frontiers | Research Topics

Novel treatments and the underlying mechanisms for diabetic foot and related diseases

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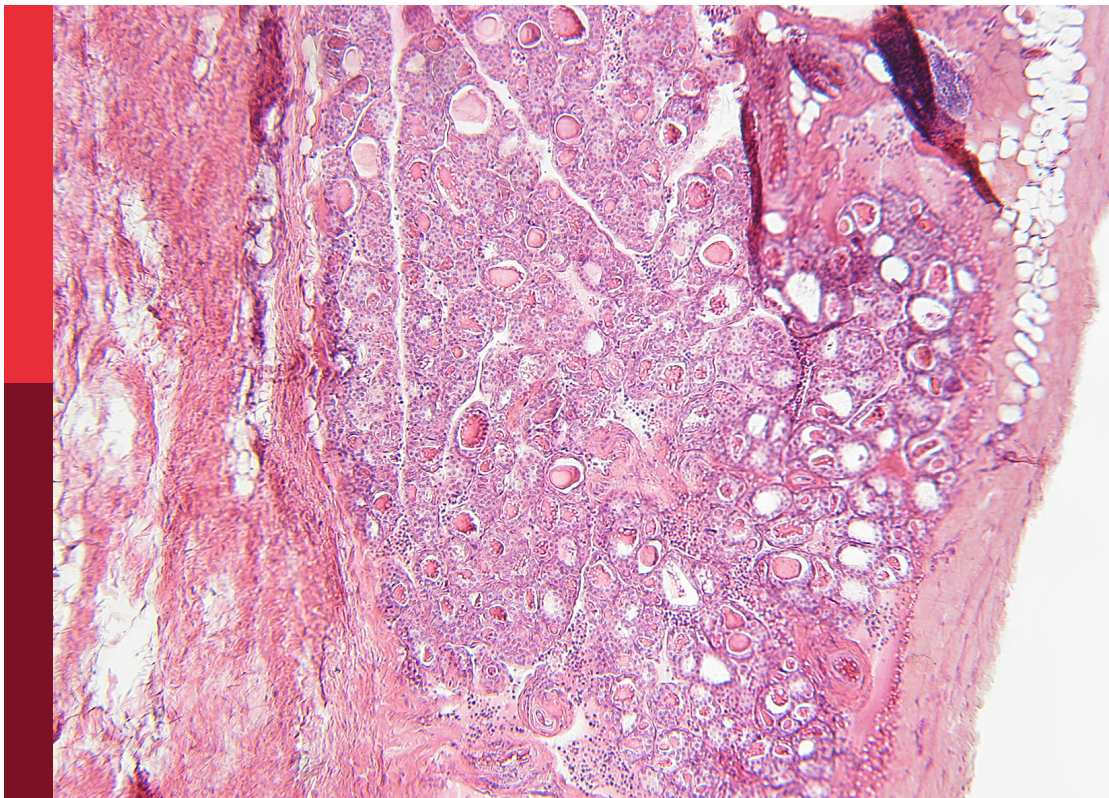
Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-3763-3
DOI 10.3389/978-2-8325-3763-3

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Novel treatments and the underlying mechanisms for diabetic foot and related diseases

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Citation

Liu, F., Aiping, W., Boey, J., Chen, A. F., Tang, Z., Wu, J., eds. (2023). *Novel treatments and the underlying mechanisms for diabetic foot and related diseases*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3763-3

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frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 20 November 2023
DOI 10.3389/fendo.2023.1323323

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 17 October 2023
ACCEPTED 18 October 2023
PUBLISHED 20 November 2023

CITATION
Boey J (2023) Editorial: Novel treatments and the underlying mechanisms for diabetic foot and related diseases.
Front. Endocrinol. 14:1323323.
doi: 10.3389/fendo.2023.1323323

Editorial: Novel treatments and the underlying mechanisms for diabetic foot and related diseases

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KEYWORDS

diabetic neuropathy, diabetic foot ulcer (DFU), diabetic limb salvage, mesenchymal stem cell (MSC), amputation

Editorial on the Research Topic
Novel treatments and the underlying mechanisms for diabetic foot and related diseases

The world is facing an epidemic of diabetes mellitus (DM), in which the International Diabetes Federation (IDF) estimates an increase of 50% to 783 million cases by 2045 (1). The prevalence of DM is higher in those above 75 years of age and in urban cities of middle-incomes countries (1). Approximately 6.7 million people died from diabetes-related complications in 2021 (1). The surge in DM and related complications have been linked to burgeoning global healthcare expenditure, reaching $1 trillion USD in 2021, and it is expected to increase significantly in the next few decades (1).

Diabetic peripheral neuropathy (DPN) is among the common diabetes-related complications that affects approximately 50% of individuals diagnosed with DM (2). Dong et al. found that abnormal vibration perception threshold (VPT) was a significant predictor for altered dynamic gait pattern and stability ($P<0.01$). In addition, the alteration to the function of the central nervous system have been implicated in the development of DPN. Neuroimaging of the cerebral cortex demonstrated significant changes to the cerebral morphology and function in the early stages of subclinical DPN (Zhao et al.). As DPN progresses, grey matter volume in the orbitofrontal cortex, the region which is involved in emotion and cognitive processing such as decision-making, decreases (Zhao et al.). The loss of protective sensation and vibration perception due to DPN predisposes diabetic foot ulcerations (DFUs) (3). Effective offloading modalities are essential to the management and prevention of DFUs. Hemler et al. designed specialized footwear with automated conformable insoles that actively adapt to patients' feet according to their plantar pressure distribution map. Further clinical trials will be necessary to evaluate the usefulness of this product.

It has been accepted that there is a 25% lifetime risk of developing DFUs (3). The prognosis of diabetes-related foot complication are often grim, and the 5-year mortality rate is comparable to that of cancer (4). The pathophysiology of DFUs is multi-factorial and it is believed that structural foot deformity and sensorimotor deficit give rise to elevated plantar pressure which lead to foot ulceration. A nomogram model for the prediction of

DFUs in elderly patients validated that age, DPN, lactate dehydrogenase, high-density cholesterol, total serum cholesterol, and smoking strongly influenced the development of DFUs (Shao et al.). Patients with DFUs require close monitoring and appropriate wound care management by multi-disciplinary foot care teams. The emergence of telemedicine and digital therapeutics has revolutionized remote patient monitoring, which has benefited both the patient and clinician during the recent COVID-19 pandemic. Keegan et al. observed an average reduction of 41.6% in wound area (p=0.005) and wound healing rate of 12% (3/25) through the use of a smartphone application.

The persistent inflammatory response exacerbates the release proteases and reactive oxygen species (ROS) that culminate in the impairment of fibroblast, growth factors, and extracellular matrix proteins (Zhu et al.). As a result, DFUs often result in lower extremity amputation (LEA) due to infection with or without a background of peripheral arterial disease (PAD) and/or infection. Laboratory investigation in patients with DM who had undergone LEA revealed reduced levels of serum albumin and poor ankle-brachial index (ABI) but elevated inflammatory biomarkers, including white blood cells (WBC), C-reactive protein (CRP), and fibrinogen (Gong et al.). Among the risk factors explored, a history of previous amputation (OR 10.194; P=0.001), gangrene (OR 6.466; P=0.010), and ABI (OR 0.791; P= 0.032) were significantly associated with LEA. The management of osteomyelitis revolves around effective source control using treatment options varying between antibiotic regimens and surgical resection of infected areas. The outcome of pharmacotherapy depends on of appropriate culture-directed antibiotic regimens. In a retrospective study by Xu et al., the authors revealed that specimens taken from DFO grew predominately gram-negative bacilli but there were low concordance rates (19.3%) between deep tissue and bone specimens. Nonetheless, only 15.7% (9/57) of specimens were negative in both pathology and bacterial culture after conservative debridement. This group of nine patients healed significantly faster than those with residual infection after conservative debridement.

The concept of angiosome theory has been used widely in diabetic limb salvage. Similar to dermatome, the perfusion of an area of soft tissue, known as an angiosome, is provided by source arteries, but a single source artery can perfuse multiple overlapping angiosomes along its path (5). This principle is demonstrated in a case report of a non-healing posterior heel ulcer complicated by osteomyelitis and extensive PAD (Chen et al.). Balloon angioplasty was performed in all primary source arteries except the posterior tibial artery (PTA). In this case, the peroneal artery (choke vessel) was able to provide perfusion to the posterior heel despite occlusion in the PTA (primary source artery). Therefore, these choke vessels function as a compensatory safety mechanism. Secondly, tibial transverse transport (TTT) has been performed increasingly in the recent decades for the treatment of DFUs with advanced PAD, owing their ability to improve local vascularity and stimulate cellular proliferation (Hu et al.). In particular, the healing and limb salvage rate after TTT is 0.96 and 0.98, respectively. TTT significantly improved wound healing (OR: 10.43; p< 0.001) and limb salvage rates (OR: 9.65; p>0.001) when compared with a control group (Hu et al.). The common complications encountered with TTT were pin-associated infection (8%), DFU recurrence (2.9%), and fracture at the transportation site (2%). Nonetheless, there remains much heterogenicity in the clinical protocol of TTT. Another promising adjunct treatment for the management of DFUs is the use of mesenchymal stem cells (MSC). The potential of MSC lies in their pluripotent ability, secreting growth factors and cytokines that stimulate the proliferation and migration of fibroblast and endothelial cells and promoting angiogenesis (Yu et al.). In addition, MSCs have also been found to exert immunomodulatory effects by inducing macrophages to express anti-inflammatory M2 phenotype (Yu et al.). Despite these cellular effects, the variable clinical efficacy from the heterogeneity of MSCs poses considerable challenge to formulating a treatment protocol appropriate for its clinical application (Yu et al.).

In this Research Topic, the authors illustrated the global efforts to deepen our understanding of diabetes-related foot disease and the relentless search for novel treatment modalities. With globalization, the changes in lifestyle, dietary patterns, and physical activity is driving an uptrend in chronic diseases such as obesity and DM. The focus in our fight against DM goes beyond treatment but prevention through education. It takes more than an individual and community to agree upon the strategy to mark our path towards a diabetes-free world. It would be just be an imagination if we all don't act now.

Author contributions

JB: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The author declares 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

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 24 November 2022
DOI 10.3389/fendo.2022.1069437

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 13 October 2022
ACCEPTED 08 November 2022
PUBLISHED 24 November 2022

CITATION
Zhao L-M, Chen X, Zhang Y-M, Qu M-L, Selvarajah D, Tesfaye S, Yang F-X, Ou C-Y, Liao W-H and Wu J (2022) Changed cerebral function and morphology serve as neuroimaging evidence for subclinical type 2 diabetic polyneuropathy. *Front. Endocrinol.* 13:1069437. doi: 10.3389/fendo.2022.1069437

Changed cerebral function and morphology serve as neuroimaging evidence for subclinical type 2 diabetic polyneuropathy

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Introduction: Central and peripheral nervous systems are all involved in type 2 diabetic polyneuropathy mechanisms, but such subclinical changes and associations remain unknown. This study aims to explore subclinical changes of the central and peripheral and unveil their association.

Methods: A total of 55 type-2 diabetes patients consisting of symptomatic (n = 23), subclinical (n = 12), and no polyneuropathy (n = 20) were enrolled in this study. Cerebral morphology, function, peripheral electrophysiology, and clinical information were collected and assessed using ANOVA and post-hoc analysis. Gaussian random field correction was used for multiple comparison corrections. Pearson/Spearman correlation analysis was used to evaluate the association of the cerebral with the peripheral.

Results: When comparing the subclinical group with no polyneuropathy groups, no statistical differences were shown in peripheral evaluations except amplitudes of tibial nerves. At the same time, functional connectivity from the orbitofrontal to bilateral postcentral and middle temporal cortex increased significantly. Gray matter volume of orbitofrontal and its functional connectivity show a transient elevation in the subclinical group compared with the symptomatic group. Besides, gray matter volume in the orbitofrontal cortex negatively correlated with the Neuropathy Symptom Score ($r = -0.5871$, $p < 0.001$), Neuropathy Disability Score ($r = -0.3682$, $p = 0.009$), and Douleur

Neuropathique en 4 questions (r = -0.4403, p = 0.003), and also found correlated positively with bilateral peroneal amplitude (r > 0.4, p < 0.05) and conduction velocities of the right sensory sural nerve(r = 0.3181, p = 0.03). Similarly, functional connectivity from the orbitofrontal to the postcentral cortex was positively associated with cold detection threshold (r = 0.3842, p = 0.03) and negatively associated with Neuropathy Symptom Score (r = -0.3460, p = 0.01).

Discussion: Function and morphology of brain changes in subclinical type 2 diabetic polyneuropathy might serve as an earlier biomarker. Novel insights from subclinical stage to investigate the mechanism of type 2 diabetic polyneuropathy are warranted.

KEYWORDS

type 2 diabetic polyneuropathy, preclinical, neuropathic pain, cortical volume, functional connectivity, resting-state functional MRI

Introduction

Type 2 diabetic polyneuropathy (DPN) is one of the most common complications of diabetes. It could lead to various sensorimotor deficits (1), even significant disability and diminished quality of life (2). Pharmacotherapy remains the mainstay of treatment but often has poor efficacy and is limited by unwanted side effects (3). Early intervention is proposed as the most promising method to improve treatment outcomes and prevent DPN and its cascade of devasting sequelae (4). Thus, improving insights into the early mechanism may improve patient management.

Peripheral and central nervous systems have been demonstrated to be involved in the mechanism of clinical DPN (5). Tight associations of central with peripheral changes were also unveiled in many previous studies (6–12). However, whether a connection and to what extent exists between subclinical central and peripheral neuropathy remain controversial. Regarding the earliest stages in diabetes patients without neuropathy, some researchers insist that cortical atrophy is impacted by independent diabetes effects (13, 14). Early central conduction abnormalities were also found independent of peripheral nerve changes (15). In contrast, early alterations on the spinal cord (16) during subclinical DPN and its tight association with peripheral severity demonstrated a subclinical connection between the spinal cord and peripheral, increasing attention on the subclinical stage. There is a possibility from these studies that central and peripheral neuropathy with different stages suffers from separate pathological connections. Further support for this theory comes from studies showing a discrepancy in cerebral functional and structurally dynamic change and even plasticity (17) at different severity of DPN, and dysfunction along somatosensory efferent ways relies on the stage of DPN (18) has also been proven. Therefore, a key point of truly unveiling relationships between central and peripheral alterations lies in the enrollment of different stages of DPN, and no similar studies have been reported.

In this study, we underscore the importance of a subclinical stage of DPN. We performed cerebral structural and functional analysis and examined multi-phase central and peripheral nervous system alterations. We then aim to explore the possible connections between central and peripheral neuropathy in different stages and determine how cerebral function and morphology react to peripheral changes.

Methods

All subjects acquired written informed consent before attending the study and got prior approval from the Medical Research Ethics Committee of Xiangya Hospital, Central South University (201709981).

Subjects

Patients with type-2 diabetes were recruited from the Department of endocrinology at Xiangya Hospital, Central South University in China. The inclusive criteria were: 1) 3 years after a type-2 diabetes diagnosis was confirmed, 2) right-handedness, and 3) age between 18 and 70 years. We excluded subjects for the following criteria: 1) HbA1c (%, mmol/mol)was over 11%, 2) deficiency of clinical or imaging data, 3) evident intracranial lesions such as retinopathy, cerebrovascular disease, psychiatric diseases, brain trauma, tumors, white matter

aberrance, or any other primary intracranial disease and history of surgery, 4) alcoholism or drug abuse, 5) patients with claustrophobia or other contraindication to MRI, and 6) images with artifacts (Figure 1).

Clinical and neuropathic assessments

Participants in this study underwent routine clinical evaluation, such as gender, age, body mass index (BMI), diabetic duration, HbA1c [%(mmol/mol)], and years of education.

All patients were examined with neuropathological assessments: 1) standard questionnaires such as the neuropathy symptom score (NSS), neuropathy disability score (NDS), and douleur neuropathique 4 questions (DN4). 2) neurophysiology testing consisting of quantitative sensory testing (QST, TSA II) and nerve conduction testings (NCTs, Nihon Kohden MEB-9400). Specifically, QST mainly detected cold and warm detection thresholds (CDT/WDT) from the dorsal surface of the bilateral foot with the standard technique (19). NCTs were performed at a stable skin temperature of 31°C and a room temperature of 24°C. The nerves measured were: 1) sensory nerve conduction velocities (SCV) and amplitudes of sural sensory nerve, 2) motor nerve conduction velocities (MCV) and amplitudes of common peroneal and tibial motor nerves.

Subjects with type-2 diabetes were divided into three groups based on neuropathy assessments above (20, 21): 1) type-2 diabetes without polyneuropathy (noDPN) group: subjects of no clinical symptoms and signs and abnormal neurophysiological assessments. 2) subclinical type 2 diabetic polyneuropathy (subDPN) group: subjects with neither clinical symptoms nor signs but at least one abnormality on neurophysiological assessments. 3) type 2 diabetic polyneuropathy group: subjects with clinical symptoms or/and signs and at least two abnormalities of neurophysiological assessments.

Image acquisition

All image data were collected from the same 3.0 T MRI scanners (Magnetom Prisma, Siemens, Germany) with a 64-channel head coil. The acquisition sequences of anatomy data were three-dimensional T1-weighted MRI scans through magnetization prepared rapid acquisition gradient-echo sequence with the following parameters. Thickness/gap:1.0/0 mm, repetition time: 2,300 ms, echo time: 2.98 ms, inversion

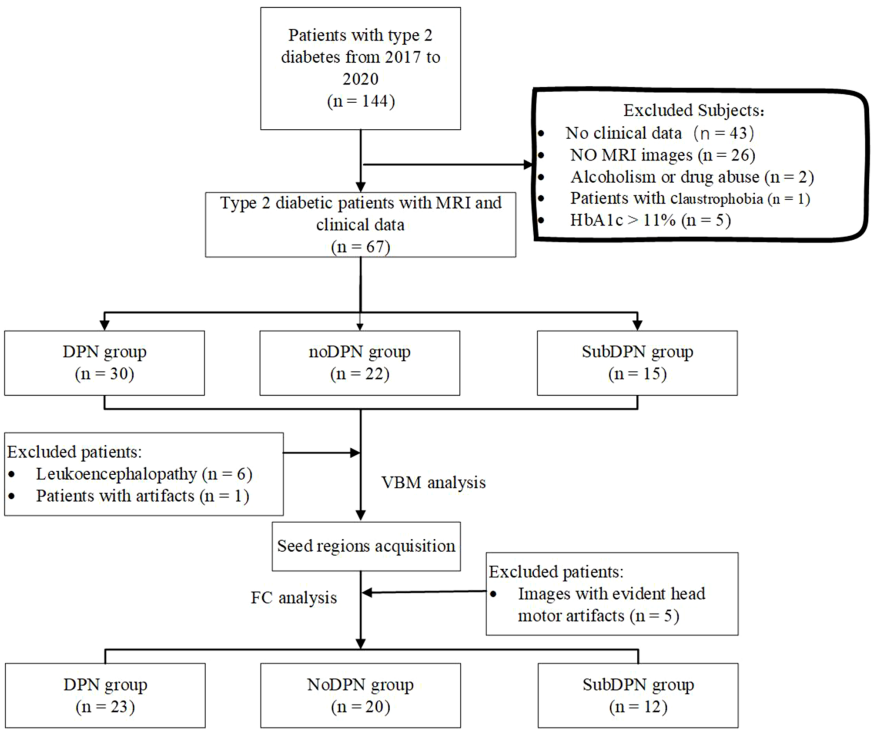

FIGURE 1
Flowchart diagram for subject selection. DPN, type 2 diabetic polyneuropathy; subDPN, subclinical type 2 diabetic polyneuropathy; noDPN,type 2 diabetes without polyneuropathy; MRI, magnetic resonance imaging; VBM, voxel-based morphometry; FC, functional connectivity.

time: 900 ms, 176 sagittal slices, the field of view: 256 mm × 256 mm, matrix: 256 × 256, flip angle: 9°, voxel size: 1.0 mm × 1.0 mm × 1.0 mm, sequence scan time: 5.09 min.

The resting-state functional data were acquired from echo-planar imaging sequences with the following parameters: repetition time: 2,000 ms, echo time: 30 ms, volumes: 240, slice thickness: 2, flip angle: 66°, in-plane pixel dimensions: 2.34 mm × 2.34 mm, total acquisition time: 8.15 min. Subjects were all asked to keep their eyes closed and awake without thinking about anything. Besides, the study collected T2-weighted fluid-attenuated inversion recovery sequence to exclude evident cerebral lesions.

Image preprocessing analysis

Before structural and functional data preprocessing, all data were transferred to NIfTI format. Structural data were preprocessed in the VBM 8 toolbox (http://dbm.neuro.uni-jena.de/vbm8/) embedded with Statistical Parametric Mapping 8 (SPM8, Wellcome Department of Cognitive Neurology, London, UK. www.fil.ion.ucl.ac.uk/spm/software/spm8). The preprocessing steps included segmentation, normalization, and smoothness. These images were split into gray matter, white matter, and cerebrospinal fluid with the segmentation algorithm. Diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) (22) algorithm was used for spatial coregistration to the Montreal neurological institute(MNI) space, and images with 1.5 mm × 1.5 mm × 1.5 mm voxel were acquired for subsequent steps. The smoothed images with a Gaussian kernel of 8 mm full-width at half-maximum were acquired for subsequent analysis. All those steps were finished in the pipeline "Estimate & Write" of VBM8.

The resting-state functional MRI data were preprocessed with Data Processing & Analysis for Resting-State Brain Imaging (DPABI, http://rfmri.org/dpabi) based on SPM8 and Matlab R2013b (23). After the first ten time points were removed, the subsequent preprocessing steps were as follows: slice timing, realignment, spatially normalization, smoothness, regression, filter, and detrending. Specifically, slice timing was carried out with corresponding slice orders and the reference order. Realignment requires head motion parameters displacement, mainly translation and rotation computed by a linear transformation with a six-parameter (rigid-body), within (x,y, or z-direction) 2 mm and 2 degrees (24). Then realigned images were normalized to MNI space with 3 mm × 3 mm × 3 mm voxel using DARTEL registration (22). Smoothness was done with a Gaussian kernel of 6 mm full-width at half-maximum, and detrending was performed to eliminate the thermal noise caused by the MRI machine. In addition, to avoid the effects of physiological low-frequency and high-frequency noise, we applied a filter to ensure the frequency was at 0.01- 0.08 Hz. Besides, other covariate nuisances were regressed, including white matter and cerebrospinal fluid signal, six head motion parameters, and global mean signal. The preprocessed images were applied for subsequent analysis.

Image analysis

One-way analysis of variance analysis (ANOVA) and *post-hoc* analysis was applied to compare differences in gray matter volume (GMV) among three groups, with Gaussian random field (GRF) multiple comparisons correction at voxel $p < 0.005$ and cluster $p < 0.05$. The brain areas with differences in cortical volume were selected as seed regions for subsequent functional connectivity analysis.

Whole-brain voxel-based functional connectivity (FC) was performed by Pearson correlation between the seed regions and the rest of the brain regions at a voxel level using DPABI. The correlation coefficients were then normalized to Z-scores using Fisher to Z transform. ANOVA analysis was applied to compare FC among groups. *Post-hoc* analysis was used to compare in pairs.

Besides, the specific values of FC and gray matter volumes were extracted from ROIs using the DPABI pipeline "ROI Signal Extractor" for subsequent correlation analysis.

Statistical analysis

Statistical analyses were performed in SPSS 26.0 (SPSS Inc., Chicago, IL) and DPABI. Continuous variables were shown as the mean and standard deviation, while the discrete variables were presented as numbers (percentages). ANOVA and *post-hoc* analysis were used for comparisons of group differences. Specifically, in SPSS, the homogeneity of variance was analyzed with Levene analysis. Besides, those with homoscedasticity underwent the Least Significance Difference test. In contrast, variables with heteroscedasticity underwent the nonparametric Kruskal-Wallis test. The significance level for the comparisons was a two-tailed p-value < 0.05.

In DPABI, each contrast was entered into ANOVA and *post-hoc* analysis regarding sex, age, and intracranial volume as covariates. Results were first thresholded at voxel-wise $p < 0.005$ and then corrected at the cluster level $p < 0.05$ for multiple comparisons using GRF correction.

Pearson and Spearman correlation analysis, with corresponding normal and non-normal distribution circumstances, was used to examine associations of parameters from brain structure and function with individual attributes of clinical assessments. The analysis was completed on SPSS 26.0. A two-tailed p-value less than 0.05 was conceived as a significance level.

Results

Demographic findings

The study cohort consisted of 20 type 2 diabetic patients with no DPN, 12 type 2 diabetic patients with subclinical DPN, and 23 type 2 diabetic patients with DPN. Gender (p = 0.944), education year (p = 0.053), BMI (p = 0.454), HbA1c (p = 0.624), and diabetes duration (p = 0.080) (Table 1) among the three groups show no statistical significance. The mean age of DPN patients was (56.96 ± 9.49) years, older than noDPN participants by (48.85 ± 9.06) years (p = 0.004).

Neuropathic assessments results

Bilateral cold and warm thresholds detection with no statistically significant were observed among individuals (Table 1). When comparing the DPN group with other groups, DN4, NSS, and NDS in subjects were highest, while MCV of right peroneal and left tibial nerves and SCV of right sural nerves were lowest (p < 0.05). Nevertheless, when comparing the subDPN group with the noDPN group, a lower amplitude of the right tibial nerve was the only significant one observed (p = 0.006), and other parameters showed no significant difference. When comparing the clinical DPN group with the noDPN group, amplitudes of bilateral

TABLE 1 Results of demographic characteristics and intracranial volumes.

	noDPN (n = 20)	subDPN (n = 12)	DPN (n = 23)
Gender			
Male	14 (70.0)	9 (75.0)	17 (73.9)
Female	6 (30.0)	3 (25.0)	6 (26.1)
Age (years)	48.85 ± 9.06	54.42 ± 7.01	56.96 ± 9.49**
Education (years)	13.18 ± 3.43	14.25 ± 1.98	11.00 ± 3.79
BMI (kg/m^2)	25.08 ± 2.75	23.52 ± 3.61	23.89 ± 3.76
HbA1c [%(mmol/mol)]	7.73 ± 2.29	8.46 ± 1.35	7.85 ± 2.03
Disease duration (years)	4.40 ± 3.57	7.61 ± 5.09	8.55 ± 6.26
DN4	0.17 ± 0.39	0.20 ± 0.42	3.90 ± 2.00***†††
NSS	0.53 ± 1.13	0.33 ± 0.65	6.61 ± 1.53***†††
NDS	0.20 ± 0.41	0.55 ± 1.04	2.52 ± 1.95***††
L_PN_MCV (m/s)	47.83 ± 4.39	45.35 ± 3.07	41.69 ± 6.48**
L_PN_Am (mV)	7.64 ± 2.69	6.02 ± 2.28	4.44 ± 2.47**
R_PN_MCV (m/s)	48.86 ± 4.17	46.56 ± 5.20	42.29 ± 6.53**†
R_PN_Am (mV)	7.03 ± 2.93	6.59 ± 3.15	4.64 ± 2.45*
L_TN_MCV (m/s)	47.92 ± 4.71	45.89 ± 5.45	40.88 ± 7.34**†
L_TN_Am (mV)	20.24 ± 5.18	16.62 ± 4.34	13.96 ± 8.12**
R_TN_MCV (m/s)	48.58 ± 5.71	45.83 ± 4.59	41.73 ± 6.53**
R_TN_Am (mV)	21.11 ± 5.59	15.08 ± 5.04**	12.46 ± 5.67***
L_SN_SCV (m/s)	56.28 ± 6.99	52.77 ± 3.43	49.08 ± 8.18**
L_SN_Am (uV)	18.66 ± 10.7	12.18 ± 8.71	10.85 ± 8.65*
R_SN_SCV (m/s)	54.66 ± 5.22	52.73 ± 4.06	48.22 ± 4.99**†
R_SN_Am (uV)	18.24 ± 11.02	12.11 ± 5.39	9.79 ± 7.16**
L_CDT (°C)	27.05 ± 3.58	25.77 ± 10.25	28.26 ± 2.99
R_ CDT (°C)	27.78 ± 3.01	25.94 ± 6.7	26.93 ± 6.39
L_ WDT (°C)	39.94 ± 3.48	41.88 ± 5.06	40.79 ± 3.54
R_WD T (°C)	41.80 ± 4.41	42.25 ± 4.67	40.25 ± 3.07
TIV (cm^3)	1403.78 ± 124.78	1424.42 ± 130.81	1421.49 ± 112.66
gGMV (cm^3)	640.07 ± 52.21	657.68 ± 50.64	634.40 ± 64.58
gWMV (cm^3)	525.48 ± 65.03	521.44 ± 70.52	519.32 ± 52.94

Values were given as a number (ratio) for discrete parameter and mean and standard deviation for continuable parameters. *p < 0.05, ** p < 0.01, ***p < 0.001 compared with noDPN groups, $^{\dagger}p$ < 0.05, $^{\dagger\dagger}p$ < 0.01, $^{\dagger\dagger\dagger}p$ < 0.001 compared with subDPN group.
BMI, body mass index; DN4, Douleur Neuropathique en 4 questions; NSS, Neuropathy Symptom Score; NDS, Neuropathy Disability Score; L, left; R, right; MCV, motor conduction velocity; SCV, sensory nerve conduction velocities. Am, amplitude; PN, the common peroneal nerve; TN, tibial nerve; SN, sural nerve; CDT, cold detection threshold; WDT, warm detection threshold; TIV, intracranial volume;gGMV, global matter volume; gWMV, global white matter volume; DPN, type 2 diabetic polyneuropathy; subDPN, subclinical type 2 diabetic polyneuropathy; noDPN, type 2 diabetes without polyneuropathy.

common peroneal nerves, left tibial nerves, and bilateral sural sensory nerves were significantly lower. Lower MCV of the left common peroneal nerve, right tibial nerve, and SCV of the left sural nerve were all also observed ($p < 0.05$) in the clinical DPN versus the noDPN group (Table 1 and Figure S1).

Image findings

There was no significant difference in intracranial volume, global GMV (gGMV) and white matter volume (gWMV) (Table 1). GMV within the right orbitofrontal cortex (OFC) showed statistically significant among three groups (GRF: voxel $p < 0.005$, cluster $p < 0.05$), with 523 voxels and peak MNI coordinate X/Y/Z: 46.5/45/-16.5 (Figure 2B). A transient increase of GMV within right OFC was found in subclinical subjects compared with clinical subjects ($p < 0.001$) and noDPN patients ($p = 0.06$). Decreased GMV in the DPN group was also observed compared with the noDPN group (Figure 2A).

The voxel-wise functional connectivity from right OFC to the bilateral middle temporal gyrus (MTG)/calcarine/thalamus and bilateral postcentral gyrus/superior parietal cortex (Figure 3C) showed a statistically significant increase in the subDPN group in comparison to the other two groups (GRF: voxel p <0.005, cluster p <0.05). Specifically, positive connectivity in the subclinical group was observed (Figures 3A, B).

Correlation results

Negative correlations of GMV of the OFC could be found with NSS (r = -0.5871, $p < 0.001$) (Figure 2H), NDS (r = -0.3682, $p = 0.009$)

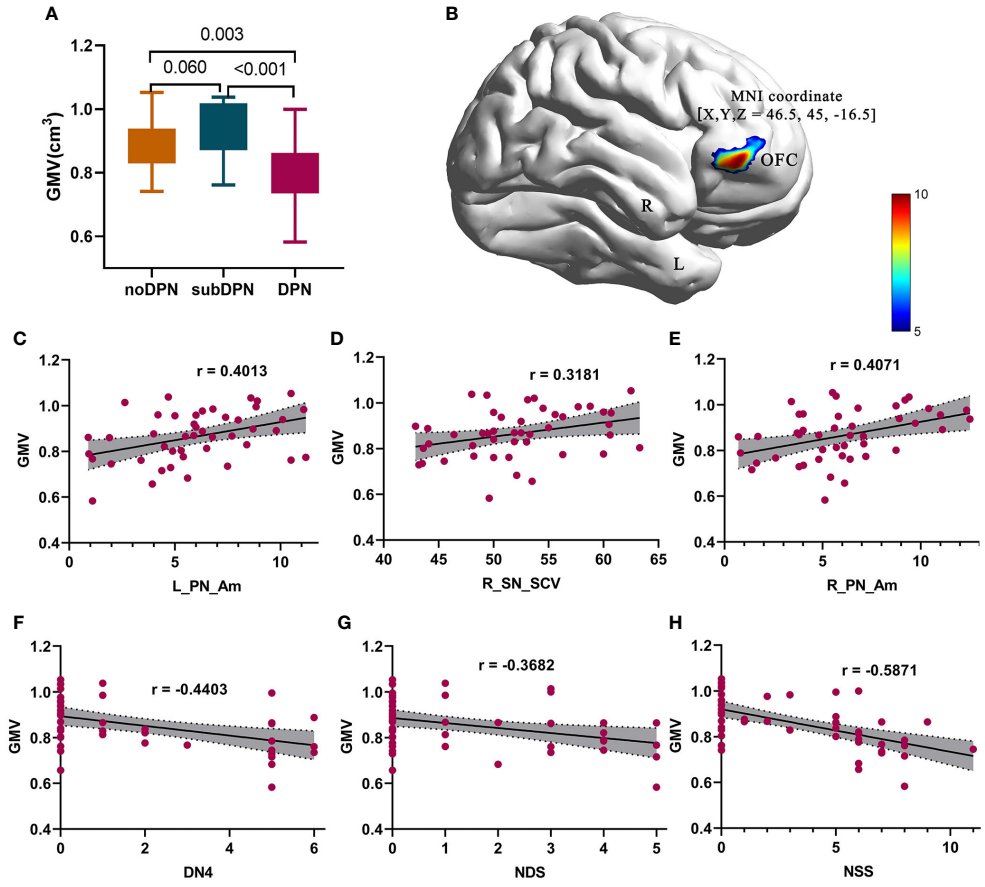

FIGURE 2
Gray matter volume difference presentation and related correlations results. **(A)** *post-hoc* results of regional gray matter volume in a bar graph. **(B)** significant difference of brain regions within the orbitofrontal cortex. **(C–E)** positive correlations of GMV with L_PN_Am **(C)**, R_SN_SCV **(D)**, and R_PN_Am **(E)** were shown in points and lines. **(F-H)** negative correlations of GMV with DN4 **(F)**, NDS **(G)**, and NSS **(H)** were shown in points and lines. The color bar denotes the corresponding T-values. DPN, type 2 diabetic polyneuropathy; subDPN, subclinical type 2 diabetic polyneuropathy; noDPN,type 2 diabetes without polyneuropathy; GMV, gray matter volume; OFC, the orbitofrontal cortex; L, left; R, right; NSS, the Neuropathy Symptom Score; DN4, Douleur Neuropathique en 4 questions; NDS, Neuropathy Disability Score; SCV, sensory nerve conduction velocities. Am, amplitude; PN, the common peroneal nerve; SN, sural nerve.

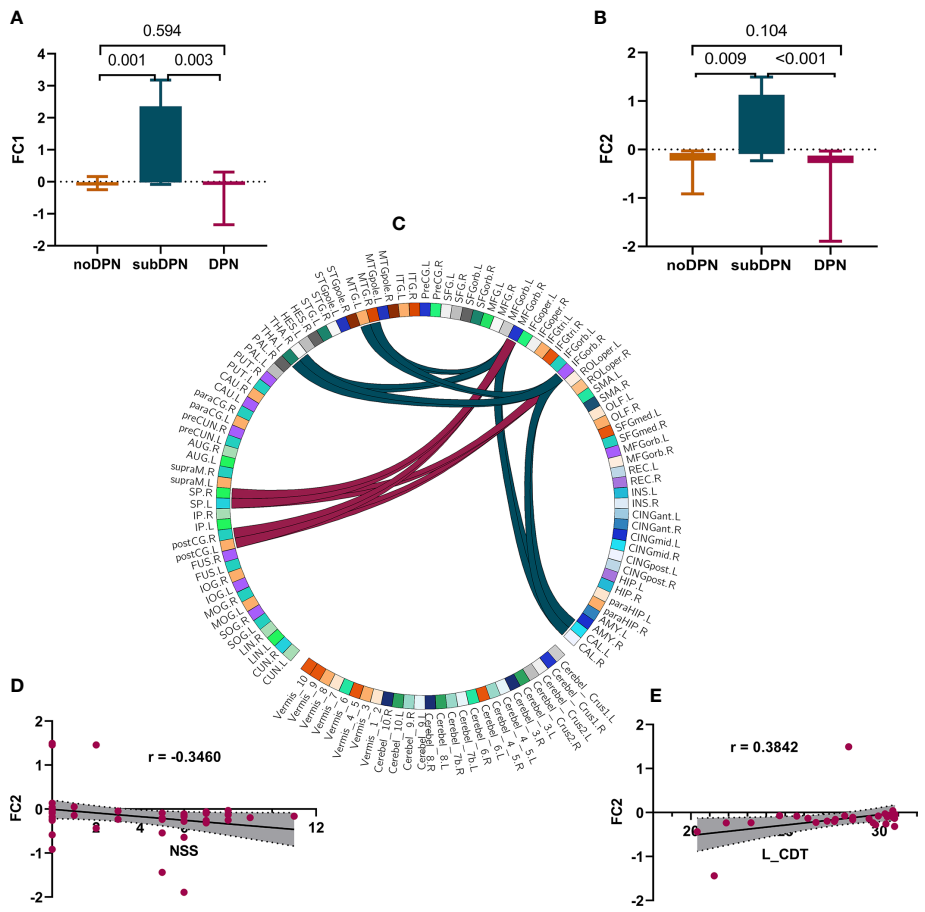

FIGURE 3
Presentations of functional connectivity differences among groups and related correlations. **(A, B)**. *post-hoc* results of FC1 **(A)** and FC2 **(B)**. **(C)** a sketch diagram denoted a significant difference in functional connectivity between cerebral regions. The dark green and red link lines denote FC1 and FC2, respectively. **(D, E)**. correlations of FC2 with NSS**(D)** and left CDT **(E)**. FC, functional connectivity values after fisher-Z transformation; FC1. connectivity of OFC with bilateral MTG/THA/CAL. FC2. connectivity of OFC with bilateral postCG/SP. L, left; R, right. OFC, the orbitofrontal cortex; MTG, middle temporal gyrus; THA, thalamus; CAL, calcarine gyrus; postCG, the postcentral gyrus; SP, superior parietal gyrus; NSS, the Neuropathy Symptom Score; CDT, cold detection threshold; DPN, type 2 diabetic polyneuropathy; subDPN, subclinical type 2 diabetic polyneuropathy; noDPN, type 2 diabetes without polyneuropathy.

(Figure 2G), and DN4 (r = -0.4403, $p = 0.003$) (Figure 2F). In contrast, positive correlations of GMV of OFC with bilateral peroneal amplitude [L: r = 0.4013, $p = 0.0063$ (Figure 2C); R: r = 0.4071, $p = 0.0055$ (Figure 2E)] and SCV of the right sensory sural nerve (r = 0.3181, $p = 0.03$) (Figure 2D) were also observed. At the same time, functional connectivity of right OFC with bilateral postcentral gyrus/superior parietal gyrus was positively associated with CDT (r = 0.3842, $p = 0.03$) (Figure 3E) and negatively associated with NSS (r = -0.3460, $p = 0.01$) (Figure 3D).

Discussions

The study examined cerebral changes in subjects with a subclinical phase of DPN, which is an extension of previous studies of DPN. The novel observation from our study included two folds: a. Compared with the noDPN group, subjects of the subDPN group presented significant functional alterations in the brain, and a transient elevation exists in both cerebral morphology and function. b. Cerebral morphology changes were related to peripheral motor and sensory abnormalities, while cerebral functional changes were only associated with peripheral sensory abnormality. Closer examination also suggested cortical function alterations were primarily in regions more related to sensory perception and structure regions located more related to neuropathic pain. This study demonstrated sensitivity in brain function changes during a subclinical stage and suggested that the brain would also play a critical role in an early phase of DPN.

Early sensory function impairment is always a major concern for DPN. QST is a quantitative method of assessing sensations

such as temperature, vibration and stimulation with a limit. Similar to a previous study (25), the QST results in our study showed no significant difference when comparing clinical DPN with the other two groups. Besides, as a psychosomatic test, QST results can be affected by many confusion factors (26), and differences in sensory tolerance might also contribute to differences in results declared by other studies (27, 28). In contrast, the cerebral morphology and function results in this present study showed an underlying potential sensitive biomarker.

Minor structural and functional changes in the cerebra of clinical DPN have been well confirmed for many years, particularly for gray matter volume and functional connectivity (6, 8, 17). However, subjects with subclinical DPN were not included in those previous studies. A principal strength of this study was the inclusion of a subclinical cohort, which was a salient complement to previous studies. Based on subclinical DPN cohorts, we found a gray matter volume reduction within the right OFC in subjects with DPN compared with subclinical and noDPN subjects. The OFC is classically involved in the emotion, reward, and cognitive processes such as decision-making (29). Notably, the involvement of OFC in the sensory process (30) and affective components of pain has been extensively demonstrated (31), yet little is known regarding its role in neuropathy. This study in DPN first directly reveals a possible correlation of OPC with neuropathic pain indexes like NSS, NDS and DN4, indicating its important role in neuropathic pain. This idea is further supported by a study that focused on the modulatory role of OFC in the nociception process, which reported projection from OFC to the ventrolateral periaqueductal gray matter (vlPAG) (32). Given that the vlPAG can receive and modify the information from the cortex, most previous studies found the connectivity of the primary somatosensory cortex (S1) with vlPAG. However, these studies are from a clinical cohort, and when subclinical DPN subjects were enrolled in this study, OFC-S1/superior parietal connectivity was also observed. Hence, we postulated that there might be S1-OFC-vlPAG circuits engaged in neuropathic pathogenesis. Nevertheless, further studies were demanded to unveil complete circuits from the periphery to the spinal cord and then the brain, as the inability of this study to access a simultaneous spinal-brain MRI.

Intriguingly, the GMV and functional connectivity tended to be consistently a transient elevation during the subclinical phase. Increased activation of somatosensory cortices appeared in the early neuropathic pain (33) while not observable in the late phase, and such changes were considered a regulation of the neuron circuit (34). Given the physical and spatial close relationship between brain structure and function (35), this study may give a structural explanation that variations of functional connectivities were accompanied by structural reorganization of DPN (17). Furthermore, one trait of the brain is that greater GMV and functional connectivities were associated with better intravenous lidocaine response (36). Our findings of subclinical DPN suggest that the subclinical phase may play a significant role in paresthesia treatment response. This could be further supported by results from the present study that showed significant correlations of GMV and functional connectivities with sensory parameters like CDT and SCV of the sural nerve.

Several limitations in this present study need to be addressed. Firstly, this study cannot interpret the causality of the brain and the peripheral nerves due to a cross-sectional investigation being designed. However, enrollment of DPN patients with different severity may help establish a better understanding of the disease progression and prepare for the subsequent longitudinal investigation. In addition, the correlations between cognition and cerebral alteration were not performed as this was not assessed. Although cognition assessment compromised an important part of DPN, in this study, we care more about the early brain structure and function changes and their relationship with peripheral nerves. More detailed cognition data are expected in future studies to determine early cognition alterations and their associations with the brain during the early phase of DPN. Lastly, this exploratory clinical trial has a relatively small sample size with inter-group differences, limiting the ability to test the results of a subgroup of clinical DPN (such as the painful and painless group). Future studies of a larger cohort would be warranted to produce a stable and comprehensive performance.

In conclusion, this present study provides evidence of significantly altered brain morphology and function in mild peripheral neuropathy at the subclinical stage. These discrepant findings suggest a critical role of central regulation on DPN and give a novel underlying mechanism. Furthermore, early cerebral function changes may interact more with sensory deficits. In contrast, cerebral morphology reacts to both motor and sensory abnormalities, giving novel insights into a specific biomarker for a different stage.

Data availability statement

The data supporting the findings of this present study are available from the corresponding authors upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Research Ethics Committee of Xiangya Hospital, Central South University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

W-HL, JW contributed to the study conceptualization. L-MZ and Y-MZ contributed to the development of methodology and data analysis. L-MZ, XC, C-YO, and F-XY contributed to data collection and image quality insurance, L-MZ and XC contributed to writing the manuscript. W-HL, JW, DS, and ST contributed to reviewing and editing the manuscript. W-HL and JW contributed to the funding acquisition. W-HL and JW were guarantors of this work, had full access to all the data, and took responsibility for the integrity and accuracy of data. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China, Grant/Award Numbers: 82170849, 82071894, 91959117; Projects of International (Regional) Cooperation and Exchanges of the National Natural Science Foundation of China, Grant/Award Numbers: 81911530222; Science and Technology Innovation Program of Hunan Province, Grant/Award Numbers: 2020RC4007, the Key Research & Development Plan, Hunan, China, Grant/Award Numbers: 2020SK2066, Science and Technology Program of Changsha, China, Grant/Award Numbers: kh2003010; National Clinical Research Center for Geriatric Disorders Foundation, Grant/Award Number 2021LNJJ04.

Acknowledgments

The authors thank all subjects who joined this study. The authors acknowledge the support from all team members, students, and technical specialists.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1069437/full#supplementary-material

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TYPE Systematic Review
PUBLISHED 04 January 2023
DOI 10.3389/fendo.2022.1095361

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 11 November 2022
ACCEPTED 05 December 2022
PUBLISHED 04 January 2023

CITATION
Hu X-x, Xiu Z-z, Li G-c, Zhang J-y, Shu L-j, Chen Z, Li H, Zou Q-f and Zhou Q (2023) Effectiveness of transverse tibial bone transport in treatment of diabetic foot ulcer: A systematic review and meta-analysis. *Front. Endocrinol.* 13:1095361. doi: 10.3389/fendo.2022.1095361

Effectiveness of transverse tibial bone transport in treatment of diabetic foot ulcer: A systematic review and meta-analysis

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Background: Diabetic foot ulcerations (DFUs) are a common but highly morbid complication of long-standing diabetes, carrying high rates of associated major amputation and mortality. Transverse tibial bone transport (TTT) has recently been applied for treatment of DFUs with the aim of accelerating wound healing. This study was performed to evaluate the effectiveness and safety of TTT in patients with DFUs.

Methods: Two authors independently retrieved the platforms of PubMed, Embase and CENTRAL, to identify studies associated with treatment of DFUs with TTT. Quantitative meta-analyses were performed to pool all available outcomes about the effectiveness and complications of TTT operation, with fixed- (I^2<50%) or random-effect (I^2>50%) model according to I^2.

Results: A total of 7 studies, involving 818 participants, were included, with 661 participants treated with TTT operation. The pooled healing rate and limb salvage rate were 0.96 (95%CI: 0.93~0.98) and 0.98 (95%CI: 0.95~1.00) respectively after treatment with TTT. The pooled mean healing time was 15.03 (95%CI: 9.05~21.00) months. When compared with the pre-operative baseline values, the ankle-brachial index (ABI, MD: 0.23; 95%CI: 0.03~0.44; p<0.001), skin temperature (MD: 1.56; 95%CI: 0.30~2.81; p<0.001), and visual analogue scale (VAS, MD: 3.70; 95%CI: 1.97~5.44; p<0.001) were significantly improved at the final follow-up. When compared with non-TTT group, the TTT group was associated with higher healing rate (OR: 10.43; 95%CI: 3.96~27.43; p<0.001) and limb salvage rate (OR: 9.65; 95%CI: 3.30~28.20; p<0.001). Concerning the complications of the TTT process, the pooled risks of fracture at transportation site and pin-site infection were 0.02 (95%CI: 0.00~0.04) and 0.08 (95%CI: 0.00~0.22), respectively; and the DFU recurrence rate in TTT group was significantly lowered comparing to that of the non-TTT group (RR: 0.18; 95%CI: 0.06~0.49; p=0.001).

Conclusions: TTT operation was associated with high healing rate and limb salvage rate, and could significantly improve the ABI, skin temperature, and VAS after operation. When compared with the control group, TTT group provided significantly higher healing rate and limb salvage rate. However, TTT operation should be conducted with caution concerning the incidences of fracture at tibia, infection at pin channels and necrosis of skin overlying the anterior tibia.

KEYWORDS

transverse tibial bone transport, meta-analysis, ulceration healing, neovascularization, diabetic foot ulceration

Introduction

Diabetes has gradually emerged as one of the most globally challenging chronic diseases, its prevalence has increased significantly over the past few decades, resulting in disabling and costly complications, life-threatening conditions, and reducing life expectancy (1, 2). The IDF (International Diabetes Federation) Diabetes Atlas, 10th edition, showed that by 2021, 1 in 10 adults worldwide will have diabetes and the number of people with diabetes will continue to increase rapidly in the future (1). Diabetic foot is an infection, ulceration and deep tissue destruction of the foot caused by neuropathy and vascular disease of the lower limbs in diabetic patients (3). About 19% - 34% of people with diabetes are likely to have diabetic foot ulcers (DFUs) in their lifetime (4, 5). DFU is a common but severely prevalent complication of long-term diabetes with high rates of associated amputation and mortality (2, 4, 6, 7). The global burden of DFUs is steadily increasing as the global prevalence of diabetes rises (8). It is well known that the outcomes of diabetes and DFUs depend heavily on the social determinants of health, with worse outcomes for ethnic minorities and socio-economically disadvantaged groups (2).

Tibial bone transverse transport (TTT) is an extension of the Ilizarov technique (9). Being different from the longitudinal transport of the osteotomy segment according to the Ilizarov external fixation technique, this procedure involves transverse traction of the tibial osteotomy segment. The primary goal of TTT is not osteogenesis, but local vascular tissue regeneration (10, 11). Originated in the law of "stress-tension", continuous distraction of the tibial cortex promotes cellular metabolism, accelerates tissue regeneration, reestablishes microcirculation and restores blood oxygen to the lower limbs. This technique is mainly used for the treatment of chronic ischaemic diseases of the lower extremities, at this stage (12).

China's Qu et al. (13) firstly applied TTT to clinical practice in China, which not only introduced TTT to China, but also initiated the exploration of TTT among Chinese scholars. Ou et al. (3) found that TTT can improve blood circulation in the affected limb, promote wound healing in diabetic feet, reduce amputation rates, and significantly increase the expression of early serum angiogenic factors, which may contribute to the mechanism of accelerated healing of diabetic foot wounds. Additionally, Nie et al. (14) found that TTT is an effective treatment for refractory non-diabetic lower extremity ulcers compared to conventional surgery.

To our knowledge, TTT has been used many times in recent years to treat diabetic foot. With the continuous development of orthopaedic procedures, the use of TTT for diabetic foot has become more and more mature, and several clinical studies have shown that this method has significant efficacy in treating diabetic foot with less adverse effects (15–18), but there is still no relevant publication on evidence-based rationale. Accordingly, we conducted this quantitative meta-analysis to thoroughly evaluate the clinical efficacy and safety of TTT in the treatment protocols of DFUs.

Materials and methods

This systematic review and meta-analysis was performed according to the guideline outlined in Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement. The PRISMA checklist is presented in Appendix 1.

Abbreviations: TTT, Transverse Tibial Bone Transport; DFU, diabetic foot ulcerations; ABI, ankle-brachial index; VAS, visual analogue scale; IDF, International Diabetes Federation; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analysis; CENTRAL, Cochrane Central Register of Controlled Trials; RCTs, randomized controlled trials; HbA1C, glycosylated hemoglobin; NOS, Newcastle-Ottawa scale; JBI-MAStARI, JBI Meta-Analysis of Statistics, Assessment, and Review Instrument; MD, mean difference; OR, odds ratio; RR, risk ratio; VSD, vacuum sealing drainage; ABC, antibiotic bone cement; α-SMA, α-Smooth Muscle Actin; SDF-1, stromal cell-derived factor-1.

Data source and study searching

Two authors retrieved the electronic databases, including PubMed, Embase and the Cochrane Central Register of Controlled Trials (CENTRAL) from the inception dates to Nov. 2022, independently. The keywords used for study searching include "diabetic foot ulcer", "diabetic foot", "transverse tibial bone transport", and so on. We combined the subject terms and free terms together to ensure full coverage of the potential eligible studies. The list of the searching strategies of three databases is available in Appendix 2. The related studies in the references list of each included study were also hand-searched and included for analysis.

Inclusion and exclusion criteria

After exporting the literature records from the databases, two authors screened all of them one by one to identify eligible studies, according to the inclusion criteria as follows: (1) patients were diagnosed with DFU (type-I or -II diabetes)DFU; (2) patients were operated with TTT on the DFU affected leg; (3) studies observed the treatment outcomes of the TTT surgery, such as healing time, healing rate, ABI, skin temperature, VAS pain scale, complications, and so on; (4) clinical studies designed as randomized controlled trials (RCTs), cohort studies, case-control studies, or case series. The publication language was restricted in English.

Studies would be excluded when meeting the following criteria: (1) duplicated studies; (2) patients operated for foot ulcer derived from non-diabetic diseases (such as occlusive vascular disease); (3) studies designed as case report, literature review, systematic review/meta analysis and letter to editors.

Study screening

The initially retrieved records were imported into EndNote version 20.2.1 (Clarivate Analytics, Philadelphia, USA), and the duplicated studies were merged together. After then, we screened the title and abstract of each record to assess the eligibility and excluded the obviously non-related studies. The full-text of the remained studies were finally reviewed to identify the final eligible studies. The whole process of study screening was conducted by two authors independently, according to the inclusion and exclusion criteria.

Data extraction and quality assessment

According to the PICOS principle, we perused all of the included studies, and extracted the items as follows: (1) Participants (P): patients number, drop-out patients, age, sex, body mass index (BMI), type of diabetes, lengths of diabetes history and DFU history, ulceration grade, ulceration area, glycosylated hemoglobin (HbA1C), and ankle-brachial index (ABI); (2) Interventions (I): detailed treatment protocol, perioperative management, anesthesia method, site of TTT, bone window size, fixations of external fixator and bone block, and detailed bone transportation protocol; (3) Comparisons (C): when a control group was set, the information about the treatment process was extracted; (4) Outcomes (O): healing rate, healing time, limb salvage rate, ABI, skin temperature, visual analogue scale (VAS) pain scale, and complications such as fracture at transportation site, pin-site infection, DFU recurrence; (5) Study (S): lead author's name, publication year, country, study period, study design, and follow-up period. The process of data extraction was conducted according to the checklist of data collection which was proposed by the Cochrane Collaboration. The data were extracted by two individual reviewers independently, and cross-checked.

The quality of the RCTs, case-control studies, case series was assessed using the Cochrane Collaboration tool for assessing risk of bias, Newcastle-Ottawa scale (NOS), and JBI Meta-Analysis of Statistics, Assessment, and Review Instrument (JBI-MAStARI) scale. This process was performed by two authors independently, and the disagreement was solved by the third author.

Statistical analysis

(1) For outcomes such as healing rate, limb salvage rate, fracture incidence at transport site, and pin-site infection risk in the TTT group, single proportional meta-analyses were performed to calculate the pooled proportions, with the "PRAW" model; (2) for outcomes such as healing time of TTT group, meta-analyses of single-group continuous data were performed to calculate the pooled mean values, with the "MRAW" model; (3) for comparisons between pre-operative and post-operative ABI, skin temperature, and VAS, meta-analyses of continuous data were conducted, with effect size of mean difference (MD); (4) for comparisons of healing rate and limb salvage rate between TTT and control groups, meta-analyses of binary data were performed with effect size of odds ratio (OR); (5) for comparison of the DFU recurrence risk between TTT and control group, meta-analyses of binary data were performed with effect size of risk ratio (RR).

The heterogeneity was tested with I^2, and random- or fixed-effect model would be employed, when presenting with or without significant heterogeneity (I^2>50%). Z test was used to test the statistical significance of the pooled results. Funnel plot and Egger's/Begg's tests ($p<0.1$ and $p<0.05$ indicate significant publication bias for Egger's and Begg's tests, respectively) were

used to detect the risk of publication bias when five or more studies were included in a meta-analysis. If significant publication bias was detected, non-parameter trim-and-fill method was used to adjust the bias. Sensitivity analyses were performed when significant heterogeneity was evident in meta-analyses with five or more studies. The statistical significance was defined as a two-side P value of less than 0.05. The statistical procedures were completed using R 4.1.3 for Windows (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study searching and selecting

The flowchart of the study searching and selecting is presented in the Figure 1. In total, 99 articles were retrieved through databases and manual searching. The titles/abstracts of 72 articles were reviewed after removing 27 duplicates. A total of 35 studies not related to this topic were excluded after reviewing the titles and abstracts. Then, the full-text of 37 studies were screening for final eligibility. A total of 7 studies (3, 15–20) were finally included for analysis.

