumed to affect the adjacent DRG neurons, largely complicating the origin of discogenic pain.

Previous studies mainly used rats as experimental models to distinguish the distribution and innervation patterns of the disk (2, 5, 14, 19–22). However, the types and origins of sensory nerve terminals in the lower lumbar disks in mice remain elusive. Moreover, transgenic tools and genetic manipulations are limited in rats, conferring a disadvantage in uncovering the mechanistic clues of the disease. Using disk microinjection and nerve retrograde tracing methods, the present study was conducted to get a better understanding of the nerve types and neural pathways of the lower lumbar disk in mice.

Materials and methods

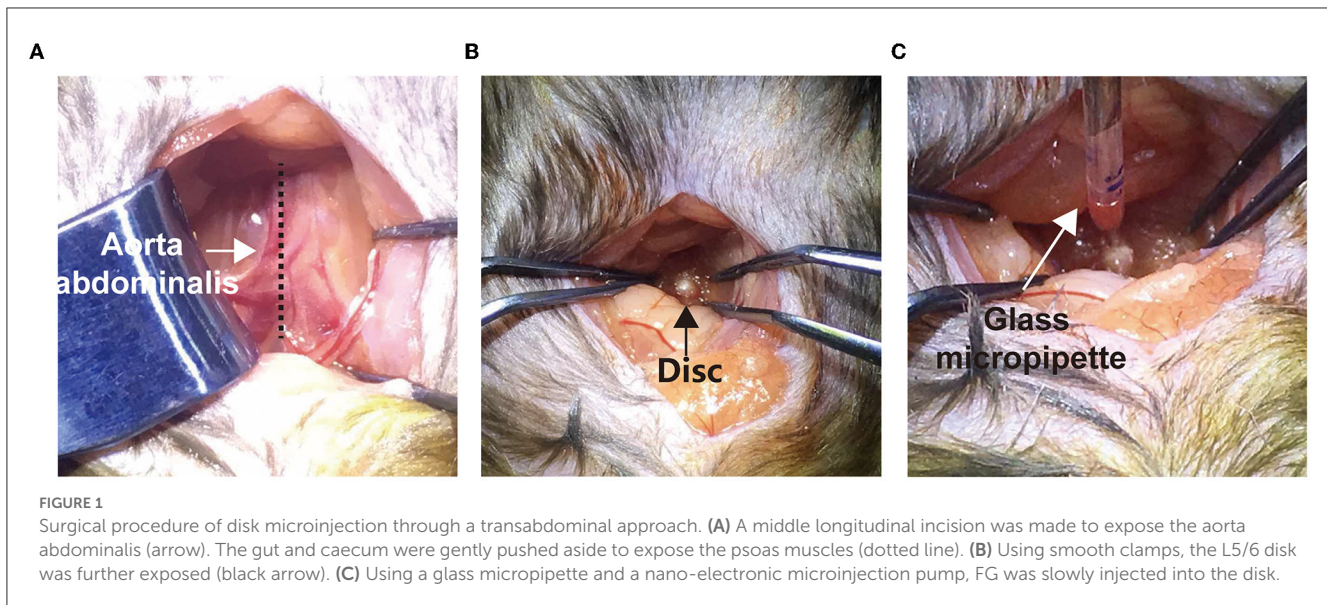
Animals

Adult C57BL/6J mice (males, 8–12 weeks) were used in the present study. All animals were housed in the Lab Animal Center at Zhejiang University, and the group was housed on a 12-h light/dark cycle with water and food available. The use and care of animals in all experiments followed the guidelines of The Tab of Animal Experimental Ethical Inspection of the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China (No. 2017054). For each experiment, at least three mice per group were used.

Disk injection

To minimize the disk injury, we connected the 10 μ L Hamilton syringe (Cat# 701N) with a glass needle (diameter $\approx$ 20 μ m) before disk injection. Before the experiments, the mice were fasted for 8 h prior to disk injection. After being anesthetized with sodium pentobarbital (100 mg/kg) intraperitoneally, the mice were placed and fixed in a supine position on a warming pad. Under the sterile surgical condition, a 1–1.5 cm midline longitudinal incision in the lower abdomen region was made in mice. Under a stereoscopic microscope (SZX7, Olympus), the gut, connective tissues, vessels, and psoas major muscles were gently retracted to expose the ventral aspect of the L5/6 and L6/sacral 1 (S1) intervertebral disk (Figures 1A, B). The weight/volume (w/v) ratio of 1% FG was infused using the homemade Hamilton syringe with a glass needle and driven by a pressure microinjector (KD Scientific) at a rate over at least 5 min (Figure 1C). The injection depth was 1 mm, and the total injection volume was 100 nL, which avoided breaking through the posterior annulus. To allow diffusion and reduce backflow, the needle remained in the disk for 5 min. The incision was then closed with 5–0 silk sutures.

Abbreviations: AF, Annulus fibrosus; CGRP, Calcitonin gene-related peptide; CSF1, Colony stimulating factor 1; DAPI, 4',6-diamidino-2-phenylindole; DRG, Dorsal root ganglion; EDTA, Ethylene diamine tetra-acetic acid; FG, Fluorogold; HCN, Hyperpolarization-activated cyclic nucleotide-gated channel; HE, Hematoxylin and Eosin; L, Lumbar; P2X3, Purinergic receptor P2X; PFA, Paraformaldehyde; PV, Parvalbumin; MMPs, Metalloproteases; MRGPR, D MAS-related GPR family member D; NF160/200, Neurofilament 160/200; NEFH, Neurofilament heavy polypeptide; NGF, Nerve growth factor; NP, Nucleus pulposus; PGP9.5, Protein gene product 9.5; S, Sacral; SEM, Means $\pm$ standard errors of the means; SP, Substance P; TH, Tyrosine hydroxylase; Th, Thoracic; TNF, Tumor necrosis factors; TRP, Transient receptor potential.



Disk and DRG dissection

Ten days after the surgery, mice were anesthetized and perfused with saline and subsequently 4% paraformaldehyde (PFA). The lumbar spine and DRGs were dissected and post-fixed in 4% PFA for at least 6 h. After decalcifying in 10% ethylene diamine tetra-acetic acid (EDTA) for 14 days and dehydrating in 30% sucrose for 2 days, the lumbar spine was sagittally sectioned with 10- μ m thickness. DRGs were sectioned in transverse with 15- μ m thickness.

Counting of FG+ neurons

All DRG sections from bilateral Th13-L6 levels were used in counting FG⁺ neurons. FG was visualized as granular particles within neurons. The FG-positive neurons were defined as the white-blue rounded cells under a fluorescence microscope (BX53, Olympus), whose fluorescence intensity was much higher than the background. To avoid double counting of FG-labeled neurons, the second one in the adjacent section was ignored if FG-labeled neurons appeared at the same location in two adjacent sections. The number of FG⁺ neurons was mounted by each DRG level.

Hematoxylin and Eosin (HE) staining

After rehydrated in alcohol, the sagittal lumbar spine sections were washed with distilled water. The sections were stained with hematoxylin for 1 min and differentiated with acid alcohol. After that, sections were stained with eosin for 1 min and dehydrated in ascending alcohol. Rinse the staining with xylene to make it transparent and mount. The figures were acquired using a microscope (BX53, Olympus).

Immunofluorescence and immunohistochemical analysis

To label different types of nerve terminals in disks, the midsagittal sections of the lumbar spine were stained using a specific rabbit (Cat# K401011) and mouse (Cat# 400611) IHC Kit (DAKO) according to the manufacturer's instruction. Shortly, sections were antigen-retrieved in citrate buffer (10 mM sodium citrate, 0.05% Tween-20, pH 6.0) at 95°C for 20 min and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. After being blocked with 10% (wt/vol) BSA, sections were incubated overnight at 4 °C with the following primary antibodies: mouse anti-NF160/200 (1:2000, Cat# n2912, Sigma-Aldrich), mouse anti-CGRP (1:1000, Cat# sc-57053, Santa Cruz Biotechnology), mouse anti-parvalbumin (PV, 1:1000, Cat# P3088, Sigma-Aldrich), rabbit anti-tyrosine hydroxylase (TH, 1:1000, Cat# AB152, Millipore), and rabbit anti-fluorogold (FG, 1:1000, Cat# 52-9600, Fluorochrome). To label disk nerve terminals, sections were washed in TBS with 0.5% tween (TBS-T) and incubated with HRP-conjugated secondary antibodies (1:1000) for 1 h at room temperature. Images were acquired using a microscope (BX53, Olympus). For DRG and spinal cord slides, sections were incubated with fluorophore-conjugated secondary antibodies for 2 h at room temperature. 4',6-diamidino-2-phenylindole (DAPI, C1005, Beyotime, China) was used to label cell nuclei. Images were acquired using a confocal microscope (FV1200, Olympus).

Statistical analysis

The number of FG⁺ neurons from Th13 to L6 DRGs was counted under a fluorescent microscope (BX53, Olympus, Japan). The percentage of DRG neurons per lumbar level was calculated as the number of DRG neurons in each lumbar level divided by the total number of FG⁺ neurons. Data are presented as means $\pm$ standard errors of the means (SEM).

TABLE 1 Number of FG⁺ neurons in DRGs.

Mouse	Th13	L1	L2	L3	L4	L5	L6	Total
1	6	19	8	3	2	4	0	42
2	2	5	2	2	3	4	3	21
3	6	22	5	7	8	9	2	59
4	12	9	4	10	9	30	13	87
5	0	8	1	1	0	3	4	17
Total	26	63	20	23	22	50	22	226

One-way ANOVA followed by Bonferroni's *post hoc* tests was used to compare the distribution of FG⁺ neurons among DRG levels. Significance was considered with a *p*-value of < 0.05.

Results

Segmental distribution of FG-labeled DRG neurons in mice

To label DRGs that innervate the L5/6 disk, the disk with FG injection and bilateral lumbar DRGs were acquired and observed under fluorescent microscopy. Compared with the disk that underwent sham surgery, HE staining demonstrated that the glass needle puncture did not induce disk degeneration 10 days after surgery (Figure 2A). As shown in Figure 2B, FG was visualized as white-blue colors. First, we recognized that FG was dispersed but limited in the L5/6 disk, without penetrating the vertebral bodies (Figure 2B). Although FG⁺ neurons were present from Th13 through L6 DRGs (Figure 2B, Table 1), FG⁺ neurons were most frequently observed in L1 DRGs (27.9% of all labeled neurons), followed by L5 DRGs (22.1%) (Figure 2C). Only 18.8% of FG⁺ neurons were identified in L5 DRG in mice. About 10% of FG⁺ neurons were found in Th13, L2, L3, L4, or L6 DRGs, respectively (Figure 2C). These data suggested that sensory fibers in the L5/6 disk in mice were segmentally innervated by Th13–L6 DRGs.

Types and distributions of nerve terminals in the intervertebral disks in mice

To identify types of DRG neurons whose nerve fibers project to the disk, we co-labeled FG with different neuronal markers, which are thought to cover major portions of sensory neurons (23). Consistent with previous studies, CGRP, the marker of small peptidergic neurons with A δ - or C-type fibers, was observed in the outer layer of the AF (Figure 3A). NF160/200, the marker of large peptidergic neurons with A β -type fibers, was also identified in the superficial region of AF (Figure 3B). Intriguingly, we found that PV⁺ fibers (Figure 3C), the marker of proprioceptors, were densely aligned within AF, demonstrating that intervertebral disks may sense proprioception, such as position and movement. However, we were unable to identify

TH⁺ fibers (Figure 3D), which indicated unmyelinated C-low threshold mechanoreceptors (C-LTMRs) and sympathetic nerve fibers, in the AF. Immunofluorescent staining of the DRG sections demonstrated that FG in DRGs was partially co-localized with NF160/200, CGRP, and PV, but not TH in mice (Figure 4). Together, our data demonstrated that disks were innervated with different types of sensory nerve fibers, which were susceptible to sensing mechanical and chemical stimuli in the internal environment.

Discussion

Although there have been several reports about the sensory innervation of lumbar disks in rats, our study describes the distribution patterns of sensory nerves in the mouse lumbar disk and their origins in DRG neurons. Consistent with rats, the sensory fibers of the L5/6 disk were shown to be derived from DRGs at Th13 through L6. Moreover, immunohistochemical staining demonstrated that there were diverse types of nerve terminals, including NF160/200, CGRP, and PV, in the outer layers of AF, suggesting that such neural structures of disks may be susceptible to sense stimuli by mechanical or chemical mediators after herniation and degeneration, and further contributed to discogenic pain.

There have been efforts trying to identify the distribution patterns of disk innervations in rats and humans. Anatomically, the lumbar disk is innervated by sinuvertebral nerves consisting of spinal sensory fibers from the adjacent DRG and postganglionic sympathetic fibers, which demonstrates that the lumbar disk is innervated by DRG at corresponding levels and upper DRGs (24). However, using retrograde tracing methods in rats, the ventral portion of the L5–L6 disk was innervated predominantly by L1 and L2 DRGs (14), whereas the dorsal portion of the L5/6 disk was innervated extensively by L1–L6 DRGs (2). To avoid FG leakage, we used the microinjection pump with a tiny tip to deliver FG. Consistent with previous studies, our findings support the multi-segmental innervation patterns of L5/6 disk in mice: the origin of nerves was thought to enter the paravertebral sympathetic trunks and ascended to L1 and L2 DRGs, whereas some fibers passed through the sinuvertebral nerves and reached L3–L6 DRGs (15). Indeed, the human L4/5 disk, which is related to the L5/6 disk in rodents, may be innervated predominantly by L1 DRG ventrally and by L2 DRG dorsally (25). As L1 and L2 DRGs innervate the inguinal region, it may explain why patients with lower lumbar intervertebral disk lesions are suffered from inguinal pain. Moreover, blocking L2 DRG alleviated some but not all of the low back pain, inguinal pain, and buttock pain in patients (17).

Nerve terminals in the outer layers of disks, which penetrated deeper after disk degeneration or herniation, provide a morphologic basis for discogenic pain (5). Disk injury and degeneration result in the upregulation of pain-related molecules, including CGRP and Substance P in DRGs, which are known to irritate the spinal nerve roots and probably also the nerve terminals to promote discogenic pain. Other than CGRP, the present study revealed that there were other types of nerve terminals in the outer layers of disks, including PV and NF160/200. Peripheral pain perception is thought to be mediated primarily by nociceptive C-fiber neurons and thinly myelinated A δ fibers (soma diameter

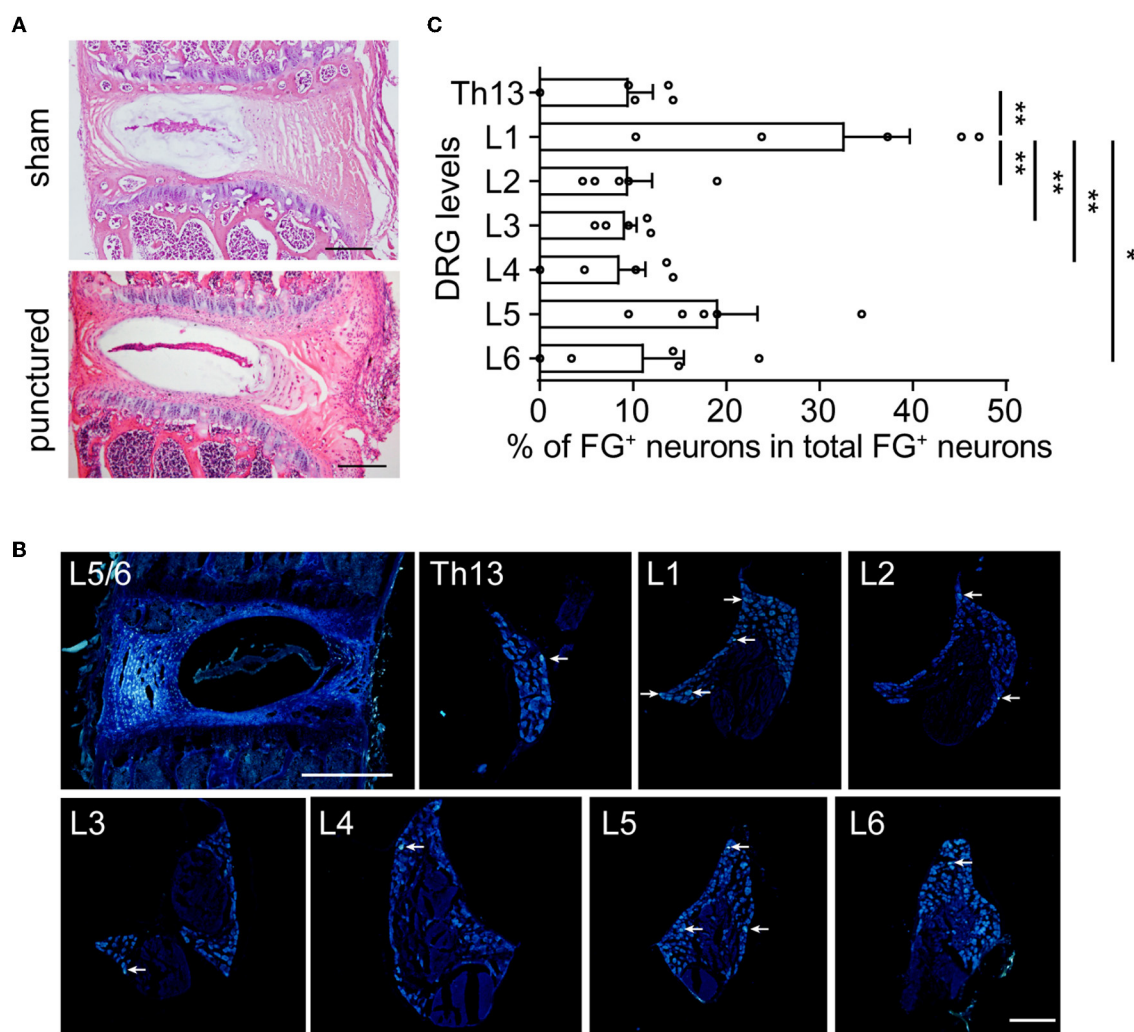


FIGURE 2
In mice, the L5/6 disk was multi-segmentally innervated by Th13–L6 DRGs (mainly L1 and L5 DRGs). **(A)** HE staining of L5/6 disks at 10 days after sham surgery (upper) or glass needle puncture (bottom). Scale bar: 200 μ m. **(B)** Representative images of the disk and DRG after FG injection. Arrows indicate FG+ DRG neurons. **(C)** The distribution of FG+ DRG neurons of different spinal levels ($n = 5$ mice). The percentage represents the number of FG+ neurons for each level of DRG to total FG+ neurons from Th13 to L6 ($n = 5$ mice). Scale bars: 200 μ m for DRG and 500 μ m for disk. Values are means $\pm$ SEM. * $p < 0.05$ and ** $p < 0.01$, one-way ANOVA followed by Bonferroni's *post-hoc* tests.

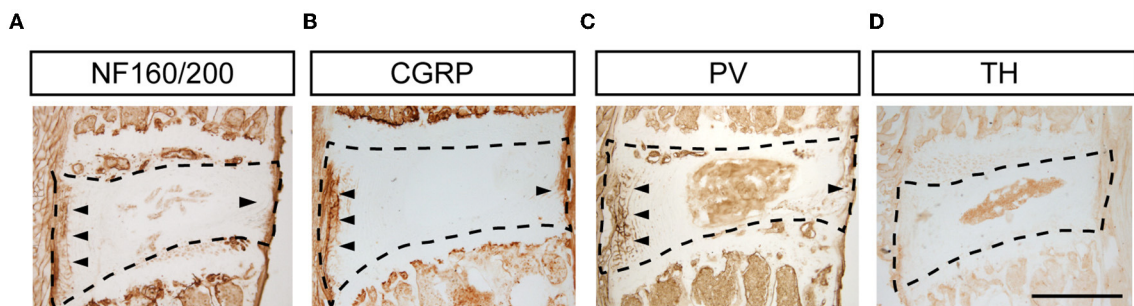
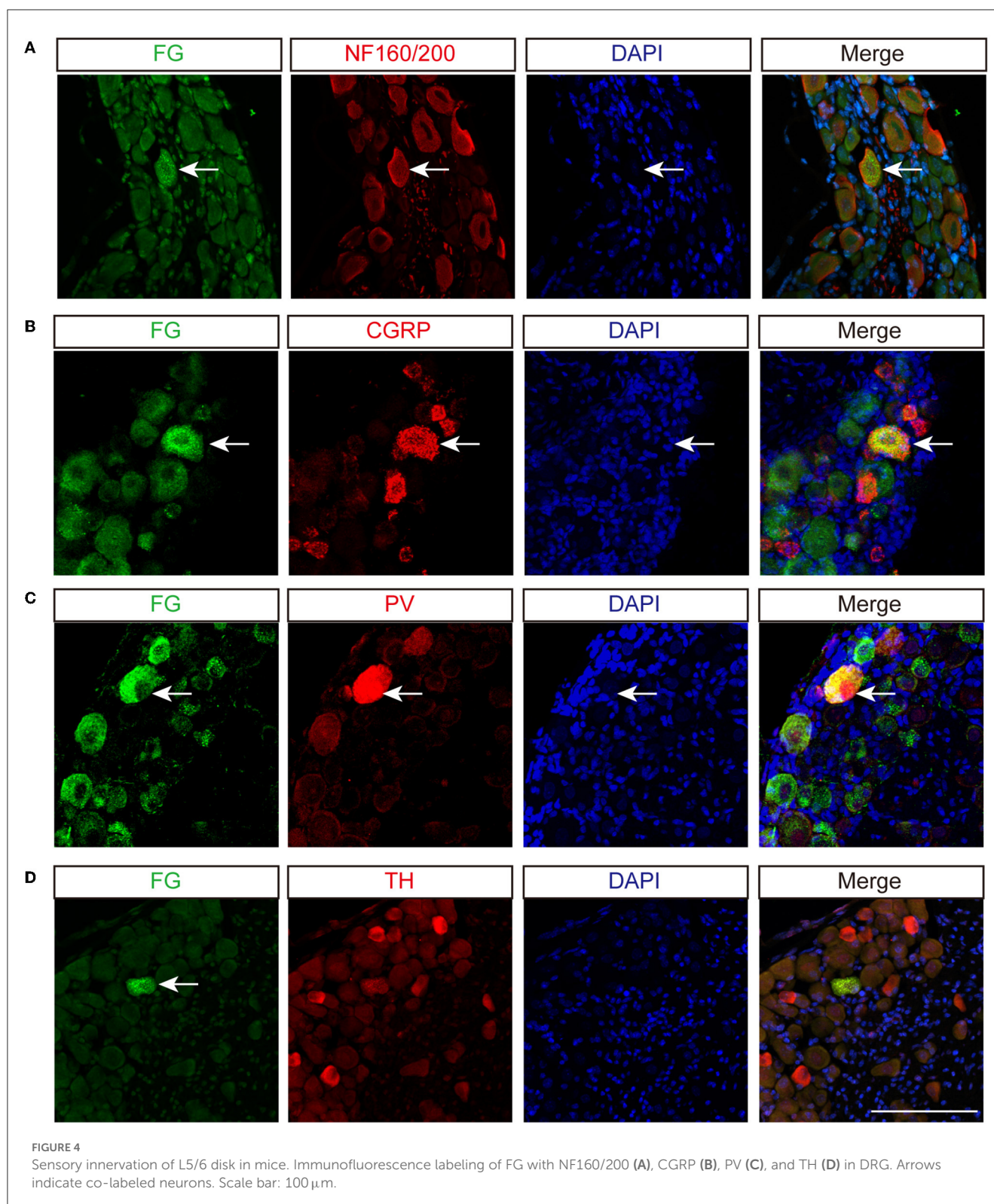


FIGURE 3
Types of nerve terminals in L5/6 disk in mice. Representative images of NF160/200+ **(A)**, CGRP+ **(B)**, PV+ **(C)**, and TH+ **(D)** staining in disks. Scale bar: 500 μ m.

< 30 μ m) (26). Growing evidence indicated that CGRP, one of the inflammatory mediators and the marker for nociceptive C fibers, was upregulated in DRG neurons after disk degeneration, as well as in aging-induced degenerated disks (5). These findings



suggest that increased innervation by C-fibers is associated with discogenic pain.

Intriguingly, we observed the density of NF160/200- and PV-positive nerve fibers in AF. As NF160/200 and PV are regarded as markers of myelinated nerve fibers and mechanoreceptors that

are derived from large-sized DRG neurons, they are responsible for transducing vibratory and position sensation (27). It is noteworthy that myelinated fibers have been implicated in neuropathic pain, largely through the activation of sodium channels (27). Blocking A-fiber neuron activity alleviated neuropathic pain (28). Intriguingly,

our recent study demonstrated that mTOR activation in large-sized DRG neurons promoted nociceptor excitability and neuropathic pain (29). PV is a calcium-binding protein that presents in proprioceptors and low-threshold mechanoreceptors (30, 31). The mean PV expression was 25% of L4 or L5 DRG neurons, and this was unchanged 2 weeks after peripheral nerve injury (30). More recently, hyperpolarization-activated cyclic nucleotide-gated channel (HCN) was found on PV⁺ neurons, and the excitability of proprioceptive afferents may contribute to the sensorimotor properties (32). Although the presence of mechanoreceptors was more frequent in diseased disk patients with pain (7, 33), further studies are warranted to define the functional role of myelinated fibers in discogenic pain. It should be noted that, other than four markers used in the present study, there are more nerve subtypes, including MRGPRD (MAS-related GPR family member D), P2X3 (purinergic receptor P2X, ligand-gated ion channel, 3), and TRP (transient receptor potential) family, have been proved to play a role in pain development and maintenance (34–36). Further studies are needed to distinguish these nerve subtypes in the disks and examine their roles in discogenic pain.

The innervation of the disk is the structural base for inducing discogenic pain. Growing evidence suggested that disk degeneration can induce increased innervation of CGRP protein gene product 9.5 (PGP9.5, a broad neuronal marker) fibers (37). Although our findings and previous findings indicated that the number of DRG neurons innervated disk is limited (2, 14), disk degeneration has been proven to induce the upregulation of neurotrophins and neuropeptides, such as nerve growth factor (NGF), CSF1, CGRP, and NPY in both disks and DRG neurons, and activate microglia and astrocytes in the spinal dorsal horn (5, 13, 38). These findings indicated that disk degeneration upregulates various pain-related neuropeptides in DRG neurons as well as immune responses in the spinal dorsal horn. Moreover, these changes contribute to neuronal plasticity, which may enhance nociceptive signaling and lead to discogenic pain (3, 5, 26, 39).

There are some limitations in the present study. First, the distribution and innervation patterns of the ventral and dorsal portions of the lumbar disk in mice should be separately studied. However, due to the limited efficiency of FG retrograde tracing (2), the percentage of FG⁺ neurons to a total number of DRG was relatively low. Although myelinated fibers have been found in healthy disks, the changes in distribution patterns after disk degeneration and herniation remain elusive. Moreover, the quantification of subtype DRG neurons that innervate disks is needed. In addition, it is important to elucidate the roles of myelinated fibers in discogenic pain. Further electrophysiologic, optogenetics, chemical genetics, and clinical studies are required to elucidate the functional implications of this study. Although there are differences between humans and rodent nociceptors in pain sensitization, rodents serve as important models for back pain research. Further studies, such as single-cell RNA sequencing, are warranted to investigate similarities and differences between humans and rodents regarding cellular and molecular aspects of sensory neurobiology (40).

In conclusion, our current study demonstrated that sensory neurons, which multi-segmentally innervated the L5/6 disk, existed

in Th13 to L6 DRGs in mice. Such an innervation pattern may explain why patients with lower disk degeneration experience inguinal and anterior thigh pain corresponding to the upper levels (L1 and L2) of DRGs. Using retrograde tracing methods, we also observed different types of nerve terminals derived from DRG neurons in mouse disks, including CGRP, NF160/200, and PV. The densely innervated structures of the lumbar disk may be responsible for sensing internal mechanical and chemical stimuli, and further contribute to discogenic pain.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the Tab of Animal Experimental Ethical Inspection of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Author contributions

LC, XL, YW, and ZG designed experiments and wrote the manuscript. LC, XL, and QJ performed experiments and analyzed the data. YW supervised the project.

Funding

This manuscript was supported by the Medicine and Health Science and Technology Plan in Zhejiang Province (2023RC019 to LC), the National Natural Science Foundation of China (82201358 to LC), Project of National and Regional Medical Center (G2022010-103 to LC), and Science Technology Department of Zhejiang Province (2020C03042 to YW).

Acknowledgments

We thank Drs. Bing Zhang and Liyao Qiu for the help with retrograde tracing. We are grateful to the research assistant Sanhua Fang at the Core Facilities of Zhejiang University Institute of Neuroscience.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or

claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Chen L, Battisti MC, Yuan Y, Yang G, Chen Z, Wang Y. Lumbar vertebral endplate defects on magnetic resonance images: prevalence, distribution patterns, and associations with back pain. *Spine J.* (2020) 20:352–60. doi: 10.1016/j.spinee.2019.10.015
- Ohtori S, Takahashi K, Chiba T, Yamagata M, Sameda H, Moriya H. Sensory innervation of the dorsal portion of the lumbar intervertebral discs in rats. *Spine.* (2001) 26:946–50. doi: 10.1097/00007632-200104150-00020
- Lyu F-J, Cui H, Pan H, Mc Cheung K, Cao X, Iatridis JC, et al. Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions. *Bone Res.* (2021) 9:7. doi: 10.1038/s41413-020-00125-x
- Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? *Spine.* (2006) 31:2151–61. doi: 10.1097/01.brs.0000231761.73859.2c
- Miyagi M, Millecamps M, Danco AT, Ohtori S, Takahashi K, Stone LS, et al. Prize winner: Increased innervation and sensory nervous system plasticity in a mouse model of low back pain due to intervertebral disc degeneration. *Spine.* (2014) 39:1345–54. doi: 10.1097/BRS.0000000000000334
- Groh AMR, Fournier DE, Battisti MC, Séguin CA. Innervation of the human intervertebral disc: A scoping review. *Pain Med.* (2021) 22:1281–304. doi: 10.1093/pm/pnab070
- Yang L, Yang C, Pang X, Li D, Yang H, Zhang X, et al. Mechanoreceptors in Diseased Cervical Intervertebral Disc and Vertigo. *Spine.* (2017) 42:540–6. doi: 10.1097/BRS.0000000000001801
- Johnson WEB, Evans H, Menage J, Eisenstein SM, El Haj A, Roberts S. Immunohistochemical detection of Schwann cells in innervated and vascularized human intervertebral discs. *Spine.* (2001) 26:2550–7. doi: 10.1097/00007632-200112010-00007
- Freemont AJ, Peacock TE, Goupille P, Hoyland JA, O'Brien J, Jayson MIV. Nerve ingrowth into diseased intervertebral disc in chronic back pain. *Lancet.* (1997) 350:178–81. doi: 10.1016/S0140-6736(97)02135-1
- Feng Z, Hu X, Zheng Q, Battisti MC, Chen Z, Wang Y. Cartilaginous endplate avulsion is associated with modic changes and endplate defects, and residual back and leg pain following lumbar discectomy. *Osteoarthritis Cartilage.* (2021) 29:707–17. doi: 10.1016/j.joca.2021.01.010
- Jacobsen HE, Khan AN, Levine ME, Filippi CG, Chahine NO. Severity of intervertebral disc herniation regulates cytokine and chemokine levels in patients with chronic radicular back pain. *Osteoarthritis and Cartilage.* (2020) 28:1341–50. doi: 10.1016/j.joca.2020.06.009
- Kang JD, Stefanovic-Racic M, McIntyre LA, Georgescu HI, Evans CH. Toward a Biochemical Understanding of Human Intervertebral Disc Degeneration and Herniation. *Spine.* (1997) 22:1065–73. doi: 10.1097/00007632-199705150-00003
- Yang G, Chen L, Gao Z, Wang Y. Implication of microglia activation and CSF-1/CSF-1R pathway in lumbar disc degeneration-related back pain. *Mol Pain.* (2018) 14:15. doi: 10.1177/1744806918811238
- Morinaga T, Takahashi K, Yamagata M, Chiba T, Tanaka K, Takahashi Y, et al. Sensory innervation to the anterior portion of lumbar intervertebral disc. *Spine.* (1996) 21:1848–51. doi: 10.1097/00007632-199608150-00002
- Takahashi Y, Ohtori S, Takahashi K. Peripheral nerve pathways of afferent fibers innervating the lumbar spine in rats. *J Pain.* (2009) 10:416–25. doi: 10.1016/j.jpain.2008.10.012
- Edgar MA. The nerve supply of the lumbar intervertebral disc. *J Bone Joint Surg Br.* (2007) 89B:1135–9. doi: 10.1302/0301-620X.89B9.18939
- Nakamura S, Takahashi K, Takahashi Y, Yamagata M, Moriya H. The afferent pathways of discogenic low-back pain - Evaluation of L2 spinal nerve infiltration. *J Bone Joint Surg Br.* (1996) 78B:606–12. doi: 10.1302/0301-620X.78B4.0780606
- Kim SH, Na HS, Kwangsup S, Jin MC. Effects of sympathectomy on a rat model of peripheral neuropathy. *Pain.* (1993) 55:85–92. doi: 10.1016/0304-3959(93)90187-T
- Fagan AB, Sarvestani G, Moore RJ, Fraser RD, Vernon-Roberts B, Blumbergs PC. Innervation of anulus tears: an experimental animal study. *Spine.* (2010) 35:1200–5. doi: 10.1097/BRS.0b013e3181c02812
- Coppes MH, Marani E, Thomeer RTWM, Groen GJ. Innervation of “painful” lumbar discs. *Spine.* (1997) 22:2342–9. doi: 10.1097/00007632-199710150-00005
- Fagan A, Moore R, Vernon Roberts B, Blumbergs P, Fraser R, ISSLS. prize winner: The innervation of the intervertebral disc: a quantitative analysis. *Spine.* (2003) 28:2570–6. doi: 10.1097/01.BRS.0000096942.29660.B1
- Ni S, Ling Z, Wang X, Cao Y, Wu T, Deng R, et al. Sensory innervation in porous endplates by Netrin-1 from osteoclasts mediates PGE2-induced spinal hypersensitivity in mice. *Nat Commun.* (2019) 10:5643. doi: 10.1038/s41467-019-13476-9
- Usoskin D, Furlan A, Islam S, Abdo H, Lönnerberg P, Lou D, et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat Neurosci.* (2015) 18:145–53. doi: 10.1038/nn.3881
- Murata Y, Takahashi K, Masatsune, Yamagata, Takahashi Y, Shimada Y, et al. Origin and pathway of sensory nerve fibers to the ventral and dorsal sides of the sacroiliac joint in rats. *J Orthopaed Res.* (2001) 19:379–83. doi: 10.1016/S0736-0266(00)90017-2
- Takahashi Y, Chiba T, Kurokawa M, Aoki Y, Takahashi K, Yamagata M. Stereoscopic structure of sensory nerve fibers in the lumbar spine and related tissues. *Spine.* (2003) 28:871–80. doi: 10.1097/01.BRS.0000058717.43888.B9
- Gold MS, Gebhart GF. Nociceptor sensitization in pain pathogenesis. *Nat Med.* (2010) 16:1248–57. doi: 10.1038/nm.2235
- Hong S, Wiley JW. Altered expression and function of sodium channels in large DRG neurons and myelinated A-fibers in early diabetic neuropathy in the rat. *Biochem Biophys Res Commun.* (2006) 339:652–60. doi: 10.1016/j.bbrc.2005.11.057
- Xu ZZ, Kim YH, Bang S, Zhang Y, Berta T, Wang F, et al. Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. *Nat Med.* (2015) 21:1326–31. doi: 10.1038/nm.3978
- Chen L, Hu Y, Wang S, Cao K, Mai W, Sha W, et al. mTOR-neuropeptide Y signaling sensitizes nociceptors to drive neuropathic pain. *JCI Insight.* (2022) 7:e159247. doi: 10.1172/jci.insight.159247
- Medici T, Shortland PJ. Effects of peripheral nerve injury on parvalbumin expression in adult rat dorsal root ganglion neurons. *BMC Neurosci.* (2015) 16:1–10. doi: 10.1186/s12868-015-0232-9
- Ichikawa H, Deguchi T, Nakago T, Jacobowitz DM, Sugimoto T. Parvalbumin, calretinin and carbonic anhydrase in the trigeminal and spinal primary neurons of the rat. *Brain Res.* (1994) 655:241–5. doi: 10.1016/0006-8993(94)91620-9
- Madden JF, Davis OC, Boyle KA, Iredale JA, Browne TJ, Callister RJ, et al. Functional and molecular analysis of proprioceptive sensory neuron excitability in mice. *Front Mol Neurosci.* (2020) 13:36. doi: 10.3389/fnmol.2020.00036
- Roberts S, Eisenstein SM, Menage J, Evans EH, Ashton IK. Mechanoreceptors in intervertebral discs. *Spine.* (1995) 20:2645–51. doi: 10.1097/00007632-199512150-00005
- Wang C, Gu L, Ruan Y, Geng X, Xu M, Yang N, et al. Facilitation of MrgprD by TRP-A1 promotes neuropathic pain. *FASEB J.* (2018) 33:1360–73. doi: 10.1096/fj.201800615RR
- Rosenbaum T, Morales-Lázaro SL, Islas LD, TRP. channels: a journey towards a molecular understanding of pain. *Nat Rev Neurosci.* (2022) 23:596–610. doi: 10.1038/s41583-022-00611-7
- Xia L-P, Luo H, Ma Q, Xie Y-K, Li W, Hu H, et al. GPR151 in nociceptors modulates neuropathic pain via regulating P2X3 function and microglial activation. *Brain.* (2021) 144:3405–20. doi: 10.1093/brain/awab245
- Lee S, Millecamps M, Foster DZ, Stone LS. Long-term histological analysis of innervation and macrophage infiltration in a mouse model of intervertebral disc injury-induced low back pain. *J Orthop Res.* (2019) 38:1238–47. doi: 10.1002/jor.24560
- García-Cosamalón J, Del Valle ME, Calavia MG, García-Suárez O, López-Muñiz A, Otero J, et al. Intervertebral disc, sensory nerves and neurotrophins: who is who in discogenic pain? *J Anat.* (2010) 217:1–15. doi: 10.1111/j.1469-7580.2010.01227.x
- Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci.* (2009) 32:1–32. doi: 10.1146/annurev.neuro.051508.135531
- Rostock C, Schrenk-Siemens K, Pohle J, Siemens J. Human vs. mouse nociceptors—similarities and differences. *Neuroscience.* (2018) 387:13–27. doi: 10.1016/j.neuroscience.2017.11.047



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University,
China

REVIEWED BY

Vittoria Nazzi,
IRCCS Carlo Besta Neurological Institute
Foundation,
Italy
Federica Ginanneschi,
University of Siena,
Italy

*CORRESPONDENCE

Jianjun Chang
✉ cjhr@126.com

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 15 December 2022

ACCEPTED 14 March 2023

PUBLISHED 06 April 2023

CITATION

Zheng D, Wu Z, Li L, Chen S and
Chang J (2023) Research advances and trends
in the surgical treatment of carpal tunnel
syndrome from 2003 to 2022: A CiteSpace-
based bibliometric analysis.
Front. Neurol. 14:1124407.
doi: 10.3389/fneur.2023.1124407

COPYRIGHT

© 2023 Zheng, Wu, Li, Chen and Chang. This is
an open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Research advances and trends in the surgical treatment of carpal tunnel syndrome from 2003 to 2022: A CiteSpace-based bibliometric analysis

Daqiang Zheng¹, Zhiming Wu², Lu Li¹, Sichao Chen¹ and
Jianjun Chang^{1*}

¹Third Hospital of Shanxi Medical University, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Taiyuan, China, ²Department of Orthopedics, Shanxi Bethune Hospital, Shanxi Academy of Medical Sciences, Tongji Shanxi Hospital, Third Hospital of Shanxi Medical University, Taiyuan, China

Background: Carpal Tunnel Syndrome (CTS) is one of the most common peripheral neuropathies. The typical symptoms are tingling and numbness in the median nerve distribution of the hand. Current treatment for CTS includes general conservative treatment and surgical treatment. Surgical treatment plays a crucial role in the management of CTS, but little bibliometric analysis has been conducted on it. Therefore, this study aimed to map the literature co-citation network using CiteSpace (6.1 R4) software. Research frontiers and trends were identified by retrieving subject headings with significant changing word frequency trends, which can be used to predict future research advances in the surgical treatment of CTS.