Study characteristics of the included studies

Summary of the characteristics of the eligible studies is shown in Table 1. All of the studies were published after 2019, in China. The study designs include single-arm RCT (n=1) (3), case-control study (n=4) (16, 18–20), and case-series study (n=2) (15, 17). A total of 818 participants were included, with 661 participants treated with TTT operation. The mean age was ranged between 40.0 ± 11.0 to 70.4 ± 6.0 years, and the male percentage was ranged between 52.6% to 83.3%. The type of diabetes was reported in 4 studies (3, 16, 17, 19), with an overall percentage of type II diabetes of 98.91% (type I: 8; type II: 727). Data about Wagner and TEXAS ulceration grades were reported in 4 (3, 15, 18, 20) and 3 (16, 17, 19) studies, respectively. In TTT

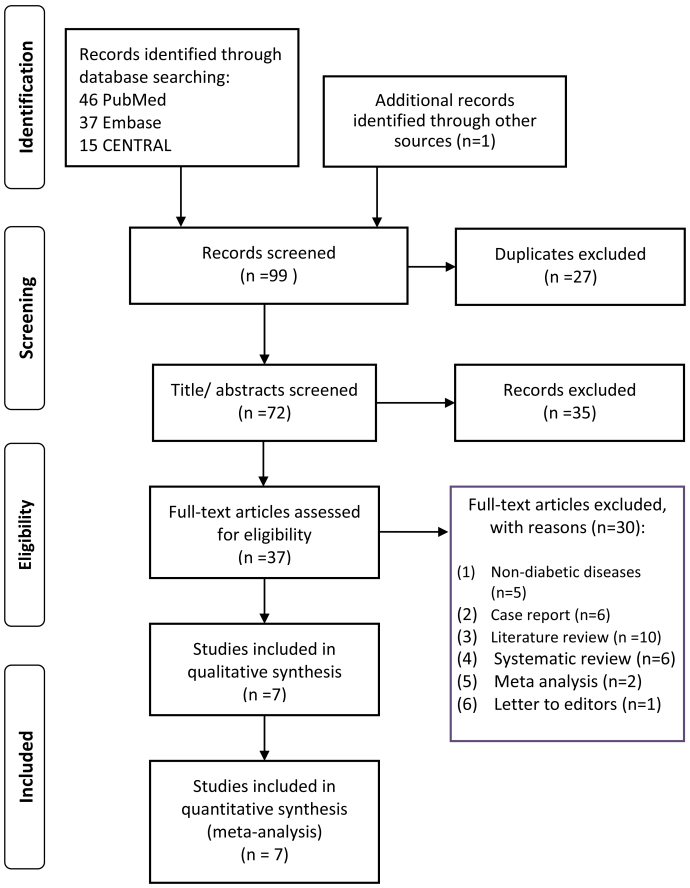

FIGURE 1
PRISMA flowchart of study searching and selecting.

TABLE 1 Summary of the study characteristics in the eligible studies.

Study ID	Country	Study period	Study design	Groups	N	Age	Male %	BMI	Diabetes types (I/II)	Length of diabetes	Length of DFU	Ulceration grade	Ulceration area (cm2)	HbA1C (%)	ABI	Follow-up	Drop-out
Fan ZQ, 2020 (15)	China	2015.03-2018.03	case series	TTT	30	40.0±11.0	70.0	NA	NA	NA	17.5y	Wagner 2/3/4: 8/16/6	NA	NA	NA	16.5m	0
Ding XF, 2022 (16)	China	2016.11-2019.11	case-control study	TTT_1	115	70.4±6.0	67.8	NA	2/113	NA	3.8±0.4m	TEXAS 3D/4D: 26/89	NA	NA	0.29 ±0.30	12.6m	0
				TTT_2	128	68.9±8.0	69.5	NA	2/126	NA	4.3±0.1m	TEXAS 3D/4D: 32/96	NA	NA	0.32 ±0.31		0
Ou SJ, 2022 (3)	China	2017.01-2019.10	single-arm Quasi-RCT	TTT	18	67.0±11.9	52.6	21.7±2.5	0/18	NA	median: 1	Wagner 4: 18	median: 2.0	7.1±0.5	NA	14.0m	1
Yuan YS, 2021 (17)	China	2016.01-2.19.10	case series	TTT	201	68.3±7.1	53.2	Median:23.5	0/201	median: 93m	NA	TEXAS 2C/2D/3D: 139/36/26	NA	Median: 10.0	NA	12m	0
Zeng ZS, 2019 (18)	China	2015.12-2017.02	case-control study	TTT non-TTT	12*	55.0±7.0	83.3	NA	NA	NA	NA	Wagner 3/4:7/5	$<25cm^2$:7; $>25cm^2$:5	NA	NA	8w	0
Chen Y, 2019 (19)	China	2014.07-2017.03	case-control study	TTT	136	61.0±10.0	70.0	23.0±3.2	2/134	21±9y	NA	TEXAS 2B/2C/2D/3B/3C/3D: 5/7/35/6/11/72	44±10	9.7±3.7	0.37 ±0.06	2y	1
				non-TTT	137	60.0±11.0	64.0	23.0±3.4	2/135	20±7y	NA	TEXAS 2B/2C/2D/3B/3C/3D: 11/10/37/7/8/64	41±9	9.5±3.2	0.35 ±0.05		2
Fan ZQ, 2022 (20)	China	2017.03-2019.03	case-control study	TTT	21	Median: 52(42-65)	76.2	23.5±4.5	NA	median:14.5y	NA	Wagner 2/3/4: 2/14/4	NA	NA	0.45 ±0.13	>1y	1
				Healthy control	20	Median: 51(40-64)	75.0	23.7±6.5	–	–	–	–	–	–	–	–	0

N, patients number; TTT, Transverse Tibial Bone Transport; ABI, ankle brachial index; BMI, body mass index; HbA1C, glycosylated hemoglobin; DFU, diabetic foot ulcer; NA, not available. *a total of 12 patients were included in this study, and the operated leg was matched with the contralateral leg in analysis.

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and non-TTT groups, 3 and 2 patients were lost to follow-up respectively. The quality assessment result of the studies is presented in Supplementary Table S1.

Detailed operation process

The detailed operation process is summarized in Table 2. A routine debridement on the ulceration site was performed at the same time in most of the studies (15–17, 19, 20). Vacuum sealing drainage (VSD) and antibiotic bone cement (ABC) were applied at ulceration site in 2 (17, 20) and 1 (16) studies, respectively. In the peri-operative period, the managements mainly include the following four aspect: (1) antibiotics treatment according to drug sensitivity test; (2) debridement and drainage; (3) blood sugar controlling; and (4) dressing changing and disinfection. The operation was performed under general anesthesia (15, 20), nerve block anesthesia (3, 16, 17, 19), or lumbar anesthesia (15, 19, 20). The transportation site mainly located at the anteromedial area of the tibia, but the heights of the bone window were divergent among these studies. In Fan et al. (15), bone window was located at 10-20 cm below knee as they stated. In Ding et al. (16), Yuan et al. (17), and Chen et al. (19), bone window was located at the proximal tibia. In Ou et al. (3) and Zeng et al. (18), transportation site was located at middle or distal tibia. The size of bone window was reported in three studies (3, 18, 19), with different sizes. Generally, two individual pins were applied at the bone block to transport it and the tibia shaft to fixing the external fixator, respectively. All of the studies initiated the transportation at the time of 3-5 days post-operatively. Two different protocols to transport the bone block were reported: (1) 1mm per day for 14 days (3, 15, 16, 20); (2) 0.25 mm per 6h for 14 days (17, 19). Three different protocols to reset the bone block were reported: (1) 1mm per day for 14 days (15, 20); (2) 0.25 mm per 6h for 14 days (19); (3) 2 mm per day for 7 days (3, 16, 17).

Results of quantitative meta-analyses

Figure 2 shows the treatment outcomes of the TTT operation at the final follow-up. The pooled healing rate was 0.96 (95% confidence interval [95%CI]: 0.93~0.98; see Figure 2A), using a fixed-effect model. The pooled limb salvage rate was as high as 0.98 (95%CI: 0.95~1.00; see Figure 2B) after treatment with TTT. The pooled mean healing time was 15.03 (95%CI: 9.05~21.00; see Figure 2C) months.

When compared with the pre-operative baseline values, the ABI (random-effect model; MD: 0.23; 95%CI: 0.03~0.44; p<0.001; see Figure 3A), skin temperature (random-effect model; MD: 1.56; 95%CI: 0.30~2.81; p<0.001; see Figure 3B), and VAS-pain scale (random-effect model; MD: 3.70; 95%CI: 1.97~5.44; p<0.001; see Figure 3C) were all significantly improved at the final follow-up.

When compared with non-TTT group, the TTT group was associated with higher healing rate (OR: 10.43; 95%CI: 3.96~27.43; p<0.001; see Figure 4A) and limb salvage rate (OR: 9.65; 95%CI: 3.30~28.20; p<0.001; see Figure 4B) as shown in Figure 4.

Concerning the complications of the TTT process, (1) the pooled risk of fracture at the transportation site was 0.02 (95%CI: 0.00~0.04; see Figure 5A); (2) the pooled pin-site infection incidence was 0.08 (95%CI: 0.00~0.22; see Figure 5B); (3) the DFU recurrence rate in TTT group was significantly lowered comparing to that of the non-TTT group (RR: 0.18; 95%CI: 0.06~0.49; p=0.001; see Figure 5C).

Sensitivity analysis, publication bias test and trim-and-fill method

The forest plot of sensitivity analysis for healing rate in TTT group is presented in Supplementary Figure S1. One study (Yuan et al. (17)) was found to cause instability on the pooling result, thus it was omitted from the final pooling (see the final forest plot in Figure 2).

The forest plot of sensitivity analysis for limb salvage rate in TTT group is presented in Supplementary Figure S2. There was no study was found to cause instability on the pooling result. Significant publication bias was detected according to Egger's (p = 0.019) and Begg's test (p = 0.327). Thus, non-parameter trim-and-fill method was performed to adjust the bias (see Supplementary Figure S3), in which three studies were filled. The adjusted effect size was 0.99 (95%CI: 0.96~1.00).

The forest plot of sensitivity analysis for mean healing time in TTT group is presented in Supplementary Figure S4. There was no study was found to cause instability on the pooling result. No significant publication bias was detected according to Egger's (p = 0.125) and Begg's test (p = 0.624).

Discussion

The main findings of the current systematic review include TTT was associated with higher healing rate and limb salvage rate when compared with control group; following operation the ABI, skin temperature, and VAS pain scale were all significantly improved; concerning the safety aspect, the TTT was associated with relatively low risks of fracture at transportation site (2%), pin-site infection (8%) and DFU recurrence (2.9%).

TABLE 2 The detailed operation process of the included studies.

Study ID	Treatment groups	Detailed treatment protocol	Perioperative management	anesthesia method	Transportation site	Bone window size	External fixation details	Bone transportation protocol
Fan ZQ, 2020 (15)	TTT	TTT+ debridement	1.continuous closed NPD (n = 12);2.antibiotics according to drug sensitivity test;3.wound dressings changing	general anesthesiaor lumbar anesthesia	medial tibial cortex about 10 to 20 cm below knee	NA	1.Fixation: 2 half nails;2.Transportation: 2 half nails	began time: 3 to 5 days; transport: 1mm per day for 14 days;reset: 1 mm per day in the reverse direction for 14 days
Ding XF, 2022 (16)	TTT_1	TTT+ debridement	1.emergency debridement, drainage, and foot care;2.antibiotics;3.nail passageway disinfection;4.blood sugar controlling	nerve block anaesthesia	anteromedial area of the proximal tibia	NA	1.Fixation: two 4.0 Steinmann pins;2.Transportation: two 3.0 Steinmann pins	began time: 3 days;transport: 1mm per day for 14 daysReset: 2mm per day in the reverse direction for 7days
	TTT_2	TTT+ABC						
Ou SJ, 2022 (3)	TTT	TTT	1.blood sugar /lipids /pressure, and hypoproteinemia controlling;2.wound dressing changing and disinfection	nerve block anesthesia	anterior medial part of the middle and lower leg	7*1.8*1.5 cm	1.Fixation: two pins;2.Transportation: two pins	1.began time: 4 days;2.transport: 1mm per day for 14 days3.Reset: 2mm per day in the reverse direction for 7days
Yuan YS, 2021 (17)	TTT	mTTT+ debridement +VSD	1.IV antibiotics based on drug susceptibility testing;2.complete debridement and removing infected bone surgically, antibiotic bone cement implantation;3. continuous closed negative pressure drainage;4.blood glucose controlling;5.dressing changing and disinfection	nerve block anaesthesia	anteromedial area of the proximal tibia	NA	1.Fixation: two 4.0 Steinmann pins;2.Transportation: two 3.0 Steinmann pins	1.began time: 3 days;2.transport: 0.25 mm per 6h for 14days;3.reset: 2 mm per day for 7 days
Zeng ZS, 2019 (18)	TTT	TTT-foot	NA	NA	middle of the tibia	3.5*1.5cm (two windows)	1.Transportation: A 60*4-mm Shashi needle on each window2.Fixation: two 120*4-mm needles	NA
	non-TTT	Contralateral foot						
Chen Y, 2019 (19)	TTT	TTT+ debridement	1.antibiotics based on drug susceptibility testing;2.Standard daily wound care and off-loading casts;	spinal anesthesia or femoral nerve block	located below the tibial tuberosity	5*1.5cm	1.Fixation: two pins;2.Transportation: two pins	1.began time: 4 days;2.transport: 0.25 mm per 6h for 14 days;3.reset: 0.25 mm per 6h for 14 days
	non-TTT	standard surgical treatments*						
Fan ZQ, 2022 (20)	TTT	TTT+ debridement +VSD	1.blood glucose controlling;2.necrotic tissue debridement;3.antibiotics based on drug sensitivity testing	general or lumbar anesthesia	medial tibial cortex, approximately 10–20 cm distal to knee joint	NA	1.Fixation: two half-nails;2.Transportation: two half-nails	1.began time: 3-5 days;2.transport: 1 mm per day for 14 days;3.reset: 1 mm per day for 14 days
	Healthy control	Healthy control without any treatment	–	–	–	–	–	–

*standard surgical treatments include: debridement, revascularization, local or free flap or skin equivalent, or graft reconstruction along with negative pressure wound therapy. TTT, Transverse Tibial Bone Transport; mTTT, modified Transverse Tibial Bone Transport; NA, not available; NPD, negative-pressure drainage; ABC, antibiotics bone cement; VSD, vacuum sealing drainage.

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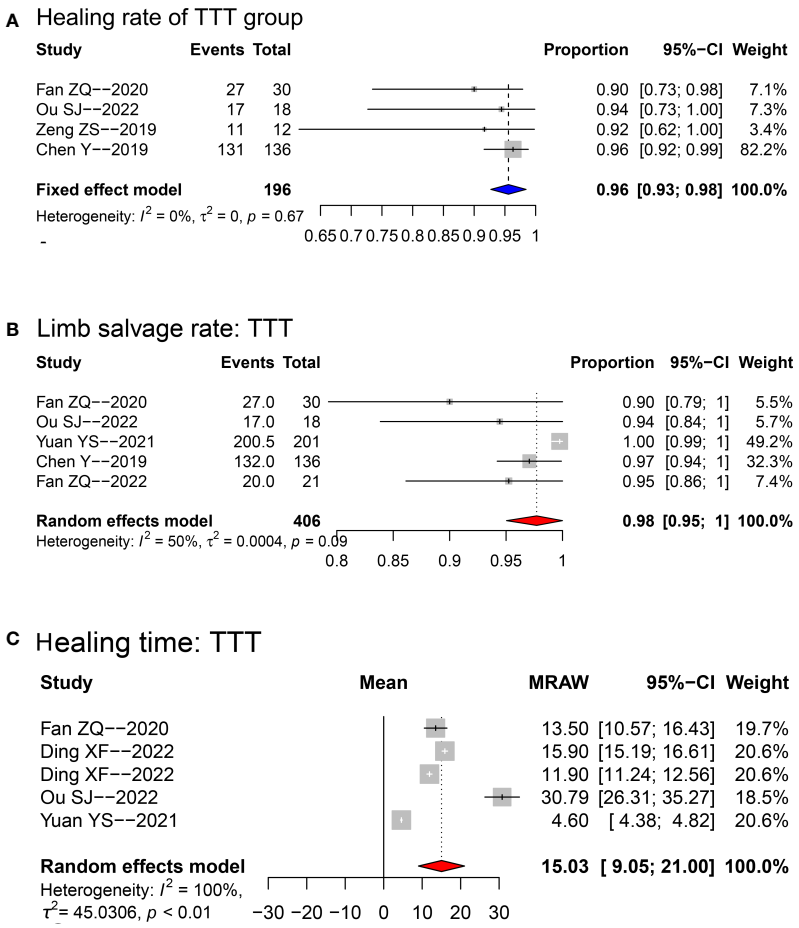

FIGURE 2
Forest plots for the meta-analyses of healing rate **(A)**, limb salvage rate **(B)** and mean healing time **(C)** in TTT group. Fixed-effect model was applied for healing rate, while random-effect model was applied for limb salvage and healing time. TTT, transverse tibia bone transportation.

The effectiveness of TTT procedure

In patients with diabetic foot, the peripheral neuropathy and vascular disease are frequently encountered, which would develop to ulceration and even amputation. The DFU, as a terminal complication of the diabetes, is of quite complex pathogenesis, being derived from a combined action of ischemia, mechanical injury, infection, and so on. Usually, the ischemia and hypoxia status is caused by damage of the small blood vessels which could not be rescued by vascular surgery (21). TTT, as a novel developed technique which was based on the Ilizarov tension-stress law, has been recently used for treatment for DFU patients with primarily satisfied success rate (18–20). These studies demonstrated that repeated mechanical stretching of the tibia bone block could stimulate the regeneration of blood vessel and accelerate the ulceration healing.

Yang et al. (22) explored the biological mechanism of the TTT procedure in rat model, and demonstrated that TTT was associated with higher blood flow in the wound area according to laser speckle imaging, and enhanced neovascularization according to double immune-labelling of CD31 and α-Smooth Muscle Actin (α-SMA). Previous studies have shown that bone distraction could enhance neovascularization through a pathway involving chemokine stromal cell-derived factor-1 (SDF-1), which is a key factor responsible for homing and migration of endothelial progenitor cells (23). In a case-control study by Chen et al. (19), they compared the treatment outcome of severe and recalcitrant DFUs with TTT and solitary standard operation, showing that tibial transverse distraction group had higher healing rate, limb salvage rate, density of small vessels, blood flow and blood volume, compared with the control group. In our results, the healing rate and limb salvage rate were demonstrated to be as high as 96% and 98% at final follow-up, and significant improvements on ABI (MD = 0.23), skin temperature (MD = 1.56) and VAS (MD = 3.70) were identified. When compared with control group, the healing rate

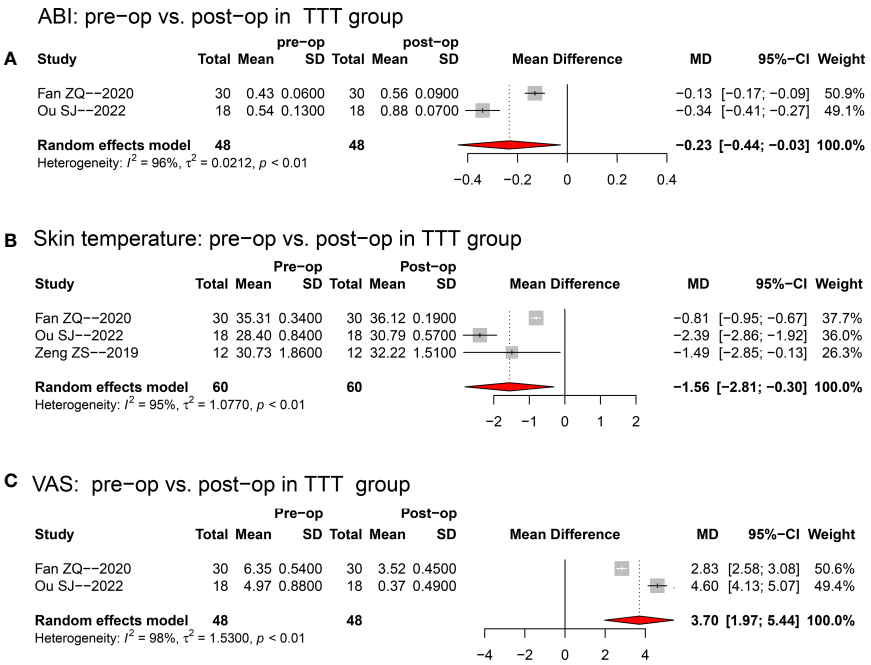

FIGURE 3
Forest plots for the comparisons between pre-operative and post-operative ABI **(A)**, skin temperature **(B)**, and VAS **(C)** in TTT group. Random-effect model was selected for the three comparisons. TTT, transverse tibia bone transportation, ABI, ankle brachial index, VAS, visual analogue scale, MD, mean difference, pre-op, pre-operative; post-op, post-operative.

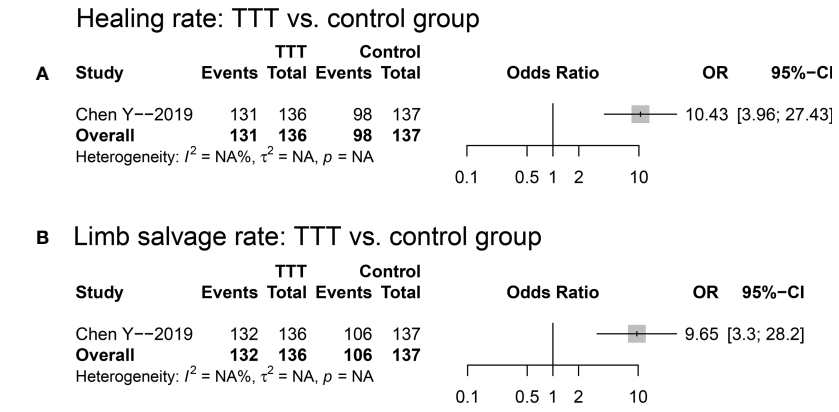

FIGURE 4
Forest plots for the comparisons of healing rate **(A)** and limb salvage rate **(B)** between TTT and non-TTT group. TTT, transverse tibia bone transportation.

(OR = 10.43) and limb salvage rate (OR = 9.65) were both obviously increased. These findings all confirmed the acceleration effect on neovascularization. The ABI and skin temperature is directly related with the microcirculatory perfusion of foot soft tissue. With an improved blood perfusion, sufficient oxygen and nutrition supplies can be guaranteed for ulceration healing.

However, though TTT was proven to be effective in promote ulceration healing, DFU is a multi-disciplinary condition which is difficult to be completely solved by sole TTT operation (24, 25). Many assistance procedures were applied at the same time, including debridement of the ulceration lesion (15–17, 19, 20), antibiotic bone cement filling (16), and vacuum sealing drainage (17, 20), which have all been proven to be valuable in promoting

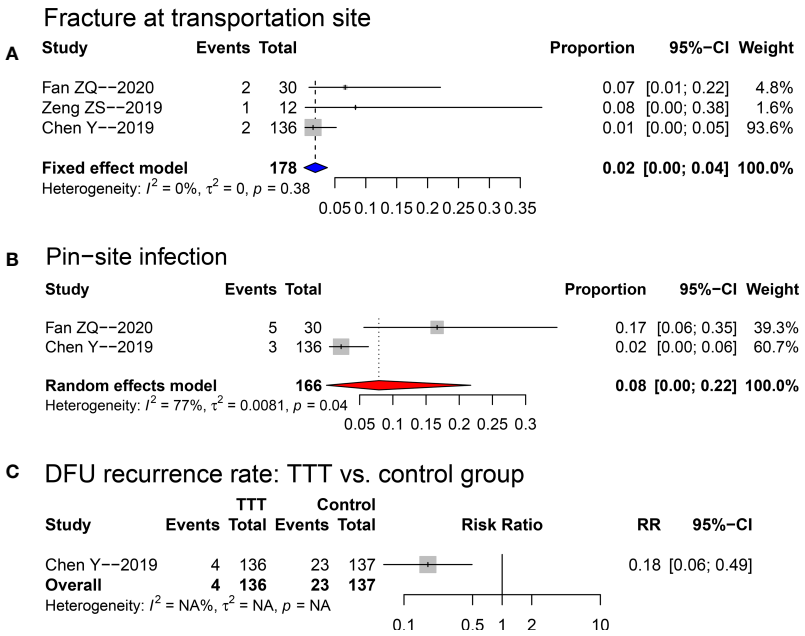

FIGURE 5
Forest plots for the risks of fracture at transportation site **(A)**, and pin-site infection **(B)** in the TTT group, and comparison of DFU recurrence rate **(C)** between TTT and non-TTT group. TTT, transverse tibia bone transportation, RR, risk ratio.

healing of ulcer wounds. At the peri-operative period, blood sugar controlling is the basic requirement to guarantee a hypoglycaemic condition for tissue repairing. Antibiotics (intravenous or per oral) according to drug sensitivity testing is also essential to control the infection and ensure the healing process. It had been reported that adequate foot care can prevent 80% of DFUs in diabetes patients (26) and effectively prevent amputation caused by ulcerations (27). Thus, it is of importance to continue standard wound care (dressing changing and disinfection) and off-loading casts in peri-operative period.

The safety of TTT procedure

The procedure of TTT, however, is related with some potential complications, especially the fracture at the tibial bone window (15, 18, 19), infection of the pin site (15, 19) and skin necrosis at surgical site (10). Our results showed a total of 5 tibia fracture among 178 patients (pooled proportion: 2%), and 8 pin-site infections among 166 patients (pooled proportion: 8%). In Fan et al. (15), the authors suggested to avoid the fracture risk by establishing standard tibial osteotomy criteria and performing post-operative education on falling prevention. They also recommended to narrow the bone window for those patients with short stature. We firmly in favour of their proposal. Additionally, those with severe osteoporosis especially among the postmenopausal older women should be referred to standard osteoporosis treatments to prevent risk of fracture at osteotomy site. Moreover, it is of great importance to let the patients return for regular follow-up after operation. To avoid the risk of infection of pin channel, peri-operative antibiotics and daily wound care (especially pin site disinfection) are mostly important. The necrosis of local soft tissue is another major concerning during TTT, which is mainly caused by long-term continuous pressure on the skin overlying the anterior tibia (28, 29). The surgeons should try their best to preserve the blood supply of the skin flap and avoid excessive interference to the soft tissue. Post-operatively, close attention should be applied on the skin status, and termination of transportation is indicated if signs of ischemia or necrosis are evident.

In 2020, with the concerted efforts of experts in various disciplines, the “Expert Consensus on the Treatment of Diabetic Foot Ulcers Using Tibial Transverse Transport” (30) was published in China. It has emphasized the importance of further simplification of the external fixator aiming to reduce the risk of complications. During the operation period, tourniquet should not be applied, to protect the blood supply of lower limb. It is of vital significance to narrow the incision and bone window sizes and preserve the periosteum, as far as possible. Through these strategies, incidence of adverse events could be significantly reduced without addition on the difficulty of surgery process.

Limitation

This study, nevertheless, has some limitations that must be pointed out here. Firstly, as the TTT technique was applied for DFU treatment in the most recent years, the available publications in this field are scarce with generally small sample size and retrospective design. Thus, more prospective studies with larger sample size are required in the future. Then, since the TTT procedure is predominately conducted in China, data from non-Chinese patients are not available at this stage. Thus, some further studies are required to verify the effectiveness of this operation in patients around the world.

Conclusions

The TTT operation was demonstrated to be with high healing rate and limb salvage rate, and could significantly improve the ABI, skin temperature, and VAS after operation. When compared with the control group, TTT group provided significantly higher healing rate and limb salvage rate. However, TTT operation should be conducted with caution concerning the incidences of fracture at tibia, infection at pin channels and necrosis of skin overlying the anterior tibia.

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.

Author contributions

X-XH and Z-ZX contributed equally to this study. X-XH, Z-ZX, and G-CL: methodology, validation, formal analysis, data extraction, data curation, writing-original draft, writing-reviewing and editing, and project administration. J-YZ, L-JS, and ZC: investigation, and data processing. X-XH and HL: validation, writing-reviewing and editing. Q-FZ, and QZ: project administration. 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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1095361/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 08 February 2023
DOI 10.3389/fendo.2022.1092764

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 08 November 2022
ACCEPTED 30 December 2022
PUBLISHED 08 February 2023

CITATION
Dong L, Hu Y, Xu L, Zeng H, Shen W, Esser P, Dawes H and Liu F (2023) Abnormal vibration perception threshold alters the gait features in type 2 diabetes mellitus patients.
Front. Endocrinol. 13:1092764.
doi: 10.3389/fendo.2022.1092764

Abnormal vibration perception threshold alters the gait features in type 2 diabetes mellitus patients

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Objective: It is generally believed that gait characteristics of diabetic neuropathic patients differ from those of non-diabetic ones. However, it is still unclear how the abnormal foot sensation influences the gait during walking in type 2 diabetes mellitus (T2DM). For the purpose of gaining a better insight into the alterations of detailed gait parameters and figuring out important aspects in the gait indexes by peripheral neuropathy in elder T2DM patients, we compared the gait features in participants with normal glucose tolerance (NGT) controls and diabetic individuals complicated by peripheral neuropathy or not.

Subjects and methods: Gait parameters were observed during the 10-m walk on flat land among different conditions of diabetes in 1,741 participants from three clinical centers. Subjects were divided into four groups: persons with NGT were taken as the control group; patients with T2DM included three subgroups: DM control (no chronic complications), DM-DPN (DM complicated by only peripheral neuropathy), and DM-DPN+LEAD (DM complicated by both neuropathy and artery disease). The clinical characteristics and gait parameters were assessed and compared among these four groups. Analyses of variance were employed to verify possible differences of gait parameters between groups and conditions. Stepwise multivariate regression analysis was performed to reveal possible predictors of gait deficits. Receiver operating characteristic (ROC) curve analysis was employed to find any discriminatory power of diabetic peripheral neuropathy (DPN) for the step time.

Results: In participants burdened with DPN, whether complicated by lower extremity arterial disease (LEAD) or not, step time increased sharply ($p < 0.05$). Stepwise multivariate regression models showed that independent variables of gait abnormality were sex, age, leg length, vibration perception threshold (VPT), and ankle-brachial index (ABI) ($p < 0.01$). Meanwhile, VPT was a significant independent predictor of step time, spatiotemporal variability (SD_A), and temporal variability (SD_B) ($p < 0.05$). ROC curve analysis was explored to find the discriminatory power of DPN for the occurrence of increased step time. The area under the curve (AUC) value was 0.608 (95% CI: 0.562–0.654, $p < 0.01$), and the cutoff point was 538.41

ms accompanied by a higher VPT. A significant positive association was observed between increased step time and the highest VPT group [odds ratio (OR) = 1.83, 95% CI: 1.32–2.55, $p< 0.01$]. In female patients, this OR value elevated to 2.16 (95% CI: 1.25–3.73, $p< 0.01$).

Conclusions: In addition to sex, age, and leg length, VPT was a distinct factor that associated with altered gait parameters. DPN is associated with increased step time, and the step time increases with worsening VPT in type 2 diabetes.

KEYWORDS

gait, diabetic peripheral neuropathy, vibrating perception threshold, diabetic, complication, type 2 diabetes

Introduction

Gait analysis provides an objective means of measuring walking (1) and presents biomechanical differences depending on individual characteristics, such as morphological nature, physical activity, age, and the presence of some diseases. In a recent report, an altered gait pattern is apparently observed among individuals with diabetic peripheral neuropathy (DPN) or other diabetic complications, even in diabetes alone, including slower gait speed, shorter stride length, increased cadence, and high gait variability (2–6).

It is generally believed that up to 50% of people with diabetes will develop significant peripheral neuropathies (7). The presence of DPN, leading to an increased number of repetitive falls compared with individuals without diabetes (7), also significantly reduces walking ability and causes the alterations of foot posture and function (8) and could cause abnormal gait during walking. Several recent studies implicated that the main abnormalities in gait parameters among DPN include decreased walking speed, shorter steps, and greater variability of step timing (9), exhibiting a more conservative gait pattern, which resulted from peripheral sensory loss rather than from vision deficiency or decreased lower-limb muscle strength, and the differences were particularly evident on an irregular surface. However, different studies yielded controversial results because of various examining devices and diverse subjects. For instance, de Mettelinge et al. (10) reported that gait patterns did not differ significantly between diabetes complicated by neuropathy and diabetes not complicated by neuropathy.

Although most emerging evidence focused on the association of specific DPN and foot plantar pressure, understanding the effects of other diabetic conditions on gait still showed their importance, and a wealth of studies have been designed to investigate their possible relationships. It has been well documented that the gait pattern can be dramatically altered in persons with diabetes, including slowed gait speed, shorter steps, prolonged double support time, and increased step width, as well as gait variability (11). However, a cross-sectional study of diabetes mellitus (DM) patients with DPN (n = 20), without DPN (n = 26), and age-/gender-/Body Mass Index (BMI)-matched healthy control subjects (n = 20) that was conducted by Yavuzer et al. (12) showed that diabetic patients with DPN had slower gait, shorter steps, limited knee and ankle mobility, and lower plantar flexion moment and power than the healthy control group. There was also a trial that proved no difference between diabetic patients with neuropathy and diabetic patients without neuropathy (10).

Although dictated by the specific matching procedure, the relatively small sample size could be considered as a limitation of these studies. Moreover, a comprehensive study focused on gait characteristics has never been accomplished in healthy controls and patients with type 2 diabetes, DPN, or DPN complicated by lower extremity arterial disease (LEAD) in the same clinical trial. Based on these considerations and those controversies, we believe that it is particularly urgent to explore the impact of different glucose conditions and chronic diabetes complications on gait. Thus, the aim of the study was to determine how gait components were affected by diabetic neuropathy.

Subjects and methods

Subjects

A cross-sectional observational study was conducted. All of the individuals were diagnosed as having normal glucose tolerance (NGT) and type 2 diabetes mellitus (T2DM) based on American Diabetes Association 2020 standards (13). DPN was screened and confirmed if the vibration perception threshold (VPT) was >25 volts (V) in combination with a positive Neuropathy Deficit Score (NDS) (14). LEAD was diagnosed if the ankle-brachial index (ABI) was <0.9 (15). For comorbid conditions, inclusion criteria for chronic heart disease (CHD): 1) history of myocardial infarction; 2) coronary stents or coronary artery bypass grafting is excluded. The inclusion criteria for cerebral infarction were as follows: 1) a history of old cerebral infarction; 2) physical activity was not affected by cerebral infarction. Patients with acute cerebral infarction within 3 months and any other cerebrovascular accident were excluded. Other exclusion criteria were the presence of any orthopedic, visual, neurological, or other disturbance that might affect gait, including current pain, injury, a history of diabetic foot, Parkinson's disease, moderate and severe lumbar disease, active ulceration or amputation and diabetic ketoacidosis, hyperosmolar hyperglycemia syndrome, and other acute diabetic complications. The study was approved by the Ethics

Committee of the Shanghai Sixth People's Hospital (ethical approval number: ChiCRT-DDD-16009531), and written informed consents were obtained from all of the participants.

A total of 1,741 individuals (868 men and 873 women) were enrolled from the Shanghai Clinical Medical Center of Diabetes, the First Affiliated Hospital of Anhui Medical University, and West China Hospital of Sichuan University; 49.9% were men, with a mean age of 60.95 ± 9.25 years. Participants were recruited and assigned into one of four groups: subjects with normal diabetic tolerance (NGT, n = 282); T2DM without peripheral neuropathy or LEAD (DM, n = 1,266); T2DM complicated by only peripheral neuropathy (DPN, n = 144); T2DM with both DPN and LEAD (n = 49).

Procedures

All subjects' sex, age, BMI, diabetes duration, hypertension (HP), CHD, and cerebral infarction were collected. BMI was calculated as body weight (in kg) divided by the square of the height (in m). The history of smoking was recorded based on self-report of all subjects. Levels of fasting plasma glucose (FPG) and 2-h postprandial blood glucose (PPG) were estimated by the glucose oxidase method. Glycosylated hemoglobin (HbA1c) was determined by high-pressure liquid chromatography using the VariantÔ II machine (Bio-Rad Inc., Hercules, CA, USA).

A neuropathic assessment of VPT was performed by the same technician using a neurothesiometer (Bio-Thesiometer; Bio-Medical Instrument Co., Newbury, OH, USA). The operational approaches were based upon the International Working Group on the Diabetic Foot of the International Diabetes Federation. The higher value of VPT in the limb was selected for our analysis. The ABI, the ratio of ankle systolic pressure to arm systolic pressure, was performed according to the standard protocols of the International Diabetes Federation. The lower value of ABI in the limb was opted for our analysis.

For the gait data collection, a smart portable wireless gait measurement instrument named gait crasher was provided by our research partners from Brooks University. In brief, a commercially available LPMS (LP-RESEARCH Motion Sensor, 400 Hz, Japan) was attached over the skin of the fourth lumbar vertebra. Participants were asked to walk over a 10-m walkway free of obstacles at their comfortable walking pace. Participants started at a static position at the 0 point, came to a complete stop at the 10-m line. Two successful walks were conducted for each participant. All parameters of the gait cycle are registered and can be analyzed using Vicon 512 Motion Analysis System (Oxford Metrics Ltd., Oxford, England) in great detail. Descriptive statistics of the spatiotemporal gait parameters for both 10-m level walks were calculated and analyzed: cadence, stride length, walking speed, duty-factor_double stance, step time, and walk ratio (step length–cadence ratio). Phase plot description of gait included spatiotemporal variability (SD_A), temporal variability (SD_B), A ratio, and symmetry (Δangleβ). SD_A means the spatial and temporal variability of the vertical trunk movement during walking but also is influenced by the magnitude of vertical trunk movement. SD_B reflects the symmetry of trunk movement from stride to stride. A ratio was described by the ratio between SD_A and SD_B. Δangleβ was calculated as the angle difference between the SD_A vector and 45°. We collected and analyzed the spatiotemporal and phase plot variables per group in the middle section of the walkway, avoiding acceleration and deceleration periods during gait.

Data analysis

Categorical variables were expressed as percentages, and continuous variables were given as mean ± SD values. Comparison of continuous variables among the four groups was performed using one-way analysis of variance (ANOVA). Nonparametric testing was accomplished by the Kruskal–Wallis test. Associations between gait parameters and other variables were evaluated with stepwise multiple regression analysis. Logistic regression analysis was performed to evaluate the odds ratio (OR) and associated factors. The OR (95% CI) was calculated in two logistic regression models: a non-adjusted model and an age-adjusted model. A receiver operating characteristic (ROC) curve was employed to find a cutoff of step time for the presence of DPN. Statistical analyses were performed using SPSS version 24.0 software (SPSS Inc., Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics

Basic characteristics of the participants were listed in Table 1. There were overall significant differences among the four groups in age, sex, height, leg length, and diabetes duration ($p < 0.01$). Although not the tallest, participants with DPN showed the longest leg length ($p < 0.05$). Compared with NGT groups, diabetic participants exhibited a poorer condition of health and living habits; for instance, they were more likely to have HP, CHD, and cerebral infarction (all $p < 0.05$), as well as a higher proportion of smokers ($p < 0.05$). The highest value of VPT (36.43 V) was detected among patients complicated by both DPN and LEAD ($p < 0.05$), followed by DPN individuals. In general, FPG, PPG, and HbA1c gradually increased with the aggravation of the disease ($p < 0.05$), and these groups showed an obvious trend of increasing age, higher prevalence of complications, and relatively worse condition.

Alterations of gait parameters among different groups

Spatiotemporal analysis was conducted for cadence, stride length, walking speed, walk ratio, duty-factor_double stance, and step time (Figure 1). Coefficient of variation (CoV) (SD/mean x 100) was used to assess the variability in these spatiotemporal parameters. As illustrated, no notable differences of cadence, stride length, walking speed, walk ratio, and related CoVs were seen, and no obvious trend was exhibited (all $p > 0.05$) among subjects with all groups. Compared with individuals with abnormal glucose metabolism, subjects with NGT showed a considerably lower duty-factor_double stance-CoV (46.3% ± 1.4% vs. an average of 52.5% ± 0.6%, $p < 0.05$, Figure 1C). In

TABLE 1 Comparison of basic characteristics among groups with and without diabetes and complications.

	NGT	DM	DM-DPN	DM-DPN+LEAD	*p*
Patients (n,%)	282, 16.2	1,266, 72.7	144, 8.3	49, 2.8	–
Male (n, %)	104, 36.9	639, 50.5	93, 64.6	32, 65.3	<0.001
Age (years)	61.07±7.93	60.41±9.59	64.32±6.37*[a]	63.91±9.34[b]	<0.001
Height (cm)	162.52±7.44	164.67±8.19*	165.24±8.11*[a]	165.52±7.58*[a]	<0.001
Leg length (cm)	91.17±4.76	91.93±5.34*	93.61±5.69*[a]	92.80±5.21*[a]	<0.001
BMI (kg/m^2)	24.16±3.03	24.67±3.31	24.99±3.13	24.19±3.61	0.099
DM duration (years)	–	9.35±7.03	11.18±7.59[a]	14.81±7.11[ab]	<0.001
Smokers (n,%)	20, 7.0	353, 27.9*	53, 36.6*[a]	23, 46.5*[ab]	<0.001
Comorbidities (n,%)					
HP	27.0	46.4*	60.7*[a]	58.1*[a]	<0.001
CHD	1.8	13.2*	22.3*[a]	14.0*[b]	<0.001
Cerebral infarction	3.9	11.1*	18.8*[a]	4.7[ab]	<0.001
VPT(V)	12.00±1.01	14.30±4.30*	31.98±8.83*[a]	36.43±13.26*[ab]	<0.001
ABI	1.10±0.12	1.12±0.09	1.13±0.11	0.74±0.25*[ab]	<0.001
HbA1c (%)	–	7.49±1.60	7.24±0.96	7.52±2.26[ab]	0.252
FPG (mmol/l)	5.50±0.73	8.23±2.41*	8.58±2.61*[a]	8.90±2.57*	<0.001
PPG (mmol/l)	–	11.45±3.55	12.08±4.15	12.58±6.32[ab]	0.076

Data were presented as mean ± SD or n (%) as appropriate.
*$p < 0.05$ compared with NGT; [a]$p < 0.05$ compared with DM; [b]$p < 0.05$ compared with DPN. Data marked with the same letter mean no significant difference between groups.
NGT, normal glucose tolerance; VPT, vibration perception threshold; ABI, ankle-brachial index; CHD, chronic heart disease; HP, hypertension; BMI, body mass index; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; PPG, 2-h postprandial blood glucose. DM, diabetes mellitus; DPN, diabetic peripheral neuropathy; LEAD, lower extremity artery disease.

participants burdened with DPN, step time increased sharply (548.7 ± 4.8 ms in DPN vs. 527.7 ± 2.7 ms in NGT vs. 530.6 ± 1.3 ms in diabetes, $p < 0.05$, Figure 1C). Furthermore, DPN individuals were subdivided into symptomatic DPN (n = 77, 53.5%) and non-symptomatic DPN (n = 67, 46.5%); no difference was detected between the two groups (544.6 vs. 551.8 ms, $p > 0.05$).

Phase plot analysis was performed for SD_A, SD_B, A ratio, and Δangleβ (Figure 2). The results displayed lower SD_A (1.32 ± 0.09 vs. an average of 1.57 ± 0.01 in NGT and DM, $p < 0.01$) and lower SD_B (0.38 ± 0.03 vs. an average of 0.51 ± 0.01 in NGT and DM, $p < 0.01$) in subjects with both DPN and LEAD. Moreover, trend analysis revealed that SD_A and SD_B gradually decreased in the three DM groups ($p < 0.05$). No significant difference was found for A ratio and Δangleβ among the four groups ($p > 0.05$).

Factors associated with altered gait parameters

Stepwise multivariate regression models used to predict gait characteristics are shown in Table 2. For gait parameters, the significant independent variables were sex and/or age ($p < 0.01$). Leg length turned out to be another important index that could influence stride length, walking speed, walk ratio, SD_A, and SD_B ($p < 0.01$). VPT was a significant independent predictor of step time (B = 0.654, $p = 0.000$), SD_A (B = -0.007, $p = 0.005$), and SD_B (B = -0.002, $p = 0.000$).

Comparison of gait parameters when sex and age were matched

Based on the results above, the impact of sex and age on gait parameters needed to be clarified further, which was summarized in Figures 3A–C. In order to match the other factors between the groups, we selected 1,420 individuals and divided them into four groups according to their sex and age. Group 1 was defined as men, aged <65 years (56.1 ± 5.2 years), n = 372; Group 2, men, aged ≥65 years (69.7 ± 3.2 years), n = 190; Group 3, women, aged <65 years (56.5 ± 5.8 years), n = 571; Group 4, women, aged ≥65 years (69.7 ± 3.3 years), n = 287. As shown in Figures 3A–C and Table 3, male participants were found to have fewer steps/min than female participants in both ≤65 and ≥65 years of age (114.1 ± 0.8 steps/min vs. 120.9 ± 1.19 steps/min; 111.4 ± 0.58 steps/min vs. 116.7 ± 0.90 steps/min, $p < 0.05$), and the step length was significantly increased (1.37 ± 0.01 m/s vs. 1.25 ± 0.01 m/s; 1.36 ± 0.01 m/s vs. 1.23 ± 0.01 m/s, $p < 0.05$); they were walking faster (1.31 ± 0.01 m/s vs. 1.27 ± 0.01 m/s; 1.27 ± 0.01 m/s vs. 1.19 ± 0.01 m/s, $p < 0.05$), spent more time per walk (542.6 ± 2.07 ms vs. 518.3 ± 2.06 ms; 549.4 ± 2.42 ms vs. 526.1 ± 2.21 ms, $p < 0.05$), and showed greater SD_A (1.71 ± 0.02 vs. 1.48 ± 0.01; 1.66 ± 0.02 vs. 1.38 ± 0.02, $p < 0.05$) and SD_B (0.48 ± 0.01 vs. 0.42 ± 0.01; 0.47 ± 0.01 vs. 0.40 ± 0.01, $p < 0.05$). Moreover, male individuals had an increased walk ratio [6.10 ± 0.04 mm/(steps/min) vs. 5.33 ± 0.04 mm/(steps/min); 6.13 ± 0.05 mm/(steps/min) vs. 5.31 ± 0.04 mm/(steps/min), $p < 0.05$] and duty-factor_double stance (30.25% ± 0.36% vs. 28.1% ± 0.36%; 30.6% ± 0.49% vs. 28.56% ± 0.38%, $p < 0.05$). Elder male participants had significantly slower walking (1.27 ± 0.01 m/s vs. 1.31 ±

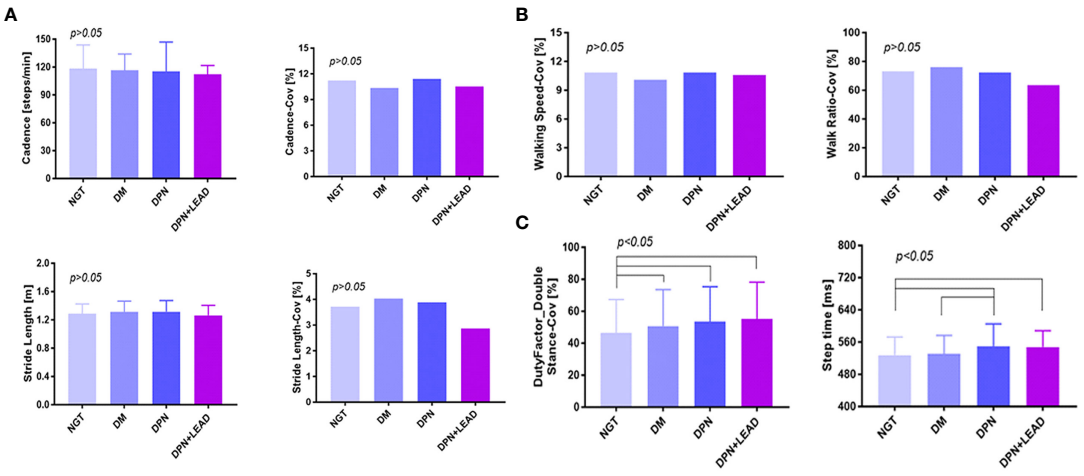

FIGURE 1
The comparison of gait spatiotemporal variables among different groups. **(A–C)** Duty-factor_double stance-CoV was different between the non-diabetic group and the diabetic subgroups, and the step time was different between the non-diabetic group and the complication groups. Variables were cadence, stride length, walking speed, walk ratio, duty-factor_double stance, step time, and CoVs (CoV = SD/mean x 100). The difference of all gait variables between groups was significant ($p < 0.05$). Subjects with abnormal blood glucose control showed a considerably higher duty-factor_double stance-CoV (52.50% vs. 46.30%, $p < 0.05$). In participants burdened with DPN, whether complicated by LEAD or not, step time increased sharply ($p < 0.05$).

0.01 m/s, $p < 0.05$) and smaller SD_A (1.66 ± 0.02 vs. 1.71 ± 0.02, $p < 0.05$). A larger part of distinction was observed only in female subjects, such as less steps/min (116.7 ± 0.90 steps/min vs.120.9 ± 1.19 steps/min, $p < 0.05$), shorter stride length (1.23 ± 0.01 m vs. 1.25 ± 0.01 m, $p < 0.05$), more time spent per walk (526.1 ± 2.21 ms vs. 518.3 ± 2.06 ms, $p < 0.05$), and smaller SD_A (1.38 ± 0.02 vs. 1.48 ± 0.01, $p < 0.01$), SD_B (0.40 ± 0.01 vs. 0.42 ± 0.01, $p < 0.05$), and A ratio (3.55 ± 0.04 vs. 3.68 ± 0.04, $p < 0.05$).

Discriminatory power

Since step time was an independent correlative factor for DPN, ROC curve analysis was explored to find any discriminatory power and sensitivity and specificity of step time for the occurrence of DPN (Figure 4). The area under the curve (AUC) value was 0.608 (95% CI: 0.562–0.654, $p < 0.01$). In total, the cutoff point was 538.41 ms. The

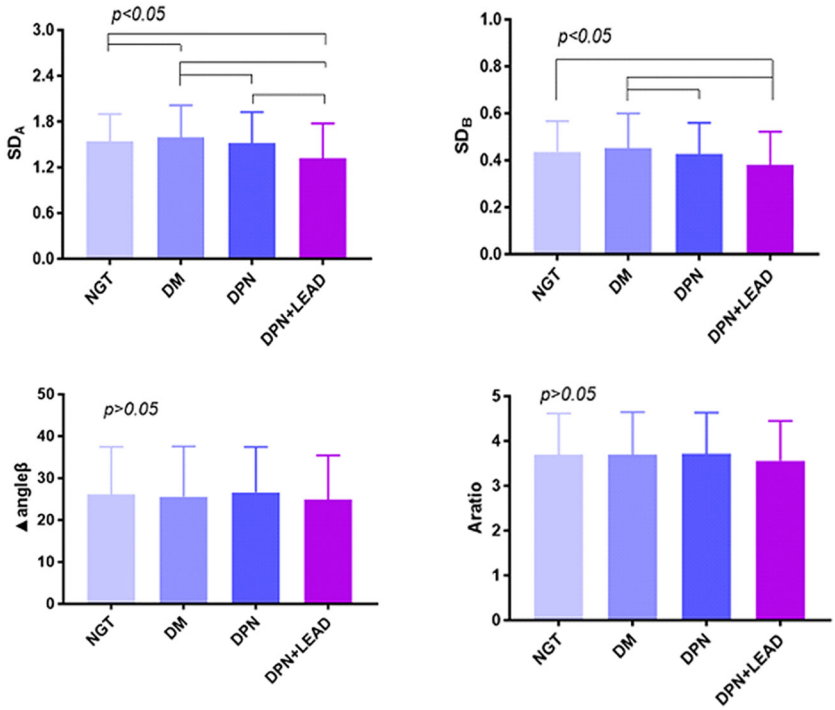

FIGURE 2
The comparison of gait phase plot variables among the different groups. Variables were SD_A, SD_B, A ratio, and Δangleβ. The difference of all gait variables between groups was significant ($p < 0.05$). Trend analysis. The results displayed lower SD_A (1.32 vs. 1.57, $p < 0.01$) and SD_B (0.38 vs. 0.51, $p < 0.01$) in subjects with both DPN and LEAD. Moreover, trend analysis revealed gradually decreased SD_A and SD_B in the four DM groups ($p < 0.05$).

10.3389/fendo.2022.1092764

TABLE 2 Regression coefficient summary for independent variables included in multivariate regression models for gait characteristic dependent variables–Spatiootemporal analysis and phase plot analysis.

Dependent variables	Predictors	Regression coefficient (B)	95% CI	Adjusted R^2	*p* value
Spatiotemporal analysis					
Cadence	Sex	6.584	4.415 ~ 8.753	0.049	0.000
(strides/min)	Age	-0.354	-0.448 ~ -0.286	0.034	0.000
	Constant	128.802	122.685 ~ 134.320		
Step time	Sex	-19.681	-25.057 ~ -14.305	0.038	0.000
(ms)	VPT	0.654	0.290 ~ 1.018	0.054	0.000
	Age	0.418	0.132 ~ 0.703	0.058	0.000
	Constant	524.993	507.707 ~ 542.280		
Stride length	Leg length	0.009	0.008 ~ 0.011	0.199	0.000
(m)	Sex	-0.074	-0.092 ~ -0.057	0.242	0.000
	ABI	0.088	0.002 ~ 0.142	0.247	0.002
	Constant	0.460	0.282 ~ 0.639		
Walking speed	Age	-0.004	-0.005 ~ -0.003	0.041	0.000
(m/s)	Leg length	0.009	0.007 ~ 0.011	0.066	0.000
	Constant	0.706	0.478 ~ 0.926		
Walk ratio	Sex	-0.655	-0.739 ~ -0.571	0.175	0.000
(mm/steps/min)	Leg length	0.038	0.030 ~ 0.046	0.206	0.000
	Constant	3.114	2.297 ~ 3.932		
Duty factor	Sex	-1.945	-2.824 ~ -1.066	0.015	0.000
-Double Stance	ABI	-4.135	-7.296 ~ -1.066	0.020	0.010
	Constant	36.794	33.039 ~ 40.548		
Phase plot analysis					
SD_A	Sex	-0.211	-0.262 ~ -0.160	0.097	0.000
	VPT	-0.007	-0.010 ~ -0.004	0.123	0.005
	Leg length	0.009	0.004 ~ 0.013	0.133	0.007
	Age	-0.004	-0.006 ~ 0.002	0.140	0.011
	ABI	0.204	0.046 ~ 0.363	0.144	0.015
	Constant	1.233	0.696 ~1.769		
SD_B	Sex	-0.052	-0.070 ~ -0.033	0.044	0.000
	VPT	-0.002	-0.003 ~ -0.001	0.057	0.000
	Leg length	0.002	0.001~ 0.004	0.062	0.000
	ABI	0.059	0.002 ~ 0.116	0.065	0.002
	Constant	0.275	0.090 ~ 0.461		
△angleβ	Sex	-0.993	-1.976 ~ -0.009	0.001	0.000
	Constant	27.367	25.766 ~ 8.968		
A ratio	Age	-0.005	-0.008 ~ 0.002	0.004	0.000
	Sex	-0.10	-.208 ~ -0.052	0.008	0.000
	Constant	4.181	3.971 ~ 4.391		

VPT, vibration perception threshold.; ABI, ankle-brachial index. CI, confidence interval; SDA, spatiotemporal variability; SDB, temporal variability.

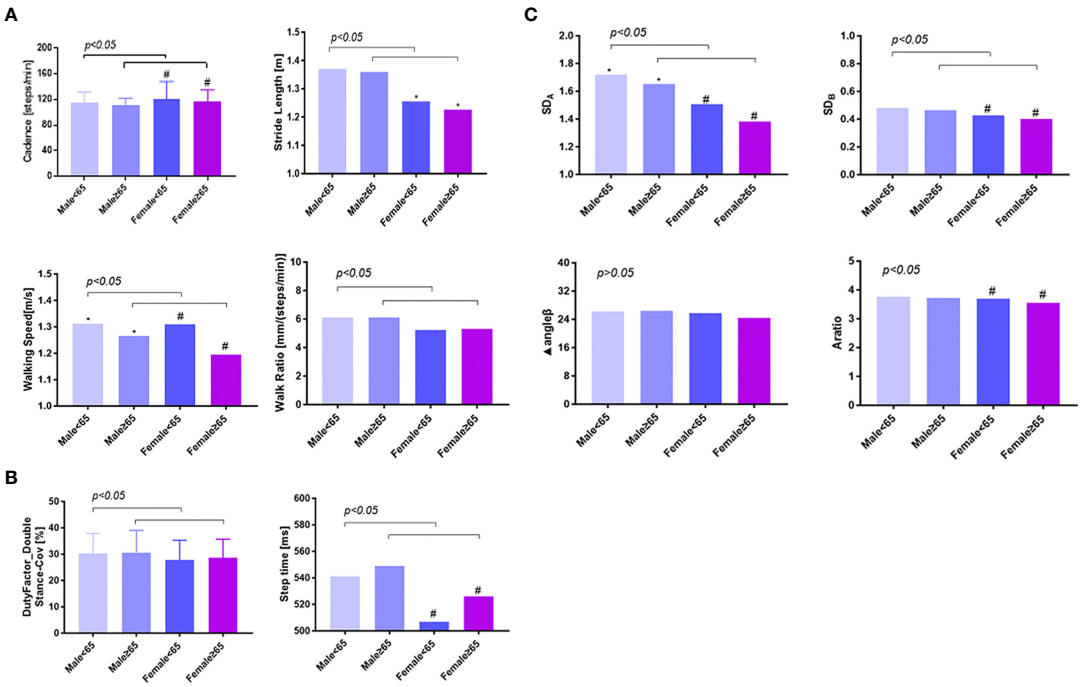

FIGURE 3
The gender and age difference of gait variables among type 2 diabetics. Panels **(A, B)** showed the comparison of spatiotemporal variables between different age and sex groups, and panel **(C)** showed the comparison of phase plot variables. A total of 1,420 individuals were selected and divided into four groups according to their sex and age and showed an obvious difference between men and women, young and elder patients. * $p < 0.05$, comparison between men of different age ranges; # $p < 0.05$, comparison between women of different age ranges; $p < 0.05$, comparison between different sex groups at the same age range. In the age range ≤65 years and ≥65 years, male participants were found to have fewer steps/min than female participants, but step length was significantly increased, walking faster, spent more time per walk, and showed greater SD_A and SD_B. A larger part of distinction was observed only in female subjects, such as less steps/min, shorter stride length, more time spent per walk, and smaller SD_A, SD_B, and A ratio. Men<65 means men aged <65 years (56.1 ± 5.2 years), n = 372; Men≥65 means men aged ≥65 years (69.7 ± 3.2 years), n = 190; Women<65 means women aged <65 years (56.5 ± 5.8 years), n = 571; Women≥65 means women aged ≥65 years (69.7 ± 3.3 years), n = 287.

TABLE 3 Comparison of dependent variables of gait characteristics among young and elder diabetic patients with different genders.

	Men<65	Men≥65	Women<65	Women≥65	*p*
Age (years)	56.1±5.2	69.7±3.2	56.5±5.8	69.7±3.3	–
Patients (n, %)	372,26.2%	190,13.4%	571,40.2%	287,20.2%	–
Cadence [steps/min]	114.1±0.8^{a}	111.4±0.58^{b}	120.9±1.19$^{a\#}$	116.7±0.90$^{b\#}$	<0.05
Stride length [m]	1.37±0.01^{a}	1.36±0.01^{b}	1.25±0.01$^{a\#}$	1.23±0.01$^{b\#}$	<0.05
Walking speed [m/s]	1.31±0.01^{a*}	1.27±0.01^{b*}	1.27±0.01$^{a\#}$	1.19±0.01$^{b\#}$	<0.05
Walk ratio [mm/(steps/min)]	6.10±0.04^{a}	6.13±0.05^{b}	5.33±0.04^{a}	5.31±0.04^{b}	<0.05
Duty Factor_Double Stance [%]	30.25±0.36^{a}	30.6±0.49^{b}	28.1±0.36^{a}	28.56±0.38^{b}	<0.05
Step time [ms]	542.6±2.07^{a}	549.4±2.42^{b}	518.3±2.06$^{a\#}$	526.1±2.21$^{b\#}$	<0.05
SD_A	1.71±0.02^{a*}	1.66±0.02^{b*}	1.48±0.01$^{a\#}$	1.38±0.02$^{b\#}$	<0.05
SD_B	0.48±0.01^{a}	0.47±0.01^{b}	0.42±0.01$^{a\#}$	0.40±0.01$^{b\#}$	<0.05
ΔAngle β	26.28±0.54	26.48±0.64	25.57±0.51	24.42±0.58	>0.05
A ratio	3.76±0.04	3.73±0.05	3.68±0.04$^{\#}$	3.55±0.04$^{\#}$	<0.05

Men<65 means men aged <65 years; Men≥65 means men aged ≥65 years; Women<65 means women aged <65 years; Women≥65 means women aged ≥65 years. Data were presented as mean ± SD or n (%) as appropriate.
*$p < 0.05$, Group 2 compared with Group 1; $^{\#}p < 0.05$, Group 4 compared with Group 3; $^{a}p < 0.05$, Group 3 compared with Group 1; $^{b}p < 0.05$, Group 4 compared with Group 2. Data marked with the same letter indicate significant differences between groups.

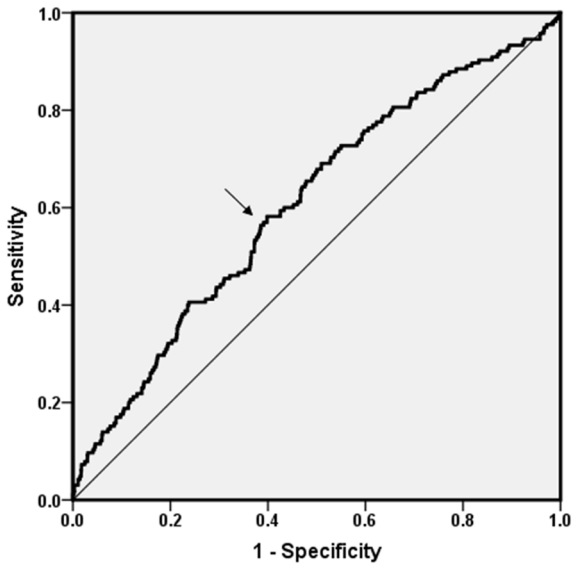

FIGURE 4
ROC curve analysis. ROC curve analysis was explored to find any discriminatory power of DPN for step time. The AUC value was 0.608 (95% CI: 0.562–0.654, $p < 0.01$). Cutoff point was 538.41 ms. The Youden index was 0.184; its sensitivity was 58.20% and the specificity was 39.81%.

Youden index at this level was 0.184; its sensitivity was 58.20% and the specificity was 39.81%.

Association of vibration perception threshold with increased step time

We chose an age range of NGT individuals from 46 to 75 years (average 56 years) as control and calculated the normal range of step time in the NGT elders (mean 527.32 ms, SD 45.01 ms). We used the mean ± twice the standard deviation as the normal range; >617.34 ms was classified as increased step time. As shown in Table 4, logistic regression analysis was used to analyze the relationship between VPT and increased step time, and there was a significant correlation between increased step time and increased levels of the highest VPT group (OR = 1.83, 95% CI: 1.32–2.55, $p < 0.01$). The OR value of female patients increased to 2.16 (OR = 2.16, 95% CI: 1.25–3.73, $p < 0.01$). This positive association persisted after adjustment for age (men: OR = 1.58, 95% CI: 1.02–2.46; women: OR = 2.17, 95% CI: 1.22–3.88, all $p < 0.05$).

Discussion

Most recently, research has focused on the measurement of gait parameters to illustrate the role of diabetes, DPN, or other diabetes-related complications (2–6), while results were discrepant due to different populations, various devices and methods of examination, diverse analyses, and so on. For the purpose of verifying the effect of different conditions of diabetes on gait alterations during shod walking. The present study investigated the changes during walking among different diabetic individuals with controls for the first time and provided the first step of the whole diabetic population toward substantiating the impact of diabetes and diabetic lower extremity complications on gait.

Our results demonstrated that both the incidence of elevated blood glucose and the occurrence of lower extremity disease indicate the appearance of alterations during both spatiotemporal and phase plot analyses. Higher duty-factor_double stance-CoV was observed among individuals with elevated blood glucose. There are also other data available on the association between impaired blood glucose and abnormal gait. In a study by Almurdhi et al. (5), subjects with impaired glucose tolerance displayed a significantly higher dynamic mediolateral sway during walking, suggesting that alterations in gait may occur very early, even in the prediabetes phase. As these previous studies documented, the impairment of gait could be observed as early as the emergence of impaired glucose tolerance (IGT). This seems to suggest that gait abnormalities can be detected early, so the presence of gait abnormalities may help us detect diabetes early.

As documented in other studies (5, 6), diabetes alone could induce gait alterations, such as slower walking speed, shorter stride length, increased cadence, and high gait variability. In this study, there was no significant difference in walking speed and walk ratio among the four groups, which was inconsistent with some reports in Caucasians, Indians, Koreans, etc. We believe that these may be the following reasons: 1) Although the total sample size of this study was large, there was a large difference in sample size between groups, which may have contributed to the disappearance of the difference; 2) Walking speed and walk ratio can be compensated by walking posture, which also explains the changes of walking posture and gait status earlier than the changes of walking speed and walk ratio.

To our knowledge, there are no previous studies examining the alteration of phase plot within such an extended range of diabetes subjects. The trend analysis during phase plot assessment revealed that the decline of variability and symmetry appears as long as diabetes occurs. Many other investigations had offered explanations for abnormal gait features in diabetes. For instance, Almurdhi et al. (16) recently noted that diabetic patients had a significant reduction in proximal and distal leg muscle strength and a proximal reduction in muscle volume, with a further contribution of brain atrophy and cognitive impairments that were related with dysregulation of glycemic control (17). Some indications that a deprivation of nerve growth factors in subjects with diabetes have been demonstrated (18). Our findings in this pronouncedly larger sample of older adults contradict the results of previous studies concerning diabetes and gait in adults primarily older than 60 years. By doing so, it further enhances the insight into the relationship that gait deficits occurred as a consequence of diabetes.

It is widely recognized that DPN affects peripheral sensory and motor nerves (2) and then the sensorimotor system is gradually affected, resulting in a decreased sensation of pain, tissue damage, loss of muscle strength, changes in foot structure, and eventually abnormal gait (19). Lowered cadence, modified stride length, and decreased gait speed were reckoned as characteristics of an impaired gait performance among DPN groups (2). Contradicting to those results, the present study showed no significant difference in these parameters mentioned above but increased step time. Stepwise regression analysis revealed that VPT was an independent risk factor for decreased SD_A and SD_B. All of these findings in our

TABLE 4 Odds ratio analysis of VPT for increased step time.

VPT (V)	Non-adjusted model						Age-adjusted model					
	Overall		Male		Female		Overall		Male		Female	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
1~15	1	/	1	/	1	/	1	/	1	/	1	/
16~24	1.73 (0.85-3.55)	0.134	1.31 (0.50-3.38)	0.583	2.51 (0.84-7.53)	0.100	1.46 (0.71-.99)	0.301	1.08 (0.43-2.81)	0.881	2.08 (0.70-0.18)	0.186
≥25	1.83 (1.32-2.55)	0.000	1.63 (1.08-2.46)	0.021	2.16 (1.25-3.73)	0.006	1.82 (1.29-2.58)	0.001	1.58 (1.02-2.46)	0.042	2.17 (1.22-3.88)	0.009

VPT (V)	Multiple factors-adjusted model					
	Overall		Male		Female	
	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
1~15	1	/	1	/	1	/
16~24	1.001 (0.998-1.005)	0.368	0.999 (0.995-1.004)	0.797	1.003 (0.998-1.008)	0.187
≥25	1.007 (1.003-1.011)	0.001	1.005 (1.000-1.011)	0.042	1.007 (1.001-1.014)	0.031

Multiple factors-adjusted model was adjusted for other associated factors including age, leg length, and ABI.
OR, odds ratio; CI, confidence interval; VPT, vibration perception threshold.

research demonstrated that DPN participants manifested a moderate modification of gait while walking to adapt sensorimotor system abnormality, much earlier than the presence of remarkable lower cadence, modified stride length, and decreased gait speed.

Several factors were considered to be responsible for the distinct results in our participants, such as sample size and control selection, 10-m level walking, eliminated process of acceleration and deceleration, and self-selected gait speed. Although the findings from previous studies were seemingly consistent (9, 20, 21), the sample size was strikingly small (approximately 50~100 cases), and usually, healthy individuals were picked as controls. However, the discrepancy disappeared as long as we extended the population to a huge sample and different diabetic complications. With respect to the impact of walking style demanded, the results varied apparently. It is meaningful to observe differences in various gait parameters while walking on challenging surfaces. For instance, Allet et al. (22) reported the difficulty of diabetic patients while changing from a tarred surface to cobblestones. Menz et al. (9) observed shorter step length of DPN patients when walking on irregular surfaces, and other evidence suggested that an irregular terrain accentuates differences in step time variability between older women with peripheral neuropathy and older women without peripheral neuropathy (23). Walking speeds (average 4.5–4.8 km/h) in the present study were within the range of values recorded in previous studies on comparable surfaces (3.4–5.1 km/h) (24) but showed no noticeable difference between groups. There was another trial that found a slower walking speed by patients with DPN when compared to control peers during the self-selected speed test (21). It has also been reported that both initiation and termination of gait were more complex procedures than steady-state walking (25). Except for all of these discussed reasons above, Gates et al. (26) noted that the sensory loss in these neuropathic patients was not complete; there are still retained proximal somatosensory inputs as well as visual and vestibular feedback information. Thus, we believe that there was no such difference in gait spatiotemporal analysis among various diabetic patients when performing a short-distance, non-weight-bearing, and level walking, even compared with non-DM ones.

Although majority of previous studies recruited DPN participants, only few studies excluded patients with LEAD particularly. In the present study, we excluded patients complicated by only LEAD in order to eliminate the impact of lower limb ischemia on the gait. In this large-sample and wide-range trial of elder individuals, we were able to examine potential explanatory factors (sex, age, BMI, height, leg length, complications, DM duration, diabetic comorbidities, smoking, VPT, and ABI) of the altered gait parameters. The key observation was that stepwise multivariate analyses identified that sex, age, and leg length were more significant independent predictors of gait parameters, in addition to VPT and ABI. In the present investigation, age-deteriorated changes existed almost in every gait feature. The elderly walked slower with lower cadence and shorter stride length as age increased. Slow walking speed is highly prevalent in men and women above age 65 years (27). Reduced walking speed appears to be a compensatory strategy adopted by the elders to maintain trunk stability, and it is associated with an increased risk of all-cause mortality, impaired gait efficiency, and an increased risk of disability (28). As de Mettelinge et al. (10) illustrated, older participants with diabetes walked slower, took shorter strides during simple, counting backward by 3 from 40, reciting animal names conditions when compared with controls, and showed more gait variability during dual-task conditions. Declined nervous system and musculoskeletal system because of aging may affect gait control (29). Age-deteriorated changes in the production of sex steroids and cortisol and in the secretion of the growth hormone and insulin-like growth factor-1 have also been identified in the pathogenesis of weakness during gait (30, 31). Sex-related changes in our investigation were popular too, which is consistent with other conclusions. As one South Korea trial described, women have a shorter stride length and walked slower than men mostly due to their shorter height, and the researchers assume that the difference is due to gender features of the gait-related anatomy and habits (32). The rapid

reduction of estrogen in postmenopausal women and the gradual decline of testosterone in men lead to a decrease in muscle mass and strength (30). Moreover, we illustrated the influence of leg length on gait indexes, which should not be ignored during gait analysis. Decades ago, the locomotor advantages of longer lower limbs have been documented (33). Recently, 18 male healthy subjects were enrolled in a walking gait analysis (34), and Fazreena et al. maintained that the mean contact forces for all joints (ankle, knee, hip, and pelvis) in the short leg were increased. Researchers also claimed that gait impairments are associated with age, sex, diabetes, hypertension, and history of cerebrovascular accidents, of which greater age and female sex were listed to be associated with slower gait speed and shorter stride length (28).

In this investigation, ROC curve analysis was employed to seek the predictive value of step time in indicating the presence of DPN, and our research may be the first effort to find an optimal cutoff point of step time for predicting DPN (538.41 ms). However, the AUC was not so large enough for clinical practice, and we believe that the combined effect of different gait indexes, such as step time, stride length, and gait variability, must be more telling. Taking normal-VPT group as the referent, the positive association between increased step time and higher VPT group was observed in non-adjusted and age-adjusted models, and the association was more prominent in women, which means that women suffer from a much higher risk of increased step time than men.

There are some limitations in the present study that should be identified. This research was a multiple-center but cross-sectional analysis of outpatients with type 2 diabetes. Additional prospective studies are further required to determine the role of all diabetic conditions on the alterations of gait. This was a study of relatively older adults; therefore, the findings may not be suitable to assess younger samples or to individuals. In this study, plantar pressure could not be directly assessed but could only be indirectly analyzed by analyzing the foot status and gait of patients. Despite that this research was a multiple-center research, there is still a large difference in the number of people in different groups, which may be the reason why there is no difference in walking time, cadence, and stride length in this study. Although these limitations exist, we believe that the novel findings of the present research are generalizable to the large number of elder diabetic outpatients in the clinic.

Conclusions

To date, current literature supports the role of DPN in altering gait parameters. However, the present investigation extended the participants to varied diabetic individuals, accompanied by lower extremity complications or not. We identified some significant differences in gait among different diabetic groups, such as an increased step time in DPN and lower SD_A and SD_B of subjects with DPN. We verified a close relation between VPT and gait alterations among elder Chinese individuals. Further hazard ratio and ROC curve analyses substantiate that the step time of walking was a simple and easier gait, which increases with worsening of DPN. Therefore, our study provides instructive significance of the impact of DPN on the alterations of gait, and the walking step time increases with the ascending VPT. The easily operated VPT screening is helpful to indicate the risk of abnormal walking mode and to prevent the fall-down, fracture, and even foot disorders in populations suffering from T2DM.

Data availability statement

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

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

LD wrote and revised the article, YH and LX analyzed and processed data and helped to collect data, HZ and WS collected clinical data and gait data, PE and HD provided us with equipment and instructed us to use it, my professor FL provided research guidance and research fundings. All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Acknowledgments

We thank all the individuals who participated in the present study. This research was supported by grants from the National Natural Science Found of China (82170827, 81770802), Shanghai Natural Science Found of Shanghai Science and Technology Committee (22ZR1450100),and Yangtze River Delta field regional cooperation projects (22002400600).

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 JW is currently organizing a Research Topic with the author FL.

Publisher's note

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frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 16 March 2023
DOI 10.3389/fendo.2023.1099310

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 15 November 2022
ACCEPTED 06 March 2023
PUBLISHED 16 March 2023

CITATION
Yu X, Liu P, Li Z and Zhang Z (2023) Function and mechanism of mesenchymal stem cells in the healing of diabetic foot wounds. *Front. Endocrinol.* 14:1099310. doi: 10.3389/fendo.2023.1099310

Function and mechanism of mesenchymal stem cells in the healing of diabetic foot wounds

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Diabetes has become a global public health problem. Diabetic foot is one of the most severe complications of diabetes, which often places a heavy economic burden on patients and seriously affects their quality of life. The current conventional treatment for the diabetic foot can only relieve the symptoms or delay the progression of the disease but cannot repair damaged blood vessels and nerves. An increasing number of studies have shown that mesenchymal stem cells (MSCs) can promote angiogenesis and re-epithelialization, participate in immune regulation, reduce inflammation, and finally repair diabetic foot ulcer (DFU), rendering it an effective means of treating diabetic foot disease. Currently, stem cells used in the treatment of diabetic foot are divided into two categories: autologous and allogeneic. They are mainly derived from the bone marrow, umbilical cord, adipose tissue, and placenta. MSCs from different sources have similar characteristics and subtle differences. Mastering their features to better select and use MSCs is the premise of improving the therapeutic effect of DFU. This article reviews the types and characteristics of MSCs and their molecular mechanisms and functions in treating DFU to provide innovative ideas for using MSCs to treat diabetic foot and promote wound healing.

KEYWORDS

Diabetic foot, Wound healing, Mesenchymal stem cells, Angiogenesis, mechanism

1 Introduction

Diabetes is a significant global public health problem (1). The number of diabetic patients in 2021 was 536.6 million, and it is expected to increase to approximately 783.2 million people by 2045 (2). With the prolongation and aggravation of the disease, patients with diabetes often present with severe lower extremity vascular disease, leading to DFU. Diabetic foot is one of the most severe complications of diabetes and is the leading cause of surgical non-traumatic amputation (3). Studies have found that approximately 25% of people with diabetes will suffer a DFU in their lifetime, and 30% of people with a diabetic foot will experience disease progression that would eventually leads to amputation (4, 5).

Currently, there is no effective clinical treatment plan for diabetic foot, as conservative medical treatment is only a routine method for diabetic foot treatment. For patients with severe ischemia and unsatisfactory effects of systemic drug treatment, vascular intervention and other operations are necessary to implement blood-flow reconstruction. However, in patients with a diabetic foot, the distal vascular outflow tract is poor, and vascular lesions of the lower extremities are diffuse and multiple. Vascular intervention can only improve stenosis of large vessels to a certain extent, and the improvement effect is limited. Studies have reported that patients with diabetic feet are prone to restenosis after the intervention, the recovery rate of peripheral blood flow is still very low, and the amputation rate is still high (6). Diabetic foot is becoming a worldwide public health problem threatening human health (7, 8). Therefore, a new method to accelerate diabetic wound healing is urgently required.

Previous studies have shown that approximately 50% of diabetic foot cases are caused by neuropathy alone, while peripheral arterial occlusive disease accounts for only 15% of cases. Furthermore, in 35% of cases, diabetic foot is caused by a combination of neuropathy and vascular disease (9, 10). In addition, microvascular diseases, biomechanical abnormalities, joint activity, and infection are increased, and multiple causes can interact (11). As a result, peripheral disease, neuropathy, deformity, previous amputation, and infection are the main factors that lead to DFU development (12).. Currently, conventional treatments—including wound dressing, hyperbaric oxygen therapy (HBOT), negative pressure wound therapy, total contact casting bracing, and wound debridement—can only relieve patients' symptoms or delay the disease progression. However, they cannot repair damaged blood vessels and nerves. An increasing number of studies have shown that MSCs can promote angiogenesis and re-epithelialization, participate in immune regulation, reduce inflammation, and finally repair DFU, rendering it an effective means of treating diabetic foot disease (13); it is a potential new method for the treatment of the diabetic foot. This article reviews stem cells' function and molecular mechanisms in treating diabetic foot, to provide innovative ideas for using stem cells to treat diabetic foot and promote wound healing.

2 Pathogenesis of DFU

Various factors cause the formation of DFU, and the common causes are poor blood sugar control, neuropathy, ischemia, nutritional dysfunction, trauma, and local infection, among others. The advanced glycation end products (AGEs) is a general term for a series of highly active end products formed by non-enzymatic glycosylation (also known as Maillard reaction) between the amino groups of proteins, fatty acids or nucleic acids, and the aldehyde groups of reducing sugars, which is highly associated with the complications of diabetes (14). In diabetic patients, due to metabolic disorders, chronic inflammation and accumulation of AGEs, vascular endothelial injury and hyperplasia, enhanced platelet adhesion, micro-thrombosis, microvascular bleeding, and exudation occur (15). In addition to abnormal glucose metabolism, diabetic patients are often accompanied by abnormal lipid metabolism, which promotes the release of inflammatory mediators, thus inducing the infiltration of macrophages and other immune cells (16). High lipid and sugar promotes the generation of inflammatory mediators, ultimately leading to sustained high inflammation in the body (17). In diabetic patients, the phagocytosis function of white blood cells and related immune cells is down-regulated. The duration of inflammatory factors in diabetic foot ulcer wounds is prolonged to compensate for the decline in white blood cell activity, leading to the downregulated function of fibroblasts and vascular endothelial cells. The formation of granulation tissue is inhibited (18, 19). Under the stimulation of a high glucose environment, the oxidative stress level of the body increases, and a high level of reactive oxygen species (ROS) will lead to the weakened antioxidant effect of the body, and inhibit the release of cytokines and growth factors and the formation of fibroblasts, collagen fibers, and new blood vessels (20, 21). Finally, capillary stenosis or obstruction exacerbates microcirculation disturbance.

Furthermore, metabolic disorders of diabetes lead to degeneration of peripheral nerve axons and nerve membrane cells, motor, sensory, and autonomic nerves dysfunction, resulting in further decline of limb perfusion effect, sensory dysfunction, muscle atrophy, and tendon and ligament sclerosis (22), followed by foot deformities and increased pressure on the forefoot. Metabolic products cannot be excluded, while extremal ischemia and hypoxia, bacterial growth, extremal ulceration, wound healing is challenging, and foot infection can become worsened (23). As blood flow is impaired, it is often difficult for drugs to reach the affected area, and DFU can progress from a simple infection to widespread gangrene (24). The occurrence and development of DFU involve various pathophysiological processes, and these complex processes often transform and superimpose each other, which renders the treatment of DFU a challenge.

3 Conventional treatment for DFU

Since the occurrence of DFU, people have been looking for the best treatment method. Conventional treatments of DFU mainly include wound debridement, wound dressing, hyperbaric oxygen therapy, negative pressure wound therapy, and off-loading.

Debridement is the most commonly used method, and the widely used types include surgical debridement, enzyme debridement, biological debridement, and ultrasonic debridement (25). The clearance goals include removing deactivated, necrotic, and infected tissue from the ulcer and retaining healthy, blood supply-rich tissue. In addition, debridement promotes healing through the surrounding healthy granulation tissue by eliminating infected tissue, senescent cells, and bacterial biofilms (26). Debridement is the most basic method in the treatment of DFU.

Negative pressure wound therapy involves placing a vacuum device on the ulcer wound after debridement. This vacuum device can collect large amounts of exudate, keep the wound clean and dry, and reduce the frequency of dressing replacement (27). In addition,

continuous negative pressure drainage can also provide an irrigation solution to promote wound healing.

Hyperbaric Oxygen Therapy (HBOT) can be divided into two methods: local delivery of oxygen to ulcers and systemic delivery of oxygen. HBOT can improve local tissue perfusion, stimulate collagen synthesis, growth factor production, and neovascularization (28). In DFU patients, local oxygenation of ulcers is impaired. HBOT can also inhibit anaerobic bacteria and reduce the use of antibiotics (29, 30). However, the therapeutic value of HBOT obtained through clinical studies remains controversial. Some studies have suggested that HBOT could improve short-term but not long-term ulcer healing efficacy of DFU and could not reduce the amputation rate of DUF (31, 32).

The primary function of wound dressing is to provide a protective barrier for DFU. Meanwhile, some new bandages can inhibit bacteria and promote the speed of blood vessel and tissue regeneration (33). Hydrogels and alginate are currently used for medical dressings, and silver ions and other nanoparticles can significantly improve the therapeutic effect (34–36). For example, Tsang et al. reported that dressing containing nanocrystalline silver and manuka honey could effectively play an antibacterial role in treating DFU and inhibit the generation of drug-resistant bacteria (37). Wound dressing for various sources is constantly being improved and developed.

Shear stress and vertical pressure on the plantar as the ground surface are adverse factors for DFU healing (38). Therefore, the principle of offloading is to reduce pressure on the plantar and forefoot of the DFU (39). The several ways to relieve foot load include orthopedic walking aids and modified shoes used in DFU treatment (40). Compared with the modified shoes, the total contact casting bracing can reduce the load on the sole, mechanically help to reduce and redistribute the pressure of the DFU, and contribute to the repair of ulcers, and is considered an important means for the treatment of DFU (41, 42). However, the production of total contact casting bracing requires personalization for different patients.

Other considerations, such as glycemic control, vascular assessment, use of sensitive antibiotics, and psychotherapy in patients with DFU, have been fully considered in previous research (43, 44). In addition, amputation may be a life-saving option if the patient's condition becomes too severe to salvage a limb (45, 46). Although there are many therapeutic methods, treating DFU is still one of the thorny problems in the complications of diabetes.

4 MSCs and stem cells

MSCs are a type of pluripotent stem cells that were first discovered by FriedenStein et al. (47, 48). The term "mesenchymal" refers to the embryonic origin of cells. "Mesenchymal stem cells" were initially named fibroblast colony-forming units or bone marrow stromal cells, and can differentiate into various mesodermal tissues (49). The mesoderm is one of the three main layers formed early in embryonic development. It produces various connective tissues, such as muscle, bone, cartilage, and fat, and cells forming blood vessels, blood cells, and the urogenital system (50). In addition, it has been found that MSCs can be used as ectoderm and endoderm-derived cells, such as liver and nerve cells (51). The differentiation potential of MSCs may depend on the source of stem cells, amplification conditions, and the culture microenvironment. The differentiation process can be induced by specific hormones, growth factors, or specific differentiation agents (52). A complex interaction of genetic and epigenetic factors also controls the differentiation process. Genetic factors include the expression of particular transcription factors and signaling molecules, while epigenetic factors include histone modification, DNA methylation, and altered expression of non-coding RNA (53).

The main feature of stem cells is their diverse origin and potential for self-renewal and multi-differentiation. Moreover, MSCs promote tissue repair by releasing growth factors and cytokines, which help recruit other cells to the damaged site (54). These growth factors and cytokines also promote the formation of new blood vessels necessary for tissue repair. MSCs can also regulate immune system activity, reduce inflammation, and suppress immune responses (55), rendering stem cell therapy a new option for repairing and regenerating tissues. This property renders them promising candidates for cellular therapies for a variety of diseases, such as autoimmune diseases and graft-versus-host diseases.

Numerous studies have found that stem cell transplantation can improve various diseases, such as diabetic retinopathy and keratopathy (56, 57), congenital cataracts (58), ocular surface burns (59, 60), severe skin burns (61, 62), myocardial infarction (63, 64), Parkinson's disease (65, 66), Huntington's disease (67, 68), and DFU (48, 69). In addition, MSCs can promote wound healing (70, 71) and serve as a cell source for many tissue engineering applications, including bone regeneration (72, 73), cartilage regeneration (74, 75), neurogenesis (76, 77), myocardial regeneration (78, 79), inflammatory bowel disease (80) and DFU (81, 82).

MSCs are easy to obtain and they belong to a class of immunodeficient cells. In general, allogeneic gene transplantation does not cause immune rejection. Previous studies have shown that most stem cells express low levels of human leukocyte antigen (HLA) class I. They do not express or lower express HLA class II, nor do they express co-stimulator factor (CD40, CD80, and CD86) and surface markers of hematopoietic cells (CD34, CD45, CD79, and CD14) (83–85). This property enables stem cells to be immune-privileged without causing immunological conflict between host and transplanted cells (86). The presence of HLA class I is important because low levels of HLA class I can protect cells from natural killer (NK) cell-mediated cytotoxicity (87). It has been reported that MSCs express HLA class II after being exposed to the pro-inflammatory microenvironment of damaged tissues (86). MSCs have been reported to be highly immunogenic after transplantation into the host (88). More than 90% of undifferentiated MSCS express HLA class II when exposed to IFN-γ (89). In addition, Agudo et al. reported that Hair follicle stem cells downregulate major histocompatibility complex (MHC) class I in the static state to avoid immune surveillance (90). Changes in the immunogenicity of MSCs may depend on many factors, including cell state and microenvironment. Therefore, more studies

on the details related to the immunogenicity of MSCs are needed to help improve the efficiency of MSCs transplantation.

Compared with mononuclear cells and endothelial progenitor cells mainly derived from autologous cells, they are suitable for a wide range of clinical applications and the promotion of later stem cell products. MSCs express a series of cell surface immune markers, based on which the International Society for Cellular Therapy (ISCT) formulated a set of identification criteria for MSCs in 2006 (1): plasticity and adherence (2); expression of CD73, CD90, and CD105, and no expression of CD14, CD34, CD45, CD11b, CD79α, CD19, and HLA-DR; (3) capability to differentiate into chondrocytes, osteoblasts, and adipocytes (91). The ISCT guidelines aim to standardize mesenchymal stem cell research and promote collaboration among investigators. Generally, MSCs from different tissue sources can express the typical immunophenotypes of MSCs, but there are slight differences in the expression of the remaining immunophenotypes. It is possible that this standard will be revised in the future as research progresses and new knowledge becomes available.

4.1 Types of MSCs

There are many sources of MSCs. Current research shows that stem cells can be extracted from different tissues. There are more studies on bone marrow MSCs (BM-MSCs), human numerical core MSCs (hUC-MSCs), adipose tissue-derived MSCs (ADSCs), urine-derived stem cells (USCs), and placenta-derived MSCs (PD-MSCs).

BM-MSCs are a group of heterogeneous cells composed of pluripotent adult stem cells with the potential ability for multi-differentiation, including chondrocytic, adipocytic, or osteocytic lineages (92). It represents ~ 0.001–0.01% of bone marrow mononuclear cells (BMMNCs) and expresses CD73, CD90, and CD105 but does not express CD14, CD45, CD34, or CD11b, CD79α, CD19, or HLA-DR surface molecules (93). Due to its low abundance, extensive *in vitro* culture and amplification are required to obtain sufficient quantities for research or clinical use (94). The acquisition process of BM-MSCs is often invasive and costly. In addition, the cell quality of BM-MSCs decreased significantly with the increase in donor age.

Human umbilical cord MSCs (hUC-MSCs) were separated from Wharton's Jelly, a colloidal tissue surrounding the umbilical cord blood canal (95). It is usually discarded during childbirth; thus, the collection is non-invasive and poses few ethical problems (96). It has the characteristics of a short doubling time (97), long survival time (98), and strong anti-inflammatory ability (99), and long-term *in vitro* culture has little influence on its phenotype and genetic stability (100). Compared with BM-MSCs, hUC-MSCs have a higher proliferative ability and lower expression of HLA-ABC and HLA-DR (101).

Adipose tissue-derived MSCs (ADSCs) are rich in tissue sources. It can be obtained by minimally invasive surgery from subcutaneous white adipose tissue separated from the abdomen, thighs, or buttocks/buttocks of animals or humans (102). The isolation of ADSCs is simple, with high yield (~ 100 mL can be collected from 1000 mL adipose tissue) (103). It can differentiate in multiple lineages, including chondrogenesis, osteogenesis, cardiomyocyte, adipogenesis, neurogenic, and hepatic differentiation (104, 105). ADSCs often express CD34 in low-passage cultures, but this decreases with continuous cell passage (106, 107). Unlike BM-MSCs, ASCs do not express the sialoglycoprotein podocalyxin (PODXL) or the adhesion marker CD106 (108, 109).

Tissue sources of placenta-derived MSCs (PD-MSCs) include amniotic fluid, amniotic membrane, chorionic plate, chorionic villi, decidua basalis, complete placenta, and complete placenta (110). Stem cell-like cells in the placenta have higher differentiation potential and self-renewal ability than other tissue-derived MSCs (111). In addition, it has shown low immune properties *in vitro* and *in vivo* studies (112). PD-MSCs have also been shown to enhance the differentiation of monocytes from inflammatory M1 macrophages to M2-like macrophages (113), suggesting that PD-MSCs have the potential to improve inflammatory diseases. However, MSCS isolated from different parts of the placenta have different subtle properties. For example, the placental tissue comprises two separate individual tissues (the maternal placental tissues and the fetal). MSCs derived from fetal placental tissues have significantly stronger proliferative capacity than those derived from maternal placental tissues (114). To understand their different characteristics for better use in future research, more research data are needed to clarify the accuracy of their data further.

Zhang et al., in 2008, first identified a urine stem cell population and found that it could expand over ten generations *in vitro* (115). This stem cell population was named urine-derived stem cells (USCs). USCs are easier to obtain than MSCs. They can be extracted directly from excreted urine and are non-invasive, painless, and low-cost (116). It has the same characteristics as those of USCs isolated from the upper urinary tract. It was found that USCs showed normal karyotypes regardless of passage (117, 118). USCs can differentiate into bone, cartilage, and adipose lineages, as well as urothelial cells, smooth muscle cells, endothelial cells, kidney cells, and podocytes, showing the potential for multidirectional differentiation (119–122). USCs expressed several MSCs markers, including CD44, CD73, and vimentin (123), and also expressed adhesion markers such as CD29 and CD166, but not CD31 (124, 125). It was reported that no teratoma was formed when USCs were injected into immunodeficient mice, showing an absence of the tumorigenic phenotype (126).

Gingival mesenchymal stem cells (GMSCs) can be obtained from periodontal tissue, gingival ligaments, and dental pulp. Similar to MSCs from other sources, GMSCs have MSCs-related cell surfaces markers such as CD73, CD90, CD105, and stromal cell antigen 1 (STRO-1) (127). In addition, studies have shown that GMSCs not only have the potential to differentiate into three lines of mesoderm (adipocytes, osteocytes, and chondrocytes) but can also transdifferentiate into ectoderm and endoderm cell lineages, such as keratinocytes, endothelial cells, and nerve cells (128, 129). In addition, GMSCs also have an anti-inflammatory function and immunomodulatory ability (130, 131), and can promote the differentiation of macrophages (132). Furthermore, GMSCS are homogenous, rapidly proliferating, and not tumorigenic, and have

stable morphological and functional characteristics under higher passage (130).

Recently, scientists isolated mixed cell populations with mesenchymal and epithelial features from normal human labial minor salivary glands (133). Subsequently, it was confirmed that human labial gland-derived MSCs (LGMSCs) existed in the lamina propria of the oral mucosa (134). Wang et al. successfully isolated MSCs from adult female salivary gland cysts, identifying their characteristic MSCs expression markers, including CD29, CD44, CD73, CD90, and CD105, using flow cytometry. However, the CD34, CD45, CD106, CD117, and the salivary gland epithelium markers (CD49f) were also negative (135). LGMSCs have the potential for osteogenic and lipogenic differentiation, and their ability to differentiate into salivary gland epithelioid-like cells is stronger than that of other MSCs. However, its adipogenic differentiation ability is lower than that of ADSCs (136, 137). In addition, LGMSCs have the characteristics of a shallow glandular location, are easy to obtain, expand *in vitro*, and regulate immune function (138–140).

In addition, MSCs derived from tissues such as the pancreas and the liver are being explored, which will provide options for multi-source pathways of MSCs in the future. It should be noted that MSCs from type 1 diabetes mellitus (T1DM) donors are similar in phenotype and function to healthy donors. They can maintain normal immunomodulatory or secretory functions (141). However, MSCs from type 2 diabetes mellitus (T2DM) donors often show increased apoptosis and senescence, as well as decreased angiogenesis potential (142).

According to the source of MSCs, those used for treating DFU can be divided into autologous and allogeneic MSCs. Due to the different biological characteristics of MSCs from different tissue sources, their therapeutic mechanisms, adapted diseases, preferred lesions, and effects are also different. Furthermore, the methods used to culture MSCs in different laboratories (including enzyme digestion or tissue-advanced methods) are also different (143). Therefore, the quality and degree of cell expansion are different, and the study results may differ. Consequently, it is necessary to establish a quality control system for MSCs to ensure the stability and effectiveness of MSCs.

4.2 Route of administration for MSC therapy

MSCs are mainly used for the treatment of diabetic foot by local delivery and systemic delivery. Local delivery is divided into topical application, topical injection, scaffold, and gel, systemic delivery is divided into intravenous and arterial administration (13). Previous research has shown that BM-MSCs are most effective by intramuscular injection (144), and the best effect of PD-MSCs was obtained by intraperitoneal injection (145).

Yan et al. found that local injection and intravenous infusion of stem cells were used to treat T2DM rat ulcer models, and both administration methods significantly accelerated wound healing. Moreover, systemic administration also had the potential to ameliorate hyperglycemia (146). However, it has been proposed that MSCs be delivered through the whole body, and most of the cells remain in the lungs, with only a small percentage of the cells moving to the ulcer site (147). In addition, intradermal injection of MSCs into the edge of the ulcer significantly improved the wound healing process. However, local injection of MSCs has the disadvantages of poor cell localization, difficult control of cell density and spacing, and impaired cell vitality due to the influence of local wounds (148, 149).

Furthermore, when MSCs are injected locally into the lesion using a syringe, irreversible damage can be caused to the cell membrane, resulting in decreased cell viability (150). For DFU patients with microvascular complications or arterial occlusion, arterial administration often fails to transport MSCs well to the ulcer site, thus affecting the therapeutic effect. When MSCs are administered to the muscle near the lesion site, the muscle tissue can provide oxygen and nutrients to the injected cells, which contributes to the survival of MSCs and improves their function (148). However, the characteristics of MSCs mean their external preparations are difficult. Therefore, it has been proposed to use scaffolds loaded with MSCs as the primary cell carriers to deliver MSCs, to provide a favorable microenvironment for cell attachment, proliferation, differentiation, and guiding host cell migration, to achieve better healing effects (2). Assi et al. found that compared with the control group with an ordinary injection of MSCs, Rolled collagen scaffolds containing MSCs showed better healing ability and increased vascular endothelial growth factor (VEGF) expression and capillary density in the local ulcers; they found increased numbers of fibroblasts, macrophages, and smooth muscle cells (151).

Assis et al. reported an approach to induce angiogenesis using vascular-inducing devices (VIDs) composed of MSCs derived from healthy donors and decellularized lung-derived micro-fragments. These VIDs express and transcribe the entire library of angiogenic factors in a controlled release manner, induce proliferation of fibroblasts and endothelial cells, and induce local vascular network formation within a week after implantation of non-obese diabetic/severe combined immunodeficiency mice (152). They then transplanted the acellular micro-fragment from the bone marrow of an elderly diabetic patient suffering from lower extremity arterial disease and DFU. They found that the MSCs expressed and secreted angiogenic factors similar to those extracted from healthy individuals (153). This provides a good idea for researching and developing stem cells and scaffolds.

A large number of studies have been devoted to developing excipients that can provide support for MSCs, such as 3D printed collagen, chitosan, polyurethane scaffolds, and cell gels (13, 154, 155), to improve the effective maintenance time for topical application preparations of MSCs (Figure 1). In the actual treatment process, we can choose the most appropriate drug administration route by personalized treatment according to the actual condition of patients and the allocation of medical resources.

4.3 Mechanisms of MSCs in the treatment of diabetic foot

Cell proliferation, differentiation, and migration are crucial for the physiological processes of DFU wound repair and growth.

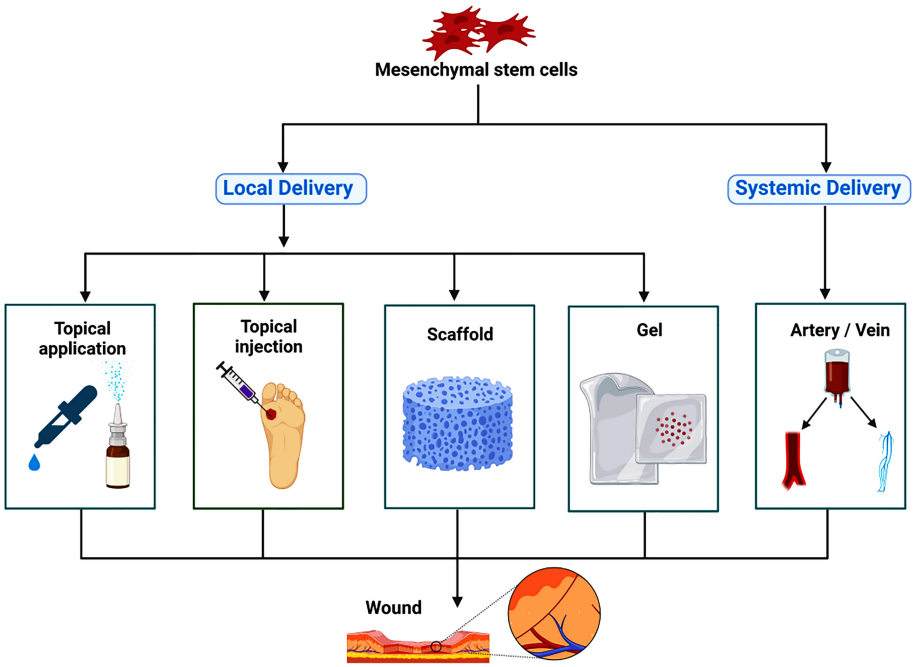

FIGURE 1
The route of administration for mesenchymal stem cells therapy. Mesenchymal stem cells are mainly used for the treatment of diabetic foot by local delivery and systemic delivery. Local delivery is divided into topical application, topical injection, scaffold, gel and so on; systemic delivery is divided into intravenous administration and arterial administration.

Wounds result from living tissue damage, and coordinating wound repair is initiated immediately upon damage to the tissue surface. During repair, growth factors and cytokines stimulate signal regulation and coordinate intercellular and intracellular signaling to promote cell proliferation, differentiation, migration, and protein synthesis. Recent studies have shown that various growth factors and molecular mechanisms play a vital role in the occurrence and development of DFU (156, 157).

4.3.1 MSCs can provide a variety of growth factors to promote angiogenesis

One of the essential reasons for diabetic foot secondary to diabetes is the damage and lesions of blood vessels. The formation and regeneration of new blood vessels in the DFU area provide nutrients for the growth of granulation tissue. Therefore, it is especially important for shrinking ulcers and promoting repair. Studies have shown that MSCs can secrete a variety of cytokines, including VEGF, basic fibroblast growth factor, stromal cell-derived factor-1 (SDF-1), keratinocyte growth factor 2, insulin-like growth factor 1, placental growth factor, and epidermal growth factor (EGF). These factors can promote angiogenesis, enhance microhemodynamics, and promote wound healing (158, 159). Among a series of factors regulating angiogenesis and repair, VEGF is the most potent (160).

Shen et al. showed that BM-MSCs could accelerate wound healing in the feet of diabetic mice by improving the activation of vascular endothelial cells and inducing angiogenesis by the paracrine VEGF and other vasoactive factors (161). After transplanting BM-MSCs into diabetic rat foot wounds, Wan et al. found that the expression of VEGF in wound tissue and angiogenesis was increased, which positively affected wound healing in diabetic rats (144). Furthermore, Badillo et al. showed that Mouse liver-derived MSCs increase local growth factor secretion, such as EGF, VEGF, and SDF-1, thus promoting neovascularization, enhancing wound cell recruitment, and improving wound contraction (162). Moreover, BM-MSCs can significantly promote the secretion of key growth factors, such as EGF and VEGF, for repairing and regenerating damaged tissues. They can increase collagen (types I–V) to promote wound healing in diabetic rats (163). Furthermore, Diao et al. demonstrated that in addition to directly promoting angiogenesis, VEGF can activate transcription factors to regulate endothelial progenitor cells (EPCs), recruit EPCs to the bone marrow, and inhibit the apoptosis of EPCs from promoting wound healing (164). These studies suggest that MSCs may directly or indirectly promote angiogenesis at the injury site *via* paracrine growth factors, improve blood flow, and promote the healing of diabetic foot wounds.

4.3.2 MSCs can promote keratinocytes to participate in wound epidermis formation and regulate the local microenvironment

In vitro studies have shown that MSCs can differentiate into epidermal cells and function as epidermal cells through different induction methods (165, 166). Kato et al. treated the foot wounds of diabetic rats and control rats with BM-MSCs. They found that the reduced phosphorylated focal adhesion kinase levels were restored when human keratinocytes were cultured in a BM-MSCs-conditioned medium containing high glucose. In addition, the

levels of matrix metalloproteinase-2, EGF, and insulin-like growth factor 1 were increased, suggesting that BM-MSCs could promote wound healing in diabetic foot model rats by improving keratinocyte function (167). Additionally, BM-MSCs-treated wounds promote the proliferation of keratinocytes and endothelial cells and promote the migration of macrophages, keratinocytes, and endothelial cells into the wounds of model mice, thereby promoting wound healing (168). Wu et al. used genetically diabetic db/db mice to conduct research and found that VEGF, Angiopoietin-1, and keratinocyte-specific protein keratin were higher in wounds treated with BMSCs. Furthermore, Bmscs significantly promoted the growth of keratinocytes at the wound site, stimulated the formation of new blood vessels, promoted epithelial regeneration at wound sites, and accelerated wound healing (169).

Furthermore, hUC-MSCs can specifically localize to the target ulcer tissue in a rat model of diabetic foot ulcer, promote the secretion of cytokeratin 19, stimulate the formation of keratinocytes and extracellular matrix, and promote epithelial regeneration in ulcerated tissues (170). Although numerous studies have confirmed that MSCs can differentiate into keratinocytes and endothelial cells, their engraftment effects remain controversial. It has been suggested that, under special circumstances, MSCs differentiate into keratinocytes but do not have the full set of expression markers that keratinocytes have (171). For example, Schneider et al. reported that BM-MSCs were cultured in air-exposed on dermal equivalents consisting of collagen types I and III with dermal fibroblasts; they found that MSCs possessed obvious vitality and three-dimensional epidermis-like growth patterns and possessed markers of early and mature epithelial cells without expression of E-cadherin or pan-cytokeratin (172).

Thus, an appropriate culture environment should be selected to cultivate BMCs to improve the success rate of differentiation. It should be noted that the current *in vivo* studies on MSCs observed by DFU models mainly focus on animal models, and the data volume of human models is still small.

4.3.3 MSCs promote cell migration to wound tissue through chemokine receptors-related signaling pathways

Recent studies have shown that various molecular mechanisms, including cell signaling pathways, play important roles in the pathophysiology and healing processes of diabetic foot (173–175). A protein-serine-threonine kinase (AKT) is a serine/threonine kinase that is an important signaling center for various cellular functions. PI13-dependent AKT activation further affects MSC survival, proliferation, migration, and angiogenesis; this pathway plays a core regulatory role (175). The Notch signaling pathway is a short-range communication sensor that regulates stem cell niche maintenance, such as cell differentiation, cell proliferation, and cell death during the development and renewal of adult tissues (176).

Hou et al. found that the conditioned medium of BM-MSCs accelerated the migration and proliferation of human umbilical vein endothelial cells. These processes were closely related to the AKT signaling pathway and independent of the extracellular signal-regulated kinases (ERK) signaling pathway (177). Jun et al. demonstrated that amniotic fluid-derived MSCs (AF-MSCs) promoted wound closure by increasing angiogenic factors while increasing epidermal cell regeneration, and it accelerated the proliferation and migration of dermal fibroblasts and accelerated wound healing through the transforming growth factor-beta (TGF-β)/SMAD2 and PI3K/AKT signaling pathways under hypoxic conditions (178). Liu et al. reported that SDF-1 and chemokine receptor four play important roles in regulating BM-MSCs to promote DFU healing (179). Interestingly, combined treatment with PRP and rat ADSCs promotes angiogenesis, triggers epidermal stem cell proliferation and recruitment by modulating the Notch pathway, and significantly accelerates the healing of experimentally induced diabetic wounds in rats (180). These phenomena suggest that the Notch signaling pathway may be a new potential therapeutic target for diabetic wounds (181, 182).

4.3.4 MSCs can participate in immune regulation and reduce inflammation and tissue damage

In addition to their ability to differentiate into different cell types, MSCs also play a regulatory role in inflammatory and immune responses. Many studies have shown that after cell or tissue injury, MSCs can be activated by inflammatory cytokines and control the process of tissue regeneration by releasing a series of factors that may promote the differentiation and proliferation of progenitor cells while participating in immune regulation and inhibiting inflammatory responses (67, 183, 184). (Figure 2).

4.3.4.1 MSCs can modulate immunity by suppressing pro-inflammatory T cells and inducing T regulatory cells

T helper cells 17 (Th17) and T helper cells 1 (Th1) can mediate inflammation (185). CD4+ cells, namely regulatory T cells (Treg), are a subset of specialized immunosuppressive T cells that can specifically express CD25 and CTLA-4 on the cell surface and the transcription factor FoxP3 in the nucleus, which can maintain homeostasis and immune self-tolerance (186) (187).. Li et al.

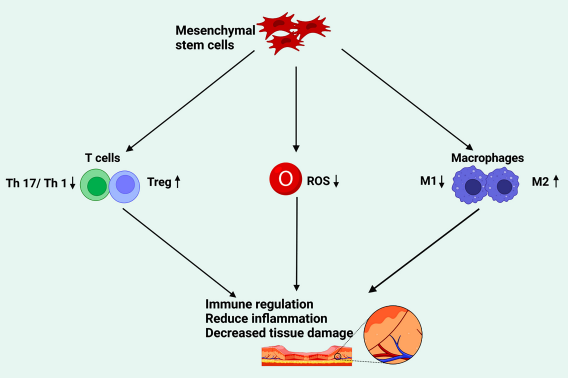

FIGURE 2
The effect of mesenchymal stem cells on tissue damage through immune regulation. Mesenchymal stem cells participate in immune regulation by inhibiting T17 and T1 cells, promoting Treg cells, downregulating ROS, and accelerating the polarization of M2, so as to reduce inflammation and repair the damage of diabetic foot. (Created in BioRender.com).

confirmed that 15 patients with diabetic foot disease received hUC-MSC transplantation under insulin treatment, after which blood glucose levels and insulin doses were decreased in all 15 patients. Four weeks after transplantation, $CD4^+CD25$ (hi) $FoxP3^+$Treg/Th17 and $CD4^+CD25$ (hi) $FoxP3^+$Treg/Th1 cell ratios increased significantly ($p < 0.01$), while Th17/Th1 cell ratios remained unchanged and VEGF serum levels peaked (188).

4.3.4.2 MSCs can play an immunomodulatory role by reducing the production of reactive oxygen species

ROS are oxygen-free radicals (189, 190). Low ROS levels are beneficial for maintaining cell proliferation, differentiation, and survival, while high ROS levels stimulate immune responses and cause oxidative damage, leading to cell damage and dysfunction (191). When tissues are damaged, phagocytes in the body phagocytose bacteria, apoptotic inflammatory cells, or cell debris to kill pathogens. However, after phagocytosis, long-lived neutrophils generate substantial ROS, causing a respiratory burst that causes tissue damage.

Some studies have suggested that antioxidant activity of MSCs may occur through cell contact or paracrine reduction of lipid peroxidation and protein oxidation (192, 193). MSCs reduce inflammation and oxidative stress in several diseases. These effects include reducing the expression of ROS-producing enzymes myeloperoxidase, inducible nitric oxide synthase, and nitrogen oxides and reducing inflammatory cytokines IL-1β, IL-4, IL-6, IL-9, tumor necrosis factor-alpha (TNF-α), and IFN-γ (194, 195). MSCs can also directly reduce ROS and myeloperoxidase in stimulated monocytes and macrophages, thereby inhibiting their pro-inflammatory phenotypes (196, 197). By enhancing the secretion and expression of stanniocalcin (STC)-1, MSCs significantly inhibited the production of mitochondrial ROS in macrophages, and inhibited nucleotide binding oligomeric domain (NOD)-like receptor pyrin domain containing 3 (NLRP3) inflammasome, Caspase-1 activation, IL-1β production, TNF-α and IL-6 transcription (197). Transplantation of PD-MSCs has also been shown to promote diabetic wound healing by reducing TNF-α, IL-6, and IL-1 pro-inflammatory cytokines and inhibiting NF-κB signal transduction (198). Li et al. showed that mesenchymal stem cell-conditioned medium could reduce the overproduction of ROS in high glucose and/or lipopolysaccharide induced keratinocytes, and reversed the downregulation of mitogen-activated protein kinase (MEK)1/2 and ERK 1/2 phosphorylation induced by high glucose and/or lipopolysaccharide, improving keratinocyte proliferation and migration in diabetes-like microenvironments (199). Raffaghello et al. found that BM-MSCs could prevent excessive or inappropriate oxidative metabolism, activate neutrophils, and inhibit their apoptosis, thereby reducing ROS production without affecting the phagocytic ability of neutrophils (200). Exosomes secreted by human ADSCs can alleviate DFU progression by preventing the senescence of EPCs and inhibiting the expression of ROS and inflammatory cytokines (201).

MSCs play an immunomodulatory role by inhibiting ROS production and enhancing mitochondrial function in macrophages and neutrophils. Therefore, in the future, the role of MSCs in anti-ROS and immune regulation in diseases should be considered to help optimize the therapeutic effect of DFU.

4.3.4.3 MSCs can exert immunomodulatory effects by reducing classically activated M1 macrophages and increasing selectively activated M2 macrophages

M1 macrophages have traditionally been associated with proinflammatory events. M1 macrophages are defined as macrophages that produce proinflammatory cytokines, which mediate resistance to pathogens, and exhibit powerful bactericidal properties, but also cause tissue destruction and inhibit angiogenesis (202, 203). The M1 macrophages are characterized by an enhanced ability to secrete cytokines such as IL-1β, TNF, IL-12, ROS, and IL-18 (204). On the contrary, M2 macrophages are thought to have anti-inflammatory and pro-regenerative effects. The molecules expressed by M2 macrophages include IL-10, Arginase1 (Arg1), resistin-like-α (also called Fizz1), Mrc1 (also called CD206), and chitinase 3-like 3 (also called Ym1) (205). These molecules may be involved in tissue remodeling, parasitic infections, immunomodulatory functions of tumors, and promote angiogenesis (206). They represent the two ends of the macrophage activation spectrum and can transform into each other in specific microenvironments.

In the first stage of ulcer healing, pro-inflammatory M1 macrophages infiltrate the ulcer to remove bacteria, dead cells, and foreign bodies from the ulcer (207). When tissue begins to repair an acute wound, the M1 macrophage population changes to an M2 phenotype, resulting in anti-inflammatory and regenerative effects (208). In chronic wounds, if proinflammatory macrophages persist with the M1 phenotype, the transformation to the M2 anti-inflammatory phenotype is impeded, which leads to impaired tissue repair (209, 210). A persistent high glucose environment *in vivo* stimulates macrophages to secrete pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and ROS, leading to a vicious cycle of persistent M1 macrophage phenotypes and a persistently higher state of inflammation in DFU (211). Therefore, it can be inferred that M2 macrophages can promote the healing of DFU. Thus, transforming M1 macrophages into adequate M2 macrophages in the wound-healing process of DFU may be an effective therapeutic idea.