Methods: Publications on the surgical treatment of CTS in the Web of Science database were collected between 2003 and 2022. CiteSpace software was applied to visualize and analyze publications, countries, institutions, journals, authors, references, and keywords.

Results: A total of 336 articles were collected, with the USA being the major publishing power in all countries/regions. JOURNAL OF HAND SURGERY AMERICAN VOLUME was the journal with the most published and co-cited articles. Based on keyword and reference co-citation analysis, keywords such as CTS, surgery, release, median nerve, and diagnosis were the focus of the study.

Conclusion: The results of this bibliometric study provide clinical research advances and trends in the surgical treatment of patients with CTS over the past 20years, which may help researchers to identify hot topics and explore new directions for future research in the field.

KEYWORDS

surgery, carpal tunnel syndrome (CTS), bibliometric analysis, CiteSpace, nerve injuries

1. Introduction

Carpal tunnel syndrome (CTS) is one of the most common peripheral neuropathies, and it is generally accepted that the median nerve is damaged by pressure as it passes through the carpal tunnel (1). However, the exact cause of this neuropathy is still unknown. Typical symptoms of CTS include nocturnal pain with tingling and numbness in the distribution of the median nerve in the hand, and further progression without proper treatment may result in atrophy of the interosseous muscles of the hand, with end-stage hand disability and loss of work capacity (2). This can lead to disability and incapacity, seriously affecting the patient's quality of life.

Non-surgical conservative treatments include acupuncture, electrotherapy and medication and are generally used in the early stages of CTS (3). However, it is worth noting that, on the one hand, there are no studies that point to the existence of the best treatment or the best combination of treatment measures (4), on the other hand, conservative treatments can only delay the progression of the disease in the short term. Studies have shown that the release of the carpal tunnel and the reinnervation of the skin associated with it are significantly associated with improved symptoms and function (5). For this reason, surgical treatment remains an important tool in the treatment of moderate to severe CTS, with its unique advantages (6, 7). The traditional surgical treatment is open transverse carpal ligament release. The traditional surgical treatment is open transverse carpal ligament release, which remains the standard of care for CTS because of its effectiveness (8). However, with the development of new devices, the traditional open treatment is gradually being replaced by minimally invasive techniques with shorter operative times, less trauma, smaller postoperative scars and shorter incision healing times (9, 10).

Bibliometrics refers to the cross-cutting science of quantitative analysis of all knowledge vehicles using mathematical and statistical methods and is a statistical analysis and quantitative tool for research publications (11). In the past, studies have confirmed the safety and effectiveness of surgical treatment of CTS. However, little bibliometric analysis of studies related to the surgical treatment of CTS has been reported. Therefore, there is a need to understand the current state of research on the surgical treatment of CTS as a whole. This study aimed to assess research trends in the surgical treatment of CTS and to provide a macro review of the extensive academic literature over the past decade through a bibliometric analysis.

CiteSpace is a visual literature analysis software based on the JAVA language environment created by Professor Chaomei Chen and is now widely used in many fields such as information science, education and medicine (12). It features a co-occurrence network graph of countries, institutions, authors, keywords, cited journals, cited authors and cited references (13, 14). In this study, CiteSpace was used to conduct a bibliometric analysis to explore global advances and research trends in the surgical treatment of CTS over the past 20 years (2003 to 2022), with all eligible literature obtained from the Web of Science database.

2. Data and methods

2.1. Data collection

All data in this study were obtained from the Web of Science database on December 1, 2022. The literature search measurement

strategy was ((TS = (carpal tunnel syndrome)) AND TS = (treat)) AND TS = (surgery), with literature publication, dates from 1 January 2013 to 1 December 2022; in addition, the country, category and language of publication were not restricted. The final search yielded 336 documents: exported as "plain text files" with the record "full record with cited references."

2.2. Inclusion criteria

Inclusion criteria were: (1) Peer-reviewed published original articles on surgical treatment of CTS, including basic and clinical research; (2) reviews on surgical treatment of CTS; and (3) articles published from 2003 to 2022.

2.3. Exclusion criteria

Exclusion criteria were: (1) articles collected by hand and telephone; (2) articles not officially published; (3) conference abstracts and corrigendum documents; (4) repeated publications; and (5) unrelated articles.

2.4. Methods

The documents were analyzed econometrically using CiteSpace (6.1R4). Node types were selected for country, institution, author, keyword, cited journal, cited author and cited reference, Time Slicing was set to Form 2003 JAN to 2022 DEC, time partitioned to 1 year, number of Years Per Slice (1), all options in Term Source were selected, one node type was selected at a time, Selection Criteria (g-index, $k = 25$), and Pruning (Pathfinder). The visual knowledge graph consists mainly of nodes and links. Each node in the map represents an element, such as an author, keyword, institution, etc. The size of the nodes usually indicates the frequency of occurrence or citation, and the different colors of the nodes indicate different years, with different colored circles running from the inside to the outside of the nodes indicating the years 2003 to 2022. In addition, lines between nodes indicate collaboration, co-occurrence, or co-referencing relationships. The purple circles represent centrality, and nodes with high centrality are often considered to be key points in a particular domain.

3. Results and discussion

3.1. Global publishing trends

3.1.1. Countries and regions

A total of 336 articles that met our search criteria were retrieved from the Web of Science database. The volume of articles published in the field of surgical treatment of CTS research by country and region worldwide was analyzed (Figure 1A). The USA contributed the most articles in this area (114, 34.03%), followed by the UK (25, 7.46%), Japan (21, 6.27%), the Netherlands (21, 6.27%), and China (18, 5.37%). The data was used in CiteSpace software for visual analysis.

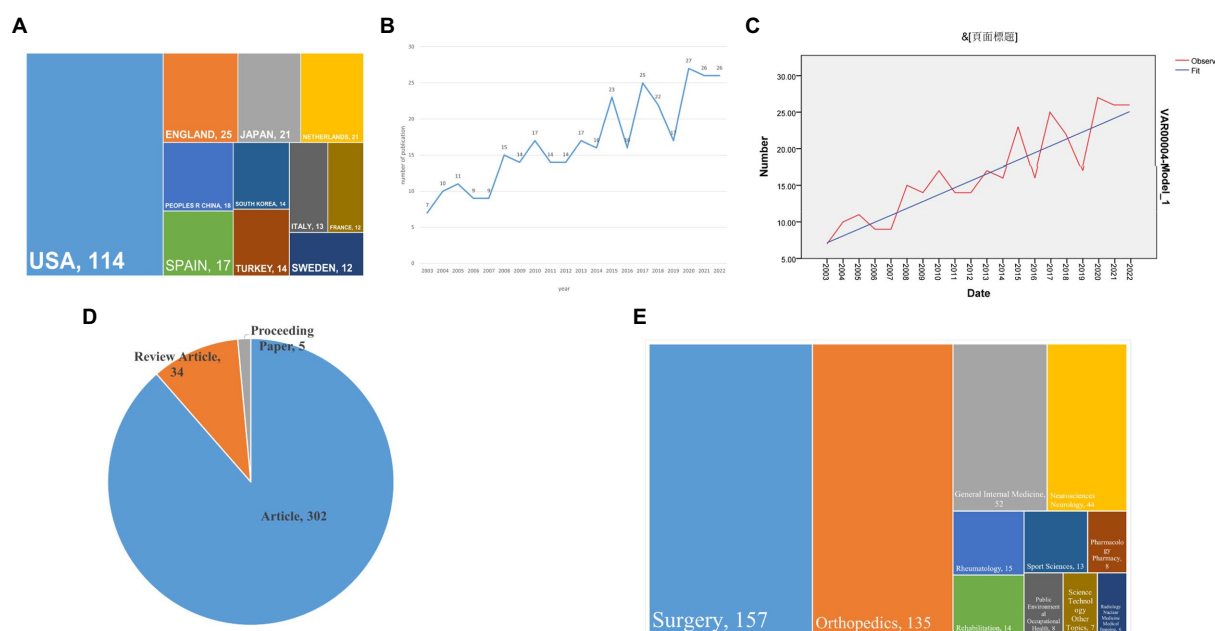


FIGURE 1

Global characteristics in the surgical treatment of CTS. (A) Volume of publications on surgical treatment of CTS by country and region from 2003 to 2022. (B) Annual volume of publications on surgical treatment CTS from 2003 to 2022. (C) Future publication forecast curve. (D) Article-genre breakdown on surgical treatment of carpal tunnel syndrome from 2003 to 2022. (E) WoS catalog classification of research areas.

3.1.2. Annual publication volume

A search within the Web of Science database by subject term revealed a total of 336 articles included in it, along with a count of the number of articles published in each 1-year period (Figure 1B).

The study found a steady upward trend in the number of publications from 2003 to 2022, with the highest number of publications (27) in 2020 and a plateau in the last 3 years. The trend in publications suggests that the surgical treatment of CTS has been gaining increasing attention from researchers over the last 20 years. It can be predicted that the annual number of publications in this field will continue to trend upwards.

3.1.3. Forecasting global publishing trends

The trend extrapolation trend analysis using SPSS software predicts future publishing trends in the field (Figure 1C). Indicates an upward trend in the number of publications in the future.

3.1.4. Type of literature

Dividing all articles by category (Figure 1D), the highest of these 336 articles was Article (302, 89.88%), followed by Review Article (34, 10.12%), and Proceeding Paper (5, 1.5%).

3.1.5. Areas of research

In the Web of Science database, the study covered 32 fields (Figure 1E), with the majority of the literature concentrated on “Surgery” (157; 46.73%), “Orthopedics” (135; 40.18%), “General Internal Medicine” (52; 15.48%) and “Neurosciences Neurology” (44; 13.10%), (135; 40.18%), “General Internal Medicine” (52; 15.48%) and “Neurosciences Neurology” (44; 13.10%), indicating that the Surgical treatment of CTS is a multifaceted and multidisciplinary field that covers a wide range of societal benefits.

3.1.6. Published journals

All 336 articles were published in 38 journals. The top 10 journals in terms of publication volume are shown in Table 1, among which JCR Q1 4, Q2 1, Q3 3, Q4 2, and the overall publication quality is high.

3.2. Collaborative network analysis

After importing 336 articles into the CiteSpace (6.1 R4) software, we deduplicated and cleaned the data and found zero duplicates, and a total of 336 articles were extracted for analysis. All articles were published in 179 journals by 316 researchers from 157 countries/regions.

3.2.1. Analysis of national/regional collaboration networks

The national collaboration networks from 2003 to 2022 were visualized through CiteSpace software and mapped (Figure 2A), showing countries that published at least 10 articles. The country with the highest number of publications is the USA with 114 articles, accounting for 33.93% of total publications, and maintains collaborative relationships with 17 other countries, with the top 5 also including the UK (25 articles), Japan (21 articles), the Netherlands (21 articles), and China (19 articles). China is the only developing country among them.

Higher centrality values, also known as mediated centrality, indicate the more active and closer role that nodes play in collaborative relationships with other nodes, and individuals with high centrality are marked with a purple halo in the graph. The USA (0.66) has a high intermediary centrality (Figure 3), indicating that it plays an important bridging role in the network of countries cooperating in this area.

TABLE 1 The TOP-10 journals with the largest number of publications.

Rank	Journal	Count	IF	JCR (2021)
1	JOURNAL OF HAND SURGERY AMERICAN VOLUME	47	2.342	Q3
2	PLASTIC AND RECONSTRUCTIVE SURGERY	13	5.169	Q1
3	BMC MUSCULOSKELETAL DISORDERS	8	2.562	Q3
4	HAND SURGERY REHABILITATION	7	1.419	Q4
5	JOURNAL OF HAND SURGERY EUROPEAN VOLUME	7	2.206	Q3
6	JOURNAL OF ORTHOPEDIC SPORTS PHYSICAL THERAPY	6	6.276	Q1
7	JOURNAL OF PLASTIC SURGERY AND HAND SURGERY	6	1.295	Q4
8	MUSCLE NERVE	6	3.852	Q2
9	CLINICAL ORTHOPEDICS AND RELATED RESEARCH	5	4.755	Q1
10	COCHRANE DATABASE OF SYSTEMATIC REVIEWS	5	12.008	Q1

Japan (0.00) and China (0.01) do not have high centrality, although they have a higher number of publications.

3.2.2. Author collaboration network analysis

With all 336 articles originating from 316 researchers, we generated an author collaboration network for this research study (Figure 2B). In the author collaboration network, each node represents an author, the line between the nodes represents a collaborative relationship between two individuals, and the color of the line represents the year of collaboration. As shown in the figure, there is less collaboration between researchers and between teams of researchers in this research area. At the same time, some scholars have formed collaborative networks centered on themselves and have a more consistent article output (Figure 2C). Overall, there is a lack of collaboration between many scholars and teams, with some important teams with a large number of early publications even phasing out of the field.

3.2.3. Analysis of journal collaboration networks

The names and number of publications in the last 10 years involving surgical treatment of CTS are shown in Table 1, with JOURNAL OF HAND SURGERY AMERICAN VOLUME being the most productive journal with 47 articles, followed by PLASTIC AND RECONSTRUCTIVE SURGERY with 13 articles, both of which are

specialist journals in the neighborhood of the specialty. We also used CiteSpace to generate a citation co-occurrence graph for journals (Figure 2B). The nodes in the graph represent journals, and the connections between the nodes represent citation relationships. In addition, the purple ring outside the node represents the centrality of the journal, and nodes with high centrality are considered to be key journals. The top ranked journals for frequency and centrality are JOURNAL OF HAND SURGERY AMERICAN VOLUME and JOURNAL OF NEUROLOGY NEUROSURGERY AND PSYCHIATRY, respectively. Of all the citations in JOURNAL OF HAND SURGERY AMERICAN VOLUME, one of the articles that has received more attention is a questionnaire analysis of members of the American Society for Surgery and Hand Surgery, in which the authors investigate the location of the procedure, the use of preoperative electrophysiological aids to diagnosis, the use of preoperative antibiotics, the choice of surgical incision, the choice of anesthesia, and the management of the patient postoperatively. The questionnaire survey is, in our opinion, of interest to researchers worldwide studying the surgical treatment of CTS.

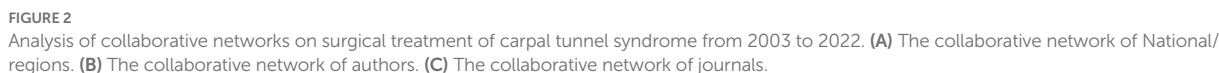
3.3. Keyword co-word analysis

3.3.1. High frequency keywords

Keyword co-occurrence plots (Figure 4A) were obtained from keyword analysis in the literature using CiteSpace software. Modularity $Q=0.7602$, with modularity 0.4–0.8 usually forming small natural network clusters that satisfy the analysis requirements. Weighted mean silhouette $S=0.7579$, when $S \geq 0.5$, clustering is generally considered more reasonable and meets the analysis requirements. Each node represents a keyword, the size of the node represents the frequency of the keyword, the linkage of two nodes represents the co-occurrence of two keywords, the color of the linkage represents the time, and the thickness represents the degree. Based on frequency and centrality, we found the following popular keywords: carpal tunnel syndrome, surgery, release, median nerve, diagnosis, questionnaire, decompression, complication, prevalence carpal tunnel release, diagnosis, risk factor, breast cancer, carpal tunnel, complication, decompression, and release (Table 2). It can be seen that the main focus of research on surgical treatment of CTS is on the release of carpal tunnel, changes in median nerve function, prognosis and the mechanism of CTS development.

The keywords were analyzed by clustering (Figure 4B) and the keywords were clustered into 12 LABELS with the clustering labels derived from the LSI/LLR/MI algorithm and the number of clusters was inversely proportional to the size of the clusters. These clustered keywords can reflect the research hotspots in surgical treatment of CTS which can guide the development pattern of this research and new directions of research.

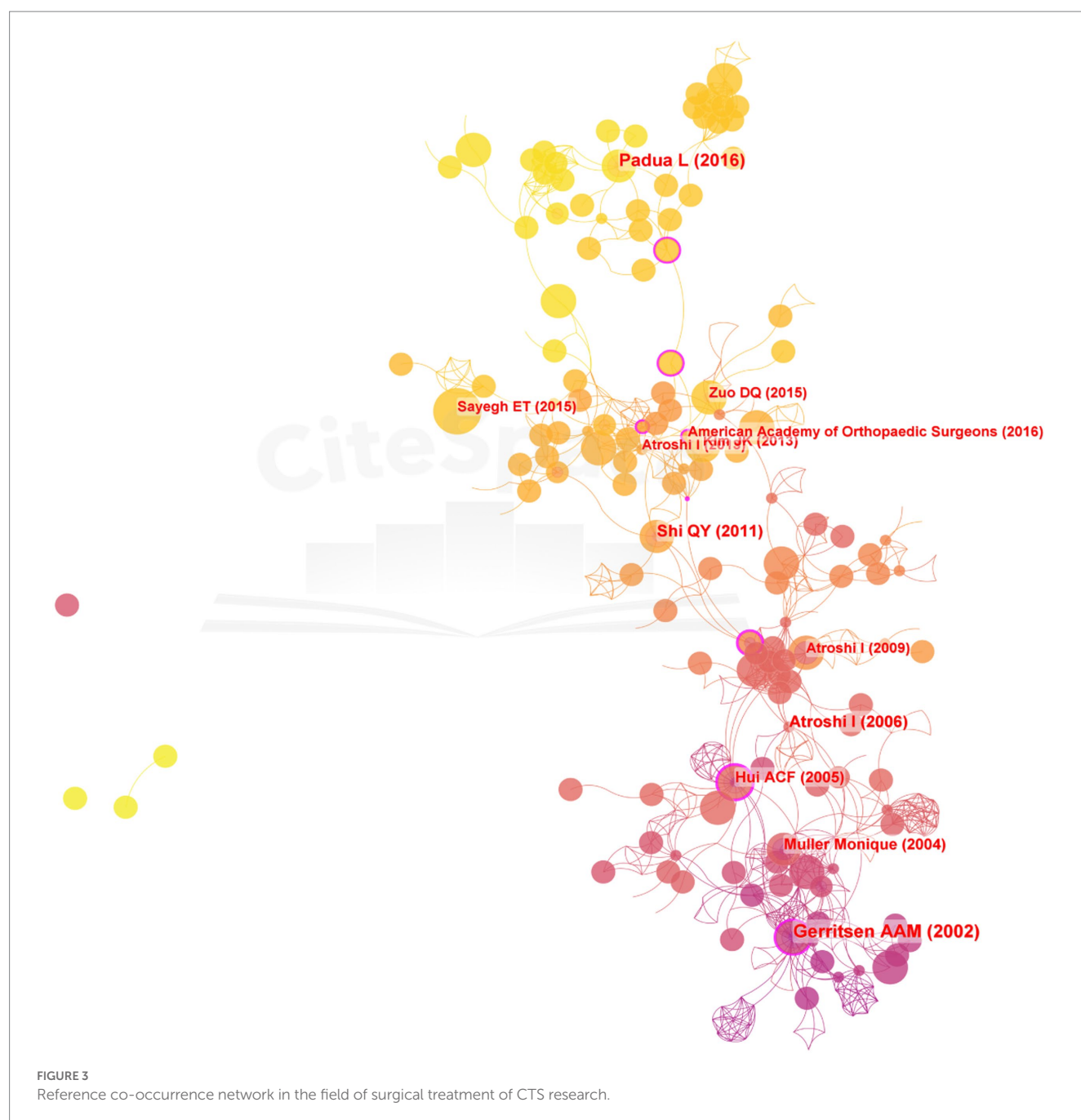
The keyword timeline analysis of the literature was obtained using CiteSpace software to obtain the keyword timeline mapping (Figure 4C). The analysis showed that the pre-research direction mainly focused on research related to the pathogenesis of CTS, with keywords around CTS, surgery, release, median nerve, and diagnosis, which we believe is since after the third technological revolution, especially since 2000, human production and lifestyle have The significant increase in the incidence of soft tissue disorders



The keyword bursts in the literature were analyzed using CiteSpace software to obtain the 10 keywords with the highest number of citation bursts (Figure 4D). The analysis showed that the keywords decompression (3.22), conduction (2.8), prevalence (4.62), management (2.91), and hand (2.75) had a long duration, and prevalence (4.62), cubital tunnel syndrome (3.38), and decompression (3.22) had high burst intensities indicating that these keywords were research hotspots in the corresponding

3.4. Reference co-citation analysis

The top ten most frequently co-cited articles in the field of surgical treatment of CTS research were compiled and listed by CiteSpace software to obtain co-citation and highlighting plots of the literature (Figure 3 and Table 4). In a randomized controlled trial by Gerritsen et al. (19), a non-invasive approach to the treatment of CTS only improved patients' symptoms and did not change outcomes. This provided direction for those who followed to study the treatment of CT. In a study by Hui et al., the efficacy of decompression surgery and steroid injections for CTS was compared in terms of nerve conduction velocity, symptoms (5 dimensions: pain, numbness, sensory abnormalities, weakness/clumsiness, nocturnal arousal) and grip strength, noting that steroid injections only provided short-term relief of symptoms and did not relieve the degeneration of the median nerve from mechanical compression (20).



4. Discussion

4.1. Analysis of global publication trends

A total of 336 articles were obtained from the Web of Science database according to our selection criteria. After importing the 336 articles into the CiteSpace (6.1 R4) software, we performed deduplication and cleaning of the data and found zero duplicates, and a total of 336 articles were extracted for analysis. All articles were published in 179 journals by 316 researchers from 157 countries/regions.

Through bibliometric and visual analysis, we found that the number of publications has increased each year between 2003 and

2022, while more predictive analysis shows that the field is now in a growth phase. Future publication trends are positive.

Research on national/regional cooperation networks can help to promote teamwork and global cooperation in specific areas. In this study, we can see that the country with the highest number of publications is the USA ($n=114$), followed by the UK ($n=24$) and Japan ($n=21$). Furthermore, the USA has the highest centrality at 0.66, indicating that it plays an important role as a bridge in inter-country cooperation. Based on this current situation, strengthening communication and cooperation between national institutions is of profound importance in promoting further development in this field.

The analysis of author collaboration networks and author co-citations helps to analyze the direction of authors' research and provides further guidance. All 336 articles originated from 316

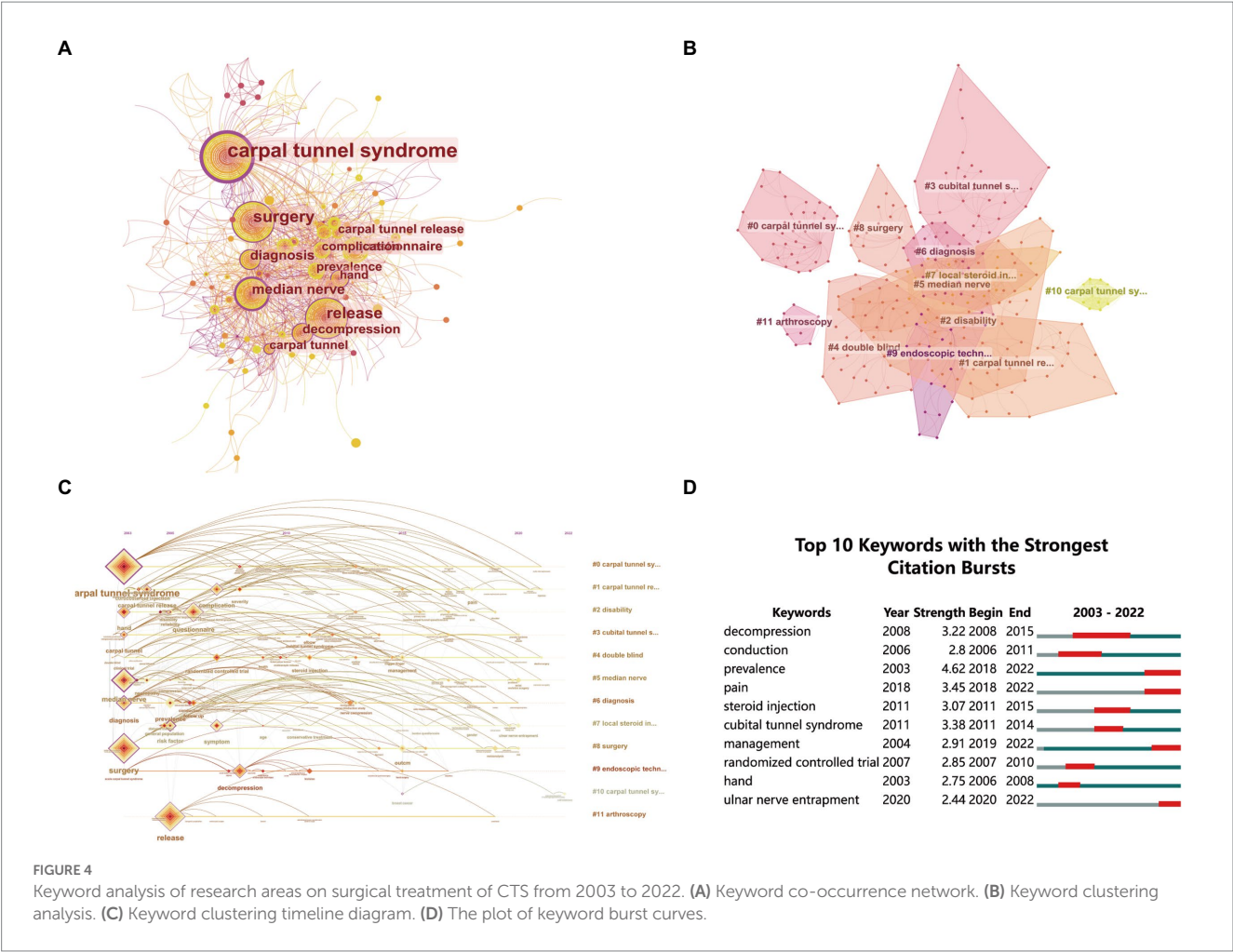


FIGURE 4 Keyword analysis of research areas on surgical treatment of CTS from 2003 to 2022. (A) Keyword co-occurrence network. (B) Keyword clustering analysis. (C) Keyword clustering timeline diagram. (D) The plot of keyword burst curves.

TABLE 2 TOP-10 of frequency, TOP-10 of centrality.

Rank	Frequency	Label	Rank	Centrality	Label
1	192	Carpal tunnel syndrome	1	0.51	Median nerve
2	76	Surgery	2	0.45	Carpal tunnel syndrome
3	57	Release	3	0.26	Diagnosis
4	36	Median nerve	4	0.23	Surgery
5	28	Diagnosis	5	0.21	Risk factor
6	25	Questionnaire	6	0.2	Breast cancer
7	23	Decompression	7	0.17	Carpal tunnel
8	20	Complication	8	0.16	Complication
9	20	Prevalence	9	0.14	Decompression
10	19	Carpal tunnel release	10	0.13	Release

Keyword co-occurrence network for research areas on surgical treatment of carpal tunnel syndrome from 2003 to 2022.

researchers. The study shows that many scholars have formed collaborative networks centered on themselves and have a more consistent article output. Overall, there is a lack of collaboration between scholars and teams, and enhanced communication and cooperation between authors could have far-reaching implications in promoting further development in the field. Future author collaboration could produce more good quality articles.

An analysis of journals can help researchers choose the right journal to submit their papers. Of the top 10 journals in terms of the number of publications, JCR Q2 and above accounted for five, with the highest IF being COCHRANE DATABASE OF SYSTEMATIC REVIEWS (IF = 12.008). The study shows that surgical treatment of CTS research has greater clinical value and is favored by many high-quality, high-impact journals.

TABLE 3 Keyword clustering labels for research areas on surgical treatment of carpal tunnel syndrome from 2003 to 2022.

Cluster-ID	Size	Label (LSI)	Label (LLR)	Label (MI)	Average year
#0	34	Carpal tunnel syndrome	Manual therapy (26.91, 1.0E-4)	National trend (2.03)	2013
#1	23	Carpal tunnel release	Longitudinal open incision (19.45, 1.0E-4)	Ulnar nerve (0.09)	2008
#2	21	Carpal tunnel syndrome	Sex difference (21.81, 1.0E-4)	Carpal tunnel syndrome (0.12)	2001
#3	20	Carpal tunnel syndrome	Long-term follow-up (24.07, 1.0E-4)	Parallel-group trial (0.28)	2011
#4	20	Short-term effect	Short-term effect (21.22, 1.0E-4)	Treatment decision (0.4)	2017
#5	20	Median nerve	Micro-invasive us-guided carpal tunnel release (20.91, 1.0E-4)	Carpal tunnel syndrome (0.11)	2016
#6	19	Sonographic follow-up	Sonographic follow-up (40.31, 1.0E-4)	Electrodiagnostic studies (0.16)	2004
#7	18	Carpal tunnel syndrome	Conservative management (24.54, 1.0E-4)	Carpal tunnel syndrome Symptom severity (0.12)	2003
#8	15	Carpal tunnel syndrome	Surgical treatments-a (13.86, 0.001)	Open carpal tunnel release (0.12)	2006
#9	14	Carpal tunnel syndrome	Occupational disease (21.81, 1.0E-4)	Carpal tunnel syndrome (0.11)	2000
#10	12	Double-blind placebo-controlled trial	Double-blind placebo-controlled trial (21.34, 1.0E-4)	Carpal tunnel syndrome (0.11)	2006
#12	6	Evaluation of patients with carpal tunnel syndrome treated by endoscopic technique	Evaluation (9.82, 0.005)	Carpal tunnel syndrome (0.17)	2010

4.2. Analysis of the current state of research on surgical treatment of CTS

Surgery is currently the mainstay of the response to moderate to severe CTS. Pressure on the median nerve is relieved by releasing the contents of the carpal tunnel by severing the transverse carpal ligament (21). This gain can be achieved both through open surgery and through small incisions, and later endoscopic release of the carpal tunnel has also been shown to be efficacious. Between 2003 and 2022, researchers have worked to find the most appropriate means of treating CTS. Through nerve conduction velocity, symptoms [the efficacy of different procedures, or surgical versus non-surgical procedures, has been compared in terms of nerve conduction velocity, symptoms (5 dimensions: pain, numbness, sensory abnormalities, weakness/clumsiness, nocturnal arousal)], and grip strength, as well as the most appropriate treatment modalities for different risk factors (20, 22, 23). Thus, the study of the efficacy of different treatment modalities for CTS has been a hot topic in the field.

4.3. Prospective analysis of research on surgical treatment of CTS

Current research on surgical treatment of CTS relies mainly on randomized controlled trials, and there is a lack of effective animal

models and *in vitro* trials. If future researchers can establish reliable animal models, the mechanism of the development of CTS will be clearer and will better assist surgeons in their clinical decisions. In addition, little information has been reported on the relationship between histological analysis and single-cell sequencing of CTS and surgery, which we believe could better explain the pathogenesis of CTS and provide guidance to clinicians.

4.4. Limitations

In this study, we only used CiteSpace software for visual analysis of the Web of Science database; therefore, this strategy may have missed papers published in other databases. Furthermore, the search strategy was created to obtain the most comprehensive data possible, which does not guarantee that all included articles are fully relevant to the study topic. It is known that older literature has a higher chance of being cited and confirmed, which may result in a time lag where recent groundbreaking research has not received sufficient attention. In addition, the software is temporarily unable to capture all authorship information and distinguish the order of authors, which may lead to a less accurate assessment of the contributions of researchers in the field. Therefore, we need to take a dialectical view

TABLE 4 Top-10 most frequently cited articles in the field of surgical treatment of CTS research in total.