Dayan et al. proposed that co-culture of human BM-MSCs and hUC-MSCs with macrophages reduced the overall macrophage/monocyte levels, including decreased pro-inflammatory M1 macrophages. In contrast, the level of alternately activated anti-inflammatory M2 macrophages was significantly increased (212). In addition, human GMSCs can induce M2 polarization of macrophages to play an immunomodulatory role, thereby enhancing wound repair (213). Yu et al. found that rat ADSCs reduce the number of M1 macrophages and increase the number of CD163 (+) M2 macrophages, delaying the progression of diabetes and its complications (214). Chen et al. used 3D nanofiber scaffolds loaded with mouse BM-MSCs to act on the wounds of diabetic mice. The ratio of alternately activated M2/classically activated M1 macrophages was significantly increased, promoting wound healing in diabetic mice (215). PGE2 secreted by hUC-MSCs rescues

endothelial cell dysfunction and improves the local microenvironment of vascular endothelial cells IL-10 and VEGF. It improves angiogenesis to promote wound healing by regulating M1-to-M2 macrophage polarization in diabetic wounds (216).

These reports on the promotion of the polarization of M1 macrophages into M2 macrophages and promotion of the healing of DFU have brought good news to patients; however, they need to be further studied.

4.3.5 MSCs/MSCs-derived exosomes can promote ischemic tissue repair and angiogenesis in the diabetic foot *via* microRNA

With the rapid development of "cell-free therapy," MSC-derived small extracellular vesicles (EVs) have become a research hotspot for treating various diseases. Exosomes are the smallest extracellular vesicles in the range of 30–150 nm in diameter, with a bilayer structure and disc-like morphology. They mediate signal transduction between adjacent cells, distant cells, and organs by delivering noncoding RNAs, proteins, and DNA (217). Chen et al. found that TNF-α, interleukin 6 (IL-6), and vascular cell adhesion molecule 1 (VCAM-1) induced heterogeneous secretion of exosomes from MSCs. Furthermore, they defined a novel pro-angiogenic miRNA by RNA sequencing, miRNA-21-5p, a novel mechanism and novel biomarker by which exosomes can promote angiogenesis and ischemia tissue repair in DFU (218). In contrast, Chen et al. found that TNF-α and IL-6 down-regulated angiogenic-related miRNA in MSCs-exo, suggesting that the angiogenesis potential of MSCs-exo decreased after TNF-α and IL6 stimulation (219). Moreover, MSCs-EVs can upregulate the expression of the VEGF gene through miRNA-210-3p and activate key pro-angiogenic proteins, such as ERK and AKT, to improve microcirculation and promote angiogenesis (220). In addition, BM-MSCs downregulate the target genes TRAF6 and IRAK1 through exosomal miR-146a, reducing the expression of NF-κB, IL-6, and MIP-2, thereby inhibiting the inflammatory response and promoting the repair of diabetic wounds (221). Finally, Yu et al. demonstrated that BM-MSCs-derived exosomes enhanced the biological function of endothelial cells through the exosomal miRNA-221-3p-mediated AKT/eNOS pathway, thereby promoting the repair of diabetic wounds (222). This suggests that MSCs-EVs can promote angiogenesis and wound healing in treating DFU, but inflammatory factors may inhibit the potential of MSCs-EVs to promote angiogenesis.

In conclusion, MSCs can accelerate the repair of diabetic foot wounds by synergistic effects, such as immunomodulation, upregulation of anti-inflammatory factors, or downregulation of pro-inflammatory factors to reduce the inflammatory response, increase blood supply to ulcers, promote granulation tissue formation, stimulate epidermal regeneration (223), and finally increase the limb salvage rate in diabetic foot patients (224, 225) (Figure 3, Table 1). However, in the context of hyperglycemia and chronic inflammation in DFU patients, AGEs lead to a decline in the survival rate of MSCs and seriously reduce the repair efficiency of MSCs. In addition, inflammatory factors may inhibit the ability of MSCs to promote vascular regeneration and repair. Therefore, good blood glucose control and inflammation control must be considered in treating DUF by MSCs (148, 219, 227). Although the current study has achieved relatively positive clinical results, optimal efficacy still needs to be explored.

4.4 MSC-related derivatives

With the continuous in-depth research and elucidation of the mechanism of action, exploring MSCs or other cell derivatives with a more clear mechanism of action for DFU treatment has become a current research hotspot. The use of these derivatives to treat DFU shows efficacy and characteristics similar to those of MSCs. MSCs or cell derivatives reported in related studies include exosomes, exosome gels, conditioned medium, growth factors, platelet lysates, and platelet-rich plasma (PRP). Among these derivatives, research on exosomes is currently hot. Li et al. suggested that AD-MSCs exosomes could significantly improve inflammation in rat DFU wounds and reduce the expression of oxidative stress-related proteins in the wound while promoting tissue regeneration, the proliferation of EPCs, angiogenesis, and growth factor expression (201).

Yang et al. proposed that the efficient delivery and enhanced exosome capacity of hUC-MSCs-derived exosomes in a Pluronic F-127 hydrogel could accelerate diabetic wound healing. Therefore, MSCs-derived exosome therapy may be a new treatment for chronic wound skin regeneration (226). Dash et al. found that autologous implantation of BM-MSCs in patients with diabetic foot accelerated the lower extremity wound healing process and significantly improved clinical symptoms (228). Furthermore, some research has found that injection of an MSC-conditioned medium promotes wound closure in diabetic mice (156, 169). Growth factors promote wound healing in patients with DFU. Transgenic *Lactobacillus merceris* secretes platelet-derived growth factor-BB, a dimeric peptide that binds to platelet-derived growth factor receptors and stimulates cell proliferation and survival. This could be a cost-effective method for patients and be used in regenerative medicine strategies to promote tissue repair (229). The combination of MSCs and PRP has been found to enhance wound healing (230). A human clinical study reported that PRP was significantly better than topical antiseptic dressings in cleaning diabetic ulcers and found healing rates of up to 86%, which significantly improved the 68% healing rate of antimicrobial ointment dressings (231). PRP contains growth factors that promote cell proliferation and matrix synthesis and could be considered a candidate treatment for the nonhealing of DFU (232). In conclusion, MSC-related derivatives are a promising new method for treating DFU; however, their exact efficacy remains to be confirmed by further studies.

4.5 MSCS treatment of diabetic foot-related clinical trials

As mentioned above, conventional treatment of DFU has been mentioned. However, conventional treatment is not always effective. For example, patients with DFU have lower limb artery lesions involving the lower leg arteries and may face amputation if

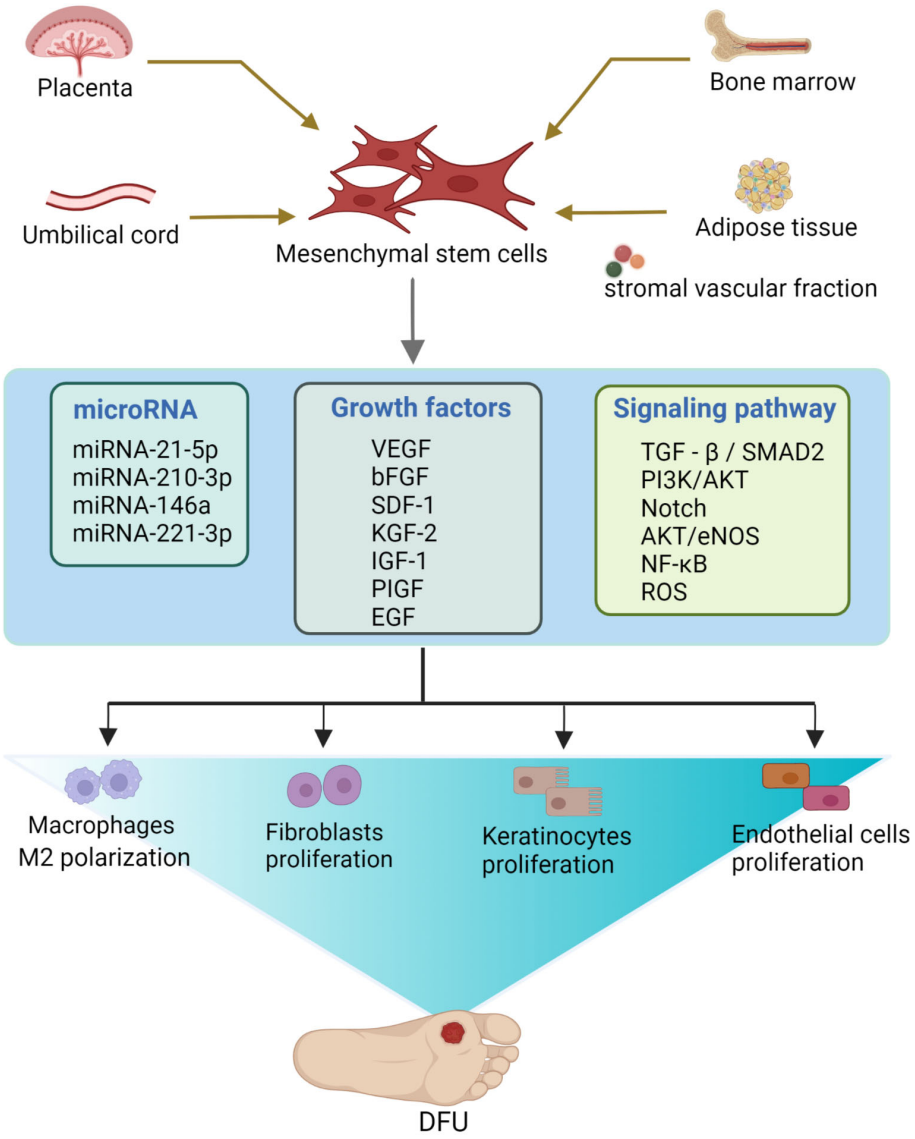

FIGURE 3
The therapeutic mechanism of mesenchymal stem cells (MSCs) for diabetic foot. MSCs are mainly derived from bone marrow, umbilical cord, adipose tissue, placenta, and other parts. During the treatment of diabetic foot, mesenchymal stem cells repair diabetic foot mainly through the proliferation of fibroblasts, keratinocytes and endothelial cells, as well as angiogenesis and polarization of macrophage M2. In this process, paracrine growth factors, related signaling pathways and microRNAs are involved. (Created in BioRender.com).

TABLE 1 Potential role of MSCs in the healing of diabetic foot.

Stem cell type	Method of administration	Changes at the molecular level	Changes Histology or clinical manifestation	References
BM-MSCs	Topical application	VEGF and NGF ↑	Promote angiogenesis and accelerate wound healing	(161)
BM-MSCs	Intramuscular injection	VEGF ↑	Promote granulation tissue formation and re-epithelialization, enhance angiogenesis, and accelerates wound closure	(144)
Mouse liver-derived MSCs	Topical application	VEGF, EGF, TGFβ-1 and SDF-1α ↑	Improve neovascularization and promote wound contraction	(162)
BM-MSCs	Topical application	TGF-β, KGF, EGFand VEGF ↑, collagen Type I-V ↑	Increase wound breaking strength (WBS) of fascial wounds and accelerate repair of damaged tissue	(163)

(Continued)

TABLE 1 Continued

Stem cell type	Method of administration	Changes at the molecular level	Changes Histology or clinical manifestation	References
BM-MSCs	Topical cell injection	pFAK, MMP2, EGF and IGF-1 ↑	Enhance epithelialization improve delayed wound healing	(167)
BM-MSCs	Subcutaneous injection	EGF, KGF, IGF-1, VEGF-α and EPO ↑	Enhance new blood vessel formation	(168)
BM-MSCs	Topical cell injection	Ang-1 and VEGF-α ↑	Enhance epithelialization and increase angiogenesis	(169)
hUC-MSCs	Left femoral artery injection	Collagen I and III ↑, Cytokeratin 19 ↑	Enhance epithelization and increase granulation tissue	(170)
BM-MSCs	Topical application	VEGF ↑	Accelerate angiogenesis, restore blood supply to wounds and promote wound healing	(161)
ADSCs	Topical cell injection	ß Integrin, Notch, DLL4, Jag1, Hes1 and Hey1 ↑	Enhance angiogenesis	(180)
hUC-MSCs	Intramuscular injection	VEGF ↑, $CD4^+CD25(hi)FoxP3^+$Treg/Th17 and $CD4^+CD25(hi)FoxP3^+$Treg/Th1 ↑	Improve symptoms such as numbness, pain, coldness and intermittent claudication	(188)
PD-MSCs	Subcutaneous injection	TNF-α, IL-6, IL-1 and NF-κB↓	Promote dermal wound healing in a diabetic Goto-Kakizaki rat model	(198)
ADSCs-exos	Topical cell injection	IL-1β, IL-6, TNF-α and ROS ↓	Promote angiogenesis, increase granulation tissue formation and significantly reduce the ulcer area of wounds	(201)
BM-MSCs	Scaffold's implantation	Formation of M1 macrophages ↓, IL-6 and TNF-α↓, formation of M2 macrophages ↑, IL-4 and IL-10 ↑	Enhance granulation tissue formation, promote angiogenesis and accelerate collagen deposition.	(215)
hUC-MSCs	Subcutaneous injection	M2 macrophages ↑	Increase angiogenesis and accelerate wound healing	(216)
hUC-MSCs-exos	Intramuscular injection	VEGFR, AKT and MAPK ↑	Promote ischemic tissue repair and angiogenesis	(218)
BM-MSCs	Intradermal injection	miRNA-146a ↑, IRAK1, TRAF6, NF-κB, IL-6 and MIP-2↓	Reduce inflammatory response and enhance wound repair	(221)
BM-MSCs-exos	Multipoint injection	miRNA-211-3p, p-AKT and p-eNOS ↑	Increase angiogenesis and accelerate wound regeneration	(222)
hUC-MSCs-exos	Topical application	VEGF and TGFβ-1↑	Accelerate wound closure rate	(226)

MSCs, mesenchymal stem cells; BM-MSCs, bone marrow mesenchymal stem cells; hUC-MSCs, human umbilical cord mesenchymal stem cells; ADSCs, adipose tissue-derived mesenchymal stem cells; ADSCs-exos, exosomes from adipose tissue-derived mesenchymal stem cells; hUC-MSCs-exos, exosomes from human umbilical cord mesenchymal stem cells; BM-MSCs-exos, exosomes from bone marrow mesenchymal stem cells; VEGF, vas-cular endothelial growth factor; NGF, nerve growth factor; EGF, epidermal growth factor; TGFβ-1:transforming growth factor beta-1; SDF-1α,stromal cell-derived factor-1α; TGF-β, transforming growth factor beta; KGF, ker-atinocyte growth factor; pFAK, phosphorylated focal adhesion kinase; MMP2, matrix met-alloproteinase-2; IGF-1, insulin-like growth factor 1; VEGF-α, vas-cular endothelial growth factor-α; EPO, Erythropoietin; Ang-1, Angiopoietin-1; DLL4, Delta-like canonical Notch ligand 4; Hes1, Hairy Enhancer of Split-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; TNF-α, tumor necrosis factor alpha; ROS, Reactive oxygen species; IL-4, Interleukin-4; IL-10, Interleukin-10; VEGFR, vascular endothelial growth factor receptor; MAPK, mitogen-activated protein kinase; IRAK1, IL-1 receptor-associated kinase 1; TRAF6, TNF receptor-associated factor 6; NF-κB, nuclear factor-κB; MIP-2, macrophage inflammatory protein-2; p-eNOS, phosphorylation-endothelial nitric oxide synthase.
"↑" represents up-regulation/increased expression, "↓" represents down-regulation/decreased expression.

serious vascular diseases occur in the affected limb. MSCs promote tissue repair and regeneration by increasing extracellular matrix, repairing cell activity, promoting angiogenesis at ulcer sites, secreting growth factors, and forming new keratinocytes (233, 234). To date, MSCs have become a hot spot for DFU, and more clinical research has been widely carried out.

4.5.1 Autologous stem cells

Transplanting autologous stem cells into DFU enhances ulcer healing and reduces amputation rates. They include BM-BMSCs, peripheral blood mononuclear cells (PBMNCs), BMMNCs, ADSCs, and an adipose tissue-derived stromal vascular fraction (SVF).

Yuyama et al. reported autologous BMMNCs transplantation for angiogenesis in patients with limb ischemia (235). A significant proportion of DFU patients suffer from vascular disease. Claeys et al. proposed that percutaneous partial pressure of oxygen could be used as a predictive parameter of DFU associated with vascular disease (236). Kirana et al. included 22 patients with DFU treated with autologous BMMNCs, which resulted in improved wound healing and transcutaneous pressure of oximetry in the affected limb (237). Xu et al. used recombinant human granulocyte colony-stimulating factor (G-CSF) 5–10 μg/kg/day for the proliferation of BM-MSCs in DFU patients for 4–5 consecutive days to promote their release into peripheral blood and then took peripheral blood

MSCs and injected them around or at the bottom of ulcers. After 4 weeks of follow-up, the ulcers gradually healed. Digital subtraction angiography (DSA) detection revealed that abundant collateral circulation was established around the lesions of the DFU (238). Huang et al. also used G-CSF to mobilize PBMNCs in treating patients with DFU accompanied by critical limb ischemia (CLI) and achieved significant clinical effect (239). This provides a reference for the diversity of treatment modalities. G-CSF is a growth factor that stimulates bone marrow and mobilizes EPCs, thereby increasing their numbers to cure DFU (240). Lu et al. conducted clinical trials, in which patients with type 2 diabetic feet were given BM-MSCs, BMMNCs, or normal saline (NS). The results illustrated that in promoting the healing of patients with DFU, the BM-MSCs treatment group could be more effective than the BMMNCs treatment group. They also found that the BMMSCs of diabetic patients secret more VEGF, FGF-2, and angiopoietin-1 than BMMNCS under normoxic and hypoxic conditions. Therefore, they believed that BMMSCS is better than BMMNCs in the local vascular generation (241). Procházka et al. performed clinical trials, dividing 96 CLI and DFU patients into two groups. The first group of patients received local treatment of autologous BM-MSCs, while the second group of patients received the standard treatment of medical care. The results suggested that BM-MSCs local treatment can save 79% of the limbs of CLI and DFU patients. Among the 21% of amputation, lymphocytes and platelet reduction may be potentially pathogenic. The primary amputation rate of the control group is 44%. Experiments confirmed that BM-MSCs can greatly improve the prognosis of DFU and reduce the amputation rate. This study found that the low platelet count and the low VEGF level are related to poor healing in bone marrow concentrate. Low platelet and $CD34^+$ cell concentrations were present in most unhealed patients, but moderate platelet and $CD34^+$ cell concentrations were present in most healed patients rather than either of the two extremes. However, for patients with low platelet counts, if they are accompanied by VEGF with high local concentration, the wound healing was satisfactory. Most amputations that are still not saved after using autologous BM-MSCS for treatment are secondary infections. These treatments are proposed to emphasize the importance of debridement and anti-infection (242). Scatena et al. treated 38 patients with DFU and no-option critical limb ischemia (NO-CLI) with intramuscular and perifocal injections of PBMNCs. Patients treated with PBMNCs had a significantly lower rate of amputation than those (38 patients) treated with standard care under the International Working Group on the Diabetic Foot (IWGDF) guidelines (243), and 86.6% of patients in the PBMNCs group recovered during the 2-year follow-up, compared with only one patient in the control group. The results showed that PBMNCs significantly reduced the amputation rate of DFU with NO-CLI (244). In a recent meta-analysis of autologous MSCs in treating DFU, it was also reported that BMMNCs were more effective in healing foot ulcers in DFU than repeated percutaneous transluminal angioplasty (245, 246).

Adipose tissue-derived SVF is a heterogeneous cell fraction. They include mesenchymal progenitor/stem cells, T cells, pericytes, endothelial cells, and macrophages (247). It has also been specifically used to treat DFU. Han et al. were the first to use uncultured processed lipoaspirate cell autografts to treat diabetic ulcers to stimulate the diabetic fibroblasts of activity and obtained a 100% cure rate of DFU (248). Carstens et al. used adipose-derived SVF in treating 10 patients with non-reconstructive peripheral vascular disease in DFU and achieved good results. They also followed the patients for 6 years and found five patients still showed a consistent clinical benefit (249, 250). Subsequent studies have shown that adipose tissue-derived SVF can induce neovascularization in ischemic conditions in treating chronic DFU, increased transcutaneous partial oxygen pressure, and cutaneous microvascular blood flow (251, 252).

Among autologous MSCs, BM-MSCs and PBMNCs are the most commonly used cell types in DFU studies. Mobilized PBMNCs are preferred over BM-MSCs because of the ease of collection and the avoidance of pain and anesthesia associated with bone marrow biopsy. The ease of execution and good clinical efficacy of adipose-derived SVF brings a new choice for treating DFU. However, further studies are needed to explore the exact use of adipose-derived SVF.

4.5.2 Allogeneic stem cells

Allogeneic stem cells are isolated from an individual of the same species rather than from the recipient, including pluripotent mesenchymal stromal cells from allogeneic sources such as the placenta, umbilical cord, amniotic membrane (148).

Qin et al. included a group of Fontaine II-V DFU patients (28 patients, 34 limbs) with varying degrees of lower extremity arterial disease treated with intravascular infusion and peri-ulcerative injection of the hUC-MSCs after angioplasty. After 3 months of follow-up, the results showed increased neovascularization at the ulcer, ulcer healing, skin temperature, transcutaneous oxygen tension, the ankle-brachial pressure index, and claudication distance were improved noticeably (253). Their study suggests that hUC-MSCs transplantation after angioplasty is a potentially safe and effective clinical treatment for severe DFU. Moon et al. conducted clinical trials, incorporated 59 patients with diabetic foot ulcers, and randomly distributed them to the hydrogel-based allogeneic ADSCs sheets group (n = 30) or the control group of polyurethane film treatment (n = 29). They observed the closure of wounds in the treatment group and control group at weeks 8 and 12, and the median time for the treatment group and the control group was 28.5 days and 63.0 days, respectively. In the 2-year follow-up study, two subjects had a recurrence 6 months after the stem cell therapy ulcer trial, which was different from the site at the beginning of the previous trial. The recurrence was at the toe tip and the plantar foot, susceptible to stress. Later recovery through therapeutic intervention (254). Therefore, in general, they achieved satisfactory results. In addition, there were no serious adverse events related to the treatment of hydrogel-based allogeneic ASC sheets. Therefore, it is proved that hydrogel-based allogeneic ADSCs sheets may be effective and safe for treating DFU (254). Rodríguez et al. launched clinical trials, allowing 28 patients with DFU patients to accept allogeneic BM-MSCs derivatives (n = 12), BM-MSCs (n = 6), or conventional treatment (PolyMem® dress, Ferris, Fort Worth, TX, USA) (n = 10). They conducted a macro assessment of the wound healing process until the ulcers were

closed entirely. As a result, no adverse events were reported. Compared with patients receiving conventional treatment, the wound closure rate of DFU patients treated with allogeneic BM-MSCs derivatives or allogeneic BM-MSCs was higher (255). Uzun et al. reported a study that divided 20 patients with DFU accompanied by chronic ulcers into two groups. Patients in the standard group (10 cases) received standard treatment with sterilization, debridement, and dressing coverage. In the study group (10 cases), in addition to routine disinfection and debridement, allogeneic ADSCs were injected into the dermo-epidermal junction and the entire wound surface using intralesional. The results showed that nine patients in the study group had wound healing, while eight patients in the control group had wound healing. The wound healing time of the study group was 31.0 ± 10.7 days, and the wound healing time of the control group was 54.8 ± 15.0 days. In the end, one patient in the study group and two in the control group had their limbs amputated. Allogenic ADSCs were safe for local injection of DFU ulcers with no significant adverse events (256). Their study showed that allogenic ADSCs have a positive therapeutic effect on chronic ulcers of DFU and are superior to standard conventional therapy.

Through the above studies, we can conclude that MSCS and their derivatives and stents delivering MSCs have achieved optimistic clinical effects in the treatment of DFU. However, the role of post-healing patient care, footwear selection, and health education should be considered in preventing the recurrence of ulcers. Furthermore, most of the above clinical trials have a limitation: the sample volume was relatively small. Therefore, multi-center random clinical trial research is recommended to expand the sample volume effectively to obtain more accurate evidence (Table 2).

4.6 Potential disadvantages of MSCs in diabetic foot treatment

Following previous studies in humans and animals, MSCs have achieved encouraging efficacy in treating DFU (241, 254, 257). However, with an increase in research, from the results of recent clinical studies, common side effects of MSCs in DFU are diarrhea, fever, increased serum creatinine level, urticaria, nausea, and vomiting (258, 259). After the passage of stem cells for many times *in vitro*, the multidirectional differentiation potential and paracrine ability may also be reduced, leading to the decline of clinical effect (234). Embryonic stem cells have strong proliferative ability and low differentiation maturity. The introduction of these cells may cause immune rejection and stimulate tumor formation. Therefore, embryonic stem cells should be avoided from DFU treatment as much as possible (260–262). In addition, it has been reported that increasing the number of stem cells applied locally to improve repair efficiency may also increase tumorigenicity (263). Although there may be some side effects of stem cell therapy for diabetic foot, overall, in animal experiments and human studies, BM-MSCs transplantation has achieved positive results in DFU treatment. MSCs transplantation may be a new method that can be used to treat diabetic foot, but the precise utilization of stem cells to control the local microenvironment of DFU to maximize the healing effect is still unknown.

5 Conclusions and prospects

These stem cells, which have clear research results, have a largely positive effect on the treatment of DFU while also having the advantage of being used in combination with other treatments to better exert their effects in the treatment of refractory DFU (234, 264). Although stem cells derived from synovium, urine, amniotic fluid, liver, lung, and gingiva have only been reported in sporadic experiments in the treatment of diabetic foot, these stem cells may still be a potential choice for the treatment of diabetic foot in the future. Researchers can explore the characteristics of MSCs derived from different tissues based on *in vivo* and *in vitro* studies. They are expected to clarify their advantages and disadvantages and elucidate the full impact of their therapeutic effects in future studies.

As the first MSCs to be studied for the treatment of DFU, BM-MSCs are relatively convenient to isolate and extract and have achieved good therapeutic effects in many clinical practice applications. Their safety has also been affirmed. After repeated studies, BM-MSCs may be the best choice for treating diabetic foot (265). However, MSCs also have certain shortcomings; for example, the differentiation potential and proliferation ability of BM-MSCs decrease with age (266), and the repair ability is negatively correlated with the number of cell passages (267). This requires us to standardize stem cell therapy for the diabetic foot in the future to maximize its advantages and minimize its disadvantages. So far, different clinical studies have been launched to evaluate the safety and efficacy of MSCs on DFU (NCT03370874, NCT04464213, NCT05610865, NCT04104451, ChiCTR2000036933). It is crucial to standardize the therapeutic efficacy of MSCs products before initiating clinical trials, and this need is driving efforts to develop improved *in vitro* efficacy assays.

Different MSCs can self-renewal and multi-directional differentiation, which brings hope for the treatment of many intractable diseases, such as Parkinson's disease, myocardial infarction, and bone defects. It is also expected to obtain gratifying clinical effects in basic and clinical application research on treating diabetic foot. Although stem cell therapy's efficacy and safety in treating diabetic foot have been preliminarily confirmed, further research is needed regarding the treatment mechanisms, efficacy judgments, individual choices of stem cell source, and promotion norms.

In conclusion, DFU treatment with MSCs is a potential, relatively safe, and effective treatment method, among which BM-MSCs may be an ideal choice. However, in the initial treatment plan of the treatment of DFU, it is necessary to select a certain stem cell to specify the specific treatment method according to the characteristics of each stem cell, such as local applications, meridian transmission, local injection, and intravenous application. This is a key step in obtaining the ideal effect, which is still a considerable challenge facing researchers.

TABLE 2 MSCs treatment of diabetic foot related clinical trials.

Stem cell type	Method	Participants	Outcomes	Method of administration	References
BM-MSCs	Patients randomized to BM-MSCs along with standard wound dressing or standard wound dressing	24	Accelerate healing process and significantly improve clinical parameters in the treatment group	Topical application	(228)
BMMNCs	Patients randomized to BMMNCs or PBMNCs	47	Significantly improve ABI, transcutaneous oxygen pressure, rest pain and pain-free walking time in the BMMNCs group	Intramuscular injection	(235)
BMMNCs	Patients randomized to BMMNCs or expanded bone marrow cells enriched in CD90+ cells	22	Both kinds of cell transplantation are safe and feasible; Improve microcirculation and complete wound healing	Intramuscular injection or intraarterial infusion	(237)
PBMNCs	Patients randomized to PBMNCs mobilized by G-CSF or Filgrastim	127	Promote the establishment of collateral circulation and improve the ischemic area of the patients	Intramuscular injection	(238)
PBMNCs	Patients randomized to a control group or PBMNCs mobilized by G-CSF	28	Significantly improve angiographic scores in the PBMNCs group	Intramuscular injection	(239)
BM-MSCs	Patients randomized to BM-MSCs, BMMNCs, or NS	41	Promote healing of foot ulcers in the BM-MSCs treatment group	Intramuscular injection	(241)
BM-MSCs	Patients randomized to BM-MSCs or standard medical care	96	Improve the prognosis of DFU and reduce amputation rate in the treatment group	Topical application	(242)
PBMNCs	Patients randomized to PBMNCs or a control group	76	Reduce the amputation rate and improve survival and wound healing in the PBMNCs group	Intramuscular injection	(244)
BMMNCs	Patients randomized to BMMNCs with percutaneous transluminal angioplasty or a control group	54	Improve amputation free survival rate and promote ulcers healing	Intramuscular injection	(246)
Adipose-derived SVF	Single arm study and followed the patients for six years	10	Improve tissue perfusion, neovascularization and ABI	Intramuscular injection	(249, 250)
Adipose-derived SVF	Single arm study	10	Increase transcutaneous partial oxygen pressure and cutaneous microvascular blood flow	Subcutaneous injection	(251)
Adipose-derived SVF	Single arm study (Phase I clinical trial)	63	At 12 months, 50 subjects had 100% DFU healing and 4 subjects had ≥85% healing. Promote vascular repair and/or angiogenesis with a good safety	Topical cell injection	(252)
hUC-MSCs	Patients randomized to hUC-MSCs or a control group	53	Increase neovessels and ulcer completely or gradually heal in the hUC-MSCs group	Endovascular infusion and topical cell injection	(253)
ADSCs	Patients randomized to ADSCs or a control group treated with polyurethane film	59	The wound closure rate was increased and the median wound closure time was shortened in the treatment group	Topical application	(254)
BM-MSCs	Patients randomized to BM-MSCs derivatives, BM-MSCs or conventional treatment (PolyMem dress). (Phase 1/2 clinical trial)	28	The wound closure rate was increased in the treatment group	Intradermal injection	(255)
ADSCs	Patients randomized to ADSCs or a standard group (Phase I/2 safety study)	20	Have a positive therapeutic effect on chronic ulcers of DFU and are superior to conventional standard therapy	Intradermal injection	(256)

ADSCs, adipose tissue-derived mesenchymal stem cells; BM-MSCs, bone marrow mesenchymal stem cells; DFU, diabetic foot ulcer; BMMNCs, bone marrow mononuclear cells; NS, normal saline; ABI, ankle-brachial index; G-CSF, granulocyte colony-stimulating factor; SVF, stromal vascular fraction.

Author contributions

XY, PL and ZL conceived and drafted the manuscript. XY, PL, ZL and ZZ proofread the manuscript and made revisions. XY, PL and ZZ collected the references. ZZ directed the overall design of the manuscript. All authors read and approved the submitted version. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by the National Natural Science Foundation of China (82073539); the Project of Sichuan Provincial Department of Science and Technology (2022JDRC0133); the Foundation of The First Affiliated Hospital of Chengdu Medical College (CYFY-GQ35); the Foundation of Chengdu Medical College (CYZYB21-12); and Special Research Project of Sichuan Medical Association (Hengrui) Scientific Research Fund (2021HR56).

Acknowledgments

The authors would like to thank Mr. Jinhao Zhang for his help with the literature search. Figures were created with BioRender.com.

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

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 31 March 2023
DOI 10.3389/fendo.2023.1144806

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 15 January 2023
ACCEPTED 16 March 2023
PUBLISHED 31 March 2023

CITATION
Gong H, Ren Y, Li Z, Zha P, Bista R, Li Y, Chen D, Gao Y, Chen L, Ran X and Wang C (2023) Clinical characteristics and risk factors of lower extremity amputation in the diabetic inpatients with foot ulcers. *Front. Endocrinol.* 14:1144806. doi: 10.3389/fendo.2023.1144806

Clinical characteristics and risk factors of lower extremity amputation in the diabetic inpatients with foot ulcers

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Objectives: To analyze clinical characteristics of the diabetic inpatients with foot ulcers and explore the risk factors of lower extremity amputation (LEA) in West China Hospital of Sichuan University.

Methods: A retrospective analysis was performed based on the clinical data of the patients with diabetic foot ulcer (DFU) hospitalized in West China Hospital of Sichuan University from January 1, 2012 to December 31, 2020. The DFU patients were divided into three groups: non-amputation, minor amputation, and major amputation groups. The ordinal logistic regression analysis was used to identify the risk factors for LEA.

Results: 992 diabetic patients (622 males and 370 females) with DFU were hospitalized in the Diabetic Foot Care Center of Sichuan University. Among them, 72 (7.3%) (55 minor amputations and 17 major amputations) cases experienced amputation, and 21(2.1%) refused amputation. Excluding the patients who refused amputation, the mean age and duration of diabetes of and HbA1c the 971 patients with DFU, were 65.1 ± 12.3 years old, 11.1 ± 7.6 years, and 8.6 ± 2.3% respectively. The patients in the major amputation group were older and had longer course of diabetes for a longer period of time than those in the non-amputation and minor amputation groups. Compared with the non-amputation patients (55.1%), more patients with amputation (minor amputation (63.5%) and major amputation (88.2%)) suffered from peripheral arterial disease (P=0.019). The amputated patients had statistically lower hemoglobin, serum albumin and ankle brachial index (ABI), but higher white blood cell, platelet counts, fibrinogen and C-reactive protein levels. The patients with amputation had a higher incidence of osteomyelitis (P = 0.006), foot gangrene (P < 0.001), and a history of prior amputations (P < 0.001) than those without amputation. Furthermore, a history of prior amputation (odds ratio 10.194; 95% *CI*, 2.646-39.279; P=0.001), foot gangrene (odds ratio 6.466; 95% *CI*, 1.576-26.539;

P=0.010) and ABI (odds ratio 0.791; 95% *CI*, 0.639-0.980; *P* = 0.032) were significantly associated with LEAs.

Conclusions: The DFU inpatients with amputation were older with long duration of diabetes, poorly glycemic control, malnutrition, PAD, severe foot ulcers with infection. A history of prior amputation, foot gangrene and a low ABI level were the independent predictors of LEA. Multidisciplinary intervention for DFU is essential to avoid amputation of the diabetic patients with foot ulcer.

KEYWORDS

diabetic foot ulcer, lower extremity amputation, foot gangrene, minor amputation, prior amputation, risk factor

1 Introduction

Diabetic foot ulcer (DFU), a severe and devastating complication of diabetes mellitus, typically presented as ulcers, infection, or destruction of tissues of the foot (1). The global diabetic foot ulcer prevalence of DFU was about 6.3% (2). DFU has always been the leading cause of non-traumatic lower extremity amputation (LEA) in the world. The rate of LEA in the diabetes was were more than five times higher than those without diabetes (3). The LEA rates were quite different in the different countries. A study in China indicated that the overall LEA rate among the DFU patients was about 19.03%, with major and minor amputation rates of 2.14% and 16.88%, respectively (4). Between 2001 and 2010, the LEA rate of the hospitalized patients with DFU in the United States was approximately 16.5% (34.8% for major and 61.2% for minor amputations) (5). In Africa, about 15% of the DFU patients underwent major amputation (6). In France, a prospective study of 347 patients with the new-onset DFU from 2001 to 2003 showed that the rates of major and minor amputation at one year were 10% and 19%, respectively (7). Furthermore, disability after LEA had a negative impact on the quality of life of the DFU patients.

An investigation revealed that the patients who had experienced diabetic foot–related complications were 79% more likely to rank LEA as their greatest fear when compared with death (8). Therefore, correctly identifying risk factors and strengthening risk prevention and control, were very important for the diabetic patients. Peripheral arterial disease (PAD), osteomyelitis, gangrene, increased inflammatory biomarkers, and low hemoglobin (Hb) levels were considered as the risk factors of LEA (9, 10). However, the risk factors for LEA of the diabetic patients in different studies were not completely consistent. Therefore, we collected clinical information of the diabetic patients with foot ulcers admitted in the Diabetic Foot Care Center of West China Hospital during Jan 1,2012 and Dec 31,2020 to analyze the clinical characteristics of the DFU inpatients with LEA (major and minor amputations) and explore the potential risk factors of LEA.

2 Patients and methods

2.1 Research objects

This is a retrospective study. The clinical data of all consecutive patients who were admitted to the Diabetic Foot Care Center in West China Hospital of Sichuan University between Jan 1, 2012 and Dec 31, 2020, were collected. The study has been approved by the Institutional Review Board Committee of West China Hospital of Sichuan University Hospital (No.2012-119). The diabetic patients who had foot ulcers met the diagnostic criteria for diabetic foot (Wagner grade 1 to 5) according to International Working Group on Diabetic Foot (IWGDF) guidelines were included in the study (11). The diabetic patients with lower limb ulcers above the ankle joint, hand ulcers, gouty ulcers and cancerous ulcers were excluded. In addition, the foot ulcers were caused by long-term use of glucocorticoids and other non-diabetic related were also excluded. Major and minor amputations referred to amputation above and below the ankle, respectively (12).

2.2 Data collection and processing

Electronic medical records of all patients were reviewed. All data were collected from hospital information system. The clinical information of the patients with DFU consisted of age, sex, course of diabetes, body mass index (BMI), diabetic medication history, smoking and drinking history, previous foot ulcer and amputation history, diabetic chronic complications, comorbidities and physical examinations. The severity of the foot ulcers was classified on the basis of the Wagner grading system.

Baseline laboratory data – including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), blood routine (Hb, platelet (PLT), white blood cell (WBC) count, neutrophil granulocyte percentage (NEUT)), coagulation routine, liver function, serum lipid profiles, serum uric acid (UA), serum creatinine, estimated glomerular filtration rate (eGFR) and serum C-reactive protein (CRP) were collected. The ankle brachial index

(ABI), the ratio of the systolic pressure measured at the ankle to that measured at the upper arm, were recorded.

2.3 Definitions of diabetic chronic complications and comorbidities

Diabetic retinopathy was diagnosed by the optometrist or through ophthalmological reports. The diagnosis of PAD was confirmed based on ABI ≤ 0.9 and/or results of doppler ultrasound of lower extremities. Diabetic peripheral neuropathy (DPN) was diagnosed based on neuropathic symptoms (such as numbness, tingling, or burning feeling, muscle weakness, etc.) and physical examination (pinprick, temperature sensation, vibration perception, proprioception, 10-g monofilament, and ankle reflexes) (13, 14). Cardiovascular autonomic neuropathy (CAN) was determined by resting tachycardia (>100 bpm), orthostatic hypotension (a fall in systolic blood pressure >20 mmHg and/or diastolic pressure >10mmHg within 3 minutes of standing) in the absence of an appropriate heart rate response (15). Diagnosis of gastrointestinal autonomic neuropathy should be reserved for patients with gastrointestinal symptoms (e.g. gastroparesis, constipation, diarrhea) and normal gastrointestinal examination (16). The clinical diagnosis of bladder autonomic neuropathy was based on the presence of lower urinary tract symptoms (e.g. dysuria, frequency, urgency, nocturia, recurrent cystitis, as well as stress and urgency urinary incontinence) with a bladder color doppler ultrasound for residual urine, and urological conditions such as benign prostatic hypertrophy in men or gynecological disorders in women must be ruled out by appropriate testing (14). Chronic kidney disease (CKD) was classified into five stages based on the eGFR (G1: eGFR ≥ 90 mL/min per 1.73 m^2, G2: 60 to 89 mL/min per 1.73 m^2, G3: 30 to 59 mL/min, G4: 15 to 29 mL/min per 1.73 m^2, G5: < 15 mL/min per 1.73 m^2) (17). Coronary heart disease was defined as myocardial infarction, angina, percutaneous coronary intervention or bypass surgery. Diagnosis of osteomyelitis was usually based on imaging(foot X-ray and foot MRI) and probe-to-bone test, and bone biopsy or microbial cultures can be used if necessary (18).

2.4 Statistical analysis

Statistical analysis was performed using IBM SPSS 26.0 software for Windows (IBM Corp., 2019). Continuous variables were reported as mean ± standard deviation or median (interquartile range). Differences among three groups were assessed using one-way ANOVA with Bonferroni post-test or Kruskal–Wallis test when inhomogeneity of variance existed. Categorical variables were expressed as frequencies with percentage (%) and compared with the chi-squared test or Fisher's exact test. Multivariate stepwise ordinal logistic regression was used to identify potential predictors for LEA. Validity of the ordinal logistic regression model was assessed with the test of parallel lines, and significance was confirmed by −2 log likelihood. For each of the candidate predictors, the odds ratio (*OR*) for the likelihood of amputation was calculated. The ABI was adjusted by multiplying by 10 so as to fit the clinical convention when the odds ratio was calculated and interpreted. For all tests, statistical significance was set at $P < 0.05$.

3 Results

3.1 Baseline characteristics

992 diabetic patients (622 males and 370 females) with DFU were admitted in the Diabetic Foot Care Center of West China Hospital during 2012 and 2020. Among them, 72 cases were amputated and 21 refused amputations. Excluding the DFU patients who refused amputation, 971 patients with DFU were analyzed in the study. Of the 72 patients with LEA, 55 cases (76.4%) received minor amputation and 17(23.6%) experienced major amputation, respectively. The mean age of the DFU patients was 65.1 ± 12.3 years old and the mean course of diabetes was 11.1 ± 7.6 years. The patients with major amputation were older and had a longer duration of diabetes than those with non-amputation and minor amputation. Only two of the patients with major amputations were female, and nearly two-thirds of the amputees were men. Approximately half of the non-amputated and minor-amputated patients smoked previously or currently, while in the major groups, the percentage rose to 76.5% (Table 1).

PAD was more frequent in the patients with minor (63.5%) and major (88.2%) amputations than those without amputation (55.3%) ($P = 0.014$). More than 95% of the DFU patients suffered from DPN. There was no statistically difference in the incidence of coronary heart disease, hypertension, diabetic retinopathy, DPN, CKD, hyperlipidemia and hyperuricemia among the three groups (Table 2).

Nearly half of the foot ulcers belonged to the neuro-ischemic foot ulcers. The first (23.5%) and fifth toes (13.3%) were the main sites of the foot ulcers, followed by heel (12.3%) and dorsum (11.6%) of feet. The foot ulcer size in the minor amputated patients (10.0(3.0-32.4) cm^2) and the major amputated patients (10.3(3.3-30.0) cm^2) were significantly larger than that in the non-amputated patients (4.0(1.3-12.0) cm^2, P=0.005). Approximately two thirds of the amputees had foot or toe gangrene. 78(8.0%) of the amputated patients had a history of previous amputations. Osteomyelitis ($P = 0.006$), foot gangrene ($P < 0.001$) and a history of previous amputations ($P < 0.001$) were more common in the patients with amputation than those without amputation. The proportion of patients with Wagner grade 4 and grade 5 foot ulcers in the non-amputation, and minor amputation and major amputation groups were 29.3%, 70.9% and 82.4%, respectively. No DFU patient with Wagner grade 1 and grade 2 was amputated during hospitalization (Table 3).

3.2 Laboratory tests

The mean HbA1c and FBG levels of the patients with DFU were 8.6 ± 2.3% and 9.0 ± 4.0mmol/L, respectively. The mean Hb, serum albumin and total cholesterol (TC) levels were 113 ± 22.7g/L, 36.2 ±

6.2g/L, and 3.5 ± 1.4 mmol/L, respectively. Compared with the non-amputated patients, the Hb ($P = 0.004$), serum albumin ($P < 0.001$), TC ($P = 0.016$) and UA(P=0.001) levels in the amputated patients were statistically lower. Compared with the non-amputated patients, the amputated patients had higher levels of PLT, FIB, WBC counts, NEUT, eGFR and serum CRP, which were the highest in the major-amputated patients. Compared with patients with the non-amputation (0.97 ± 0.28) and minor amputation (0.85 ± 0.33), the patients with major amputation had lower ABI levels (0.76 ± 0.31). In additon, ABI values of 13 cases in the minor amputation group were normal (0.9-1.3). All of the minor amputated patients with normal ABI had osteomyelitis or gangrene, and the sizes of the foot ulcers in more than half of them were larger than 6cm^2 (Table 1).

TABLE 1 Baseline demographic and laboratory data among the non-amputation, minor amputation and major amputation groups.

Factor	Non-amputation (N=899)	Minor Amputation (N=55)	Major Amputation (N=17)	*P* value
Demographics				
Age, yr	65.1 ± 12.3	62.9 ± 12.4	69.4 ± 9.9	0.146
Sex				0.086
Male	563	33	15	
Female	336	22	2	
BMI, kg/m^2	23.3 ± 3.4†(n=780)	23.2 ± 3.3†(n=45)	23.3 ± 3.4	0.118
Smoking (current or ever)	462	27	13	0.113
Drinking (current or ever)	346	18	10	0.155
Hospital stays (day)	30(1-244)	57(8-251)	47(18-114)	<0.001
Diabetes-related characteristics				
Duration of diabetes, yr	11.1 ± 7.6	10.9 ± 7.3	8.5 ± 4.7	0.416*
ABI	0.97 ± 0.28†(n=539)	0.85 ± 0.33†(n=23)	0.76 ± 0.31†(n=9)	0.012
Ulcer area, cm^2	4.0(1.3-12.0)†(n=710)	10.0(3.0-32.4)†(n=45) [a]	10.3(3.3-30.0)†(n=13)	0.005*
Laboratory results				
FBG, mmol/L	9.0 ± 4.1†(n=680)	9.2 ± 3.8†(n=37)	9.9 ± 4.1†(n=13)	0.666
HbA1c, %	8.6 ± 2.3†(n=803)	8.8 ± 2.4†(n=50)	8.7 ± 1.9†(n=16)	0.897
Hb, g/L	114 ± 22†(n=854)	104 ± 25 [a]	109 ± 23	0.004
PLT, ×10^9/L	232 ± 108†(n=851)	266 ± 104	304 ± 143 [a]	0.001
FIB, g/L	4.4 ± 1.5†(n=827)	4.9 ± 1.7 (n=54)	5.0 ± 1.8 (n=16)	0.019
WBC count, ×10^9/L	7.8 ± 3.7†(n=853)	9.6 ± 5.1† (n=54)	11.6 ± 6.1	0.001*
NEUT, %	68.5 ± 12.5†(n=806)	71.4 ± 16.8	81.0 ± 9.3†(n=15) [a]	<0.001*
Albumin, g/L	36.4 ± 6.1†(n=892)	33.1 ± 7.3[a]	32.8 ± 6.1[a]	<0.001
TG, mmol/L	2.1 ± 1.5†(n=887)	2.2 ± 1.6	1.6 ± 1.0	0.315
TC, mmol/L	3.5 ± 1.4†(n=888)	3.0 ± 1.4 [a]	3.0 ± 1.2	0.016
LDL-C, mmol/L	2.2 ± 1.0†(n=888)	1.9 ± 0.9†(n=54)	1.9 ± 1.0	0.148
HDL-C, mmol/L	1.09(0.87-1.42)†(n=888)	1.03(0.67-1.48)	0.93(0.69-1.29)	0.308*
UA, μmol/L	322 ± 110†(n=888)	276 ± 123†(n=54) [a]	255 ± 133 [a]	0.001
Creatinine, μmol/L	81.0(63.5-108.0)†(n=892)	68.5(55.5-91.8)†(n=54)	70.6(53.5-103.5)	0.152
eGFR, mL/mL.1.73m^2	76.4 ± 31.4†(n=864)	86.3 ± 32.7†(n=53)	85.5 ± 36.2†	0.047
CRP, mg/L	10.0 (3.3-30.2)†(n=547)	24.3(4.9-105.9)†(n=28)	74.6(9.7-146.5)†(n=13) [a]	0.002*

Values are presented as number, median (IQR), or mean ± standard deviation. BMI, body mass index. ABI, ankle-brachial index, FBG, fasting blood glucose. HbA1c, glycosylated hemoglobin. Hb, hemoglobin. PLT, platelet. PT, prothrombin time. FIB, fibrinogen. WBC, white blood cell, NEUT, neutrophil granulocyte percentage. TG, triglyceride. TC, total cholesterol. LDL-C, low-density lipoprotein cholesterol. HDL-C, high-density lipoprotein cholesterol. UA, uric acid. eGFR, estimated glomerular filtration rate. CRP, C-reactive protein. †Some cases are lacking data and the number of patients was shown in brackets. *, Kruskal–Wallis test. a, statistical significance compared with non-amputation group with Bonferroni post-test.

10.3389/fendo.2023.1144806

TABLE 2 Comparison of diabetic complications and comorbidities among the non-amputation, minor amputation and major amputation groups.

Factor	Non-amputation (N=899)	Minor Amputation (N=55)	Major Amputation (N=17)	*P* value
Retinopathy				0.970
Yes	351	20	6	
No	474	28	9	
Diabetic peripheral neuropathy				0.372
Yes	848	54	17	
No	44	1	0	
PAD				0.014
Yes	473	33	15	
No	383	19	2	
Cardiac autonomic neuropathy				0.243
Yes	607	37	15	
No	248	12	2	
Gastrointestinal autonomic neuropathy				0.706
Yes	230	13	3	
No	642	39	14	
Bladder autonomic neuropathy				0.192
Yes	431	27	12	
No	460	28	5	
Albuminuria				0.147
Yes	485	38	9	
No	320	14	8	
CKD				0.730
Yes	591	39	11	
No	308	16	6	
Hypertension				0.238
Yes	633	33	11	
No	266	22	6	
Coronary heart diseases				0.528
Yes	215	10	3	
No	684	45	14	
Hyperuricemia				0.263
Yes	116	6	0	
No	783	49	17	
Hyperlipidemia				0.511
Yes	231	17	3	
No	668	38	13	

Values are presented as number. PAD, peripheral arterial disease. CKD, chronic kidney disease.

TABLE 3 Comparison of foot-related characteristics among the non-amputation, minor amputation and major amputation groups.

Factor	Non-amputation (N=899)	Minor amputation (N=55)	Major amputation (N=17)	*P* value
Prior ulcer				0.372
Yes	263	21	5	
No	636	34	12	
Prior amputation				<0.001
Yes	61	12	5	
No	838	43	12	
Deformities				0.544
Yes	133	7	1	
No	766	48	16	
Callus				0.087
Yes	310	11	6	
No	589	44	11	
Osteomyelitis				0.006
Yes	422	38	7	
No	429	15	9	
Foot gangrene				<0.001
Yes	263	39	14	
No	636	16	3	
Wagner grade				<0.001
1	72	0	0	
2	170	0	0	
3	393	16	3	
4	250	33	9	
5	14	6	5	

Values are presented as number.

3.3 Risk factors associated with LEAs

Results of the ordinal logistic regression models are shown in Table 4. After adjustment of the baseline predictors, a history of prior amputations (*OR*, 5.380; 95% *CI*, 1.847-15.668, *P* = 0.002), foot gangrene (*OR*, 6.854; 95% *CI*, 2.246-20.915, *P* = 0.001) and ABI (*OR*, 0.853; 95% *CI*, 0.733-0.992, *P* = 0.038) significantly associated with LEAs. In addition to eGFR and CRP, a history of prior amputations (*OR*, 10.709; 95% *CI*, 2.871-39.938, *P* = 0.001), foot gangrene (*OR*, 5.625; 95% *CI*, 1.448-7.510, *P* = 0.013) and ABI (*OR*, 0.794; 95% *CI*, 0.649-0.971, *P* = 0.029) significantly associated with LEAs in the Model 2. Finally, in the full Model 3, a history of prior amputations (*OR*, 10.194; 95% *CI*, 2.646-39.279; *P*=0.001), foot gangrene (*OR*, 6.466; 95% *CI*, 1.576-26.539; *P*=0.010) and ABI (*OR*, 0.791; 95% *CI*, 0.639-0.980; *P* = 0.032) were the independent risk factors of LEAs. The ordinal logistic regression model was assessed validity with the test of parallel lines ($P > 0.05$), and significance was confirmed by −2 log likelihood ($P < 0.001$).

3.4 Prognosis during hospitalization

The mean hospital stay was 31 (18–56) days, and which of minor (47 (37-63) days) and major amputation groups (57 (38-95) days) were longer than those of non-amputation group (30 (15-55) days, P< 0.001). On discharge, foot ulcers in 240(26.7%) and 94 (9.5%) patients with non-amputation were completely healed and poorly healed, respectively. Foot ulcers of 11(20.0%) and 4(23.5%) patients healed in minor and major amputation group, respectively. 10 (1.0%) patients died during the hospitalization. The main of death causes were myocardial infarction (3 cases), heart failure (3 cases) and respiratory failure (3 cases). One of these died of septic shock after major amputation.

TABLE 4 The ordinal logistic regression analysis of major and minor amputation risks in patients with diabetic foot ulcers.

	Model 1 *OR* (95%*CI*)	Model 2 *OR* (95%*CI*)	Model 3 *OR* (95%*CI*)
Prior amputation	5.380(1.847-15.668) *	10.709 (2.871-39.938) *	10.194(2.646-39.279) *
Osteomyelitis	1.254(0.434-3.629)	1.744(0.405-7.510)	1.926(0.443-8.364)
Foot gangrene	6.854(2.246-20.915) *	5.625 (1.448-7.510) *	6.466 (1.576-26.539) *
Ulcer area	1.007(0.996-1.018)	1.011(0.996-1.027)	1.012(0.998-1.027)
Hb	1.010(0.990-1.031)	1.014(0.988-1.041)	1.000(0.994-1.006)
PLT	0.998(0.993-1.003)	0.998(0.993-1.004)	0.998(0.993-1.004)
FIB	/	/	0.642(0.387-1.064)
NEUT	1.015(0.971-1.061)	1.029(0.968-1.094)	1.044(0.975-1.118)
Albumin	0.952(0.873-1.037)	0.950(0.836-1.079)	0.927(0.814-1.002)
TC	0.843(0.595-1.195)	0.778(0.476-1.274)	0.773(0.474-1.260)
UA	0.996(0.991-1.001)	0.993(0.986-1.001)	0.993(0.985-1.002)
eGFR	/	1.025(1.000-1.051)	1.022(0.994-1.011)
CRP	/	0.995(0.984-1.007)	0.999(0.987-1.011)
ABI (per 0.1)	0.853(0.733-0.992) *	0.794(0.649-0.971) *	0.791(0.639-0.980) *

OR, odds ratio; CI, confidence interval; ABI, ankle brachial pressure index. Amputation was defined as an ordinal variable with major amputation, minor amputation and non- amputation. The stepwise ordinal logistic regression was used to identify potential predictors for major and minor amputation and to calculate OR, using the "non-amputation" subgroup as a baseline. Model 1 was adjusted for Hb, PLT, NEUT, albumin, TC, UA, ABI, ulcer area, and the presence of prior amputation, osteomyelitis, foot gangrene. Model 2 was adjusted for eGFR, CRP on the basis of Model 1. *P < 0.05. Model 3 was adjusted for FIB on the basis of Model 2.

4 Discussion

This study showed a comparatively low rate of LEA among the hospitalized patients with DFU in the Diabetic Foot Care Center of a tertiary hospital (7.3%) in China. The previous amputation, foot gangrene and decreased ABI value were independent predictors of LEA. Therefore, it is a great challenge for the practitioners to avoid amputation and re-amputation in the diabetic patients, especially in the elderly and poorly glycemic controlled patients with a previous history of foot ulcer or amputation.

A history of prior foot ulceration was considered as a significant risk factor for amputation (19–21). Furthermore, a prior history of amputation was linked to an increased risk of major adverse limb events (22). One meta-analysis about risk of major amputation in the DFU patients showed that hypertension, ischemic heart disease, cerebrovascular disease and peripheral vascular disease were identified as the predisposing factors for major amputation (10).The FIELD study indicated that previous cardiovascular disease, microvascular disease, previous non-traumatic amputation or skin ulcer, smoking, and longer duration of diabetes were more frequent in the amputated patients than in the non-amputated patients (23). Therefore, the diabetic patients experienced non-traumatic lower-limb amputations were multifactorial.

It appears that PAD was more common in the minor (63.5%) and major (88.2%) amputated patients than the non-amputated patients (55.3%) (*P*=0.014) in this study. A study consisting of 3892 type 2 diabetes patients with a first-time diagnosis of diabetic foot syndrome in German showed that the presence of PAD was the strongest independent predictor of LEA in the DFU patients (*HR*, 5.13; *CI*: 4.27–6.16) (24). Another prospective single-center study in German showed that perfusion status of foot, and ulcer extent and depth were the risk factors of LEA according to the PEDIS classification (25). Lower extremity artery stenosis or occlusion was considered as a risk factor for amputation in the DFU patients (26, 27). ABI was a simple and non-invasive method to screen PAD. In this study, the mean values of ABI in the major and minor amputation groups were 0.76 and 0.85, respectively. The decreased ABI value was a strong predictor for LEA. Another prospective single-center study in China also suggested that low ABI were significantly associated with an increased risk of LEA (28). The SEASON study in Japan suggested that ABI <0.4 was the strongest risk factor for amputation of the diabetic patients with PAD (29). In the FIELD study, ABI >0.52 increased a rate of limb preservation in the patients with chronic limb-threatening ischemia (23). Thus, IWGDF recommended that a screening ABI should be performed in the diabetic patients who had symptoms or signs of PAD or who were over than older than 50 years old (30). Actually, ABI was not completed reliable on diagnosis of PAD in the diabetic patients. ABI could falsely elevate due to calcification of arterial media (31). Falsely high ABI was an independent predictor of major amputation in the patients with chronic limb ischemia (32). In addition, our study showed that LEA occurred even in the DFU patients with normal ABI values, especially in the minor amputated patients. A Korean study found that 28.7% of patients had normal ABI ranging from 0.91 to 1.40 but were diagnosed with PAD using color doppler ultrasonography (33). Our previous study showed that 19.8% of limbs in the patients with diabetic foot disease had

normal ABI values (0.91-1.3). However, digital subtraction arteriography showed that 72.2% of the lower limbs with normal ABI had occlusion of at least one artery below knees (34). This could be explained by extensive distribution and multiple segments of atherosclerotic lesions in below-the-knee arteries or formation of collaterals. Therefore, ABI could underestimate PAD in the DFU patients and color doppler ultrasound was usually necessary for further diagnosis of PAD in the diabetic patients with foot ulcers.