Rank	Count	Centrality	Author	Year	Reference	Source
	28	0.13	Gerritsen AAM	2002	Splinting vs. surgery in the treatment of carpal tunnel syndrome 10.1245	JAMA-JAM MED ASSOC
2	21	0.26	Hui ACF	2005	A randomized controlled trial of surgery vs. steroid injection for carpal tunnel syndrome	NEUROLOGY
3	21	0.04	Agarwal V	2005	A prospective study of the long-term efficacy of local methylprednisolone acetate injection in the management of mild carpal tunnel syndrome	RHEUMATOLOGY
4	20	0.03	Muller Monique	2004	Effectiveness of hand therapy interventions in primary management of carpal tunnel syndrome: a systematic review	J HAND THER
5	17	0.1	Aroori Somaiah	2008	Carpal tunnel syndrome	ULSTER MED J
6	17	0.05	Baysal O	2006	Comparison of three conservative treatment protocols in carpal tunnel syndrome	INT J CLIN PRACT
7	17	0.04	Atroshi I	2007	The SF-6D health utility index in carpal tunnel syndrome	J HAND SURG-BRIT EUR
8	17	0.02	Greenslade JR	2004	Dash and Boston questionnaire assessment of carpal tunnel syndrome outcome: what is the responsiveness of an outcome questionnaire?	J HAND SURG-BRIT EUR
9	16	0.05	Atroshi I	2013	Methylprednisolone injections for the carpal tunnel syndrome	ANN INTERN MED
10	16	0.02	Bland JDP	2001	Do nerve conduction studies predict the outcome of carpal tunnel decompression?	MUSCLE NERVE

of the strengths and limitations of this approach compared to traditional reviews.

Author contributions

DZ conceived the study and carried out the data analysis, interpretation, and manuscript writing. ZW helped to draft the manuscript. LL and SC helped to check the data. JC contributed to methodology and writing—review and editing. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors would like to express their appreciation to Prof. Chaomei Chen for inventing the CiteSpace and making it free to use.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Middleton SD, Anakwe RE. Carpal tunnel syndrome. *BMJ*. (2014) 349:g 6437. doi: 10.1136/bmj.g6437
- Aslani HR, Alizadeh K, Eajazi A, Karimi A, Karimi MH, Zaferani Z, et al. Comparison of carpal tunnel release with three different techniques. *Clin Neurol Neurosurg*. (2012) 114:965–8. doi: 10.1016/j.clineuro.2012.02.017
- Wiperman J, Goerl K. Carpal tunnel syndrome: Diagnosis and management. *Am Fam Physician*. (2016) 94:993–9.
- Jiménez Del Barrio S, Bueno Gracia E, Hidalgo García C, Estébanez de Miguel E, Tricás Moreno JM, Rodríguez Marco S, et al. Conservative treatment in patients with mild to moderate carpal tunnel syndrome: A systematic review. *Neurologia*. (2018) 33:590–601. doi: 10.1016/j.nrl.2016.05.018
- Baskozos G, Sandy-Hindmarch O, Clark AJ, Windsor K, Karlsson P, Weir GA, et al. Molecular and cellular correlates of human nerve regeneration: ADCYAP1/PACAP enhance nerve outgrowth. *Brain J Neurol*. (2020) 143:2009–26. doi: 10.1093/brain/awaa163
- Karamanos E, Jillian BQ, Person D. Endoscopic carpal tunnel release: Indications, technique, and outcomes. *Orthop Clin North Am*. (2020) 51:361–8. doi: 10.1016/j.ocl.2020.02.001
- Vasiliadis HS, Georgoulas P, Shrier I, Salanti G, Scholten RJ. Endoscopic release for carpal tunnel syndrome. *Cochrane Database Syst Rev*. (2014) 1:CD008265. doi: 10.1002/14651858.CD008265.pub2
- Kim PT, Lee HJ, Kim TG, Jeon IH. Current approaches for carpal tunnel syndrome. *Clin Orthop Surg*. (2014) 6:253–7. doi: 10.4055/cios.2014.6.3.253
- Padua L, Coraci D, Erra C, Pazzaglia C, Paolasso I, Loreti C, et al. Carpal tunnel syndrome: Clinical features, diagnosis, and management. *Lancet Neurol*. (2016) 15:1273–84. doi: 10.1016/S1474-4422(16)30231-9
- Trung DT, Ngoc TM, Gia DH, Ngoc SD, Le Manh S, Dinh TD, et al. Endoscopic carpal tunnel release surgery: A case study in Vietnam. *J Orthop Surg Res*. (2019) 14:149. doi: 10.1186/s13018-019-1192-z
- Cooper ID. Bibliometrics basics. *J Med Libr Assoc*. (2015) 103:217–8. doi: 10.3163/1536-5050.103.4.013
- Chen C. Searching for intellectual turning points: Progressive knowledge domain visualization. *Proc Natl Acad Sci U S A*. (2004) 101:5303–10.
- Belter CW. Bibliometric indicators: Opportunities and limits. *J Med Libr Assoc*. (2015) 103:219–21. doi: 10.3163/1536-5050.103.4.014
- Chen C, Ibekwe-San Juan F, Hou J. The structure and dynamics of cocitation clusters: A multiple-perspective cocitation analysis. *J Am Soc Inf Sci Technol*. (2010) 61:1386–409. doi: 10.1002/asi.21309
- Walker-Bone K, Cooper C. Hard work never hurt anyone: Or did it? A review of occupational associations with soft tissue musculoskeletal disorders of the neck and upper limb. *Ann Rheum Dis*. (2005) 64:1391–6. doi: 10.1136/ard.2003.020016
- Okutsu I, Ninomiya S, Takatori Y, Ugawa Y. Endoscopic management of carpal tunnel syndrome. *Arthroscopy*. (1989) 5:11–8. doi: 10.1016/0749-8063(89)90084-4
- Agee JM, McCarroll HR Jr, Tortosa RD, Berry DA, Szabo RM, Peimer CA. Endoscopic release of the carpal tunnel: A randomized prospective multicenter study. *J Hand Surg Am*. (1992) 17:987–95. doi: 10.1016/S0363-5023(09)91044-9
- Chow JC. Endoscopic release of the carpal ligament: A new technique for carpal tunnel syndrome. *Arthroscopy*. (1989) 5:19–24. doi: 10.1016/0749-8063(89)90085-6
- Gerritsen AA, de Vet HC, Scholten RJ, Bertelsmann FW, de Krom MC, Bouter LM. Splinting vs. surgery in the treatment of carpal tunnel syndrome: A randomized controlled trial. *JAMA*. (2002) 288:1245–51. doi: 10.1001/jama.288.10.1245
- Hui AC, Wong S, Leung CH, Tong P, Mok V, Poon D, et al. A randomized controlled trial of surgery vs. steroid injection for carpal tunnel syndrome. *Neurology*. (2005) 64:2074–8. doi: 10.1212/01.WNL.0000169017.79374.93
- O'Connor D, Marshall S, Massy-Westropp N. Non-surgical treatment (other than steroid injection) for carpal tunnel syndrome. *Cochrane Database Syst Rev*. (2003) 2003:CD003219. doi: 10.1002/14651858.CD003219
- Orhurhu V, Orman S, Peck J, Urits I, Orhurhu MS, Jones MR, et al. Carpal tunnel release surgery—a systematic review of open and endoscopic approaches. *Anesthesiol Pain Med*. (2020) 10:e112291. doi: 10.5812/aapm.112291
- Michelotti B, Romanowsky D, Hauck RM. Prospective, randomized evaluation of endoscopic versus open carpal tunnel release in bilateral carpal tunnel syndrome: An interim analysis. *Ann Plast Surg*. (2014) 73:S157–60. doi: 10.1097/SAP.0000000000000203



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan Medical
College, China

REVIEWED BY

Viktor Aleinikov,
National Center for Neurosurgery, Kazakhstan
Hu Qingfeng,
Zhejiang University, China
Xiangyang Wang,
The Second Affiliated Hospital and Yuying
Children's Hospital of Wenzhou Medical
University, China

*CORRESPONDENCE

Xiaozhang Ying
✉ yxz991010@zcmu.edu.cn
Qi Zheng
✉ 13656642008@163.com

[†]These authors have contributed equally to this work

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 17 November 2022

ACCEPTED 14 February 2023

PUBLISHED 11 April 2023

CITATION

Zhang P, Jin Y, Zhu B, Zheng M, Ying X and
Zheng Q (2023) Unilateral biportal endoscopic
foraminotomy and discectomy combined with
piezosurgery for treating cervical spondylotic
radiculopathy with neuropathic radicular pain.
Front. Neurol. 14:1100641.
doi: 10.3389/fneur.2023.1100641

COPYRIGHT

© 2023 Zhang, Jin, Zhu, Zheng, Ying and
Zheng. This is an open-access article
distributed under the terms of the [Creative
Commons Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Unilateral biportal endoscopic foraminotomy and discectomy combined with piezosurgery for treating cervical spondylotic radiculopathy with neuropathic radicular pain

Peng Zhang[†], Yanghui Jin[†], Bo Zhu, Mingfeng Zheng,
Xiaozhang Ying* and Qi Zheng*

Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine, Hangzhou, China

Objective: Unilateral biportal endoscopy (UBE) represents a relatively recent development in minimally invasive spine surgery. This study aimed to evaluate the efficacy and safety of UBE foraminotomy and discectomy combined with piezosurgery for treating cervical spondylotic radiculopathy (CSR) with neuropathic radicular pain.

Methods: We retrospectively analyzed the outcomes in 12 patients with CSR who underwent UBE foraminotomy and discectomy combined with piezosurgery. The intraoperative blood loss, operative time, visual analog scale (VAS) scores for the neck and arm, neck disability index (NDI) scores, and complications were recorded.

Results: Postoperative VAS scores of the neck and arm and NDI scores were significantly improved. Additionally, a postoperative CT scan revealed adequate enlargement of the cervical canal and nerve root. No specific complications occurred during surgery and the immediate postoperative period.

Conclusions: This primary study indicated that the UBE foraminotomy and discectomy with piezosurgery is a promising technique for treating cervical spondylotic radiculopathy with neuropathic radicular pain.

KEYWORDS

cervical spondylotic radiculopathy, neuropathic radicular pain, unilateral biportal endoscopy, piezosurgery, foraminotomy and discectomy

Introduction

With the arrival of an aging society, the number of patients with cervical spondylotic radiculopathy (CSR), which is one of the degenerative spinal diseases, is increasing rapidly (1). CSR is the most common form of cervical spondylosis, mainly due to cervical nerve root compression caused by disc degeneration, herniation, segmental instability, or other conditions, mainly manifested as neuropathic radicular pain. CSR, the most prevalent form of cervical spondylosis, is caused by cervical nerve root compression due to disc degeneration, herniation, segmental instability, or other conditions, mainly manifested as neuropathic radicular pain (2). Although anterior cervical discectomy and fusion (ACDF) is the gold standard surgical strategy for the treatment of CSR, it has several disadvantages, including adjacent segment degeneration, incision and implant-related complications, and

TABLE 1 Biodemographic data of patients with CSR who underwent UBE foraminotomy and disectomy combined with piezosurgery.

Variables	Mean (SD)	Frequency (%)
Age (years)		
30–39		1 (8.3)
40–49		4 (33.3)
50–59		5 (41.7)
60–70		2 (16.7)
Gender		
Male		7 (58.3)
Female		5 (41.7)
Chief complaint		
Neck pain		2 (16.7)
Arm pain		4 (33.3)
Both		6 (50.0)
Level, No.		
C4–C5		3 (25.0)
C5–C6		4 (33.3)
C6–C7		5 (41.7)
Operative time (min)	251.2 (14.6)	
Blood loss (ml)	50.2 (9.5)	
Follow-up (month)	16.8 (1.8)	

high hospitalization costs (3). In addition, it is reported that anterior open surgery is closely associated with a high risk of postoperative dysphagia (4). Consequently, minimally invasive spinal surgeries have emerged with similar clinical outcomes, which have several advantages, including less injury, shorter hospital stay, and fewer postoperative complications (5).

Unilateral biportal endoscopy (UBE) is a minimally invasive spinal surgery with minimal injury to the surrounding structures, thereby greatly increasing the stability of the operated segment (6). Currently, UBE has gradually been applied for cervical disorders with remarkable efficiency and convenience (6, 7).

However, whether piezosurgery can be used in UBE surgery has not been reported. This study aimed to evaluate the efficacy and safety of UBE foraminotomy and disectomy combined with piezosurgery for treating CSR with neuropathic radicular pain

Materials and methods

Clinical data

We retrospectively followed a group of 12 patients suffering from CSR and received UBE foraminotomy and disectomy with piezosurgery between May 2020 and June 2021. The average age of patients was 50.2 ± 9.5 years, seven of them were men and five were women. The demographic and clinical characteristics of patients were shown in Table 1. This research was approved by the Ethics Committee of Affiliated Hangzhou Chest Hospital,

Zhejiang University School of Medicine. Each patient provided written informed consent before being administered any treatment.

The CSR patients were initially diagnosed according to a combination of the patient's clinical history, radicular symptoms, and radiographic scan. Inclusion criteria were as follow: no improvement in radicular symptoms after at least 3 months of conservative treatments with oral NSAIDs and neurotrophic drugs, no previous cervical surgery, 1-level disk herniation, no large disk repress cervical myelopathy, and the patient refused conventional open surgeries. Exclusion criteria included: disc herniation with no radicular symptoms, accompanying spinal instability, and coexisting spinal infection or malignancy.

Surgical procedure

Following general anesthesia, the patient underwent surgery in a prone position on a chest pad, and the neck was placed in slight flexion using a horseshoe-shaped headrest. With the guidance of a C-arm fluoroscope, the target interlaminar space was identified and marked. Two 1.5 cm skin incisions about 1 to 1.5 cm ipsilateral to the spinous process line were created at 1 cm above and below the marker line. Next, a series of dilators were used to split the paraspinal muscle and created the two viewing and working portals. The viewing portal was used to provide surgical vision, while the working portal was used for instrument handling.

Under endoscopic guidance, the soft tissue was removed from the bone surface with a bipolar radiofrequency probe until the "V" point is exposed. Thereafter, foraminotomy was carefully performed on the inferior lamina, superior lamina, interlaminar spaces, and the medial portion of the facet joint. An oval hole about 2 cm in the bone was made with a high-speed spherical drill and Piezosurgery device (XD880A, SMTP Technology Co., Ltd.) (Figure 1). The use of Piezosurgery device can safely and efficiently remove the bone around the nerve root, thereby fully releasing the nerve root.

After the cervical nerve root and herniated disc were seen under the endoscope, a disectomy was performed to remove the herniated nucleus pulposus tissue, thus releasing the nerve root. Subsequently, complete decompression of the spinal cord and nerve root was confirmed. Finally, the endoscopy and instrument were withdrawn, a drainage tube was placed, and then the wound was closed. The surgical procedure is shown in Figure 2.

Evaluation

Intraoperative blood loss, operative time, visual analog scale (VAS) score for the neck and arm, Neck Disability Index (NDI) scores and complications were documented. Radiographic examinations including MRI and CT were performed to access the extent of disc herniation, the status of the spinal cord and nerve root, and relevant bony structures preoperatively and at the follow-up periods.



FIGURE 1
SMTP Piezosurgery device with hand-piece and different inserts.

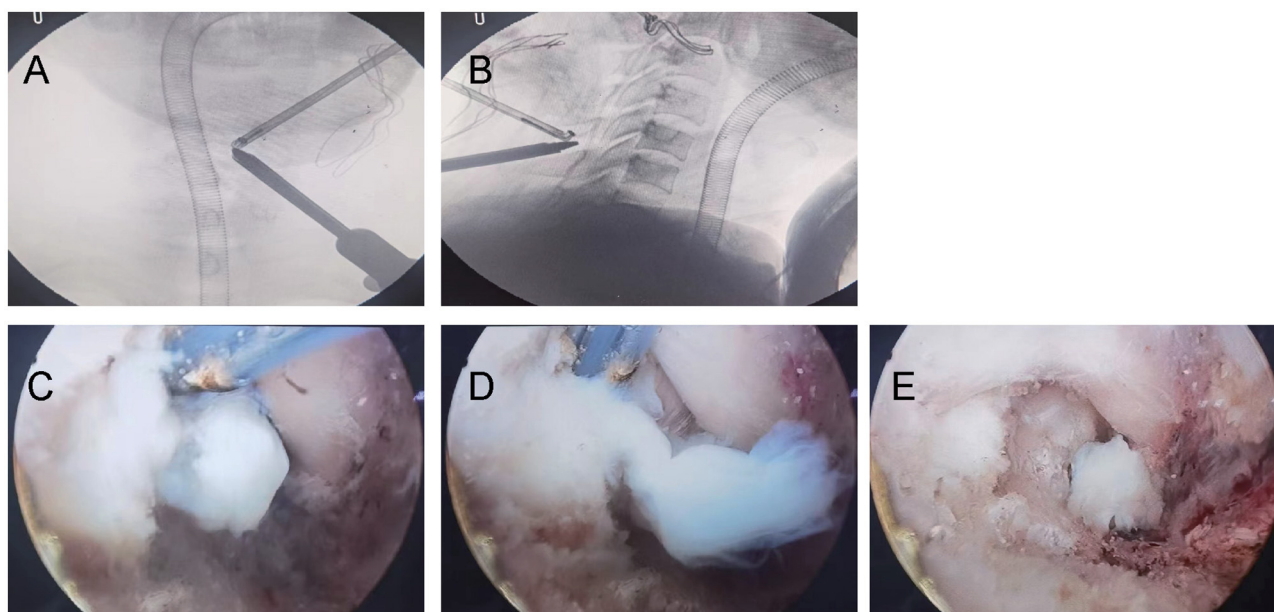


FIGURE 2
(A, B) Radiographic anteroposterior represented the locations of viewing and working portals. (C–E) Discectomy was performed.

Statistical analysis

Statistical analyses were conducted on all data employing SPSS 16.0 (IBM, USA). Results were presented as mean $\pm$ standard deviation (SD). Herein, Fisher's exact probability test, independent-samples *t*-test, and one-way ANOVA analysis were conducted employing SPSS. $p < 0.05$ was judged significant.

Results

Table 1 shows the mean operation time and intraoperative blood loss. Postoperative CT scan revealed adequate enlargement of the cervical canal and nerve root (Figure 3). At the final follow-up, VAS scores of the neck and arm and NDI scores were significantly improved compared to the pre-operation (Figure 4).

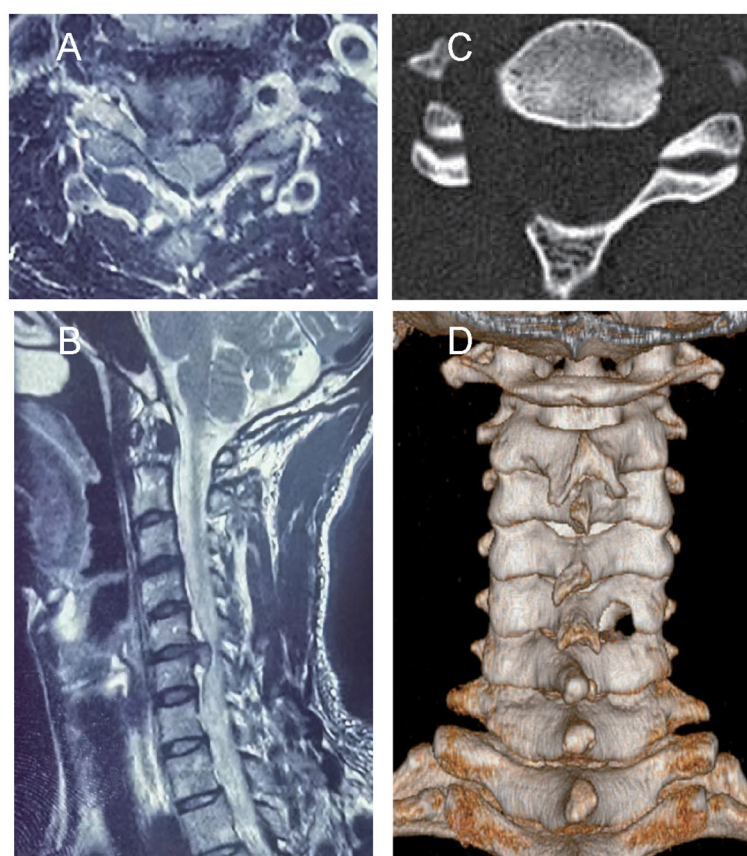


FIGURE 3

A 40-year-old man had cervical 5–6 intervertebral disc herniation, presenting with neck pain and numbness in the right upper limb. (A, B) Preoperative axial and sagittal magnetic resonance images showed C5/6 disk protrusion on the right subarticular zone. (C) Postoperative axial computed tomography scan showed the location of the unilateral foraminotomy. (D) Postoperative 3-dimensional computed tomography scan showed widening of the spinal canal after surgery.

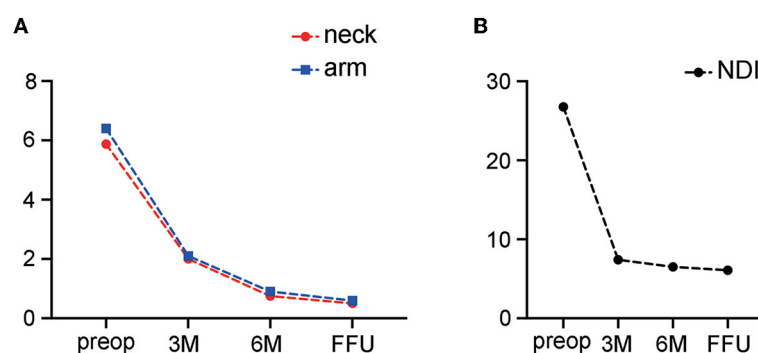


FIGURE 4

At the final follow-up, VAS scores of the neck and arm (A) and the Neck Disability Index (NDI) scores (B) were significantly improved compared to the pre-operation ($P < 0.05$). preop, preoperative; M, month; FFU, final follow-up.

No specific complications occurred during surgery and the immediate postoperative period. No patient required additional surgery for sustained or aggravated symptoms during postoperative hospitalization. No patient had the nerve or vascular injury during surgery, and no patient experienced persistent or aggravated symptoms during the postoperative hospitalization.

Discussion

CSR, mainly manifested as neuropathic radicular pain, is one of the common spinal disorders and usually requires only conservative treatment. However, when conservative treatment fails, surgery is necessary to relieve the symptoms by

removing the compression (8). Generally, ACDF is the primary surgical strategy for CSR with good outcomes, however, it is accompanied by several limitations, including limited neck mobility, adjacent segment degeneration, and high reoperation rate (9–11). In addition, there is conflicting evidence regarding the efficacy of anterior foraminotomy, with reported success rates of 52–99%, but recurrence of radicular symptoms is as high as 30% (12).

With the development of new technology and devices, the application of minimally invasive endoscopic surgery to treat CSR is gradually increasing, with better outcomes and fewer complications compared to ACDF (13–15). A decade ago, percutaneous endoscopic cervical discectomy using a small-sized single portal was introduced, which was reported to not only minimize surgical trauma but also effectively relieve compression (16, 17). However, working and viewing in the same narrow portal manifested several difficulties, such as disorientation and difficulty in maneuvering (18, 19).

Currently, UBE technology represents a relatively recent development in minimally invasive spine surgery (20, 21). Compared with uniportal endoscopic surgery, UBE with two portals has important advantages in improving the scope of the surgical field and the freedom of working devices. Through independent viewing and working portals, the operator can maneuver comfortably with working devices, such as piezosurgery and drills, to expand the range of exploration and decompression. Moreover, independent control of the 30-degree endoscope can enlarge the surgical field of view, which is conducive to the observation of tiny blood vessels and ligaments, thereby reducing iatrogenic blood vessels and neurological injuries. At the same time, the use of continuous irrigation not only provides a clearer view to preventing intraoperative complications but also flushes away inflammatory factors to reduce postoperative pain and the risk of infection (22).

As we all know, the vessels and nerves around the spine are dense and complex. During foraminotomy and discectomy, there is a risk of unintended injury to the nerve root, blood vessel, and dura. In particular, rotary drills are commonly used to remove bone tissue, however, they undoubtedly increase the risk of neurovascular structures due to their rotational power and limited operating space (23). Piezosurgery technique is based on micro-vibrations, which selectively cuts bone tissue and preserve surrounding soft tissue structures (24). Piezosurgery device performs bone cutting by controlled scraping motion without rotational power, which provides precise and safe bone removal procedures with reduced risk to adjacent soft tissue (25). To our knowledge, the present study first combined Piezosurgery device and UBE for cervical foraminotomy and discectomy to optimize surgical precision and reduce surgical risks. A series of foraminotomy and discectomy in 12 patients with CSR was performed, which showed that the postoperative VSA and NDI scores were significantly improved at the final follow-up. No nerve or vascular injury occurred during surgery and the immediate postoperative period. Meanwhile, no patient experienced persistent or aggravated symptoms during the postoperative hospitalization.

Limitations

The current study has several limitations: (1) It was a retrospective non-randomized comparative study; (2) Need a larger number of patients and a longer follow-up period to further confirm the outcomes; (3) The disadvantage of UBE surgery is that it is relatively difficult and requires a long learning curve for the surgeon to gain proficiency.

Conclusions

In summary, UBE foraminotomy and discectomy with piezosurgery is a promising technique for the treatment of cervical spondylotic radiculopathy, which has several advantages, including improvement of pain and functional disability, less blood loss, low hospitalization cost, and preservation of spinal tissue.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Affiliated Hangzhou Chest Hospital, Zhejiang University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: YJ. Methodology: BZ. Validation: MZ. Writing original draft preparation: PZ. Writing-review and editing: XY. Project administration: QZ. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer HQ declared a shared affiliation with the authors at the time of review.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Cook C, Roman M, Stewart KM, Leithe LG, Isaacs R. Reliability and diagnostic accuracy of clinical special tests for myelopathy in patients seen for cervical dysfunction. *J Orthop Sports Phys Ther.* (2009) 39:172–8. doi: 10.2519/jospt.2009.2938
- Hu JH, Chen F, Qiu GX, Sun TS, Yang HL, Shen HY, et al. Jingshu Keli for treating cervical spondylotic radiculopathy: the first multicenter, randomized, controlled clinical trial. *J Orthop Translat.* (2020) 27:44–56. doi: 10.1016/j.jot.2020.10.010
- Jung SB, Kim N. Biportal endoscopic spine surgery for cervical disk herniation a technical notes and preliminary report. *Medicine.* (2022) 101:e29751. doi: 10.1097/MD.00000000000029751
- Puvanesarajah V, Jain A, Shimer AL, Singla A, Shen F, Hassanzadeh H. Complications and mortality following one to two-level anterior cervical fusion for cervical spondylosis in patients above 80 years of age. *Spine.* (2017) 42:E509–14. doi: 10.1097/BRS.0000000000001876
- Yuan H, Zhang XF, Zhang L, M, Yan YQ, Liu YK, Lewandrowski KU. Comparative study of curative effect of spinal endoscopic surgery and anterior cervical decompression for cervical spondylotic myelopathy. *J Spine Surg.* (2020) 6:S186–96. doi: 10.21037/jss.2019.11.15
- Kim J, Heo DH, Lee DC, Chung HT. Biportal endoscopic unilateral laminotomy with bilateral decompression for the treatment of cervical spondylotic myelopathy. *Acta Neurochir.* (2021) 163:2537–43. doi: 10.1007/s00701-021-04921-0
- Zhu CY, Deng XQ, Pan H, Zhang W. Unilateral biportal endoscopic laminectomy with lateral mass screw fixation for treating cervical spinal stenosis. *Acta Neurochir.* (2022) 164:1529–33. doi: 10.1007/s00701-022-05212-y
- Park JH, Jun SG, Jung JT, Lee SJ. Posterior percutaneous endoscopic cervical foraminotomy and discectomy with unilateral biportal endoscopy. *Orthopedics.* (2017) 40:e779–83. doi: 10.3928/01477447-20170531-02
- Epstein NE, A. review of complication rates for anterior cervical Discectomy and fusion (ACDF). *Surg Neurol Int.* (2019) 10:100. doi: 10.25259/SNI-191-2019
- Chung JY, Kim SK, Jung ST, Lee KB. Clinical adjacent-segment pathology after anterior cervical discectomy and fusion: results after a minimum of 10-year follow-up. *Spine J.* (2014) 14:2290–8. doi: 10.1016/j.spinee.2014.01.027
- Shousha M, Alhashash M, Allouch H, Boehm H. Reoperation rate after anterior cervical discectomy and fusion using standalone cages in degenerative disease: a study of 2,078 cases. *Spine J.* (2019) 19:2007–12. doi: 10.1016/j.spinee.2019.08.003
- Tong Y, Huang Z, Hu C, Fan Z, Bian F, Yang F, et al. A comparison study of posterior cervical percutaneous endoscopic ventral bony decompression and simple dorsal decompression treatment in cervical spondylotic radiculopathy caused by cervical foraminal and/or lateral spinal stenosis: a clinical retrospective study. *BMC Musculoskelet Disord.* (2020) 21:290. doi: 10.1186/s12891-020-03313-2
- Bonaldi G, Minonzio G, Belloni G, Dorizzi A, Fachinetti P, Marra A, et al. Percutaneous cervical discectomy: preliminary experience. *Neuroradiology.* (1994) 36:483–6. doi: 10.1007/BF00593690
- Chiu JC, Hansraj KK, Akiyama C, Greenspan M. Percutaneous (endoscopic) decompression discectomy for non-extruded cervical herniated nucleus pulposus. *Surg Technol Int.* (1997) 6:405–11.
- Knight MT, Goswami A, Patko JT. Cervical percutaneous laser disc decompression: preliminary results of an ongoing prospective outcome study. *J Clin Laser Med Surg.* (2001) 19:3–8. doi: 10.1089/104454701750066875
- Ruetten S, Komp M, Merk H, Godolias G. Full-endoscopic cervical posterior foraminotomy for the operation of lateral disc herniations using 59-mm endoscopes: a prospective, randomized, controlled study. *Spine.* (2008) 33:940–8. doi: 10.1097/BRS.0b013e31816c8b67
- Ruetten S, Komp M, Merk H, Godolias G. A new full-endoscopic technique for cervical posterior foraminotomy in the treatment of lateral disc herniations using 69-mm endoscopes: prospective 2-year results of 87 patients. *Minim Invasive Neurosurg.* (2007) 50:219–26. doi: 10.1055/s-2007-985860
- Ahn Y. Percutaneous endoscopic decompression for lumbar spinal stenosis. *Expert Rev Med Devices.* (2014) 11:605–16. doi: 10.1586/17434440.2014.940314
- Minamide A, Yoshida M, Yamada H, Nakagawa Y, Maio K, Kawai M, et al. Clinical outcomes of microendoscopic decompression surgery for cervical myelopathy. *Eur Spine J.* (2010) 19:487–93. doi: 10.1007/s00586-009-1233-0
- Choi CM, Chung JT, Lee SJ, Choi DJ. How i do it? biportal endoscopic spinal surgery (BESS) for treatment of lumbar spinal stenosis. *Acta Neurochir.* (2016) 158:459–63. doi: 10.1007/s00701-015-2670-7
- Ahn JS, Lee HJ, Choi DJ, Lee KY, Hwang SJ. Extraforaminal approach of biportal endoscopic spinal surgery: a new endoscopic technique for transforaminal decompression and discectomy. *J Neurosurg Spine.* (2018) 28:492–8. doi: 10.3171/2017.8.SPINE17771
- Soliman HM. Irrigation endoscopic discectomy: a novel percutaneous approach for lumbar disc prolapse. *Eur Spine J.* (2013) 22:1037–44. doi: 10.1007/s00586-013-2701-0
- Chanda A, Nanda A. Retrosigmoid intradural suprameatal approach: advantages and disadvantages from an anatomical perspective. *Neurosurgery.* (2006) 59:ONS1–6. doi: 10.1227/01.NEU.0000220673.79877.30
- Vercellotti T. Technological characteristics and clinical indications of piezoelectric bone surgery. *Minerva Stomatol.* (2004) 53:207–14.
- Vercellotti T. Piezoelectric surgery in implantology: a case report—a new piezoelectric ridge expansion technique. *Int J Periodontics Restorative Dent.* (2000) 20:358–65.



OPEN ACCESS

EDITED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan
Medical College, China

REVIEWED BY

Emmanuel Estrella,
University of the Philippines Manila, Philippines
Jie Ma,
Shanghai University of Traditional
Chinese Medicine, China

*CORRESPONDENCE

Zuobing Chen
✉ czb1971@zju.edu.cn

[†]These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 30 October 2022

ACCEPTED 13 March 2023

PUBLISHED 17 April 2023

CITATION

Li H, Chen J, Wang J, Zhang T and
Chen Z (2023) Review of rehabilitation
protocols for brachial plexus injury.
Front. Neurol. 14:1084223.
doi: 10.3389/fneur.2023.1084223

COPYRIGHT

© 2023 Li, Chen, Wang, Zhang and Chen. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Review of rehabilitation protocols for brachial plexus injury

Haijun Li[†], Jinxiu Chen[†], Juehan Wang[†], Tianfang Zhang and Zuobing Chen*

Department of Rehabilitation Medicine, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Brachial plexus injury (BPI) is one of the most serious peripheral nerve injuries, resulting in severe and persistent impairments of the upper limb and disability in adults and children alike. With the relatively mature early diagnosis and surgical technique of brachial plexus injury, the demand for rehabilitation treatment after brachial plexus injury is gradually increasing. Rehabilitation intervention can be beneficial to some extent during all stages of recovery, including the spontaneous recovery period, the postoperative period, and the sequelae period. However, due to the complex composition of the brachial plexus, location of injury, and the different causes, the treatment varies. A clear rehabilitation process has not been developed yet. Rehabilitation therapy that has been widely studied focusing on exercise therapy, sensory training, neuroelectromagnetic stimulation, neurotrophic factors, acupuncture and massage therapy, etc., while interventions like hydrotherapy, phototherapy, and neural stem cell therapy are less studied. In addition, rehabilitation methods in some special condition and group often neglected, such as postoperative edema, pain, and neonates. The purpose of this article is to explore the potential contributions of various methods to brachial plexus injury rehabilitation and to provide a concise overview of the interventions that have been shown to be beneficial. The key contribution of this article is to form relatively clear rehabilitation processes based on different periods and populations, which provides an important reference for the treatment of brachial plexus injuries.

KEYWORDS

brachial plexus injury, rehabilitation, neuropathic pain, neonatal brachial plexus injury, treatment

1. Introduction

Brachial plexus injury (BPI) is one of the most serious peripheral nerve injuries, with severe physical disabilities and long-term financial and psychological consequences. Epidemiological studies conducted in both developing and developed countries have shown that brachial plexus injuries are very common in productive young men. Closed injury is the most common type of injury, caused primarily by strains, fractures, and compression injuries, medical injuries are also quite common (1). When a newborn sustains a brachial plexus injury, it is referred to as a neonatal brachial plexus injury (NBPI), which is caused primarily by a birth incident. The brachial plexus nerve is a complex neural component that arises from the anterior branches of the cervical (C5-8) and thoracic (T1) segments of the spinal cord and creates an interlocking network that innervates the muscles of the upper extremities and transmits cutaneous sensation. It is challenging to pinpoint the damage precisely because of the intricate structure of brachial plexus nerve. Surgery and neurological rehabilitation employ distinct methods and struggle to

provide consistent outcomes. Myelography methods and neurophysiological exams have steadily evolved in recent years. Simultaneously, surgical procedures such as nerve repair and grafting have increasingly matured. There is currently no comprehensive rehabilitation program available for individuals with various degrees of brachial plexus injury. To create a more comprehensive treatment plan, we will incorporate the most relevant and successful rehabilitation therapy approaches for various timeframes. To serve as a reference for the clinical management of brachial plexus injury, we will also briefly discuss the fundamental rehabilitation treatments for each period.

2. Brachial plexus injury: Neural localization and clinical symptoms

Brachial plexus injuries (Figure 1) fall under many categories. However, functional placement is more advised since sensory-motor reconstruction and muscle balance restoration are the main goals of therapy after brachial plexus injury. When the affected nerve is C5 and C6, it is categorized as “superior trunk dissection,” which is characterized by impaired shoulder and elbow flexion, abduction, and external rotation while hand function is preserved, and is also known as Duchenne-Erb syndrome. When the involved nerve is C7, it is categorized as “middle trunk dissection.” When the involved nerve is C8 and T1, it is categorized as “inferior trunk dissection,” which is characterized by the impaired hand and wrist function, also known as Dejerine-Klumpke syndrome. Complete brachial plexus palsy (C5-T1) affects the function of the entire arm, resulting in a fully flaccid arm. When this condition is coupled with an eye injury, it is referred to as Horner's syndrome.

3. Adult brachial plexus injury rehabilitation

The management of BPI depends on the type, location, and severity of the injury. It is usually determined by magnetic resonance imaging and/or computed tomography myelography. Surgeons now agree that the best time for surgery is 3–6 months after injury; traction injuries usually heal spontaneously within 3–6 months, and surgical exploration may cause secondary injury, so surgical intervention is recommended if nerve function has not recovered by the end of the spontaneous recovery period (2). Complete tears, ruptures, and lacerations typically do not recover within this time range; hence, early surgical intervention is advised in place of this. Unsurprisingly, rehabilitation, including neurotrophic therapy, is the best course of action before surgery. Following surgery, rehabilitation treatment can aid in the restoration of neurological function. Early intervention for rehabilitation is necessary since brachial plexus injury, with or without surgery, since BPI will cause some degree of muscle loss in the upper extremity and thus can inevitably culminate in fast muscular atrophy. Promoting nerve regeneration and functional recovery while safeguarding and regaining function in joints and intact muscles is the main objective of brachial plexus injury rehabilitation. Based on this premise, subsequent abnormalities such as muscle atrophy, joint stiffness, and limited range of motion are all minimized to the greatest extent feasible.

3.1. Rehabilitation in the spontaneous recovery period

Axonal growth occurs at a pace of approximately 1 mm every day. The basic objectives of the spontaneous recovery period following brachial plexus injury are to ensure adequate muscle nutrition, preserve muscle function, and promote nerve regeneration until the nerve reaches the periphery because it takes a long time for nerves to reinnervate.

3.1.1. Pain management

Pain is present at all stages of brachial plexus injury, including acute neuromuscular injury pain, postoperative pain in the operative area, and chronic neuropathic pain, and it significantly reduces patients' rehabilitation compliance, mental health, and quality of life. It should be addressed as soon as it is identified. A review by Tom et al. for the functional assessment of brachial plexus injury recommends the use of the Patient Reported Outcome Measure (PROM) preferably for those patients who can report pain autonomously, and the commonly used Visual Analog Scale (VAS) and Numeric Rating Scale (NRS) are also recommended (3). However, there is no efficacy analysis of different pain assessment scales for brachial plexus injury. The various types of pharmaceuticals listed in the overview of pain management after complicated nerve injury (4), such as central analgesics, psychiatric medications, narcotics, and hormones, can be used by both surgical and non-surgical patients. Oral and local injections are just two examples of administration methods. Given that pain is subjective and frequently accompanied by psychological illnesses, psychotherapy, and psychotropic medications may occasionally provide further advantages with lingering effects (5). Yun et al. have demonstrated that continuous interscalene brachial plexus block (CISB) has better effective analgesia, better sleep quality and causes fewer opioid-related complications than single interscalene brachial plexus block (SISB) (6). There have not been many studies comparing the efficacy of various drugs and administration methods. Psychotherapy, along with physiotherapy, exercise therapy, invasive surgery, and others, is one of the non-pharmacological strategies that, when combined with pharmacotherapy, form a comprehensive pain treatment. It is critical to understand that medication is not a substitute for non-drug approaches. Furthermore, while chronic neuropathologic pain is commonly challenging to manage with medication, it can still be used to provide short-term relief in acute nerve injury pain or postoperative pain. Neurostimulation has become increasingly effective for chronic neuropathic pain in recent years, in addition to surgical treatments such as nerve transfer and neuroma excision. Thalamic DBS, for example, has proven to be an effective treatment option for patients suffering from severe and medically incurable pain (7). However, studies on this kind of neural stimulation typically do not include a lot of instances, and there is not a widely accepted standard stimulation paradigm due to the heterogeneity of different stimulation parameters. Future research should be expanded upon, it is advised.

3.1.2. Motor reeducation

Strong external fixation support of the damaged limb is necessary before commencing activities. When shoulder and elbow movements are compromised, shoulder straps and figure-eight bandages can be utilized to restrict the uncontrolled movement of paralyzed joints.

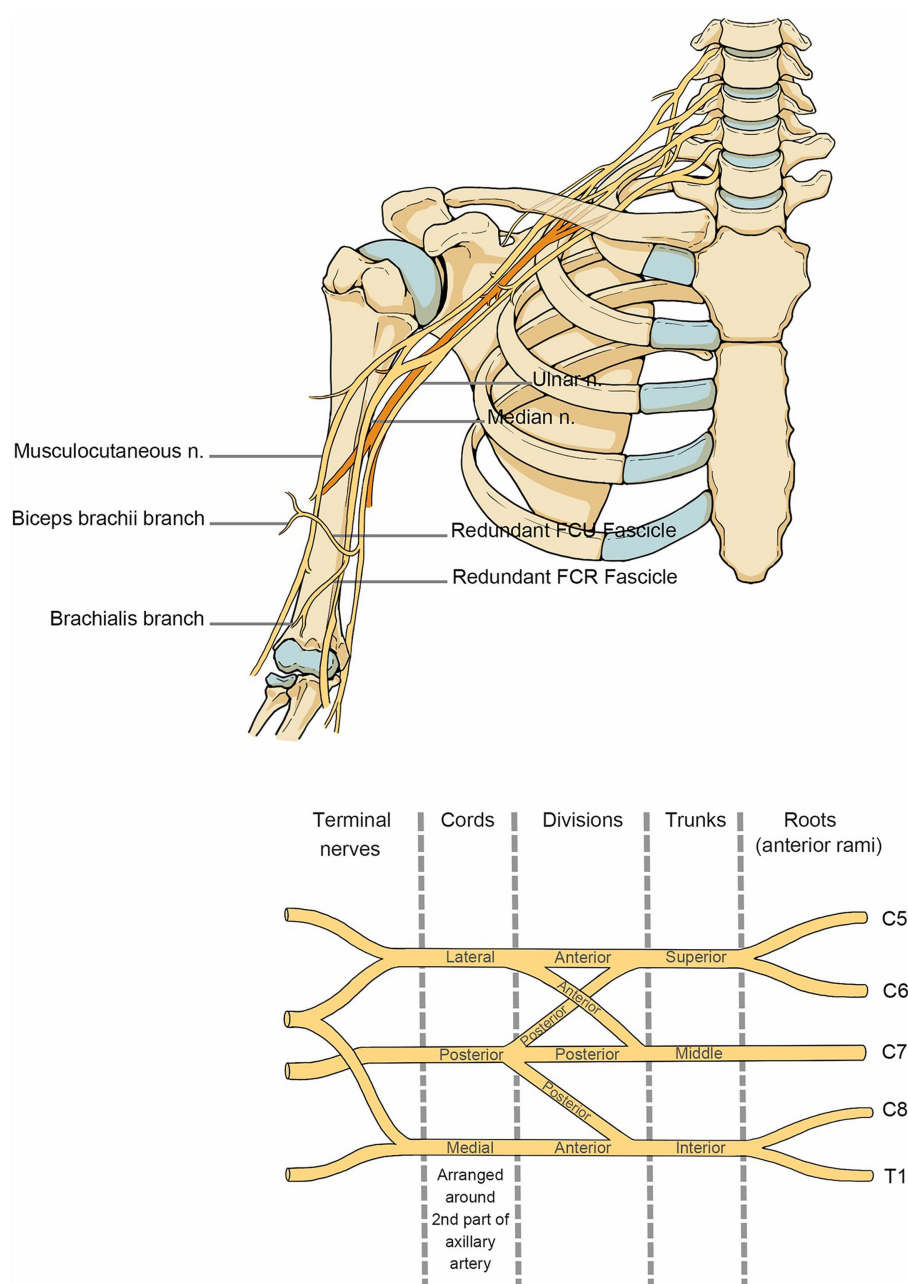


FIGURE 1

Anatomy of the brachial plexus nerve. The anatomical course of the brachial plexus nerve in the shoulder, neck and upper arm.

When the wrist joint is affected, a splint can be utilized to maintain the wrist in an extended position for 10–20 degrees, assisting in the prevention of joint contracture and easing the patient's pain (8). The advantages of various types of external fixation have not been studied; thus, the choice may be determined based on the medical profile and tolerance level of the patient.

There is a large amount of literature on motor re-education after brachial plexus injury. Typical therapies include passive range of motion, active-assisted range of motion, active range of motion, and strength, according to a systematic review based on proprioceptive neuromuscular facilitation (PNF) theory (9). This is a progressive procedure that might start with a determination of the strength of the

muscles in the upper extremities and the choice of an adequate range-of-motion recovery based on strength grading. The therapeutic concepts indicated in various treatment reports are summarized as follows (10, 11): When the patient has no muscular contraction, a passive range of motion is employed. When the patient maintains sufficient muscular contraction, an active-assisted range of motion is employed. As muscular strength grows, the therapist's function in joint range of motion is eventually superseded by the patient, and resistance rises.

In comparison to simulative activities or pointless exercise, a systematic study revealed that involvement in purposeful activities for upper extremity motor therapy regularly leads to a greater quantity

and quality of muscle building (12). Occupational therapy is frequently utilized after brachial plexus injuries and is based on deliberate and targeted occupational exercises to enhance the patient's capacity to adapt to daily life and expedite the patient's return to family and society. A prospective longitudinal study confirmed that postoperative specialist occupational therapy increased motor recovery and upper extremity function in patients following BPI, involving occupational therapy such as folding towels, drinking water, lifting plastic bags, cutting with utensils, etc. (13). The profession of occupational therapy for patients with brachial plexus injuries is not standardized. Nonetheless, it would be beneficial to design more occupational forms that incorporate bending the elbow, bending the shoulder, and extending the wrist, as many doctors expect, which will help the patient regain their capacity to live independently (14). Furthermore, the therapist presses and pulls on the affected limb, or the patient voluntarily touches the object, providing some tactile stimulation that contributes to activity-based sensory recovery. Better outcomes will come from including exposure to different objects at various temperatures during the movement treatment.

3.1.3. Sensory reeducation

Brachial plexus injury can cause sensory deficiencies in the upper extremities, including sensory anomalies and hypoesthesia. Standardized tests can evaluate sensory thresholds and functions in both children and adults including the two-point discrimination test, skin pressure thresholds, vibration perception, and assessments of pain and tactile sensations using needles and cotton (15). Patrick et al. summarized seven rehabilitation techniques for peripheral nerve injuries based on the theory of cortical remodeling. The sensory reeducation component comprises traditional sensory reeducation, activity-based sensory reeducation, mirror visual feedback, cross-modal sensory substitution, and selective deafferentation (16). The above was a description of activity-based sensory re-education. Selective deafferentation is not recommended because it could affect the sensory input of the healthy side.

Traditional sensory re-education involves reestablishing impaired afferent sensory pathways through interactions with the environment to subsequently restore more discriminative senses. The European Consensus on Sensory Retraining in Peripheral Hand Neuropathy provides a systematic strategy to promote reeducation of tactile cognition by interacting with different textures, temperatures, forms, and objects (17). Although sensory abnormalities following brachial plexus injury are not just present in the hand, it is still feasible to employ the traditional methods of sensory re-education mentioned above. Traditional sensory re-education has mostly been proven to be successful as a participating component in comprehensive rehabilitation treatment, but there are unfortunately no clinical investigations on its effectiveness with sufficient sample numbers.

Mirror visual feedback (MVF) is the process of superimposing the motion picture of the healthy hand on the location of the affected hand using a mirror that has been placed vertically on a table. This creates a motor-sensory connection between the hands (18). Such visual input promotes upper extremity mobility and sensory rehabilitation in children with Duchenne-Erb syndrome, according to a randomized controlled trial in children ages 6–12 years (19). It is possible to treat adults with the same technique. However, a randomized controlled trial with insufficient information on sensory or motor recovery found that MVF combined with TDCS over

3 months may lessen neuropathic pain after brachial plexus injury (20). Chen et al. demonstrated the effectiveness of MVF in sensory and motor reeducation, observing improved learning in hand and finger dexterity compared to traditional sensory. Additionally, patients who used MVF demonstrated increased activation in ipsilateral brain areas and the multimodal association cortex on fMRI (21). Unfortunately, Chen only examined 6 cases, which is not representative, and it is advised that future research include more cases and additional follow-up. MVF is a potential therapy for sensory re-education with brachial plexus injury in adults, although its efficacy has not been explicitly validated and requires additional investigation.

Cross-modal sensory replacement translates tactile information across several modalities using sensing technologies (22). Patients must acquire extra sensor systems, which are not commonly employed in the treatment of brachial plexus injuries. It appears to be a possibility to explore persistent sensory abnormalities with brachial plexus injury. We are delighted to see sensing technologies advance and become more widely used.

3.1.4. Neurotrophic treatment

Once brachial plexus injury has been diagnosed, neurotrophic treatment will be used continually and regularly. B vitamins, mecobalamin (23), citicoline, and other pharmaceuticals that are routinely used in clinical practice are the principal neurotrophic medications. Exogenous neurotrophic factors are also a research hotspot in animal studies due to their extensive effectiveness, low risk of side effects, and high level of safety. It has been demonstrated that topical anti-NGF therapy may reduce chronic neuropathic pain, whereas early topical administration of NGF and CNTF-like medicines may assist in preventing the degeneration of injured nerves (24). Regrettably, there have been fewer exogenous neurotrophic factor clinical medication studies overall, and only enkephalin has received marketing approval. A more comprehensive list of neurotrophic factors with potential neuroprotective effects on motor neurons has been published in research and may be a viable pharmaceutical therapy for brachial plexus nerve injury (25). Furthermore, additional clinical investigations are needed to prove the effectiveness of medications such as gangliosides, hormones, Chinese herbs, etc.

3.1.5. Modalities

Numerous studies have demonstrated the effectiveness of early modalities in reducing swelling, promoting edema absorption, relieving pain, and releasing adhesions. This therapy can be applied in a variety of settings, including ultrashort wave, infrared, and low-energy laser irradiation (15).

Electrical nerve stimulation promotes nerve healing and regeneration and is a useful tool. Depending on the stimulation site, neural electrical stimulation can be classified as deep brain stimulation (DBS), transcutaneous neuromuscular electrical stimulation (NMES), spinal cord stimulation, and transcranial direct current stimulation (tDCS) (26). Neuromuscular electrical stimulation, which is frequently utilized for motor and sensory reeducation in the treatment of children and adults with brachial plexus injuries, has been demonstrated to excite both motor nerve fibers and afferent sensory nerve fibers (27). In contrast, DBS, TDCS, and spinal cord stimulation are more commonly applied to alleviate neuropathic pain. The stimulation techniques mentioned above may need to rely on relatively

unharmful nerve-down routes to effectively stimulate peripheral nerves, particularly in individuals with diminished fine hand function following brachial plexus injury. Given the aforementioned, there is a huge market and development potential for an implanted peripheral nerve stimulator that could work directly on nerve branches in the axilla while also providing long-term relief from peripheral neuropathic pain following brachial plexus injury (28).

In addition, transcranial magnetic stimulation (TMS) is often used to collect motor-evoked potentials to assess neurological activity or to treat neuropathic pain as a complement to Noninvasive electrical brain stimulation (29). It is worth mentioning that the strong magnetic field generated by TMS can be employed for peripheral neuromuscular regulation in addition to agitating the central nervous system. Consequently, TMS has the potential to replace transcutaneous neuromuscular electrical stimulation with a technique that has a higher safety profile, and it may also represent the future of neuromagnetic stimulation research.

The benefit of neuro-electromagnetic stimulation in the treatment of neuropathic pain, which may be regularly utilized in pain management, should not be overlooked. However, further clinical investigations and systematic assessment research are required to better understand the suggested paradigms due to the diversity of stimulation paradigms resulting in varying effectiveness.

3.1.6. Traditional Chinese medicine treatment

Due to their capacity to stimulate the repair and regeneration of injured nerves, enhance local blood circulation in the treated area, and lessen postoperative pain, acupuncture and tui na are widely used in traditional Chinese medicine to treat brachial plexus nerve injury. Whereas using tui-na or acupuncture alone is preferable to using medication isolated, the combined rehabilitation impact of both with other treatment modalities is noticeably superior to using tui-na or acupuncture alone. Direct comparisons of various tui na massage or acupuncture point therapies are also scarce. Electroacupuncture was initially shown to be more successful than medication therapy alone in treating 54 individuals with peripheral nerve damage in 1995 (30). A further animal investigation revealed that electroacupuncture therapy may protect against brachial plexus injury by lowering the expression of nNOS and slowing the degeneration of injured neurons (31). A series of case reports that were conducted afterward also supported this finding, making the efficacy more solid. Electroacupuncture can be suggested as a conservative treatment for brachial plexus injury in the absence of pertinent contraindications.

3.1.7. Psychosocial support

Emotional and social factors may influence rehabilitation after brachial plexus injury, particularly in a productive young male population. Their high expectations of efficacy lead to an inability to accept the possibility of permanent disability and the long recovery time required for neurological recovery, making them prone to psychological disengagement, denial, and venting behaviors in the later stages of treatment, and highly cooperative in the earlier stages. As a consequence, it is essential to regularly monitor patients' adherence to treatment during the later phases of recovery, prevent dangerous behaviors, and promptly offer psychosocial assistance such as psychotherapy, psychiatric medication, and family support. For the time being, there is no systematic psychological rehabilitation strategy

for brachial plexus injury, and few studies on psychological rehabilitation have been conducted. Studies on psychological aspects in brachial plexus injury patients have demonstrated that these individuals typically employ adaptive coping strategies and self-distraction in the early phases of their recovery to deal with negative psychology (32). Therefore, we investigated pertinent psychological rehabilitation models and came to the conclusion that active positive mindfulness therapy, such as meditation, body scanning, and other forms of active positive mindfulness, can support the above-mentioned positive psychological strategies and lead to a positive psychological state, which reduces stress and eradicates depression. Future research on the effects of various social support measures on the functional rehabilitation and psychological condition of brachial plexus injury patients is advised.

3.2. Rehabilitation in the acute postoperative period

Before starting a structured rehabilitation program after surgery, the muscles and nerves must have had enough time to recover. Controlling pain and edema in the acute postoperative period is the main therapy objective (9). Postoperative patients with complaints of pain might involve pain management because quick pain relief can enhance the patient's psychological circumstance and speed up recovery. Potential pain relief options include opioids, topical analgesic patches, antiepileptic drugs, antidepressants, and nerve electrical stimulation, according to a systematic review of pain management for complicated nerve injuries (4). There is limited graded pain evaluation after brachial plexus injury, resulting in a wide range of pain management therapy options. Procedures for recommended pain rehabilitation management are not arranged in a hierarchical order. It is critical to highlight that pain control must be utilized throughout the brachial plexus injury treatment procedure.

Additionally, there is general agreement to start early passive mobility of the proximal and distal joints of the trauma and to locally immobilize the nerve anastomosis site in the acute postoperative phase. The purpose is to safeguard the surgical site, promote nerve gliding, and eventually reduce pain and encourage functional recovery. The best period for postoperative fixation is not universally agreed upon; however, it is crucial to hold off until the nerve anastomosis site is tension-free, particularly after repairing a nerve with significant local tension. Based on randomized controlled research of comprehensive postoperative therapy in patients with brachial plexus injuries, the passive motion of the joint within an unrestricted range of motion is advised and needs to be combined flexibly depending on the type of surgery. In conclusion, it is critical to consider the functional recovery of the primary injury site as well as the remaining neuromuscular functional compensation after a surgical transfer, such as after a phrenic nerve transfer to the anterior branch of the superior brachial plexus, which requires both flexions of the elbow joint and deep inspiration (33). Numerous randomized controlled studies have advised thorough rehabilitation following surgery using therapeutic modalities that do not significantly diverge from those performed in spontaneous recovery (34).

3.3. Rehabilitation in the sequela period

Even with complete rehabilitation and satisfactory neurological recovery, associated complications, including pain, muscle atrophy, restricted joint mobility, and subsequent abnormalities, are still possible (15). Considering that complete alleviation of brachial plexus injury is challenging with rehabilitation alone in the sequela period, surgical procedures such as neuroma removal to reduce pain and latissimus dorsi tendon grafting to the rotator cuff to address shoulder mobility limitations are typically employed (35). Postoperative rehabilitation strategies for sequelae are not contradictory and may be adapted to acute postoperative rehabilitation for brachial plexus injuries. The distinction is that patients need to be reevaluated for postoperative immobilization duration and joint range of motion since they are generally related to a loss in muscle performance and joint function. Regrettably, there is no clinical consensus on these specifics (36). Treatment methods may rely on the expertise of experienced physicians and therapists. Power-assisted orthoses can offer a functional replacement in the end phase and have some effectiveness in motor re-education when the surgical benefit is not preferably factored in (37). Rehabilitation of brachial plexus injuries in the sequela period seems to be a neglected area that needs further development.

4. Neonatal brachial plexus injury rehabilitation

Clinical rehabilitation and research on brachial plexus injuries in children have been widely conducted, particularly in children aged 1–12 years. However, the rehabilitation treatment of neonatal brachial plexus injury (NBPI) confronts challenges such as poor patient compliance, difficult efficacy assessment, and high family participation, resulting in slow advancement and becoming an insurmountable barrier for brachial plexus injury. It's the fact that some demographics indicate a gradually declining frequency of brachial plexus injury following a neonatal birth injury, because of fewer forceps used (38). First, neonatal brachial plexus injuries can cause long-term chronic damage and necessitate early intervention for rehabilitation; second, a high level of home rehabilitation demands specific rehabilitation instructions for parents in preparation. The basic stage in diagnosing NBPP is clinical evaluation and physical examination by an expert doctor. Next, fewer intrusive procedures, frequently magnetic resonance imaging (MRI) and ultrasound scanning (US), are needed. If the diagnosis is still elusive, electrodiagnosis can be utilized (39). The anatomy of a neonate (0–28 days) is less developed than that of an adult; thus, precise localization is more crucial than in adults. Knowing the location and severity of a nerve injury can also help to accurately guide reconstruction procedures and provide advice to families.

For neonates with a clear diagnosis, early referral to an integrated multidisciplinary management model is advised by several systematic reviews of NBPP rehabilitation. The fundamental rehabilitation strategies advised are parallel to those for adults, which include treatments such as passive/active motor exercises, smooth joint motor, sensory stimulation, electrical stimulation, and botulinum toxin injections (40). To serve as a reference, we evaluated the available literature and collected the therapy recommendations that, when

applied to newborn brachial plexus injury rehabilitation: firstly, cooperation between the family and the newborn should be considered when selecting a rehabilitation process. As the family rehabilitation model predominates and is more suitable for the care of the infant, simple, straightforward, and repeatable treatments can be selected to allow parents to perform rehabilitation therapy numerous times during the day; Secondly, rehabilitation should intentionally select to encourage active movement to restore function to the injured limb rather than compensating for the healthy side by emphasizing developmental neglect and misuse related to neonatal growth and development (41). In conclusion, there is variability in the efficacy of different treatment methods, which calls for more study to clarify. The agreement on a comprehensive rehabilitation process for NBPP continues to be in the early stages of development.

5. Discussion

The functional recovery of brachial plexus injury is a worldwide challenge. This study compiled the various rehabilitation modalities for clinicians, arranged them into rehabilitation protocols from the time a brachial plexus patient was seen until clinical remission (Figure 2) and enabled the systematic management of BPI patients. A fairly comprehensive evaluation of physical therapy for brachial plexus injuries has been conducted (42). On this basis, we have closely followed the latest advances in current clinical research on brachial plexus injury rehabilitation, and we summarize its intervention strategies and outcomes, details see Table 1.

Currently, a significant proportion of patients with brachial plexus injury are young adults and newborns, and these patients usually have an expectation of immediate recovery. However, brachial plexus injury typically requires a lengthy recovery period due to the complexity of the injury and the slow recovery tempo. During long-term rehabilitation, the degree of neurological recovery (including motor and sensory recovery, etc.), psychological status, and complications of the affected limb vary widely, requiring the clinician to develop an individualized rehabilitation program, which requires a great deal of experience. Therefore, patients may be in various stages of rehabilitation at the time of consultation, and there is no clinical consensus on how to manage patients in each stage in an integrated manner, making it easy to overlook the various aspects of rehabilitation principles that require attention at various stages of disease development. We will do some sorting later to fill in the gaps in this section.

The efficacy of comprehensive rehabilitation therapy is well established. For patients with brachial plexus who do not need emergency surgery and proceed to the spontaneous recovery stage of rehabilitation. Local external fixation support, motor and sensory re-education, and neurotrophic therapy are common alternatives. When specific treatment requirements are met, physical factor therapy, traditional Chinese medicine treatment, psychosocial support, and other supplementary treatment methods can be applied. For patients undergoing postoperative acute rehabilitation postemergency surgery, passive movement of the afflicted limb and nerve removal site, with suggested mobility within the unrestricted joint range, can be used to reduce pain and edema. For further rehabilitation, conservative therapy options available throughout the spontaneous recovery period may be selected. It is impossible to

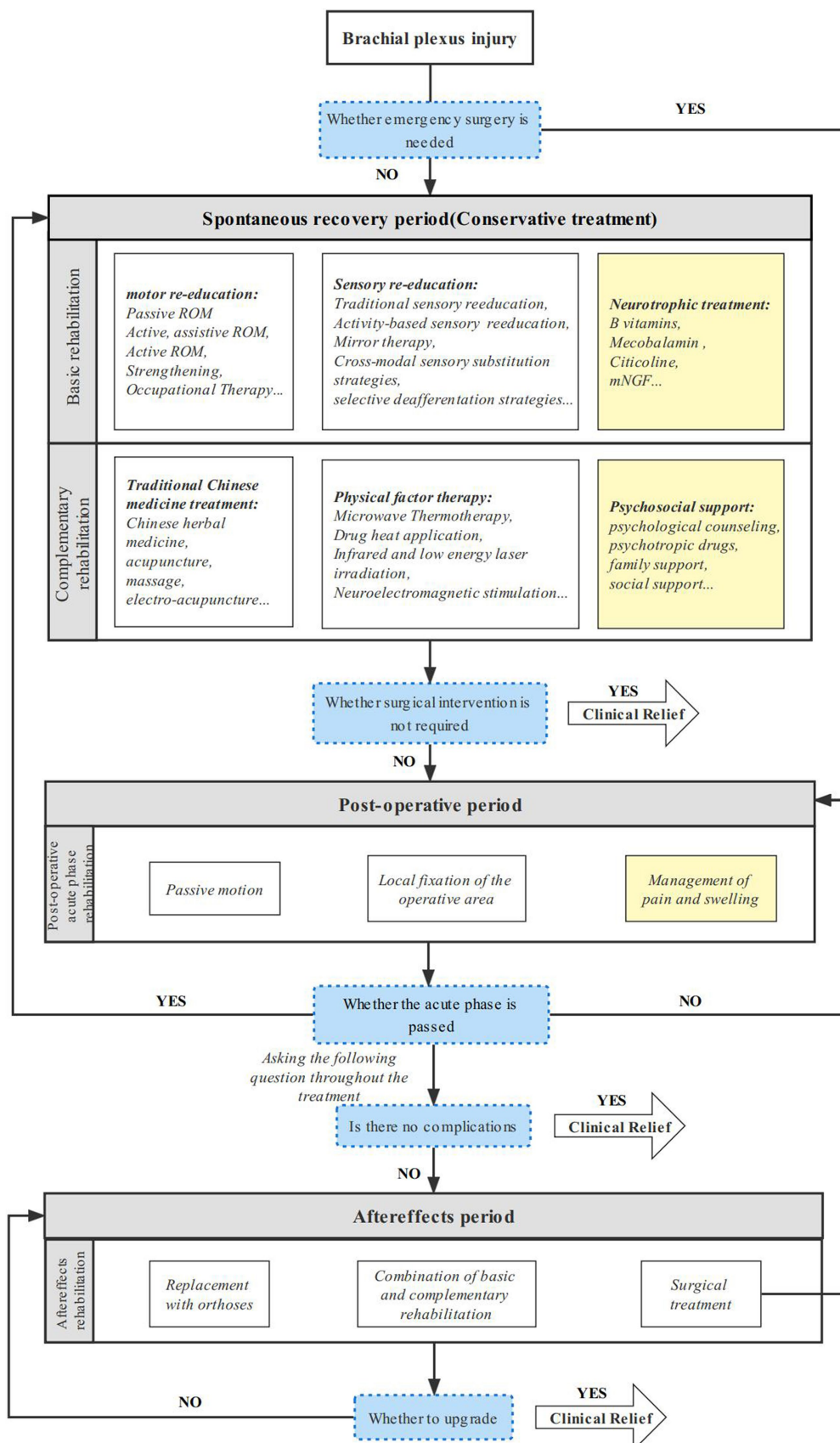


FIGURE 2

Comprehensive rehabilitation protocol for brachial plexus injury. A sustainable and comprehensive rehabilitation protocol for adult brachial plexus injury in different periods.

TABLE 1 Characteristics of the clinical studies on rehabilitation of brachial plexus injuries published in recent years.

Author/year	Study location	Study design/ population	Level/time of injury	Intervention	Main outcomes
(46)	Institute for Nursing and Interprofessional Research, Children's Hospital Los Angeles, Los Angeles, CA	Randomized crossover trial $n = 21$ (11 males, 10 females) Age = 25 ± 10.3 mo	Unilateral NBPP uninformed	Group CMT: 3 weeks of casting the unaffected limb followed by 5 weeks of transference activities. Group standard: usual occupational therapy for 8 weeks	Assisting Hand Assessment (AHA) The Pediatric Motor Activity Log-Revised (PMAL-R) Group CMT is favored over group standard care for bimanual activity performance.
(47)	University Institute of Physical Therapy, University of Lahore, Lahore, Pakistan	Prospective randomized clinical trial $n = 66$ Unrealized	Uninformed <3-month	Group 1: ultrasound therapy 2 days a week for 6 weeks (using a pulsed mode 0.8 W/cm^2 and frequency 1 MHz), wrist splinting, tendon gliding exercises Group 2: ultrasound, splinting, tendon gliding exercises, a neuromobilization technique.	ROM, manual muscle strength, pain at NPRS, functional status on the Boston Carpal Tunnel Syndrome Questionnaire (BCTQ) -symptom severity scale (SSS), functional status scale (FSS) Group 2 showed a statistically more significant increase in flexion, extension, decrease in pain, decrease in SSS, decrease in FSS, and BCTQ as compared to the group 1.
(21)	School and Graduate Institute of Physical Therapy, College of Medicine, National Taiwan University, Taipei, Taiwan	Prospective randomized clinical trial $n = 6$ Uninformed	Median and/or ulnar nerve injury Uninformed	Group 1: mirror-therapy (using mirror reflection of the unaffected hand in order to train the affected hand) Group 2: sensory-relearning	<u>Clinical</u> : Semmes Weinstein Monofilaments (SWM) test, static 2-point discrimination test (S-2PD), grip strength, the Disabilities of the Arm, Shoulder and Hand (DASH) scores <u>Radiographic</u> : fMRI Group 1 had better outcomes in finger dexterity and manual dexterity, and fMRIs showed greater activation in the multimodal association cortices and ipsilateral brain areas during motor tasks.
(48)	School of Physiotherapy, King Edward Medical University, Lahore	Prospective double blinded randomized control trial $n = 66$ Age = 37.79 ± 5.91 years	Carpal tunnel syndrome <3-month	Group 1: ultrasound therapy, wrist splinting, tendon-gliding exercises Group 2: ultrasound therapy, wrist splinting, tendon-gliding exercises, neuromobilisation. Wrist splinting: the wrist was repeatedly moved into and out of stretch by performing a few degrees of flexion and extension at the wrist.	Boston Carpal Tunnel Syndrome Questionnaire (BCTSQ)--symptom severity scale (SSS), functional status scale (FSS) Group 2 showed better index of BCTSQ compared to Group 1
(45)	Department of Rehabilitation, Leiden University Medical Center, Leiden, Netherlands	Randomized controlled trial $n = 55$ Unrealized	Elbow flexion contracture of $\geq 30^\circ$ uninformed	Group 1: night-worn dynamic orthosis for 1 year Group 2: serial casting for 1 year	Elbow flexion contracture Goal attainment scaling Both treatment options showed a comparable reduction in the elbow flexion
(49)	Mexican Faculty of Medicine of La Salle University, Mexico City, Mexico	Prospective, longitudinal, nonrandomized, self-controlled before and after study $n = 10$ Age = 34 ± 16.85 years	BPI of traumatic etiology, high level of injury (proximal third of the upper extremity), and refractory neuropathic pain (medical treatment with at least 2 different analgesic drugs during 3 months of management) Uninformed	Surgical neurolysis	Visual Analog Scale (VAS) British Medical Research Council (BMRC) scale 9 patients showed improvement in the continuous and paroxysmal pattern of pain.
(6)	Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, Korea	Prospective non-randomized controlled trial $n = 134$ Uninformed	Uninformed	Group 1: Continuous interscalene brachial plexus block (CISB) after arthroscopic surgery for rotator cuff repair Group 2: single interscalene brachial plexus block (SISB) combined with intravenous patient-controlled analgesia (IV-PCA) after arthroscopic surgery for rotator cuff repair	Quality of recovery (QoR-40) score CISB showed a higher quality of recovery score than SISB with IV-PCA in arthroscopic rotator cuff repair

(Continued)

TABLE 1 (Continued)

Author/year	Study location	Study design/ population	Level/time of injury	Intervention	Main outcomes
(50)	The Division of Anesthesia, Intensive Care, and Pain Management, Tel Aviv Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel	Randomized Clinical Trial $n = 59$ Uninformed	Uninformed	Group 1: perioperative oral pregabalin (Lyrica, twice daily starting the evening before surgery, for a total of 4 doses) Group 2: single-shot ISBPB (20 mL of bupivacaine 0.25%)	Median self-reported pain score (on a visual analog scale of 0 to 100) pain during activity, postoperative opioid consumption, opioid-related adverse effects, quality of recovery, and pain satisfaction score Perioperative use of pregabalin in adults undergoing arthroscopic RC repair provided analgesia comparable to that of ISBPB for 10 days after surgery.
(51)	Physical Medicine and Rehabilitation, Health Science University Antalya Training and Research Hospital, Antalya, Turkey	Randomized, prospective clinical study $n = 143$ Uninformed	Clinical features (such as pain and/or numbness in the median nerve distribution or nocturnal pain) >3-month	Group 1: low level laser therapy (LLLT) Group 2: corticosteroid injection	Numbness and pain, QuickDASH questionnaire, grasping tests, Tinel and Phalen tests <u>electrophysiological tests</u> ; Nerve conduction studies (NCS) <u>Radiographic</u> ; MRI
(52)	Centre for Interdisciplinary Research in Rehabilitation of Greater Montreal, Institut universitaire sur la réadaptation en déficience physique de Montréal, Centre intégré universitaire	Randomized controlled study $n = 30$ Age = 54.2 ± 9.2 years	Diagnostic of CTS <i>via</i> an electrodiagnostic test Uninformed	Group 1: four-week home-based neuromobilization exercise group (NEP) Group 2: standard care	<u>Feasibility</u> ; recruitment, attrition, adherence, satisfaction, and safety <u>efficacy metrics</u> ; median nerve integrity and neurodynamics, tip pinch grip, pain, and upper limb functional abilities novel preoperative neuromobilization exercise program has limited beneficial effect.
(53)	Department of Physical Medicine and Rehabilitation, Tri-Service General Hospital, School of Medicine, National Defense Medical Center, Neihu, Taipei, Taiwan, Republic of China	Randomized Double-Blind Clinical Trial $n = 35$ Unrealized	Mild or moderate CTS >3-month	Group 1: one ultrasound-guided perineural injection of 2.5 mL hyaluronic acid (HA) Group 2: 2.5 mL normal saline injection through in-plane, long-axis approach to separate the median nerve from the flexor retinaculum <i>via</i> nerve hydrodissection	Boston Carpal Tunnel Syndrome Questionnaire (BCTQ) scores, e numeric rating scale (NRS), electrophysiological domains, and the cross sectional area of the median nerve A single ultrasound guided perineural HA injection may have short-term therapeutic efficacy for mild or moderate CTS.
(54)	Department of Physiotherapy, Babol University of Medical Sciences, Babol, Iran	Randomized clinical trial $n = 60$ Unrealized	Mild or moderate CTS Uninformed	Group 1: 1500 shocks on the carpal tunnel and conventional physiotherapy for 10 sessions Group 2: 1500 shocks on the carpal tunnel and median nerve pathways and conventional physiotherapy for 10 sessions Group 3: conventional physiotherapy for 10 sessions	Pain and paresthesia intensity sensory and motor distal latency Radial shockwave combined with conventional physiotherapy is an effective noninvasive treatment for mild-to-moderate carpal tunnel syndrome.
(55)	Department of Physical Medicine and Rehabilitation, Tri-Service General Hospital, School of Medicine, National Defense Medical Center, No. 325, Sec. 2, Cheng-Kung Road, Neihu District, Taipei, Taiwan, Republic of China.	Prospective randomized, single-blind trial $n = 47$ Unrealized	Mild or moderate CTS Uninformed	Group 1: short-axis hydrodissection Group 2: long-axis hydrodissection	Boston Carpal Tunnel Syndrome Questionnaire (BCTQ) score, the cross-sectional area of the median nerve and electrophysiological studies. Both short-and long-axis hydrodissection are effective for patients with mild-to moderate CTS
(56)	Department of Rehabilitation, Radboud university medical center, Donders Institute for Brain, Cognition and Behavior, Nijmegen, the Netherlands	Randomized Controlled Trial $n = 27$ Unrealized	Neuralgic amyotrophy (NA) caused by auto-immune inflammation of nerves in the brachial plexus territory Uninformed	Group 1: multidisciplinary rehabilitation Group 2: usual care multidisciplinary rehabilitation: a 16-week outpatient multidisciplinary rehabilitation program involved 1 h of physical therapy and 1 h of occupational therapy per day. 4-weekly treatment sessions followed by two sessions every other week and two monthly sessions.	<u>Clinical</u> : a hand laterality judgment task <u>Radiographic</u> : task-based functional MRI.

totally eliminate complications, and when they do arise, the patient moves into the sequelae phase of rehabilitation. These complications include muscular atrophy, joint stiffness, chronic neuropathic pain,

etc. To accomplish functional compensation and improve the patient's quality of life, an end-stage rehabilitation approach might be adopted, mostly employing vigorous replacement therapy. Neurotrophic

therapy, pain and swelling control, and psychosocial support can all be consistently employed at different phases because pain and psychiatric illnesses might flare up at various periods. Complete recovery from brachial plexus injury is exceptional, but when clinical remission—that is, increased performance and quality of life—is attained, the patient feels sufficiently better to jump their treatment protocol and return to home rehabilitation. This page also provides an overview of the fundamental principles of rehabilitation for generally slow-progressing neonatal brachial plexus injuries, which can help to hasten the development of treatment approaches to this specific demographic problem.