We found that the hospitalized DFU patients with foot gangrene had an approximately 6.5-fold higher risk of amputation. Foot gangrene was caused by deficient blood supply to tissues due to arterial stenosis or occlusion that further led to localized necrosis and tissue death. Mortality rate was significantly high after major amputation. A study in Tanzania revealed that the overall mortality rates for amputees and non-amputees were similar (29%), but patients with severe foot ulcers (Wagner grade ≥ 4) who did not undergo surgery had the highest mortality rate (54%) during hospitalization (35). Another retrospective study in Finland showed that after a major amputation, the one- and five-year overall survival rates of the diabetic patients with foot infection were 41.7% and 8.3%, respectively (36). Rapid revascularization, either endovascular or open vascular surgery, could reduce the risk of amputation in DFU patients with the PAD (37). The incidence of gangrene decreased from 14.7% to 11.3% (P<0.001) with a concomitant increase in vascular interventions (6.2% to 19.5%, P<0.001). Therefore, it is critical to take effective measures to improve blood supply of the gangrene foot early as much as possible in order to effectively reduce the amputation plane of the patients with severe foot ulcers, even avoid major amputation.

Prothrombotic state was more pronounced in the amputated patients than those in the non-amputated patients, which implied increased coagulation, impaired fibrinolysis, and endothelial dysfunction (38). This was illustrated by higher fibrinogen levels in the amputated patients compared with the non-amputated patients from this study and other studies (39, 40). Wang et al. suggested that fibrinogen was an independent risk factor of LEA in the DFU patients (39). Plasma fibrinogen level >300.4 mg% (100% sensitivity, 99.2% specificity) was correlated with a high risk of amputation in DFU (41). Another study showed a fibrinogen cut-off value of 5.13g/L indicated the possible amputation with a sensitivity of 81.8% and a specificity of 78.9% (positive predictive value 78.6%, negative predictive value 89.0%) (40). Therefore, early anticoagulant treatment undoubtedly improve prognosis of DFU.

Foot ulcer infection was closely associated with the increased amputation rate. In routine clinical practice, WBC, PLT, and CRP levels were used to determine procession of DFU (42). A prospective study in Turkey showed that 33.2% of 126 cases with diabetic foot infection (DFI) underwent amputation (43). Approximately 50% of DFU patients could develop DFI, which was diagnosed on the basis of clinical characteristics (44). Inflammatory biomarkers such as WBC, Neutrophils, CRP, IL-6, PCT and ESR could be used to distinguish between non-infection and mild infection, indicate severity of foot ulcer infection and monitor response of anti-infective therapy. Therefore, the inflammatory markers were reported to be a strong predictor of amputation (45, 46). In our study, compared with the non-amputated patients, the DFU patients with minor and major amputations had higher levels of WBC counts, NEUT, and serum CRP, which were higher in the major amputees than the minor amputees. Foot gangrene and osteomyelitis affected roughly one-third and one-half of the amputees, respectively. One meta-analysis showed that osteomyelitis (OR: 4.5), neuro-ischemic DFI (OR: 3.06), severe infection (OR: 3.12), leukocytosis (OR: 1.76), mean ESR (SMD: 0.5), mean CRP (SMD: 0.8), tissue culture positivity (OR: 1.61), and isolation of Gram-negative bacteria from tissue culture (OR: 1.5) were predictors of amputation in DFI (19). PCT was a diagnostic marker of bacterial infection. Another meta-analysis revealed that PCT>0.5 ng/ml was an independent predictor of major amputation (OR 3.3) and mortality (OR 4.13) in the DFI patients with CLI (47). WBC, ESR and CRP were non-specific inflammatory biomarkers. Therefore, testing for the inflammatory biomarkers in the DFU patients could help early identify diagnosis of DFI and monitor therapeutic response after anti-infective treatment.

The process of wound healing required adequate nutrient supply to the tissue, which could be hampered by circulatory compromise and rapid protein loss (48, 49). Malnutrition is highly prevalent among the DFU patients (50). Serum albumin and Hb were used to evaluate the nutritional status of human body. Compared with the non-amputated patients, the amputated patients had significantly lower Hb, serum albumin and TC levels. A study enrolling 3654 patients with DFU revealed that Hb and plasma albumin were the independent factors of major amputation (21). There was no definitive evidence to confirm the close relationship between malnutrition and amputation in the DFU patients, but protein-energy wasting was common in the DFU patients with severe infection. Thus, the clinicians should focus on the nutritional status of the DFU patients and correct their anemia and hypoalbuminemia as soon as possible in order to improve general conditions of the patients and promote wound healing.

A multicenter study revealed that the diabetic patient with even moderate CKD(eGFR<60ml/min per 1.73m^2) had an increased risk for DFU and LEA (51). The eGFR<30ml/min per 1.73m^2 in DFU patients with osteomyelitis was an independent predictor for amputation and healing failure (52). However, in this study, we found the mean eGFR value in the amputated patients was over 60 mL/min per 1.73 m^2, which was higher than that in the non-amputees. Although we could not fully explain why the amputated patients had higher eGFR compared with the non-amputated patients, glomerular hyperfiltration due to hyperglycemia and adequate rehydration for the amputated patients was the possible reason.

Most of patients in our study had eventually good therapeutic effects with low amputation and mortality rates, highlighting the importance of the multidisciplinary intervention. However, the study had several limitations. It was a retrospective study from single medical center, which could lead to selection bias. Clinical data of some patients were incomplete. The number of LEA outcomes was low which was good for the patients, but reduced our sample size. In addition, treatment strategies, e.g.,

revascularization (surgical or endovascular), statin therapy, was not considered, which may render some of risk estimates unstable.

5 Conclusion

The DFU inpatients with LEA were older with long duration of diabetes, poorly glycemic control, malnutrition, high prevalence of PAD, severe foot ulcers and infection, and longer hospital stays. A history of prior amputation, foot gangrene and a low ABI level were the independent predictors of LEA. However, normal ABI could not exclude PAD and LEA was caused by multiple factors which should be concerned. Therefore, multidisciplinary diagnosis and treatment of DFU is essential to avoid amputation of the DFU patients.

Data availability statement

The original contributions presented in the study are included in the article. 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 West China Hospital, Sichuan University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HG ontributes in the proposal preparation, analysis and writes up of the manuscript. HG and CW revised the manuscript. CW contributed to study design and analysis. DC, YG, and XR contributed to the design of the research protocol. HG, YR, ZL, PZ, YL, RB, and LC contributed to the collection of the clinical data. All authors contributed to and approved the final manuscript for publication.

Funding

This work was supported by Sichuan Province Science and Technology Support Program, China (2018JY0608) and the 1.3.5 Project for Disciplines of Excellence, West China Hospital, Sichuan University (ZYGD18025).

Acknowledgments

We thank contribution diabetic foot care center, West China Hospital, Sichuan University, contribution to this study.

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

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 04 April 2023
DOI 10.3389/fendo.2023.1107830

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 29 November 2022
ACCEPTED 07 March 2023
PUBLISHED 04 April 2023

CITATION
Shao Z, Wang Z, Bi S and Zhang J (2023) Establishment and validation of a nomogram for progression to diabetic foot ulcers in elderly diabetic patients. *Front. Endocrinol.* 14:1107830. doi: 10.3389/fendo.2023.1107830

Establishment and validation of a nomogram for progression to diabetic foot ulcers in elderly diabetic patients

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Background: Many diabetic patients develop and progress to diabetic foot ulcers, which seriously affect health and quality of life and cause great economic and psychological stress, especially in elderly diabetic patients who often have various underlying diseases, and the consequences of their progression to diabetic foot ulcers are more serious and seriously affect elderly patients in surgery. Therefore, it is particularly important to analyze the influencing factors related to the progression of elderly diabetic patients to diabetic foot, and the column line graph prediction model is drawn based on regression analysis to derive the influencing factors of the progression of elderly diabetic patients to diabetic foot, and the total score derived from the combination of various influencing factors can visually calculate the probability of the progression of elderly diabetic patients to diabetic foot.

Objective: The influencing factors of progression deterioration to diabetic foot in elderly diabetic patients based on LASSO regression analysis and logistics regression analysis, and the column line graph prediction model was established by statistically significant risk factors.

Methods: The clinical data of elderly diabetic patients aged 60 years or older in the orthopedic ward and endocrine ward of the Third Hospital of Shanxi Medical University from 2015-01-01 to 2021-12-31 were retrospectively analyzed and divided into a modeling population (211) and an internal validation population (88) according to the random assignment principle. Firstly, LASSO regression analysis was performed based on the modeling population to screen out the independent influencing factors for progression to diabetic foot in elderly diabetic patients; Logistics univariate and multifactor regressions were performed by the screened influencing factors, and then column line graph prediction models for progression to diabetic foot in elderly diabetic patients were made by these influencing factors, using ROC (subject working characteristic curve) and AUC (their area under the curve), C-index validation, and calibration curve to initially evaluate the model discrimination and calibration. Model validation was performed by the internal validation set, and the ROC curve, C-index and calibration curve were used to further evaluate the

column line graph model performance. Finally, using DCA (decision curve analysis), we observed whether the model could be used better in clinical settings.

Results and conclusions: (1) LASSO (Least absolute shrinkage and selection operator) regression analysis yielded a more significant significance on risk factors for progression to diabetic foot in elderly diabetic patients, such as age, presence of peripheral neuropathy, history of smoking, duration of disease, serum lactate dehydrogenase, and high-density cholesterol; (2) Based on the influencing factors and existing theories, a column line graph prediction model for progression to diabetic foot in elderly diabetic patients was constructed. The working characteristic curves of subjects in the training group and their area under the curve (area under the curve = 0.840) were also analyzed simultaneously with the working characteristic curves of subjects in the external validation population and their area under the curve (area under the curve = 0.934), which finally showed that the model was effective in predicting column line graphs; (iii) the C-index in the modeled cohort was 0.840 (95%CI: 0.779-0.901) and the C-index in the validation cohort was 0.934 (95%CI: 0.887-0.981), indicating that the model had good predictive accuracy; the calibration curve fit was good; (iv) the results of the decision curve analysis showed that the model would have good results in clinical use; (v) it indicated that the established predictive model for predicting progression to diabetic foot in elderly diabetic patients had good test efficacy and helped clinically screen the possibility of progression to diabetic foot in elderly diabetic patients and give personalized interventions to different patients in time.

KEYWORDS

diabetic foot, elderly, diabetes, nomogram, prediction

1 Introduction

Diabetes mellitus is a common complication associated with disorders of glucose and insulin metabolism (1). Diabetes mellitus is a series of metabolic disorders characterized by hyperglycemia (2). As a very common chronic disease, diabetes is of increasing concern to patients and physicians worldwide. Some studies show that currently, 3.882 billion people have diabetes, and the total number in the world is increasing (3). Public health economic pressures from diabetes are also increasing, and the cause of these is more likely to come from complications of diabetes, with diabetics at higher risk of microvascular complications such as nephropathy, neuropathy and retinopathy. Complicated cardiovascular diseases such as ischemic heart disease, stroke and peripheral arterial disease. One study found that nearly 20% of diabetic patients will eventually develop a diabetic foot during their lifetime. The diabetic foot, in turn, can bring further more serious consequences and complications (4). More urgently, many elderly patients have many underlying diseases themselves, such as hypertension, diabetes and cerebral infarction, etc. Elderly patients with concomitant diabetes should receive more attention and more and better preventive measures for the complications brought about by diabetes. Among them, the consequences of developing diabetic foot in elderly diabetic patients are more serious and require more urgent treatment or timely prevention. Foot complications are among the most serious and costly complications of DM, eventually leading to amputation of the lower extremity or part of it due to foot ulcers (5). The diabetic foot is one of the most serious complications of diabetes and is defined as a group of syndromes of neuropathy, ischemia and infection leading to tissue breakdown and possible amputation. If a foot ulcer is untreated and fails to heal, it can become infected and 5-24% of foot ulcers will result in limb amputation within 6 to 18 months of the first evaluation (6, 7). More than half of the non-traumatic amputations are due to diabetic foot, which shows that the harm and economic pressure caused by diabetic foot is great, and the persecution of diabetic patients should not be ignored, and the subsequent family and psychological impact should not be underestimated as well. Moreover, the life expectancy of elderly diabetic foot patients is further affected by amputation, and the mortality rate is greatly increased.

Although a number of scholars have conducted studies on the progression of diabetes and diabetic foot, the mechanisms and risk factors of how elderly diabetic patients progress to diabetic foot step by step have not been conclusively established, and few studies have been conducted on the development of diabetic foot in this group of

elderly patients. One study, a national survey in the United States, showed that smoking was more common in white Americans and American Indians than in other racial groups such as blacks and Asians (8). Previous studies have identified smoking as a risk factor for diabetic foot ulcers because daily tissue hypoxia may lead to vascular and neuropathic disease in the lower extremities of diabetic patients (9). A number of studies have concluded that there are differences in age, duration of diabetes, BMI distribution, hypertension, and diabetic retinopathy between patients with and without diabetic foot ulcers, and these findings suggest some of the potential influencing factors for the development of diabetic foot in patients with diabetes. The magnitude of the effect of a patient's obesity on the risk of developing ulcers in the diabetic foot is inconclusive. Given previous studies suggesting that obesity may be associated with diabetic foot ulcers (10, 11). Some prospective studies have concluded that there is no significant relationship between BMI and the development of diabetic foot (12), There are also findings showing that patients with BMI <25 kg/m2 and BMI ≥45 kg/m2 are associated with a higher risk of developing diabetic foot ulcers (13). Studies have shown that impaired microcirculation in diabetic patients may lead to dysfunctional vasodilation leading to secondary complications in the lower extremities, and diabetic foot patients with retinopathy have higher levels of diabetic biomarkers (14–16), These results suggest an association between retinopathy and diabetic foot ulcers. In addition, some studies have found that men with diabetes are more likely to develop diabetic foot than women with diabetes, and this gender variability has been suggested to be related to more labor and physical activity in men (17, 18).

2 Objects and methods

2.1 Patients

299 diabetic patients, aged 60 years or older, were selected from January 2015 to December 2021 in the Department of Orthopedics and Endocrinology, Third Hospital of Shanxi Medical University. Retrospective analysis of 299 patients hospitalized for diabetes mellitus or diabetic foot from January 2015 to December 2021. These 299 patients were randomly divided internally into a modeling population and a validation population. We randomly collected a total of 299 elderly diabetic patients, of which 211 patients randomly collected at the beginning were the modeling population and 88 patients randomly collected later were the validation population according to time allocation. In the modeling population, there were 102 male patients and 109 female patients; there were 53 patients with diabetic foot and 158 patients with non-diabetic foot. The internal validation population consisted of 88 patients, of whom 28 were diabetic foot patients, while a total of 60 were non-swollen patients, and other specific information can be found in Table 1.

TABLE 1 Patient demographic characteristics of the modeled population.

Features	Modeling population (n=211)/n (%)	Validation population (n=88)/n (%)	P value
Age (%)			0.266
60-65	92 (43.6)	48(54.5)	
66-75	77 (36.5)	29(33.0)	
>75	42 (19.9)	11(12.5)	
Sex (%)			0.533
Male	102 (48.3)	42(47.7)	
Female	109 (51.7)	46(52.3)	
Duration of Diabetes/year (%)			0.455
<5	55 (26.1)	25(28.4)	
5~10	74 (35.0)	33(37.5)	
>10	82 (38.9)	30(34.1)	
Alcohol (%)			0.166
YES	19 (9.0)	82(93.2)	
NO	192 (91.0)	6(6.8)	
Triglycerides (%)			0.312
0~1.7	163 (77.3)	65(73.9)	
>1.7	48 (22.7)	23(26.1)	

(Continued)

TABLE 1 Continued

Features	Modeling population (n=211)/n (%)	Validation population (n=88)/n (%)	P value
BMI (%)			0.123
<18.5	23 (10.9)	11(12.5)	
18.5-23.9	165 (78.2)	60(68.2)	
>24.0	23 (10.9)	17(19.3)	
Lactate dehydrogenase (%)			0.335
≤245	185 (87.7)	76(86.4)	
>245	26 (12.3)	12(13.6)	
Peripheral neuropathy (%)			0.563
YES	47 (22.3)	62(70.5)	
NO	164(77.7)	26(29.5)	
Smoking (%)			0.566
YES	160 (75.8)	17(19.3)	
NO	51 (24.2)	71(80.7)	
Total cholesterol (%)			0.344
≤6.0mmoI/L	161 (76.3)	21(23.9)	
>6.0mmoI/L	50(23.7)	67(76.1)	
High-density cholesterol (%)			
≤2.0mmol/L	123 (58.3)	50(56.8)	
>2.0mmol/L	88 (41.7)	38(43.2)	
High blood pressure (%)			0.243
NO	129 (61.1)	58(65.9)	
YES	82 (38.9)	30(34.1)	
Diabetic foot (%)			0.169
NO	158 (74.9)	60(68.2)	
YES	53 (25.1)	28(31.8)	

Both the modeling and validation populations passed informed consent.

The study of this columnar map prediction model was reviewed and approved by the ethics committee of the Third Affiliated Hospital of Shanxi Medical University.

The following diagnostic criteria were selected to meet: the criteria for diabetic foot established by the World Health Organization for retrospective analysis of the occurrence of DF, and relevant clinical data were collected for the analysis.

The inclusion criteria were as follows.

1. meeting the diagnostic criteria for diabetes mellitus established by the World Health Organization. 2. patients with complete case data and clinical examination data, and those who gave informed consent and voluntarily participated in the survey.

Exclusion criteria were as follows.

1. patients with malignancy, myocardial infarction or other serious infectious diseases and cognitive dysfunction. 2. those without clear diagnostic findings

2.2 Methods

Information about patients in the modeling and validation populations was collected by reviewing electronic medical records.

General information: including age, gender, height, BMI, etc.; ② Clinical information: duration of disease, presence of hypertension, etc.

2.2.1 Main observation indicators

(i) the results of univariate and multifactorial logistics regression analysis of the development of diabetic foot in elderly diabetic patients; (ii) the construction of the column line graph prediction model; (iii) the evaluation results of the column line graph prediction model; (iv) the internal validation of the column line graph prediction model.

2.2.2 Statistical analysis

LASSO (Least absolute shrinkage and selection operator) regression analysis was first performed using the (glmnet) package of R language software (version 4.0.5) to screen out statistically significant variables, i.e., age, presence of peripheral neuropathy, history of smoking, duration of disease, serum lactate Dehydrogenase, high-density cholesterol and other 10 variable factors were influential risk factors for progression of diabetes to diabetic foot ulcers in the elderly. A multifactorial logistics regression analysis was then performed using SPSS (version 25.0). Further, column line graph prediction models were drawn using the six screened risk factors by R language software "car", "rms". In order to evaluate the accuracy of the developed prediction models, the models were also evaluated simultaneously by producing C-index, calibration curves and using receiver operating characteristic (ROC) curves by R language software. Finally, in order to make the established model can be better used in clinical work, it is validated with DCA (Decision curve analysis) decision curve to determine whether the model has better performance in clinical work. In this case, the calibration curve has a 45° diagonal line, and the closer the other line produced is to this diagonal line, the better the model is. In addition, the ROC curve is calculated mainly by its AUC area, and different ranges of AUC area represent different meanings, where the model has no predictive power: AUC < 0.50; moderate accuracy: 0.50 < AUC ≤ 0.70; moderate to high accuracy: 0.70 < AUC ≤ 0.90; high accuracy: > 0.90.

3 Results

3.1 Analysis of the number of participants The medical records of the 211 modeled populations were analyzed

The number of participants and groups included in the observation, the number of participants and groups entered into the outcome analysis, and whether there was any shedding, if so please explain why.

The flow chart of the trial can be seen in Figure 1.

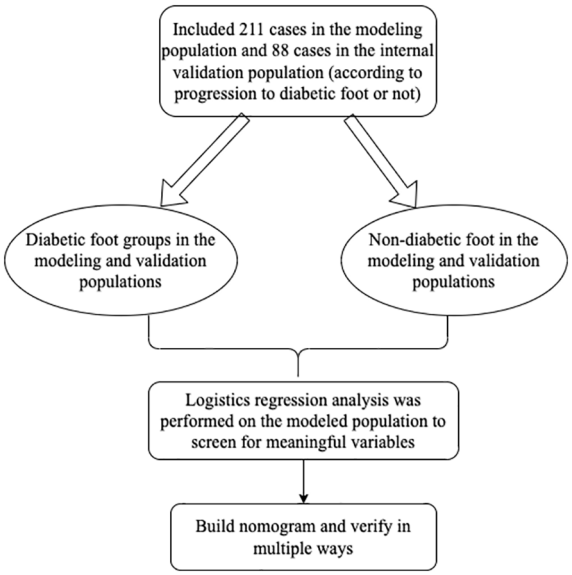

FIGURE 1
Flow chart.

3.2 Baseline information

We found that 48.3% of the patients in the modeled population were male and 51.7% were female. The majority of patients (78.2%) had a BMI between 18.5 and 23.9, and 38.9% had a disease duration of more than 10 years, while this data for the validation population showed that 34.1% of patients had a disease duration of more than 10 years. Specific information for all modeled and validated populations is shown in Table 1. And there was no significant comparison of baseline information between the two groups.

3.3 Screening of risk factors for progression to diabetic foot ulcers in elderly diabetic patients

The clinical data collected from elderly diabetic patients (diabetic foot and non-diabetic foot) were subjected to LASSO regression analysis, and 10 factors were found to be important influences on the progression to diabetic foot ulcers in elderly diabetic patients, including age, presence of peripheral neuropathy, smoking or not, high-density cholesterol, lactate dehydrogenase, total serum cholesterol, history of alcohol consumption, age, presence of hypertension, and triglycerides factors, as shown in Figure 2 of the LASSO regression analysis.

3.4 Results of logistics regression analysis of progression to diabetic foot ulcers in elderly diabetic patients

Table 2 shows the results of the univariate and multifactorial logistics regression for the modeled population, which reveals statistically significant effects of age, presence of peripheral neuropathy, presence of smoking, high-density cholesterol, lactate dehydrogenase, and total serum cholesterol. The details can be seen in Table 2.

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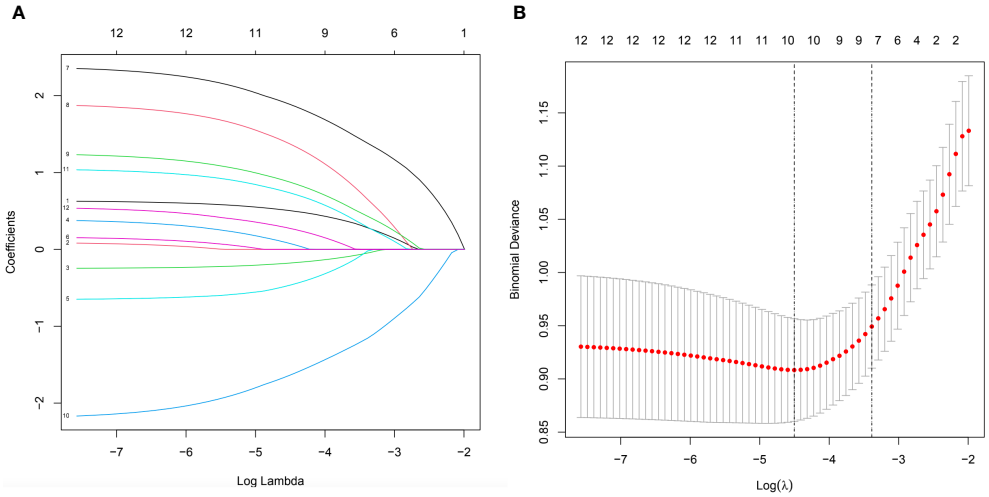

FIGURE 2
(A) All parameters were included in the LASSO analysis and coefficient curves were plotted. **(B)** Binomial deviations were plotted using the LASSO binary logistic regression model, which ultimately showed that 10 parameters were significant.

TABLE 2 Logistics regression univariate and multifactor analysis.

Variables	Univariate-analysis	P value	Multivariate-analysis	P value
	OR (95%CI)		OR (95%CI)	
Age	1.894 (1.219- 3.045)	0.006	1.882 (<0.001-3.352)	0.025
sex	1.149 (0.616-2.148)	0.661	1.099 (<0.001-2.508)	0.823
Duration of diabetes	0.685 (0.451-1.021)	0.07	0.780 (<0.001-1.260)	0.314
alcohol	1.878 (0.594-8.315)	0.333	1.484 (<0.001-8.361)	0.612
Triglycerides	0.669 (0.332-1.386)	0.267	0.519 (<0.734-1.311)	0.164
BMI	0.637 (0.414-0.990)	0.04	1.179 (<0.001-2.174)	0.582
dehydrogenase	6.400 (2.725-15.725)	<0.001	10.855 (<0.001-38.268)	<0.001
Peripheral neuropathy	2.228 (0.981-5.751)	0.07	6.702 (<0.001-23.160)	0.001
Smoking	3.943 (1.603-11.905)	0.006	3.506 (<0.001-12.677)	0.035
Total cholesterol	1.099 (0.788-1.531)	0.001	0.110 (<0.001-0.450)	0.007
High-density cholesterol	2.454 (1.260-5.009)	0.01	2.867 (<0.001- 6.947)	0.016
High blood pressure	1.324 (0.697-2.579)	0.398	1.739 (<0.001-4.304)	0.219

3.5 Establishment of a nomogram to predict the risk of progression of diabetic foot ulcers in elderly diabetic patients

Ultimately, a nomogram was drawn based on the six statistically significant risk factors of age, presence of peripheral neuropathy, presence of smoking, high-density cholesterol, lactate dehydrogenase, and total serum cholesterol of the patients (Figure 3). Figure 3 predicts the probability of progression to diabetic foot ulcers in older diabetic patients. In the nomogram we created, the effect of each variable on the endpoint event is reflected in the respective row length and the corresponding score. Individualized scores are available for different patients. The total score associated with each variable constitutes the probability of progression to a diabetic foot ulcer in older diabetic patients. We found that of all the factors included, the patient's total cholesterol, presence of peripheral neuropathy, and lactate dehydrogenase had a more significant effect on progression to diabetic foot ulcers in elderly diabetic patients, followed closely by age and whether or not they smoked also contributed significantly to the development of swelling. Different patients have different individualized scores, giving clinicians more over treatment decisions or better interventions to take.

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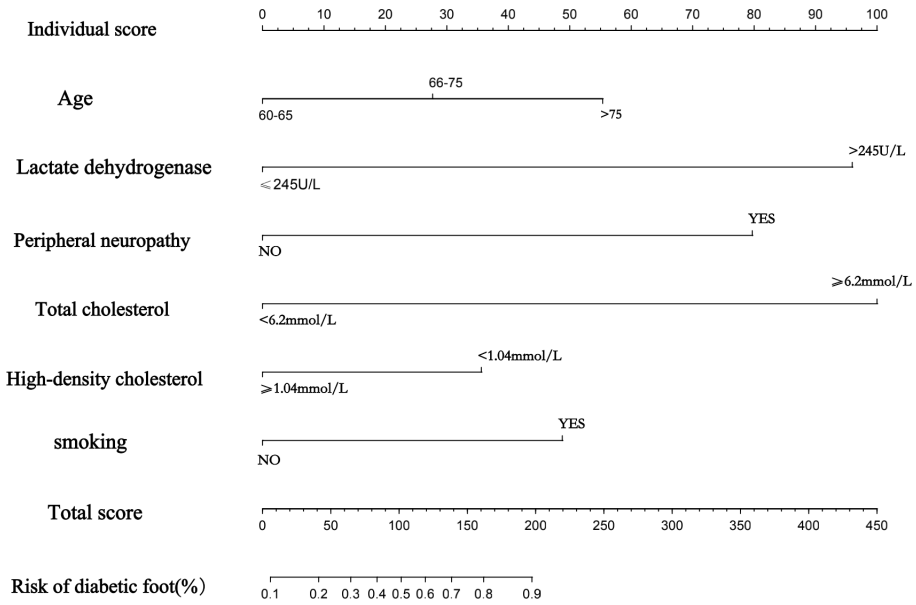

FIGURE 3
Nomogram drawn with 6 variables.

3.6 ROC curve to assess the accuracy of the prediction model

Receiver Operating Characteristic (ROC) curves were used to evaluate the prediction performance of the column line graphs, and the ROC curve and its AUC area (AUC=0.840) for the training group were calculated by R language software, and the ROC curve and its AUC (AUC=0.934) for the validation group were also analyzed at the same time, which finally showed that the model was predictive ability of the line graph was effective. Figures 4A, B show the ROC curves of the modeling group and the validation group nomogram, respectively.

3.7 Graphical calibration method and C-index to validate the predictive ability of the column line graph model

The calibration graphs of the training and validation groups (Figure 5) were also used to assess the accuracy of the prediction results of the column line graphs relative to the actual occurrence. Ideally, the calibration curve is a diagonal line; at this point, the predicted probability is equal to the true probability. The calibration curve confirms the good agreement between the actual and predicted values (Figure 5). Figure 5 shows the calibration curve for the column line graph; the blocks are close to the 45 degree line,

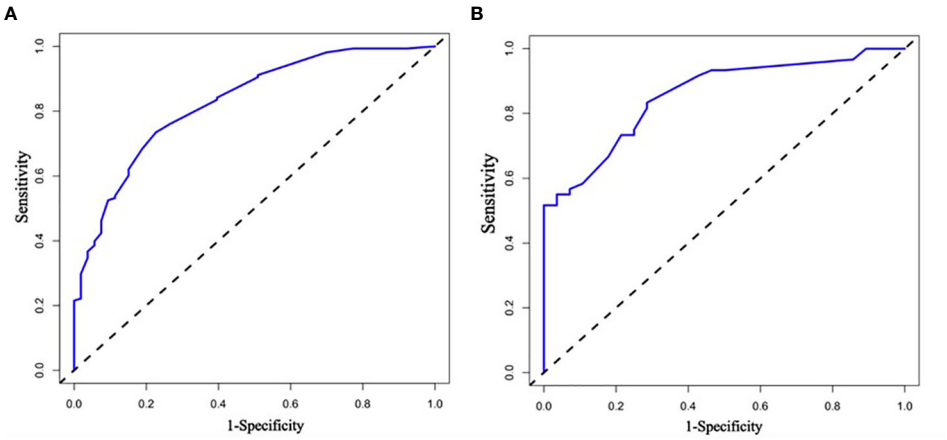

FIGURE 4
(A, B) Are the ROC curves of nomogram of modeling group and verification group respectively. ROC curve is the subject work characteristic curve; AUC is the effective area under the curve. the calculation of ROC curve is mainly expressed by its AUC area, and different ranges of AUC area represent different meanings.

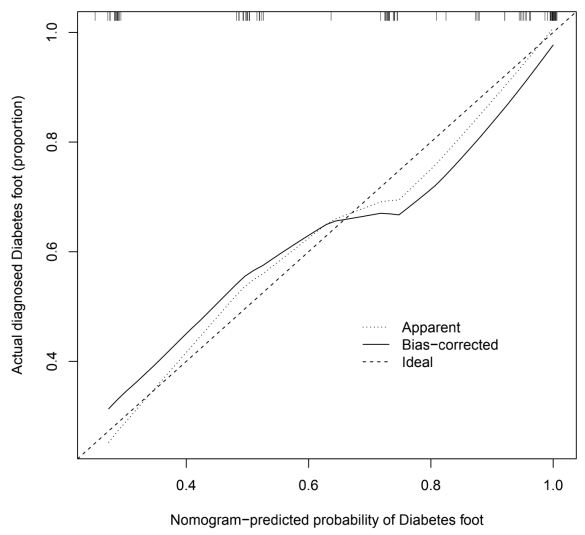

FIGURE 5
Calibration diagram. The calibration graph is a way to assess the accuracy of the prediction model for the column line graph. The significance of the calibration chart is that the closer the actual line in the chart is to the 45 degree diagonal the more accurate the prediction model is, and this image reflects the better accuracy of the prediction model we have built.

indicating that the survival nomogram is well calibrated in the training set. In addition, we also calculated a C-index of 0.840 (95% CI:0.779-0.901) in the training modeling cohort and 0.934 (95% CI:0.887-0.981) in the validation group, indicating that the prediction accuracy of the model was quite good.

3.8 Decision curve DCA validation for clinical applicability

The DCA curves show that the predictive models derived from the modeled population for the column line plots are clinically useful (Figure 6).

4 Discussion

Older patients with diabetic foot should receive more attention because they are always accompanied by other diseases and also, some studies have found that older diabetic patients are at higher risk of developing diabetic foot ulcers and their incidence may be higher (19). The prevalence of diabetic foot is not low, roughly 6% (17). And many studies have been conducted to analyze the risk factors for the progression of diabetes to diabetic foot ulcers, and these studies point out that the development of diabetic foot ulcers is associated with Hb1AC, foot trauma, obesity and overweight, smoking or not, duration of diabetes, and increasing age (20–24).

This study established novel, convenient, and highly accurate columnar line graphs for predicting the probability of progression to diabetic foot ulcers in older adults with diabetes, and to our knowledge, no study has ever analyzed and produced a columnar line graph prediction model on predicting progression to diabetic foot ulcers in older adults with diabetes. Columnar line plots also showed satisfactory agreement in both the modeled and validated populations, suggesting good clinical applicability. Columnar line plots are widely used for the prediction of various diseases or complications, mainly because of their ability to reduce statistical prediction models to estimates of simple numbers of event probabilities (e.g., diagnosis or recurrence) tailored to the profile of individual patients. It can inform clinical decision making (25). Column line graphs meet the need for integrated biological and clinical models and the quest for personalized medicine that can provide individualized predictions for individual patients (26).

From the results of our study, age, presence of peripheral neuropathy, smoking or not, high-density cholesterol, lactate dehydrogenase, and total serum cholesterol were the most relevant variable factors affecting the progression of diabetes to diabetic foot ulcers in older patients with diabetes. This also has similarities with some of the previous studies done above. First, the age of diabetes in elderly patients can influence the development of diabetic foot, probably because the longer the duration of the disease, the greater the accumulation of long-term progression of diabetes and the chance of complications, which can be verified from some later studies, which also suggests that clinicians should start to intervene or treat the progression of diabetes at an early stage when guiding patients, as the duration of diabetes and patient's age, it may bring about drawbacks similar to diabetic foot ulcers and the like. Then, clinicians do not recommend that patients delay treatment while ensuring that they receive the best possible treatment. Then, the effect of whether or not to smoke on the progression of diabetes to diabetic foot is also supported by many studies on the effect of smoking on diabetic microangiopathy, where smokers have diminished vasodilatation of different stimuli (mainly endothelium-dependent) to the skin microvasculature, further reducing the already reduced blood flow in the diabetic microcirculation, which in turn leads to impaired vascular activity eventually progressing or exacerbating diabetic foot ulcers, (27–29) Smoking also leads to the production of reactive oxidants in leukocytes, leading to a local inflammatory response that affects the development of the diabetic foot (30, 31). And our study, further proves that smoking is also an important risk factor for the development of diabetic foot ulcers in older diabetic patients, and even we believe that smoking has a greater impact on the progression to diabetic foot in older diabetic patients than in younger diabetic patients, because the microvasculature is more fragile and more prone to problems in older patients, so older diabetic patients who have a history of chronic and persistent smoking are only more susceptible to complications of diabetes. Peripheral neuropathy in elderly diabetic patients has also been shown to be one of the important influencing factors in the progression to diabetic foot through our study, which also found in numerous previous studies that peripheral neuropathy accounts for about 30% of diabetic patients and even more than half of type 2 diabetic patients over 60 years of age (32, 33). Nearly 80% of patients with diabetic foot ulcers have peripheral neuropathy (34). Oxidative stress is considered to be the ultimate mechanism of cellular damage in diabetic neuropathy, which is characterized by high levels of sustained generation of reactive oxides (ROS) and ultimately damages the nervous system leading to diabetic foot (35, 36). Similar to our findings, the close correlation

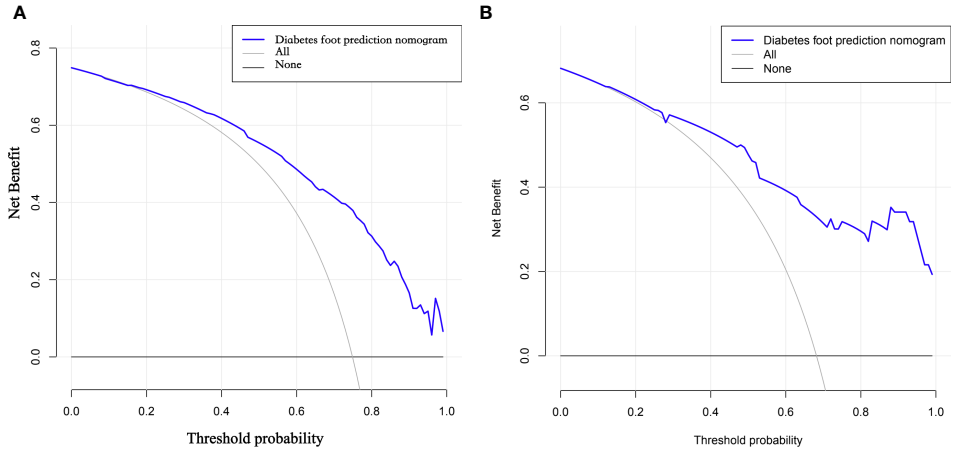

FIGURE 6
(A, B) DCA curves of the modeling group and validation group nomogram. DCA curves refer to decision curve analysis, which allows assessing the efficacy of the model for clinical use. The decision curve shows that if the probability of swelling occurs between 29% and 70%, the net benefit level of applying the columnar line graph is significantly higher than other options with better clinical benefit. Therefore, the DCA curves we plotted indicate that the predictive model of column line graphs derived from the modeled population is clinically useful.

between peripheral neuropathy and diabetic foot ulcers has also been demonstrated (35, 37). Moreover, our study further demonstrates that peripheral neuropathy is also a risk factor for progression to diabetic foot in the older age group of diabetic patients and is closely associated with its development. Our study suggests that clinicians need to pay extra attention to peripheral neuropathy in older diabetic patients and intervene early to prevent further progression to diabetic foot ulcers in older diabetic patients. Finally, our study also found that high-density cholesterol, lactate dehydrogenase, and total serum cholesterol were all associated with progression in elderly diabetic patients, similar to our findings, and it has also been suggested that low levels of HDL contribute to the development of diabetic foot ulcers by increasing the risk of diabetic peripheral neuropathy (38). Whereas our findings suggest a correlation between lactate dehydrogenase and total serum cholesterol and the progression of diabetes mellitus to diabetic foot ulcers in the elderly, there are indeed fewer studies of this type, but a few scholars have also performed the effect of lactate dehydrogenase or total serum cholesterol on diabetes mellitus, and there are those who believe that diabetes mellitus and diabetic foot ulcers are essentially the result of disorders of blood glucose and lipids, so their relationship with the development of diabetic foot cannot be ruled out. The relationship between them and the development of diabetic foot cannot be ruled out (39, 40). This seems to require further findings and studies in later prospective clinical trials.

The analysis of the ROC curves and their AUC areas revealed that the predictive model of the column line graphs we have drawn has good accuracy. Moreover, we also used the C-index and calibration plots to again validate the good predictive accuracy of the column line plot for the progression of diabetes to diabetic foot ulcers in the elderly.

The limitation of this study is that a multicenter study was not conducted, and cooperation of multiple centers can be sought in subsequent studies to further improve the accuracy of the model.

For future studies, we hope that more scientific results may be obtained by prospectively investigating the progression of geriatric diabetes to diabetic foot ulcers.

5 Conclusion

We have developed and are validating a new nomogram for predicting the risk of developing diabetic foot ulcers in older diabetic patients. After our study, we found that Age, dehydrogenase, Peripheral neuropathy, Smoking, Total cholesterol, and High-density cholesterol are meaningful risk factors for the development of diabetic foot ulcers in elderly diabetic patients after multiple modalities. validation showed that the nomogram we established has good accuracy and precision and has good predictive value. This is particularly useful for those working with diabetic foot wounds and care, and our study could suggest some hypotheses about the occurrence of diabetic ulcers for clinicians to accept. It could provide a more effective protocol for intervening in the progression to diabetic foot ulcers in older diabetic patients.

6 Limitations

This study has limitations. This is a single center retrospective study that affects patients and produces selection bias. Finally, the cases in this study are small samples from the same hospital. We suggest that in the follow-up study, it is better to conduct a prospective study with a large sample from multiple centers.

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 Medical Ethics Committee of the Third Hospital of Shanxi Medical 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

ZS and JZ designed and conceived the study, ZS wrote this paper. ZW and SB revised the article. 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.

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 14 April 2023
DOI 10.3389/fendo.2023.1109456

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Clinical Diabetes, a section of the journal Frontiers in Endocrinology

RECEIVED 27 November 2022
ACCEPTED 27 February 2023
PUBLISHED 14 April 2023

CITATION
Zhu Y, Lu J, Wang S, Xu D, Wu M, Xian S, Zhang W, Tong X, Liu Y, Huang J, Jiang L, Guo X, Xie S, Gu M, Jin S, Ma Y, Huang R, Xiao S and Ji S (2023) Mapping intellectual structure and research hotspots in the field of fibroblast-associated DFUs: a bibliometric analysis.
Front. Endocrinol. 14:1109456.
doi: 10.3389/fendo.2023.1109456

Mapping intellectual structure and research hotspots in the field of fibroblast-associated DFUs: a bibliometric analysis

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Background: Diabetic foot ulcers (DFUs) are one of the most popular and severe complications of diabetes. The persistent non-healing of DFUs may eventually contribute to severe complications such as amputation, which presents patients with significant physical and psychological challenges. Fibroblasts are critical cells in wound healing and perform essential roles in all phases of wound healing. In diabetic foot patients, the disruption of fibroblast function exacerbates the non-healing of the wound. This study aimed to summarize the hotspots and evaluate the global research trends on fibroblast-related DFUs through bibliometric analysis.

Methods: Scientific publications on the study of fibroblast-related DFUs from January 1, 2000 to April 27, 2022 were retrieved from the Web of Science Core Collection (WoSCC). Biblioshiny software was primarily performed for the visual analysis of the literature, CiteSpace software and VOSviewer software were used to validate the results.

Results: A total of 479 articles on fibroblast-related DFUs were retrieved. The most published countries, institutions, journals, and authors in this field were the USA, The Chinese University of Hong Kong, Wound Repair and Regeneration, and Seung-Kyu Han. In addition, keyword co-occurrence networks, historical direct citation networks, thematic map, and the trend topics map summarize the research hotspots and trends in this field.

Conclusion: Current studies indicated that research on fibroblast-related DFUs is attracting increasing concern and have clinical implications. The cellular and molecular mechanisms of the DFU pathophysiological process, the molecular mechanisms and therapeutic targets associated with DFUs angiogenesis, and the measures to promote DFUs wound healing are three worthy research hotspots in this field.

KEYWORDS

diabetic foot ulcers (DFUs), fibroblast, bibliometric analysis, pathophysiological process, therapeutic targets

1 Introduction

Diabetes is a severe long-term disease that significantly impacting the lives of individuals, families, and societies globally (1). Globally, 463 million people are living with diabetes worldwide, and this number is predicted to increase by 25% in 2030 and 51% in 2045 (1). Diabetic foot ulcers (DFUs) are among the most frequent and severe complications of diabetes, which typically occur in response to neuropathy, peripheral vascular disease, and decreased resistance to infection (2). It is reported that the lifetime risk of developing DFUs in people with diabetes is potentially as high as 19-34% (3). DFUs are a primary contributor to hospitalizations and amputations in patients with diabetes, placing a significant demand on healthcare systems. The DFUs market alone is estimated to rise from USD 7.03 billion in 2019 to USD 11.05 billion by 2027 (4). In patients with diabetes, persistent hyperglycemia damages the nerves in the foot and ankle, leading to peripheral neuropathy. Combined with the narrowing of the arteries due to fatty deposits with subsequent decreased perfusion and tissue ischemia, this leads to peripheral arterial disease (5). These complications of diabetes can diminish sensation in the foot, leaving the patients more susceptible to injury and complications from DFUs. As DFUs are consistently non-healing, it may eventually lead to amputation, thus causing tremendous physical and psychological pain to the patient. Current DFUs wound care standards include unloading, infection control, debridement, and dressing coverage. As well as adjunctive therapies used in the event of DFU progression, such as hyperbaric oxygen and negative pressure wound therapy (6). However, although the treatment of DFUs has achieved some benefits, no satisfactory solution has been achieved so far. Many patients still have suffered amputations of lower limbs from further wound deterioration.

The tricky part of DFU treatment is that it is a chronic non-healing wound. The natural wound healing process consists of inflammatory, proliferative, and remodeling phases (7). In contrast, in DFUs, wound repair is stalled in the inflammatory phase, resulting in the inability of the wound to heal appropriately (5). Macrophages play an important role in this process. Several previous studies have shown that excessive activation of the M1 phenotype of macrophages and impaired M1 to M2 conversion are important mechanisms leading to non-healing of DFU wounds (8–10). However, the role of fibroblasts in DFU wounds cannot be ignored. Dermal fibroblasts are the key cells in wound healing (11). Following the end of the inflammatory phase, the fibroblasts migrate to the wounds in response to various cytokines released from the wound surface (12). They contribute dramatically to wound healing and control wound contraction by forming an extracellular matrix and secreting multiple cytokines (13). More importantly, increased apoptosis and functional disruption of fibroblast-related DFUs led to decreased production of cytokines and extracellular matrix and reduced proliferation and migration capacity, thus hindering wound healing. Therefore, insight into research hotspots and development trends in this area is critical to advancing molecular mechanisms of fibroblast-associated DFUs. More importantly, this may contribute to the potential therapeutic goals of accelerating DFU wound healing, avoiding amputation, and preventing DFU recurrence. In recent years, the explosion and popularity of bioinformatics, especially second-generation sequencing technologies and single-cell sequencing, have allowed researchers to study diabetic fibroblasts in depth and detail. Previous studies have shown that compared to normal fibroblasts, diabetic fibroblasts have a decreased ability to produce, assemble and remodel the extracellular matrix (14) and secrete the vascular endothelial growth factor VEGF. In addition, they have inhibited motility, proliferation, migration, and collagen synthesis (15, 16) and advanced cellular senescence (17), as well as alterations in metabolic memory associated with epigenetics (18).

Bibliometrics has become a popular methodology that assists in rapidly identifying research hotspots, trends, and frontiers in a specific research field based on statistics, network structures, and text analytics (19). Recently, it has been used extensively in multiple research areas, such as coronavirus disease, obesity, triple-negative breast cancer (TNBC), and pancreatic cancer (20–22). These contribute substantially to discovering the latest research hotspots and guiding clinical treatment. From among that, they have identified the newest research hotspots on TNBC, such as immunotherapy, targets, PARP inhibitors, TNBC protein, and receptors. They have taken a significant step forward in addressing drug resistance and tolerance issues to finding the best chemotherapy regimen. Although various meta-analyses and systematic reviews have explicitly addressed research on

fibroblast-related DFUs, there still needs to be bibliometric research providing the developing trends and research hotspots in this domain. Therefore, we compiled the scientific literature on the study of fibroblast-related DFUs since the 21st century derived from the Web of Science (WoSSC) database. Furthermore, Biblioshiny software, VOSviewer, and CiteSpace were used to visually analyze the retrieved literature to identify research hotspots and trends in this field (23–25). As there is no comparable bibliometric analysis of research on fibroblast-related DFUs, our work provided a research foundation, frontiers, future trends, and future research hotspots in this field.

2 Materials and methods

2.1 Search strategy

The Web of Science (WOS) is the greatest global database for collecting and retrieving publications from multiple academic disciplines. Searches were performed based on the WOS Core Collection (WOSCC) database to obtain literature in the Science Citation Index Expanded (SCI-EXPANDED) on April 27th, 2022. The literature retrieval strategy was as follows. literature type=article, year=2000-2022. (((TS=diabetic foot ulcer) OR (TS= diabetic foot)) AND ((TS= fibroblast) OR (TS=fibroblasts))). After excluding literature that did not meet the language and article type requirements, we selected the rest of the literature by assessing the title and abstract of articles to determine whether they should be included or excluded. The raw data can be found in Supplementary Material (Supplemental File 1). To avoid frequent database update bias, all literature searches and data extractions were performed on April 27th, and all results were imported into Bibliometrics analysis tools for further analysis.

2.2 Data analysis

VOSviewer (24) and Citespace (25) are tools commonly used in knowledge mapping and visualization analysis of scientific literature. Bibliometrix is an open-source tool for performing bibliometric analysis, comprehensive visualization, and knowledge mapping analysis (23). The original data retrieved from WOSCC were analyzed using the bibliometrix package in R version 4.2.0 (Institute for Statistics and Mathematics, Vienna, Austria; www.r-project.org). Biblioshiny software was primarily performed to visualize all retrieved literature and generate visual maps. A visual analysis of annual scientific output and average citation counts provides access to trends in the field. The impact of countries, institutions, authors, and journals is estimated through visual analysis of various bibliometric indicators such as production, citation counts, and H-index. H-index is commonly utilized to evaluate a scholar's scientific influence and outputs concisely and usefully. Inter-country and inter-author collaboration analyses were also performed, and country collaboration network and author collaboration network maps were generated. Subsequently, high-frequency keywords and highly cited literature analyses were performed. A keyword clustering network map and a historical direct citation network map were constructed to summarize the research hotspots in the field. Based on the analysis of the thematic map, trend topics map, and historical direct citation network map, we outlined the research frontiers and development of fibroblast-related DFUs. CiteSpace software version 6.1.2R was also performed to validate the analysis results (25).

3 Results

3.1 The growth of fibroblast-related DFUs is steadily increasing and arousing increasing concern

The total number of publications (NP) over a given period could quantitatively and objectively reflect the general development trend of a specific field. A total of 479 articles on fibroblast-related DFUs were published in the WOSCC from January 1st, 2000 to April 27th, 2022. The annual publications and the average number of annual citations are presented in Figures S1A, S1B. The overall trend in the number of documents related to fibroblast-related DFUs has gradually increased since 2000, despite some fluctuations during this period. The growth has been rapid since 2011 and maintained a high level after 2016. Additionally, the number of annual citations is increasing rapidly. These findings generally indicated that the research on fibroblast-related DFUs has gradually stabilized. It also meant that fibroblast-related DFUs are arousing growing concern and have significant clinical significance and potential for essential experimental development.

3.2 The USA and China were the most two influential and contributing countries in fibroblast-related DFUs research

The country scientific production map showed the distribution and numbers of publications by countries/regions worldwide (Figure S2A). The USA had the most publications (n=441), and its total citation is 4260 (Figure 1A), followed by China (321 records cited 2081 times) and Japan (116 records cited 1070 times). This indicated that the USA had the highest publication production and citations and is the leading prolific and impactful country for fibroblast-related DFUs research. According to the visualization country cooperation map (Figure 1B), the USA had the most significant central connection point, which indicated that the USA had the most collaborations with other publishing countries. While the line between the USA and China was the widest, it was noted that these two countries collaborated closely on fibroblast-related DFUs research. In contrast, the strength of research and inter-country partnerships in other countries can be further developed. Besides, Table 1 and Figure 1C illustrate the number of single-country and multi-country publications for the top 20 most productive countries/regions.

All institutions involved in the fibroblast-related DFUs were ranked based on the number of publications. Figure S2B shows the top 20 institutions with 326 relevant publications. The Chinese University of Hong Kong published the maximum

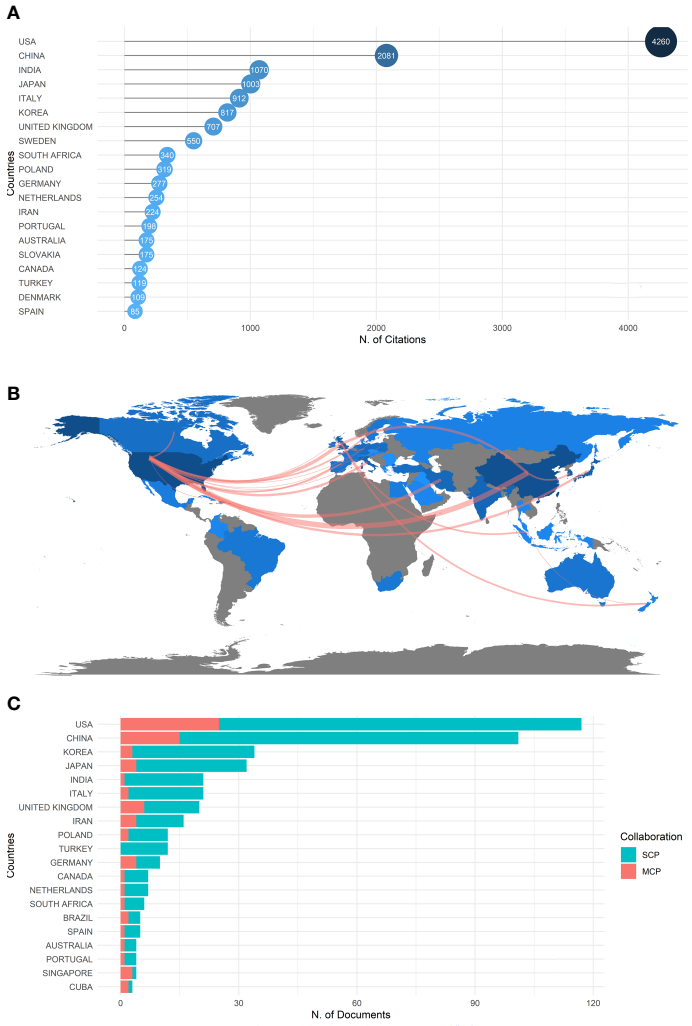

FIGURE 1
Central countries/regions of fibroblast-related DFUs research production and collaboration. The USA and China were the most two influential and contributing countries in fibroblast-related DFUs research. **(A)** The top 20 countries/regions of fibroblast-related DFUs research with the highest number of publications. **(B)** Countries/Regions production and collaboration world map of fibroblast-related DFUs research. **(C)** Single-country and multi-country publications for the top 20 most productive countries/regions.

number of publications (n=39), followed by Harvard University (n=59) and Shahid Beheshti University Medical Sciences (n=52). These three institutions are from China, the USA, and Iran. In summary, we hypothesized that the USA and China were the two most influential and contributing countries in fibroblast-related DFUs research.

3.3 The journal of wound repair and regeneration was a critical pathway to access the research frontiers and crucial information of fibroblast-related DFUs research

Since 2000, 243 sources have published articles on fibroblast-related DFUs research. Based on Bradford's Law, 19 high-production journals were classified as core sources based on the number of publications (Figure 2A) (26). The total number of articles published in the top 20 academic journals is 168 (Figure 2B), with 326 total citations (Figure S2C). The academic journal Wound Repair and Regeneration published the maximum number of articles (n=36), and its full citation is 664. Followed by the Journal of Wound Care (11 records cited 119 times), Wounds A Compendium of Clinical Research & Practice (11 records), International Wound Journal (10 records cited 156 times), and Acta Biomaterialia (9 records cited 139 times). These productive journals are essential sources of knowledge in this field. Wound Repair and Regeneration has the most publications and total citations, indicating its significant impact on fibroblast-related DFUs. Following this journal enables more rapid access to the research frontiers and crucial information in this field. Moreover, Figure 2C showed the growth in productivity with time for the top six most productive journals, which indicated that the number of publications per period of these journals increased rapidly.

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TABLE 1 Top 20 most productive countries/regions for fibroblast-related DFUs research.

Rank	Country	Publications	Proportion of Publications (%)	SCP	MCP	Proportion of MCP (%)
1	USA	117	24.48%	92	25	21.37%
2	China	101	21.13%	86	15	14.85%
3	Korea	34	7.11%	31	3	8.82%
4	Japan	32	6.70%	28	4	12.50%
5	India	21	4.39%	20	1	4.76%
6	Italy	21	4.39%	19	2	9.52%
7	United Kingdom	20	4.18%	14	6	30.00%
8	Iran	16	3.35%	12	4	25.00%
9	Poland	12	2.51%	10	2	16.67%
10	Turkey	12	2.51%	12	0	0.00%
11	Germany	10	2.09%	6	4	40.00%
12	Canada	7	1.46%	6	1	14.29%
13	Netherlands	7	1.46%	6	1	14.29%
14	South Africa	6	1.26%	5	1	16.67%
15	Brazil	5	1.05%	3	2	40.00%
16	Spain	5	1.05%	4	1	20.00%
17	Australia	4	0.84%	3	1	25.00%
18	Portugal	4	0.84%	3	1	25.00%
19	Singapore	4	0.84%	1	3	75.00%
20	Cuba	3	0.63%	1	2	66.67%

SCP, single-country publications; MCP, multiple-country publications.