Artificial intelligence and rehabilitation techniques have been developed in the last 3 years. An electroencephalography (EEG)-based human-machine interface combined with contralateral C7 transfer surgery can treat brachial plexus injury, but further research is required to understand how it promotes cortical remodeling (43). Virtual reality and robotics combined to facilitate upper extremity pain management and sensory recovery after brachial plexus injury (44). The very first mention of dynamic orthoses as having a role in motor re-education came in 2018 (37), but more recent research has revealed that dynamic nocturnal orthoses are equally effective as continuous cast immobilization in treating elbow flexion contractures in kids with brachial plexus nerve birth injuries (45). As children's upper extremities move less than those of adults, dynamic orthoses may only serve as a fixation device in children with brachial plexus injuries while offering more motor support in adults. Individual rehabilitation is a constant topic, and AI rehabilitation has a higher degree of adjustability, sure AI involvement will become an unstoppable trend.

The literature review deserves some essential conclusions and insights to be presented. The lack of sufficient case studies, inadequate follow-up time, the lack of a recognized control group, and the propensity to employ comprehensive rehabilitation as an intervention to improve treatment results are more or fewer defects of the literature on brachial plexus injury treatment methods. Therefore, techniques like stem cell therapy that remain at the level of animal trials are not included in the clinical approach presented in this work. Our work excludes the mention of efficient treatment methods in case reports involving a small number of patients but keeps useful comments. This takes into account treatment approaches that have all been shown in numerous case reports to be successful. Randomized controlled studies reporting effective rehabilitation treatment modalities and clinical studies with a long-term follow-up process were considered first. The rehabilitation protocols described in this article are experimental and can be supplemented with additional clinical treatment modalities that show benefits. Unfortunately, due to a lack of quantitative assessment methods, this article only develops a comprehensive

treatment protocol rather than offering a suitable rehabilitation therapy approach for individuals with varying injury severity and locations. The determination of the optimal treatment approach for a specific patient is still left to the discretion of qualified medical staff and therapists. In summary, it is strongly advised that future research focus on the efficiency of potential comprehensive rehabilitation programs in the management of BPI.

6. Conclusion

The efficacy of comprehensive rehabilitation therapy is well established. Physical therapy, traditional Chinese medicine treatment, psychosocial support, and other supplementary treatment methods can be applied when someone undergoes a brachial plexus injury, in addition, artificial intelligence and rehabilitation techniques are popular in these years which need further research. All the rehabilitation protocols we described are experimental and they show benefits as supplemented with additional clinical treatment modalities. In summary, we strongly advised that future research focus on the efficiency of potential comprehensive rehabilitation programs in the management of BPI, which will facilitate the return of patients, physical activity levels.

Author contributions

HL: visualization and methodology. JC: writing—review and editing, conceptualization. JW: writing—original draft, visualization. TZ: supervision. ZC: authors may have contributed in multiple roles. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Kaiser R, Waldauf P, Ullas G, Krajcova A. Epidemiology, etiology, and types of severe adult brachial plexus injuries requiring surgical repair: Systematic review and meta-analysis. *Neurosurg Rev.* (2020) 43:443–52. doi: 10.1007/s10143-018-1009-2
2. Yang LJ, Chang KW, Chung KC. A systematic review of nerve transfer and nerve repair for the treatment of adult upper brachial plexus injury. *Neurosurgery.* (2012) 71:417–29; discussion 429. doi: 10.1227/NEU.0b013e318257be98
3. Quick TJ, Brown H. Evaluation of functional outcomes after brachial plexus injury. *J Hand Surg Eur.* (2020) 45:28–33. doi: 10.1177/1753193419879645
4. Davis G, Curtin CM. Management of Pain in complex nerve injuries. *Hand Clin.* (2016) 32:257–62. doi: 10.1016/j.hcl.2015.12.011
5. Razak I, Chung TY, Ahmad TS. A comparative study of two modalities in pain management of patients presenting with chronic brachial neuralgia. *J Altern Complement Med.* (2019) 25:861–7. doi: 10.1089/acm.2019.0052
6. Yun S, Jo Y, Sim S, Jeong K, Oh C, Kim B, et al. Comparison of continuous and single interscalene block for quality of recovery score following arthroscopic rotator cuff repair. *J Orthop Surg.* (2021) 29:23094990211000142. doi: 10.1177/23094990211000142

7. Abdallat M, Saryyeva A, Blahak C, Wolf ME, Weigel R, Loher TJ, et al. Centromedian-parafascicular and somatosensory thalamic deep brain stimulation for treatment of chronic neuropathic pain: A contemporary series of 40 patients. *Biomedicine*. (2021) 9:731. doi: 10.3390/biomedicine9070731
8. Marsh JL, Mahoney CR, Steinbronn D. External fixation of open humerus fractures. *Iowa Orthop J*. (1999) 19:35–42.
9. Kinlaw D. Pre-/postoperative therapy for adult plexus injury. *Hand Clin*. (2005) 21:103–8. doi: 10.1016/j.hcl.2004.10.003
10. de Oliveira LAS, Pedron CA, de Andrade FG, Horsczaruk CHR, Martins JVP. Motor recovery after bilateral brachial plexus injury using motor irradiation: A case report. *Int J Ther Rehabil*. (2019) 26:1–12. doi: 10.12968/ijtr.2017.0170
11. Chagas ACS, Wanderley D, Barboza PJM, Martins JVP, de Moraes AA, de Souza FHM, et al. Proprioceptive neuromuscular facilitation compared to conventional physiotherapy for adults with traumatic upper brachial plexus injury: A protocol for a randomized clinical trial. *Physiother Res Int*. (2021) 26:e1873. doi: 10.1002/pri.1873
12. Collis JM, Signal N, Mayland E, Wright-St Clair VA. Influence of purposeful activities on upper extremity motor performance: A systematic review. *OTJR*. (2020) 40:223–34. doi: 10.1177/1539449220912187
13. Cole T, Nicks R, Ferris S, Paul E, O'Brien L, Pritchard E. Outcomes after occupational therapy intervention for traumatic brachial plexus injury: A prospective longitudinal cohort study. *J Hand Ther*. (2020) 33:528–39. doi: 10.1016/j.jht.2019.08.002
14. Moore AM, Novak CB. Advances in nerve transfer surgery. *J Hand Ther*. (2014) 27:96–104; quiz 5. doi: 10.1016/j.jht.2013.12.007
15. Smania N, Berto G, La Marchina E, Melotti C, Midiri A, Roncarì L, et al. Rehabilitation of brachial plexus injuries in adults and children. *Eur J Phys Rehabil Med*. (2012) 48:483–506.
16. Zink PJ, Philip BA. Cortical plasticity in rehabilitation for upper extremity peripheral nerve injury: A scoping review. *Am J Occup Ther*. (2020) 74:7401205030p1–7401205030p15. doi: 10.5014/ajot.2020.036665
17. Jerosch-Herold C. Sensory relearning in peripheral nerve disorders of the hand: A web-based survey and Delphi consensus method. *J Hand Ther*. (2011) 24:292–9. doi: 10.1016/j.jht.2011.05.002
18. Ramachandran VS, RogersRamachandran D. Synaesthesia in phantom limbs induced with mirrors. *Proc R Soc B Biol Sci*. (1996) 263:377–86. doi: 10.1098/rspb.1996.0058
19. Yeves-Lite A, Zuñi-Escobar JC, Martinez-Cepa C, Romay-Barrero H, Ferri-Morales A, Palomo-Carrión R. Conventional and virtual reality mirror therapies in upper obstetric brachial palsy: A randomized pilot study. *J Clin Med*. (2020) 9:3021. doi: 10.3390/jcm9093021
20. Ferreira CM, de Carvalho CD, Gomes R, Bonifacio de Assis ED, Andrade SM. Transcranial direct current stimulation and mirror therapy for neuropathic pain after brachial plexus avulsion: A randomized, double-blind, controlled pilot study. *Front Neurol*. (2020) 11:568261. doi: 10.3389/fneur.2020.568261
21. Chen Y-H, Siow T-Y, Wang J-Y, Lin S-Y, Chao Y-H. Greater cortical activation and motor recovery following mirror therapy immediately after peripheral nerve repair of the forearm. *Neuroscience*. (2022) 481:123–33. doi: 10.1016/j.neuroscience.2021.11.048
22. Mendes RM, Barbosa RI, Salmon CEG, Rondinoni C, Escorsi-Rosset S, Delsim JC, et al. Auditory stimuli from a sensor glove model modulate cortical audiotactile integration. *Neurosci Lett*. (2013) 548:33–7. doi: 10.1016/j.neulet.2013.04.019
23. Waikukul S, Hirunyachote P. Effects of methylcobalamin in neurotisation after brachial plexus injury - a controlled study. *Clin Drug Investig*. (1999) 17:179–84. doi: 10.2165/00044011-199917030-00002
24. Anand P, Terenghi G, Birch R, Wellmer A, Cedarbaum JM, Lindsay RM, et al. Endogenous Ngf and Cntf levels in human peripheral nerve injury. *Neuroreport*. (1997) 8:1935–8. doi: 10.1097/00001756-199705260-00028
25. Chu T-H, Wu W. Neurotrophic factor treatment after spinal root avulsion injury. *Cent Nerv Syst Agents Med Chem*. (2009) 9:40–55. doi: 10.2174/187152409787601914
26. Gordon T, Chan KM, Sulaiman OAR, Udina E, Amirjani N, Brushart TM. Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury. *Neurosurgery*. (2009) 65:A132–44. doi: 10.1227/01.Neu.0000335650.09473.D3
27. Cobo-Vicente F, San Juan AF, Larumbe-Zabala E, Estevez-Gonzalez AJ, Donadio MVE, Perez-Ruiz M. Neuromuscular electrical stimulation improves muscle strength, biomechanics of movement, and functional mobility in children with chronic neurological disorders: A systematic review and meta-analysis. *Phys Ther*. (2021) 101:pzab170. doi: 10.1093/ptj/pzab170
28. Stevanato G, Devigili G, Eleopra R, Fontana P, Lettieri C, Baracco C, et al. Chronic post-traumatic neuropathic pain of brachial plexus and upper limb: A new technique of peripheral nerve stimulation. *Neurosurg Rev*. (2014) 37:473–9; discussion 479–80. doi: 10.1007/s10143-014-0523-0
29. Bonifacio de Assis ED, Martins WKN, de Carvalho CD, Ferreira CM, Gomes R, de Almeida Rodrigues ET, et al. Effects of rTMS and tDCS on neuropathic pain after brachial plexus injury: A randomized placebo-controlled pilot study. *Sci Rep*. (2022) 12:1440. doi: 10.1038/s41598-022-05254-3
30. Hao J, Zhao C, Cao S, Yang S. Electric acupuncture treatment of peripheral nerve injury. *J Tradit Chin Med*. (1995) 15:114–7.
31. Luo H, Cheng X, Tang Y, Ling Z, Zhou L. Electroacupuncture treatment contributes to the downregulation of neuronal nitric oxide synthase and motoneuron death in injured spinal cords following root avulsion of the brachial plexus. *Biomed Rep*. (2014) 2:207–12. doi: 10.3892/br.2013.212
32. Sachar R, Landau AJ, Ray WZ, Brogan DM, Dy CJ. Social support and coping strategies in patients with traumatic brachial plexus injury. *HSS J*. (2020) 16:468–74. doi: 10.1007/s11420-020-09814-z
33. Zhou JM, Gu YD, Xu XJ, Zhang SY, Zhao X. Clinical research of comprehensive rehabilitation in treating brachial plexus injury patients. *Chin Med J*. (2012) 125:2516–20. doi: 10.3760/cma.j.issn.0366-6999.2012.14.022
34. Kahn LC, Moore AM. Donor activation focused rehabilitation approach: Maximizing outcomes after nerve transfers. *Hand Clin*. (2016) 32:263–77. doi: 10.1016/j.hcl.2015.12.014
35. Safoury YA, Eldesoky MT, Abutaleb EE, Atteya MR, Gabr AM. Postoperative physical therapy program for latissimus dorsi and teres major tendons transfer to rotator cuff in children with obstetrical brachial plexus injury. *Eur J Phys Rehabil Med*. (2017) 53:277–85. doi: 10.23736/S1973-9087.16.03910-1
36. Steeg AMT, Hoeksma AF, Dijkstra PF, Nelissen RGHH, Jong BAD. Orthopaedic sequelae in neurologically recovered obstetrical brachial plexus injury: Case study and literature review. *Disabil Rehabil*. (2009) 25:1–8. doi: 10.1080/09638280210142185
37. Chinchalkar SJ, Larocerie-Salgado J, Cepek J, Grenier ML. The use of dynamic assist orthosis for muscle reeducation following brachial plexus injury and reconstruction. *J Hand Microsurg*. (2018) 10:172–7. doi: 10.1055/s-0038-1642068
38. Gupta R, Cabacungan ET. Neonatal birth trauma: Analysis of yearly trends, risk factors, and outcomes. *J Pediatr*. (2021) 238:174–180.e3. doi: 10.1016/j.jpeds.2021.06.080
39. van der Looven R, Le Roy L, Tanghe E, van den Broeck C, de Muynck M, Vingerhoets G, et al. Early electrodiagnosis in the management of neonatal brachial plexus palsy: A systematic review. *Muscle Nerve*. (2020) 61:557–66. doi: 10.1002/mus.26762
40. Frade F, Gomez-Salgado J, Jacobsohn L, Florindo-Silva F. Rehabilitation of neonatal brachial plexus palsy: Integrative literature review. *J Clin Med*. (2019) 8:980. doi: 10.3390/jcm8070980
41. Yang LJ. Neonatal brachial plexus palsy--management and prognostic factors. *Semin Perinatol*. (2014) 38:222–34. doi: 10.1053/j.semper.2014.04.009
42. de Santana Chagas AC, Wanderley D, de Oliveira Ferro JK, Alves de Moraes A, Morais de Souza FH, da Silva Tenorio A, et al. Physical therapeutic treatment for traumatic brachial plexus injury in adults: A scoping review. *PM R*. (2022) 14:120–50. doi: 10.1002/pmrj.12566
43. Zhang M, Li C, Liu SY, Zhang FS, Zhang PX. An electroencephalography-based human-machine interface combined with contralateral C7 transfer in the treatment of brachial plexus injury. *Neural Regen Res*. (2022) 17:2600–5. doi: 10.4103/1673-5374.335838
44. Snow PW, Dimante D, Sinisi M, Loureiro RCV. Virtual reality combined with robotic facilitated movements for pain management and sensory stimulation of the upper limb following a brachial plexus injury: A case study. *Int Conf Rehabil Robot*. (2022) 2022:1–6. doi: 10.1109/ICORR55369.2022.9896552
45. Op de Coul LS, Bleeker S, de Groot JH, Nelissen R, Steenbeek D. Elbow flexion contractures in neonatal brachial plexus palsy: A one-year comparison of dynamic orthosis and serial casting. *Clin Rehabil*. (2023) 37:72–85. doi: 10.1177/02692155221121011
46. Werner JM, Berggren J, Loisele J, Lee GK. Constraint-induced movement therapy for children with neonatal brachial plexus palsy: A randomized crossover trial. *Dev Med Child Neurol*. (2021) 63:545–51. doi: 10.1111/dmnc.14741
47. Ijaz MJ, Karimi H, Ahmad A, Gillani SA, Anwar N, Chaudhary MA. Comparative efficacy of routine physical therapy with and without neuromobilization in the treatment of patients with mild to moderate carpal tunnel syndrome. *Biomed Res Int*. (2022) 2022:2155765. doi: 10.1155/2022/2155765
48. Ijaz MJ, Karimi H, Gillani SA, Ahmad A, Chaudhary MA. Effect of median nerve neuromobilization on functional status in patients with carpal tunnel syndrome: A double blinded randomized control trial. *J Pak Med Assoc*. (2022) 72:605–9. doi: 10.47391/JPMA.2212
49. Armas-Salazar A, Tellez-Leon N, Garcia-Jeronimo AI, Villegas-Lopez FA, Navarro-Olvera JL, Carrillo-Ruiz JD. Neuropathic pain relief after surgical neurolysis in patients with traumatic brachial plexus injuries: A preliminary report. *Pain Res Manag*. (2022) 2022:5660462. doi: 10.1155/2022/5660462
50. Farladansky E, Hazan S, Maman E, Reuveni AM, Cattani A, Matot I, et al. Perioperative oral pregabalin results in postoperative pain scores equivalent to those of interscalene brachial plexus block after arthroscopic rotator cuff repair: A randomized clinical trial. *Arthroscopy*. (2022) 38:31–7. doi: 10.1016/j.arthro.2021.05.022
51. Badil Guloglu S, Bilgiliyoz Filiz M, Kilic KK, Koldas Dogan S, Toslak IE, Toraman NF. Treatment of carpal tunnel syndrome by low-level laser therapy versus corticosteroid injection: A randomized, prospective clinical study. *Lasers Med Sci*. (2022) 37:2227–37. doi: 10.1007/s10103-021-03489-6
52. Paquette P, Higgins J, Danino MA, Harris P, Lamontagne M, Gagnon DH. Effects of a preoperative neuromobilization program offered to individuals with carpal tunnel syndrome awaiting carpal tunnel decompression surgery: A pilot randomized controlled study. *J Hand Ther*. (2021) 34:37–46. doi: 10.1016/j.jht.2019.12.012

53. Su YC, Shen YP, Li TY, Ho TY, Chen LC, Wu YT. The efficacy of hyaluronic acid for carpal tunnel syndrome: A randomized double-blind clinical trial. *Pain Med.* (2021) 22:2676–85. doi: 10.1093/pm/pnab109
54. Habibzadeh A, Mousavi-Khatir R, Saadat P, Javadian Y. The effect of radial shockwave on the median nerve pathway in patients with mild-to-moderate carpal tunnel syndrome: A randomized clinical trial. *J Orthop Surg Res.* (2022) 17:46. doi: 10.1186/s13018-022-02941-9
55. Chen SR, Ho TY, Shen YP, Li TY, Su YC, Lam KHS, et al. Comparison of short- and long-axis nerve hydrodissection for carpal tunnel syndrome: A prospective randomized, single-blind trial. *Int J Med Sci.* (2021) 18:3488–97. doi: 10.7150/ijms.63815
56. Lustenhouwer R, Cameron IGM, van Alfen N, Toni I, Geurts ACH, van Engelen BGM, et al. Cerebral adaptation associated with peripheral nerve recovery in neuralgic amyotrophy: A randomized controlled trial. *Neurorehabil Neural Repair* (2023) 37:3–15. doi: 10.1177/15459683221145149



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University, China

REVIEWED BY

Shusen Cui,
Jilin University, China
Yifei Yao,
Shanghai Jiao Tong University, China

*CORRESPONDENCE

Gang Zhao
✉ zhaogangmd@suda.edu.cn
Yongjun Rui
✉ wxswkryj@163.com

RECEIVED 27 October 2022

ACCEPTED 30 March 2023

PUBLISHED 25 April 2023

CITATION

Zhang F, Jiang H, Lu Z, Yang H, Zhang Q, Mi J, Rui Y and Zhao G (2023) The significance of wrist immobilization for endoscopic carpal tunnel release. *Front. Neurol.* 14:1081440. doi: 10.3389/fneur.2023.1081440

COPYRIGHT

© 2023 Zhang, Jiang, Lu, Yang, Zhang, Mi, Rui and Zhao. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

The significance of wrist immobilization for endoscopic carpal tunnel release

Fei Zhang¹, Hong Jiang², Zhenfeng Lu¹, Haoyu Yang¹, Qian Zhang¹, Jingyi Mi³, Yongjun Rui^{4*} and Gang Zhao^{1*}

¹Department of Hand Surgery, WuXi 9th People's Hospital Affiliated to Soochow University, Wuxi, China, ²Suzhou Medical College of Soochow University, Soochow University, Suzhou, China, ³Department of Sport Medicine, WuXi 9th People's Hospital Affiliated to Soochow University, Wuxi, China, ⁴Department of Orthopaedics Surgery, WuXi 9th People's Hospital Affiliated to Soochow University, Wuxi, China

Background: Over the years, endoscopic carpal tunnel release (ECTR) has gained significant interest as an alternative to surgery. However, no consensus has been reached on the necessity of postoperative wrist immobilization. This study aims to compare the outcomes of wrist immobilization for a period of 2 weeks to immediate wrist mobilization after ECTR.

Methods: A total of 24 patients with idiopathic carpal tunnel syndrome undergoing dual-portal ECTR from May 2020 to Feb 2022 were enrolled and randomly divided into two groups postoperatively. In one group, patients wore a wrist splint for 2 weeks. In another group, wrist mobilization was allowed immediately after surgery. The two-point discrimination test (2PD test); the Semmes–Weinstein monofilament test (SWM test); the occurrence of pillar pain, digital and wrist range of motion (ROM); grip and pinch strength; the visual analog score (VAS), the Boston Carpal Tunnel Questionnaire (BCTQ) score; the Disabilities of the Arm, Shoulder, and Hand (DASH) score; and complications were evaluated at 2 weeks and 1, 2, 3, and 6 months after the surgery.

Results: All 24 subjects finished this study with no dropouts. During the early follow-up, patients with wrist immobilization demonstrated lower VAS scores, lower occurrence of pillar pain, and higher grip and pinch strength compared with the immediate mobilization group. No significant difference was obtained between these two groups in terms of the 2PD test, the SWM test, digital and wrist ROM, BCTQ, and the DASH score. In total, two patients without splints reported transient scar discomfort. No one complained of neurapraxia, injury of the flexor tendon, median nerve, and major artery. At the final follow-up, no significant difference was found in any parameters between both groups. The local scar discomfort mentioned above disappeared and left no serious sequelae.

Conclusion: Wrist immobilization during the early postoperative period demonstrated significant pain alleviation along with stronger grip and pinch strength. However, wrist immobilization yielded no obvious superiority regarding clinical outcomes at the final follow-up.

KEYWORDS

carpal tunnel syndrome, median nerve, transverse carpal ligament, endoscopic carpal tunnel release, immobilization

Introduction

Carpal tunnel syndrome (CTS) is the most common peripheral compressive neuropathy worldwide (1). Carpal tunnel release (CTR) represents an alternative approach indicated when conventional intervention fails or symptoms recur (2). The surgery can be undergone via the traditional open or endoscopic approach (3–5). Although endoscopic carpal tunnel release (ECTR) yields similar clinical outcomes compared to open release, ECTR has gained significant attention worldwide for its improved aesthetics and fast recovery (5–9).

However, postoperative immobilization is still subject to much controversy. Earlier studies showed comparable outcomes and even worse prognosis of splint immobilization after open release compared with initial active motion (10–19). According to the 2016 American Academy of Orthopedic Surgeons (AAOS) evidence-based guidelines, postoperative immobilization yields no benefit (20). There are no valid data and associated criteria on splint immobilization after ECTR until now. This study sought to evaluate the outcomes of splint immobilization or initial active motion after ECTR and assess the significance of postoperative wrist immobilization.

Materials and methods

This study was approved by the Ethics Committee of WuXi No.9 People's Hospital Affiliated to Soochow University. All subjects provided informed consent before this study started.

Subjects

Outpatients at the WuXi No.9 People's Hospital Affiliated to Soochow University from May 2020 to Feb 2022 diagnosed with CTS clinically or by electrodiagnostic testing were recruited in our study. Inclusion criteria were severe idiopathic CTS with an electromyography (EMG) test and failure of conventional intervention or symptom recurrence. We defined severe CTS

according to the AAEM grading criteria: prolonged median motor and sensory distal latencies, with either an absent SNAP or mixed NAP or low amplitude or absent thenar CMAP. Needle examination often reveals fibrillations, reduced recruitment, and motor unit potential changes (21). Exclusion criteria were slight or moderate CTS, mass-like cyst in the carpal tunnel, other wrist disorders, such as arthritis, wrist fracture within 3 months, CTS revision, and local skin or subcutaneous tissue disorders. Ultimately, 24 subjects according to the inclusion and exclusion criteria were enrolled and randomly divided into two groups, each containing 12 subjects. In total, five subjects had bilateral CTS and received ECTR concomitantly.

Intervention

All subjects received one sealed envelope prepared by a person who was not involved in this trial after inclusion. The envelope contained the subject's sequential number and group and was opened just before the skin preparation of the surgical area. All subjects underwent dual-portal ECTR by the same operator. After surgery, one group was provided with a plaster splint put in the volar side of the wrist and half of the forearm and wrapped with bandage gauze. The wrist was immobilized in the neutral position for 2 weeks while the metacarpophalangeal joint and elbow could be moved immediately after surgery. Thereafter, the splint and bandage were removed after 2 weeks at the outpatient. For another group, an elastic bandage was applied to cover the incision, with no limitation of active wrist motion. All subjects were discharged on the first or second postoperative day with no analgesics and were required to visit the outpatient clinic five times at 2 weeks and 1, 2, 3, and 6 months.

Outcome measure

Outcome parameters contained the 2PD test, SWM tests, the occurrence of pillar pain, digital and wrist range of motion, grip and pinch strength, VAS for pain, BCTQ, DASH score,

TABLE 1 Group characteristics.

Characteristics	All patients (<i>n</i> = 24)	WI group (<i>n</i> = 12)	AM group (<i>n</i> = 12)	<i>P</i> -value
Gender (<i>n</i>)				
Male/female	6/18	2/10	4/8	<0.01*
Age (years)				
Mean ± SD	49.96 ± 11.67	51.00 ± 11.54	48.92 ± 12.22	0.71
Affected hand (<i>n</i>)				
Left/right/bilateral	7/12/5	4/5/3	3/7/2	0.76
Dominant hand (<i>n</i>)				
Left/right	1/23	0/12	1/11	0.16
Length of symptom (months)				
Mean ± SD	12.63 ± 24.28	9.83 ± 9.36	15.42 ± 33.58	0.295

WI, wrist immobilization group; AM, active motion group; SD, standard deviation; n, number. * denotes results with statistical significance.

TABLE 2 Outcome measures of strength and flexibility.

	Pre M ± S	2 weeks M ± S	1 month M ± S	2 months M ± S	3 months M ± S	6 months M ± S
D-ROM (°)						
WI	96.73 ± 7.91	88.40 ± 6.36	93.73 ± 6.03	97.07 ± 7.64	98.00 ± 7.21	98.40 ± 6.38
AM	95.14 ± 5.80	91.00 ± 4.67	92.86 ± 4.79	95.57 ± 5.33	96.43 ± 5.17	96.64 ± 5.53
P-value	0.55	0.22	0.67	0.55	0.51	0.44
W-ROM (°)						
WI	146.60 ± 11.98	116.60 ± 13.51	138.80 ± 11.42	146.13 ± 12.94	146.53 ± 13.08	148.40 ± 11.28
AM	148.36 ± 6.95	117.57 ± 7.52	140.57 ± 5.83	148.64 ± 7.23	149.50 ± 6.75	150.57 ± 6.73
P-value	0.64	0.81	0.61	0.53	0.45	0.54
Grip (kg)						
WI	24.40 ± 7.29	17.40 ± 4.63	20.40 ± 4.72	26.67 ± 8.39	28.53 ± 7.84	29.27 ± 7.80
AM	27.93 ± 7.32	14.07 ± 2.95	18.79 ± 4.59	26.29 ± 7.25	28.36 ± 6.73	29.07 ± 6.47
P-value	0.21	0.03*	0.36	0.90	0.95	0.94
Pinch (kg)						
WI	6.83 ± 1.94	4.93 ± 1.28	5.63 ± 1.72	7.13 ± 1.90	7.80 ± 1.96	7.90 ± 2.06
AM	7.29 ± 2.40	3.71 ± 1.49	5.11 ± 2.44	7.29 ± 1.83	7.54 ± 1.88	7.64 ± 1.79
P-value	0.58	0.025*	0.51	0.83	0.71	0.72

Pre, preoperative; D-ROM, digital range of motion; W-ROM, wrist range of motion; WI, wrist immobilization group; AM, active motion group; M±S, mean ± standard deviation. * denotes results with statistical significance.

and complications. All data were measured and recorded by an independent therapist with the Jamar® Hand Evaluation kit.

Sensibility testing was measured by the 2PD and SWM tests. The 2PD test was used to evaluate the threshold capacity to distinguish two different pressure points applied on the fingertip via a particular discriminator. The SWM test was used to evaluate the touch threshold of the fingertip via five standardized filaments (size 2.83, size 3.61, size 4.31, size 4.56, and size 6.65; size 2.83 represents normal sensibility, and size 6.65 represents no protective sensibility). The tactile senses of the first to fourth digital fingertips were measured three times, and the median value was obtained.

Postoperative pain was assessed by VAS and the occurrence of pillar pain. VAS of the wrist was measured during active motion without any load with a 100 mm visual analog scale ranging from 0 to 10 (0: absence of pain and 10: worst pain).

Postoperative strength was measured by the grip and pinch strength. Grip strength was measured in a seated position with the shoulder and wrist in a neutral position, with the elbow in a 90-degree flexed position, using the Jamar dynamometer. Key pinch strength measured the pressure between the thumb pad and the middle phalanx of the index digit with a pinch gauge dynamometer. All tests were measured three times, and the best value was recorded to decrease bias.

Postoperative flexibility was assessed by digital and wrist ROM. Each digital ROM was measured with a finger goniometer, and the interphalangeal joint of thumb value was recorded for further comparison. Wrist ROM during flexion and extension was measured in a seated position with elbow flexion and the hand placed in a resting position using a wrist goniometer.

Postoperative self-evaluation was measured by the BCTQ and DASH questionnaire. The BCTQ consists of two scales: a

symptom severity scale (BCTQ-S) and a functional status scale (BCTQ-F) (22, 23). The former section contained 11 items with a score between 1 and 5 (one means normal and five means serious symptom), whereas the latter section included eight items regarding physical function with a score between 1 and 5 (one means no difficulty and five means that subject cannot perform the activity). The average value of each section was recorded for further comparison. The DASH questionnaire contained 30 items with a score between 1 and 5 (one means no difficulty and five means inability). The score was calculated with the formula $[(\text{Sum of } n \text{ responses})/n - 1] * 25$ and recorded for further comparison (24).

Statistical analysis

Data were analyzed using SPSS software, v.25 (IBM SPSS Statistics 25, USA). Digital and wrist ROM and grip and pinch strength were analyzed using a Student's *t*-test. Data were expressed in the form of mean ± standard deviation. The 2PD test, SWM test, VAS, BCTQ, and DASH score were analyzed with Wilcoxon's rank sum test. Non-parametric data were expressed as median values. The occurrence of pillar pain was analyzed with the Fisher exact test. Data were expressed in the form of occurrence. The level of significance was set at a *P*-value of < 0.05.

Results

The outcome measures were assessed in all subjects, and none were lost during the follow-up period. The subjects exhibited a

TABLE 3 Outcome measures of sensibility.

		Pre median	2 weeks median	1 month median	2 months median	3 months median	6 months median
2PD 1st digit	WI	4	4	3	3	3	3
	AM	4	3	3	3	3	3
P-value		0.82	0.82	0.84	0.65	0.64	0.95
2PD 2nd digit	WI	3	3	3	3	3	2
	AM	3.5	3	3	2.5	2	2
P-value		0.33	0.87	0.94	0.81	0.61	0.72
2PD 3rd digit	WI	4	3	3	3	3	3
	AM	3.5	3	3	3	3	3
P-value		0.98	0.67	0.70	0.88	0.68	0.92
2PD 4th digit	WI	4	3	3	3	3	3
	AM	3.5	3	3	3	3	3
P-value		0.86	0.79	0.60	0.72	0.49	0.49
SWM 1st digit	WI	3.61	3.61	3.61	3.61	2.83	2.83
	AM	3.61	3.61	3.61	3.61	3.22	2.83
P-value		0.53	0.64	0.72	0.91	0.60	0.37
SWM 2nd digit	WI	3.61	2.83	2.83	2.83	2.83	2.83
	AM	3.61	2.83	2.83	2.83	2.83	2.83
P-value		0.92	0.54	0.82	0.98	0.94	0.59
SWM 3rd digit	WI	3.61	3.61	3.61	2.83	2.83	2.83
	AM	3.61	3.61	3.61	2.83	2.83	2.83
P-value		0.52	0.32	0.37	0.40	0.53	0.16
SWM 4th digit	WI	3.61	3.61	3.61	3.61	3.61	2.83
	AM	3.61	3.22	3.22	2.83	2.83	2.83
P-value		0.75	0.49	0.60	0.65	0.58	0.60

Pre, preoperative; 2PD, two-point discrimination; WI, wrist immobilization group; AM, active motion group.

female predominance ($n = 18$, 75%) aged 26–82 years old. The duration of symptoms ranged from 1 to 120 months. In addition to gender, there was no significant difference between the two groups regarding demographic data (Table 1).

The 2PD and SWM tests showed an apparent improvement compared with preoperative values although no significant difference was found between both groups at each time point. Moreover, the wrist immobilization group yielded better VAS and lower occurrence of pillar pain compared with the wrist mobilization motion group at 2 weeks postoperatively. No difference in pillar pain was observed at 1 month postoperatively. In addition, nearly 80% of subjects were pain-free at the final follow-up in both cohorts. Moreover, grip and pinch strength decreased in early follow-up and got restored to preoperative levels at 2 months postoperatively. At 3 months postoperatively and the final follow-up, strength was slightly stronger than preoperative values in both groups. When these two groups were compared, the wrist immobilization group showed stronger grip and pinch strength than the wrist mobilization group at 2 weeks postoperatively. Subsequently, the strength of both groups

was comparable. Digital and wrist ROM showed no obvious difference between preoperative and final follow-up and in both cohorts at any time. For the BCTQ and DASH questionnaire, both groups showed decreased scores at 2 weeks postoperatively and returned to preoperative levels at 1 month. Although the DASH scores of wrist immobilization at 1 month were greater, the difference showed no statistical significance. In total, two subjects in the wrist mobilization group complained of scar discomfort in the distal wound during early follow-up, which disappeared at 1 month postoperatively. No severe complications, including wound dehiscence, lesion of the superficial palmar arch, or median nerve, occurred during the whole follow-up. All data are shown in Tables 2–5.

Discussion

This study assessed postoperative outcome measures of wrist immobilization for 2 weeks following ECTR, compared to active mobilization with no restriction. Our results demonstrated

TABLE 4 Outcome measures of VAS and self-evaluation.

	Pre median	2 weeks median	1 month median	2 months median	3 months median	6 months median
VAS						
WI	1	1	1	0	0	0
AM	1	2	1	0.50	0	0
P-value	0.71	0.03*	0.22	0.76	1.00	0.62
BCTQ-S						
WI	2.35	2.55	2.05	1.85	1.55	1.30
AM	2.25	2.50	2.15	1.80	1.50	1.30
P-value	0.75	0.73	0.75	0.80	0.56	0.98
BCTQ-F						
WI	2.10	2.35	1.90	1.75	1.45	1.25
AM	1.85	2.35	1.80	1.55	1.35	1.20
P-value	0.91	0.66	0.86	0.38	0.88	0.86
DASH						
WI	28.80	32.95	24.25	18.80	15.45	13.75
AM	35.00	37.40	29.60	21.30	17.85	14.35
P-value	0.20	0.29	0.33	0.41	0.19	0.44

Pre, preoperative; VAS, visual analog score; BCTQ-S, Boston Carpal Tunnel Questionnaire of symptom severity scale; BCTQ-F, Boston Carpal Tunnel Questionnaire of functional status scale; DASH, Disabilities of the Arm, Shoulder, and Hand score; WI, wrist immobilization group; AM, active motion group. * denotes results with statistical significance.

TABLE 5 Outcome measures regarding the occurrence of pillar pain.

	2 weeks	1 month	2 months	3 months	6 months
WI					
n (%)	1 (6.7)	1 (6.7)	0	0	–
AM					
n (%)	6 (42.9)	5 (35.7)	3 (21.4)	2 (14.3)	–
P-value	0.035*	0.08	0.10	0.22	–

WI, wrist immobilization group; AM, active motion group; n, number; –, means no pillar pain occurred. * denotes results with statistical significance.

that wrist immobilization significantly improved postoperative pain and strength rehabilitation at early follow-up, whereas there was no significant difference between the two groups regarding postoperative sensibility, flexibility, and self-evaluation. Interestingly, there was no significant difference between both groups in any outcome measure at the final follow-up.

ECTR has increasingly become popular worldwide (1–3). Though significant emphasis has been put on the strengths and limitations between ECTR and open carpal tunnel release (OCTR) (5–8), the postoperative protocol has been largely understudied. To minimize the complications, some surgeon traditionally immobilizes the wrist in question for 1–4 weeks after OCTR (25, 26). An increasing body of evidence suggests that wrist immobilization following OCTR is ineffective and adversely affects postoperative rehabilitation (10–13, 16, 17, 19). However, relevant study concerning wrist immobilization following ECTR is rare.

The published literature does not recommend wrist immobilization or not after ECTR. Due to a lack of thorough understanding, some trials allowed initial mobilization while others recommended a plaster splint casting at early stages with variable duration of immobilization (from 48 h to 2 weeks) (14, 15, 18).

In this study, the outcome measures of sensibility improved sustainably in both groups, especially 1 month after ECTR, consistent with the literature. All patients benefited from ECTR and showed no obvious indication of incomplete release. Moreover, the sensibility of both groups at every time point was comparable, which suggested compressive nerve recovery after release was not disturbed by external splint casting.

Postoperative pain peaked at 2 weeks and was initially alleviated at 1 month after ECTR in both groups. At 2 weeks after ECTR, the wrist immobilization group exhibited better outcomes in VAS and pillar pain occurrence than the active motion group. Although pillar pain occurrence between the two groups differed by almost 30% at 1 month postoperatively, this difference did not reach statistical significance, which might be attributed to inadequate sample size. Subsequently, the two groups showed comparable outcomes regarding postoperative pain and achieved satisfactory results at the final follow-up. This difference could be related to stimulation by tiny subcutaneous hematoma and palmar cutaneous branches (27). The premature active motion can reportedly delay nerve recovery and swelling regression. In contrast, wrist immobilization for 2 weeks favored alleviation of the micro-circumstances and relief of nerve edema.

The change in postoperative strength showed a similar tendency to postoperative pain (13, 28). It took nearly 2 months for both groups to recover normal strength, which

could provide a reference for the therapist to formulate accurate postoperative physiotherapy. As stated, the wrist immobilization group yielded greater strength than the active motion group at 2 weeks after ECTR. Various factors accounted for this difference although postoperative pain was the predominant cause. After pain alleviation and wound healing, wrist strength got improved 1 month after ECTR. At the final follow-up, the strength of both groups was stronger than the baseline, which was attributed to the recovery of the median nerve and the corresponding innervated muscles.

Postoperative flexibility and self-evaluation demonstrated no significant difference between these two groups in the whole follow-up. The DASH outcome measures at 2 weeks after ECTR was superior in the wrist immobilization group although this difference was not significant. This finding implied that pain and slight strength did not influence the daily routine.

No severe complications like bowstringing of the flexor tendons occurred, which showed the safety of ECTR. In total, two subjects in the wrist mobilization group complained of scar discomfort, which got worse during grip. Moreover, they both felt uncomfortable with a scar on the palm rather than the forearm. Despite a lack of theoretical support, we considered that premature active motion, especially grip, would stimulate the cutaneous nerve in this region and cause discomfort.

The outcome measures of ECTR in this study are in accordance with the literature (29–32). This is the first study to compare the outcome of wrist immobilization and active motion after ECTR, which provides novel insights. Earlier studies have demonstrated little value of wrist immobilization after OCTR, and the outcomes even worsened with early immobilization. As already mentioned, splint casting would weaken the strength and cause stiffness of the wrist, which in turn delayed rehabilitation (13). However, we found that wrist immobilization is necessary for the early stage and contributes to symptom relief at 2 weeks postoperatively. This discrepancy may be attributed to the difference in the surgical technique. Due to the limited visualization, ECTR was considered with a higher risk of nerve damage compared with OCTR (5). Early immobilization may contribute to the recovery of the nerve. However, this damage is almost reversible and transient, and the symptoms will relieve with time pass in both groups. Further studies should be carried out to explain this discrepancy clearly.

Several limitations in this study should be given attention. First, the sample size was relatively small, which could easily cause bias and error. Moreover, this is a single-center study, and the findings only represent this single-center, which limits the generalization of our conclusions. Some outcome measures assessed are subjective, while objective outcome measures, such as EMG, were not analyzed. Hence, further multiple-center studies with large sample sizes are warranted for a more comprehensive investigation of outcome measures.

Conclusion

Wrist immobilization for 2 weeks contributes to pain alleviation and improved grip and pinch strength during early

follow-up. It brings no significant benefits in terms of improvement of sensibility, flexibility, and self-evaluation. At the final follow-up, wrist immobilization yielded no obvious significance compared with initial active motion. Therefore, wrist immobilization for 2 weeks is warranted after ECTR of idiopathic carpal tunnel syndrome.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of WuXi No. 9 People's Hospital Affiliated to SooChow University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GZ and YR designed this study. HJ, ZL, HY, QZ, and JM worked on data acquisition. FZ and HJ created tables and charts. FZ analyzed the data and drafted this manuscript. GZ revised and finished this manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Subsidy Program for Young and Middle-aged Top Talents of Wuxi Municipal Health Commission, the Postdoctoral Research Funding Program of Jiangsu Province (2020Z359), and the Binhu Medical Expert Team of "Light of Binhu Program".

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

- Joshi A, Patel K, Mohamed A, Oak S, Zhang MH, Hsiung H, et al. Carpal tunnel syndrome: pathophysiology and comprehensive guidelines for clinical evaluation and treatment. *Cureus*. (2022) 14:e27053. doi: 10.7759/cureus.27053
- Genova A, Dix O, Saefan A, Thakur M, Hassan A. Carpal tunnel syndrome: a review of literature. *Cureus*. (2020) 12:e7333. doi: 10.7759/cureus.7333
- Hacquebord JH, Chen JS, Rettig ME. Endoscopic carpal tunnel release: techniques, controversies, and comparison to open techniques. *J Am Acad Orthop Surg*. (2022) 30:292–301. doi: 10.5435/JAAOS-D-21-00949
- Wu YX, Yin H, Lin JY, Jiang HJ, Han F, Liu SD, et al. Our 15-year experience of complications of Chow's technique for endoscopic carpal tunnel releasing. *Neurol Res*. (2022) 44:761–5. doi: 10.1080/01616412.2022.2051134
- Shin EK. Endoscopic versus open carpal tunnel Release. *Curr Rev Musculoskelet Med*. (2019) 12:509–14. doi: 10.1007/s12178-019-09584-0
- Williamson ERC, Vasquez Montes D, Melamed E. Multistate comparison of cost, trends, and complications in open versus endoscopic carpal tunnel release. *Hand*. (2021) 16:25–31. doi: 10.1177/1558944719837020
- Miles MR, Shetty PN, Bhayana K, Yousaf IS, Sanghavi KK, Giladi AM. Early outcomes of endoscopic versus open carpal tunnel release. *J Hand Surg Am*. (2021) 46:868–76. doi: 10.1016/j.jhsa.2021.04.030
- Michelotti BM, Vakharia KT, Romanowsky D, Hauck RM. A prospective, randomized trial comparing open and endoscopic carpal tunnel release within the same patient. *Hand*. (2020) 15:322–6. doi: 10.1177/1558944718812129
- Koong DP, An VVG, Nandapalan H, Lawson RD, Graham DJ, Sivakumar BS. Open versus single- or dual-portal endoscopic carpal tunnel release: a meta-analysis of randomized controlled trials. *Hand*. (2022). doi: 10.1177/15589447221075665. [Epub ahead of print].
- Isaac SM, Okoro T, Danial I, Wildin C. Does wrist immobilization following open carpal tunnel release improve functional outcome? A literature review. *Curr Rev Musculoskelet Med*. (2010) 3:11–7. doi: 10.1007/s12178-010-9060-9
- Martins RS, Siqueira MG, Simplicio H. Wrist immobilization after carpal tunnel release: a prospective study. *Arq Neuropsiquiatr*. (2006) 64(3a):596–9. doi: 10.1590/S0004-282X2006000400013
- Shalimar A, Nor-Hazla MH, Arifaizad A, Jamari S. Splinting after carpal tunnel release: does it really matter? *Malays Orthop J*. (2015) 9:41–6. doi: 10.5704/MOJ.1507.011
- Svegard J, Nordvall Persson A, Zetterlund C, Alkner B. Comparison of use of plaster casting versus elastic bandage following carpal tunnel release: a randomized controlled study. *J Rehabil Med*. (2021) 53:jrm00168. doi: 10.2340/16501977-2788
- Bury TF, Akelman E, Weiss AP. Prospective, randomized trial of splinting after carpal tunnel release. *Ann Plast Surg*. (1995) 35:19–22. doi: 10.1097/0000637-199507000-00004
- Logli AL, Bear BJ, Schwartz EG, Korcek KJ, Foster BJ. A prospective, randomized trial of splinting after minicarpal tunnel release. *J Hand Surg Am*. (2018) 43:775.e1–8. doi: 10.1016/j.jhsa.2018.01.016
- Cook AC, Szabo RM, Birkholz SW, King EF. Early mobilization following carpal tunnel release. A prospective randomized study. *J Hand Surg Br*. (1995) 20:228–30. doi: 10.1016/S0266-7681(05)80057-9
- Finsen V, Andersen K, Russwurm H. No advantage from splinting the wrist after open carpal tunnel release. A randomized study of 82 wrists. *Acta Orthop Scand*. (1999) 70:288–92. doi: 10.3109/17453679908997810
- Cebesoy O, Kose KC, Kuru I, Altinel L, Gul R, Demirtas M. Use of a splint following open carpal tunnel release: a comparative study. *Adv Ther*. (2007) 24:478–84. doi: 10.1007/BF02848769
- Bhatia R, Field J, Grote J, Huma H. Does splintage help pain after carpal tunnel release? *J Hand Surg Br*. (2000) 25:150. doi: 10.1054/jhsb.2000.0365
- Graham B, Peljovich AE, Afra R, Cho MS, Gray R, Stephenson J, et al. The American Academy of Orthopaedic Surgeons evidence-based clinical practice guideline on: management of carpal tunnel syndrome. *J Bone Joint Surg Am*. (2016) 98:1750–4. doi: 10.2106/JBJS.16.00719
- Stevens JC. Aaem minimonograph #26: the electrodiagnosis of carpal tunnel syndrome. American Association of Electrodiagnostic Medicine. *Muscle Nerve*. (1997) 20:1477–86. doi: 10.1002/(sici)1097-4598(199712)20:12<1477::aid-mus1>3.0.co;2-5
- Mahmood B, Chongshu C, Qiu X, Messing S, Hammert WC. Comparison of the Michigan hand outcomes questionnaire, Boston carpal tunnel questionnaire, and promis instruments in carpal tunnel syndrome. *J Hand Surg Am*. (2019) 44:366–73. doi: 10.1016/j.jhsa.2018.10.031
- Lo IN, Hsu PC, Huang YC, Yeh CK, Yang YC, Wang JC. Dynamic ultrasound assessment of median nerve mobility changes following corticosteroid injection and carpal tunnel release in patients with carpal tunnel syndrome. *Front Neurol*. (2021) 12:710511. doi: 10.3389/fneur.2021.710511
- Angst F, Schwyzer HK, Aeschlimann A, Simmen BR, Goldhahn J. Measures of adult shoulder function: Disabilities of the Arm, Shoulder, and Hand Questionnaire (DASH) and Its Short Version (Quickdash), Shoulder Pain and Disability Index (SPADI), American Shoulder and Elbow Surgeons (ASES) Society Standardized Shoulder Assessment Form, Constant (Murley) Score (CS), Simple Shoulder Test (SST), Oxford Shoulder Score (OSS), Shoulder Disability Questionnaire (SDQ), and Western Ontario Shoulder Instability Index (WOSI). *Arthritis Care Res*. (2011) 63:S174–88. doi: 10.1002/acr.20630
- McDowell D, Garwood M, Barnes D, Minott M. Endoscopic carpal tunnel release in Jamaica—seven years experience. *West Indian Med J*. (2012) 61:158–62.
- Chen Z, Liu J, Yuan TB, Cai DW, Wang XX, Qin J. Comparison of clinical outcomes between open and modified endoscopic release for carpal tunnel syndrome. *Exp Ther Med*. (2021) 22:861. doi: 10.3892/etm.2021.10293
- Mujadzic T, Friedman HI, Mujadzic MM, Gober C, Chen E, Atwez A, et al. Modified carpal tunnel release: a new approach to minimizing pillar pain. *Ann Plast Surg*. (2021) 86(6S Suppl 5):S503–9. doi: 10.1097/SAP.0000000000002885
- Schroeder J, Malige A, Rodriguez W, Liongson F, Matullo K. Outcomes of endoscopic carpal tunnel release surgery with home guided hand therapy versus no hand therapy: a prospective randomized controlled trial after endoscopic carpal tunnel release. *Hand*. (2022). doi: 10.1177/15589447221122824. [Epub ahead of print].
- Teh KK, Gunasagaran J, Choo CH, Ahmad TS. A novel suprarretinacular endoscopic carpal tunnel release: surgical technique, clinical efficacy and safety (a series of 48 consecutive cases). *J Hand Surg Glob Online*. (2021) 3:329–34. doi: 10.1016/j.jhsg.2021.06.011
- Sato K, Murakami K, Mimata Y, Takahashi G, Doita M. Suprarretinacular endoscopic carpal tunnel release: a safe alternative method for treatment of carpal tunnel syndrome. *J Orthop Surg*. (2021) 29:2309499021993407. doi: 10.1177/2309499021993407
- Wellington I, Cusano A, Ferreira JV, Parrino A. WALANT technique versus sedation for endoscopic carpal tunnel release. *Hand*. (2023) 18:214–21. doi: 10.1177/15589447211003180
- Oertel J, Schroeder HW, Gaab MR. Dual-portal endoscopic release of the transverse ligament in carpal tunnel syndrome: results of 411 procedures with special reference to technique, efficacy, and complications. *Neurosurgery*. (2006) 59:333–40. discussion 40. doi: 10.1227/01.NEU.0000223500.25131.99



OPEN ACCESS

EDITED BY

Hui Lu,
Zhejiang University, China

REVIEWED BY

Claudio Marcello Solaro,
Galliera Hospital, Italy
Vadim Byvaltsev,
Irkutsk State Medical University, Russia

*CORRESPONDENCE

Shuyu Ge
✉ gsy_zjstldyy@163.com

SPECIALTY SECTION

This article was submitted to
Neuromuscular Disorders and Peripheral
Neuropathies,
a section of the journal
Frontiers in Neurology

RECEIVED 15 September 2022

ACCEPTED 27 February 2023

PUBLISHED 02 May 2023

CITATION

Zhao X and Ge S (2023) The efficacy and safety
of gabapentin vs. carbamazepine in patients
with primary trigeminal neuralgia: A systematic
review and meta-analysis.
Front. Neurol. 14:1045640.
doi: 10.3389/fneur.2023.1045640

COPYRIGHT

© 2023 Zhao and Ge. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/).
The use, distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in this
journal is cited, in accordance with accepted
academic practice. No use, distribution or
reproduction is permitted which does not
comply with these terms.

The efficacy and safety of gabapentin vs. carbamazepine in patients with primary trigeminal neuralgia: A systematic review and meta-analysis

Xin Zhao¹ and Shuyu Ge^{2*}

¹Department of Pharmacy, Beilun People's Hospital, Ningbo, China, ²Department of Pharmacy, Tongde Hospital of Zhejiang Province, Hangzhou, China

Background: Drug therapy is the most commonly used treatment for primary trigeminal neuralgia (PTN), in which carbamazepine is the first-line drug. Recently, the anti-epileptic drug gabapentin has also been widely used in patients with PTN, but whether it can be used as a substitute for carbamazepine still needs to be verified. Our study aimed to assess the safety and efficacy of gabapentin vs. carbamazepine as a treatment for PTN.

Methods: We searched seven electronic databases for studies published as of 31 July 2022. All randomized controlled trials (RCTs) of gabapentin vs. carbamazepine on patients with PTN that met the inclusion criteria were included. Meta-analysis was conducted using Revman 5.4 and Stata 14.0, in which forest plots, funnel plots, and sensitivity analysis were performed. Mean difference (MD) and odds ratio (OR) with 95% confidence intervals (CIs) were used for the measurement indicators of continuous and categorical variables, respectively.

Results: A total of 18 RCTs with 1,604 patients were eventually identified. The meta-analysis showed that compared with the carbamazepine group, the gabapentin group significantly improved the effective rate (OR = 2.02, 95% CI 1.56 to 2.62, $P < 0.001$), reduced the adverse event rate (OR = 0.28, 95% CI 0.21 to 0.37, $P < 0.001$), and improved the visual analog scale (VAS) score (MD = -0.46, 95% CI -0.86 to -0.06, $P = 0.03$). Although the funnel plot showed evidence of publication bias, the sensitivity analysis revealed the stability of the results.

Conclusion: The current evidence showed that gabapentin may be superior to carbamazepine in relation to efficacy and safety in patients with PTN. It is crucial that more RCTs are conducted to confirm the conclusion in the future.

KEYWORDS

gabapentin, carbamazepine, primary trigeminal neuralgia, randomized controlled trial, meta-analysis

Introduction

Trigeminal neuralgia (TN) is a common peripheral neuropathy in neurology. The characteristic symptoms of TN include unilateral, paroxysmal, provokable, and without sensory loss of facial pain (1). It can be divided into primary trigeminal neuralgia (PTN, i.e., idiopathic and classical types of TN) and secondary trigeminal neuralgia (STN), of which PTN is more common (2–4). Various examinations revealed that PTN refers to pain with clinical symptoms, while without a clear etiology, and no organic lesions related to the pathogenesis (5). STN refers to pain caused by a clear etiology, such as intracranial tumors, inflammation, and abnormal blood vessels (6).

There are more women with PTN, and it is more common after the age of 40. It can be described by patients as electrocautery, tearing, acupuncture, or knife cutting. At the onset, patients often show a painful expression, and it is necessary to rub the face tightly with a towel to relieve the pain. The onset of the disease is acute, and it stops suddenly after 1–2 min and relapses after a period of time. The interval between relapses shortens as the disease aggravates (7). At present, the etiology, lesion location, and pathogenesis of PTN are not completely clear. There are clinical theories of central etiology and mechanical compression, as well as the theory of microvascular, which need further research to confirm (8).

At present, drugs can be used for nerve block, and then, neurotomy, microvascular decompression, or radiofrequency thermocoagulation can be used for treatment, but available treatments are still far from the ideal effect and pain tend to recur (9–12). Carbamazepine is the first choice for the treatment of primary trigeminal neuralgia, and it is effective for most patients, but it has many adverse reactions; blood routine, electrolytes, and liver and kidney functions should be monitored during medication (13).

Gabapentin has been used in clinics for more than 10 years as a new type of anti-epileptic drug. Its good curative effect for neuropathic pain has been reported in many literature studies (14, 15). PTN is also a neuropathic pain in mechanisms. At present, many randomized controlled trials (RCTs) comparing the safety and efficacy of gabapentin and carbamazepine for PTN have been carried out. Whether gabapentin can be used as a substitute for

carbamazepine has not been verified by evidence-based research. Our study conducted a systematic review and meta-analysis of RCTs of gabapentin and carbamazepine as a treatment for PTN, so as to provide evidence-based medicine for medical practice.

Methods

Literature search strategy

Seven electronic databases (PubMed, Web of Science, EMBASE, Chinese BioMedical Database, China National Knowledge Infrastructure, China Scientific Journal Database, and Wanfang Database) were comprehensively searched from inception to 15 August 2022 for all studies involving gabapentin vs. carbamazepine for the treatment of PTN. The combination of “gabapentin”, “carbamazepine”, and “primary trigeminal neuralgia” used the Boolean operator “AND” to carry out the specific search. Language restrictions and publication status were not considered in our literature search. A hand search of the reference lists of the original articles and previous reviews was also conducted to screen other potentially eligible studies.

Study selection

The inclusion criteria were as follows: (P) patients with primary trigeminal neuralgia (PTN); (I) intervention: the test

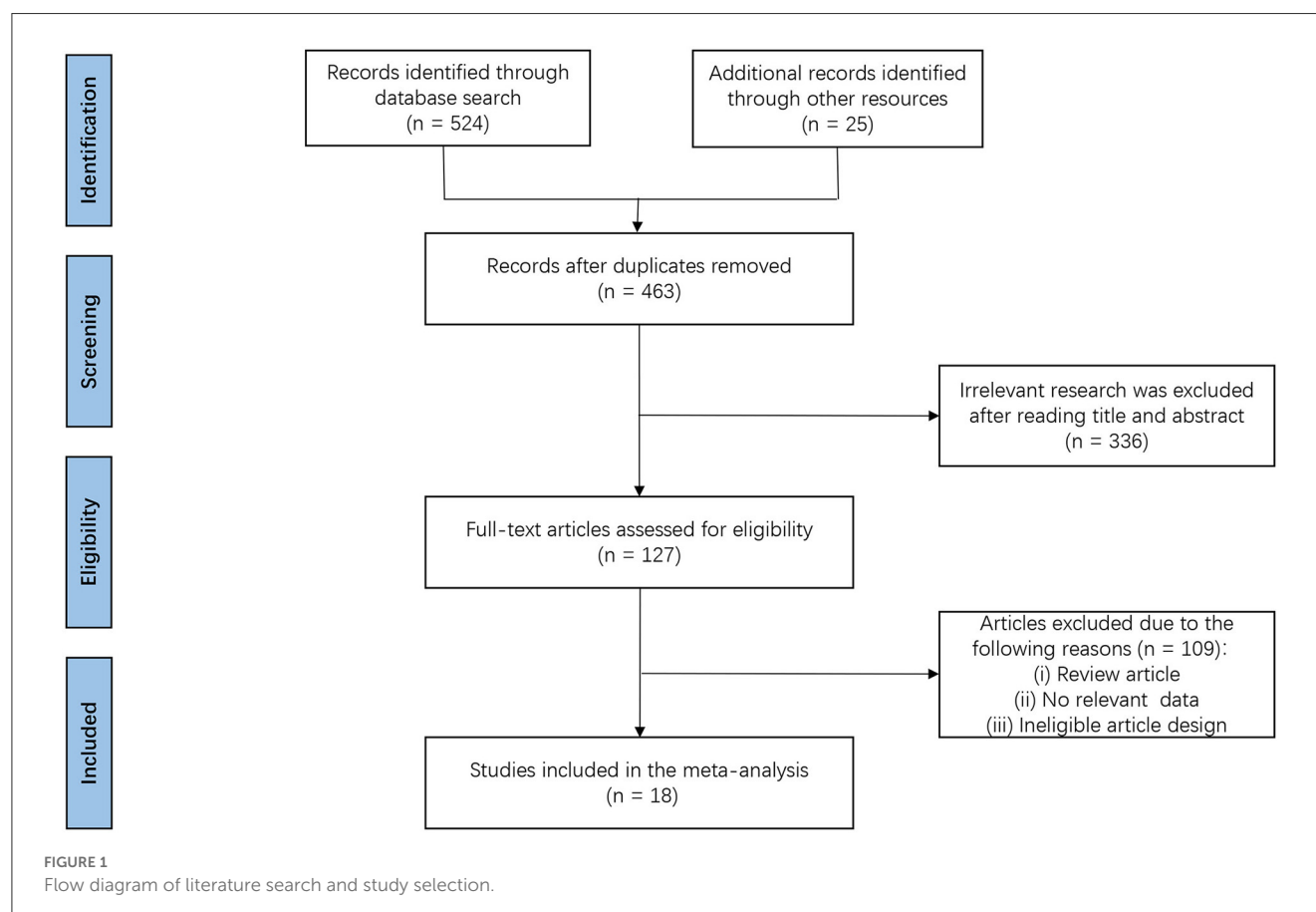
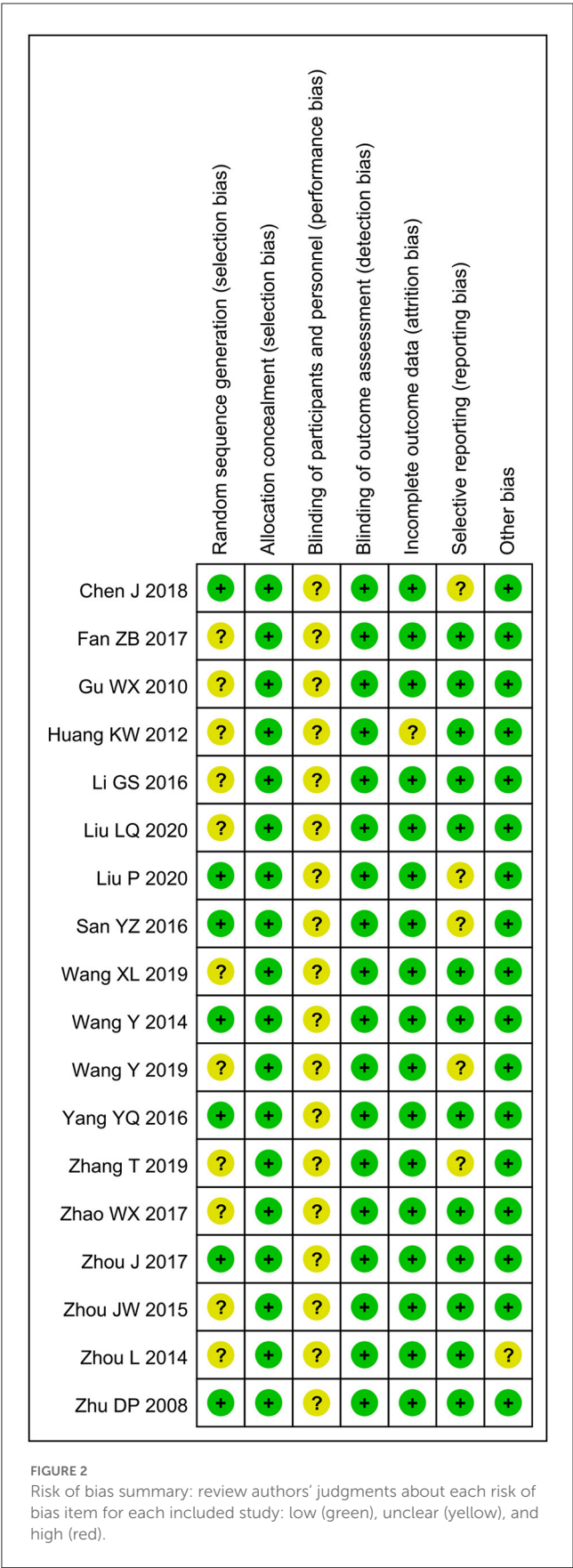


TABLE 1 Presentation of main characteristics for included studies.

Study	Treatment		Treatment time (days)	No. of patients		Gender (M/F)		Age (years)		Course of disease (months)		Years of onset
	Test	Control		Test	Control	Test	Control	Test	Control	Test	Control	
Chen (16)	GBP: 200–1300 mg, tid	CBZ: 200–500 mg, tid	28	49	49	27/22	29/20	53.8 ± 11.6	52.1 ± 11.3	NR	NR	March 2015 to March 2017
Fan et al. (23)	GBP: 100–800 mg, tid	CBZ: 100–300 mg, tid	60	31	31	18/13	19/12	52.21 ± 6.58	53.01 ± 6.21	20.14 ± 5.23	19.61 ± 6.31	February 2013 to February 2014
Gu et al. (31)	GBP: 100–800 mg, tid	CBZ: 200–300 mg, tid	70	34	34	16/18	15/19	52.13 ± 5.75	53.57 ± 5.14	NR	NR	September 2007 to April 2009
Huang et al. (28)	GBP: 300–600 mg, tid	CBZ: 100–300 mg, tid	28	48	48	22/26	23/25	56.13 ± 3.75	55.73 ± 4.97	NR	NR	February 2009 to June 2011
Li (21)	GBP: 300–1200 mg, qd	CBZ: 100–200 mg, bid	30	45	45	14/31	16/29	57.13 ± 1.06	56.09 ± 1.12	9.56 ± 1.03	9.96 ± 1.12	January 2014 to January 2015
Liu (25)	GBP: 300–1200 mg, tid	CBZ: 100–400 mg, tid	28	36	36	16/20	15/21	66.18 ± 4.92	67.01 ± 5.28	NR	NR	May 2017 to October 2018
Liu (33)	GBP: 300–800 mg, tid	CBZ: 100–400 mg, tid	28	40	40	18/22	16/24	49.2 ± 4.3	49.2 ± 4.7	NR	NR	January 2018 to December 2019
San et al. (30)	GBP: 300–600 mg, tid	CBZ: 100–300 mg, tid	35	20	20	11/9	8/12	30–77	32–75	NR	NR	April 2015 to April 2016
Wang (27)	GBP: 300–600 mg, tid	CBZ: 100–600 mg, tid	28	105	105	55/50	51/54	55.65 ± 2.59	56.02 ± 2.66	NR	NR	March 2015 to March 2019
Wang (18)	GBP: 300–800 mg, tid	CBZ: 100–300 mg, tid	60	36	36	22/14	23/13	53.4 ± 11.6	53.5 ± 11.8	22.6 ± 9.8	22.4 ± 9.6	August 2011 to August 2012
Wang (22)	GBP: 300–1200 mg, tid	CBZ: 150–600 mg, tid	28	44	44	30/14	29/15	52.21 ± 6.58	53.01 ± 6.21	20.14 ± 5.23	19.62 ± 6.31	June 2017 to December 2018
Yang (19)	GBP: 100–300 mg, tid	CBZ: 100–300 mg, tid	60	42	42	19/23	20/22	54.2 ± 5.5	55.4 ± 5.6	10.44 ± 2.58	10.45 ± 2.59	August 2013 to May 2015
Zhang and Xiao (26)	GBP: 300–1200 mg, tid	CBZ: 100–400 mg, tid	60	40	40	11/29	14/26	56.2 ± 5.1	56.7 ± 5.2	10.1 ± 2.4	10.2 ± 2.5	January 2017 to June 2018
Zhao (24)	GBP: 300–600 mg, tid	CBZ: 100–300 mg, tid	28	49	49	31/18	28/21	45 ± 1.29	44 ± 1.72	NR	NR	February 2015 to February 2016
Zhou et al. (32)	GBP: 300–1200 mg, tid	CBZ: 100–400 mg, tid	28	44	44	17/27	19/25	51.9 ± 7.2	52.6 ± 7.8	12.1 ± 6.5	11.5 ± 6.3	February 2014 to December 2015
Zhou (20)	GBP: 300–800 mg, tid	CBZ: 100–500 mg, tid	30	51	50	23/28	24/26	44 ± 1.7	45 ± 1.5	10.51 ± 2.63	10.24 ± 2.67	January 2012 to January 2014
Zhou (29)	GBP: 300–1200 mg, tid	CBZ: 100–400 mg, tid	28	43	43	20/23	18/25	56.12 ± 4.69	56.45 ± 4.71	10.51 ± 2.63	10.43 ± 2.57	September 2011 to September 2012
Zhu et al. (17)	GBP: 300–1200 mg, tid	CBZ: 100–400 mg, tid	28	45	46	23/22	22/24	61.03 ± 8.30	59.68 ± 8.72	5.57 ± 1.02	5.43 ± 0.91	January 2006 to June 2007

GBP, gabapentin; CBZ, carbamazepine; qd, one time daily; bid, twice daily; tid, three times daily; NR, not reported.



group received oral gabapentin treatment; (C) comparison: the control group received oral carbamazepine treatment; (O) main outcome indicators: the effective rate, the adverse event rate, and the visual analog scale (VAS) score; and (S) study design: randomized controlled trials (RCTs). The major exclusion criteria were as follows: (1) patients with secondary trigeminal neuralgia (STN) or other diseases; (2) non-randomized controlled studies; (3) treatment with other interventions; and (4) the relevant data not reported.

Data extraction

XZ and SG independently reviewed RCTs included in the final analysis and extracted relevant data directly from the articles. The following information was extracted from each included study: author's name, year of publication, treatment of test and control group, sample size, patients' characteristics, study's years of onset, and the results of main outcome indicators [the effective rate, the visual analog scale (VAS) score, and the adverse event rate].