3.4 Woo Kyung Kim and Jonathan A. Garlick were the most two influential and contributing countries in fibroblast-related DFUs research

The H index is predominantly used to evaluate the total influential power of a specific author (27). Since 2000, over 2650 authors have participated in publications on fibroblast-related DFUs research, and 15 authors had more than 25 publications. We identified the top 20 most productive authors, with 168 articles accounting for 35.07% of all articles. The top 20 most productive authors, the top 20 most locally cited authors, and the top 20 most locally influential authors measured by the H-index are presented in Figures S3A–C. Seung-Kyu Han had the most publications (n=18), total citations (n=74), and H-index (n=12). Woo Kyung Kim and Jonathan A. Garlick were relative leaders in each indicator. These authors contributed significantly and notably impacted fibroblast-related DFUs research. Seung-Kyu Han developed a fresh human fibroblast allograft approach and achieved positive results in clinical studies, laying a solid foundation for subsequent research (28). Additionally, according to the author's collaboration network map (Figure S4A), Jonathan A. Garlick seemed to be the author with the most significant collaborative network. Figure S4 shows that Lin Yan has been a relatively active author recently.

3.5 Analysis of high-frequency keywords and four research hotspots based on the keyword co-occurrence analysis

Keywords are highly condensed versions of the critical content of the article and can efficiently identify research hotspots and other significant points (29). Figure 3A showed the growth in frequency with time for the top 10 most frequent keywords. It indicated that the keyword "expression" frequency had risen rapidly since 2014, especially after 2019, when it jumped to the number one position. Subsequently, we identified the top 50 high-frequency keywords for fibroblast-related DFUs research with a word cloud (Figure 3B) and a tree map (Figure S5). Specifically, "expression" had the most frequency of occurrence (n=83), followed by "foot ulcers" (n=76), "diabetic foot ulcers" (n=72), "fibroblasts" (n=72), angiogenesis" (n=63), "proliferation" (n=47), "cells" (n=45), "skin" (n=44), "*in-vitro*" (n=42), and "foot" (n=39). More importantly, Biblioshiny software was performed for keyword co-occurrence analysis and categorized relevant keywords into 4 clusters, thus forming a keyword clustering network map (Figure 3C). These clusters reflected the preliminary study content and core research regions to which the keywords referred (30). Within the keyword co-occurrence network graph, each node represents a keyword, and

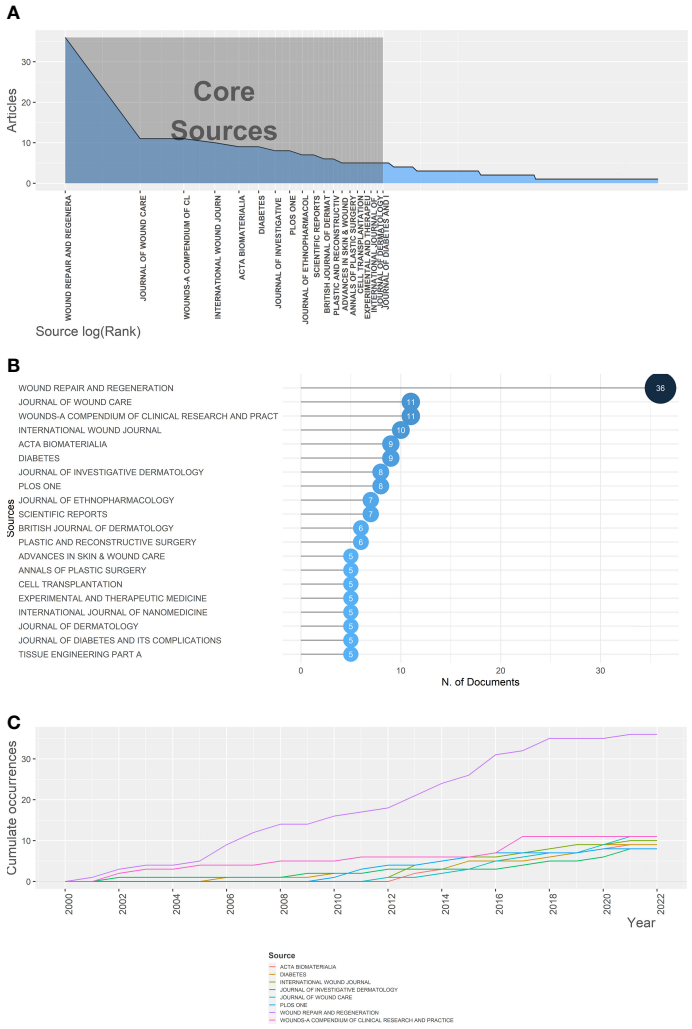

FIGURE 2
The Journal of Wound Repair and Regeneration was a critical pathway to access the research frontiers and crucial information of fibroblast-related DFUs research. **(A)** Core journals of fibroblast-related DFUs research based on Bradford's Law. **(B)** The top 20 journals on fibroblast-related DFUs research with the highest number of publications. **(C)** The six highest yielding journals growth of fibroblast-related DFUs research from 2000 to 2022.

the node size represents the popularity; the line between the nodes indicates the intimacy between the keywords.

Clusters1 (red): Mechanisms of fibroblasts in DFUs pathophysiological process and application of fibroblast-derived related materials. The crucial keywords in this group include "diabetic foot ulcers" (avg. pub. per year as of 2022. 72, 3.27 occurrences), "fibroblasts" (avg. pub. per year as of 2022. 72, 3.27 occurrences), "efficacy" (avg. pub. per year as of 2022. 26, 1.18 occurrences) and "management" (avg. pub. per year as of 2022. 26, 1.18 occurrences).

Cluster2 (blue): The molecular mechanisms and therapeutic targets associated with DFUs angiogenesis. The most recent four hot topics in this cluster include "expression" (avg. pub. per year as of 2022. 83, 3.77 occurrences), "foot ulcers" (avg. pub. per year as of 2022. 76, 3.45 occurrences), "angiogenesis" (avg. pub. per year as of 2022. 63, 2.86 occurrences) and "cells" (avg. pub. per year as of 2022. 45, 2.05 occurrences).

Cluster 3 (green): Bioengineered scaffolds for cutaneous wound healing. The most recent four hot topics in this cluster include "proliferation" (avg. pub. per year as of 2022. 47, 2.14 occurrences), "skin" (avg. pub. per year as of 2022. 44, 2 occurrences), and "foot" (avg. pub. per year as of 2022. 39, 1.77 occurrences), "migration" (avg. pub. per year as of 2022. 32, 1.45 occurrences).

Cluster 4 (purple): Validation of fibroblast differentiation-related mechanisms in DFUs in an *in vitro* model. The most recent four hot topics in this cluster include "*in-vitro*" (avg. pub. per year as of 2022. 42, 2.1 occurrences), "model" (avg. pub. per year as of 2022. 28, 1.27 occurrences), "tissue" (avg. pub. per year as of 2022. 28, 1.27 occurrences) and "differentiation" (avg. pub. per year as of 2022. 27, 1.23 occurrences).

Research on the molecular mechanisms involved in the pathophysiological process of DFUs, the potential therapeutic targets, and the value of bioengineered scaffolds in wound healing

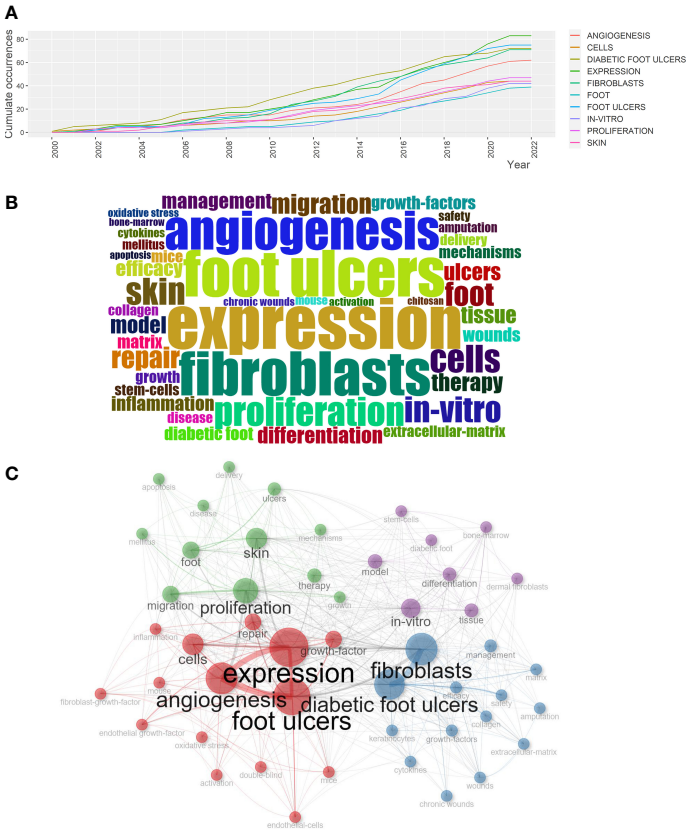

FIGURE 3
Analysis of high frequency keywords and four research hotspots based on the keyword co-occurrence analysis. **(A)** Top 10 most frequent keywords growth of fibroblast-related DFUs research from 2000 to 2022. **(B)** Visualized word cloud map based on the top 50 most frequent keywords for fibroblast-related DFUs research. **(C)** Visualized keywords co-occurrence network for fibroblast-related DFUs research. Each node indicates a keyword, and the connecting lines between nodes denote the intimacy between keywords. The four clusters were red, blue, green, and purple.

in translational medicine have been highly investigated and were the primary research directions.

3.6 Relationship between high-impact literature and historical evolution and hotspots

Overall, global citations reflect the impact of an article on the whole database, while local citations reflect the influence of a particular article in our retrieval collection. The top 20 most locally cited documents among the 479 publications were summarized in Table 2, along with their journals, authors, and years of publication. Figure 4A and Table 3 showed the top 20 most global cited documents, and 16 documents had more than 130 citations. The article of William A Marston (31) with the title "The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers," which was published in 2003 in Diabetes Care, was the most local cited article (49 citations). Followed by the article with the title "Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia" by Oren Z. Lerman in 2003 from The American Journal of Pathology with 27 local citations. Then the article "Clinical application of fresh fibroblast allografts for the treatment of diabetic foot ulcers: a pilot study" by Seung-Kyu Han in 2004 from Plastic and Reconstructive Surgery. More importantly, these articles revealed the mechanisms underlying the role of fibroblast dysfunction in non-healing DFUs wounds. The safety and efficacy of human fibroblast-derived dermal substitutes in promoting DFUs healing were demonstrated. A solid foundation has been laid to guide the clinical treatment of complex refractory DFUs.

Subsequently, to acquire the interrelationships between this literature and the historical evolution and hotspots of the field, the software performed the historical direct citation network analysis, and a visual map was generated (Figure 4B). Each node represents a piece of literature, and the lines between the nodes indicate the citation relationships between publications. Articles with similar subjects and keywords would be integrated into the same cluster. Moreover, articles with a high normalized local citation score were considered vital documents. Based on these connections, papers were grouped into five clusters representing the four research themes of fibroblast-related DFUs since the 21st century. The first cluster (red) can be traced back to 2002 (32), when scholars, represented by Loots, worked on mechanisms related to DFUs generation and healing in the diabetic

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TABLE 2 Top 20 most local cited documents for fibroblast-related DFUs research.

Rank	Title	Journal	Author	Year	Local Citations	Global Citations	LC/GC Ratio (%)
1	The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial.	DIABETES CARE	MARSTON WA	2003	49	437	11.21%
2	Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia.	AM J PATHOL	LERMAN OZ	2003	27	318	8.49%
3	Clinical application of fresh fibroblast allografts for the treatment of diabetic foot ulcers: a pilot study.	PLAST RECONSTR SURG	HAN SK	2004	21	30	70.00%
4	Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers.	WOUND REPAIR REGEN	GALKOWSKA H	2006	19	184	10.33%
5	Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls.	EUR J CELL BIOL	LOOTS MAM	2002	15	121	12.40%
6	Mechanisms involved in the development and healing of diabetic foot ulceration.	DIABETES	DINH T	2012	13	202	6.44%
7	Clinical efficacy of basic fibroblast growth factor (bFGF) for diabetic ulcer.	EUR J DERMATOL	UCHI H	2009	12	111	10.81%
8	Altered ECM deposition by diabetic foot ulcer-derived fibroblasts implicates fibronectin in chronic wound repair.	WOUND REPAIR REGEN	MAIONE AG	2016	10	39	25.64%
9	Potential of human bone marrow stromal cells to accelerate wound healing *in vitro*.	ANN PLAS SURG	HAN SK	2005	9	57	15.79%
10	Efficacy and safety of fresh fibroblast allografts in the treatment of diabetic foot ulcers.	DERMATOL SURG	HAN SK	2009	9	20	45.00%
11	Effect of human bone marrow stromal cells and dermal fibroblasts on collagen synthesis and epithelization.	ANN PLAS SURG	LEE CH	2007	8	12	66.67%
12	Investigation of the effects of Chinese medicine on fibroblast viability: implications in wound healing.	PHYTOTHER RES	LAU TW	2007	7	26	26.92%
13	Effect of human bone marrow stromal cell allograft on proliferation and collagen synthesis of diabetic fibroblasts *in vitro*.	J PLAST RECONSTR AES	KIM JB	2010	7	10	70.00%
14	Fibrin-based scaffold incorporating VEGF- and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice.	ACTA BIOMATER	LOSI P	2013	7	196	3.57%
15	Genome-wide DNA methylation analysis identifies a metabolic memory profile in patient-derived diabetic foot ulcer fibroblasts.	EPIGENETICS-US	PARK LK	2014	7	33	21.21%
16	Autologous fibroblasts to treat deep and complicated leg ulcers in diabetic patients.	WOUND REPAIR REGEN	CAVALLINI M	2007	6	18	33.33%
17	Stabilization of HIF-1alpha is critical to improve wound healing in diabetic mice.	P NATL ACAD SCI USA	BOTUSAN IR	2008	6	329	1.82%
18	The *in vivo* and *in vitro* diabetic wound healing effects of a 2-herb formula and its mechanisms of action.	J ETHNOPHARMACOL	TAM JCW	2011	6	82	7.32%
19	Overexpression of the gap junction protein Cx43 as found in diabetic foot ulcers can retard fibroblast migration.	CELL BIOL INT	MENDOZA-NARANJO A	2012	6	37	16.22%
20	Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing.	WOUND REPAIR REGEN	CIANFARANI F	2013	6	127	4.72%

GCs, Global Citations; LCs, local citations.

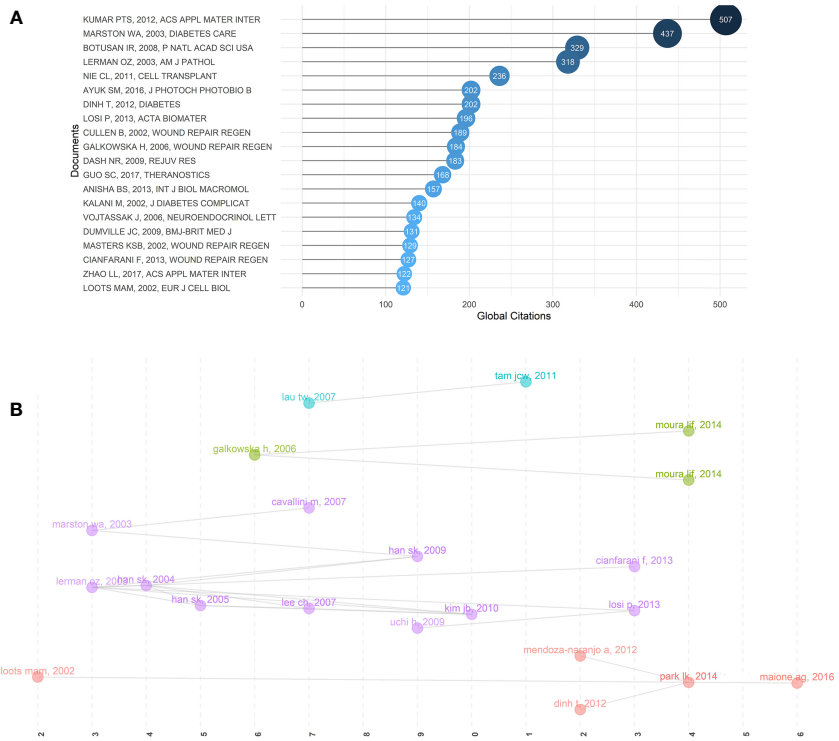

FIGURE 4
Relationship between high-impact literature and historical evolution and hotspots. **(A)** The top 20 most global cited documents of fibroblast-related DFUs research. **(B)** Visualized historical direct citation network based on the evolution trend of fibroblast-related DFUs research from 2000 to 2022. Each node represents a piece of literature, and the lines between the nodes indicate the citation relationships between publications.

microenvironment (32). Including increased expression of Cx43 in DFUs dermal fibroblasts retarded fibroblasts migration (33). Increased inflammatory response, expression of inflammatory factors, and abnormal growth factor levels are the primary factors associated with DFUs failure to heal. Targeting these factors may assist in the management of DFUs (34). The second group (purple) focused on several potential therapeutic measures to promote DFUs wound healing (31, 35–39). Clinical study results demonstrated that human fibroblast cell-derived dermal substitutes and autologous *in vitro* expanded fibroblasts are a safe and effective treatment for DFU (31). Besides, the basic fibroblast growth factor (bFGF) also promotes wound healing in patients with DFU (37).

The third cluster (green) is centralized on the mechanisms of dysfunction of fibroblast migration and release of associated growth factors in DFU and associated therapeutic measures (40–42). Reduced expression of leukocyte chemokines and growth factors at the margins of DFU wounds, resultant angiogenesis in DFU wounds, and impaired fibroblast chemotaxis may explain the poor granulation tissue formation and chronic epithelialization of ulcers (41). Neurotensin-loaded collagen dressings significantly stimulate fibroblast migration and collagen deposition by inhibiting the expression of inflammatory factors, thus promoting DFU wound healing (40, 42). The fourth cluster (blue) is centralized on the potential mechanism of Chinese herbal formula made from the herbs Radix Rehmanniae and Radix Astragali in promoting wound healing in DFU. Chinese herbal formula made from the herbs Radix Rehmanniae and Radix Astragali promotes human fibroblast proliferation and angiogenic and anti-inflammatory effects by increasing fibroblast activity in DFU patients, thereby facilitating the healing of DFU wounds (43, 44). Accordingly, we hypothesized that these research themes might indicate the evolution of research hotspots in the research field of fibroblast-related DFUs.

3.7 The research status of various hot topics on fibroblast-related DFUs

Biblioshiny software was performed to construct a two-dimensional thematic map with density as the y-axis and centrality as the x-axis (Figure 5A). Density represents the development degree of a single theme, and higher density values mean higher maturity of the theme. Centrality indicates the degree of intimacy with different themes, and high centrality means the heart of the research field.

Motor themes represent the core themes with high centrality and maturity. The crucial keywords in this group include “diabetic foot ulcers”, “fibroblasts”, and “model”. Consistent with clustering 1 in the keyword co-occurrence network, the efficacy of human fibroblast-derived skin substitutes on DFU healing was revealed. Clinical studies have shown that human fibroblast-derived skin substitutes can safely and effectively promote wound healing (45–47). This subject has long been of interest to scholars, and concrete results have been achieved. However, substantial breakthrough research is still urgently needed to

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TABLE 3 Top 20 most global cited documents on fibroblast-related DFUs research.

Rank	TiTle	Author	Journal	Year	TC	TC per Year
1	Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: *in vitro* and *in vivo* evaluation.	KUMAR PTS	ACS APPL MATER INTER	2012	507	46.0909
2	The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial.	MARSTON WA	DIABETES CARE	2003	437	21.85
3	Stabilization of HIF-1alpha is critical to improve wound healing in diabetic mice.	BOTUSAN IR	P NATL ACAD SCI USA	2008	329	21.9333
4	Cellular dysfunction in the diabetic fibroblast	LERMAN OZ	AM J PATHOL	2003	318	15.9
5	Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis.	NIE CL	CELL TRANSPLANT	2011	236	19.6667
6	The role of photobiomodulation on gene expression of cell adhesion molecules in diabetic wounded fibroblasts *in vitro*.	AYUK SM	J PHOTOCH PHOTOBIO B	2016	202	28.8571
7	Mechanisms involved in the development and healing of diabetic foot ulceration.	DINH T	DIABETES CARE	2012	202	18.3636
8	Fibrin-based scaffold incorporating VEGF- and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice.	LOSI P	ACTA BIOMATER	2013	196	19.6
9	Mechanism of action of PROMOGRAN, a protease modulating matrix, for the treatment of diabetic foot ulcers.	CULLEN B	WOUND REPAIR REGEN	2002	189	9
10	Chemokines, cytokines, and growth factors in keratinocytes and dermal endothelial cells in the margin of chronic diabetic foot ulcers.	GALKOWSKA H	WOUND REPAIR REGEN	2006	184	10.8235
11	Targeting nonhealing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells.	DASH NR	REJUV RES	2009	183	13.0714
12	Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds *via* activation of YAP in a diabetic rat model.	GUO SC	THERANOSTICS	2017	168	28
13	Chitosan-hyaluronic acid/nano silver composite sponges for drug resistant bacteria infected diabetic wounds.	ANISHA BS	INT J BIOL MACROMOL	2013	157	15.7
14	Hyperbaric oxygen (HBO) therapy in treatment of diabetic foot ulcers	KALANI M	J DIABETES COMPLICAT	2002	140	6.6667
15	Autologous biograft and mesenchymal stem cells in treatment of the diabetic foot.	VOJTASSAK J	NEUROENDOCRINOL LETT	2006	134	7.8824
16	Larval therapy for leg ulcers (VenUS II): randomised controlled trial.	DUMVILLE JC	BMJ-BRIT MED J	2009	131	9.3571
17	Effects of nitric oxide releasing poly(vinyl alcohol) hydrogel dressings on dermal wound healing in diabetic mice.	MASTERS KSB	WOUND REPAIR REGEN	2002	129	6.1429
18	Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing.	CIANFARANI F	WOUND REPAIR REGEN	2013	127	12.7
19	pH and Glucose Dual-Responsive Injectable Hydrogels with Insulin and Fibroblasts as Bioactive Dressings for Diabetic Wound Healing.	ZHAO LL	ACS APPL MATER INTER	2017	122	20.3333
20	Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls.	LOOTS MAM	EUR J CELL BIOL	2002	121	5.7619

TCs, total citations.

drive further developments. Niche themes represent isolated themes with high maturity. The critical keywords in this group include "*in-vitro*", "skin" and "tissue". These themes are dedicated to the *in vitro* validation of measures to promote diabetic foot wound healing and related mechanisms. In 1999, the International Diabetic Foot Working Group published an international consensus and guidelines on the management and prevention of diabetic foot, bringing milestones in the management of diabetic foot (48). With the tireless efforts of researchers over the past decades, the physiological knowledge of wound healing and tissue repair, as well as the mechanisms of nonhealing diabetic foot wounds, has become increasingly sophisticated (49–52), which includes an imbalance between the accumulation of ECM components and their remodeling by tissue degrading matrix metalloproteinase(MMPs) (53), reduced or impaired production of growth factors (54, 55), impaired proliferation and migration of keratinocytes and fibroblasts (56). Further, tissue engineering of skin has been developed and extensively studied (57–59). Nevertheless, most of the studies are still in the *in vitro* stage, and high-quality clinical RCT studies may be needed to achieve translation from basic to clinical.

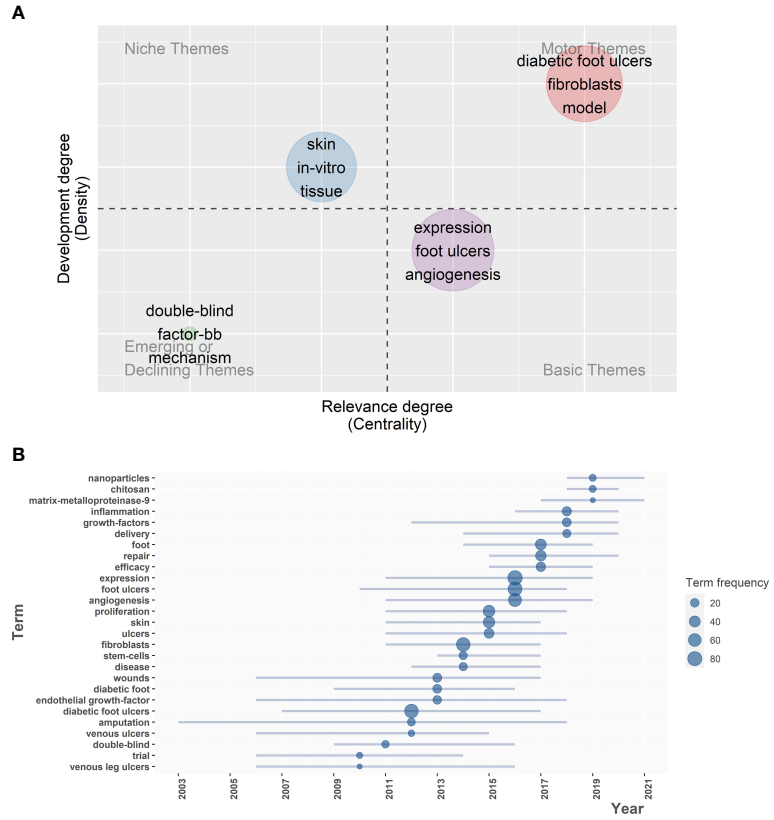

FIGURE 5
Exploring research status of various hot topics on fibroblast-related DFUs, sketching historical trajectories and revealing research frontiers. **(A)** Thematic map for fibroblast-related DFUs research. The horizontal coordinate refers to the relevance degree (centrality), and the vertical coordinate represents the development degree (density). Motor themes in the first quadrant represents core themes with high centrality and maturity, niche themes in the second quadrant represent isolated themes with increased maturity, the third quadrant represents emerging or declining themes with low centrality and high maturity, and basic themes in the fourth quadrant means popular themes with low maturity. **(B)** Trend topics map for fibroblast-related DFUs research. Showing trends in the occurrence of high frequency keywords for fibroblast-related DFUs research.

Emerging or declining themes indicate low centrality and maturity themes. The crucial keywords in this group include “double-blind”, “factor-bb”, and “mechanism”. These themes are recommended in the clinical study of human platelet-derived growth factor-BB (becaplermin) in treating patients with DFU. Platelet-derived growth factor-BB (becaplermin) is the most studied growth factor, and its local application in DFU has shown some success. Back in 1998, researchers conducted a phase III randomized, placebo-controlled, double-blind study. It investigated the efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers (60). Basic themes represent hot themes with low maturity. The empath keywords in this group include “expression”, “foot ulcers”, and “angiogenesis”. Consistent with clustering 2 in the keyword co-occurrence network, these themes are recommended as the molecular mechanisms and therapeutic targets associated with DFU angiogenesis. With the widespread availability of high-throughput sequencing technology in 2010 and the development of single-cell sequencing technology since 2013, extensive multi-omics and phenotypic correlation studies have been carried out. Consequently, researchers extensively investigated the molecular mechanisms associated with angiogenesis in diabetic foot ulcer wounds and their upstream and downstream potential therapeutic targets. This was a sacred step from basic research to translation to clinical application.

3.8 Sketching historical trajectories and exploring research frontiers through trend topics analysis

The trend topics analysis contributes to exploring research hotspots evolution, historical development trajectory, and future research directions in fibroblast-related DFUs. Biblioshiny software was performed to construct the trend topics map (Figure 5B). According to Figure 5B, it was observed that the evolution of topics related to the research of fibroblast-related DFUs is closely associated with the development of bioinformatics. In patients with diabetic foot, diabetic foot ulcers can be caused by pathogenic factors such as chronic inflammation, peripheral arterial disease, and peripheral neuropathy. However, chronic wound development caused by untreated DFU can often lead to amputation. Hence, Prior to 2010, key topics included “trials”, “venous leg ulcer” and “amputation”, etc. This was probably attributed to the fact that technologies such as high-throughput sequencing were not widespread then, and clinically relevant studies could only be conducted for complications related to diabetic foot

ulcers to resolve the patient's suffering as soon as possible. During this time, researchers have explored multiple pathophysiological mechanisms of DFU trauma and associated therapeutic targets. These include inhibition of fibroblast proliferation migration (61), decreased growth factor release, impaired angiogenesis, disrupted collagen accumulation, and increased levels of MMPs (62). Based on this, various therapeutic measures have been developed and applied to contribute significantly to the clinical management of patients with DFU. These included the use of platelet-derived growth factors, epidermal growth factors (63), inhibition of MMP release (62), and skin substitutes containing components such as collagen and fibroblasts to promote the healing of DFU wounds effectively (53). In contrast, after 2010, the explosion and popularity of second-generation sequencing (64) and single-cell sequencing technologies (64). Scientists turned to study the molecular mechanisms involved in the pathophysiological process of DFU and the potential therapeutic targets. Accordingly, the topic has gradually shifted to "expression", "angiogenesis", and "inflammation", and peaked around 2016, which coincides with the peak in annual publications production.

Additionally, critical topics in recent years were focused on "chitosan", "nanoparticles", and "matrix-metalloproteinase-9". Recent studies have shown that squilla chitosan nanosilver-metal complex and chitosan-hyaluronic acid/nano-silver antimicrobial sponges can be used as potential dressings for wounds infected with DFU-resistant bacteria and effectively inhibit infections with drug-resistant bacteria such as Staphylococcus aureus and Pseudomonas aeruginosa (65, 66). The newly developed chitosan nanoparticle drug delivery system loaded with growth factors and metal oxides effectively promotes the healing of DFU wounds. This includes the removal of pathogens in biofilm structure, a reduced inflammatory response, thorough re-epithelization, and advanced collagen deposition and maturation (67, 68). Therefore, we speculated that applying chitosan-based nanoparticles in DFUs might be a trending topic in the future.

4 Discussion

In the present study, we analyzed publications on the research of fibroblast-related DFUs from January 1, 2000 to April 27, 2022 with an information visualization approach. A total of 479 relevant articles were retrieved. The results showed that the trend of publications on the study of this field has continued to grow over time worldwide, exposing that fibroblast-related DFUs have attracted widespread attention from researchers and provided a rich basis for subsequent analyses. The top three countries with a high number of publications and citations were the United States, China, and Japan. Wound Repair and Regeneration, Journal of Wound Care, and Wounds A Compendium of Clinical Research & Practice are the top three most prolific journals. Seung-Kyu Han, Woo Kyung Kim, and Jonathan A. Garlick are the most influential authors with significant status in the field. Subsequently, Biblioshiny software was performed to analyze high-frequency keywords, highly cited documents, and keyword co-occurrence networks. Combined with the results of these analyses, we identified three research hotspots in the fibroblast-related DFUs: the cellular and molecular mechanisms of DFU pathophysiological process, molecular mechanisms and therapeutic targets associated with DFU angiogenesis, and the measures to promote DFUs wound healing. Additionally, bioengineered scaffolds for promoting DFU wound healing are potential directions for researchers to focus on. According to the National Science Foundation Workshop, scaffolds are the ideal resource for repairing, maintaining, and facilitating tissue function (69). Multiple scaffolds have recently been developed as potential materials to promote skin tissue healing (70). Among them mainly included decellularized scaffolds with collagen-rich matrices, microsphere scaffolds composed of a variety of natural polymers, hydrogel scaffolds made up of naturally derived macromolecules or synthetic polymers, and porous scaffolds composed of nanofibers (71–75). Play a unique role in tissue repair and regeneration by providing a suitable platform for supplying various factors associated with cell proliferation and differentiation (75, 76).

Subsequently, the analysis combines a historical direct citation network, a thematic map, and trend topics map. We analyzed the evolution of research hotspots in the field and speculated that applying chitosan-based nanoparticles in DFUs might be a future research direction. In a particular bibliometric analysis, keyword analysis is one of the most indispensable parts, which reflects the general contents and themes of a specific article and represents the research hotspots. The keywords' variation over time shows the evolution of the field. The research hotspots in the area of fibroblast-related DFUs were summarized as follows.

4.1 Cellular and molecular mechanisms of DFUs pathophysiological process

DFU is one of the most popular and severe complications of diabetes. The development of DFUs typically occurs in response to neuropathy, peripheral vascular disease, and decreased resistance to infection (2). The persistent non-healing of DFUs wounds may eventually evolve into serious complications such as amputation, causing significant physical and psychological damage to the patient. Thus, an adequate understanding of the mechanisms of functional alterations of DFUs is essential for finding relevant therapeutic targets to promote the healing of diabetic foot ulcers. Cutaneous wound healing is a complex time-dependent multicellular process separated into three overlapping phases: inflammation, proliferation, and remodeling. During this process, multiple cells in the skin, including fibroblasts, keratocytes, and macrophages play essential roles. However, in the diabetic foot wound microenvironment, the normal progression of these phases is impeded, and cellular functions are altered, contributing to a persistent inflammatory state and dysfunctional epithelialization of the wound, ultimately leading to chronic wounds Figures 6, 7.

4.1.1 Fibroblasts and keratocytes

As early as 1977, Rowe's laboratory (77) pioneered an *in vitro* model of diabetic fibroblasts and demonstrated reduced synthesis, proliferation, and secretion of skin fibroblasts in diabetic patients. This established a solid foundation for the study of fibroblast-related DFUs. Loots' (61) study showed similar results with reduced proliferative capacity and abnormal morphology of diabetic ulcer fibroblasts compared to the control group. In subsequent studies, the

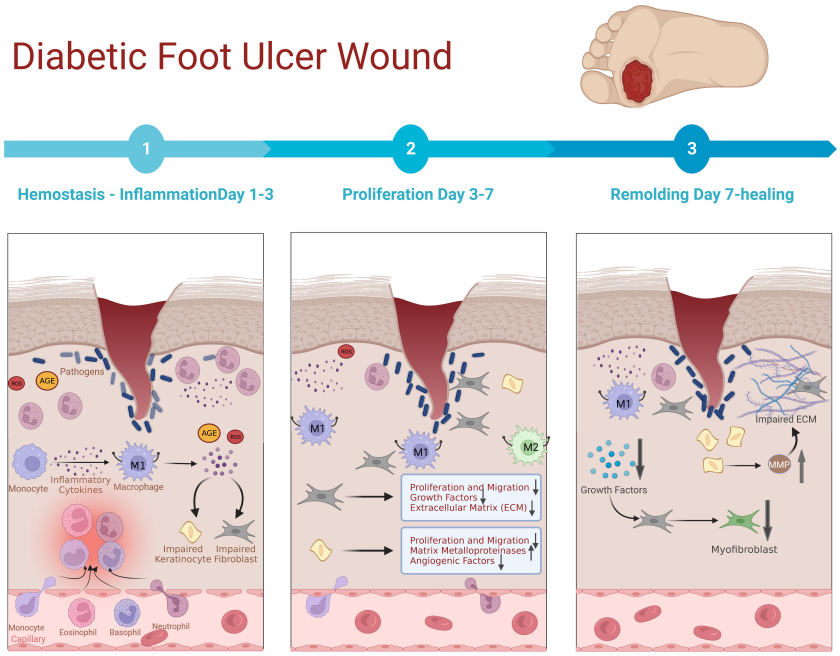

FIGURE 6
The molecular mechanisms of fibroblast and keratinocyte pathophysiological process in DFUs.The persistent non-healing of DFU wounds is the result of a combination of factors leading to a constant and excessive chronic inflammatory response. In the microenvironment of diabetic wounds, perturbations are associated with hyperglycaemia, advanced glycation end products, oxidative stress and impaired angiogenesis. These factors comprise impaired fibroblasts and disruption of their proliferation, migration, secretion of extracellular matrix and differentiation into myofibroblasts. Meanwhile, there is keratinocyte migration and proliferation, reduced angiogenesis, chronic Inflammation, and abnormal expression of MMPs. Resulting in a constant and excessive chronic inflammatory response, disrupting epithelial cell formation and eventual wound closure. Ultimately leading to the development of chronic non-healing wounds.

team further discovered that the diabetic environment reduced the ability of fibroblasts to respond to growth factors such as platelet-derived growth factor (PDGF), resulting in abnormal fibroblast function and delayed wound healing. Accordingly, the combination of growth factor PDGF-AB and insulin-like growth factor (IGF-I) treatment may promote diabetic wound healing (32). It was suggested that increased expression of Cx43 may be an underlying cause of poor fibroblast migration and reduced healing rates in diabetic ulcers. The gap junction protein Cx43 (Connexin 43) is central in wound healing (78). In the normal acute wound, keratocytes Cx43 typically downregulate within the first 24-48 hours to migrate toward the wound surface and promote healing (79). A tenfold increase in Cx43 expression in human dermal fibroblasts biopsied from DFUs, which retarded the migration of fibroblasts (33). Similarly, Pollok et al. identified that at the edges of diabetic wounds, there was increased expression of CD43, which induced impaired proliferation and migration of fibroblasts and keratinocytes (80). Besides, Xuan et al. discovered that a high glucose environment inhibited the migration of human fibroblasts in wound healing, which was achieved by inhibiting BFGF to regulate JNK phosphorylation (81). Be note, in the diabetic microenvironment, disturbances accompanied by hyperglycemia, oxidative stress, late glycosylation end products, and impaired angiogenesis. It induces impaired polarization of M2 macrophages, contributing to increased secretion of pro-inflammatory cytokines and a significant decrease in the secretion of anti-inflammatory and growth factors (82). This consequently provokes fibroblasts and keratinocyte damage, impaired proliferation and migration capacity, and wound re-epithelialization.

In addition, high levels of pro-inflammatory cytokines increase the production of matrix metalloproteinases (e.g., MMP9) along with the inhibition of matrix metalloproteinase inhibitor expression. This imbalance further exacerbates extracellular matrix degradation and deprives cells of a scaffold for migration (83). Consequently, fibroblast migration, proliferation, and collagen synthesis are impaired, as well as disrupted wound closure.

Beyond the decreased ability of fibroblasts to proliferate and migrate and secrete growth factors, the power of diabetic fibroblasts to synthesize and secrete extracellular matrix is also disrupted. It was revealed by using a three-dimensional self-assembling ECM model that DFUs-derived fibroblasts have a reduced ability to express, produce, and assemble ECM proteins compared to healthy donor-derived

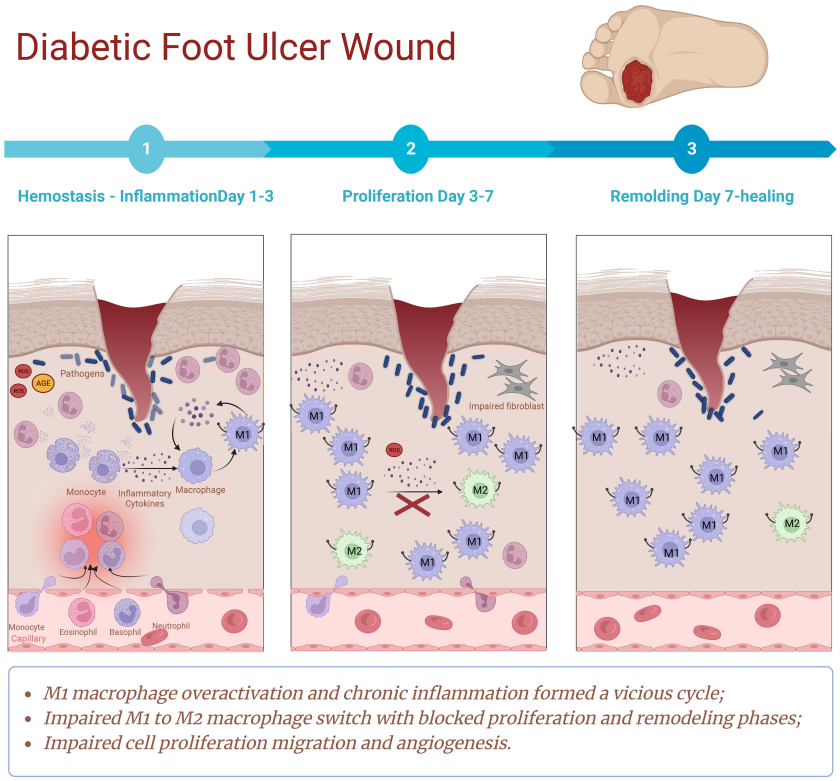

FIGURE 7
The molecular mechanisms of macrophage pathophysiological process in DFUs. Macrophages play a crucial role in routine wound healing, promoting angiogenesis, collagen deposition and wound closure. Over-activation of M1 macrophages and impaired transition from M1 to M2 phenotype are essential differences between normal and diabetic wound healing. In normal wounds, macrophages clear pathogens and cellular debris by activating a pro-inflammatory phenotype. As the inflammatory phase progresses, macrophages shift from pro-inflammatory phenotype to pro-repair phenotype. As a result, it stimulates the proliferation, differentiation and migration of keratinocytes, fibroblasts and endothelial cells by secreting cytokines and growth factors, which directly or indirectly regulate the proliferative phase of the repair process. However, in the microenvironment of diabetic wounds, perturbations associated with hyperglycaemia, advanced glycation end products, oxidative stress and impaired angiogenesis induce disturbances in the immune microenvironment of diabetic wounds, leading to phenotypic dysregulation as well as quantitative, functional, and epigenetic alterations in traumatic macrophages. As a result, M1 polarization is enhanced and the switch from M1 to M2 is severely impaired. This culminates in a situation where lower numbers of M2 macrophages and higher M1/M2 ratios release low levels of growth factors. Meanwhile, the diabetic microenvironment resulted in macrophage sensitivity to pro-inflammatory cytokines and stimulated macrophages to secrete pro-inflammatory cytokines such as IL-1, IL-6, MMP9, and TNF-a. This further exacerbates the vicious cycle of M1 macrophage polarization and chronic inflammation, causing stagnation of the inflammatory phase. Which created an excess inflammatory cytokines microenvironment, ultimately contributed to impaired fibroblast and keratinocyte migration and delayed wound healing.

fibroblasts, producing a thin, fibronectin-rich matrix that is involved in the non-healing of diabetic foot wounds leading to the development of DFUs (14). Besides, the early or excessive expression of many aging markers in type 2 Diabetes mellitus contributes to the disruption of diabetic fibroblast function. Senescence is a cellular program that instills proliferative stasis associated with morphological changes, metabolic reprogramming, increased autophagy, apoptosis resistance, and epigenetic reprogramming (84–86). Recent studies suggest that hyperglycemia/oxidative stress/mitochondrial and DNA damage may be the main drivers shaping the senescence phenotype. These adverse agents may trigger replicative senescence of fibroblasts and endothelial cells, thereby impeding DFUs wound healing (17). Wilkinson et al. Discovered that in diabetic mouse trauma, reduced polarization of M2 macrophages resulted in the production of a CXCR2-rich senescence-associated phenotype (85). This induces fibrogenic markers in fibroblasts and ultimately accelerates fibroblast senescence. In a recent study, the Notch pathway, negatively correlated with fibroblast activity, was activated in fibroblasts from the diabetic wound. Increased Notch1 activity inhibits fibroblasts' growth, migration, and differentiation into myofibroblasts (87). Notch1 signaling dictates the plasticity and function of fibroblasts in wound healing and angiogenesis, and intracellular Notch1 signaling in fibroblasts may represent a potential target for therapeutic intervention in diabetic wound healing (87).

What's more, various dysfunctions of keratocytes in the DFUs microenvironment have been suggested to be a key factor in non-healing wounds (88). These factors comprise impaired keratinocyte migration and proliferation, reduced angiogenesis, chronic inflammation and infections, oxidative stress. As well as gap junction abnormalities, and abnormal expression of MMPs (49, 89, 90). It was observed that in the diabetic environment, the proliferation and migration of keratinocytes are impaired. This appears to be associated with decreased focal adhesion kinase expression (p125FAK), which determines keratinocyte motility. In addition, elevated expression of various connexins seems to be involved in this process (91, 92).

4.1.2 Macrophages

The persistent non-healing of DFU wounds results from a combination of factors leading to a constant and excessive chronic inflammatory response, disrupting epithelial cell formation and eventual wound closure. Several previous studies have shown that macrophages recruited to the wound site are a vital component of the healing process (93, 94). Macrophages have high plasticity and perform critical roles in all phases of wound repair through host defense, cellular regulatory functions, and tissue debridement (95). Elie Metchnikoff, the father of innate and cellular immunity, first discovered Macrophages, for which he was awarded the Nobel Prize in Physiology or Medicine in 1908 (96). He formulated now generally accepted theories related to the phagocytosis of pathogens by phagocytes such as monocytes, macrophages, and neutrophils (97). Subsequently, until the 1990s, numerous researchers devoted themselves to studying the inflammatory induction and effector functions of macrophages. With the demonstration by Stein et al. in 1992 that IL-4 enhances the expression of the mannose receptor in macrophages, the alternative activation M2 macrophage phenotype came into the public eye (98). The extent and source of phenotypic and functional heterogeneity in macrophage populations and the role of tissue microenvironment in differentially regulating macrophage function have also captured the attention of researchers. All these have laid an essential foundation for unraveling the mystery of the mechanisms of macrophage pathophysiology during wound healing.

Meszaros et al. showed that during the early stages of wound healing, macrophage cleared contaminating microorganisms, apoptotic neutrophils, and cellular debris from the wound surface by phagocytosis (99). Based on the LysMCre/DTR transgenic mouse model of diphtheria toxin-induced macrophage depletion, Goren et al. demonstrated that macrophages play a crucial role in routine wound healing, promoting angiogenesis, collagen deposition, and wound closure (100). Traumatic macrophages are mainly derived from skin resident macrophage populations and bone marrow-derived monocytes (101). They have a diverse function in wound healing to ensure routine healing. During the inflammatory phase, macrophages clear pathogens and cellular debris by activating a pro-inflammatory phenotype. As the inflammatory phase progresses, macrophages engulf traumatized apoptotic cells and release chemokines (e.g., CXCL12, etc.) to facilitate the change from a pro-inflammatory to a pro-repair phenotype (102). As a result, it stimulates the proliferation, differentiation, and migration of keratinocytes, fibroblasts and endothelial cells by secreting cytokines and growth factors, which directly or indirectly regulate the proliferative phase of the repair process (103). During the remodeling phase, M2 macrophages can also digest excess ECM and remodel the structure of the wound by secreting proteases (104), thus playing a crucial role in the whole process of wound healing. However, in the microenvironment of diabetic wounds, perturbations associated with hyperglycaemia, advanced glycation end products, oxidative stress and impaired angiogenesis induce disturbances in the immune microenvironment of diabetic wounds, leading to phenotypic dysregulation as well as quantitative, functional, and epigenetic alterations in macrophages (105–107). This is manifested by a persistent chronic inflammatory response to trauma and stagnation of the repair process during the inflammatory phase, ultimately leading to chronic non-healing trauma (106).

Macrophages present highly plastic, characterized by a critical feature of their phenotype that changes in response to changes in the microenvironment. Based on their surface receptor expression, secretion characteristics and function. Macrophages are mainly classified into the classically activated M1 phenotype and the alternatively activated M2 phenotype (96). M1 macrophages are considered pro-inflammatory since they are driven by pro-inflammatory cytokines such as lipopolysaccharide (LPS) and tumor necrosis factor (TNF). They produce pro-inflammatory cytokines, including interleukin (IL)-12 and IL-23, along with reactive oxygen species (ROS) (108). In contrast, non-classical macrophages, also known as M2 macrophages, are regarded as pro-healing or resolving macrophages. They are stimulated by anti-inflammatory cytokines such as IL-4 and IL-10, and release growth factors such as the insulin-like growth factor (IGF) and transforming growth factor (TGF) (108).

Notably, over-activation of M1 macrophages and impaired transition from M1 to M2 phenotype are essential differences between normal and diabetic wound healing (109). In normal wounds, infiltrating monocytes differentiate into classically activated M1 and alternatively activated M2 macrophages. Whereas in diabetic wounds, M1 polarization is enhanced and the switch from M1 to M2 is severely impaired (105, 110). This culminates in a situation where lower numbers of M2 macrophages and higher M1/M2 ratios release low levels of the growth factors EGF, FGF, PDGF, and VEGF, as well as the anti-inflammatory cytokines IL-10, TGF-α and TGF-β, which are key contributors to the proliferation and remodeling phase (8). Notably, the hyperglycaemic environment is thought to be one of the major pathways leading to an increase in pro-inflammatory cytokines. In particular, 13 pro-inflammatory cytokines, including TNF-a, IL-1, and IL-6, which stimulate the overactivation of M1 macrophages, are upregulated in the hyperglycaemic environment (111). Interestingly, the diabetic microenvironment resulted in macrophage sensitivity to pro-inflammatory cytokines and stimulated macrophages to secrete pro-inflammatory cytokines such as IL-1, IL-6, MMP9, and TNF-a (112). This further exacerbates the vicious cycle of M1 macrophage polarization and chronic inflammation (113), causing stagnation of the inflammatory phase. However, beyond the cellular and molecular mechanisms underlying macrophage plasticity, the maturing field of epigenetics has become a new point of focus in the investigations of macrophage-mediated inflammation. Recent evidence points to the role of epigenetics in regulating macrophage function in diabetic wound healing, including DNA methylation of CpG islands and methylation of histone tails. These processes can trigger increased expression of pro-inflammatory cytokines, which in turn promote further polarization of M1 macrophages (114).

Huang et al. demonstrated that the hyperglycemic wound environment had more infiltration of M1 macrophages, which created an excess TNF-α microenvironment that upregulated TIMP1 expression in keratinocytes (8). This ultimately contributed to impaired keratinocyte migration and delayed wound healing (8). Mirza et al. showed that persistent activity of NOD-like receptor protein (NLRP)-3 inflammasomes in diabetic and mouse wounds

resulted in a sustained inflammatory response and impaired healing of the wounds. In contrast, inhibition of inflammasome activity in diabetic mice wounds when applied topically promoted wound healing, induced a shift from a pro-inflammatory phenotype to a healing-associated MP phenotype, and increased pro-healing growth factor levels (115). In addition, M1 macrophages in the wound secrete large amounts of proteases such as MMP9 and reduce inhibitors of MMPs; this imbalance further exacerbates extracellular matrix degradation and deprives cells of a scaffold for migration (83, 116). Consequently, this impairs fibroblast migration, proliferation, and collagen synthesis, disrupting wound closure (83, 116). M2 macrophages also play an essential role in wound angiogenesis and can promote wound angiogenesis through macrophage-endothelial cell adhesion paracrine effects mechanisms (117). Gibson et al. have identified reduced VEGFR1 signaling in diabetic wound tissue, which appears to be associated with impaired angiogenesis (118). Unfortunately, in DFUs wounds, there was impaired activation of M2 macrophages and over-activation of the M1 macrophage phenotype, which contributed to impaired angiogenesis.

Moreover, in the DFUs microenvironment, the number of macrophages and functional alterations are also closely associated with wound healing. The number of macrophages is also closely related to diabetes mellitus in wound healing. Barman et al. showed that diabetes induces myeloid preference in bone marrow progenitor cells in the DFUs microenvironment. This was associated with increased macrophage accumulation in wounds and impaired wound healing (119). Using macrophages isolated from diabetic mouse wounds, Savita et al. first demonstrated impaired clearance of apoptotic cells from diabetic wound macrophages, with significant impairment in efferocytosis (9). This resulted in a markedly increased load of apoptotic cells in the wound tissue, high expression of pro-inflammatory factors and low expression of anti-inflammatory cytokines. Ultimately, it prolongs the inflammatory phase and makes wound healing more difficult. Notably, the senescence-associated secretory profile (SASP) is a robust approach whereby a small number of senescent cells in tissues can exert significant local biological effects and is implicated in the pathogenesis of many chronic diseases (120). Based on a diabetic mouse model, Wilkinson et al. found that wound-derived macrophages from diabetic mice exhibited reduced M2 macrophage polarization and the production of CXCR2-rich SASP (85). This induced fibrotic markers of fibroblasts and had the potential to stimulate fibroblast senescence. In addition, wounds in diabetic mice treated with CXCR2 antagonists showed reduced macrophage senescence and local inflammation and promoted wound closure (85). Interestingly, beyond the cellular and molecular mechanisms underlying macrophage plasticity, the maturing field of epigenetics has become a new point of focus in investigating macrophage-mediated inflammation. Recent evidence points to the role of epigenetics in regulating macrophage function in diabetic wound healing, including DNA methylation of CpG islands and methylation of histone tails (114). These processes can trigger increased expression of pro-inflammatory cytokines, which in turn promote further polarization of M1 macrophages.

4.2 Molecular mechanisms and therapeutic targets associated with DFUs angiogenesis

In the current study, research on molecular mechanisms and therapeutic targets related to DFUs angiogenesis can be found in the keyword co-occurrence clustering network map, thematic map, and trend topics map. Combining the analysis of highly cited literature and the extensive related literature, we speculate that the study of molecular mechanisms and therapeutic targets related to DFUs angiogenesis is a focus of academic attention and potentially a research trend for the coming period significant for promoting diabetic wound healing. Angiogenesis refers to expanding its vascular branches by sprouting and forming vascular networks, which are essential for embryonic growth, tissue development, and wound healing (121). Inadequate arterial perfusion associated with peripheral arterial disease, along with macrovascular and microvascular disease, has been reported to be responsible for the chronicity of diabetic foot ulcers (6). Moreover, decreased nutrient supply due to poor granulation tissue angiogenesis is closely associated with impaired healing of diabetic ulcers (122). Inadequate blood perfusion combined with impaired angiogenesis complicates tissue repair in diabetes. Consequently, a thorough understanding of this topic can better reveal the molecular events that delay diabetic wound healing and potential molecular targets that promote healing. Thus, the current dilemma of non-healing DFUs and avoiding serious complications such as amputation can be avoided.

We have identified an explosion of studies related to DFUs angiogenesis starting in 2016. This may be attributed to single-cell sequencing being named "Technology of the Year" by Nature Methods in 2013 (123). It opens new perspectives on exploring molecular mechanisms and therapeutic targets related to DFUs angiogenesis. One of the most researched factors is recombinant human PDGF-BB (Becaprine gel), which has become the only growth factor authorized by the FDA for wound treatment (124). Dopamine is a primary central catecholamine neurotransmitter that controls cognition, mood, and movement and regulates cardiovascular, endocrine, renal, gastrointestinal, and immune functions (125–129). It was shown that activation of dopamine D1 receptors in dermal fibroblasts restores their production of vascular endothelial growth factor A *via* the protein kinase A pathway and consequently restores angiogenesis in subsequent diabetic skin wound tissue (130). Fibrocytes are bone marrow-derived hematopoietic stem cells integral to wound healing (131). Fibrocytes have been found to promote wound healing by facilitating cell proliferation, re-epithelialization, and angiogenesis compared to dermal fibroblasts and diabetic mice treated with PBS (132). Furthermore, Xing et al. identified that Netrin-1 levels were lowest in DFUs patients compared to healthy controls and DM patients. In *in vitro* experiments, overexpression of Netrin-1 restored the high glucose-induced impairment of the PI3K/Akt-eNOS pathway *via* restoring NO production that was significantly inhibited by high glucose, thereby improving DFUs angiogenesis (133).

Importantly, with the development of bioinformatics technologies such as high-throughput sequencing in recent years, extensive identification of Long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) by scholars has opened new doors to studying the regulation of gene expression. lncRNAs have been demonstrated

to be involved in the abnormal regulation of angiogenic genes by regulating the stability and translation of mRNAs (134). A recent study suggested that lncRNA Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is poorly expressed in DFUs patients and consistent with the expression of angiogenic factors such as nuclear factor erythroid 2-related factor 2 (NRF2), Hypoxia-inducible factor-1α (HIF-1α) and VEGF (135). Indeed, Nrf2 can positively regulate the MALAT1/HIF-1α loop and thus regulate angiogenesis, which may become a new target for treating diabetic wounds in the future (135). miRNAs are non-coding RNAs of approximately 22 nucleotides implicated in various roles in critical phases of inflammation, angiogenesis, epithelialization, and remodeling in diabetic wound healing (136, 137). Increasingly, miRNAs have been found to be associated with diabetic wound angiogenesis. In the latest study, Wang et al. (138) have discovered that, unlike non-DFUs wounds, the circulating exosome miR-181b-5p in the plasma of DFUs patients facilitates cellular senescence and inhibits angiogenesis *via* the NRF2/HO-1 pathway to impede DFUs healing. In further studies, the team used the miR-181b5p inhibitor *in vivo* experiments and found that angiogenesis was promoted, with the consequent restoration of wound healing capacity. Furthermore, other studies have shown that miR-217 (139) and miR23c (140) are overexpressed in DFUs patients and restrain angiogenesis by inhibiting the HIF-1α/VEGF pathway and targeting stromal-cell-derived factor-1 (SDF-1α). More importantly, inhibition of miR-217 and miR23c could facilitate DFUs angiogenesis by upregulating the above-mentioned corresponding pathways, thereby favoring wound healing. Another exciting study used maggot excreta/secretions to stimulate miR18a/19a overexpression. The results revealed that diabetic wound angiogenesis could be promoted by downregulating thrombochondroitin-1 (TSP-1) expression. This protein inhibits angiogenesis, which could be a new target for DFU therapy (141).

4.3 Diabetic foot ulcer management

Back in the mid-19th century, the problem of diabetic foot ulcers was first described (142). In 1852, Marchal de Calvi discovered the phenomenon that there was an association between diabetes mellitus and foot gangrene. Subsequently, in 1854, Thomas Hodgkin was similarly aware of the problem. At that time, given the limited development of science and technology, the most popular method of treating ulcers was prolonged bed rest (142). Not until the late 19th century, the genius surgeon Treves provided a landmark contribution to the treatment of the diabetic foot. He established three essential principles for treating foot ulcers: rapid debridement, offloading pressure, and foot care (143). Afterward, these principles led to today's standard of care for DFUs: surgical debridement, wound off-loading, dressing coverage, and infection control (144), as shown in Figure S6. Along with a wide range of adjunctive therapies, such as growth factors, hyperbaric oxygen (HBOT), and negative pressure wound therapy (NPWT) (145). Furthermore, in addition to these measures, multidisciplinary diabetic foot care is becoming a focal point of treatment (146).

The goal of diabetic foot treatment is to achieve tissue healing while maintaining adequate function and weight bearing to bed (145). Debridement is a gold standard in treating DFUs, including removing necrotic and inactivated tissue, surrounding callus, and foreign debris from the wound. This process contributes to the formation and re-epithelialization of granulation tissue, and reduces the pressure in the plantar region of the foot (147). Meanwhile, removing necrotic tissue destroyed the breeding ground and physical barrier for bacterial colonization, which could be instrumental in controlling traumatic infections (148). A 10-year retrospective study of standardized wound care protocols revealed that amputation rates in diabetic foot patients decreased through timely and effective debridement (149). Current debridement modalities consist of surgical (sharp debridement), biological (maggot therapy), enzymatic (clostridial collagenase), autolytic (hydrogel), mechanical (hydro surgery), and ultrasound (150–152). Of note, according to the recommendations of the Wound Healing Society (WHS), sharp debridement is the preferred approach to debridement (153). High plantar pressure has been recognized as a primary factor in the development and poor healing of DFUs. Offloading not only destresses the ulcer site, but also ensures the redistribution of shear forces which is currently an effective strategy for treating DFUs (148). Off-loading can be accomplished through various mechanisms, including shoe modifications, boots, and orthopedic walkers (148). Guidelines published by the International Working Group on the Diabetic Foot (IWGDF) suggest that in the absence of infection or ischemia, non-removable and knee-high devices (total-contact casts or non-removable walkers) are the preferred treatment for neuropathic forefoot or midfoot plantar ulcers (154). Infection is a fundamental cause of DFUs morbidity, hospitalization, impaired healing, and amputation. Compared to uninfected DFUs, infection increases the risk of lower extremity amputation by 50% (155). In DFUs wounds, factors such as stress and pressure, decreased function of immune cells (e.g., macrophages and neutrophils), and ischemia makes them more susceptible to infection. More importantly, in diabetic ulcers, the infection can spread rapidly and induce cellulitis, abscesses and osteomyelitis. It can also lead to life-threatening infectious infections when treatment is delayed (156). Effective control of traumatic infections can be achieved through surgical and pharmacological approaches, which are essential for managing DFUs. Antibiotics are considered the most effective drugs available to fight infections in clinical practice. Nevertheless, the consequent problem of drug-resistant microorganisms is increasing. Fortunately, a variety of new anti-infective measures have been developed and proven to be effective. Fortunately, a variety of new anti-infective measures have been developed and proven to be effective. These include new vehicles for drug delivery systems (e.g., multifunctional nanomaterials) (157) and Bioactive Antimicrobial Peptides (5, 158), which bring hope for the control of DFUs infections.

The dressing provides a protective barrier to the wound, not only preventing bacterial contamination and maintaining a moist environment on the wound surface; it also promotes granulation, angiogenesis, autolysis processes, and rapid migration of epidermal cells at the base of the wound to promote wound healing (159). Recently, an RCT study including 160 patients presented a shred of critical evidence. That is, the combination of recombinant epidermal growth factor and nanosilver dressings can effectively promote DFUs' wound healing and prevent infection (160). In

addition, with the development of bioengineering science and technology, wound dressings can also be used as drug delivery systems to deliver various therapeutic substances (drugs, growth factors, peptides, stem cells, and other bioactive substances) to the wound surface (161), thus promoting wound healing and performing an instrumental role in the treatment of DFUs. Notably, several studies have shown the beneficial effects of hyperbaric oxygen therapy in promoting wound healing in DFUs (162–164). A recent multicenter RCT of 73 patients with chronic DFUs revealed superior efficacy of multimodality cyclical pressure Topical Wound Oxygen compared to standard care alone at 12 weeks and 12 months (164). In the end, the education of patients on meticulous foot care and appropriate foot products through a multidisciplinary approach must be considered (145).

Nevertheless, there are still some limitations to our study. First, we only retrieved publications from the WoSCC database, which may contribute to an imperfect collection of relevant publications. Second, we only accessed publications from January 1st, 2000 to April 27th, 2022, which would cause the exclusion of some of the most recent findings as this data is continuously updated. Third, some recent critical publications may have yet to receive sufficient attention and thus may not have been explored in depth. Despite these limitations, this study comprehensively reviews the global status and research trends on fibroblast-related DFUs.

5 Conclusion

In the present study, we performed an in-depth analysis of research on fibroblast-related DFUs from a bibliometric perspective. Including an exploration of the current knowledge structure, development trends, research hotspots and future directions of the field. The present study indicated that research on fibroblast-related DFUs is growing. The cellular and molecular mechanisms of DFU pathophysiological process, molecular mechanisms and therapeutic targets associated with DFU angiogenesis, and the measures to promote DFUs wound healing are three worthy research hotspots in this field. Further research on these topics could contribute to a complete understanding of the molecular events in the pathophysiological processes of DFUs and the search for potential therapeutic targets, establishing a solid foundation for achieving clinical translation.

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 study was approved by the Ethics Committee of the First Affiliated Hospital of Naval Medical University.

Author contributions

Conception/design: YZ, JL, SW, DX, MW, SYX, WZ, XT, YL, JH, LJ, XG, SJX, MG, SXJ, RH, SCX, and SZJ. Collection and/or assembly of data: YZ, JL, SW, DX, MW, SYX, WZ, XT, YL, JH, LJ, XG, SJX, MG, SXJ, RH, SCX, and SZJ. Data analysis and interpretation: YZ, JL, SW, DX, MW, SYX, WZ, XT, YL, JH, LJ, XG, SJX, MG, SXJ, RH, SCX, and SZJ. Manuscript writing: YZ, JL, SW, DX, MW, SYX, WZ, XT, YL, JH, LJ, XG, SJX, MG, SXJ, RH, SCX, and SZJ. Final approval of manuscript: YZ, JL, SW, DX, MW, SYX, WZ, XT, YL, JH, LJ, XG, SJX, MG, SXJ, RH, SCX, and SZJ. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the National Natural Science Foundation of China (81930057, 81772076, 81971836), CAMS Innovation Fund for Medical Sciences (2019-I2M-5-076), Deep Blue Talent Project of Naval Medical University, 234 Academic Climbing Programme of Changhai hospital and Achievements Supportive Fund (2018-CGPZ-B03), Shanghai Rising-Star Program (Sailing Special Program) (No. 23YF1458400).

Acknowledgments

We thank the Web of Science™ (WOS, http://www.webofknowledge.com) team for allowing us to use their data.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1109456/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 24 May 2023
DOI 10.3389/fendo.2023.1157518

OPEN ACCESS

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RECEIVED 02 February 2023
ACCEPTED 09 May 2023
PUBLISHED 24 May 2023

CITATION
Keegan AC, Bose S, McDermott KM, Starks White MP, Stonko DP, Jeddah D, Lev-Ari E, Rutkowski J, Sherman R, Abularrage CJ, Selvin E and Hicks CW (2023) Implementation of a patient-centered remote wound monitoring system for management of diabetic foot ulcers.
Front. Endocrinol. 14:1157518.
doi: 10.3389/fendo.2023.1157518

Implementation of a patient-centered remote wound monitoring system for management of diabetic foot ulcers

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Background: Regular clinical assessment is critical to optimize lower extremity wound healing. However, family and work obligations, socioeconomic, transportation, and time barriers often limit patient follow-up. We assessed the feasibility of a novel, patient-centered, remote wound management system (Healthy.io Minuteful for Wound Digital Management System) for the surveillance of lower extremity wounds.

Methods: We enrolled 25 patients from our outpatient multidisciplinary limb preservation clinic with a diabetic foot ulcer, who had undergone revascularization and podiatric interventions prior to enrollment. Patients and their caregivers were instructed on how to use the digital management system and asked to perform one at-home wound scan per week for a total of 8 weeks using a smartphone application. We collected prospective data on patient engagement, smartphone app useability, and patient satisfaction.

Results: Twenty-five patients (mean age 65.5 $\pm$ 13.7 years, 60.0% male, 52.0% Black) were enrolled over 3 months. Mean baseline wound area was 18.0 $\pm$ 15.2 cm^2, 24.0% of patients were recovering from osteomyelitis, and post-surgical WiFi stage was 1 in 24.0%, 2 in 40.0%, 3 in 28.0%, and 4 in 8.00% of patients. We provided a smartphone to 28.0% of patients who did not have access to one that was compatible with the technology. Wound scans were obtained by patients (40.0%) and caregivers (60.0%). Overall, 179 wound scans were submitted through the app. The mean number of

wound scans acquired per patient was 0.72 ± 0.63 per week, for a total mean of 5.80 ± 5.30 scans over the course of 8 weeks. Use of the digital wound management system triggered an early change in wound management for 36.0% of patients. Patient satisfaction was high; 94.0% of patients reported the system was useful.

Conclusion: The Healthy.io Minuteful for Wound Digital Management System is a feasible means of remote wound monitoring for use by patients and/or their caregivers.

KEYWORDS

diabetic foot ulcer (DFU), diabetes, smartphone application (app), telemedicine, technology, smartphone, diabetic foot

Introduction

Chronic lower extremity wounds are a major source of global morbidity, disability, and healthcare utilization (1–3). Diabetic foot ulcers (DFU) represent an increasingly common and difficult to treat subset of lower extremity wounds (2, 4). In the United States, diabetes affects 37.3 million persons, of whom 19% to 33% will develop a DFU during their lifetime (5, 6). Complications of DFU are common and morbid, including up to a 60% occurrence of diabetic foot infection and a 15% to 20% risk of subsequent lower extremity amputation (4). Both incident DFU and poor healing disproportionately affect socioeconomically vulnerable populations, persons with complex medical needs, and/or persons with limited access to high-quality wound care (7).

In-person multidisciplinary diabetic foot and wound management is standard of care for the treatment of DFU (8, 9). However, the model of multidisciplinary care typically requires frequent in-person wound assessments, which may not be achievable for patients due to numerous barriers. Patients with DFU and their caregivers consistently identify time constraints (e.g., difficulty finding available appointment times, conflicts with occupational and care-giving responsibilities), financial insecurity, mobility deficits, and lack of access to safe transportation as barriers to accessing treatment (10, 11). Remote wound care offers a potential approach to overcoming these barriers.

In response to the COVID-19 pandemic, the use of telemedicine has expanded exponentially in the United States (12) Telemedicine strategies have been applied to the management of DFU with mixed results (13). Patients and physicians have expressed enthusiasm for remote wound monitoring solutions, but most current systems rely on trained healthcare providers (e.g., home care nurses) or non-expert clinicians in the home for execution (14, 15). There is a paucity of data on the feasibility, compliance, and outcomes of a remote wound monitoring system that relies on patients and their caregivers to perform their own wound scans.

The Minuteful for Wound Digital Management System (Healthy.io, Tel Aviv, Israel) is a novel, remote wound monitoring system that captures wound measurements and analyzes tissue distribution in real-time through use of a smartphone application. Use of this digital management system by clinicians has been shown to be successful in non-US healthcare settings such as England (16), but a newer patient-facing version of the technology has recently been developed. We conducted a pilot study of patients with DFU to assess patient engagement, reliability, and satisfaction with the Minuteful for Wound Digital Management System.

Methods

Patient population

We enrolled 25 patients who presented to the Johns Hopkins Hospital multidisciplinary diabetic limb preservation clinic with an active DFU between July 1 and November 30, 2022. Patients were considered for enrollment in the study if they were proficient in English, ≥18 years of age, had an active DFU, had completed any planned revascularization and/or wound debridement procedures, and were willing and able to use a smartphone to capture weekly wound scans for an 8-week study period. For patients with multiple wounds, the largest wound that was accessible for imaging was designated to be monitored using the device throughout the study. Patients were excluded from the study if the wound was too large to capture in a single wound scan, if the wound was in a location that was not accessible to the patient or their caregiver, or if they were unable to operate the smartphone application. Patients who wished to participate but did not have access to a smartphone were loaned a smartphone with the app pre-installed for the duration of their participation in the study.

The Johns Hopkins University Institutional Review Board approved the study, and all patients provided written informed consent to participate.

Minuteful for wound digital management system

The Minuteful for Wound Digital Management System (Healthy.io, Tel Aviv Israel) consists of dedicated calibration

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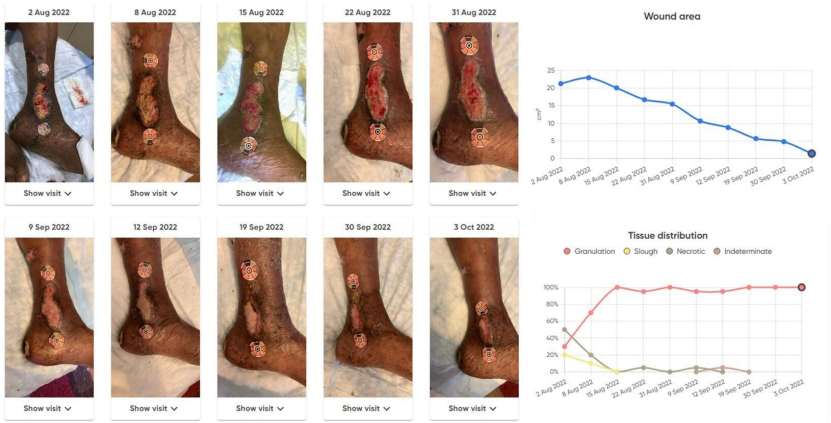

FIGURE 1
Example wound snapshots showing wound progression over the 8-week study period, along with associated wound area and tissue distribution plots, as provided by the Healthy.io Minuteful for Wound Digital Management System.

markers (stickers), a smartphone application (Minuteful for Wound app), and a web-based Portal (Minuteful for Wound Portal) that turns any smart mobile device into a wound care management tool (Figure 1). The use of calibration markers helps the application identify the wound area and controls for different lighting conditions and camera types. The Minuteful for Wound app guides patients through the process of collecting clinical data and capturing scans of their wound using the embedded smartphone camera. The captured scan is transferred to a cloud-based server, where a set of distinct algorithms is used to analyze and translate it into a set of measurements for each wound. The measurements are securely displayed in the cloud-based Minuteful for Wound Portal to assist healthcare professionals in managing and monitoring the wound healing process (Figure 2).

Study protocol

Following informed consent, the patients and their caregivers were instructed how to download and log into the Minuteful for Wound app on their smartphone. The primary user (patient or caregiver) was then provided with a box of calibration stickers specifically for use with the Minuteful for Wound app and taught how to apply the stickers and scan the wound. The primary user was given the opportunity to ask questions and practice scanning the wound and, once they were proficient, completed the first scan for upload in the clinic. Written information about the study and use of the application, including a user manual and a brochure, were also provided to the patient and their caregiver.

Once trained to use the app, the primary user was asked to obtain weekly at-home wound scans during regular dressing changes. Users were asked to capture a minimum of one wound scan per week to allow for flexibility in scanning, but were encouraged to capture scans with each dressing change when possible. The quality of the scans was standardized using in-app boundary conditions, an algorithmic mechanism that enables results to be presented to the clinicians in the Portal. In cases where the environmental conditions did not meet the device's prerequisites (e.g., not enough motion during the scan or it was blurry, the lighting was too bright or too dark, or the calibration markers did not remain in the camera field for the entire scan), the patient would be prompted by the app to re-perform the scan. All remotely collected assessments were securely transmitted to the HIPAA-compliant Minuteful for Wound Portal for review by the

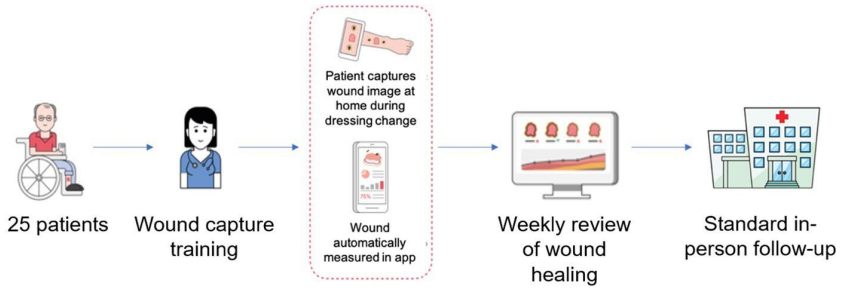

FIGURE 2
Overview of feasibility study design.

study team. All wound assessments were reviewed by members of the study team (consisting of a vascular surgeon, surgical podiatrist, and general surgery resident) once per week to assess progress. A weekly wound update was then provided to the patient by phone, and patients with concern for clinically stagnating wounds were asked to visit the clinic for an in-person assessment within the next week. Primary users who did not complete a weekly wound scan were called by the study team with a reminder. A Healthy.io engagement team was available to support the primary user in completing remote assessments as needed. A technology support hotline was available from 8am to 6pm EST on Mondays through Fridays to provide live phone support for app use. At the end of the 8-week study period primary users were asked to complete a useability and satisfaction survey to assess ease of use and usefulness of the Minuteful for Wound Digital Management System. The survey was developed using a mixed Likert scale and open-ended question design through iterative processing by the study team (Supplementary Table 1).

Patient data

We captured age, sex, race, ethnicity, insurance status, area deprivation index and comorbidities for each patient through direct review and abstraction of the electronic medical records. Area deprivation index, which is a comprehensive measure of neighborhood socioeconomic deprivation, was calculated using each patient's complete address and Neighborhood Atlas mapping (17, 18). Hypertension was defined as systolic blood pressure >160 mmHg on 3 separate visits or current use of antihypertensive medications. Hyperlipidemia was defined as a cholesterol level >200mg/dL, LDL level >130 mg/dL, or use of cholesterol-lowering medications. Coronary artery disease was defined as a documented history of myocardial infarction or previous coronary revascularization. Congestive heart failure was defined based on a documented diagnosis, echocardiogram findings, or the Framingham criteria (19). Peripheral artery disease was defined as an ankle-brachial index (ABI) of ≤0.8, toe pressure of <70 mmHg, or a history of a revascularization procedure on the affected limb. Chronic kidney disease was defined as an eGFR of <90 mL/min/1.73m^2.

The wound characteristics for each patient were documented by study staff at the time of study enrollment including wound location, size, vascular studies, Wound, Ischemia and foot Infection (WIfI) score (20), and previous revascularization and podiatric surgical interventions. WIfI classification was assigned based on post-revascularization and wound debridement characteristics to determine wound stage at the time of enrollment.

Study outcomes

The primary outcomes were patient engagement and satisfaction with the Minuteful for Wound Digital Management System. Patient engagement was determined by the recorded number of successful scans performed over the study period. 'Optimally engaged patients' were defined as patients completing 100% of study scans (equivalent to at least one scan every week). 'Highly engaged patients' were defined as patients meeting a threshold of 75% to 99% of study scans (equivalent to at least one scan every other week and a half). 'Engaged patients' were defined as patients meeting a predefined threshold of 50% to 74% of study scans (equivalent to at least one scan every other week, which is equivalent to the expected frequency for standard in-person wound care visits). 'Not engaged patients' were defined as patients completing 25% to 49% (equivalent to at least one scan per month). Patients who completed <25% of wound scans (i.e. <2 scans over 8 weeks) were considered to be study failures.

Patient and caregiver satisfaction with the application was determined by survey responses to questions about ease of use and overall usefulness. A patient was determined to be satisfied if they provided a response of 4 or 5 ("Agree" or "Strongly Agree," respectively) on the Likert Scale.

Secondary outcomes were the proportion of scans that led to a change in patient management, including changes to wound care plan or a change in planned next in-person visit; number of reminder phone calls made to patients; change in wound area from study enrollment to study completion; and the proportion of patients who achieved wound healing. To evaluate the scanning experience of the patients, the number of boundary condition alerts and scan attempts were recorded for each patient assessment.

Statistical analysis

Baseline patient demographics, comorbidities, wound information, and study outcomes were tabulated and reported using means (standard deviations) or percent (N) as appropriate. Change in wound area over the course of the study was compared using paired t-tests, with $P<0.05$ denoting statistical significance. Qualitative data collected from open-ended questions on the patient survey were analyzed by two reviewers who used open coding, resolved discrepancies with triangulation, and applied thematic analysis.

Results

Patient cohort

We enrolled 25 patients over the month study period. Mean age was 65.5 (SD, 13.7) years, 60.0% of our participants were male, and 52.0% self-identified as non-Hispanic Black adults. The most common comorbidities were hypertension (68.0%), chronic kidney disease (68.0%), and peripheral artery disease (56.0%) (Table 1).

There were a wide variety of wound locations (Table 2). Mean wound area at baseline was 18.0 cm^2 (SD, 15.2), 36.0% of wounds were severe (WIfI stage 3 or 4), 64.0% of patients had undergone lower extremity revascularization, and 80.0% had undergone surgical debridement prior to enrollment. Home health was involved in the care of 60.0% of patients, and a wide range of wound dressing treatment strategies were used (Table 2).

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TABLE 1 Baseline characteristics of patients included in the feasibility study.

Characteristic	Overall % (N=25)
Age, years (SD)	65.5 (13.6)
Female sex	40.0% (N=10)
Race/Ethnicity	
Non-Hispanic Black	52.0% (N=13)
Non-Hispanic white	48.0% (N=12)
Insurance Status	
Medicare	76.0% (N=19)
Medicaid	16.0% (N=4)
Private/Self Pay/Other	8.00% (N=2)
Other	4.00% (N=1)
Area deprivation index	
Quartile 1 (least deprived)	32.0% (N=8)
Quartile 2	56.0% (N=14)
Quartile 3	4.00% (N=1)
Quartile 4 (most deprived)	8.00% (N=2)
Functional Status	
Independent	72.0% (N=18)
Partially dependent	28.0% (N=7)
Diabetes type	
No medications	32.0% (N=8)
Type 1	NA (N=0)
Type 2 on oral medications	24.0% (N=6)
Type 2 on insulin	44.0% (N=11)
Comorbidities	
Hypertension	68.0% (N=17)
Dyslipidemia	16.0% (N=4)
Coronary artery disease	24.0% (N=6)
Congestive heart failure	12.0% (N=3)
Peripheral artery disease	56.0% (N=14)
Chronic kidney disease	68.0% (N=17)
Dialysis	16.0% (N=4)
Smoking status	
Current	NA (N=0)
Former	72.0% (N=18)
Never	28.0% (N=7)

NA, Not Applicable.

TABLE 2 Baseline wound characteristics and related surgical procedures of the study population.

Characteristic	Overall % (N=25)
Wound location	
Lateral/forefoot	44.0% (11)
Lower leg/ankle	32.0% (8)
Heel	12.0% (3)
Plantar foot	8.00% (2)
Toe	4.00% (1)
Wound area, mean cm^2 ± SD	18.0 ± 15.2
Osteomyelitis	24.0% (6)
WIfI Classification	
1	24.0% (6)
2	40.0% (10)
3	28.0% (7)
4	8.00% (2)
Toe pressure, mean mmHg ± SD (N= 17)	79.6 ± 45.0
Ankle Brachial Index, mean ± SD (N= 16)	1.0 ± 0.2
Related revascularization procedure	
Endovascular	32.0% (8)
Open	32.0% (8)
No. revascularization procedures, mean ± SD	1.1 ± 1.5
Podiatric interventions of the affected limb*	
None	20.0% (5)
Bone resection/debridement	56.0% (14)
Biologic coverage	48.0% (12)
Minor amputation	40.0% (10)
Skin graft	16.0% (4)
Home care	60.0% (15)
Wound dressing type	
Collagen	24.0% (6)
Negative pressure wound therapy	16.0% (4)
Wound hydration	24.0% (6)
Enzymatic debridement	12.0% (3)
Filler	24.0% (6)

WIfI, Wound, Ischemia, and foot Infection.
*Sum equals greater than 100% because some patients received more than one podiatric intervention.

TABLE 3 Summary of Healthy.io Minuteful for Wound smartphone app useability.

Characteristic	Overall % (N=25)
Borrowed smartphone	28.0% (N=7)
Did not have smartphone	NA (N=0)
Personal smartphone not compatible	28.0% (N=7)
Primary wound scanner	
Patient	40.0% (N=10)
Caregiver	60.0% (N=15)
No. phone calls per patient during study period, mean (SD)	
Reminder calls from study team	2.28 (2.25)
Technical calls from Healthy.io team	6.32 (5.18)
No. scans completed overall per patient, mean (SD)	5.80 (5.30)
No. scans per week per patient, mean (SD)	0.72 (0.63)

NA, Not Applicable.

Study participation

The primary user was a caregiver in 60.0% of cases and the patient in 40.0%. Home health was not involved in wound scanning for this feasibility study. Twenty-eight percent of users borrowed a smartphone for the purposes of study participation.

Overall, patients submitted a mean number of 5.80 (SD, 5.30) wound scans over the total 8-week study period, equal to a mean of 0.72 (SD, 0.63) wound scans per week. Patients received a mean of 2.28 (SD, 2.25) reminder phone calls to submit wound scans from the study team during the study period (Table 3). Patients made or received a mean of 6.32 (SD, 5.18) technical calls from the Healthy.io technical team.

TABLE 4 Patient outcomes related to Healthy.io Minuteful for Wound smartphone app use.

Outcome	Overall % (N=25)
Study engagement	
Optimally engaged (100% weekly scans)	20.0% (N=5)
Highly engaged (≥ 75% weekly scans)	28.0% (N=7)
Engaged (≥ 50% weekly scans)	12.0% (N=3)
Not engaged (≥ 25% weekly scans)	12.0% (N=3)
Study failure (< 25% weekly scans)	28.0% (N=7)
Reasons for study failure	
Communication/information flow	16.0% (N=4)
Caregiver availability	12.0% (N=3)
Change in management as a result of scan	
Change in wound care	20.0% (N=5)
Earlier clinic appointment	16.0% (N=4)
Mean wound area at study completion, cm^2 (SD)	10.8 (11.9)
Mean wound area change from enrollment to completion, cm^2 (SD)	7.67 (9.72)
Wound healed at conclusion of study	12.0% (N=3)

Clinical study outcomes

App engagement was variable, with 20.0% of patients completing 100% of the weekly wound scans (optimally engaged), 28.0% completing 75-99% of weekly wound scans (highly engaged), 12.0% completing 50-74% of weekly wound scans (engaged), and 28.0% completing <25% weekly wounds scans (i.e., study failure) (Table 4). Study failures were investigated and classified as communication difficulties in 16.0% of patients and lack of caregiver availability for assistance with the scans in 12.0% of patients. Overall, 21/25 (84.0%) patients completed at least one in-home wound scan.

Thirty-six percent of patients were advised that they should undergo an early change in their wound management plan at least once during the study based on weekly review of their wound scan by the study team. Treatment changes included a change in wound treatment in 20.0% of patients and initiation of an earlier appointment for in-person clinic evaluation in 16.0% of patients. There were no instances where use of the digital management system resulted in a delayed diagnosis of wound deterioration.

At the conclusion of the study, there was a mean decrease in wound area of 7.67 cm^2 (SD, 9.72) per patient (P=0.005), equivalent to a mean decrease of 41.6% (SD, 15.8%). Complete wound healing was achieved in 12.0% of patients (3/25).

Patient satisfaction

Primary users who completed at least one at-home wound scan during the study period were asked to take part in a post-study

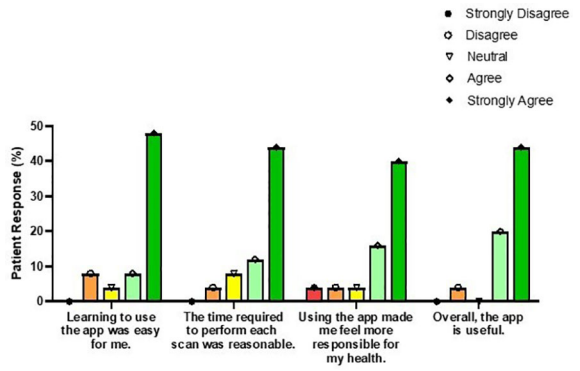

FIGURE 3
Post-study primary user survey results summarizing patient satisfaction with the Healthy.io Minuteful for Wound smartphone app.

survey, with a response rate of 81.0% (17/21). Of the four participants who did not complete the survey, three were unable to be reached by telephone after completion of the study and one declined to participate. Of the survey respondents, 88.2% agreed or strongly agreed that the Minuteful Wound app was easy to use. Overall, 94.1% of patients found the digital wound management system to be useful, with the large majority noting they felt more involved in their wound care, more responsible for their health, and more able to access healthcare services (Figure 3). One patient even stated that they felt "empowered [to be accountable for their health]."

Common themes that recurred throughout the patient surveys included appreciation for close wound monitoring without the need for travel to the clinic. However, many respondents expressed room for improvement with "more instantaneous feedback." Despite the asynchronous design of the app use and study team evaluation, 100% of patients felt confident that the information they sent to the study team was received.

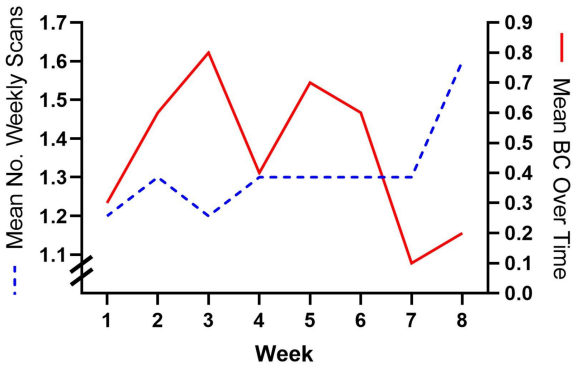

FIGURE 4
Mean number of weekly scans submitted (dotted blue line) and mean number of boundary condition notifications received (solid red line) by the Healthy.io Minuteful for Wound Digital Management System over the 8-week study period.

Usage data outcomes

Among the 179 wound scans attempted by patients and/or their caregiver, 145 (81.0%) were free of boundary condition violations and successfully submitted to the Portal *via* the app. Eleven patients did not receive any boundary condition notifications during the study period, meaning they produced satisfactory and clinically valid wound documentation on all first wound scan attempts throughout the study period. Of the 34 scans that did not meet the prerequisite boundary conditions, 19 (55.9%) were rectified after a single user notification. The boundary conditions that were violated for these patients were much more common during the beginning of the study and decreased over the course of 8 weeks (Figure 4).

Discussion

As our healthcare system has been adapting to the ever-changing climate of the COVID-19 pandemic, telemedicine advancement has become a priority for medical technology companies. We aimed to determine whether a novel digital wound monitoring system could be effectively used by patients and/or their caregivers to provide clinicians with high-quality wound data to guide care. We found that patients and caregivers could successfully learn how to use the Minuteful for Wound smartphone app, and the majority successfully engaged in its use. Study participants found the digital wound management system useful, and more than a third of patients benefited from its use in the form of early treatment modification.

A number of software companies have developed smartphone apps to measure and record wound size, including for DFU (15, 21–25). Remote wound monitoring programs may be helpful in the management of chronic wounds, particularly as a communication tool between patients and their healthcare providers when close follow-up is necessary (26). However, most of the applications are developed with physicians and nurses being the intended users. A recent study enrolled patients from a rural Veteran's Affairs wound care clinic in a remote wound telemedicine program and showed excellent wound healing outcomes, but the wound telemedicine was facilitated by a trained telepresenter (27). Similarly, a meta-analysis of telemedicine versus in-person management of DFU showed similar or possibly improved wound healing, amputation, and mortality outcomes for patients managed *via* telemedicine (13). However, all telemedicine studies identified in the meta-analysis involved use of a trained nurse or similar healthcare provider to facilitate the telemedicine communication between the patient and physician. Our study is unique in that it assessed a remote wound monitoring system designed to be patient-facing, where patients and their caregivers had total responsibility for capturing and submitting remote wound scans on a repeated basis.

Patient engagement in our study was high compared to prior studies of telemedicine use. In a study of emergency department patients undergoing acute laceration repair or incision and drainage procedures, 58% of patients sent at least one picture of their wound

through a Mobile Post-operative Wound Evaluator (mPOWEr) smartphone app (25). In our study, 84.0% of patients submitted at least one at-home wound scan. However, overall engagement was lower because we defined patient engagement as capturing ≥50% of expected weekly scans. The need for repeated wound scans places a larger burden on the patients and their caregivers than a single wound capture but was designed to simulate the frequency of standard in-person wound monitoring in the clinic. Reminder phone calls from the study team were required approximately twice per patient over the course of the 8-week study. Whether this burden is sustainable for larger numbers of patients is unclear. A prior study also demonstrated that telemedicine costs for managing DFU patients by telemedicine are approximately $2222 USD lower per patient compared to standard in-person monitoring (28).

Primary users in our study reported high rates of satisfaction with the Healthy.io Minuteful for Wound Digital Management System. Patients are generally in favor of remote wound monitoring based on data from prior studies, including studies specific to DFU (29–31). In a scoping review of telemedicine solutions for DFU, four main maps emerged: "A whole human not merely a hole in a human," "Less of a burden on the family, the community, and the environment," "Competences and continuity of care are essential for high-quality care" and "The quality and modality of the technology." Consistent with these concepts, our patients reported less frequent in-person appointments, better continuity of care, and more accountability with care as benefits. We also observed some drawbacks to the technology. Specifically, primary users felt that more instantaneous feedback about the wound would be helpful. Future iterations of the app will involve a 2-way in-app communication tool that will allow patients to receive feedback more synchronously and remove the burden of the weekly phone call.

In addition to patients' desire for more timely wound feedback, we encountered other challenges in our study. While our rate of study completion was high (72.0%), seven patients failed to complete the study. One of the major concerns around the use of remote wound monitoring systems is how they can be utilized by socioeconomically disadvantaged patients. Nearly one third of patients in our study required a borrowed smartphone because they lacked a smartphone with the specifications needed to run the app; most patients in our study had a preexisting smartphone, but many were older models not compatible with this technology. Both smartphone and reliable internet access are barriers to implementation in vulnerable populations (13, 32). Our multidisciplinary diabetic limb preservation clinic serves a large number of patients from socially disadvantaged backgrounds, however, patients enrolled in this study resided in less disadvantaged neighborhoods than a typical patient in our clinic (33). Making remote wound monitoring technology accessible to a wide range of populations will be important for successful adoption moving forward.

There are a number of limitations to this study. We enrolled only a small number of patients in this pilot study, and we did not have a control group for comparison. We were not able to evaluate hospital financial data associated with the implementation and continued use of the app, but plan to do so in the future. Finally, our study was not designed or powered to assess wound healing outcomes, and due to the feasibility design we did not attempt to alter patient care based on the wound images provided. However, our findings did lead to the successful initiation of a now ongoing randomized controlled trial comparing use of the Minuteful for Wound Digital Management System compared to standard of care in-person monitoring (34).

Conclusion

The Healthy.io Minuteful for Wound Digital Management System is a feasible means of remote wound monitoring for use by patients and their caregivers. We were able to show good patient engagement, satisfaction, and usage data in a pilot study design. Our results suggest the feasibility of patient-facing technology for the remote wound app monitoring of diabetic foot ulcers. This study is the impetus for a new randomized controlled trial designed to study wound healing efficacy for remote wound app monitoring vs. standard in-person clinic visits for the treatment of lower extremity wounds, in which we hope to show the barriers that often interfere with in-person follow up visits will no longer interfere with proper wound care.

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 Johns Hopkins University Institutional Review Board. 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

AK and CH, project conceptualization, patient enrollment, trial coordination, wound scan review, data analysis, manuscript development, and review. SB and EL-A, data analysis, manuscript development, and review. KM, MS, DS, CA, and ES, manuscript development and review. DJ, project conceptualization, data analysis, manuscript development, and review. RS, patient enrollment, wound scan review, manuscript development, and review. JR, patient enrollment and trial coordination. All authors contributed to the article and approved the submitted version.

Funding

K23DK124515 (NIH/NIDDK) R03DK133557 (NIH/NIDDK).

Conflict of interest

Authors DJ and EL-A were employed by Healthy.io 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1157518/full#supplementary-material

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frontiers | Frontiers in Endocrinology

TYPE Case Report
PUBLISHED 16 June 2023
DOI 10.3389/fendo.2023.1198818

OPEN ACCESS

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RECEIVED 02 April 2023
ACCEPTED 29 May 2023
PUBLISHED 16 June 2023

CITATION
Chen Y, Yang H, Wang W, Chen Y, Jiang D, Li Y, Li L, Yang W and Wang A (2023) Staged management of a large ischemic heel ulcer in a diabetes patient: a case report.
Front. Endocrinol. 14:1198818.
doi: 10.3389/fendo.2023.1198818

Staged management of a large ischemic heel ulcer in a diabetes patient: a case report

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Heel ulcer is one of the severe complications of patients with diabetes mellitus, which poses a high risk for foot infection and amputation, especially in patients with peripheral arterial disease and neuropathy. Researchers have searched for new treatments for treating diabetic foot ulcers in recent years. In this case report, we demonstrated the treatment of large ischemic ulcers for the first time in a diabetic patient. The overall treatment goal of this patient was designed to improve blood supply to her diseased lower extremities and close the ulcer. This two-stage reconstruction approach resulted in an ulcer-free, stable, plantigrade foot at postoperative follow-up.

KEYWORDS

heel ulcer, ischemic, vascular reconstruction, peroneal artery perforator flap, staged

1 Introduction

Diabetes mellitus (DM) is a chronic, demanding metabolic disease that affects individuals, their families, and society worldwide (1). Approximately 463 million people suffer from DM in the world, and this number is expected to rise by 25% by 2030 (2). Among all complications, diabetic foot ulcers (DFUs) are one of the serious and refractory complications of DM. The latest definition of DFUs refers to infection, ulcer, or destruction of foot tissue caused by lower limb neuropathy and/or peripheral arterial disease in patients with DM (3). Studies have found that the prevalence rate of diabetic foot is 4%–10%, the annual population-based incidence rate is 1.0%–4.1%, and the lifetime incidence rate of diabetic patients may be as high as 25%, which brings a heavy economic burden to patients and their families (4). Patients suffering from DM account for almost 60% of all whole-limb amputations (5). Among these, heel ulcer is one of the severe complications of patients with diabetes, which poses a high risk for foot infection and amputation, especially in patients with peripheral arterial disease (PAD). In this case report, we demonstrated the treatment of large ischemic ulcers for the first time in a diabetic patient.

2 Case presentation

2.1 The case

A 59-year-old female patient with a 17-year history of DM was transferred to our Diabetic Foot Center due to a non-healing heel ulcer after 2 months of scalding. In the early stage of the disease, a large blister burst with much light-yellow exudation on the skin of the lesion site. Further, the amount of exudate decreased gradually, and the ulcer became black and failed to heal even with some conventional treatments such as dressing change and using antibiotics prescribed by another hospital.

2.2 Physical examination

On both of her feet, the skin temperature was low, and the pulse of the dorsalis pedis artery and posterior tibial artery could not be palpated. There was a large, round ulcer of approximately 5 cm × 5 cm in diameter and unclear in depth. Its surface was covered with large black scabs with a little yellowish exudation visible around it. The tissue around it was cool, dark red, and swollen (Figure 1A). Laboratory tests showed her peripheral blood white blood cell (WBC) was 9.1 * 10^9/L, of which neutrophils accounted for 79.9% and lymphocytes for 12.2%. Meanwhile, the erythrocyte sedimentation rate (ESR) was 79 mm/h, C-reactive protein (CRP) was 12 mg/dl, and procalcitonin (PCT) was 0.41 ng/ml. MRI of the diseased foot revealed local osteomyelitis on the calcaneal surface. B-scan ultrasound of her lower limb vessels showed diffuse moderate-to-severe stenosis in the middle and lower segments of the bilateral femoral artery. Computed tomography angiography (CTA) examination further indicated mild-to-severe stenosis in multiple segments of her bilateral femoral and popliteal arteries, as well as complete occlusion of her right femoral artery and posterior tibial artery (Figure 1B).

2.3 Diagnosis

There are more than 10 classification methods for diabetic foot, such as Meggitt–Wagner, Texas, PEDIS, SINBAD, and WIfI. The contents and indications of these classification methods are different, and each of them has its own advantages and disadvantages (6, 7). Among them, the WIfI classification has been proposed for the threatened lower limb, based on the three main factors that have an impact on limb amputation risk: wound (W), ischemia (I), and foot infection ("fI"). This classification is commonly used in patients with ischemic diabetic foot, with the advantage of clear evaluation indicators that can help guide the diagnosis and treatment of DFUs, but with the disadvantage of lacking evaluation of diabetic neuropathy. The patient in this case report was evaluated and diagnosed using the WIfI classification. The person in this case report was found to have a large ulcer on the heel with approximately 25 cm^2 in area, accompanied by severe limb ischemia and heel osteomyelitis by MRI but no systemic inflammatory response syndrome (SIRS). Therefore, the DFUs of this patient were rated as diabetic foot W3 I3 fI2.

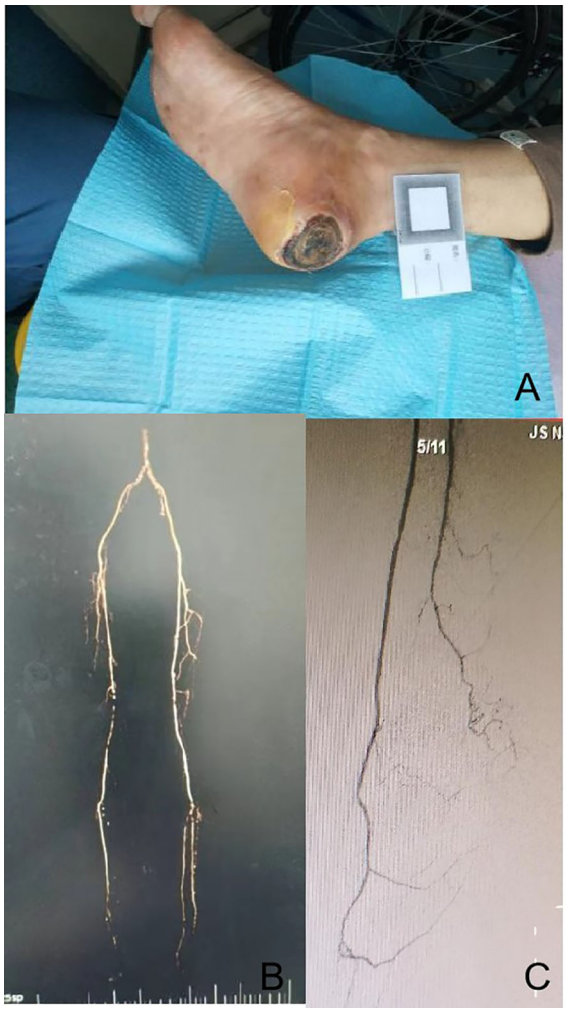

FIGURE 1
Physical examination of the wound, preoperative CTA examination of lower limb vessels, and angiography after lower limb arterial reconstruction. It was a round ulcer of approximately 5 cm × 5 cm in diameter, which was covered with large black scabs, and the tissue around it was cool, dark red, and swollen **(A)**. Long segment occlusion of the inferior segment of the right superficial femoral artery with segmentary severe stenosis **(B)**. The vascular lumen of the superficial femoral artery, popliteal artery, anterior tibial artery, peroneal artery, and some arterial arches of the foot were unobstructed in the affected limb after lower limb arterial reconstruction **(C)**. CTA, computed tomography angiography.

2.4 Treatment

First, the surgery of balloon dilation under local anesthesia was planned for this patient to revascularize her right superficial femoral artery and peroneal artery. Preoperative angiography confirmed long segment occlusion of the inferior segment of her right superficial femoral artery with segmentary severe stenosis (Figure 1B). Intraoperatively, the superficial femoral artery, popliteal artery, anterior tibial artery, and peroneal artery were successfully revascularized, but the posterior tibial artery was not successfully re-opened. Postoperative angiography showed that the vascular lumen of the superficial femoral artery, popliteal artery, anterior tibial artery, peroneal artery, and some arterial arches of the foot were unobstructed

in the affected limb (Figure 1C). Subsequently, the patient was then allowed several weeks for the recovery of local blood supply to the wound, while the wound was periodically debrided and dressed in a small range. In the fourth week after surgery, the peroneal perforator flap was transferred and repaired under general anesthesia. Postoperative nursing was also very important. For postoperative nursing, we took the following measures: psychological counseling, keeping the non-weight-bearing state of the limb, keeping the limb warm, raising the limb, and closely observing the blood transport and the color change of the flap. In the 7th week, stitches were removed, and the flap was alive (Figure 2G). At the 12th week, the affected limb could be loaded with moderate weight bearing. In the 24th week, the patient was able to walk freely with this limb in which the flap appearance and texture were perfect.

3 Discussion

Heel ulcer and ischemia are both predisposing factors for major amputation in diabetic foot patients, and the combination of these two factors increases the risk of this amputation to 20%, which is much higher than that of non-heel ulcer (6.9%) or non-ischemia ulcer (<5.1%) (8). In this case, the overall treatment goal was designed to improve blood supply to her diseased lower extremities and close the ulcer. First of all, we developed a set of treatment procedures for these patients (Figure 3), which were implemented in four stages: to revascularize the occluded large vessels of the affected lower limb, to improve the local blood supply of the ulcer, to debride and repair the ulcer, and to remodel the closed ulcer. If the target vessels leading to the heel, namely, the posterior tibial and peroneal arteries, are successfully reconstructed, which could improve the local blood supply, the overall survival rate of the free flap grafted to this site can reach 73%, and the limb salvage rate of the patient can be greatly increased (9, 10).

Under the guidance of the concept of "angiosome", wound-oriented revascularization surgery is very important for ulcer healing and limb rescue (11–13). In this patient, the ulcer was located in the middle posterior aspect of her right heel, and the preoperative angiography confirmed long segment occlusion of the inferior segment of her right superficial femoral artery with segmentary severe stenosis. The posterior tibial artery and peroneal artery of her right lower extremity should be opened theoretically. However, as shown in Figure 1C, the anterior tibial artery, the peroneal artery including the posterior communicating branch, and part arterial arch of the foot were re-opened successfully, but the posterior tibial artery failed to be recanalized. Because the posterior communicating branch of the peroneal artery can compensatively supply blood to the posteromedial heel of the heel, thereby overcoming the adverse effects of the unsuccessful recanalization of her posterior tibial artery, it was believed that the patient still has the possibility of further complex surgeries to repair the wound at this time.

Studies have shown that as skin oxygen tension increases and carbon dioxide pressure decreases progressively for several weeks after a successful percutaneous transluminal angioplasty (PTA), the likelihood of ulcer healing increases dramatically. Therefore, the best time for surgical wound management should be 3–4 weeks after successful PTA revascularization and 1–2 weeks after bypass revascularization (14, 15). Note that during this period, local infection of the wound may worsen following the gradual recovery of blood flow, which needs much more observation and even debridement in advance if necessary. This patient experienced a waiting period of approximately 4 weeks after the vascular intervention, until the blood supply to the injured heel was restored, and the granulation tissue at the base of the wound was bright red and bleeding after scraping.

The following repair process for diabetic foot wounds is a combination of a series of medical methods, which is complicated. As a core component of traditional DFU treatment, debriding the wound not only removes inactive bone or soft tissue that may pose a risk of bacterial colonization and infection but also effectively repairs the wound to the acute stage or hemostasis/coagulation stage, which is conducive to its later healing (16). Some appropriate methods from the four-layer "pyramid" techniques of repairing soft tissue include negative pressure wound therapy/primary closure, local random flaps/skin grafting/bioengineered tissue alternatives, pedicle flaps/local muscle flaps, and free tissue transfer to cover the ulcer (17). Among them, pedicle flaps/local muscle flaps and free tissue transfer

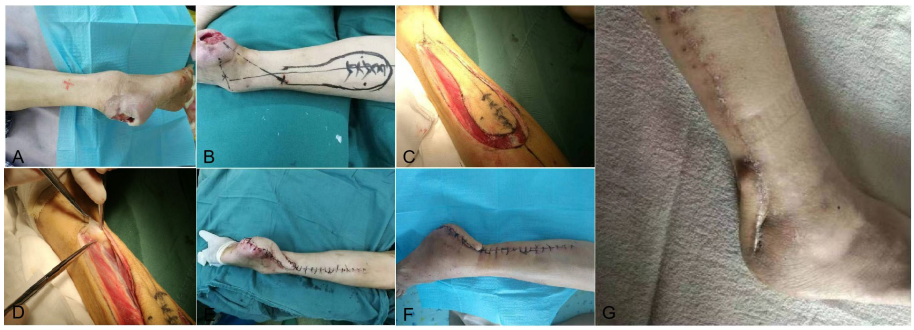

FIGURE 2
The repair of the wound and postoperative follow-up. After the blood supply improved, the wound repair procedure began. Wound debridement began approximately 4 weeks after the vascular intervention, with fresh granulation tissue hyperplasia at the base of the wound and bleeding after scraping **(A)**. The location of perforating artery of peroneal artery perforator flap **(B)**. Peroneal artery perforator flap design during operation **(C, D)**. The wound condition after the operation **(E)** and dressing change of the wound post-operation **(F)**. At the postoperative follow-up, the patient's wound healed completely approximately 7 weeks after the operation **(G)**.

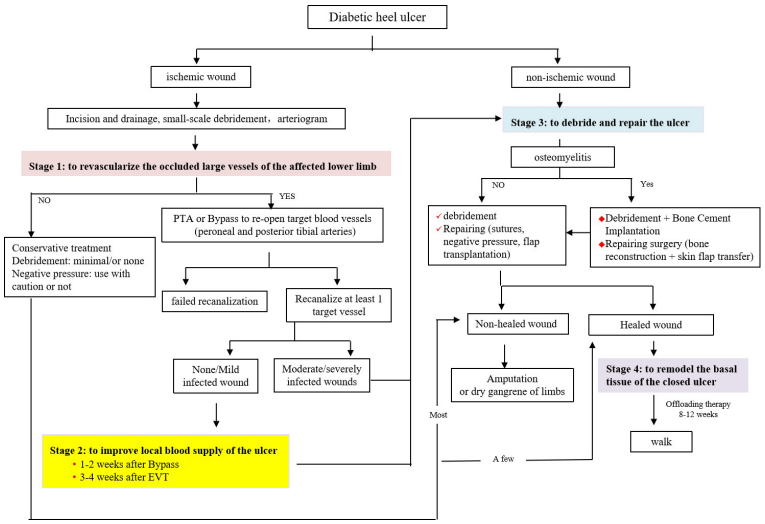

FIGURE 3
Treatment procedures for heel ulcers. For ischemic diabetic heel ulcer, treatment procedures were implemented in four stages: i) revascularize the occluded large vessels of the affected lower limb, ii) improve the local blood supply of the ulcer, iii) debride and repair the ulcer, and iv) remodel the closed ulcer.

are the most difficult and risky and have high technical requirements for the operator, but they are still increasingly used in the treatment of diabetic foot ulcers because of their high effectiveness (18). With the restoration of local blood supply to the heel by recanalizing the peroneal artery successfully, the heel ulcer of this patient was cleaned clearly and closed with a highly challenging ultra-microsurgical peroneal artery perforator flap completely (Figure 2).

It is generally known that wound healing is the beginning of its basal tissue remodeling phase. During the healing process of diabetic foot wounds, irregular or irregularly arranged collagen fibers are gradually replaced by newly synthesized, more regular, and elastic collagen fibers until the original scar tissue on the wound becomes soft, has a lighter color, and becomes smooth and orderly. The entire wound remodeling process can last for months or even years until the tensile strength of the tissue within the wound is restored as much as possible. We followed up the patient approximately 7 weeks after the operation, and the wound was completely healed (Figure 2G). Moreover, offloading treatment at the wound-healing stage is very important for a DFU patient (19). The female patient was given adequate offloading therapy, such as

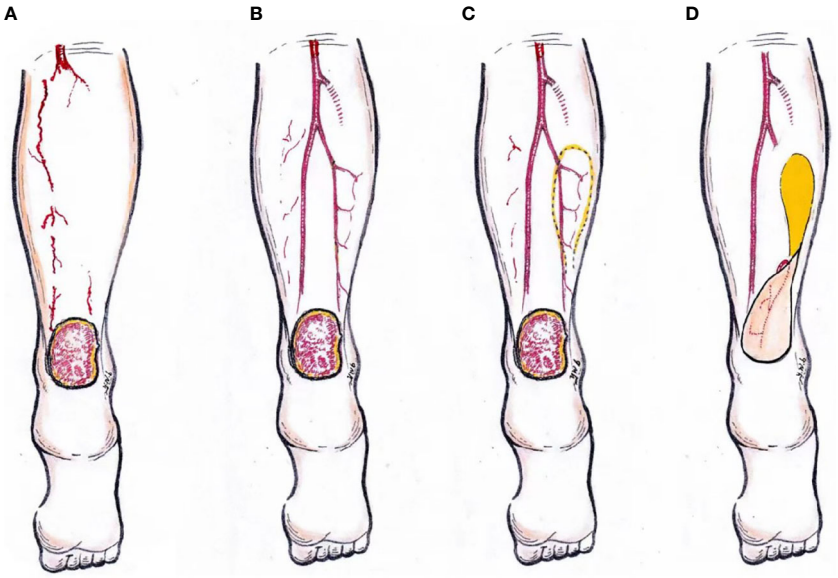

FIGURE 4
A schematic representation of the surgical management of ischemic diabetic heel ulcer. Assessment of the lower limb blood supply **(A)**. Reconstruction of the target artery vessels **(B)**. Design and operation of peroneal artery perforator flap transfer surgery **(C, D)**.

non-weight bearing for up to 8 weeks after flap transplantation. Ultimately, the patient was able to walk gently with a well-healed flap at the 12th week and with full weight bearing at the 24th week.

4 Conclusions

This case report demonstrates that the management of large ischemic heel ulcers in diabetic patients has its particularity. The diagnosis of diabetic foot requires not only evaluation for ulceration, ischemia, and infection but also differentiation from other causes of heel ulcers. Subsequently, this type of ulcer should be treated in stages according to a certain sequence, as shown in Figure 4, to assess and revascularize the occluded large vessels of the affected lower limb, to improve the local blood supply of the ulcer, to debride and repair the ulcer, and finally to remodel the closed ulcer using flap surgery. Among them, the opening of at least one target blood vessel in the heel is the premise, the successful transplantation of the skin flap to cover the wound is the key, and offloading measures such as walking without weight-bearing are also very important in the later stage. In conclusion, as one of the risk factors of major amputation, massive ischemic heel ulcer needs to be carefully diagnosed and treated according to a special procedure.

Data availability statement

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

Ethics statement

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

Author contributions

YDC was responsible for study conception and design. HY wrote the first draft of the manuscript. YCC, AP edited the manuscript. WW, DJ, LL, WY reviewed the last version of the manuscript. All authors contributed to the article and approved the submitted version

Funding

This work was supported by the National Natural Science Foundation of China (No. 81770810). The authors gratefully acknowledge the contribution of the participants in this study and their families.

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

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TYPE Review
PUBLISHED 04 July 2023
DOI 10.3389/fendo.2023.1166513

OPEN ACCESS

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RECEIVED 15 February 2023
ACCEPTED 19 June 2023
PUBLISHED 04 July 2023

CITATION
Hemler SL, Ntella SL, Jeanmonod K, Köchli C, Tiwari B, Civet Y, Perriard Y and Pataky Z (2023) Intelligent plantar pressure offloading for the prevention of diabetic foot ulcers and amputations. *Front. Endocrinol.* 14:1166513. doi: 10.3389/fendo.2023.1166513

Intelligent plantar pressure offloading for the prevention of diabetic foot ulcers and amputations

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The high prevalence of lower extremity ulceration and amputation in people with diabetes is strongly linked to difficulties in achieving and maintaining a reduction of high plantar pressures (PPs) which remains an important risk factor. The effectiveness of current offloading footwear is opposed in part by poor patient adherence to these interventions which have an impact on everyday living activities of patients. Moreover, the offloading devices currently available utilize primarily passive techniques, whereas PP distribution is a dynamically changing process with frequent shifts of high PP areas under different areas of the foot. Thus, there is a need for pressure offloading footwear capable of regularly and autonomously adapting to PPs of people with diabetes. The aim of this article is to summarize the concepts of intelligent pressure offloading footwear under development which will regulate PPs in people with diabetes to prevent and treat diabetic foot ulcers. Our team is creating this intelligent footwear with an auto-contouring insole which will continuously read PPs and adapt its shape in the forefoot and heel regions to redistribute high PP areas. The PP-redistribution process is to be performed consistently while the footwear is being worn. To improve adherence, the footwear is designed to resemble a conventional shoe worn by patients in everyday life. Preliminary pressure offloading and user perceptions assessments in people without and with diabetes, respectively, exhibit encouraging results for the future directions of the footwear. Overall, this intelligent footwear is designed to prevent and treat diabetic foot ulcers while enhancing patient usability for the ultimate prevention of lower limb amputations.