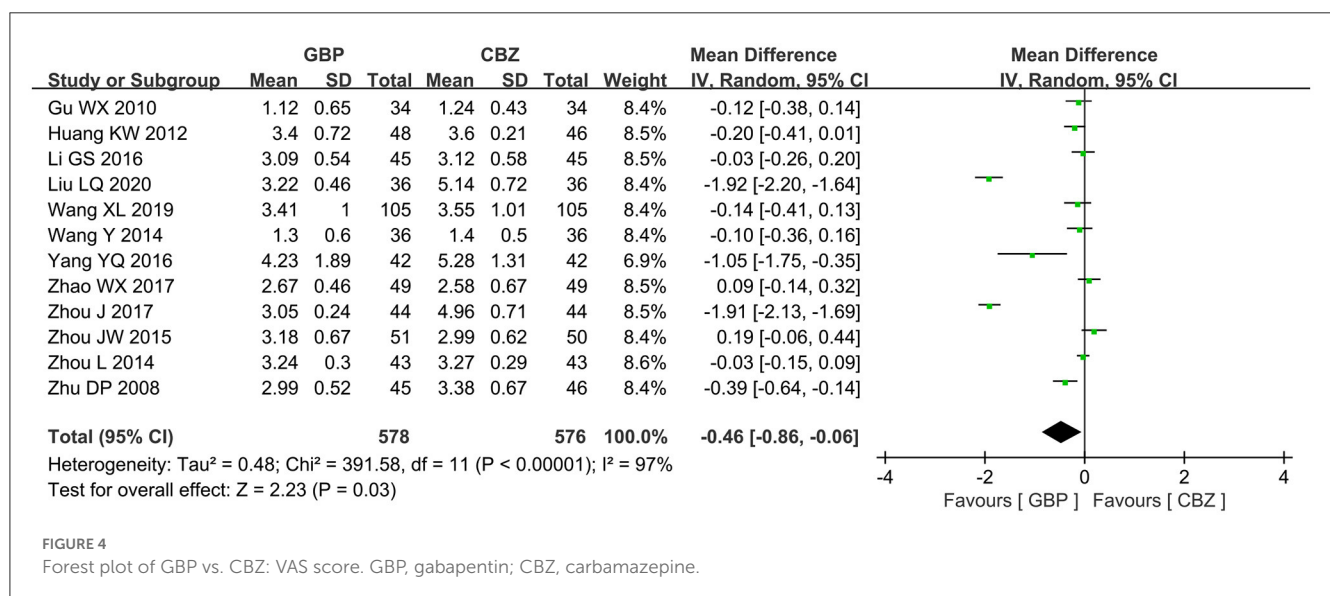
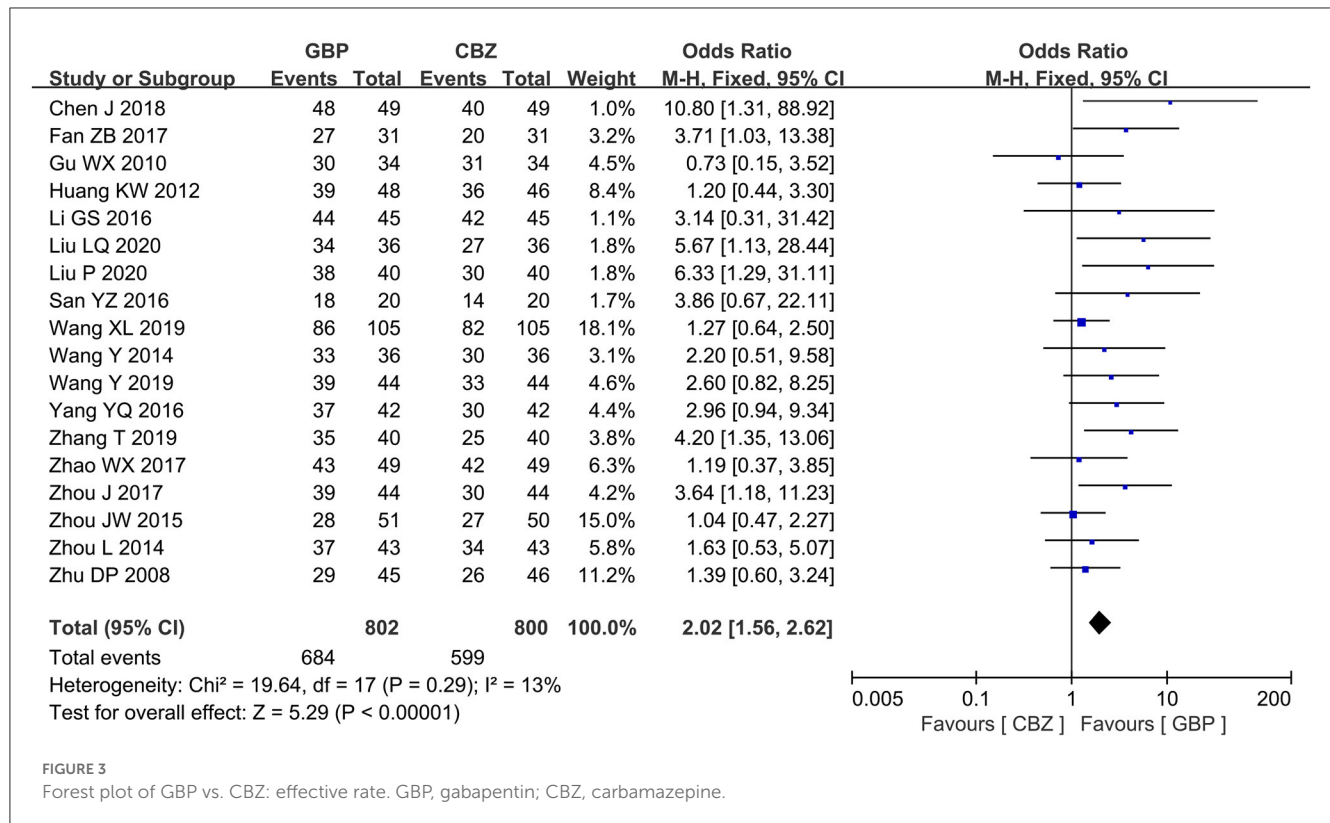
The VAS score was used to assess the pain degree of patients, with a total score of 10 points. In order to categorize the patients, the VAS score was divided into three categories: mild or no pain (0–2), moderate pain (3–6), and severe pain (7–10). Compared with before treatment, the VAS score decreased by >50% after treatment indicated effective treatment; otherwise, it was considered ineffective.

Quality assessment

Quality assessment of RCTs was performed using the Cochrane bias risk assessment tool (The Cochrane Collaboration) by the following items: random sequence generation, allocation concealment, blinding (performance bias and detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias.

Statistical analysis

All statistical analyses were performed by using Review Manager, version 5.4, software (The Nordic Cochrane Centre) and Stata 14.0 (STATA Corp., College Station). Specifically, data for effect sizes of continuous outcomes were calculated as mean difference (MD) with a 95% confidence interval (CI), and odds ratio (OR) was used for categorical outcomes. Heterogeneity between studies was assessed using the I² statistic the Cochrane Q-test. Statistical heterogeneity will determine the choice of model (fixed or random effect). An assessment of possible publication bias was carried out using funnel plots and Egger's test. If I² is >50% or the p-value of publication bias is < 0.05, the potential source of heterogeneity or publication bias would be tested by sensitivity analysis, which was evaluated by removing study after study from the pooled results using the leave-one-out method.



Results

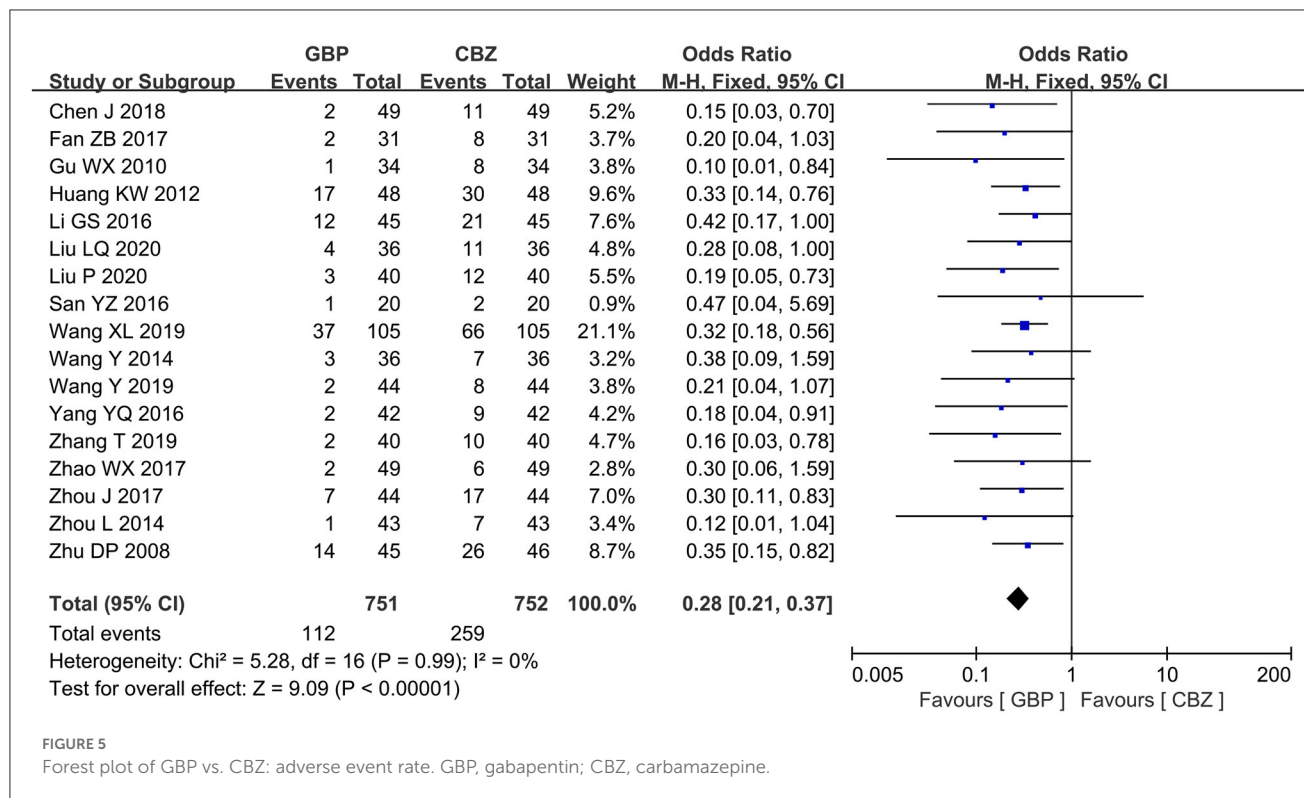
Search process

A total of 549 potentially relevant articles were identified by the searches. After the removal of duplicates, 463 articles were identified. After going through the titles and abstracts, another 336 articles were excluded. Full-text articles from the remaining 127 studies were assessed for eligibility, and 109 studies were further excluded because they did not meet the inclusion criteria.

Eventually, 18 RCTs were deemed eligible and were included in the final meta-analysis (16–33). Figure 1 illustrates the search process and application of the study inclusion/exclusion criteria.

Characteristics of the included studies

The detailed characteristics of these 18 eligible studies are summarized in Table 1. This study included 18 RCTs consisting of 1,604 cases, of which 802 were in the test group and 802 were in



the control group. The sample size was between 40 and 210, the treatment time ranged from 28 days to 60 days, and the studies were published between 2008 and 2020.

Results of quality assessment

The included RCTs were assessed for bias using the Cochrane Risk of Bias Assessment tool (Figure 2). Among them, 11 articles had selection bias as they did not use the random number table for grouping, all articles did not mention the use of blinding, one article had cases lost to follow-up and had attrition bias, five articles had reported bias due to fewer outcome variables, and another article had other biases due to the lack of comparability between the two groups. Overall, none of the included RCTs show a high risk of bias, and their quality was acceptable.

Results of meta-analysis

Effective rate

All the included studies measured the effective rate after treatment. The included studies showed no notable heterogeneity ($I^2 = 13\%$, $P = 0.29$), so a fixed-effects model was selected for analysis, which revealed that the gabapentin group showed a high effective rate compared with the carbamazepine group after treatment (OR = 2.02, 95% CI: 1.56 to 2.62, $P < 0.00001$) (Figure 3).

VAS score

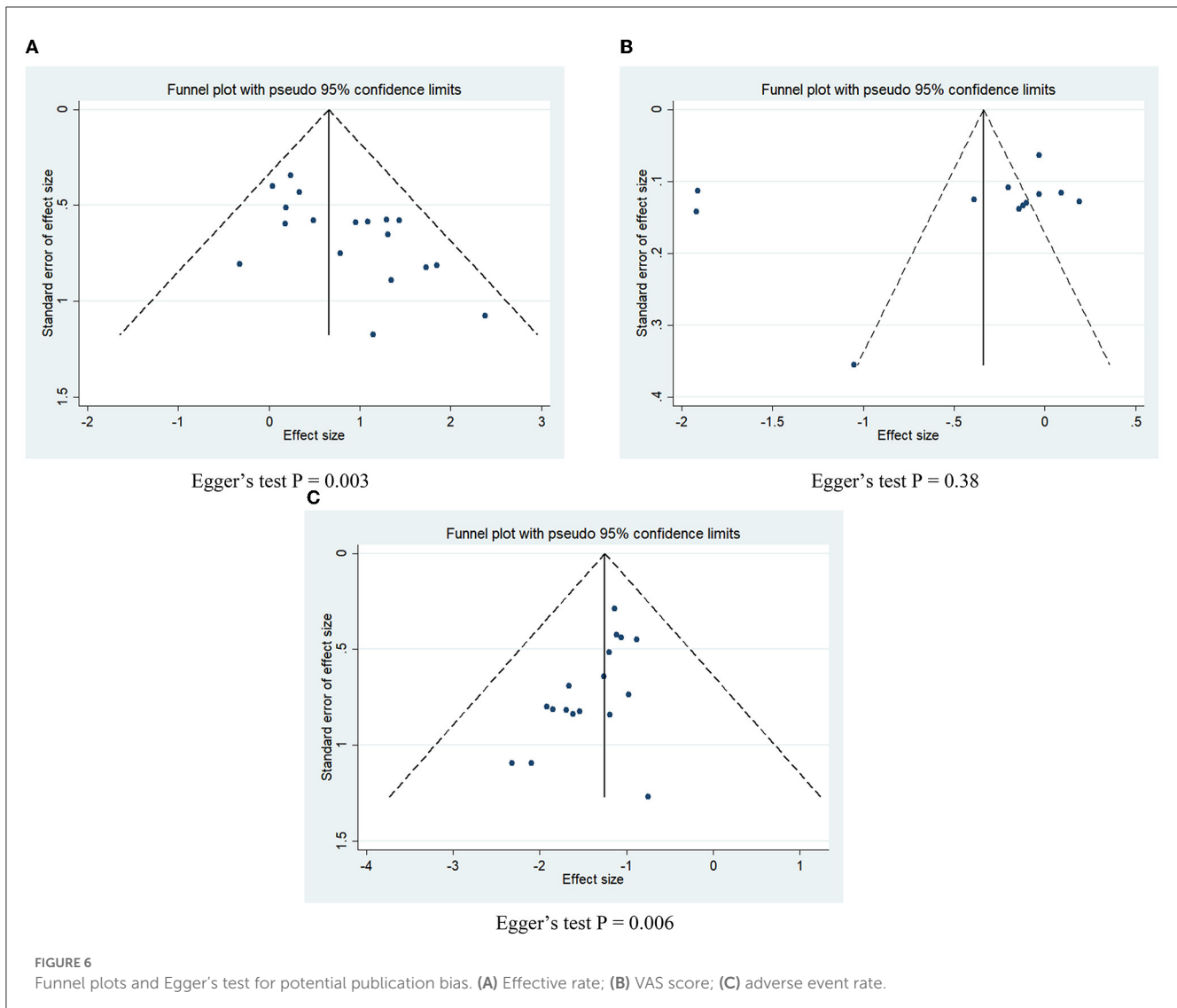
A total of 12 articles reported the VAS score of gabapentin vs. carbamazepine in treating patients with PTN. The included studies had marked heterogeneity ($I^2 = 97\%$, $P < 0.00001$), so the random effects model was selected for further analysis, and it showed that the gabapentin group had a lower VAS score than the carbamazepine group (MD = -0.46, 95% CI: -0.86 to -0.06, $P = 0.03$) (Figure 4).

Adverse event rate

A total of 17 studies had data available to assess the adverse event rate after treatment. No heterogeneity was found among studies ($I^2 = 0\%$, $P = 0.99$). A significant decrease in adverse event rate was observed in the gabapentin group when compared with the carbamazepine group (OR = 0.28, 95% CI: 0.21 to 0.37, $P < 0.00001$) (Figure 5).

Publication bias

The funnel plots corresponding to the three outcome variables were visually asymmetric (Figure 6), and the p -values of Egger's test for the effective rate and the adverse event rate were <0.05 ($P = 0.003$ for the effective rate, Figure 6A; $P = 0.006$ for the adverse event rate, Figure 6C), indicating that there may be potential publication bias between the two variables included in the literature; the p -value of Egger's test for VAS score was 0.38 (Figure 6B), indicating that no obvious publication bias was present.



Sensitivity analysis

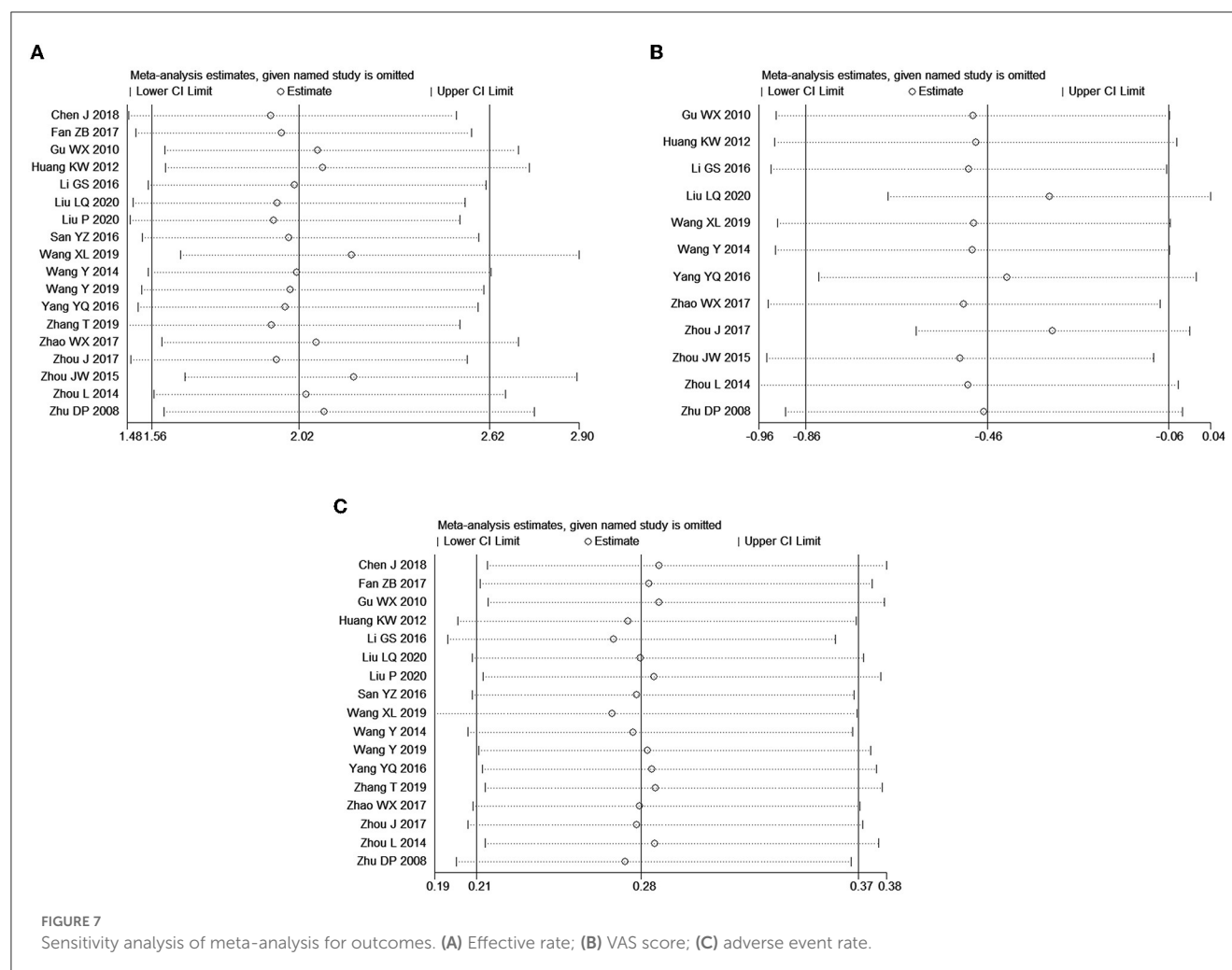
Since there was publication bias among the included literature on the effective rate and adverse reaction rate ($P < 0.05$), and significant heterogeneity existed in the included literature for VAS score, we conducted a sensitivity analysis on the meta-analysis results of the three variables (Figure 7). Arbitrary deletion of an included article did not change the significance of meta-analysis results for the effective rate (Figure 7A) and adverse event rate (Figure 7C), which indicated that the meta-analysis results of these two variables were stable, while after deletion of Liu (25), the combined results of VAS scores (Figure 7B) become statistically insignificant, which indicated that the meta-analysis of VAS was less stable.

Discussion

An episode of PTN is often triggered by normal facial activities such as speaking, chewing, brushing, and washing, or by touching an area of the mouth or face called a “trigger point” (34). In order

to avoid attacks, the patient should try to reduce the actions such as speaking, chewing, brushing their teeth, and washing their face, so as to avoid physical and psychological suffering. At present, the etiology of TN is not completely clear. The classic theory holds that the occurrence of TN is due to the compression of abnormally twisted blood vessels such as the superior cerebellar artery and basilar artery near the pons of the trigeminal nerve root. The nerve root is compressed by an aneurysm or arteriovenous malformation (8). Studies have shown that vascular compression at the root of the trigeminal nerve can be found in 95% of patients with PTN (35).

In the present meta-analysis, we comprehensively reviewed and pooled results from 18 RCTs with a total of 1,604 patients who compared gabapentin and carbamazepine therapy for PTN. The results revealed that for patients with PTN, the gabapentin group resulted in a significant improvement in the effective rate compared with the carbamazepine group with an OR of 2.02 and a significant decrease in adverse event rate with an OR of 0.28. The pooled result of the VAS score in the gabapentin group was significantly lower than the carbamazepine group.



There was a meta-analysis to evaluate the safety and efficacy of gabapentin for diabetic peripheral neuropathy. The results showed that gabapentin has a significant effect in the treatment of diabetic peripheral neuropathy with good safety, and its efficacy was comparable to that of carbamazepine or oxcarbazepine. The effective rate was even lower than that of carbamazepine or oxcarbazepine (36). There were also studies on the safety and efficacy of gabapentin for post-herpetic neuropathy (37, 38). Evidence showed that gabapentin was effective for post-herpetic neuropathy and had a high treatment retention rate. These studies confirmed that gabapentin was effective in treating a wide variety of neuropathic pain symptoms. Maria's study conducted a systematic review of the efficacy of pregabalin and gabapentin for pain and disability due to acute sciatica and adverse events associated with their clinical use and found that gabapentin compared with placebo in assessing leg pain, low back pain, and function. There was no statistical difference in the remaining period of disability, suggesting that gabapentin may be less effective in acute neuropathic pain (39).

The chemical name of gabapentin is 1-(aminomethyl)-cyclohexanecarboxylic acid, and its molecular structure is related to the neurotransmitter γ -aminobutyric acid. It has been widely used for TN in recent years, but its analgesic mechanism has

not been fully elucidated. Its molecular structure is similar to that of γ -aminobutyric acid, and it is a voltage-gated calcium channel $\alpha_2\delta_1$ subunit blocker (40, 41). At present, it is believed that it can act on peripheral nerve nociceptors, spinal pain conduction pathway, cerebral cortex, and other targets, regulate voltage-gated calcium channel receptors, and reduce the release of excitatory neurotransmitters to achieve the analgesic effect. Therefore, gabapentin may have a therapeutic effect on PTN (42).

Studies have found that inflammatory factors are closely related to the occurrence and progression of TN. Due to changes in nerve demyelination, intraneural mast cells, macrophages, and vascular endothelial cells are damaged and inflammatory responses are induced (43). Tumor necrosis factor- α (TNF- α) can promote the aggregation and activation of inflammatory cells and induce neuropathic pain. Interleukin-6 (IL-6) has a variety of cellular functions and has the effect of promoting inflammation and inducing the production of acute response protein (44). Gabapentin has been confirmed to reduce the levels of TNF- α and IL-6 and inhibit the inflammatory response of patients, thereby achieving the effect of relieving pain and alleviating clinical symptoms (33).

This study had the following limitations: (1) The allocation sequence concealment and risk of blinding bias in most studies

were unclear, and there may be limitations; (2) although all included studies had the same purpose, each included study differed in terms of patient disease severity and drug dosage. There were different degrees of differences in terms of time and intervention time, and these differences may lead to heterogeneity of outcomes. (3) The currently included studies were all published in Chinese, which may affect the extrapolation of the results of this study. Therefore, the final conclusion is still needed to be further verified by more large-sample, multi-center high-quality RCTs.

Conclusion

Existing evidence shows that gabapentin is effective in the treatment of PTN, and it is even better than carbamazepine in terms of therapeutic effect and safety. Gabapentin may be a better choice in cases where first-line drugs such as carbamazepine are ineffective in controlling pain or have severe side effects. In addition, the findings of this review are limited by the quality and limitation of the included studies, and more high-quality studies subdivided research should be further carried out.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

References

- Nicola NM, Giulio C, Di BR, Mario M, Eduardo F, Fabio P. Advances in diagnosis and treatment of trigeminal neuralgia. *Ther Clin Risk Manage*. (2015) 11:289–99. doi: 10.2147/TCRM.S37592
- Cruccu G, Finnerup NB, Jensen TS, Scholz J, Nurmikko T. Trigeminal neuralgia: new classification and diagnostic grading for practice and research. *Neurology*. (2016) 87:220–8. doi: 10.1212/WNL.0000000000002840
- Asan F, Gündüz A, Tütüncü M, Uygungölu U, Savrun FK, Saip S, et al. Treatment of multiple sclerosis-related trigeminal neuralgia with onabotulinumtoxin A. *J Head Face Pain*. (2022) 62:1322–8. doi: 10.1111/head.14414
- Headache Classification Committee of the International Headache Society (IHS) the International Classification of Headache Disorders, 3rd edition. *Cephalalgia*. (2018) 38:1–211. doi: 10.1177/0333102417738202
- Peker S, Sirin A. Primary trigeminal neuralgia and the role of pars oralis of the spinal trigeminal nucleus. *Med Hypotheses*. (2017) 100:15–8. doi: 10.1016/j.mehy.2017.01.008
- Di Stefano G, Maarbjerg S, Truini A. Trigeminal neuralgia secondary to multiple sclerosis: from the clinical picture to the treatment options. *J Headache Pain*. (2019) 20:20. doi: 10.1186/s10194-019-0969-0
- Stine M, Frauke W, Aydin G, Jes O, Lars B. Significance of neurovascular contact in classical trigeminal neuralgia. *Brain*. (2015) 138:311–9. doi: 10.1093/brain/awu349
- Smith CA, Paskhover B, Mammis A. Molecular mechanisms of trigeminal neuralgia: a systematic review. *Clin Neurol Neurosurg*. (2021) 2021:106397. doi: 10.1016/j.clineuro.2020.106397
- Ziegeler C, Brauns G, May A. Characteristics and natural disease history of persistent idiopathic facial pain, trigeminal neuralgia, and neuropathic facial pain. *Headache*. (2021) 61:1441–51. doi: 10.1111/head.14212
- Di Stefano G, De Stefano G, Leone C, Di Leonardo A, Di Pietro G, Sgro E, et al. Real-world effectiveness and tolerability of carbamazepine and oxcarbazepine in 354 patients with trigeminal neuralgia. *Eur J Pain*. (2021) 25:1064–71. doi: 10.1002/ejp.1727
- Sabourin V, Mazza J, Garzon T, Head J, Ye D, Stefanelli A, et al. Internal neurolysis with and without microvascular decompression for trigeminal neuralgia: case series. *World Neurosurg*. (2020) 6:206. doi: 10.1016/j.wneu.2020.06.206
- Khadilkar SV, Patil VA. Medical management of trigeminal neuralgia. *Neurol India*. (2021) 168:399–401. doi: 10.4103/0028-3886.315996
- Zhang Y, Wang Y, Xia W, Song A. Triple puncture for primary trigeminal neuralgia: a randomized clinical trial. *Curr. Med. Sci*. (2019) 39:638–44. doi: 10.1007/s11596-019-2085-4
- Flickinger JC, Kim H, Kano H, Greenberger JS, Arai Y, Niranjan A, et al. Do carbamazepine, gabapentin, or other anticonvulsants exert sufficient radioprotective effects to alter responses from trigeminal neuralgia radiosurgery? *Int J Radiat Oncol Biol Phys*. (2012) 83:e501–6. doi: 10.1016/j.ijrobp.2012.01.016
- Di Stefano G, Truini A, Cruccu G. Current and innovative pharmacological options to treat typical and atypical trigeminal neuralgia. *Drugs*. (2018) 78:1433–42. doi: 10.1007/s40265-018-0964-9
- Chen J. Study the feasibility and safety of gabapentin in the treatment of primary trigeminal neuralgia. *Contemp. Med*. (2018) 24:142–3.
- Zhu DP, Zhang YN, Xu PR. Randomized control trial of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *West China Med J*. (2008) 23:790–2.
- Wang Y. Feasibility and safety of gabapentin in the treatment of primary trigeminal neuralgia. *Rural Health China*. (2014) 54:36–7.
- Yang YQ. Comparison of application value between gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *J. Aerospace Med*. (2016) 27:749–51.
- Zhou JW. Clinical efficacy and side effects of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Chin J Prac Nerv Dis*. (2015) 22:127–8.
- Li GS. Comparison of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *J China Pres Drug*. (2016) 9:69–70.

Author contributions

SG designed the study. XZ performed a database search, data extraction, and quality evaluation. SG and XZ performed the statistical analysis, verified the data, and drafted the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Zhejiang Chinese Medical and Health Technology Project (20212A039).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

22. Wang Y. Comparative analysis of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Modern Med Health Res.* (2019) 3:23–4.
23. Fan ZB, Chen XP, Zhou XP. Comparative analysis of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Jilin Med.* (2016) 37:103–4.
24. Zhao WX. Comparative analysis of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Cardiovas Dis J Int Trad Chin West Med.* (2017) 5:80.
25. Liu LQ. Clinical efficacy and side effects of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Northern Pharm.* (2020) 17:162–3.
26. Zhang T, Xiao Q. Comparison of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Contemp Med Treatise.* (2019) 17:113–4.
27. Wang XL. A comparative study of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Heilongjiang Trad Chin Med.* (2019) 48:178–9.
28. Huang QW, Sun GY, Cheng JX, A. comparative study of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Chin J Prac Nerv Dis.* (2012) 15:33–5.
29. Zhou L. A comparative study of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *Chin J Prac Nerv Dis.* (2014) 17:59–61.
30. San YZ, Zhao WF, Cui KG, Zhang LK. Clinical study of gabapentin in treating trigeminal neuralgia. *J Clin Med.* (2016) 3:6081–4.
31. Gu WX, Chen H, Ye XR, Zhu J, Chen XH. Comparative study of gabapentin in the treatment of primary trigeminal neuralgia. *Mod Diagn Treat.* (2010) 21:8–10.
32. Zhou J, Yao S, Wu YH. Comparison of efficacy and safety between carbamazepine and gabapentin in the treatment of trigeminal neuralgia. *J Shenyang Med College.* (2017) 19:224–5.
33. Liu P. Effect of carbamazepine and gabapentin on trigeminal neuralgia. *China Hygiene Standard Manage.* (2020) 11:94–6.
34. Wei W, Wang Y, Han Z, Liu Z, Liu Y, Chen M. Personalized tooth-supported digital guide plate used in the treatment of trigeminal neuralgia with balloon compression. *Annal Trans Med.* (2022) 10:628–38. doi: 10.21037/atm-21-4827
35. Fromm GH, Terrence CF, Chattha AS. Baclofen in the treatment of trigeminal neuralgia: double-blind study and long-term follow-up. *Ann Neurol.* (1984) 15:240–4. doi: 10.1002/ana.410150306
36. Zhang XL, Yuan HY, Zhang XH. Effects and safety of gabapentin for treatment of diabetic peripheral neuropathic pain: a Meta-analysis. *Drug Eval Res.* (2015) 38:557–62.
37. Ding Y, Xie H, Ge WH. Meta analysis of the efficacy and safety of pregabalin and gabapentin in the treatment of postherpetic neuralgia. *Pharm Clin Res.* (2019) 27:61–4.
38. Zhu L, Jiang ZB. Meta analysis of the efficacy of gabapentin sustained release tablets in the treatment of postherpetic neuralgia. *J Clin Anesthesiol.* (2012) 28:317–20.
39. Giménez-Campos MS, Pimenta-Fermission-Ramos P, Díaz-Cambronero JJ, Carbonell-Sanchis R, López-Briz E, Ruiz-García V, et al. systematic review and meta-analysis of the effectiveness and adverse events of gabapentin and pregabalin for sciatica pain. *Atención Primaria.* (2022) 54:102144. doi: 10.1016/j.aprim.2021.102144
40. Lemos L, Flores S, Oliveira P, Almeida A. Gabapentin supplemented with ropivacain block of trigger points improves pain control and quality of life in trigeminal neuralgia patients when compared with gabapentin alone. *Clin J Pain.* (2008) 24:64–75. doi: 10.1097/AJP.0b013e318158011a
41. Ding W, You Z, Shen S, Yang J, Lim G, Doheny JT, et al. An improved rodent model of trigeminal neuropathic pain by unilateral chronic constriction injury of distal infraorbital nerve. *J Pain.* (2017) 18:899–907. doi: 10.1016/j.jpain.2017.02.427
42. Ta PCP, Dinh HQ, Nguyen K, Lin S, Ong YL, Ariyawardana A. Efficacy of gabapentin in the treatment of trigeminal neuralgia: a systematic review of randomized controlled trials. *J. Inv. Clin. Dent.* (2019) 10:12448. doi: 10.1111/jicd.12448
43. Zhang YN. Clinical efficacy and adverse reactions of gabapentin and carbamazepine in the treatment of primary trigeminal neuralgia. *China Hygiene Stand Manage.* (2017) 8:75–6.
44. Sun YC. Efficacy analysis of carbamazepine and gabapentin in the treatment of recurrent trigeminal neuralgia and their effects on serum 5-HT, HCY and CRP. *Clin Med Res Prac.* (2016) 1:2.



OPEN ACCESS

EDITED BY

Lei Xu,
Fudan University, China

REVIEWED BY

Shizhang Ling,
The First Affiliated Hospital of Wannan Medical
College, China
Yongjie Wang,
Hangzhou Normal University, China

*CORRESPONDENCE

Yue Wang
✉ wangyuespine@zju.edu.cn

RECEIVED 22 November 2022

ACCEPTED 12 June 2023

PUBLISHED 28 June 2023

CITATION

Jin Q, Chang Y, Lu C, Chen L and
Wang Y (2023) Referred pain: characteristics,
possible mechanisms, and clinical
management.
Front. Neurol. 14:1104817.
doi: 10.3389/fneur.2023.1104817

COPYRIGHT

© 2023 Jin, Chang, Lu, Chen and Wang. This is
an open-access article distributed under the
terms of the [Creative Commons Attribution
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is
permitted which does not comply with these
terms.

Referred pain: characteristics, possible mechanisms, and clinical management

Qianjun Jin, Yuxin Chang, Chenmiao Lu, Lunhao Chen and
Yue Wang*

Department of Orthopedic Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Purpose of this review: Referred pain is a common but less understood symptom that originates from somatic tissues. A comprehensive recognition of referred pain is important for clinicians when dealing with it. The purpose of this study is to summarize the current understanding of referred pain, including its pathogenesis, characteristics, diagnosis, and treatment.