KEYWORDS

diabetes mellitus, pressure offloading, foot ulcers, amputations, medical device, human factors, smart insole

1 Introduction

There are currently 537 million adults with diabetes mellitus representing 10.5% of the global adult population (1). On average, 19-34% of people with diabetes will develop at least one ulcer in their lifetime (2–4) and 84% of amputations in people with diabetes are due to an ulcer (5). Foot plantar ulcers typically form due to elevated plantar pressures (PPs) as a consequence of peripheral sensorimotor neuropathy (6). Peripheral neuropathy is characterized by the lack of protective pain sensation (the "gift of pain" (7)) and affects up to 50% of individuals with type 2 diabetes (8). Furthermore, ill-fitting footwear has been identified as the root cause of 21-76% of ulcers and/or lower extremity amputations in people with diabetes (9). Effectively implementing pressure offloading interventions is essential for treating and preventing diabetic foot ulcers.

There are many wearable offloading interventions with varying efficacy and with limitations (Figure 1). Non-removable interventions effectively aid ulcer healing because of their forced adherence (10–13). However, these interventions are used in less than 2% of diabetic foot centers and may increase pre-existing challenges such as gait and balance impairments (14–16), low-weight bearing activities (14), restrictions of daily activities, low quality of life (17), and stigmatization (16). Removable interventions include a range of foot enclosures that allow the patient to have autonomy for removal during treatment. In general, removable interventions have been preferred by patients for convenience among other factors (18). Such interventions include knee-high or ankle-high offloading devices and types of footwear and insoles. These interventions vary in effectiveness for reducing peak PP at the ulcer location (10) and thus, aiding in ulcer healing (19). Knee- and ankle-high offloading devices (i.e., cast shoes, half-shoes, and forefoot offloading shoes) have been shown to have higher effectiveness in healing ulcers than conventional or custom-insole footwear (10, 11).

However, knee- and ankle-high offloading devices often limit mobility or have negative rocker outsoles which may induce balance problems (11). Therefore, once the ulcer is healed, lower-height modalities (e.g., custom footwear) which support more natural gait and mobility may be a more practical, long-term solution for ulcer prevention for this population for whom there may already be limited joint mobility (11, 13, 20, 21).

There are a range of therapeutic footwear interventions that have shown varied efficacy in ulcer treatment and prevention. Footwear interventions may include fully customized footwear (i.e., custom insoles in custom-made shoes), semi-customized footwear (i.e., custom insoles in extra-depth shoes), or un-customized footwear (i.e., prefabricated insoles in normal shoes) (22, 23). Custom-insoles are designed to offload high pressure areas for preventing ulcers, and/or to offload known ulcer areas. However, there is varied efficacy in how much pressure reduction occurs in different types of custom insoles (22, 24). Other research has shown a lack of difference in peak PP reduction between custom and prefabricated insoles (23). Overall, there are wide-ranging levels of PP reduction in various types of interventions.

Pressure biofeedback insoles have been used to actively detect and display high pressure areas under the foot. In current systems, users are instructed to adjust their gait to reduce the PPs in the identified regions. These insoles have been shown to be helpful for redistributing pressures, though the method of redistribution involves patient's active participation which is difficult in the long-term (25–27). In one of these studies, the learning response took 12 weeks of wear (25). Thus, there is a need for a footwear strategy that actively senses and instantly offloads the high plantar regions without cognitive input from the user.

Therapeutic footwear could have high adherence if designed according to the desires of the patient. Offloading intervention adherence is associated with ulcer healing (28) and wearing offloading footwear for the majority of the day has been shown to reduce the risk for foot re-ulceration (29, 30). However, studies have shown that less than 50% of patients wear their therapeutic footwear for more than 60% of daytime hours (31, 32). Furthermore, one study showed that among the most important footwear features for patients with diabetes, style was a priority compared to comfort as a priority in people with other diseases such as rheumatoid arthritis (33). Important factors influencing therapeutic footwear dissatisfaction for people with diabetes and neuropathy are the weight (31, 34) and comfort of the footwear (31), and the perceived opinion of others (35). Thus, improving the design and style of therapeutic footwear to be adapted to the patients' desires, is mandatory to improve long-term adherence.

Overall, there is a need for user-friendly, offloading footwear that reduces high PP to prevent and treat foot ulcers. The aim of this article is to present a review of the advancements toward this goal made by a multi-centered team from the Geneva University Hospitals (HUG), University of Geneva (UNIGE), and École polytechnique fédérale de Lausanne (EPFL). The team is

	Needs for Ulcer Prevention & Treatment			
Wearable Interventions	Active Offloading	Adaptable to daily life	Long-term efficacy	Minimal resources required
Non-removable cast walkers			✓	
Removable knee- & ankle-high devices			~	~
Custom/prefabricated insoles & footwear		✓	~	~
Pressure-feedback & gait retrainment	✓			
Our Intelligent Pressure-Offloading Footwear	✓	✓	~	✓

FIGURE 1
Offloading interventions and the ulcer prevention and treatment needs which each fulfill with proven success (green check marks) or varied/inconsistent success (yellow tilde), or which they do not fulfill (blank orange). Our intelligent footwear plans will meet all needs and will be tested for long-term efficacy.

developing intelligent offloading footwear that is designed to use a pressure feedback loop to automatically sense and redistribute PPs to prevent and treat diabetic foot ulcers (36).

2 Intelligent footwear design

2.1 Summary of pressure-offloading

The intelligent footwear presented in this article consists of outer and inner (removable insole system) parts (Figure 2). Within the removable insole system, there is a pressure-sensing system coupled with miniaturized pressure-offloading modules. The system is designed to automatically detect the location of high PPs and correspondingly adjust the contour of the insole according to the user's individual pressure needs (Figure 3).

2.2 Intelligent insole system

Inside the footwear, there is a removable, intelligent insole system (Figure 2 - inner), which is made of several components working together to redistribute high PP. The system consists of a housing insole in which the pressure-offloading modules, batteries, and control electronics rest, and above which the comfort insole and pressure-sensing insole sit. The pressure-offloading modules operate independently and are connected to the control electronics via flex PCB through channels on the underside of the housing insole. Each pressure-offloading module consists of three primary parts: 1) Top - deformable bellow filled with magnetorheological (MR) fluid and top plug, 2) Middle – flow channels and valve, and 3) Bottom - deformable reflow membrane and auxiliary reservoir (Figure 4) (37). The pressure-sensing insole consists of piezoresistive sensors (dynamic range: 0-800kPa; sampling frequency: 200 Hz) aligned directly over each corresponding pressure-offloading module. The thin-protective layer is the interface between the foot and the removable insole system with a goal of providing a moisture-absorbing and comfortable barrier between the mechanics and the foot.

The design of the removable insole system is such that when pressure is applied by the foot to the area above a module (e.g., from standing or walking), the module will be triggered to operate in one of two states: 1) valve *off*, or 2) valve *on*. When the valve is *off*, the MR material remains in its fluid state and can move through the flow channels and the annular gap in the valve to be dispensed into the auxiliary reservoir (Figure 5A). The resultant movement of the MR material results in a maximum module compression of 2.5 mm. When the force is removed, the reflow membrane forces the fluid to return into the deformable bellow above. However, when the valve is *on*, the exciting magnetic field (magnetic flux) causes the MR material to solidify in the valve channels and prevents the fluid from traveling to the auxiliary reservoir (Figure 5B). In the *on* state, there is a maximum module compression of 0.5 mm due to mechanical stabilization.

A baseline decision algorithm (based on the maximum peak pressure sensed above a module) will be employed to determine which modules will be turned *off* according to the user's pressure needs (Figure 6). There will be a set number of modules that may be turned *off* at one time (X_{red}) to redistribute pressures. In the future, a trained and validated machine learning algorithm will be used to intelligently control which modules are *off* or *on*.

The design of the inner components is based on previous research and tailored to be suitable for footwear conditions. The size of the region above each module that can be deformed to achieve the intended pressure redistribution is a compromise between the complexity of the control system (in terms of both the hardware and the algorithm) and the accuracy in the determination of the

FIGURE 2
Schematic of the outer and inner (removable insole system) parts of the shoe. The removable insole system includes the housing insole which contains the batteries, computing device, and pressure-offloading modules, and the comfort insole, pressure-sensing insole, and protective insole which rest on top of the housing insole.

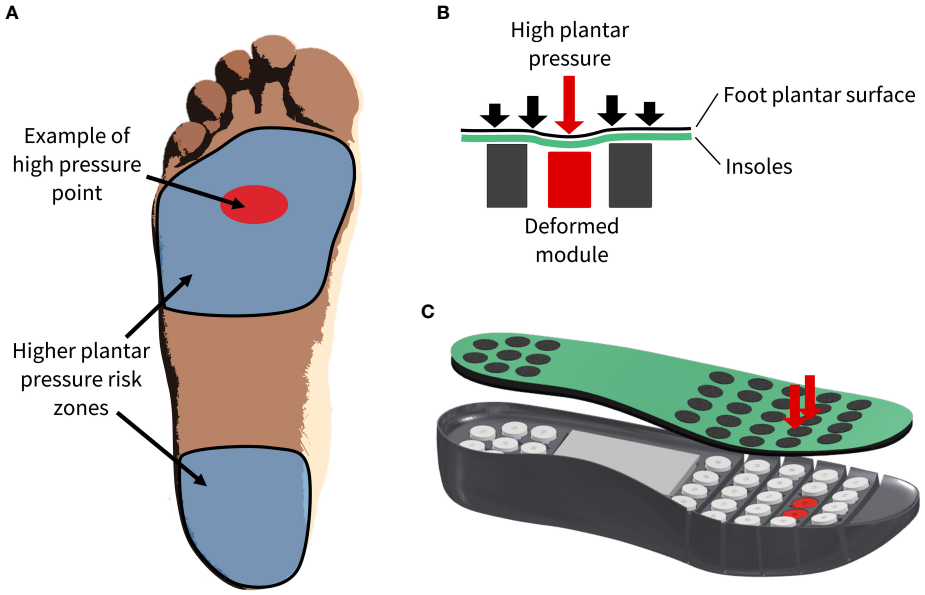

FIGURE 3
(A) High pressure regions of interest and example high pressures on the plantar surface of the foot. **(B)** 2D, **(C)** 3D schematics of high plantar pressures (downward red arrows) which guide the automation and deformation of specific modules (in red).

magnitude and location of the peak PPs. In this respect, previous research (38) underlined that in most cases, a sensor having a surface area of 1 cm^2 is sufficient (accuracy of ≈ 90%) to define the position and the proportions of the peaks of PPs. Thus, the reference value for the surface area exposed to loading (above each pressure-offloading module) is fixed to 1.6 cm^2, being a compromise between sufficient sensing/actuation resolution and system complexity. Each of the modules is waterproof and future designs will incorporate this same quality for all other electrical components. Further tests will also address safety features such as componentry heating and falls risk due to the elevated height of the footwear (measurement of ground to foot plantar height = 6.3 cm).

The module's performance and the system's ability to reduce PP across the insole have been tested. In the module's *on* state, it could sustain a load of 55N, which corresponds to 357 kPa (39) with a residual deformation of only 0.5 mm. Thus, the performance of the module while *on* meets performance standards; with a PP of this magnitude, the module would likely be turned *off* to offload that region to prevent a foot ulcer. When the module was turned *off* during the tests, the module instantaneously deformed to 1.5 mm

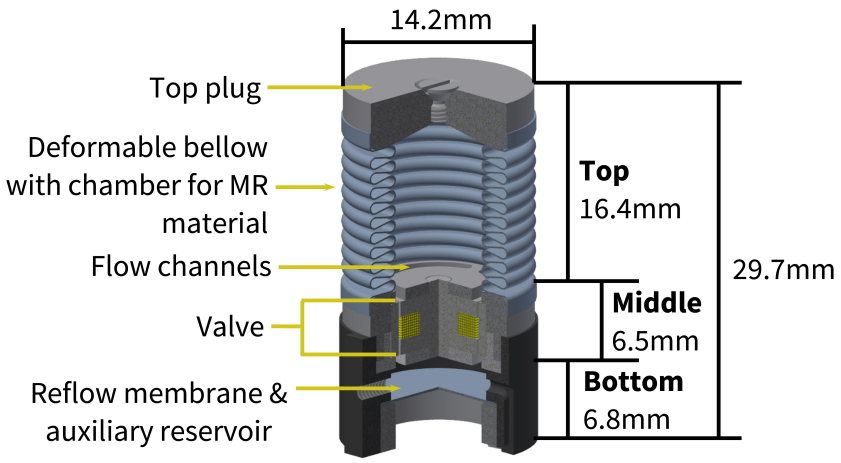

FIGURE 4
The pressure-offloading module frame and dimensions.

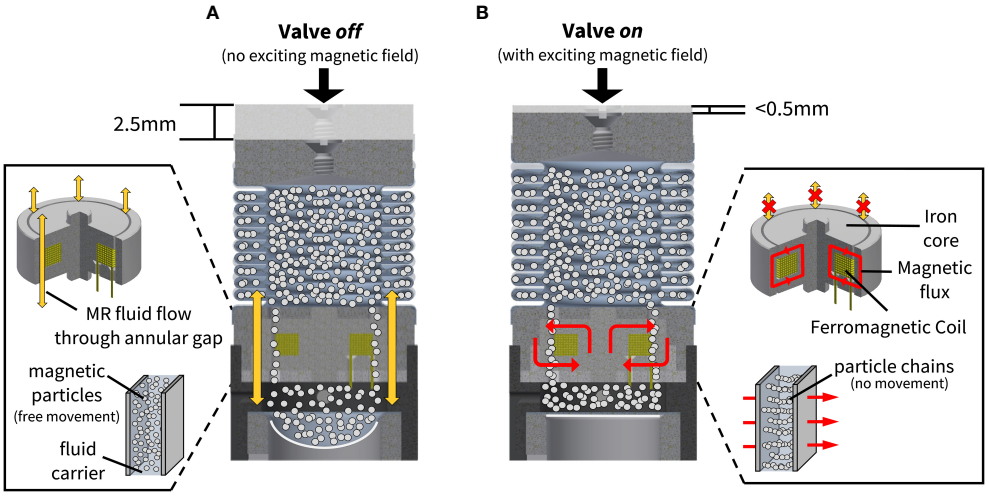

FIGURE 5
(A) When the valve is *off* and an external force is applied to the module, the fluid remains in its liquid state and is pressed from the deformable bellow through the flow channels and annular gap in the valve to the auxiliary reservoir below (downward yellow arrows). **(B)** When the valve is *on*, the fluid is solidified by the magnetic flux (cyclical red arrows) such that there is no fluid flow from the deformable bellow to the auxiliary reservoir below.

which was the maximum deformation allowed for this test, and the force was reduced to 30 N (corresponding to 214 kPa) (39). This final force was linked to the features of the deformable bellow and the hydraulic resistance. Furthermore, a preliminary walking study with four, healthy, male adults was conducted to assess the PP reduction. Participants wore the prototype of the footwear with surrogate modules as they walked 10m at a comfortable walking speed. The deformation of the module between the *on* and *off* states allowed for a maximum reduction of PP of 18-24% directly over the module and 6-10% reduction in the area around the module when the peak starting PP ranged from 273-607 kPa (40). Furthermore, for cases with an initial peak PP above 400 kPa, there was a 20-32% reduction in peak PP. The present study was approved by the University Commission for Ethical Research in Geneva (CUREG 2022-07-78).

3 Design for patient use and adherence

Adherence is an essential aspect of medical device use. To understand the user perceptions and potential adherence barriers to this footwear, a pilot, in-person questionnaire and a larger, online

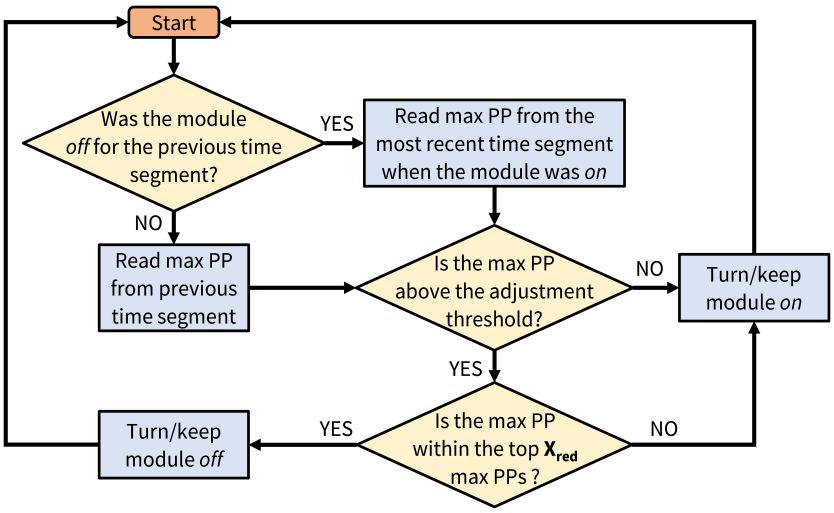

FIGURE 6
Flow diagram of the baseline decision algorithm (mPP – the maximum of the sensor's peak PP; X_{red} – the maximum number of modules that would be turned *off* at one time).

questionnaire were conducted concerning the intelligent footwear presented in this article (41). Ethical approval was obtained to conduct the questionnaires (CUREG 2022-03-35). Across the two questionnaires, people with diabetes (n=48), caregivers of people with diabetes (n=10), and healthcare professionals working with people with diabetes (n=65) from 30 countries on 6 continents gave important insight regarding the functionality, potential adherence, self-image, and aesthetics of the footwear. The questionnaires addressed the potential use and barriers to using this intelligent footwear based on previous work (42, 43); questionnaires were administered and processed by the researchers with aid from clinicians. Generally, 95% of respondents thought that it would be beneficial to use the footwear and over 70% in each role stated that they would use the footwear or recommend it to their patients when available. Several parts of the questionnaire addressed self-image while wearing the footwear and perceived efficacy of using the footwear. The results informed aspects of this intelligent footwear design such as implementation of a sports shoe design which was the most preferred style among others (41). Future designs of this footwear will include styles of shoes for all occasions for men and women.

One of the limitations of other "smart" offloading footwear is the need for the patient to interact with a device and alter gait to relieve areas of high PPs (25–27, 44). To lessen the required user-involvement and thus, increase the likelihood of adherence, this intelligent footwear will have an autonomous, pressure-redistribution algorithm. As the individual wears the shoes, the insole will regularly read the PPs and automatically change the contour of the insole eliminating the need for the patient to interact directly with the device during the day. The only required interaction is the need to charge the shoes each day after wear. With a current of 0.7A, activating the *on* state of a module (200 ms) for 5,000 steps (recommended daily step count per foot for people with diabetes (45, 46)) would require 195 mAh per module. One shoe of size EU 43 has 31 modules which would require a total of ~6,000 mAh. Therefore, a battery with 9,000mAh of energy (footwear has the capacity to house two batteries) is sufficient to provide a day's worth of charge for each shoe. To apprehend complications with charging that could possibly reduce adherence, the footwear is designed to have a charging mechanism similar to technology that users may already operate (e.g., cellphones, tablets). Furthermore, assessments of other adherence parameters have been performed and are ongoing in order to increase footwear adherence (41).

4 Conclusion

The presented intelligent footwear is designed to automatically and autonomously redistribute high PP under the feet of people with diabetes and specifically those with neuropathy. The mechanisms to offload the pressures use an intelligent, removable insole system which will actively adapt to the person's foot while they are wearing the intelligent footwear. The footwear is designed to improve adherence through simplicity of user involvement and aesthetics resembling footwear not associated with a medical condition. Future versions will improve upon the technical and human factors aspects of the footwear to enhance flexibility, durability, battery life, usability, and aesthetics. The technological and adherence aspects of the footwear will continue to be tested and improved through clinical trials.

Author contributions

YP and ZP conceived of the original idea. KJ, SN, CK and BT developed the technological aspects of the idea (modules, electronics, removable insole system, etc.). YC, SH, and ZP developed the structural design of the footwear. YC, CK, YP and ZP supervised and guided the project. SH wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the Swiss National Science Foundation (BRIDGE Discovery Grant #40B2-0_181020).

Acknowledgments

The authors would like to acknowledge Paolo Germano (EPFL) as well as Paul Giglio and Corentin Laffont (Giglio Orthopédie SA - Geneva) for their research and development collaboration.

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

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frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 02 August 2023
DOI 10.3389/fendo.2023.1165305

OPEN ACCESS

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RECEIVED 13 February 2023
ACCEPTED 17 July 2023
PUBLISHED 02 August 2023

CITATION
Xu J, Chen W, He L, Feng S, Zhang J and Chang B (2023) Most postoperative reserved "normal" metatarsal stumps of diabetic foot osteomyelitis are infected but have healing potential.
Front. Endocrinol. 14:1165305.
doi: 10.3389/fendo.2023.1165305

Most postoperative reserved "normal" metatarsal stumps of diabetic foot osteomyelitis are infected but have healing potential

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Background: Although the pathology and bacterial status of the "normal" bone stump after operation of diabetic foot osteomyelitis (DFO) are of great significance for the prognosis of foot wounds, there are only a few studies on this topic; hence, it is clinically relevant and urgent to study this topic.

Methods: The data of 57 inpatients with DFO from June 2021 to April 2022 were collected, all of whom had DFO in the forefoot and underwent conservative surgery. After the surgical removal of necrotic bone, bone biopsies were taken from the necrotic phalangeal bone and the reserved "normal" metatarsal stump. They were cultured, after which antibiotic susceptibility test and pathological screening were carried out. According to clinical judgment, inpatients' wounds were divided into metatarsal affected group and metatarsal unaffected group. We then compared and analyzed the pathological and bacterial characteristics of preserved "normal" bone stump and its effect on wound healing and prognosis.

Results: The poor concordance rate between deep soft tissue culture and infected phalange culture was only 19.3%. The deep soft tissue (72.6%), infected phalange (70.7%), and metatarsal stump (71.4%) were mainly infected with gram-negative Bacillus. The proportion of *Enterococcus spp.* increased significantly in bone tissue. *Acinetobacter baumannii* had the highest drug resistance (88%, 22/25). There was no significant difference in several clinical characteristics and wound healing regardless of whether their metatarsal stumps were affected. Most reserved "normal" metatarsal stumps (84.2%, 48/57) were positive by pathological diagnosis and bacterial culture testing; only 15.7% (9/57) samples were truly sterile. Only 8.3% (4/48) of the former patients healed within 6 months; whereas, all the latter (9/9) patients healed within 6 months. However, the majority (89.6%, 43/48) could heal. There was no difference in operations, skin grafting, negative pressure wound therapy, and mortality between the two groups.

Conclusion: The most reserved "normal" metatarsal stumps have been invaded by bacteria. However, the majority stumps can be preserved, and the wound will eventually be healed according to the pathological and bacterial culture results.

KEYWORDS

diabetic foot osteomyelitis, reserved metatarsal stump, infection, conservative surgery, wound healing

1 Introduction

According to the diabetes map of International Diabetes Federation (10th version), there are 578 million diabetic patients in the world (1). Diabetic foot is a common chronic complication of diabetes. According to the International Working Group on Diabetic Foot (IWGDF) report, one patient will lose a leg because of diabetic foot every 20 s (2). Diabetic foot is associated with serious financial and health-related burden to the affected patients, their families, and society. Diabetic foot infection is one of the main causes for hospitalization of diabetic patients. If the infection is not treated promptly and appropriately, then it will often lead to amputation or even death (3–6). Diabetic foot osteomyelitis (DFO) has always been an important topic in clinical practice, both in diagnosis and treatment. In the past, clinicians often pointed out that infected bone should be completely removed. However, recent studies have reported that the DFO in toe can heal without amputation and with only using antibiotics (7). Further studies recommend the use of conservative surgery, in that, after complete removal of the necrotic bone, even if the adjacent bone is infected, it can be retained provided that it is "normal" after the operation and it is hard with healthy, red bone marrow and with no obvious purulent necrosis (8). The question remains whether it is necessary to conduct bone culture and pathology for the preserved "normal" stump by clinical observation? In addition, is the preserved "normal" stump truly sterile? Finally, in case of residual infection, is there any difference between the postoperative treatment and wound healing and the truly sterile stump. To our knowledge, there is a scarcity of research around these questions, and, hence, it is clinically relevant and urgent to study this topic.

2 Materials and methods

Patients hospitalized in the Department of Diabetic Foot Chu Hsien-I Memorial Hospital, Tianjin Medical University, from June 2021 to April 2022 were selected if they met the following criteria: (i) Patients with a diagnosis of diabetes based on the WHO criteria; (ii) those with diabetic foot who met the IWGDF guidelines and had a grading of their infectious severity; (iii) the infection was mainly in the phalanges and/or the metatarsal bones; (iv) all osteomyelitis cases were confirmed by biopsy of the affected phalangeal bone during the operation, and at least one positive bacterial culture or diagnostic bone histopathology confirmed phalangeal osteomyelitis; (v) after amputation of the infected and necrotic phalanges, the preserved metatarsal stumps were sampled by bone biopsy, including bacterial culture and histopathology; and (vi) patients voluntarily agreed to participate in this study.

According to the above inclusion criteria, 57 patients with DFO were enrolled for the final analysis.

The following exclusion criteria were applied: (i) Patients with DFO who were only treated with antibiotic treatment and simple debridement without amputation; (ii) patients with DFO in the heel; (iii) pregnant patients; and (iv) those who were unable to cooperate with the study.

All patients' demographic characteristics; duration of diabetes and diabetic foot; and complications such as coronary heart disease, stroke, hypertension, dyslipidemia, hyperuricemia, or gout were recorded. Glycosylated hemoglobin A1c; biochemical indicators; and infectious indicators such as white blood cell count and neutrophils percentage, C-reactive protein, procalcitonin, and erythrocyte sedimentation rate were measured by the hospital laboratory. Peripheral arterial disease was diagnosed by foot artery palpation, ultrasound, or transcutaneous oxygen pressure. Diabetic peripheral neuropathy was diagnosed by a 10-g monofilament and 128-Hz tuning fork.

The enrolled patients with DFO in the forefoot, as well as those of diabetic complication, were treated with antiglycemic medicine or insulin, antibiotics, and debridement. We also provided basic treatment for hypertension, dyslipidemia, hyperfibrinogenemia, elevated D-dimer levels, and cessation of smoking. Deep tissue for bacterial culture was collected from all patients at admission. Because their toes were severely infected and necrotic and could not be preserved, they were all amputated. The management of the adjacent metatarsal bones involved two situations. One was when the metatarsals were unaffected upon clinical observation. However, after treatment of the metatarsophalangeal joint capsule, the metatarsal bones were not conducive to granulation growth because of the presence of the joint surface. The conventional method is to remove the joint surface and expose the metatarsal head, which is conducive to wound healing. The other was that the adjacent metatarsal bone was damaged and needed surgical debridement until the surgeon deemed the reserved metatarsal stump as being "normal." All infected phalanges and the reserved "normal" metatarsal stump were subjected to bone biopsy. The former was to determine the diagnosis of osteomyelitis, and the latter was to determine whether there was infection and whether the infected stump had an impact on wound healing. The use of

antibiotics was guided by the results of bone culture and the reaction of the wound after the operation. The treatment of negative pressure drainage, various growth factors, and skin grafting was carried out according to the situation of the wound (9).

All patients were followed up at the diabetic foot outpatient clinic for at least 6 months after discharge. On average, patients came to the outpatient clinic for a follow-up visit every 2–4 weeks based on condition of their wounds.

The first author and the corresponding author completed all the surgical procedures of this study; the third author completed all the bacterial cultures; and a pathologist from the Department of Pathology interpreted all histopathological findings. This study was approved by the institutional ethics committee, and all patients signed the informed consent form.

SPSS 28.01.1 (IBM Corporation, Armonk, NY, USA) was used for data analysis. Levene test was used for normality testing of quantitative data. F-test or t-test was used for normally distributed quantitative data, and Mann–Whitney U-test was used for non-normally distributed quantitative data. Qualitative data were compared using $\chi 2$ test, but, if the expected value of the cell was <5, then Fisher's exact test was used. $P < 0.05$ was considered to indicate statistically significant differences.

3 Results

3.1 Bacterial composition of diabetic foot wound

In the foot wounds of 57 patients, one fungus and 83 bacteria were cultured in the deep soft tissue, one fungus and 74 bacteria were cultured in the phalanges, and 28 bacteria were cultured in the metatarsal stumps. The composition of specific bacteria is presented in Figure 1.

Among the deep soft tissue, gram-positive cocci accounted for 25%, gram-negative bacilli accounted for 72.6%, and one gram-positive bacilli (*Corynebacterium striatum*) and one fungus (*Candida smoothens*) accounted for 1.2%, respectively. The top three bacteria were *Staphylococcus aureus* (nine strains), *Klebsiella pneumoniae* (eight strains), and *Pseudomonas aeruginosa* (eight strains). Among the gram-positive cocci, the first three were *Staphylococcus aureus* (nine strains), *Streptococcus lactis* (three strains), and *Staphylococcus epistaphylum* (three strains). Among gram-negative bacilli, the first three were *K. pneumoniae* (eight strains), *P. aeruginosa* (eight strains), and *Acinetobacter baumannii* (seven strains). In the phalanges, gram-positive cocci accounted for 28%, gram-negative bacilli accounted for 70.6%, and one fungus accounted for 1.3%. The top three bacteria *were A. baumannii* (14 strains), *P. aeruginosa* (six strains), and *Enterobacter cloacae* (five strains). Among gram-positive cocci, the top three were *Staphylococcus aureus* (three strains), *Enterococcus faecalis* (three strains), and *Enterococcus avium* (three strains). The sequence of gram-negative bacilli was the same as the total sequence. In the metatarsal stumps, gram-positive cocci accounted for 28.6%, and gram-negative bacilli accounted for 71.4%. The top three bacteria were *A. baumannii* (four strains), *Enterococcus avium* (four strains), and *Enterobacter cloacae* (three strains). Among the gram-positive cocci, the first three were *Enterococcus avium* (four strains), *Enterococcus faecalis* (four strains), *Staphylococcus aureus* (one strain), and *Enterococcus faecium* (one strain). Among the gram-negative bacilli, *A. baumannii* (four strains), *Enterobacter cloacae* (three strains), and two strains each of *P. aeruginosa*, *Citrobacter freundii*, and *Proteus mirabilis* were found.

From the above bacterial distribution, the following results can be obtained: Deep soft tissue or bone tissue was mainly infected with gram-negative bacilli.

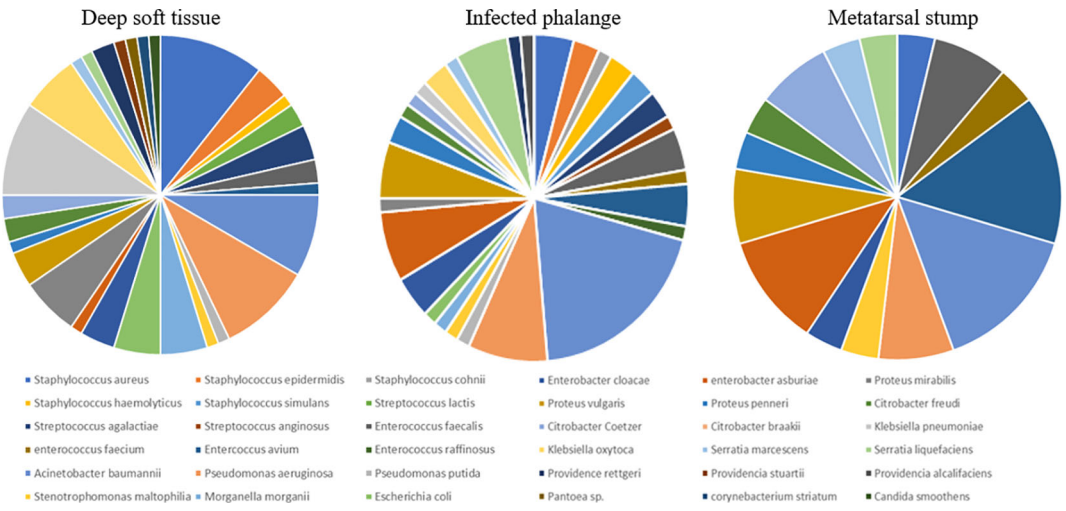

FIGURE 1
The composition of bacteria in deep soft tissue, infected phalange, and "normal" reserved metatarsal stump. Different colors represented different bacteria. The larger was the share, the more was the number of bacteria in the corresponding group. At least 28 species of bacteria had been cultivated from the three kinds of tissue samples. In addition, the specific proportion of bacteria was different, but most of them were Gram-negative bacillus, all were more than 70%.

3.2 Distribution of antibiotics resistant bacteria

The antibiotic resistant rate of deep soft tissue, phalange, and metatarsal stump was not statistically significant (all P > 0.05) (Table 1). Overall, *A. baumannii* had the highest antibiotic resistance (88%, 22/25)

3.3 Distribution of bacterial species

The distribution of bacterial species in different tissues is shown in Figure 2. Only 54.4% of the clinically preserved "normal" metatarsal stumps were sterile.

3.4 Concordance of bacteria culture from deep soft tissues, phalanges and metatarsal stumps

3.4.1 Concordance of bacteria culture from deep soft tissues and phalanges

The concordance of isolated bacteria between the deep soft tissues and phalanges was low, and only 10 wounds (17.5%) had the same bacteria. The proportion of Enterococcus spp. in bone increased, and most of the corresponding soft tissue did not exist (Table 2).

3.4.2 Concordance of bacteria culture from phalanges and metatarsal stump

Although all the reserved metatarsal stumps in the operation were considered "normal," there were two situations requiring different management: In one, the metatarsal bone was unaffected, whereas, in the other, the metatarsal bone was affected. The patients were divided into two groups for comparison of the clinical outcomes.

Table 3 shows that, even if the infected and necrotic part of the affected metatarsal bone was removed in the operation, the negative rate of the retained "normal" metatarsal stump was only 42.4% (14/33). Only 24.2% of the metatarsal and phalangeal bacteria were completely consistent, and bacteria changed in 33% of all metatarsal stumps. Although the unaffected metatarsal bone group was considered be sterile, there was only 62.5% sterility. The metatarsal stump still had bacteria, but it was consistent with the phalange. However, there was no statistically significant difference in the distribution of bacteria between the two groups (Fisher's exact test: 4.822, P = 0.155).

3.5 Analysis of metatarsal stumps

Table 4 shows that the proportion of osteomyelitis in the affected vs. unaffected metatarsal groups was 60.6% (20/33) vs. 29.2% (7/24), respectively, only by bacterial culture diagnosis, and the proportion of osteomyelitis was 93.9% (31/33) vs. 62.5% (15/24), respectively, only by pathological diagnosis. The differences were statistically significant (all P < 0.05). The positive rate of pathology was significantly higher than that of bacterial culture, but it did not reach 100%.

3.6 Clinical characteristics and prognosis

3.6.1 Clinical characteristics

Table 5 shows that the procalcitonin level of patients with affected metatarsal was higher than that of patients with unaffected metatarsal. The positive rate of X-ray was higher in the affected metatarsal group than that in the unaffected metatarsal group. There was no statistical difference between other indicators.

TABLE 1 Distribution of antibiotic resistant bacteria.

	Deep soft tissue				Infected phalange				Metatarsal stump			
	n	First	Second	Third	n	First	Second	Third	n	First	Second	Third
G (+)	1	MRSA1			1	MRSA1			0			
G (−)	10	CRAB6	*Escherichia coli* ESBL 1, *Klebsiella pneumoniae* ESBL 1, *Proteus mirabilis*, CRE 1, *Proteus vulgaris* CRE 1		18	CRAB 13	*Proteus mirabilis* CRE2	*Escherichia coli*, ESBL 1, CRPA 1, *Proteus vulgaris* CRE 1	5	CRAB3	*Proteus mirabilis* CRE 1, *Proteus vulgaris* CRE 1	
Gram (+) antibiotic resistance rate	4.8% (1/21)				4.8% (1/21)				0			
Gram (−) antibiotic resistance rate	16.4% (10/61)				34.0% (18/53)				25% (5/20)			
Total Antibiotic resistance rate	13.4% (11/82)				25.7% (19/74				17.9% (5/28)			

The antibiotic resistance rate in deep soft tissue, infected phalangeal, and metatarsal stump was no statistical significance (all P > 0.05).
CRE, carbapenem-resistant Enterobacteriaceae; ESBL, extended-spectrum beta-lactamase; MASR, methicillin-resistant Staphylococcus aureus.
G (+), gram positive coccus; G (−), gram negative bacilli.

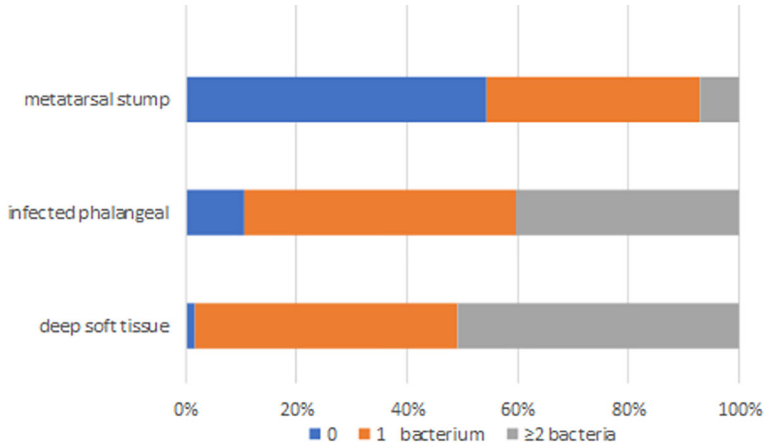

FIGURE 2
The distribution of bacteria species in deep soft tissue, infected phalange, and "normal" reserved metatarsal stump. 0 represented sterile, 1 represents one kind of bacteria, and ≥ 2 represented two or more different kinds of bacteria in sample.

3.6.2 Comparison of operation and prognosis

It can be seen from Table 6 that there was no statistical difference in wound healing, mortality, amputation, and negative pressure use, but patients in the group with clinically affected metatarsal needed more skin grafts than those in the group with clinically unaffected metatarsal (P = 0.007). Three patients died because of poor control of foot infection, difficult wound healing, and heart and multi-organ failure, all of which were related to the foot infection.

3.7 Clinical characteristics and outcomes of patients with true metatarsal negative

3.7.1 Clinical characteristics

There were positive (infected) metatarsal stumps (84.2%, 48/57) in the reserved "normal" metatarsals stump based on surgical diagnosis, regardless of whether the metatarsal was affected on clinical observation. However, there were also true sterile stumps with negative pathology and bacteria. There were eight patients in the clinically unaffected metatarsal group and one in the clinically affected metatarsal group. Table 7 shows the comparison of differences in clinical characteristics and prognosis between these nine patients with true negative metatarsal stumps and other patients.

There was no difference in clinical characteristics between the two parts from Table 7.

3.7.2 Outcome and prognosis

We found that the nine patients healed faster than other patients (P < 0.05) (Table 8). There was no difference in surgical procedure, skin grafting, negative pressure use, and mortality.

TABLE 2 Concordance of bacteria in deep soft tissues and phalanges.

No. 57	Concordance	Difference 47		Bone negative
		Completely difference	Partial difference	
	10 (17.5%)	19	23	5

Completely different meant that there were no same bacteria between the wound deep soft tissue and phalange. Partial difference meant there was at least one species of bacteria consistent between deep soft tissue and phalange. Bone negative meant phalangeal bone culture was negative (but DFO was still established, and its pathology was positive).

TABLE 3 Concordance of bacteria in phalanges and metatarsal stumps.

	Concordance	Difference		Bone negative
		Total difference	Partial difference	
Affected metatarsal "normal" stumps 33	8	2	9	14
Unaffected metatarsal "normal" stumps 24	7	0	2	15

TABLE 4 Analysis of metatarsal stump.

	No.	Bacterial culture (+)	Pathology (+)	Bacterial culture + Pathology (+)	Bacterial culture + Pathology (−)	Metatarsal bone positive by X ray	Bacterial culture (−)	Pathology (−)
Clinically affected metatarsal group	33	1	12	19	1	20	13	2
Clinically unaffected metatarsal group	24	1	9	6	8	0	17	9
Fisher's exact test	11.592					–	5.509	–
P-value	0.004					0.003	0.019	0.005

(+) meaning positive. (-) meaning positive.
The results of Fisher's exact test only have P value; -, do not have statistics value.

TABLE 5 Clinical characteristics between clinically affected and unaffected metatarsal groups.

	No.	Gender (m/f)	Age (year)	Duration of diabetes (years)	HbA1c (%)	Duration of diabetes (months)	Severity of infection (moderate/severe)	CRP (mg/L)	ESR (mm/h)	Procalcitonin (>0.05 μg/L positive)
Clinically affected metatarsal group	33	22/11	62.7 ± 10.6	12.7 ± 8.4	8.2 ± 2.3	2.89 ± 3.16	20/13	67.6 ± 78.3	48.2 ± 17.4	7/26
Clinically unaffected metatarsal	24	16/8	65.5 ± 9.1	14.5 ± 9.2	7.9 ± 1.9	1.97 ± 2.27	20/4	53.9 ± 67.4	48.0 ± 15.4	0/24
Statistics		0.0	−1.030	−0.758	0.46	1.419	3.429	0.689	0.053	–
P-value		1.0	0.307	0.452	0.647	0.162	0.064	0.494	0.958	0.017

	WBC ($\times10^9$/L)	Neutrophils (%)	IWGDF infection severity grade (moderate/severe)	$TcPO_2$ (mmHg)	eGFR (ml/min)	Fibrinogen (g/L)	D-dimer (mg/L)	Albumin (g/L)	hemoglobin (g/L)	DR (±)
Clinically affected metatarsal group	12.55 ± 8.42	73.81 ± 9.92	15/18	24.5 ± 18.6	82.4 ± 28.5	6.07 ± 1.90	1.119 ± 0.89	32.3 ± 5.2	100.0 ± 24.5	23/10
Clinically unaffected metatarsal	9.66 ± 6.64	71.45 ± 10.08	17/7	28.8 ± 17.4	80.4 ± 20.1	5.43 ± 1.76	1.27 ± 1.10	33.5 ± 2.9.0	109.4 ± 22.4	18/6
Statistics	1.395	0.801	3.635	−0.891	0.414	1.28	−0.568	−1.057	−1.473	0.194
P-value	0.169	0.426	0.057	0.377	0.68	0.206	0.572	0.295	0.146	0.660

	CHD (±)	Stroke (±)	Hypertension (±)	Dyslipidemia (±)	Hyperuricemia or gout (±)	Probe to bone test (±)	X ray (soft tissue +/phalange +/metatarsal bone+)
Clinically affected metatarsal group	18/15	12/21	17/16	20/13	6/27	28/5	5/9/20
Clinically unaffected metatarsal group	15/9	11/13	17/7	15/9	5/19	19/5	6/17/0
Statistics	0.361	0.518	2.154	0.021	–	–	26.858
P-value	0.548	0.472	0.142	0.885	1.000	0.727	0.000

HbA1c, glycated hemoglobin A1c; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell; $TcPO_2$, transcutaneous oxygen pressure; eGFR, estimated glomerular filtration rate; DR, diabetic retinopathy; CHD, coronary heart disease.
The results of Fisher's exact test only have P value; -, do not have statistics value.

TABLE 6 Operation and prognosis between clinically affected and unaffected metatarsal groups.

	Wound healing					death	Amputation			Negative pressure wound therapy	Skin grafting
	Healing time < 6 months	Healing time ≥ 6 months	Unhealing	Recurrence	New wound		Primary minor amputation1	Secondary minor amputation	Major amputation		
Clinically affected metatarsal group	7	20	3	2	1	1	26	4	3	21	9
Clinically unaffected metatarsal group	6	12	2	1	3	2	21	3	0	15	0
Statistics	2.385					–	1.977			0.008	–
P-value	0.716					0.567	0.418			0.93	0.007

The results of Fisher's exact test only have P value; -, do not have statistics value.

4 Discussion

Given the increasing incidence of diabetes worldwide, the number of patients with diabetic foot is also increasing year by year. More than 50% patients with diabetic foot are further complicated with infections (10, 11). Typically, patients with DFO need urgent medical treatment or even hospitalization. Given the complexity of treating DFO, the IWGDF infection guidelines (2019

TABLE 7 Clinical characteristics between patients with true negative metatarsal stump with others.

	No.	Gender (m/f)	Age (years)	Duration of diabetes (years)	HbA1c (%)	Duration of diabetes (months)	Severity of infection (moderate/severe)	CRP (mg/L)	ESR (mm/h)	Procalcitonin (>0.05 μg/L positive)
Patients with true negative metatarsal stump	9	5/4	61.0 ± 10.3	13.4 ± 8.5	7.5 ± 1.5	1.8 ± 1.7	7/2	50.7 ± 53.1	47.1 ± 18.2	0/9
others	48	33/15	64.4 ± 10.0	13.5 ± 8.9	8.2 ± 2.2	2.7 ± 3.0	33/15	66.9 ± 77.1	48.3 ± 16.3	7/41
Statistics		–	0.934	0.009	0.906	0.781	–	0.490	0.192	–
P-value		0.463	0.354	0.993	0.369	0.438	0.71	0.626	0.848	0.582
	WBC ($\times10^9$/L)	Neutrophils (%)		$TcPO_2$ (mmHg)	eGFR (ml/min)	Fibrinogen (g/L)	D-dimer (mg/L)		Albumin (g/L)	Hemoglobin (g/L)
Patients with true negative metatarsal stump	8.11 ± 2.44	68.98 ± 9.35		28.3 ± 15.8	75.9 ± 21.8	5.54 ± 2.26	1.35 ± 1.61		34.6 ± 3.2	110.4 ± 31.0
Others	11.94 ± 8.31	73.53 ± 9.88		25.9 ± 18.6	82.6 ± 25.8	5.85 ± 1.79	1.15 ± 0.84		32.5 ± 4.5	102.7 ± 22.5
Statistics	1.362	1.28		−0.369	0.730	0.457	−0.57		−1.341	−0.886
P-value	0.179	0.206		0.714	0.468	0.649	0.571		0.185	0.38
	CHD (±)	Stroke (±)		Hypertension (±)	Dyslipidemia (±)	Hyperuricemia or gout (±)	Probe to bone test (±)		DR (±)	
Patients with true negative metatarsal stump	7/2	4/5		8/1	5/4	2/7	7/2		7/2	
Others	26/22	19/29		26/22	30/18	9/39	40/8		34/14	
Statistics	–	–		–	–	–	–		26.858	
P-value	0.277	1.0		0.069	0.722	1.0	0.65		1.0	

HbA1c, glycated hemoglobin A1c; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell; $TcPO_2$, transcutaneous oxygen pressure; eGFR, estimated glomerular filtration rate; DR, diabetic retinopathy; CHD, coronary heart disease.
The results of Fisher's exact test only have P value; -, do not have statistics value.

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TABLE 8 Operation and prognosis between patients with true negative metatarsal stump with others.

	No.	Wound healing					Death	Amputation			Negative pressure wound therapy	Skin grafting
		Healing time < 6 months	Healing time ≥ 6 months	unhealing	recurrence	New wound		Primary minor amputation	Secondary minor amputation	Major amputation		
Patients with true negative metatarsal stump	9	9	0	0	0	0	0	9	0	0	3/6	0
Others	48	4	32	5	3	4	3	38	7	3	33/15	9
Fisher's exact test		27.333					–	1.283			–	–
P-value		0.000					1.0	0.755			0.063	0.328

The results of Fisher's exact test only have P value; -, do not have statistics value.

edition) have introduced some changes, in that, in the case of moderate and severe infection and if osteomyelitis exists, osteomyelitis must be specifically marked "O" followed by moderate or severe infection (2). This is because the diagnosis and treatment of DFO are more difficult than that of soft tissue infection.

The treatment of DFO has always been a hot topic of research and debate. In recent years, some studies have suggested that osteomyelitis of the forefoot is caused by only neuropathy with good blood supply, provided that there is no serious destruction of the joint capsule, rather only bone exposure. DFO can heal through antibiotic treatment and appropriate debridement, and toe amputation can be avoided (12, 13). However, in clinical practice, the proportion of such patients is limited. Although 90% of osteomyelitis occurs in the forefoot, in many cases, the infection range is large, as the phalangeal bone is broken, and the metatarsophalangeal joint capsule is damaged. Hence, toe amputation is often required. Understandably, the optimum approach to treat has been controversial. If the metatarsal bone is damaged and purulent, then it will be removed, but, generally, the entire metatarsal bone will not be taken off (unless the entire metatarsal bone is damaged and the infection extends to the midfoot or even the hindfoot). Instead, it will be retained up to the residual end of the metatarsal bone that the surgeon considers "normal." However, it is impossible to immediately determine whether these stumps are truly sterile based on pathology or bacteria culture. The IWGDF guidelines state that the relationship between the true infection of these "normal" stumps and prognosis should be urgently studied. Clinically, for cases where the phalangeal bone is completely necrotic, part of the metatarsophalangeal joint capsule is involved, but the side connected with the metatarsal bone is normal are also encountered, but these conditions are not conducive to granulation growth and wound healing. For the purpose of promoting growth, experts have reached a consensus that it is necessary to open the joint capsule and expose the metatarsal head to facilitate the growth of granulation tissue and accelerate wound healing (14–16). However, it is yet unclear whether this part of the metatarsus is affected.

All 57 patients selected in this study had osteomyelitis of the forefoot, and the diagnosis was confirmed by intraoperative bone biopsy. We found that the proportion of gram-negative bacilli was significantly higher than that of gram-positive cocci in the soft tissue and bone tissue (all >70%). The prevalence of gram-positive cocci (43.4%) was lower than that of gram-negative bacilli (52.4%) in China (17). In terms of antibiotic-resistant bacteria, we found *A. baumannii* had the highest drug resistance rate. Enterococcus spp. had more infection in bone, and there was no infection in the corresponding soft tissue. Because the choice of antibiotics for Enterococcus spp. has certain characteristics, more attention should be paid to it in clinical practice.

The consistency rate of bacteria cultured in soft tissue and bone tissue was very low, <20% in this study. Senneville et al. reported the overall concordance was 22.5% (18). Because our diabetic foot centers are in a tertiary-care hospital, most patients used antibiotics, and we did not stop antibiotic use before collecting samples. All patients in this study had osteomyelitis of the phalanges. On the basis of clinical judgment, patients were divided into the affected metatarsal group and unaffected metatarsal group. However, the metatarsal bone sample that we obtained was the "normal" stump per clinical judgment. These reserved stumps were not truly sterile. Even in the unaffected group, 37.5% samples tested positive for bacterial growth, but the consistency with the phalanges was good. In the affected metatarsal group, only 42.4% stumps were truly sterile, despite being judged as "normal" by the surgeons. Moreover, the bacteria in the metatarsal bone were not consistent with those in the phalangeal bone, which merits further investigation. It should be noted that the infection rate of the retained metatarsal stump judged by pathology and bacterial culture was <100%, although the positive rate of pathology was higher than that of bacterial culture. Hence, it is recommended to carry out both tests at the same time.

We found that there was no significant difference in many clinical characteristics between the two groups regardless of whether their metatarsal bones were involved before the operation. On the one hand, the cardiovascular risk factors such as hypertension, diabetes, dyslipidemia, smoking, hyper D-dimer, and hyperfibrinogenemia and cardiovascular events such as

coronary heart disease and stroke existed in both groups and showed no differences. We prescribed standard, routine treatment as needed. Some patients with acute myocardial infarction, heart failure, and stroke had a poor wound healing, including bone healing, but there was no difference between the two groups. On the other hand, patients with cardiovascular risk factors were given comprehensive treatment, which would not occur the cardiovascular events, and, then, the wound healing and bone healing were better. There were more procalcitonin-positive patients in the affected group than that in unaffected metatarsal group. In addition, the affected metatarsal group had a higher number of X-ray findings than the unaffected metatarsal group. After surgical treatment, there was no difference between the two groups in terms of wound healing, minor amputation times, death, major amputation, and negative pressure use. The affected metatarsal group needed more skin grafts than the unaffected group, which was related to the large wound size. On the basis of these findings, we point out that the surgical methods used in this study were effective for the above two groups of patients.

Among these patients, only nine patients' metatarsal stumps were negative by both pathology and bacterial culture. These patients had true normal metatarsal stumps, only accounting for 15.8% (9/57). Unfortunately, there was no significant clinical difference between them and other patients. Eight of these patients were from the unaffected metatarsal group and one from the affected metatarsal group. The only difference between these nine patients and other patients was that the healing time was fast, as they all healed within 6 months. The other 40 patients (excluding the three deaths) finally healed. The causes of three deceased patients were myocardial infarction (two patients) and COVID-19 (one patient). The main reason for the five of the 48 patients who did not heal was the presence of severe peripheral arterial disease and the inability or failure of revascularization.

This study has the following strengths: First, our diabetic foot center has 60 beds in the ward, all of which are used to treat patients with diabetic foot. The researchers are all diabetic foot professionals. Two surgeons (the first author and corresponding author of this study) completed the operation in and collected the bone samples from all 57 patients. There was no operating or sampling error. Second, to clarify the bacterial condition of soft tissue, samples were taken from phalangeal bone tissue and the retained metatarsal stump in diabetic foot wounds; additionally, the bone tissue was biopsied for pathological evaluation. All 57 phalangeal osteomyelitis cases were diagnosed on the basis of the gold standard technique. Third, the fifth key controversy in the IWGDF infection guideline (2019) was: "In diabetic foot osteomyelitis cases, is obtaining a specimen of residual or marginal bone after surgical resection useful for deciding which patients need further antibiotic or surgical treatment?" The residual stump that clinically is considered not to be affected has a certain proportion of infection. Antibiotic and surgical debridement should be carried out for these infected stumps. Only a small part of patients needed secondary minor amputation; most wounds patients eventually healed.

This study also has some limitations. First, this study had a single-center design. Although the 57 patients exceeded Tianjin, including some other cities, the number of cases is relatively small. Second, because the researchers work in tertiary hospitals, most patients often had a history of antibiotic use in the early stage, so the sampling was affected to some degree. Third, this study did not adopt percutaneous bone biopsy, rather used the method of intraoperative sampling.

In conclusion, we studied the characteristics of bacteria in the wound of patients with DFO. On the basis of the need for healing, the metatarsal bone was treated until the "normal stump" was exposed, regardless of whether the metatarsal bone was affected. It was confirmed that most of the retained "normal" stumps showed bacterial growth (84.2%), but these "normal" stumps can heal after treatment. The true sterile stumps (n = 9) healed quickly. Therefore, we should pay attention to the retained bone stumps, which is helpful for subsequent treatment decisions.

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 Medical Ethics Committee of Chu Hsien-I Memorial Hospital Tianjin Medical 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

Conception and design: JX and BC. Collection and assembly of data: JX, LH, SF, and JZ. Data analysis and interpretation: SF and JZ. Manuscript writing: JX. Final approval of manuscript: All authors. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Natural Science Foundation of China (81973614). Tianjin Key Medical Discipline (Specialty) Construction Project (TJYXZDXK-032A).

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

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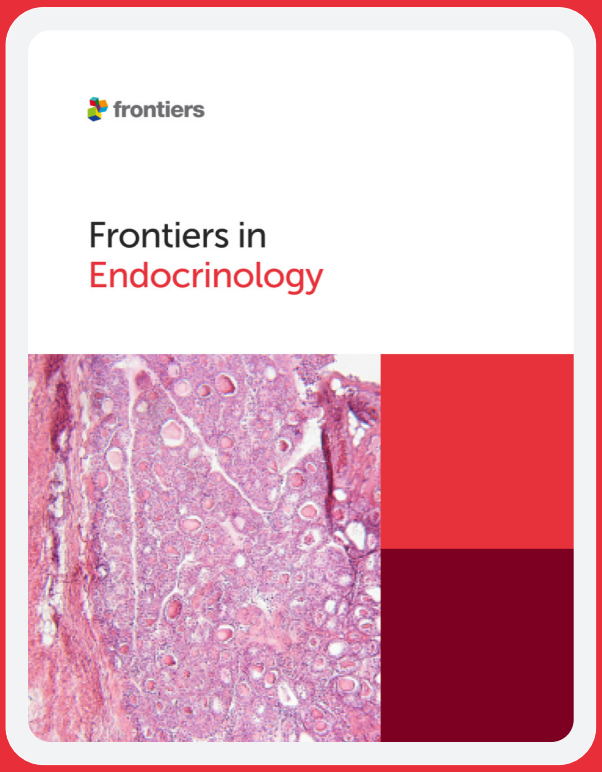

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