Recent findings: Referred pain arises not only from pathologies primarily involving local tissue but also from lesions in distant structures. Central sensitization of convergent neurons and peripheral reflexes of dichotomizing afferent fibers are two theories proposed to explain the pathological mechanism of referred pain. Because syndromes related to referred pain of different origins overlap each other, it is challenging to define referred pain and identify its originating lesions. Although various approaches have been used in the diagnosis and treatment of referred pain, including conservative treatment, blockade, radiofrequency, and surgery, management of referred pain remains a clinical challenge.

Summary: Unlike radicular pain and neuropathic pain, referred pain is a less studied area, despite being common in clinics. Referred pain can derive from various spinal structures, and blockade helps identify the primary pathology. Due to the heterogeneity of referred pain, treatment outcomes remain uncertain. Further studies are needed to improve our understanding of referred pain.

KEYWORDS

referred pain, somatic, spinal, neuropathic pain, central sensitization

Introduction

In the field of somatic pain, most clinicians are familiar with radicular pain and neuropathic pain, but little is known about referred pain. Referred pain occurs in an area far from the primary lesion (1, 2) and is sometimes associated with secondary hyperalgesia and trophic changes in the referred areas (3). Although clinically common, the nature of referred pain remains an enigma (4–6). In most cases, pain in the dermatome regions, which are innervated by specific peripheral nerves, is simply regarded as radicular pain or neuropathic pain (1, 7). Referring to this phenomenon, the International Association for the Study of Pain (IASP), citing the classic treatise on pain, stated long ago in 2011 that “Pain in the lower limb should be described specifically as either referred pain or radicular pain” [sic] (2). In addition to the lower limbs, the diagnosis of pain in other somatic areas should also follow this guideline. Some

researchers have even suggested that if the nature of pain is not clear, the diagnosis should not be jumped to Vulfsons et al. (7).

A better understanding of referred pain may help physicians in clinical diagnosis and decision-making, thus improving clinical outcomes. To this end, this review characterizes referred pain in various conditions and discusses possible pathological mechanisms and therapeutic measures based on the contributions of previous studies.

Definition and epidemiology of referred pain

Somatic pain arises not only from pathologies primarily involving local tissues, but also from dysfunction in distant tissues, including spinal, neuromuscular, and other somatic structures (8, 9). The term “referred pain” has been documented to describe pain spreading to the somatic regions far from the site of noxious stimulation (10), which is not caused by nerve root stimulation.

Referred pain can be caused by autogenous dysfunction or triggered by external stimuli. For example, referred pain can even be stimulated by hypertonic saline in healthy adults (11, 12). However, referred pain is not directly caused by mechanical or inflammatory stimulation of the nerves or nerve roots (1), nor is it caused by neuromas or lesions of the peripheral nerves or central nervous system (13). On the contrary, referred pain has even been induced by stimulating adjacent structures in patients with denervated or missing limbs in special cases of spinal cord injury (14), plexus avulsion (15, 16), and amputation (13, 17). In another typical example, referred pain has been triggered in areas of neuropathic pain by scraping the earlobe in patients with postherpetic neuralgia (18).

It is clear that referred pain is common and variable. Spinal referred pain, for example, reportedly occurs in 17%–84% of patients with low back pain (19, 20). While referred pain has been a focus of research for a long time, multicenter studies are lacking, and the incidence of referred pain in the general population largely remains unknown.

Possible pathophysiology of referred pain

The cortical reorganization theory has long been proposed to explain the pathophysiology of referred pain (21). Contrary to the cortical reorganization theory, however, referred pain has also been found in areas with segregated cortical activation (22). In general, most researchers accept convergence projection theory. In this theory, the convergence of nociceptive afferents on second-order neurons in the spinal cord leads to the occurrence of pain in different somatic areas, like “crossed telephone lines” (9, 10, 23, 24) (Figure 1). While the convergence of sensory afferents at the subcortical level has been confirmed in an animal study (25), referred pain can be considered the central sensitization of convergent neurons. A classic example is the convergence of C1–C3 spinal nerves, where trigeminal input results in migraine and cervicogenic headache (26).

In another theory, it has been proposed that there are dichotomizing afferent fibers that ramify and distribute to regions of primary dysfunction and referred areas (27–30). While primary

lesions stimulate afferent fibers in deep regions, these afferent fibers trigger the activation of a reflex arc toward the muscle via somatic efferent fibers. This theory can be summarized as the pathomechanism underlying physiological reflexes (8). Using double-labeling with fluorescent tracers, dichotomizing axons between the lumbar intervertebral disc and the groin region have been reported (30), supporting this theory (Figure 2). Another example is visceral referred pain, which is regarded as a pathological combination of nociceptive processing pathways for visceral and somatic sensory afferent neurons. As such, visceral referred pain is also called a “viscerosensory reflex” (9, 31).

Referred areas for lesions in various spinal structures

Reported referred pain can originate from almost all spinal structures (5, 31). Among them, the intervertebral disc (31), facet joint (32, 33), and sacroiliac joint (34) are the most common structures involved in spinal referred pain. Referred pain is usually felt in some designated dermatome areas in patients with various spinal disorders, including spinal injuries, disc degeneration, spinal canal stenosis, and lumbar spondylolisthesis (34–36). For example, the pain usually occurs in the distant lumbosacral regions in patients with traumatic or osteoporotic vertebral fractures (37, 38), suggesting that pain may be referred outward and downward from the fracture site according to some predictable patterns. Kellgren et al. first reported that spinal referred pain may be distributed in a segmental pattern (39, 40). However, it is difficult to “map” referred areas because referred pain of different origins often overlaps (12). Moreover, referred pain does not follow dermatomes or patterns of nerve root distribution (11, 41).

While referred pain in the chest, shoulder, and upper limb usually originates from the cervical and thoracic spine (11, 42, 43), referred pain related to lumbar spine diseases tends to spread to the back, abdomen (24), buttocks (12, 31, 44, 45), groin (12, 46, 47), thigh (12, 34), and even the distal limb below the knee (48). Among them, the buttocks and leg are the most common sites of lumbar referred pain (12, 28, 31, 34, 49). Moreover, the distribution patterns for lumbar referred pain vary according to anatomical structures. For example, patients with disc degeneration may feel pain at the dermatome region innervated by the corresponding dorsal root ganglion (DRG) neurons (1, 19, 31, 48, 50), with greater intensity of referred pain being associated with more severe disc degeneration (31, 48).

Referred pain originating from the facet joints is also segmentally distributed, as it may be related to segmental DRGs. For cervical facet joints, the neck and shoulder are the most commonly involved sites of referred pain (42, 51). Referred pain in the back and iliac crest usually originates from the thoracic facet joints (43). For lumbar facet joints, pain may be referred to as the region between the hip and thigh. It has been reported that pathology of the superior facet joints typically induces referred pain to the flank, hip, and lateral thigh regions, while the inferior facet joints usually refer to the posterior thigh (12, 33, 52).

Referred pain from the sacroiliac joint mainly distributes to the lower back and buttocks, in addition to the thigh, groin, leg, and even foot (31, 53, 54). Nevertheless, there is considerable overlap in referral areas, although the pain has been irradiated from various spinal

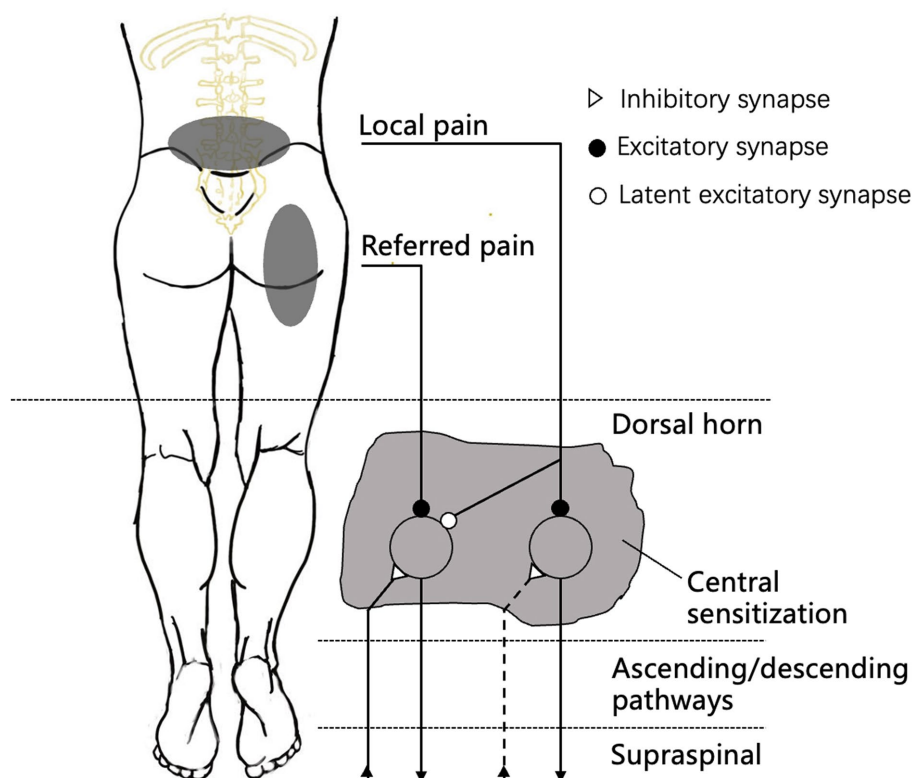


FIGURE 1

The neurophysiological schematic model of “central sensitization” in humans. Local pain will excite the pathway mediating pain upward to the dorsal horn, while a sensitization process is initiated at the same time. The release of sensitizing substances will cause the opening of latent synaptic connections to the neurons mediating pain from the referred area. Both the direct excitation of the involved neurons and the facilitation of afferent input in the referred area will result in the perception of referred pain (5).

elements (54). A clear map specifying the relationship between anatomical structures and referred regions is currently lacking.

Differentiation of referred pain from radicular pain

Referred pain usually occurs after local pain has persisted for a certain period (5, 55). Typically, referred pain is described as dull, aching, gnawing, annoying, drilling, or pressing (1, 55). Sometimes, referred pain is associated with secondary hyperalgesia and trophic changes (3). Once present, referred pain tends to become fixed in a particular region, depending on the referral pattern (1, 55). Unlike the explicit pathophysiology of radicular pain, it is difficult to locate the exact original site or boundaries of the affected area for referred pain as the convergence of afferent neurons is rather complicated (24, 34, 40, 44). However, referred pain can be identified in a wide area with an identified center or core, although its boundary is difficult to define (11). Although a number of studies have described the segmental patterns of spinal referred pain, these patterns are not consistent among patients and dysfunctions (11, 31, 34, 43, 44). Importantly, referred pain is not dermatomally distributed, which is markedly different from radicular pain (11, 41).

Neuropathic pain is a symptom and also a consequence of various diseases affecting the somatosensory nervous system (13, 56).

Typically, neuropathic pain is described as allodynia and hyperalgesia and is usually distributed along the dermatome of peripheral nerve-innervated areas (13, 24). Mechanistically, radicular pain is evoked by ectopic discharges from affected DRGs or nerves. For example, herniated disc tissues compress the spinal root and induce pain traveling along the lower limb, which may be largely resolved after the removal of the herniated disc tissue (57, 58). In many cases, radicular pain is associated with neurological deficits such as decreased muscle strength, weakened reflexes, and specific numbness in the related dermatome (59, 60). The neurophysiological relationship between radicular pain and the affected nerves has been clarified in preclinical animal studies (61–63). The distinguishing features of referred pain and radicular pain are listed in Table 1. However, how to precisely identify referred pain and distinguish it from radicular pain needs further investigation.

Diagnostic measures for referred pain

As mentioned above, referred pain can originate from various spinal elements, and there is significant overlap in referred areas and distribution patterns. It is thus difficult to locate the exact source of referred pain (24). There are two methods that can help diagnose referred pain: (1) to induce a similar pain pattern using different types of stimuli; (2) to relieve the pain with a local block (5, 31, 55, 64).

In many cases, radiographic imaging, such as CT, MR, and myelography, is not able to identify the exact source of referred pain. As early as 1939, Kellgren et al. first developed an experimental model and found that referred pain could be triggered by injection of hypertonic saline solution (39, 40, 65), as verified in investigating cases of spontaneous pain of unclear etiology (6). Extending this theory, various percutaneous procedures, namely provocative lumbar discography (66–70), facet joint injections (32), blocks (33, 52, 70), intra-articular sacroiliac joint injections (31, 53, 54), and epidural injections (70) have been developed to reproduce or block referred pain and to identify its exact source. In an inflammatory pain model, capsaicin has been used for functional spinal and supraspinal MR imaging, which has been reported to identify the origin of referred pain (18).

A multidisciplinary approach to the treatment of referred pain

Multimodal treatments for referred pain include pharmacotherapy, physical therapy, regular exercise, and psychotherapy (52). Invasive

procedures are reserved for those with a confirmed source of somatic referred pain. Invasive therapies, such as local blockage and radiofrequency, are commonly used in treating spinal referred pain. In an early Lancet report, referred pain triggered by injecting hypertonic saline solution could be successfully treated with a local block (6). Later, Hockaday et al. reported that referred pain could be abolished by a local block but was less consistently reduced by blocking the referred areas (11). For instance, intra-articular injections of corticosteroids have been used to treat facetogenic (52, 71) and sacroiliac-referred pain (72). Furthermore, disco block, inspired by discography, was once regarded as a useful approach to relieving discogenic referred pain (50, 73). Shealy et al. first described the method of applying radiofrequency to the facet joints in 1975 (74). Radiofrequency has since been acknowledged as the “gold standard” for the treatment of facetogenic and sacroiliac-referred pain. Thermal intradiscal procedures, such as intradiscal electrothermal annuloplasty and biacuplasty, have also been used to effectively treat discogenic low back pain and referred leg pain (20, 31, 75).

When treating referred pain, non-invasive treatments are usually optional (76). There are some exceptions. For example, discogenic groin pain can be significantly improved after lumbar disc surgery (50). Percutaneous vertebroplasty, or kyphoplasty, is effective in relieving lumbosacral referred pain following osteoporotic vertebral compression fractures (38). It has also been reported that patients with back pain associated with referred inguinal or leg pain had better clinical outcomes after spine surgery than those without (77), suggesting that surgical therapy for the primary conditions may be an effective approach to controlling the secondary referred pain.

Although various interventions have been reported to be effective in the treatment of referred pain, randomized controlled trials are needed to clarify the efficacy of conservative treatments, blockage, radiofrequency, and surgery. In addition, clinical guidelines and expert consensus on the management of referred pain are currently lacking.

Conclusion

In this mini-review, patterns, and characteristics of referred pain from somatic structures are discussed. The similarity between referred pain and peripheral neuropathic pain makes it difficult to approach the diagnosis of referred pain. The main hypotheses for the pathophysiological mechanism of referred pain include central sensitization and peripheral reflex, which may jointly explain the development of referred pain. Convergence of sensory afferents at the subcortical level and dichotomizing afferents have also been proposed in scientific research. The overlap of referred areas from different somatic structures makes it difficult to locate the primary origins of referred pain. In clinical practice, local blockage and radiofrequency

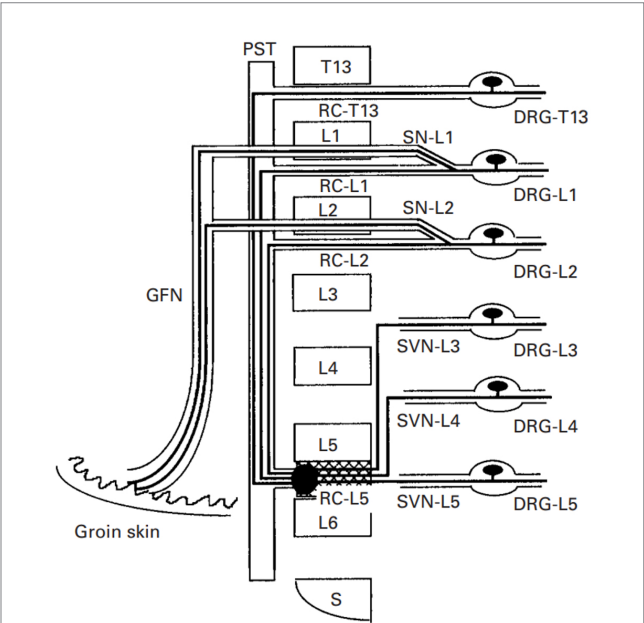


FIGURE 2 The neurophysiological schematic model of “dichotomizing axons” in rats. There are dichotomizing axons that project to the ventral portion of the L5–L6 disc and the groin skin during the sensory pathways from the ventral portion of the L5–L6 disc and neurons in the L1 and L2 DRG. GFN, genitofemoral nerve; PST, paravertebral sympathetic trunk; RC, ramus communicans; SN, spinal nerve; SVN, sinuvertebral nerve (30).

TABLE 1 Differential features of referred pain and radicular pain.

Feature	Referred pain	Radicular pain
Quality	Dull, aching, gnawing, annoying, drilling, pressing	Shooting, lancinating, shocking, electric
Concomitant symptom	Secondary hyperalgesia trophic changes	Numbness, decreased muscle strength, weakened reflexes
Distribution pattern	A wide area with a boundary that is difficult to define, but with an identified center or core	Travels along the length of the lower limb in a band no more than 2–3 inches in width

are commonly used interventions in the diagnosis and treatment of referred pain, although there is a considerably high false-positive rate for both. Given the complex pathologies, changeable manifestations, and inconsistent distribution patterns, the treatment of referred pain remains a clinical challenge.

Author contributions

QJ and YW carried out the project design and literature summary. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 82201358) and the Medicine and Health Science and Technology Plan in Zhejiang Province (grant no. 2023RC019).

References

- Bogduk N. On the definitions and physiology of back pain, referred pain, and radicular pain. *Pain*. (2009) 147:17–9. doi: 10.1016/j.pain.2009.08.020
- Merskey H, Bogduk N. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. *Pain Suppl*. (1986) 3:S1–S226.
- Vecchiet L, Vecchiet J, Giamberardino MA. Referred muscle pain: clinical and pathophysiologic aspects. *Curr Rev Pain*. (1999) 3:489–98. doi: 10.1007/s11916-999-0077-y
- Affaitati G, Costantini R, Tana C, Cipollone F, Giamberardino MA. Co-occurrence of pain syndromes. *J Neural Transm*. (2020) 127:625–46. doi: 10.1007/s00702-019-02107-8
- Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. *Scand J Rheumatol Suppl*. (2006) 122:1–43. doi: 10.1080/03009740600865980
- Whitty C, Willison RGJL. Some aspects of referred pain. *Lancet*. (1958) 272:226–31. doi: 10.1016/S0140-6736(58)90058-8
- Vulfsons S, Bar N, Eisenberg E. Back pain with leg pain. *Curr Pain Headache Rep*. (2017) 21:32. doi: 10.1007/s11916-017-0632-x
- Giamberardino MA. Referred muscle pain/hyperalgesia and central sensitisation. *J Rehabil Med*. (2003) 35:85–8. doi: 10.1080/16501960310010205
- Luz LL, Fernandes EC, Sívado M, Kokai E, Szucs P, Saffronov BV. Monosynaptic convergence of somatic and visceral C-fiber afferents on projection and local circuit neurons in lamina I: a substrate for referred pain. *Pain*. (2015) 156:2042–51. doi: 10.1097/j.pain.0000000000000267
- Merskey H, Bogduk N. *Classification of chronic pain. Descriptions of chronic pain syndromes and definition of pain terms*. Seattle: IASP Press (1994) 1–28.
- Hockaday JM, Whitty CW. Patterns of referred pain in the normal subject. *Brain*. (1967) 90:481–96. doi: 10.1093/brain/90.3.481
- McCall IW, Park WM, O'Brien JP. Induced pain referral from posterior lumbar elements in Normal subjects. *Spine (Phila Pa 1976)*. (1979) 4:441–6.
- Finnerup NB, Kuner R, Jensen TS. Neuropathic pain: from mechanisms to treatment. *Physiol Rev*. (2021) 101:259–301. doi: 10.1152/physrev.00045.2019
- Moore CI, Stern CE, Dunbar C, Kostyk SK, Gehi A, Corkin S. Referred phantom sensations and cortical reorganization after spinal cord injury in humans. *Proc Natl Acad Sci U S A*. (2000) 97:14703–8. doi: 10.1073/pnas.250348997
- Finnerup NB, Norrbrink C, Fuglsang-Frederiksen A, Terkelsen AJ, Hojlund AP, Jensen TS. Pain, referred sensations, and involuntary muscle movements in brachial plexus injury. *Acta Neurol Scand*. (2010) 121:320–7. doi: 10.1111/j.1600-0404.2009.01248.x
- Htut M, Misra P, Anand P, Birch R, Carlstedt T. Pain phenomena and sensory recovery following brachial plexus avulsion injury and surgical repairs. *J Hand Surg*. (2006) 31:596–605. doi: 10.1016/j.jhsb.2006.04.027
- Bolognini N, Olgiati E, Maravita A, Ferraro F, Fregni F. Motor and parietal cortex stimulation for phantom limb pain and sensations. *Pain*. (2013) 154:1274–80. doi: 10.1016/j.pain.2013.03.040

Acknowledgments

We would like to thank the colleagues in our spine laboratory for their assistance in this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

- Forstenpointner J, Wolff S, Stroman PW, Jansen O, Rehm S, Baron R, et al. "from ear to trunk"-magnetic resonance imaging reveals referral of pain. *Pain*. (2018) 159:1900–3. doi: 10.1097/j.pain.0000000000001279
- Mellin G, Hurri H. Referred limb symptoms in chronic low back pain. *J Spinal Disord*. (1990) 3:52–8. doi: 10.1097/00002517-199003000-00009
- Derby R, Lee SH, Seo KS, Kazala K, Kim BJ, Mi JK. Efficacy of IDET for relief of leg pain associated with Discogenic low Back pain. *Pain Pract*. (2004) 4:281–5. doi: 10.1111/j.1533-2500.2004.04401.x
- Grüsser SM, Winter C, Mühlhnickel W, Denke C, Karl A, Villringer K, et al. The relationship of perceptual phenomena and cortical reorganization in upper extremity amputees. *Neuroscience*. (2001) 102:263–72. doi: 10.1016/S0306-4522(00)00491-7
- Soler MD, Kumru H, Vidal J, Pelayo R, Tormos JM, Fregni F, et al. Referred sensations and neuropathic pain following spinal cord injury. *Pain*. (2010) 150:192–8. doi: 10.1016/j.pain.2010.04.027
- Ohtori S, Takahashi K, Chiba T, Yamagata M, Sameda H, Moriya H. Calcitonin gene-related peptide immunoreactive neurons with dichotomizing axons projecting to the lumbar muscle and knee in rats. *Eur Spine J*. (2003) 12:576–80. doi: 10.1007/s00586-003-0573-4
- Harding G, Yelland M. Back, chest and abdominal pain—is it spinal referred pain? Australian family physician. *Aust Fam Physician*. (2007) 36:422–3.
- Chen L, Lu X, Jin Q, Gao Z, Wang Y. Sensory innervation of the lumbar 5/6 intervertebral disk in mice. *Front Neurol*. (2023) 14:1084209. doi: 10.3389/fneur.2023.1084209
- Edmeads J. The cervical spine and headache. *Neurology*. (1988) 38:1874–8. doi: 10.1212/WNL.38.12.1874
- Takahashi Y, Nakajima Y, Sakamoto T, Moriya H, Takahashi K. Capsaicin applied to rat lumbar intervertebral disc causes extravasation in the groin skin: a possible mechanism of referred pain of the intervertebral disc. *Neurosci Lett*. (1993) 161:1–3. doi: 10.1016/0304-3940(93)90125-5
- Fujii T, Sakuma Y, Orita S, Inoue G, Ochiai N, Kuniyoshi K, et al. Dichotomizing sensory nerve fibers innervating both the lumbar vertebral body and the area surrounding the iliac crest: a possible mechanism of referred lateral back pain from lumbar vertebral body. *Spine*. (2013) 38:E1571–4. doi: 10.1097/BRS.0b013e3182a879cd
- Wakai K, Ohtori S, Yamashita M, Yamauchi K, Inoue G, Suzuki M, et al. Primary sensory neurons with dichotomizing axons projecting to the facet joint and the low back muscle in rats. *J Orthop Sci*. (2010) 15:402–6. doi: 10.1007/s00776-010-1465-1
- Sameda H, Takahashi Y, Takahashi K, Chiba T, Moriya H. Dorsal root ganglion neurones with dichotomising afferent fibres to both the lumbar disc and the groin skin. *J Bone Joint Surg Br*. (2003) 85:600–3. doi: 10.1302/0301-620X.85B4.13306
- Kurosawa D, Murakami E, Aizawa T. Referred pain location depends on the affected section of the sacroiliac joint. *Eur Spine J*. (2015) 24:521–7. doi: 10.1007/s00586-014-3604-4
- Ohtori S, Miyagi M, Inoue G. Sensory nerve ingrowth, cytokines, and instability of discogenic low back pain: a review. *Spine Surg Relat Res*. (2018) 2:11–7. doi: 10.22603/ssrr.2016-0018

33. Marks PRJ. Distribution of pain provoked from lumbar facet joints and related structures during diagnostic spinal infiltration. *Pain*. (1989) 39:37–40. doi: 10.1016/0304-3959(89)90173-5
34. Laplante BL, Ketchum JM, Saullo TR, Depalma MJ. Multivariable analysis of the relationship between pain referral patterns and the source of chronic low Back pain. *Pain Physician*. (2012) 2:171–8. doi: 10.36076/ppj.2012/15/171
35. Ko S. Correlations between sedimentation sign, dural sac cross-sectional area, and clinical symptoms of degenerative lumbar spinal stenosis. *Eur Spine J*. (2018) 27:1623–8. doi: 10.1007/s00586-017-5374-2
36. Mannion AF, Mutter UM, Fekete TF, Porchet F, Jeszenszky D, Kleinstuck FS. Validity of a single-item measure to assess leg or back pain as the predominant symptom in patients with degenerative disorders of the lumbar spine. *Eur Spine J*. (2014) 23:882–7. doi: 10.1007/s00586-014-3193-2
37. Clark EM, Hutchinson AP, Mcloskey EV, Stone MD, Martin JC, Bhalla AK, et al. Lateral back pain identifies prevalent vertebral fractures in post-menopausal women: cross-sectional analysis of a primary care-based cohort. *Rheumatology*. (2010) 49:505–12. doi: 10.1093/rheumatology/kep414
38. Li YFX, Pan J, Yang M, Li L, Su Q, Tan J. Percutaneous vertebroplasty versus kyphoplasty for thoracolumbar osteoporotic vertebral compression fractures in patients with distant lumbosacral pain. *Pain Physician*. (2021) 24:E349–56. doi: 10.36076/ppj.2021/24/E349
39. Kellgren JH. Observations on referred pain arising from muscle. *Clin Sci*. (1938) 3:175–90.
40. Kellgren JH. On the distribution of pain arising from deep somatic structures with charts of segmental pain areas. *Clin Sci*. (1939) 4:36.
41. Elliott FA. Tender muscles in sciatica: electromyographic studies. *Lancet*. (1944) 243:47–9. doi: 10.1016/S0140-6736(00)42562-6
42. Fukui S, Ohseto K, Shiotani M, Ohno K, Karasawa H, Naganuma Y, et al. Referred pain distribution of the cervical zygapophyseal joints and cervical dorsal rami. *Pain*. (1996) 68:79–83. doi: 10.1016/S0304-3959(96)03173-9
43. Fukui S, Ohseto K, Shiotani M. Patterns of pain induced by distending the thoracic zygapophyseal joints. *Reg Anesth*. (1997) 22:332–6. doi: 10.1016/S1098-7339(97)80007-7
44. Furman MB, Johnson SC. Induced lumbosacral radicular symptom referral patterns: a descriptive study. *Spine J*. (2019) 19:163–70. doi: 10.1016/j.spinee.2018.05.029
45. Ohnmeiss DD, Vanharanta H, Ekholm J. Relation between pain location and disc pathology: a study of pain drawings and CT/discography. *Clin J Pain*. (1999) 15:210–7. doi: 10.1097/00002508-199909000-00008
46. Yukawa Y, Kato F, Kajino G, Nakamura S, Nitta H. Groin pain associated with lower lumbar disc herniation. *Spine*. (1997) 22:1736–9.
47. Murphey F. Sources and patterns of pain in disc disease. *Clin Neurosurg*. (1968) 15:343–51. doi: 10.1093/neurosurgery/15.CN_suppl_1.343
48. Ohnmeiss DD, Vanharanta H, Ekholm J. Degree of disc disruption and lower extremity pain. *Spine*. (1997) 22:1600–5.
49. DePalma MJ, Ketchum JM, Thomas S. What is the source of chronic low back pain and does age play a role? *Pain Med*. (2011) 12:224–33. doi: 10.1111/j.1526-4637.2010.01045.x
50. Oikawa Y, Ohtori S, Koshi T, Takaso M, Inoue G, Orita S, et al. Lumbar disc degeneration induces persistent groin pain. *Spine*. (2012) 37:114–8. doi: 10.1097/BRS.0b013e318210e6b5
51. Manchikanti L, Kosanovic R, Cash KA, Pampati V, Hirsch JA. Assessment of prevalence of cervical facet joint pain with diagnostic cervical medial branch blocks: analysis based on chronic pain model. *Pain Physician*. (2020) 23:531–40. doi: 10.36076/ppj.2020.23.531
52. Van Kleef M, Vanelender P, Cohen SP, Lataster A, Van Zundert J, Mekhail N. 12. Pain originating from the lumbar facet joints. *Pain Pract*. (2010) 10:459–69. doi: 10.1111/j.1533-2500.2010.00393.x
53. Slipman CW, Jackson HB, Lipetz JS, Chan KT, Vresilovic EJ. Sacroiliac joint pain referral zones. *Arch Phys Med Rehabil*. (2000) 81:334–8. doi: 10.1016/S0003-9993(00)90080-7
54. Van der Wurff P, Buijs EJ, Groen GJ. Intensity mapping of pain referral areas in sacroiliac joint pain patients. *J Manip Physiol Ther*. (2006) 29:190–5. doi: 10.1016/j.jmpt.2006.01.007
55. Domenech-Garcia V, Palsson TS, Herrero P, Graven-Nielsen T. Pressure-induced referred pain is expanded by persistent soreness. *Pain*. (2016) 157:1164–72. doi: 10.1097/j.pain.0000000000000497
56. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol*. (2010) 9:807–19. doi: 10.1016/S1474-4422(10)70143-5
57. Kreiner DS, Hwang SW, Easa JE, Resnick DK, Baisden JL, Bess S, et al. An evidence-based clinical guideline for the diagnosis and treatment of lumbar disc herniation with radiculopathy. *Spine J*. (2014) 14:180–91. doi: 10.1016/j.spinee.2013.08.003
58. Amin RM, Andrade NS, Neuman BJ. Lumbar disc herniation. *Curr Rev Musculoskelet Med*. (2017) 10:507–16. doi: 10.1007/s12178-017-9441-4
59. Vroomen PC, de Krom MC, Wilmink JT, Kester AD, Knottnerus JA. Diagnostic value of history and physical examination in patients suspected of lumbosacral nerve root compression. *J Neurol Neurosurg Psychiatry*. (2002) 72:630–4. doi: 10.1136/jnnp.72.5.630
60. Vucetic N, Svensson O. Physical signs in lumbar disc hernia. *Clin Orthop Relat Res*. (1996) 333:192–201. doi: 10.1097/00003086-199612000-00020
61. Kim SJ, Kim WR, Kim HS, Park HW, Cho YW, Jang SH, et al. Abnormal spontaneous activities on needle electromyography and their relation with pain behavior and nerve fiber pathology in a rat model of lumbar disc herniation. *Spine*. (2011) 36:E1562–7. doi: 10.1097/BRS.0b013e318210aa10
62. Obata K, Tsujino H, Yamanaka H, Yi D, Fukuoka T, Hashimoto N, et al. Expression of neurotrophic factors in the dorsal root ganglion in a rat model of lumbar disc herniation. *Pain*. (2002) 99:121–32. doi: 10.1016/S0304-3959(02)00068-4
63. Seki S, Sekiguchi M, Konno SI. Association between neurotrophic factor expression and pain-related behavior induced by nucleus pulposus applied to rat nerve root. *Spine*. (2018) 43:E7–E15. doi: 10.1097/BRS.0000000000002223
64. O'Neill S, Graven-Nielsen T, Manniche C, Arendt-Nielsen L. Ultrasound guided, painful electrical stimulation of lumbar facet joint structures: an experimental model of acute low back pain. *Pain*. (2009) 144:76–83. doi: 10.1016/j.pain.2009.03.014
65. Lewis T, Kellgren JH. Observations relating to referred pain, visceromotor reflexes and other associated phenomena. *Clin Sci*. (1939) 4:47–71.
66. Moneta GB, Videman T, Kaivanto K, Aprill C, Spivey M, Vanharanta H, et al. Reported pain during lumbar discography as a function of annular ruptures and disc degeneration: a re-analysis of 833 discograms. *Spine*. (1994) 19:1968–74.
67. Kim HG, Shin DA, Kim HI, Yoo EA, Shin DG, Lee JO. Clinical and radiological findings of Discogenic low Back pain confirmed by automated pressure-controlled discography. *J Korean Neurosurg Soc*. (2009) 46:333–9. doi: 10.3340/jkns.2009.46.4.333
68. Lindblom K. Technique and results of diagnostic disc puncture and injection (discography) in the lumbar region. *Acta Orthop Scand*. (1951) 20:315–26. doi: 10.3109/17453675108991178
69. Derby R, Kim BJ, Lee SH, Chen Y, Seo KS, Aprill C. Comparison of discographic findings in asymptomatic subject discs and the negative discs of chronic LBP patients: can discography distinguish asymptomatic discs among morphologically abnormal discs? *Spine J*. (2005) 5:389–94. doi: 10.1016/j.spinee.2005.01.007
70. El-Khoury GY, Renfrew DL. Percutaneous procedures for the diagnosis and treatment of lower back pain: diskography, facet-joint injection, and epidural injection. *AJR Am J Roentgenol*. (1991) 157:685–91. doi: 10.2214/ajr.157.4.1832511
71. O'Leary SA, Paschos NK, Link JM, Klineberg EO, Hu JC, Athanasiou KA. Facet joints of the spine: structure-function relationships, problems and treatments, and the potential for regeneration. *Annu Rev Biomed Eng*. (2018) 20:145–70. doi: 10.1146/annurev-bioeng-062117-120924
72. Vanelender P, Szadek K, Cohen SP, De Witte J, Lataster A, Patijn J, et al. 13. Sacroiliac Joint Pain. *Pain Pract*. (2010) 10:470–8. doi: 10.1111/j.1533-2500.2010.00394.x
73. Ohtori S, Kinoshita T, Yamashita M, Inoue G, Takahashi K. Results of surgery for discogenic low back pain: a randomized study using discography versus discoblock for diagnosis. *Spine*. (2009) 34:1345–8. doi: 10.1097/BRS.0b013e3181a401bf
74. Shealy CN. Percutaneous radiofrequency denervation of spinal facets. Treatment for chronic back pain and sciatica. *J Neurosurg*. (1975) 43:448–51. doi: 10.3171/jns.1975.43.4.0448
75. Helm Ii S, Simopoulos TT, Stojanovic M, Abdi S, El Terany MA. Effectiveness of thermal annular procedures in treating Discogenic low Back pain. *Pain Physician*. (2017) 20:447–70. doi: 10.36076/ppj/447
76. Brügger A. Über vertebrale, radikuläre und pseudoradikuläre Syndrome II. *Acta Rheumatol*. (1962) 19:S9–S111.
77. Ohtori S, Orita S, Yamauchi K, Eguchi Y, Aoki Y, Nakamura J, et al. Do physical symptoms predict the outcome of surgical fusion in patients with Discogenic low Back pain? *Asian Spine J*. (2016) 10:509–15. doi: 10.4184/asj.2016.10.3.509

Frontiers in Neurology

Explores neurological illness to improve patient care

The third most-cited clinical neurology journal explores the diagnosis, causes, treatment, and public health aspects of neurological illnesses. Its ultimate aim is to inform improvements in patient care.

Discover the latest Research Topics

[See more →](#)

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact

