INNOVATION IN RHEUMATIC DISEASES

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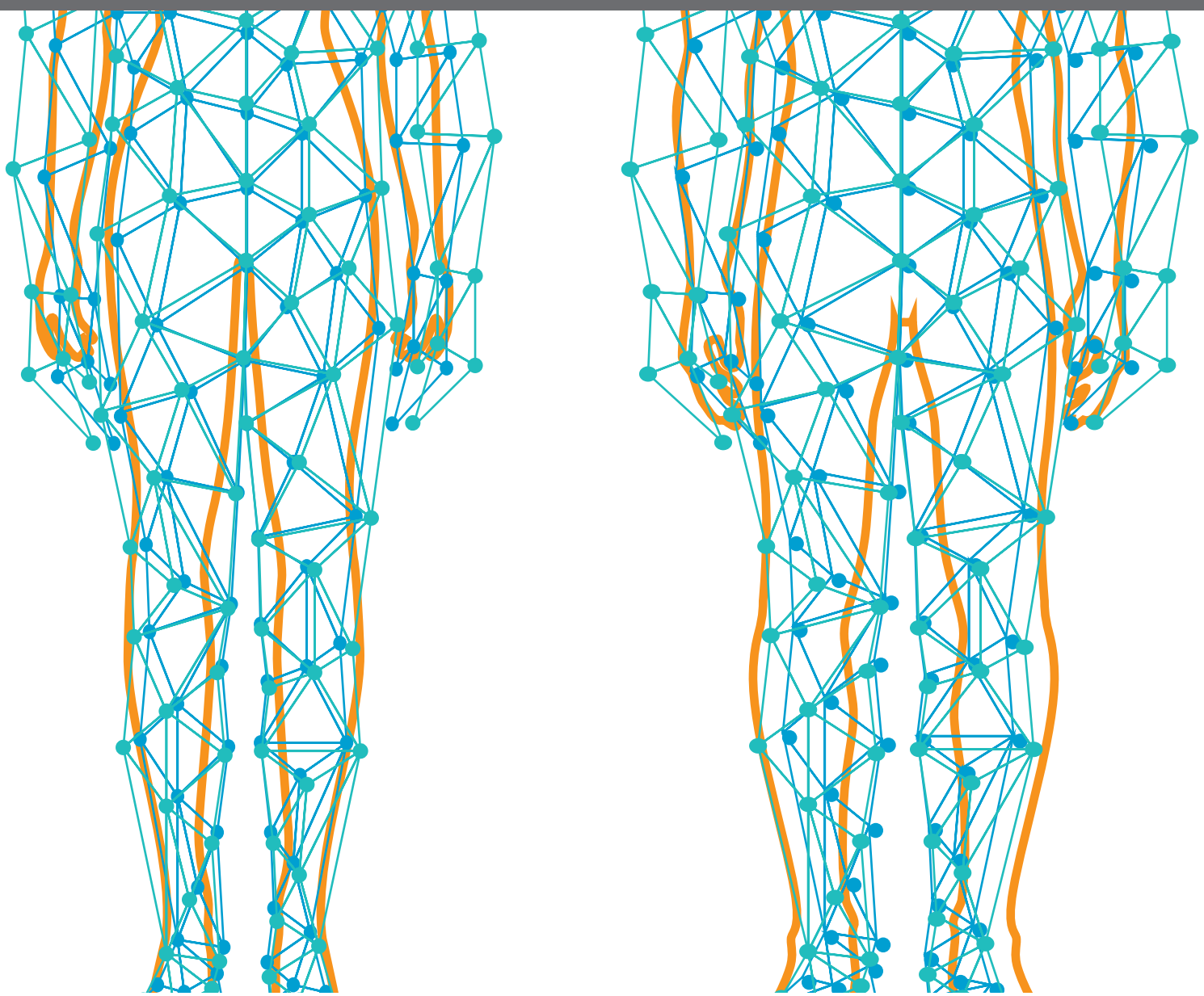

frontiers Research Topics

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INNOVATION IN RHEUMATIC DISEASES

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EDITORIAL
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Editorial: Innovation in Rheumatic Diseases

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Keywords: innovation, rheumatic diseases, lupus, rheumatoid arthritis, diagnosis, treatment

Editorial on the Research Topic

Innovation in Rheumatic Diseases

Rheumatic diseases are high prevalence diseases which affect people of all ages. Inflammatory rheumatic conditions warrant for an early diagnosis and an effective treatment so patients can better manage their disease and its effects. On another hand, osteoporosis and osteoarthritis affect elderly population the most; with the increasing number of older people in countries worldwide, it leads to a major impact on patients' well-being and on society's high economic costs due to healthcare and social resources consumption. Thus, innovation has become a hot topic that aims to solve sustainability of healthcare systems and patients' real needs. In that sense, innovation in rheumatic diseases presents a wide broad of topics, namely novel disease management and treatment approaches, new drugs, therapies, and medical devices, as well as additions to fundamental research.

All these challenging issues justify the edition of our Research Topic "Innovation in Rheumatic Diseases" which, as we expect, can bring up some of the breakthroughs and point out good practices to tackle rheumatic health needs. This group of papers has in common the fact that they cover practical approaches focused on innovation in rheumatic disorders, which include disease knowledge, diagnosis, disease management, well-being improvement, and treatment.

This Research Topic gathers 11 papers from different research groups that deal with distinct aspects of innovation in rheumatic diseases—advances in diagnosis, new mechanisms of disease, innovative technologies, artificial intelligence applications and new contributes to health innovation arena. Eight of them are original research, one clinical trial, one review and one brief research report.

On one side, the rheumatology diseases understanding reveal to be a relevant topic for 4 different research groups. Ingegnoli et al. reviewed the connection between autonomic nervous system and rheumatoid arthritis (RA), where the authors explored common cardiac and mental health-related RA comorbidities with potential relationships to systemic and joint inflammation. Chen et al. performed a population-based study to investigate the relation between anti-phospholipid syndrome (APS) and the risk of newly diagnosed systemic lupus erythematosus (SLE), which showed a significant correlation mainly within the 1st years of APS diagnosis, which highlights the need for vigilance of SLE-associated symptoms in patients recently diagnosed APS. Ma et al. successfully established biomarkers and diagnostic patterns for RA early diagnosis, using serum

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protein profiles analysis by MALDI-TOF-MS combined with magnetic beads, proteomic analysis and biomarkers identification and quantification, involving patients with different disease activity score (in remission, with low disease activity, with moderate disease activity, and with high disease activity). Finally, Gau et al. investigated the impact of anti-neutrophil cytoplasmic antibodies (ANCAs) on clinical manifestation, organ involvement and outcomes in pediatric-onset systemic lupus erythematosus patients; the authors found an indication that patients with ANCAs tend to have hematuria and an absence of typical renal immunofluorescence histology, but no difference in clinical presentations and treatment outcomes, although further studies are needed to verify those findings.

On another hand, rheumatic diseases diagnosis is also a relevant topic. Monti et al. howed that color duplex sonography of temporal arteries and large vessels, combined with a fast-track approach, contributed to a substantial reduction of permanent visual loss in giant cell arteritis, one of the most feared complications, by shortening the time to diagnosis and treatment initiation. In a different approach, Cipolletta et al. presented an automatic deep-learning algorithm for informative ultrasound image analysis for assessing metacarpal head cartilage integrity, which may contribute to the enhancement of ultrasound reliability for this task and support beginner sonographers during ultrasound training in the future.

New treatment approaches for different rheumatologic diseases were also highlighted by different researchers. Li et al. presented a cohort study that suggests that adding Chinese herbal medicines to conventional therapy may reduce subsequent risk of hearing loss in RA patients, although prospective randomized trials are still recommended to further support a causal relationship. In a different work, Wu et al. evaluated the positive efficacy and safety of a Chinese medicine formula combined with methotrexate in active RA patients' treatment, in comparison with the combination of methotrexate and leflunomide. Luo et al. compared the efficacy and safety of glucocorticoids combined with cyclophosphamide or mycophenolate mofetil in immunoglobulin G4-related diseases patients.

Regarding strategies to tackle rheumatic diseases-based needs, Quaresma et al. presented a user-friendly, easy to apply and effective quantitative support tool "OrthoRehab" that can assist physicians/therapists when choosing the most suitable ankle-foot orthosis for each patient with foot dysfunction—one of the most likely consequences of RA. In a different approach comparing to the previous described work, Jacinto et al. showed that patients and their caregivers also present a significant and high-value innovative profile, since these unusual innovators develop novel solutions to solve their own needs whenever the market isn't providing any answer to it.

We believe the papers published in this Research Topic show a comprehensive vision of relevant aspects of innovation in rheumatic diseases and can help to increase knowledge and awareness around these themes. Enjoy the reading!

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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ORIGINAL RESEARCH
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Comparison of the Effects of Cyclophosphamide and Mycophenolate Mofetil Treatment Against Immunoglobulin G4-Related Disease: A Retrospective Cohort Study

Xuan Luo[1,2†], Yu Peng[1,2†], Panpan Zhang[1,2], Jieqiong Li[1,2], Zheng Liu[1,2], Hui Lu[1,2], Xuan Zhang[1,2], Xiaofeng Zeng[1,2], Fengchun Zhang[1,2], Yunyun Fei[1,2] and Wen Zhang[1,2*]*

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Background: Although there are multiple ways to manage immunoglobulin G4–related disease (IgG4-RD), including treatment with glucocorticoids, "steroid-sparing" immunosuppressive drugs, or biologic agents, few clinical trials on IgG4-RD have been conducted. This study aimed to compare the efficacy and safety of glucocorticoids (GCs) combined with cyclophosphamide (CYC) or mycophenolate mofetil (MMF) in IgG4-RD patients. This cohort study was registered at ClinicalTrials.gov (ID: NCT01670695).

Methods: This retrospective study included 155 IgG4-RD patients who received GCs with CYC or MMF at the Department of Rheumatology at Peking Union Medical College Hospital between January 2012 and July 2018. Propensity score matching (PSM) was conducted to match two groups of patients based on their baseline clinical characteristics. Treatment response, relapse rate, and drug safety were analyzed. The treatment response was evaluated based on complete response (CR), partial response (PR), and no change (NC), and the cumulative relapse rate and adverse events in each treatment group were compared using Kaplan–Meier curves and log-rank test, respectively.

Results: Of the 155 IgG4-RD patients, 90 were treated with GCs plus CYC (group I) and 65 with GCs plus MMF (group II). After propensity score–matched (PSM) analysis, 108 patients were selected (54 in each group), 49 of whom had "definite" IgG4-RD, 8 "probable" IgG4-RD, and 51 "possible" IgG4-RD. At the last follow-up, the total response in groups I and II was 98.15 and 96.3%, respectively, and within 12 months, the cumulative relapse rate in group II was significantly higher than that in group I (14.8 vs. 3.7%, $P = 0.046$). Recurrence occurred at the paranasal sinus, lacrimal glands, skin, lung, pancreas, and bile ducts, and the relapsed patients achieved remission after switching immunosuppressants or/and increasing the GC dose.

Conclusions: In IgG4-RD patients with internal organ involvement, GCs plus CYC or MMF are both effective with similar effects in disease response, while GCs plus CYC reduced the relapse rate better than GCs plus MMF.

Keywords: cyclophosphamide, efficacy, IgG4-related disease, mycophenolate mofetil, relapse, response

INTRODUCTION

Immunoglobulin G4–related disease (IgG4-RD) is a systemic fibroinflammatory condition that affects multiple organs including the pancreas, bile duct, lacrimal gland, salivary gland, thyroid, lung, liver, gastrointestinal tract, kidney, and retroperitoneum (1). It is characterized by tissue infiltration by IgG4-positive cells, and causes prominent pathological changes in most of the affected organs, including lymphoplasmacytic infiltration, storiform fibrosis, and obliterative phlebitis, as well as tumor-like lesions and even depletion (2).

Glucocorticoids (GCs) are the first-line therapy for achieving clinical remission in active or untreated IgG4-RD patients (3). Relapse occurred in about 50% patients with GC tapering or withdrawal (4), and long-term application of GCs may increase the risk of adverse reactions. Thus far, conventional immunosuppressants including azathioprine (AZA) (5), mycophenolate mofetil (MMF), 6-mercaptopurine (6-MP), methotrexate (MTX), tacrolimus, cyclophosphamide (CYC), and leflunomide (LEF) have been used in the treatment of patients with IgG4-RD (6, 7). Data from cohort and randomized controlled trial (RCT) studies show that IgG4-RD can be better controlled with GCs plus conventional immunosuppressants than with GC monotherapy (7–10). However, data comparing the efficacy and safety of combined treatment regimens in IgG4-RD are limited.

MMF, an immunomodulatory agent with antifibrotic effects, can be used for the treatment of IgG4-RD because of its ability to inhibit the transforming growth factor beta pathway (11). CYC is a bifunctional nonspecific cell cycle alkylating agent used for treating tumors, a variety of autoimmune diseases, and IgG4-RD. Our previous studies showed that GCs plus low-dose CYC or MMF can effectively reduce the relapse rate of IgG4-RD (4, 8, 9), but we could not identify which one was more effective in treating IgG4-RD and preventing clinical relapse. Therefore, in this study, we aimed to compare the efficacy of two regimens (GCs plus low-dose CYC or MMF) by retrospectively assessing the response rate, relapse rate, and side effects in eligible IgG4-RD patients who were treated with either of them.

METHODS

This prospective observational cohort study to investigate the disease course and treatment response of IgG4-RD patients was registered on ClinicalTrials.gov (ID: NCT01670695). From January 2012 to July 2018, consecutive patients treated with GCs plus CYC or MMF were retrospectively identified for this study. The study protocol was approved by the ethics board of Peking Union Medical College Hospital, and all study subjects provided written informed consent for study participation.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows:

(1) 18 to 75 years of age.
(2) Agreement with the 2011 comprehensive diagnostic criteria for definite, probable, or possible IgG4-RD as follows: (1) characteristic diffuse/localized swelling, sclerosis, or inflammation affecting single or multiple organs; (2) elevated serum IgG4 concentrations (135 mg/dL); (3) increased number of lymphocytes, infiltrated IgG4+ plasma cells (IgG4+ cells/IgG-positive cells >40% and >10 IgG4+ plasma), and fibrosis. Patients who met criteria (1), (2), and (3) were diagnosed with definite IgG4-RD; those who met (1) and (3) were diagnosed with probable IgG4-RD; and those who met (1) and (2) were diagnosed with possible IgG4-RD (12).
(3) Presence of internal organ involvement.
(4) Use of GC and CYC or MMF for initial treatment and maintenance until the 12-month follow-up.
(5) Regularly followed up at months 1, 3, 6, and 12.

The exclusion criteria were as follows:

(1) Presenting only with IgG4-related dacryoadenitis and sialadenitis or lymphadenopathy of IgG4-RD.
(2) Serious infection.
(3) Presence of other rheumatic diseases.
(4) Presence of malignant diseases.
(5) Women with childbearing potential or currently planning a pregnancy.

Laboratory Tests, Imaging Studies, and Histological Examination

All patients underwent laboratory tests for complete blood count, erythrocyte sedimentation rate (ESR), hypersensitivity C-reactive protein (hs-CRP) levels, serum immunoglobulin, and IgG subclass concentrations, urinalysis, liver, and renal function tests; and at least one imaging examination, including ultrasonography, digital radiography (DR), computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography/computed tomography (PET-CT). In total, 78 patients (72.22%) underwent CT scan, 24 (22.22%) underwent PET scan, 33 (30.56%) underwent MRI, and 49 (45.37%) underwent other imaging examinations, including ultrasound and DR. When necessary, tissue biopsies were analyzed and reviewed by pathologists using previously described methods (12), and laboratory tests and imaging studies were performed during the follow-up period.

TABLE 1 | Baseline characteristics before and after matching on the propensity score.

Variables	Before matching		*p*-value	After matching		*p*-value
	Group I (*N* = 90)	Group II (*N* = 65)		Group I (*N* = 54)	Group II (*N* = 54)	
Male, *N* (%)	68 (75.56%)	42 (64.62%)	0.14	37 (68.52%)	37 (68.52%)	1.00
Age at onset (years)	57.50 (52.00–63.75)	55.00 (48.00–62.00)	0.11	56.50 (9.94)	53.96 (12.22)	0.24
Allergy, *N* (%)	43 (47.78%)	34 (52.31%)	0.58	23 (42.59%)	29 (53.70%)	0.25
Biopsy, *N* (%)	46 (51.11%)	36 (55.38%)	0.60	31 (57.41%)	30 (55.56%)	0.44
Imaging examination						
CT scan	61 (67.78%)	48 (73.85%)	0.41	36 (66.67%)	42 (77.78%)	0.20
MRI	23 (25.56%)	18 (27.69%)	0.77	12 (22.22%)	12 (22.22%)	1.00
PET	28 (31.11%)	15 (23.08%)	0.27	19 (35.19%)	14 (25.93%)	0.30
Others	19 (21.11%)	18 (27.59%)	0.34	11 (20.37%)	14 (25.93%)	0.49
Definite IgG4-RD, *N* (%)	35 (38.89%)	30 (46.15%)	0.37	24 (44.44%)	25 (46.30%)	0.85
Probable IgG4-RD, *N* (%)	8 (8.89%)	4 (6.15%)	0.76	5 (9.26%)	3 (5.55%)	0.72
Possible IgG4-RD, *N* (%)	47 (52.22%)	31 (47.70%)	0.58	25 (46.30%)	26 (48.15)	0.85
Number of organs	4 (2–6)	4 (3–5.5)	0.13	4.00(2.75–6.00)	4.5 (3.00–6.00)	0.23
Multiorgan disease (≥3 organs), *N* (%)	46 (51.11%)	42 (64.62%)	0.09	30 (55.56%)	37 (68.52%)	0.17
Disease duration (months)	8.50 (3.00–36.00)	12.00 (5.00–36.00)	0.15	6.50 (3.00–24.00)	12.00 (2.00–36.00)	0.23
Organ involvements, *N* (%)						
Meninges	3 (3.33%)	0	0.27	0	0	—
Pituitary glands	3 (3.33%)	2 (3.08%)	1.00	1 (1.85%)	1 (1.85%)	1.00
Orbital lesion	6 (6.67%)	4 (6.15%)	0.90	4 (7.41%)	4 (7.41%)	1.00
Lacrimal glands	32 (35.56%)	25 (38.56%)	0.71	18 (33.33%)	22 (40.74%)	0.43
Parotid glands	20 (22.22%)	10 (15.38%)	0.29	10 (18.52%)	9 (16.67%)	0.80
Submandibular glands	37 (41.11%)	32 (49.23%)	0.32	24 (44.44%)	28 (51.85%)	0.44
Nasal cavity lesions and sinusitis	18 (20.00%)	23 (35.38%)	<0.05	12 (22.22%)	21 (38.89%)	0.06
Thyroid	3 (3.33%)	2 (3.08%)	1.00	2 (3.70%)	1 (1.85%)	1.00
Lung	32 (35.56%)	24 (36.92%)	0.86	22 (40.74%)	22 (40.74%)	1.00
Lymph nodes	34 (37.78%)	30 (46.15%)	0.30	23 (42.59%)	28 (51.85%)	0.34
Aorta and large blood vessels	18 (20.00%)	7 (10.77%)	0.12	10 (18.52%)	7 (12.96%)	0.43
Heart/pericardium	2 (2.22%)	2 (3.08%)	1.00	1 (1.85%)	2 (3.70%)	1.00
Retroperitoneal fibrosis	36 (40.00%)	13 (20.00%)	0.08	16 (29.63%)	13 (24.07%)	0.52
Sclerosing mediastinitis	10 (11.11%)	6 (9.23%)	0.70	7 (12.96%)	6 (11.11%)	0.77
Sclerosing mesenteritis	0	1(1.54%)	0.42	3 (5.56%)	2 (3.70%)	1.00
Pancreas	30 (33.33%)	42 (64.62%)	<0.01	27 (50.00%)	32 (59.26%)	0.33
Liver	3 (3.33%)	2 (3.08%)	1.00	2 (3.70%)	1 (1.85%)	1.00
Bile ducts	17 (18.89%)	23 (35.38%)	<0.05	16 (29.63%)	18 (33.33%)	0.68
Skin	5 (5.56%)	2 (3.08%)	0.70	4 (7.41%)	2 (3.70%)	0.68
Kidney	13 (13.33%)	7 (10.77%)	0.50	9 (16.67%)	7 (12.96%)	0.59
Prostate	17 (18.89%)	9 (13.85%)	0.41	8 (14.81%)	9 (16.67%)	0.79
Constitutional symptoms not attributable to involvement of a particular organ[a]	10 (11.11%)	4 (6.15%)	0.29	4 (7.41%)	4 (7.41%)	1.00
Other involvement: specify[b]	24 (26.67%)	18 (27.69%)	0.89	17 (31.48%)	11 (20.37%)	0.19
Serological						
Eosinophils (10^9/L)	2.60 (0.70–5.80)	2.50 (0.70–5.20)	0.69	3.2 (1.60–5.60)	2.45 (0.53–5.83)	0.23
C-reactive protein (mg/L)	3.51 (0.81–9.43)	3.53 (1.26–11.26)	0.66	4.40 (1.73–13.88)	2.48 (0.79–8.25)	0.13

(Continued)

TABLE 1 | Continued

Variables	Before matching		*p*-value	After matching		*p*-value
	Group I (*N* = 90)	Group II (*N* = 65)		Group I (*N* = 54)	Group II (*N* = 54)	
Erythrocyte sedimentation rate (mm/h)	32.50 (9.75–61.25)	23.00 (11.75–67.25)	0.73	32.00 (13.00–65.00)	24.50 (8.25–60.50)	0.43
IgE (KU/L)	180.00 (97.23–780.00)	263.00 (78.73–631.75)	0.18	327.00 (97.23–780.00)	206.00 (102.00–644.50)	0.57
IgG4 (mg/L)	5610.00 (2252.50–19,125.00)	11,000.00 (4190.00–19,300.00)	0.12	11,000 (4183.50–20,550.00)	7510 (2925.00–20,950.00)	0.59
IgG4-RD RI	11.63 ± 6.16	11.72 ± 5.06	0.70	12.28 ± 5.87	12.50 ± 4.90	0.65
Initial GC dose (mg/day)	42.69 ± 10.32	41.16 ± 8.37	0.18	41.13 ± 8.09	41.33 ± 10.89	0.54

[a] *Weight loss, fever, and fatigue caused by active IgG4-RD.*
[b] *Prostate, breast, gallbladder involvement, and others.*

Data Collection

Clinical features, laboratory results, imaging data, adverse events, and details of treatment protocol and disease relapse were evaluated at baseline and at 1, 3, 6, and 12 months after treatment or until relapse.

Assessment of Disease Activity and Definition of Clinical Response and Relapse

Disease activity per visit was assessed by the IgG4-RD responder index (RI), with a system score of 0–4 for each site/organ system (13). An IgG4-RD RI score ≥3 was used to identify patients with active disease (14).

Three categories of clinical response were defined: (1) complete response (CR), in which IgG4-RD RI <3 and declined ≥2 points after treatment; (2) partial response (PR), in which IgG4-RD RI declined ≥2 points after treatment but still remained ≥3, and if IgG4-RD RI was 3 at initial treatment a partial response was defined as a 1-point decline after treatment; and (3) no change (NC), in which there was no significant improvement in affected organs and clinical symptoms and change of IgG4-RD RI <2 points (8, 9, 15).

Relapse was defined as the recurrence of worsened/new disease manifestations or abnormality of organ-specific imaging findings despite treatment, with or without elevated serum IgG4 (8).

Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD), median, or interquartile range (IQR), and compared by *t*-test, whereas categorical variables were compared by the chi-square test, Fisher Exact test, or Mann–Whitney test. The Kaplan–Meier curves and log-rank test were used to calculate the cumulative relapse rate. Statistical analyses were performed using IBM SPSS statistics (Version 22.0, IBM, Armonk, NY, USA).

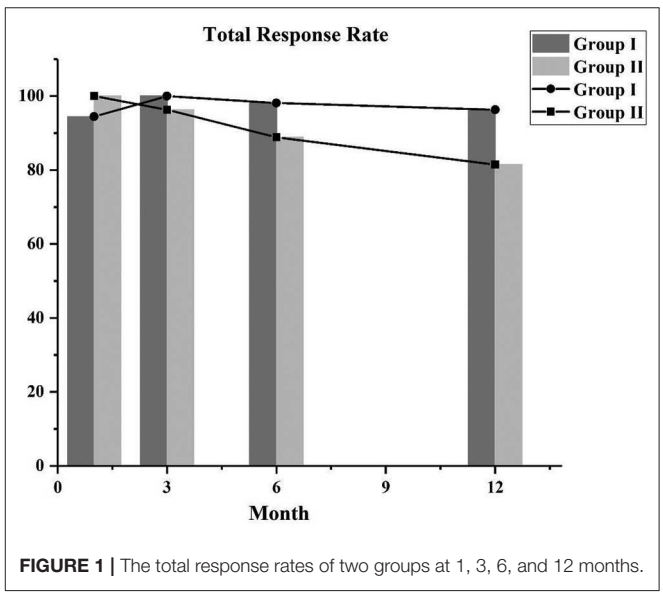

FIGURE 1 | The total response rates of two groups at 1, 3, 6, and 12 months.

RESULTS

Demographic and Baseline Features

From January 2012 to July 2018, 155 IgG4-RD patients who conformed to the inclusion criteria were selected. Among them, 90 were treated with GCs plus CYC (group I) and 65 were treated with GCs plus MMF (group II). Compared to group I, patients in group II had more paranasal sinus (35.38 vs. 20.00%, $p < 0.05$), pancreas (64.62 vs. 33.33%, $p < 0.01$), and bile duct (35.38 vs. 18.89%, $p < 0.05$) involvement.

To minimize the influence of confounders on the results of the study, we used 1:1 propensity score-matched (PSM) analysis to match group I patients with group II patients based on gender, age, involvement organs (meninges, pituitary gland, lacrimal glands, parotid glands, submandibular glands, nasal cavity lesions, thyroid, lungs, lymph nodes, aorta/large blood vessels, heart/pericardium, retroperitoneal fibrosis, sclerosing mediastinitis, sclerosing mesenteritis, pancreas, liver, bile ducts,

kidney, and skin), and other involvement and constitutional symptoms not attributable to the involvement of a particular organ (weight loss, fever, and fatigue caused by active IgG4-RD). At the end of the analysis, 108 patients were selected (54 in each group), of whom 49 were diagnosed with definite IgG4-RD (group I vs. group II, 24 vs. 25), 8 with probable IgG4-RD (group I vs. group II, 5 vs. 3), and 51 with possible IgG4-RD (group I vs. group II, 25 vs. 26). In the post-match model, the differences of baseline characteristic between group I and group II was not statistically significance. All patients were treated with prednisone, which was gradually tapered down according to the individual's clinical response. The analyses indicated that the initial prednisone dosages were similar between the two groups (41.13 vs. 41.33 mg/day, respectively; $p = 0.54$).

TABLE 2 | Response rates of group I and group II.

Period (months)	Status, *N* (%)	Group I (*N* = 54)	Group II (*N* = 54)
1	CR	5 (9.26%)	2 (3.70%)
	PR	46 (85.19%)	52 (96.30%)
	NC	3 (5.56%)	0
	Relapse	0	0
3	CR	10 (18.52%)	7 (12.96%)
	PR	44 (81.48%)	45 (83.33%)
	NC	0	0
	Relapse	0	2 (3.70%)
6	CR	18 (33.33%)	17 (31.48%)
	PR	35 (64.82%)	33 (61.11%)
	NC	0	0
	Relapse	1 (1.85%)	4 (7.41%)
12	CR	30 (55.56%)	27 (50.00%)
	PR	23 (42.59%)	25 (46.30%)
	NC	0	0
	Relapse	1 (1.85%)	2 (3.70%)

CR, complete response; NC, no change; PR, partial response.

In group I, patients received CYC at a mean initial dose of 54.75 mg/day (oral, 50–100 mg/day). They maintained the initial dose for 3 months, after which it was reduced to 50 mg per day or every other day, consistent with previous treatment regimens (9). The mean cumulative dose of CYC over 12 months was 11.30 g. Patients in group II received MMF at a mean initial dose of 1,060 mg/day (1000–1500 mg/day), which was maintained for 6 months and decreased to 500–1000 mg/day for 6 months, consistent with previous reports (8, 16, 17). The baseline characteristics of the two groups before and after matching are summarized in **Table 1**.

Treatment Response

After using the PSM approach to control covariates, we compared the response rates of the two groups. The total response rates of the patients in the two groups are shown in **Figure 1**, and the majority of the patients achieved CR or PR. In the first month follow-up period, in group I, 46 patients achieved PR, 5 attained CR, and 3 did not respond to treatment (NC), and in group II, 2 attained CR and 52 achieved PR. After follow-up at 3 months, the 3 NC patients in group I achieved PR. In the third month follow-up period, the majority of patients in group I (10 achieved CR and 44 achieved PR) and group II (7 achieved CR and 45 achieved PR) had a good response. At the 6- and 12-month follow-up, the total response rates in group I were higher than those in group II, but there was no statistical difference between the two groups (**Table 2**).

The IgG4-RD RI and GC dose at each follow-up period are shown in **Figures 2A,B**. They both decreased over time, especially during the first 6 months of treatment, after which they stabilized. At the last follow-up period, IgG4-RD RI significantly declined in both groups, and the mean (SD) IgG4-RD RI decreased to 2.39 (2.10) in group I and 2.89 (1.76) in group II, with a mean decline of 80.54 and 76.88% of baseline level, respectively. Also, the GCs dose was reduced from the initial 41.13 to 7.95 mg/day in group I, and from 41.33 to 9.18 mg/day in group II, with no significant difference between both groups.

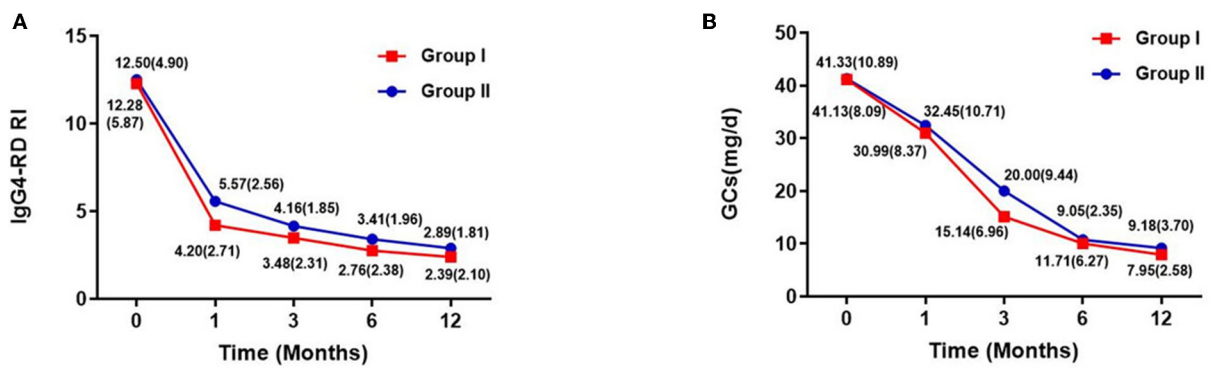

FIGURE 2 | IgG4-RD RI [mean (SD)] and GCs dose [mean (SD)] of two groups within follow-up time. **(A)** IgG4-RD RI evaluated during the follow-up period. **(B)** GC doses of each patient at baseline, and at 1, 3, 6, and 12 months. Doses of glucocorticoids are presented as means.

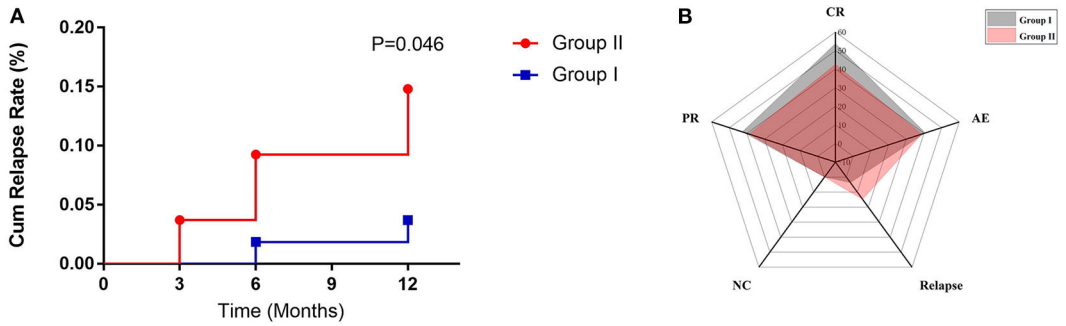

FIGURE 3 | (A) The relapse rate of two groups within follow-up time. **(B)** Radar map for evaluating the efficacy of two treatment regimens by response rate (CR, PR, and NC), relapse rate (RR), and adverse effect (AE).

TABLE 3 | Relapsed patients in Group I and Group II.

Patients	Group	Involvement organ	Relapsed organ	Time of relapse	Added treatment	Outcome
1	I	Aorta, retroperitoneal fibrosis, and pancreas	Pancreas	6	Increased GC dosage, changed CYC to MMF	PR
2	I	Submandibular glands, lymph nodes, retroperitoneal fibrosis, pancreas, and bile ducts	Lung	12	Increased GC dosage	PR
3	II	Aorta, retroperitoneal fibrosis, pancreas, and bile ducts	Skin and lung	3	GCs, MMF, and iguratimod	PR
4	II	Lacrimal glands, aorta, lymph nodes, lung, pancreas, and bile ducts	Lacrimal glands	3	Increased GC dosage, changed MMF to MTX	PR
5	II	Submandibular glands, lymph nodes, pancreas, lacrimal glands, and lung	Lacrimal glands	6	Increased GC and MMF dosage	PR
6	II	Submandibular glands, lymph nodes, pancreas, lacrimal glands, prostate, and lung	Lacrimal glands	6	Increased GC dosage and changed MMF to CYC	CR
7	II	Pancreas and bile ducts	Pancreas and bile ducts	6	Changed MMF to CYC	PR
8	II	Aorta, retroperitoneal fibrosis, and pancreas	Pancreas	6	Increased GC dosage	PR
9	II	Lacrimal gland, lung, and paranasal sinus	Lacrimal gland and paranasal sinus	12	Changed MMF to CYC	CR
10	II	Parotid glands and paranasal sinus	Paranasal sinus	12	Increased GC and MMF dosage	PR

CR, complete response; CYC, Cyclophosphamide; GC, glucocorticoid; MMF, Mycophenolate mofetil. MTX, methotrexate; PR, partial response.

Disease Relapse

In group I, 2 patients suffered relapse; one had a recurrence in the pancreas in the 6th month, which manifested as abdominal pain and the other had a worsened lung image on the 12th month with cough. In group II, 8 patients suffered relapse; 2 had recurrence on the skin, lung, and lacrimal glands at the 3rd month visit, 4 relapsed in the 6th month (lacrimal glands and pancreas), and 2 had recurrence in the 12th month (lacrimal glands and paranasal sinus). In group II, recurrence at the paranasal sinus, lacrimal glands, skin, lung, bile ducts, and pancreas accounted for 25, 50, 12.5, 12.5, 12.5, and 25%, respectively, with the lacrimal glands being the most vulnerable to recurrence. The cumulative recurrence rate in groups I and II was 3.7 and 14.8%, respectively, and group II had a higher relapse rate than group I ($p = 0.046$) (**Figure 3A**).

The follow-up data after retreatment in these 10 patients showed that they all achieved remission after the immunosuppressants were changes and/or the dose of GCs was increased according to their condition. In group I, for one patient, we increased the GCs dosage and replaced CYC with

TABLE 4 | Adverse events in response to glucocorticoid or immunosuppressant observed during treatment in two groups.

Adverse events	Number of patients		*p*-Value
	Group I	Group II	(*< 0.05)
Infection			
Upper respiratory tract infection	5	7	0.54
Pneumonia	0	2	0.50
Herpes zoster	2	1	1.00
Other	2	1	1.00
Glucose intolerance			
Newly diagnosed with diabetes mellitus	2	1	1.00
Aggravation of diabetes mellitus	2	3	1.00
Gastrointestinal reaction	6	5	0.75
Hematological system	1	0	1.00
Liver damage	1	0	1.00
Hemorrhagic cystitis	0	0	—
Other adverse reaction	0	0	—

MMF, whereas for the second patient, the GCs dosage was increased. Of the 8 relapsed patients in group II, the treatment was converted to GCs and CYC for 3, iguratimod was added for 1, GC dose was increased and MMF was replaced with MTX for 1, and previous regimens with increased dosages were maintained in 3 patients. The details of relapsed patients in group I and group II are shown in **Table 3**.

Adverse Effects

Table 4 shows the adverse effect (AEs) of therapy, with infections as the main adverse reaction caused by the two treatment regimens. In addition, a patient in group I developed persistent platelet reduction after treatment with GCs plus CYC, so CYC was replaced with MMF and the patient's platelet count was monitored. It was observed that the patient's platelet counts gradually returned to normal after CYC was replaced. Also, a 37-year-old female patient's treatment plan was changed to oral GCs plus MMF because she had amenorrhea and a gastrointestinal reaction after CYC treatment. For another patient in group I, CYC was replaced with MMF due to liver damage manifested as elevated transaminase after using CYC. In group II, MMF was discontinued in three patients or replaced with CYC due to recurrent infections. Other adverse reactions in group II were similar to those in group I, the majority of which were infections and gastrointestinal reactions. There was no significant difference in treatment-related side effects in the two groups.

Based on comprehensive assessment of disease response, relapse rate, and adverse reactions, it was suggested that GCs plus CYC had a better therapeutic effect than GCs plus MMF (**Figure 3B**).

DISCUSSION

Several immunosuppressants have been used as a steroid-sparing treatment for IgG4-RD, but there has been no head-to-head study comparing the efficacies between steroid-sparing drugs. In this retrospective cohort study, we aimed to explore the efficacies of GCs plus MMF therapy and GCs plus CTX and compare them in order to determine a better therapeutic strategy for IgG4-RD patients and prevent disease recurrence.

In this study, CYC and MMF were used because their efficacies in IgG4-RD had been proven in previous studies. In a recent study, CYC plus GCs yielded a lower relapse rate and higher response rate relative to GC monotherapy. Similar to the results of combined CYC regimens, a study from India showed clinical improvement in patients who were treated with GCs plus MMF, with no case of relapse within a median duration of 8 months. Furthermore, an RCT study from China reported that GCs plus MMF was more effective than GCs monotherapy.

In our study, IgG4-RD patients responded well to GCs plus CYC or MMF, and a majority of them experienced marked improvements within a month of treatment, which were manifested as improvement in the affected organs; reductions in the concentrations of serum ESR, CRP, IgG, and IgG4; as well as successful GC tapering. Both combined treatment regimens were efficient for IgG4-RD patients, having almost the same response rate during a year follow-up period. Comparing the ability of the two immunosuppressants to prevent flare by relapse rate, our results demonstrated that 10 patients (2 with CYC and 8 with MMF) relapsed, and relapse occurred earlier and more frequently in those receiving MMF than CYC (14.8 vs. 3.7%). Additionally, patients receiving CYC had less cumulative GCs than those receiving MMF, indicating that the steroid-sparing effect of CYC is superior to that of MMF. Therefore, the combined treatment with CYC decreased organ recurrence and maintained disease remission much better than combined treatment with MMF. Therefore, it was suggested that a low-dose CYC combination treatment regimen might be a better choice than low-dose MMF therapy in IgG4-RD patients with multiple organ involvement.

In a recent RCT study that enrolled IgG4-RD patients with multiple organ involvement, it was pointed out that lacrimal glands and paranasal sinus were most susceptible to recurrence in patients receiving low-dose MMF plus GCs (8). However, in a prospective cohort study of combined CYC treatment for IgG4-RD, it was observed that no patient experienced recurrence of superficial organs such as lacrimal glands and paranasal sinuses in the group receiving GCs plus CYC (9). In the present study, the relapse of patients in group II was more likely due to recurrence in the lacrimal glands (4/8, 50.00%) and paranasal sinus (2/8, 25.00%), and the other relapsed organs including the pancreas and lung were similar to those in group I. In accordance with the results obtained from this study, it is necessary to find

other more efficient treatments for lacrimal glands and paranasal sinus involvement.

Regarding safety issues, since low doses of CYC and MMF were applied in this study, both drugs were well tolerated, and this is consistent with previous IgG4-RD studies. The main adverse reactions in the CYC or MMF groups were infections and gastrointestinal reactions, and they did not differ between both groups (**Table 4**). Previous studies observed that the incidence of severe infection significantly increased in the Asian population compared with those of other races when MMF at 3 g/day was used in the treatment of lupus nephritis (16, 17). Based on our clinical experience, patients over 60 years of age showed vulnerability to MMF of more than 1.5 g per day, with an increased risk of opportunistic infection, while most IgG4-RD patients are older than 60 years old. In the CYC group, two patients had drug-induced liver damage and persistent reduction in platelets, respectively, but the symptoms disappeared after CYC was discontinued. When CYC is used, it is applied only in patients with internal organ involvement. We carefully monitored short-term and long-term side effects of CYC, and strictly controlled the cumulative dose for every patient, although in our experience in treating systemic rheumatic diseases, such as systemic lupus erythematosus and systemic vasculitis, Chinese patients tolerate CYC much better than patients reported from Western countries.

So far, this is the first study to compare the effects of different immunosuppressive agents in IgG4-RD, although there are some limitations. Since the study is a retrospective cohort study, unknown variables might affect the results, leaving room for potential bias such as adverse effects that were as retrieved from the patients' hospital records. However, the results are quite reliable because there were no statistically significant differences in the baseline characteristics between the two groups.

In conclusion, most IgG4-RD patients had a good response to both treatment regimens, but GCs plus CYC had a lower rate of relapse within a year than GCs plus MMF. This suggests that GCs plus CYC might be a more effective treatment option for IgG4-RD patients with internal organ involvement. However, larger prospective and RCT studies are required to understand the role of CYC and MMF in IgG4-RD.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

AUTHOR CONTRIBUTIONS

XL and YP designed the research, analyzed data, and wrote the manuscript. PZ, ZL, JL, and HL collected and analyzed data and revised the manuscript. XZh, FZ, and XZe optimized the research and proofread the paper. YF and WZ designed the study and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, et al. A novel clinical entity, igG4-related disease (IgG4RD): general concept and details. *Modern Rheumatol.* (2012) 22:1–14. doi: 10.3109/s10165-011-0508-6
2. Sekiguchi H, Horie R, Kanai M, Suzuki R, Yi ES, Ryu JH. IgG4-Related disease: retrospective analysis of one hundred sixty-Six patients. *Arthr Rheumatol.* (2016) 68:2290–9. doi: 10.1002/art.39686
3. Khosroshahi A, Wallace ZS, Crowe JL, Akamizu T, Azumi A, Carruthers MN, et al. International consensus guidance statement on the management and treatment of igG4-Related disease. *Arthr Rheumatol.* (2015) 67:1688–99. doi: 10.1002/art.39132
4. Hart PA, Kamisawa T, Brugge WR, Chung JB, Culver EL, Czako L, et al. Long-term outcomes of autoimmune pancreatitis: a multicentre, international analysis. *Gut.* (2013) 62:1771–6. doi: 10.1136/gutjnl-2012-303617
5. Venetsanopoulou A, Tzioufas A. 58-year-old patient with IgG4-related thoracic aortitis. *Mediterr J Rheumatol.* (2016) 27:191–3. doi: 10.31138/mjr.27.4.191
6. Brito-Zeron P, Bosch X, Ramos-Casals M, Stone JH. IgG4-related disease: advances in the diagnosis and treatment. *Best Pract Res Clin Rheumatol.* (2016) 30:261–78. doi: 10.1016/j.berh.2016.07.003
7. Wang Y, Zhao Z, Gao D, Wang H, Liao S, Dong C, et al. Additive effect of leflunomide and glucocorticoids compared with glucocorticoids monotherapy in preventing relapse of igG4-related disease: a randomized clinical trial. *Sem Arthr Rheum.* (2020) doi: 10.1016/j.semarthrit.2020.01.010
8. Yunyun F, Yu P, Panpan Z, Xia Z, Linyi P, Jiaxin Z, et al. Efficacy and safety of low dose mycophenolate mofetil treatment for immunoglobulin g4-related disease: a randomized clinical trial. *Rheumatology.* (2018) 58:52–60. doi: 10.1093/rheumatology/key227
9. Yunyun F, Yu C, Panpan Z, Hua C, Di W, Lidan Z, et al. Efficacy of cyclophosphamide treatment for immunoglobulin g4-related disease with addition of glucocorticoids. *Sci Rep.* (2017) 7:6195. doi: 10.1038/s41598-017-06520-5
10. Wang L, Zhang P, Wang M, Feng R, Lai Y, Peng L, et al. Failure of remission induction by glucocorticoids alone or in combination with immunosuppressive agents in igG4-related disease: a prospective study of 215 patients. *Arthr Res Ther.* (2018) 20:65. doi: 10.1186/s13075-018-1567-2
11. Roos N, Poulalhon N, Farge D, Madelaine I, Mauviel A, Verrecchia F. *In vitro* evidence for a direct antifibrotic role of the immunosuppressive drug mycophenolate mofetil. *J Pharmacol Exp Ther.* (2007) 321:583–9. doi: 10.1124/jpet.106.117051
12. Umehara H, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, et al. Comprehensive diagnostic criteria for igG4-related disease (IgG4-RD), 2011. *Moder Rheumatol.* (2012) 22:21–30. doi: 10.3109/s10165-011-0571-z
13. Carruthers MN, Topazian MD, Khosroshahi A, Witzig TE, Wallace ZS, Hart PA, et al. Rituximab for igG4-related disease: a prospective, open-label trial. *Ann Rheum Dis.* (2015) 74:1171–7. doi: 10.1136/annrheumdis-2014-206605
14. Wallace ZS, Mattoo H, Carruthers M, Mahajan VS, Della Torre E, Lee H, et al. Plasmablasts as a biomarker for igG4-related disease, independent of serum igG4 concentrations. *Ann Rheum Dis.* (2015) 74:190–5. doi: 10.1136/annrheumdis-2014-205233

15. Campochiaro C, Ramirez GA, Bozzolo EP, Lanzillotta M, Berti A, Baldissera E, et al. IgG4-related disease in italy: clinical features and outcomes of a large cohort of patients. *Scand J Rheum.* (2016) 45:135–45. doi: 10.3109/03009742.2015.1055796
16. An Y, Zhou Y, Bi L, Liu B, Wang H, Lin J, et al. Combined immunosuppressive treatment (CIST) in lupus nephritis: a multicenter, randomized controlled study. *Clin Rheumatol.* (2019) 38:1047–54. doi: 10.1007/s10067-018-4368-8
17. Appel GB, Contreras G, Dooley MA, Ginzler EM, Isenberg D, Jayne D, et al. Mycophenolate mofetil versus cyclophosphamide for induction treatment of lupus nephritis. *J Am Soc Nephrol.* (2009) 20:1103–12. doi: 10.1681/ASN.2008101028

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Chinese Herbal Formula Huayu-Qiangshen-Tongbi Decoction Compared With Leflunomide in Combination With Methotrexate in Patients With Active Rheumatoid Arthritis: An Open-Label, Randomized, Controlled, Pilot Study

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Background: Traditional Chinese Medicine is complementary and an alternative to modern medicine. The combination therapies of herbal products with disease-modifying anti-rheumatic drugs are gradually and widely adopted in the management of rheumatoid arthritis (RA) in China.

Purpose: To evaluate the efficacy and safety of Huayu-Qiangshen-Tongbi (HQT) decoction, a Chinese medicine formula, combined with methotrexate (MTX) in the treatment of patients with active RA, in comparison with the combination therapy of MTX with leflunomide (LEF).

Methods: This pilot study was a monocenter, open-label, randomized controlled trial with two parallel arms. Ninety patients with active RA were randomly allocated to receive either HQT at a dose of 250 ml twice daily or LEF at a dose of 20 mg once daily, and all participants received MTX at a dose of 10–15 mg once weekly. The primary efficacy endpoint was the proportion of patients who achieved a 20% improvement in the American College of Rheumatology criteria (ACR20) after a 24-week treatment.

Results: 84.4% (76/90) patients completed the 24-week observation. In the intention-to-treat analysis, the percentage values of patients achieving the ACR20 response criteria were 72.1% (31/43) in MTX + HQT group and 74.4% (32/43) in MTX + LEF group ($p = 0.808$). No significant difference was observed in other parameters,

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including ACR50, ACR70, clinical disease activity index good responses, European League Against Rheumatism good response, remission rate, and low disease activity rate. The results of the per-protocol analysis showed consistency with those of the intention-to-treat analysis. The mean change from baseline at week 24 for the van der Heijde modified total sharp score had no significant difference between two groups (3.59 $\pm$ 4.75 and 1.34 $\pm$ 8.67 in the MTX + HQT group and MTX + LEF group, respectively, $p = 0.613$). The frequency of adverse events was similar in both groups (11 cases in the MTX + HQT and 17 cases in the MTX + LEF, $p > 0.05$).

Conclusions: In patients with active RA, treatment with the combination of HQT and MTX was associated with improvement in signs, symptoms, and physical function. With a beneficial clinical response and acceptable tolerability, HQT or other Chinese medicine formula may be a good therapeutic option in combination with MTX for RA treatment.

Trial registration: Chinese Clinical Trails Registry, ChiCTR-INR-16009031, Registered on 15th August 2016, http://www.chictr.org.cn/enindex.aspx.

Keywords: combination therapy, Chinese medical formula, Huayu-Qiangshen-Tongbi decoction, randomized controlled clinical trial, pilot study

INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune musculoskeletal disease affecting the joints primarily, leads to structural damage including cartilage destruction and bone erosion, and brings about extra-articular harm such as cardiovascular, pulmonary, and psychological disorders (1). Disease-modifying anti-rheumatic drugs (DMARDs) are the principal choice of first-line treatments for patients with RA, among which methotrexate (MTX) is well-established as an anchor drug for both treatment and research (2). However, not all patients receiving MTX monotherapy achieved low disease activity (LDA) or clinical remission (3). Over the last two decades, the treatment of RA has been transformed, and today, in patients with insufficient response to MTX monotherapy, combination with biological DMARDs or other conventional synthetic DMARDs (csDMARDs) is an international consensus of RA therapeutic strategy (2). Although new effective treatment regimens increased the clinical response rate of achieving full or long-lasting remission, a substantial number of RA patients did not respond to the current therapeutic strategies and even suffered from adverse effects (AEs) caused by long-term treatments, such as gastrointestinal toxicity, hepatotoxicity, bone marrow suppression, tuberculosis, and infection (2, 4). Previous researches reported that the use of prednisone and certain biological DMARDs increased the risk of tuberculosis and other opportunistic infections occurring in RA patients (4–6). Due to the development of advance effects, a portion of RA patients did not benefit from these combination therapeutic regimens and discontinued the treatment (7, 8). Therefore, there is still a considerable unmet need in RA treatment, and an application of new effective and safe treatment strategies should now be the priority of research efforts.

Traditional Chinese medicine (TCM), such as herbal products and acupuncture, has been widely practiced in clinics for over thousands of years in China and has found to be effective in treating many types of diseases, such as RA. Several Chinese medical herbs and their formulas, extracts, active ingredients, and even single compounds have been used for the RA treatment. Their clinical efficacy against RA and the safety have been evidenced by clinical practices and clinical trials in RA patients (9–12). Huayu-Qiangshen-Tongbi decoction (HQT) is a Chinese medical formula used in RA treatment in Guangdong Provincial Hospital of Chinese Medicine, which is composed of the following natural materials: the root and rhizoma of *Salvia miltiorrhiza* Bunge (Danshen), the rhizoma of *Dioscorea nipponica* Makino (Chuanshanlong), the root of *Astragalus membranaceus* (Huangqi), the root of *Paeonia tacti lora Pall* (Baishao), the root, stem, and leaf of *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip (Tianshanxuelian), the bark of *Eucommia ulmoides* Oliver (Duzhong), the root and rhizoma of *Davallia mariesii* Moore ex Bak (Gusuibu), the root of *Dipsacus asperoides* C. Y. Cheng et T. M. Ai (Chuanxuduan), the earthnut of Chinese Foxglove (Shudi), and the root and rhizoma of *Glycyrrhiza*

Abbreviations: ACR, American College of Rheumatology criteria; ACR, American College of Rheumatology; ANOVA, analysis of variance; cDAIs, clinical disease activity index good responses; csDMARDs, conventional synthetic DMARDs; DAS28-CRP, 28-joint disease activity score based on C-reactive protein; DMARDs, disease-modifying antirheumatic drugs; EULAR, European League against rheumatism; ESR, erythrocyte sedimentation; HQT, Huayu-Qiangshen-Tongbi decoction; HAQ, Health Assessment Questionnaire; ITT, intention-to-treat; JSN, joint space narrowing; LDA, low disease activity; LEF, Leflunomide; mTSS, van der Heijde modified total sharp score; MTX, methotrexate; NSAIDs, Nonsteroidal anti-inflammatory drugs; PaGADA, physician's or patient's assessment of global health status; PhGADA, patient's assessment of global health status; PP, per-protocol; RA, rheumatoid arthritis; RCT, randomized controlled trial; RF, rheumatoid factor; TB, tuberculosis; TCM, Traditional Chinese medicine.

uralensis (Gancao). In our hospital, HQT has been used for RA management with the combination of csDMARDs, especially the MTX. Notably, we had undertaken a retrospective record review to evaluate the clinical response and AEs of the combination therapy of HQT and MTX in 2019. The result of the retrospective study showed that HQT combined with MTX had favorable therapeutic effects in improving the overall symptoms of RA patients with good tolerance (13). HQT may function as a kind of DMARDs, which can be used as an alternative or add-on treatment against RA. The purpose of this study is to determine the efficacy and safety of HQT in combination with MTX by performing an investigator-initiated, 24-week prospective, randomized clinical study, which might provide basic data and evidence for a further undergoing multicenter, double-blinded, randomized, placebo-controlled trial.

METHODS

Study Design

This pilot study was a 24-week, monocenter, open-label, randomized controlled trial, which was conducted in the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangdong Provincial Hospital of Chinese Medicine) between August 2016 and September 2018. All the participants were provided written informed consent, and the protocol was first approved by the Medical Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (B2016-076-01) and subsequently registered with the World Health Organization clinical trial registry (no. ChiCTR-INR-16009031).

Patients

All participants were recruited from an outpatient rheumatology clinic at the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangdong Provincial Hospital of Chinese Medicine) in Guangzhou, China. Individuals with RA were all screened in clinics based on the inclusion and exclusion criteria, which are described later. If the eligibility criteria were met, the patients would be asked if they were interested in participating in the trial. The trial coordinator contacted participants to explain the requirements and purpose of the study, and the informed consent was completed as well.

Inclusion/Exclusion Criteria

Participants should meet the following criteria in this study: (1) aged between 18 and 65 years; (2) diagnosed with RA based on the diagnostic criteria of 1987 American College of Rheumatology (ACR) (14) or the 2009 ACR criteria (15); (3) were in functional class I, II, or III (according to the 1987 American Rheumatism Association classification standard) (14); (5) Chinese medicine inclusion criteria: with a syndrome pattern including wind and damp stagnation, cold and damp stagnation, hot and damp stagnation, phlegm and stasis stagnation, and deficiency of kidney and liver (criteria of Chinese medicine symptoms assessment is shown in **Appendix 1**); (5) the 28-joint disease activity score (DAS) based on C-reactive protein (DAS28-CRP) score > 3.2 (16); (6) received a stable dose of non-steroidal anti-inflammatory drug (NSAID) during the 4 weeks before screening, or did not take NSAIDs before screening for at least 1 week; (7) did not take DMARDs (including biological DMARDs and csDMARDs) before screening during the 4 weeks; the patients who received DMARDs must have a period of DMARD washout that lasted for at least 4 weeks before the trial; (8) if patients took corticosteroids such as prednisone, the dose should be ≤ 10 mg, and they must have already taken more than 4 weeks before starting this study; and (9) agreed to participate in the trial and signed a form of informed consent.

Patients were excluded from this trial if they: (1) had a history of another autoimmune rheumatic disease, Sjögren's syndrome or systemic lupus erythematosus for instance; with joint swelling because of osteoarthritis, trauma, septic arthritis, or crystal arthritis; recent, current, or chronic infection, for example, the infection with hepatitis B or hepatitis C; evidence of any extents of *Mycobacterium tuberculosis* infection; (2) had other severe disorders, such as hematopoietic, brain, lung, or cardiovascular diseases; (3) had a hemoglobin level of <90 g/L, a platelet count of $<100 \times 10^9$/L, or a white cell count of $<3.0 \times 10^9$/L; (4) had an estimated glomerular filtration rate of ≤ 40 ml/min (evaluated by Cockcroft and Gault method); (5) with a alanine aminotransferase or aspartate aminotransferase level $>$ 1.5 times the upper normal limit; (6) had a gastritis or active gastroduodenal ulcer induced by the long-term treatment of NSAIDs; (7) were hypersensitive to medication used in the trial; (8) had participated in any other trials within 4 weeks at the time of screening; (9) women currently pregnant or who were planning on becoming pregnant during the study period; and (10) patients with mental disease.

Interventions

Eligible patients were allocated to receive either HQT (orally, twice per day, 250 ml for each time, 30 min after meals) or LEF (20 mg once daily) for 24 successive weeks. All the patients took MTX orally once a week, starting with 10 mg and increasing to 12.5 or 15 mg after a 4-week treatment. The ingredients and cooking method of HQT are shown in **Table 1**. The result observed from high-performance liquid chromatography analysis of HQT decoction is shown in **Appendix 2**. Patients were allowed to continue to receive NSAIDs and/or stable dosage of oral glucocorticoid (5–10 mg per day, prednisolone or equivalent) if the patients suffered intolerable pain (patient's assessment of pain ≥ 40 mm), folic acid, bone protection drugs such as alendronate and calcium/vitamin D, and antacids during the trial. Patients could withdraw from the trial at any time if they were not satisfied with the clinical response.

Outcomes and Measurements

Primary Outcomes

Patients were assessed concerning the outcomes and clinical parameters at baseline on weeks 4, 12, and 24 by different trained evaluators who did not know the treatments in the trial. The primary outcome was the patient's proportion achieving an ACR response of at least 20% (ACR20) at 24 weeks, according to the ACR criteria (17). To be considered as an ACR20 responder, a

TABLE 1 | Main components of HQT.

Pinyin Name	Latin Name	Doses
Danshen	*Salvia miltiorrhiza* Bunge	20 g
Chuanshanlong	*Dioscorea nipponica* Makino	30 g
Huangqi	*Astragalus membranaceus*	30 g
Baishao	*Paeonia tacti lora* Pall	20 g
Tianshanxuelian	*Saussurea involucrata* (Kar. et Kir.) Sch.-Bip	3 g
Duzhong	*Eucommia ulmoides* Oliver	20 g
Gusuibu	*Davallia mariesii* Moore ex Bak	20 g
Chuanxuduan	*Dipsacus asperoides* C. Y. Cheng et T. M. Ai	15 g
Shudi	Chinese Foxglove	15 g
Gancao	*Glycyrrhiza uralensis*	10 g

HQT, Huayu-Qiangshen-Tongbi decoction. The decoction was made in the following manner: (1) Put the herbals and the right amount of cold water in the casserole, and soak the herbals in the cold water for 30 min. (2) Add 1,200 ml of cold water in the casserole, heat to boiling, and boil for 40 min, then filter the decoction two times; (3) Add 800 ml of hot water to the casserole with the boiled herbs together, boil for 30 min, and then filter the decoction two times; (4) Mix the decoctions together twice in 1 day, 250 ml for each time.

patient should achieve ≥20% improvement in both tender and swollen joints (28 tender and 28 swollen joints were evaluated) and ≥20% improvement in following three or more parameters: the patient's assessment of pain on a visual analog scale (0–100 mm), the physician's or patient's assessment of global health status (PaGADA/PhGADA, 0–100 mm), the patient's assessment of function with a modified version of the Health Assessment Questionnaire (HAQ, scores are based on an overall mean score ranging from the highest within each group), and the serum level of CRP or erythrocyte sedimentation (ESR).

Secondary Outcomes

Secondary efficacy measures were the proportion of patients with 50 or 70% improvement, ACR50 or ACR70, at week 24, the clinical disease activity index (cDAI) good response, European League Against Rheumatism (EULAR) good and moderate responses, clinical remission, and LDA. The criteria of EULAR response were evaluated based on the individual amount of change in the DAS as well as the achieved DAS (low, moderate, or high). Moderate EULAR responses were a decrease (improvement) of >0.6 and ≤1.2 and a DAS <5.1, whereas good responses are a reduction of >1.2 and a DAS <2.6 (18). A good response for cDAI was defined when achieving ≥50% improvement or a cDAI ≤2.8 (19). The extent of disease activity was assessed based on the DAS in DAS28-CRP as remission (<2.6) and LDA (<3.2) (16).

The following clinical and laboratory indexes were also assessed: the 28-joint tender joint count, 28-joint swollen joint count, morning stiffness duration, the patient's assessment of pain on a visual analog scale, PaGADA, PhGADA, CRP, ESR, rheumatoid factor (RF), HAQ score, and DAS28-CRP. Radiographs of the hands (including wrists) were performed at the screening visit and after 24-week treatment. Radiographs of the hands (including wrists) were evaluated by the van der Heijde modified total sharp score (mTSS), which was utilized to assess radiographic joint damage progression (20) taken at baseline and after 24 weeks in the trial. Sixteen and 15 areas were included for the evaluation of erosions and joint space narrowing (JSN) in hands and wrists. The maximum score of erosion was 160, and the maximum JSN score was 120. The sum of the earlier mentioned scores (maximum 280) was the mTSS. All radiographs in the trial were scored centrally in chronological order by a professional while blinded reader.

Safety Outcomes

Measurement of safety was evaluated by patient-expressed AEs, physical examinations, and laboratory investigations, which included a routine blood test, urine analysis, renal function, and liver function. These evaluations were undertaken at each visit during the period of treatment (baseline, 4, 12, and 24 weeks). Chest X-ray examinations and electrocardiography were conducted at the screening visit and after 24-week treatment. Hepatotoxicity was defined by the abnormal increase of the hepatic enzyme level. Hematological adverse events were assessed by the changes in hematologic characteristics, such as anemia (hemoglobin < 90 g/L), leukopenia ($<3.5 \times 10^9$/L), and thrombocytopenia ($<100 \times 10^9$/L).

Sample Size

Due to the lack of previous similar trials and pilot studies to consult, the sample size of this pilot study was set as 45 cases in each group.

Randomization and Blinding

An independent statistician performed randomization. SAS 9.2 software (SAS Institute Inc., Cary, USA) was used to generate the randomization sequence. Participants were randomly assigned at a 1:1 ratio by a randomization system to the MTX + HQT group or the MTX + LEF group. Blinding and placebo tables were not available for this investigator-initiated clinical trial, and the allocation sequence was not concealed from both the researchers and participants.

Statistical Analysis

SPSS17.0 and GraphPad Prism 7 statistical software packages were used to establish the database by an independent statistician who was blinded to the group allocation. The full analysis set evaluated baseline data, and the efficacy in the two groups was assessed by both intent-to-treat (ITT) analysis and per-protocol (PP) analysis. The ITT analysis included participants who received at least 4 weeks of treatment, whereas the PP analysis only included the patients who finished 24-week treatment. The data from the patients who withdrew from the trial prematurely were considered missing, and these data were calculated using the last observation when performing the ITT analysis. Safety set analysis was used to assess the safety of two treatments, including all patients who received treatment once.

Baseline characteristics of participants were reported as the mean ± standard deviation or as numbers with corresponding percentages for categorical variables. To determine the differences in baseline characteristics between two groups, the independent *t*-tests were used for normally distributed variables, chi-square tests for categorical variables, and Mann–Whitney

U-tests for non-normally distributed variables. Analysis of the primary endpoint (the ACR20) and some secondary efficacy endpoints (the numeration data) was analyzed by using a chi-square test or Fisher's exact test, whereas the measurement data of secondary endpoints were detected by one-way repeated measures ANOVA of the mean values from baseline to weeks 4, 12, and 24 for each group. Missing values were replaced using the last observation. All statistical tests were two-sided, which were performed at the $p < 0.05$ significance level.

RESULTS

Characteristics of the Sample

Totally, 107 active RA patients were screened in this trial. Among these participants, 90 patients were eligible to be enrolled in this trial based on inclusion criteria. All of them were randomly assigned to the two groups: MTX + HQT ($n = 45$) and MTX + LEF ($n = 45$). The percentages of patients who did not finish the 24-week treatment were 13.3% in the MTX + HQT group and 17.8% in the MTX + LEF group. There were four and three patients in the MTX + HQT group and MTX + LEF group, respectively, excluded from the PP set for the protocol violation. Additionally, because of the adverse events, there were two patients in the MTX + HQT group and five patients in the MTX + LEF group who discontinued treatment (**Figure 1**).

There were no statistically significant differences in age, sex, demographics, or patient clinical characteristics between the two groups ($p > 0.05$). Demographics and clinical characteristics at baseline of patients with active RA are shown in **Table 2**. Patients received MTX at a dose of 11.84 ± 1.46 mg/week in the MTX + HQT group and 10.56 ± 1.14 mg/week in the MTX + LEF group. Concomitant medication evaluation was also performed to compare the two groups during the trial. There was no statistically significant difference in the proportion of the patients who used glucocorticoids, NSAIDs, antacids, folic acid, or calcitriol/calcium ($p > 0.05$). Besides, the mean values of the glucocorticoid doses in patients each day were 5.17 ± 0.93 mg in the MTX + HQT group and 5.0 ± 0.0 mg in the MTX + LEF group. The major concomitant medications in this study of the two groups are shown in **Table 2**. Furthermore, 85.6% (77/90) participants had a period of DMARD washout that lasted for at least 4 weeks before participating in this trial. Of them, 82.2% (37/45) participants were in the MTX + HQT group, and 88.9% (40/45) participants were in the MTX + LEF group; the rest of the participants, 17.8% (8/45) in the MTX + HQT group and 11.1% (5/45) in the MTX + LEF group, had never taken DMARDs. No significant difference between the two groups was observed in the rate of receiving DMARDs treatment before participating in this trial ($p > 0.05$).

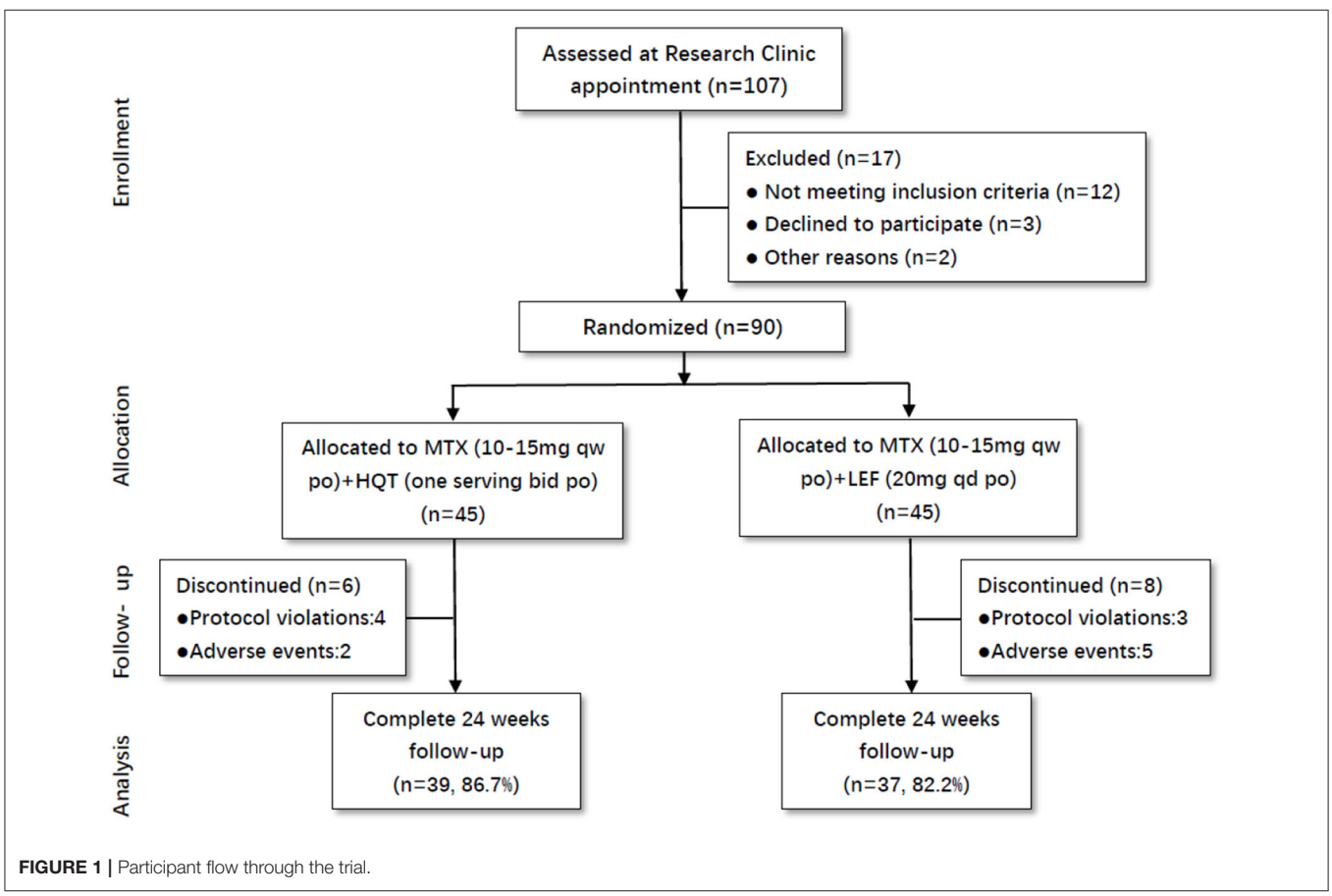

FIGURE 1 | Participant flow through the trial.

TABLE 2 | Demographic and characteristics data of RA patients at baseline in FAS.

Characteristics	MTX + HQT ($n = 45$)	MTX + LEF ($n = 45$)	P
Age (SD), years	51.67 (9.92)	47.56 (11.40)	0.099
Female, *n* (%)	35.00 (77.80)	31.00 (68.90)	0.340
Disease duration (SD), months	41.82 (45.93)	33.90 (36.53)	0.783
TJC (SD), *n*	8.62 (5.09)	9.20(6.15)	0.948
SJC (SD), *n*	6.29 (4.19)	4.67(3.02)	0.053
Patient's assessment of pain (SD), mm	65.09 (16.40)	59.78(20.17)	0.183
PhGADA† (SD), mm	61.11 (14.81)	56.33(18.84)	0.246
PaGADA† (SD), mm	62.00 (18.17)	57.56(20.47)	0.295
Morning stiffness (SD), min	47.11 (32.80)	53.89 (50.56)	0.798
HAQ, mean ± SD	0.65 (0.56)	0.92 (0.67)	0.063
hs-CRP (SD), mg/L	17.72 (19.10)	27.62 (35.15)	0.161
ESR (SD), mm/h	60.09 (27.29)	55.64 (31.39)	0.368
RF# (SD), U/ml	216.15 (298.88)	180.89 (194.82)	0.812
Anti-CCP#, positive rate	86.05% (37/43)	87.80% (36/41)	0.811
DAS28-CRP	6.02 (1.81)	5.97 (2.17)	0.620
cDAI (SD)	27.22 (8.98)	25.12 (10.06)	0.284
Concomitant treatments			
NSAIDs, *n* (%)	43 (95.6%)	44 (97.8%)	1.000
Glucocorticoid oral, *n* (%)	29 (64.4%)	27 (60.0%)	0.664
Folic acid tablet, *n* (%)	41 (91.1%)	44 (97.8%)	0.357
Calcitriol/calcium carbonate, *n* (%)	37 (82.2%)	43 (95.6%)	0.094
Antacids, *n* (%)	40 (88.9%)	42 (93.3%)	0.711

Data are presented as the mean (SD) or n (%).
FAS, full analysis set; TJC, tender joint count; SJC, swollen joint count; PhGADA, physician's global assessment of disease activity; PaGADA, patient's global assessment of disease activity; HAQ, Health Assessment Questionnaire; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide antibody; DAS28-CRP, 28-joint disease activity score- C-reactive protein; cDAI, clinical disease activity index; NSAIDs, non-steroidal anti-inflammatory drugs.
RF# was measured by immunonephelometric with a cutoff value of 20 U/ml. Anti-CCP# was measured using a commercially available second-generation ELISA kit (Abbott, USA) with a cutoff value of 25 U/ml.
†Measured on a 100-mm visual analog scale; the upper limit of normal for CRP is 0–6 mg/L.

Clinical Efficacy

In the ITT analyses after 24 weeks of treatment, there were 72.1% (31/43) and 74.4% (32/43) in the MTX + HQT and the MTX + LEF groups, respectively, who achieved the ACR20 response. Although there were more patients achieving ACR20 response in the MTX + HQT group, as compared with the MTX + LEF group, statistically, there was no difference ($p = 0.808$, **Figure 2**). ACR50, ACR70, cDAI good responses, EULAR good response, remission rate, and LDA rates of the patients at each evaluation point in the MTX + HQT group were similar to those in the MTX + LEF group (ACR50: 60.5 [26/43] vs. 60.5% (26/43); ACR70: 30.2 [13/43] vs. 30.2% [13/43]; cDAI good response: 76.7 [33/43] vs. 72.1% [31/43]; EULAR good or moderate response: 86.0 [37/43] vs. 86.0% [37/43]; EULAR good response: 51.2 [22/43] vs. 62.8% [27/43]; remission rate: 34.9 [15/43] vs. 48.8% [21/43]; LDA rate: 55.8 [24/43] vs. 67.4% [29/43]) (**Figure 2**). There was no appreciable difference between the two groups in those response rates mentioned earlier ($p > 0.05$).

In both groups, improvements in clinical symptoms (28-joint tender joint count, 28-joint swollen joint count, patient's assessment of pain, PaGADA, PhGADA, and morning stiffness duration), disease activity (DAS28-CRP), laboratory investigations (ESR, CRP, and RF), health status, and quality-of-life outcome (HAQ) were observed as early as week 4 and maintained through week 24 ($p < 0.05$). Overall, no clear differences were found between the two therapeutic regimens ($p > 0.05$). The clinical symptoms and laboratory investigations in the two groups at each point are shown in **Table 3**.

Additionally, we performed a PP analysis of the data from the patients who finished the 24-week treatment. At 24 weeks, ACR20 responses were attained in 76.9% (30/39) patients who received MTX and HQT and 75.7% (28/37) patients who received MTX and LEF, and no statistical significance was observed between the two groups ($p > 0.05$). The result of the PP analysis was in agreement with those found in the ITT analysis. Similar results of statistical analyses were seen for ACR50, ACR70, EULAR good response, cDAI good response, clinical remission, and LDA rate at week 24 in the PP analysis (**Figure 3**). A full list of the mean (standard deviation) on clinical symptoms and laboratory investigations at each point is provided in **Appendix 3**. After treatment, the clinical symptoms, laboratory investigations, HAQ score, and DAS were significantly improved compared with those before ($p < 0.05$). No statistical significance was observed in the improvement of those measures from baseline to week 24 between the two groups ($p > 0.05$).

Evaluation of the Radiographic Joint Damage

After 24-week treatment, 82.2% (37/45) of the patients in the MTX + HQT group provide the reports of radiographs at the two evaluation points and 71.1% (32/45) of the patients in the MTX + LEF group. Mean change values from baseline at week 24 were 3.59 ± 4.75 and 1.34 ± 8.67 with mTSS, 1.24 ± 2.39 and 0.63 ± 3.78 with JSN scores, 2.35 ± 2.96 and 0.72 ± 5.48 with erosions scores in the MTX + HQT and MTX + LEF groups, respectively; no significant differences were found between the two groups ($p > 0.05$). Comparing with the baseline, mTSS and erosions scores resulted in significant differences in the patients treated with two different treatment regimens at week 24 ($p < 0.05$), so as the JSN score in the MTX + HQT group ($p < 0.05$), whereas no significant differences of JSN score were observed in the MTX + LEF group ($p > 0.05$). Radiographs of the hands (including wrists) assessed by mTSS are shown in **Table 4**.

Safety and Tolerability

Safety evaluation was performed in the safety set analysis; all adverse events reported in this trial are listed in **Table 5**. In total, 28 patients (31.1%) experienced one or more adverse events (11 cases in the MTX + HQT group and 17 cases in the MTX + LEF group). The most common adverse events related to MTX + HQT were gastrointestinal discomfort, and all of the participants alleviated quickly and continued our trial after treatment with an antacid, although hepatic dysfunction, and gastrointestinal

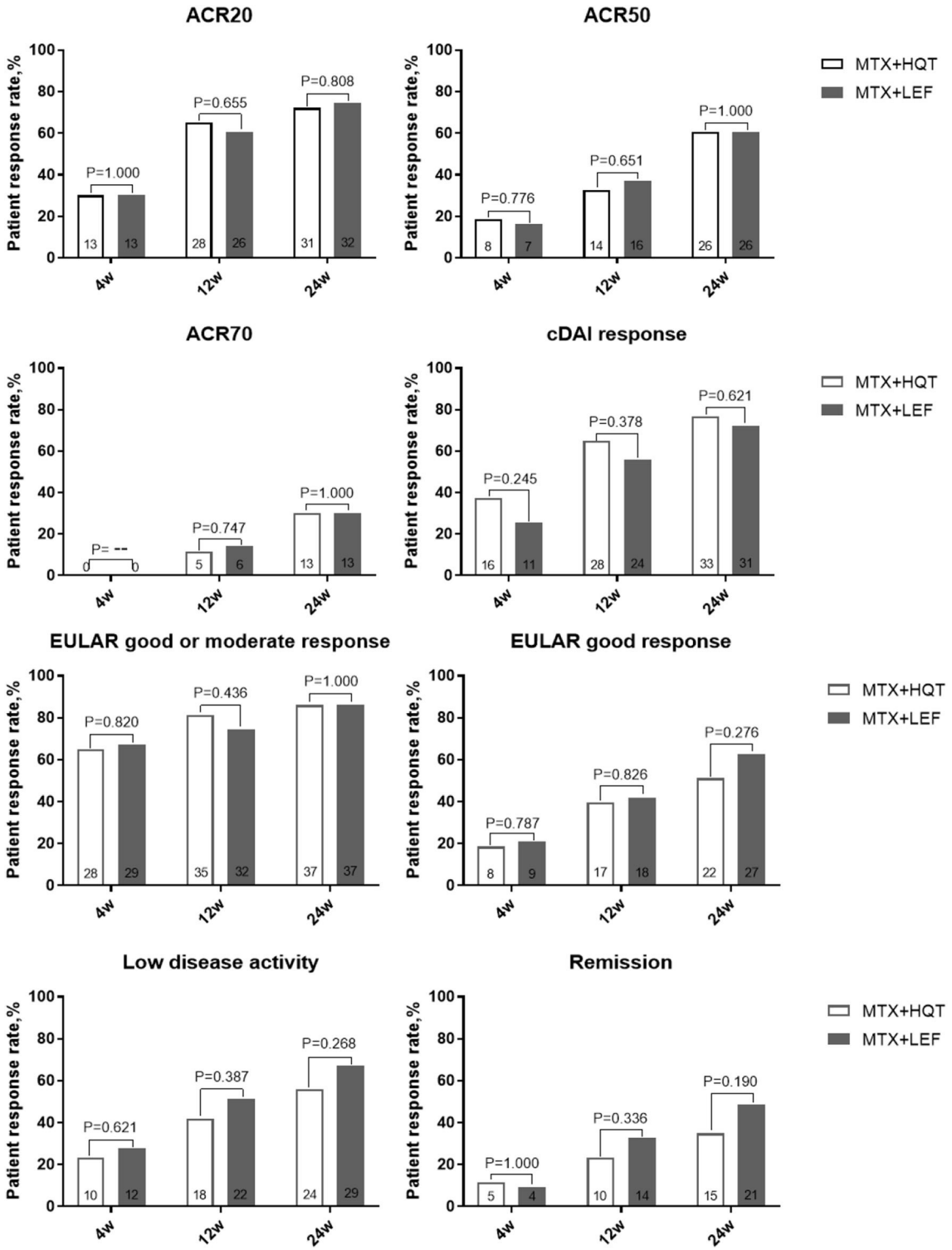

FIGURE 2 | Over time measurements of primary and secondary efficacy endpoints in the ITT analysis. A comparison between two combination treatments was performed by using the chi-square test or Fisher's exact test. ACR, American College of Rheumatology; cDAI, clinical disease activity index; EULAR, European League Against Rheumatism; ITT, intention-to-treat.

TABLE 3 | Clinical and laboratory measures of the two groups at each visit in the ITT analysis.

Measures	MTX + HQT				MTX + LEF			
	0W	4W	12W	24W	0W	4W	12W	24W
TJC, *n*	8.40 (5.10)	6.47 (4.86)	4.72 (3.53)	3.56 (4.04)	8.98 (5.92)	6.36 (5.19)	4.77 (5.02)	3.32 (5.01)
SJC, *n*	6.33 (4.28)	4.05 (3.37)	2.00 (2.40)	1.81 (3.81)	4.61 (3.03)	2.91 (2.87)	1.89 (2.53)	1.07 (1.89)
Patient's assessment of pain, mm†	65.09 (16.75)	45.81 (17.89)	30.35 (20.66)	23.44 (18.20)	60.00 (20.35)	42.45 (22.35)	29.91 (21.30)	21.48 (19.55)
PaGADA, mm†	61.86 (18.55)	39.70 (20.52)	29.53 (19.75)	19.95 (17.47)	58.18 (20.26)	44.32 (22.45)	29.32 (20.95)	20.80 (20.17)
PhGADA, mm†	61.16 (15.15)	40.81 (20.18)	28.60 (18.85)	20.70 (16.53)	56.25 (19.05)	43.18 (20.88)	30.57 (21.41)	21.25 (19.68)
Morning stiffness, min	47.91 (33.35)	26.98 (28.10)	17.84 (32.62)	19.30 (49.07)	54.20 (51.01)	35.00 (41.81)	14.55 (18.48)	11.50 (22.49)
CRP, mg/L	18.42 (19.25)	14.36 (19.21)	12.50 (15.38)	11.52 (18.39)	27.16 (35.78)	15.07 (25.27)	18.12 (55.57)	10.13 (16.96)
ESR, mm/h	60.70 (26.44)	55.05 (27.41)	51.81 (31.30)	46.91 (27.69)	54.43 (30.79)	51.95 (32.14)	44.12 (29.49)	42.09 (28.45)
RF, U/ml	204.14 (283.21)	157.91 (222.86)	153.38 (224.95)	222.09 (437.13)	182.05 (196.06)	182.21 (246.89)	123.59 (202.98)	113.37 (149.13)
HAQ	0.64 (0.57)	0.43 (0.45)	0.38 (0.53)	0.26 (0.46)	0.91 (0.68)	0.59 (0.63)	0.44 (0.56)	0.31 (0.57)
DAS28-CRP	5.98 (1.85)	4.73 (2.08)	3.69 (1.60)	3.21 (1.77)	5. 90 (2.09)	4.47 (2.04)	3.64 (2.09)	2.98 (2.05)

**Values are the mean (SD).*

TJC, tender joint count; SJC, swollen joint count; PaGADA, patient's global assessment of disease activity; PhGADA, physician's global assessment of disease activity; HAQ, Health Assessment Questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; DAS28, 28-joint disease activity score.

† Measured on a 100-mm visual analog scale.

discomfort were the most common adverse events with MTX + LEF, and five patients withdrew from the trial (four patients for hepatic dysfunction and one for hypertension). There was no statistical significance between the two groups in the incidences of all adverse events ($p > 0.05$).

DISCUSSION

Over thousands of years, TCM has been beneficial to many patients in China. Nowadays, TCM is regarded as a basic or complementary therapy for RA patients, and, therefore, a variety of TCM herbs have been used in the clinic for RA treatment. The anti-inflammatory and antiarthritic activities of many TCM herbs have been validated in arthritic models and also tested in clinical trials in patients with RA (10, 19, 21). Thus, Chinese herbals may also function as DMARDs, which could be used as an alternative or basic treatment for RA patients.

In TCM theory, RA belongs to "Bi" disease, which is a group of disorders with symptoms and signs similar to arthritis or other rheumatism defined in Western medicine (22–24). The development of "Bi" syndrome is due to an evil spirit, the pathogeny of TCM, including wind, cold, and wet that invade the human body and lead to poor circulation of Qi and blood, so-called "blood stasis" (23, 25, 26). Based on years of clinical experience and observation, "removing blood stasis" theory of TCM and the use of blood-activating herbs had efficacy in relieving clinical symptoms, signs, and indicators of inflammatory activity in RA patients (27). HQT is a Chinese herbal formula, and it is prepared for treating RA by activating blood circulation, dissipating blood stasis, and dispelling pathogenic wind, cold, and wet.

During the 24-week trial, the treatment with MTX + HQT resulted in significant improvement in clinical signs and symptoms of RA, including joint pain, joint swelling, morning stiffness duration, and measures of quality-of-life outcome, as well as in many inflammatory indicators, such as CRP, ESR, and the autoantibody RF. Comparing with MTX + LEF (a recommended therapy for refractory RA) (28–30), MTX + HQT led to a similar improvement in terms of patients achieving ACR20, ACR50, ACR70, cDAI, LDA, and remission responses and to moderate or good improvement in DAS28-CRP. In this pilot study, the combinational therapy of HQT with MTX effectively and safely alleviated symptoms and signs of patients with active RA. It is well-known that the destruction of smaller joints more frequently attacks the RA patients and that radiographs of both hands and feet are the most popular standard to evaluate structural changes, which is regarded as one of the criteria for assessing therapeutic efficacy (31). After 24-week treatment, both groups had higher mTSS than before treatment. However, X-ray analyses in our study showed no statistical difference in terms of mTSS between the two groups in the progression of radiographic joint damage. We considered that increasing mTSS might be in association with the high proportion (60.9%) of patients with disease duration of 2 years or more who enrolled in radiographic analyses set.

Our trial showed for the first time that MTX combined with Chinese herbal formula is equivalently effective as MTX combined with LEF in active RA patients. Previous studies have shown that herbal medicine monotherapy or combination therapy has efficacy in relieving clinical symptoms, signs, and indicators of inflammatory activity for RA patients (12, 32). According to previous pharmacological studies, the herbals in HQT formula are proven to have a variety of pharmacological effects, such as anti-inflammatory properties, analgesia, and immune suppression (33, 34), supporting the clinical efficacy of HQT in RA treatment. The root and rhizoma of *S. miltiorrhiza* Bunge (Danshen) and the rhizoma of *D. nipponica* Makino (Chuanshanlong) are the most important components of HQT. Previous pharmacological studies have demonstrated that *S. miltiorrhiza* injection could inhibit the proliferation of

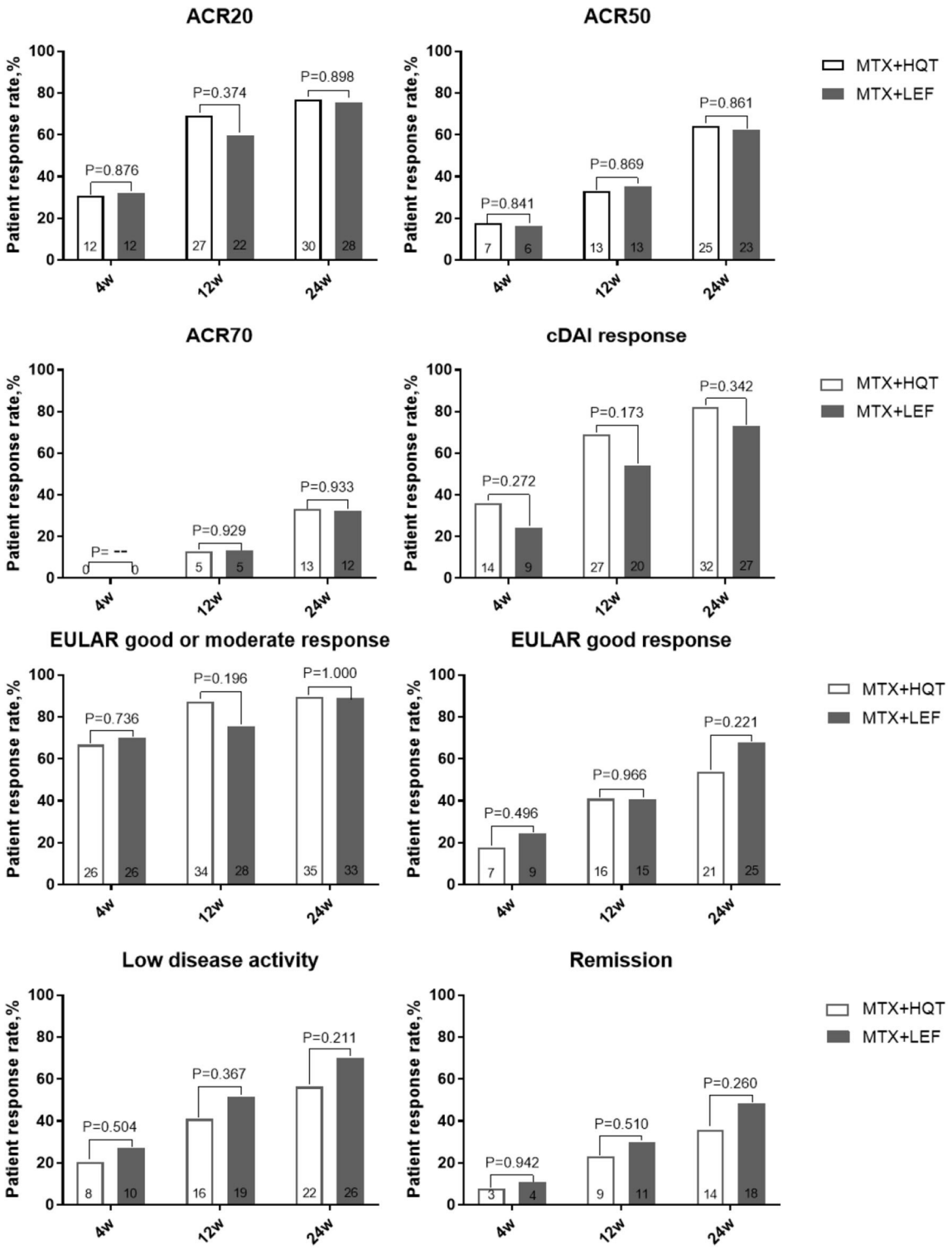

FIGURE 3 | Over time measurements of primary and secondary efficacy endpoints in the PPS analysis. A comparison between two combination treatments was performed by using the chi-square test or Fisher's exact test. ACR, American College of Rheumatology; cDAI, clinical disease activity index; EULAR, European League Against Rheumatism; PPS, per-protocol set.

TABLE 4 | Mean (SD) in the van der Heijde modified total sharp score (mTSS) in the two groups.

Joint damage	MTX + HQT (*N* = 37)	MTX + LEF (*N* = 32)	*P* (between groups)
mTSS			
Baseline, mean (SD)	50.92 (53.66)	36.44 (32.46)	0.736
Week 24, mean (SD)	54.51 (55.80)	37.78 (31.70)	0.613
P value (within the group)	<0.01	0.013	
JSN SCORE			
Baseline, mean (SD)	23.92 (25.46)	15.47 (14.87)	0.535
Week 24, mean (SD)	25.16 (26.33)	16.09 (14.35)	0.413
P value (within the group)	0.002	0.132	
EROSION SCORE			
Baseline, mean (SD)	27.00 (30.33)	20.97 (19.53)	0.928
Week 24, mean (SD)	29.35 (31.60)	21.69 (19.35)	0.814
P-value (within the group)	<0.01	0.017	

mTSS, the van der Heijde modified total sharp score; JSN, joint space narrowing; SD, standard deviation.

TABLE 5 | Summary of adverse events in the safety analysis set.

Adverse events	MTX + HQT (*n* = 45)	MTX + LEF (*n* = 45)	*P*
All	11 (24.4%)	17 (37.8%)	
ALT/AST elevation	0 (0%)	4 (8.9%)	0.117
Gastrointestinal reactions	9 (20.0%)	10 (22.2%)	0.796
Rash	1 (2.2%)	1 (2.2%)	1.000
Atrial fibrillation	1 (2.2%)	0 (0%)	1.000
Leukopenia	0 (0%)	1 (2.2%)	1.000
Hypertension	0 (0%)	1 (2.2%)	1.000

Data are presented as n (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase.

fibroblast-like synoviocytes obtained from RA patients (35, 36). Also, tanshinone VI, an abietane diterpene extracted from the root of *S. miltiorrhiza* Bunge, could improve bone loss by inhibiting osteoclastic bone resorption through inhibition of nuclear factor-κB and receptor activator of nuclear factor kappa-κ ligand pathways (37). Diosgenin, a major alkaloid monomer from the rhizoma of *D. nipponica* Makino, has a variety of pharmacological effects to relieve pain, reduce inflammation, regulate cytokine expression, and inhibit the proliferation of fibroblast-like synoviocytes, and, therefore, it has been frequently used to treat RA (38, 39). Many pharmacological studies reported that *D. mariesii* Moore ex Bak (Gusuibu), *D. asperoides* C. Y. Cheng et T. M. Ai (Chuanxuduan), and *E. ulmoides* Oliver (Duzhong) hold the potentials to prevent osteoporosis and inflammation associated with arthritis (40–42). Our previous study demonstrated that the combined therapy of HQT and MTX could significantly improve the clinical symptoms of RA patients with good tolerance (13). Furthermore, many experimental studies showed that the active ingredients of the other six herbs in HQT also exert anti-arthritic effects in both *in vivo* and *in vitro* models (43–47). Pieces of evidence from these pharmacological or mechanism studies support the clinical efficacy of MTX + HQT observed in our trial.

In our current study, all AEs were predominantly mild or moderate, with a low incidence rate. Gastrointestinal reactions and liver abnormalities were AEs. Reported AEs suggested that the most significant safety issue of MTX + LEF combination was potential liver toxicity, which was undoubtedly consistent with the real situation of LEF usage in RA treatment (48). In our study, liver abnormalities occurred in 8.9% of the MTX + LEF, which was lower than those reported in several other RCTs in RA (28, 29). The result of the previous study found that the incidence of alanine aminotransferase/aspartate aminotransferase elevations increased ∼ 2–5 folds in the combination of MTX and LEF, which depended on the dosages of MTX (48). In our study, we used the low dose of MTX (10–12.5 mg/week), reflecting the standard of the Chinses recommendations for the management of RA currently, which might be one of the reasons for the low rate of adverse events that occurred in our trial. Besides, more than 90% of patients in our trial received folic acid, which had the efficacy of lessening toxicity without altering efficacy during long-term treatment with MTX for RA (49).

Several critical factors in the course of analyzing the observational data should be considered in this work. Firstly, this trial was an open-label, monocenter, clinical trial, and the treating physicians and patients were not blinded to medication. To make an objective assessment of the efficacy and safety of the MTX + HQT combination therapy, the clinical outcomes were assessed and analyzed by evaluators and statisticians who were unaware of the therapy. However, a completely objective assessment needs to be verified in a multicenter, double-blind RCT in the future. Secondly, it was a 24-week observation trial, which may not be sufficient to show the long-term benefit of the MTX + HQT combination therapy, especially the radiographic progression. Thirdly, this trial did not compare the HQT in monotherapy with the MTX or another csDMARD in monotherapy, so the clinical efficacy of HQT itself cannot be evaluated or compared directly. Finally, due to a lack of similar clinical studies and pilot studies to reference, the sample size and the hypothesis test type (non-inferiority trial, equivalence trial, or superiority trial) in the design of this pilot study could not be pre-estimated accurately. However, the result of this pilot study can be the basic data and reference for further study.

CONCLUSION

This pilot study was the first time to evaluate the effect and safety of HQT, a Chinese medicine formula, combined with MTX, comparing with the combination of MTX and LEF. The results of this analysis indicate that the therapeutic regimen of HQT combined with MTX provides a potentially beneficial clinical response with acceptable tolerability for treating patients with active RA, which implies that HQT or other Chinese medicine formula may be a good therapeutic option in combination with MTX for RA treatment. However, it should be stressed that interpretations of the efficacy data are limited by the shortcoming mentioned earlier. A multicentric, double-blinded, preferably placebo-controlled, as well as with a longer follow-up, RCT

is motivated to definitively establish the efficacy and safety of the HQT + MTX combination therapy and even the HQT in monotherapy.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Medical Ethics Committee of the Second Affiliated Hospital of Guangzhou University of Chinese Medicine (B2016-076-01). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XiaC, YL, YZ, XiuC, YC, XW, ZW, P-JJ, QH, and RH: design and conception. JW, KG, AO, ZL, YL, YZ, and XiuC: acquisition, analysis, and interpretation. JZ, JP, ZH, XiuC, JW, KG, QH, and RH: major contributors in writing the manuscript. All authors read and approved the final manuscript. All authors contributed to the study design and review before submission according to their interests and scientific expertise.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Mcinnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med*. (2011) 365:2205–19. doi: 10.1056/NEJMra1004965
2. Sepriano A, Kerschbaumer A, Smolen JS, Van Der Heijde D, Dougados M, Van Vollenhoven R, et al. Safety of synthetic and biological DMARDs: a systematic literature review informing the 2019 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann Rheum Dis*. (2020) 7:760–70. doi: 10.1136/annrheumdis-2019-216653
3. Chatzidionysiou K, Sfikakis PP. Low rates of remission with methotrexate monotherapy in rheumatoid arthritis: review of randomized controlled trials could point towards a paradigm shift. *RMD Open*. (2019) 5:e000993. doi: 10.1136/rmdopen-2019-000993
4. Badavanis G, Pasmatzi E, Monastirli A, Tsambaos D. Biologic agents in systemic dermatotherapy: cutaneous and systemic side effects. *Curr Drug Saf*. (2017) 12:76–94. doi: 10.2174/1574886312666170518124014
5. Jick SS, Lieberman ES, Rahman MU, Choi HK. Glucocorticoid use, other associated factors, ad the risk of tuberculosis. *Arthritis Rheum*. (2006) 55:19–26. doi: 10.1002/art.21705
6. Novosad SA, Winthrop KL. Beyond tumor necrosis factor inhibition: the expanding pipeline of biologic therapies for inflammatory diseases and their associated infectious sequelae. *Clin Infect Dis*. (2014) 58:1587–98. doi: 10.1093/cid/ciu104
7. Du Pan SM, Dehler S, Ciurea A, Ziswiler HR, Gabay C, Finckh A, et al. Comparison of drug retention rates and causes of drug discontinuation between anti-tumor necrosis factor agents in rheumatoid arthritis. *Arthritis Rheum*. (2009) 61:560–8. doi: 10.1002/art.24463
8. Cohen SB, Czeloth N, Lee E, Klimiuk PA, Peter N, et al. Long-term safety, efficacy, and immunogenicity of adalimumab biosimilar BI 695501 and adalimumab reference product in patients with moderately-to-severely active rheumatoid arthritis: results from a phase 3b extension study (VOLTAIRE-RAext). *Expert Opin Biol Ther*. (2019) 19:1097–105. doi: 10.1080/14712598.2019.1645114
9. Lu MC, Livneh H, Chiu LM, Lai NS, Yeh CC, Tsai TY. A survey of traditional Chinese medicine use among rheumatoid arthritis patients: a claims data-based cohort study. *Clin Rheumatol*. (2019) 38:1393–400. doi: 10.1007/s10067-018-04425-w
10. Huang RY, Pan HD, Wu JQ, Zhou H, Li ZG, Qiu P, et al. Comparison of combination therapy with methotrexate and sinomenine or leflunomide for active rheumatoid arthritis: A randomized controlled clinical trial. *Phytomedicine*. (2019) 57:403–10. doi: 10.1016/j.phymed.2018.12.030
11. Chen XM, Wu JQ, Huang QC, Zhang JY, Pen JH, Huang ZS, et al. Systematic review and meta-analysis of the efficacy and safety of Biqi capsule in rheumatoid patients arthritis. *Exp Ther Med*. (2018) 15:5221–30. doi: 10.3892/etm.2018.6121
12. He YT, Ou AH, Yang XB, Chen W, Fu LY, Lu AP, et al. Traditional Chinese medicine versus western medicine as used in China in the management of rheumatoid arthritis: a randomized, single-blind, 24-week study. *Rheumatol Int*. (2014) 34:1647–55. doi: 10.1007/s00296-014-3010-6
13. Lv Y, Chen XH, Huang RY, Zhao Y, Wu JQ, Chen XM, et al. Clinical analysis of chinese medicine compound huayu-qiangshen-tongbi decoction combined with Methotrexate for the treatment of chinese patients with rheumatoid arthritis: a retrospective study. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. (2019) 39:547–52.
14. Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS, et al. The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum*. (1988) 31:315–24. doi: 10.1002/art.1780310302
15. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. (2010) 62:2569–81. doi: 10.1002/art.27584
16. Madsen OR. Is DAS28-CRP with three and four variables interchangeable in individual patients selected for biological treatment in daily clinical practice? *Clin Rheumatol*. (2011) 30:1577–82. doi: 10.1007/s10067-011-1847-6

17. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American college of rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum*. (1995) 38:727–35. doi: 10.1002/art.1780380602
18. Fransen J, Van Riel PL. The disease activity score and the EULAR response criteria. *Rheum Dis Clin North Am*. (2009) 35:745–57. doi: 10.1016/j.rdc.2009.10.001
19. Lv QW, Zhang W, Shi Q, Zheng WJ, Li X, Chen H, et al. Comparison of *Tripterygium wilfordii* hook F with methotrexate in the treatment of active rheumatoid arthritis (TRIFRA): a randomised, controlled clinical trial. *Ann Rheum Dis*. (2015) 74:1078–86. doi: 10.1136/annrheumdis-2013-204807
20. Van Der Heijde D. How to read radiographs according to the Sharp/van der Heijde method. *J Rheumatol*. (2000) 27:261–3. doi: 10.1097/00002281-200007000-00005
21. Venkatesha SH, Rajaiah R, Berman BM, Moudgil KD. Immunomodulation of autoimmune arthritis by herbal CAM. *Evid Based Complement Alternat Med*. (2011) 2011:986797. doi: 10.1155/2011/986797
22. Zhang C, Jiang M, Lu AP. Evidence-based Chinese medicine for rheumatoid arthritis. *J Tradit Chin Med*. (2011) 31:152–7. doi: 10.1016/S0254-6272(11)60031-9
23. Lo LC, Chen CY, Chiang JY, Cheng TL, Lin HJ, Chang HH. Tongue diagnosis of traditional Chinese medicine for rheumatoid arthritis. *Afr J Tradit Complement Altern Med*. (2013) 10:360–9. doi: 10.4314/ajtcam.v10i5.24
24. Wang M, Chen G, Lu C, Xiao C, Li L, Niu X, et al. Rheumatoid arthritis with deficiency pattern in traditional Chinese medicine shows correlation with cold and hot patterns in gene expression profiles. *Evid Based Complement Alternat Med*. (2013) 2013:248650. doi: 10.1155/2013/248650
25. Zhou X, Zhou Z, Jin M, Wang H, Wu M, Gu Q, et al. Intermediate and late rheumatoid arthritis treated by tonifying the kidney, resolving phlegm and removing blood stasis. *J Tradit Chin Med*. (2000) 20:87–92.
26. Chu YL, Jiang YQ, Sun SL, Zheng BL, Xiong WS, Li WJ, et al. The differential profiles of long non-coding RNAs between rheumatoid arthritis and gouty arthritis. *Discov Med*. (2017) 24:133–46.
27. Huang QC, Chu YL, He XH, Huang RY. Regulatory roles of compound danshen in the downstream path of cyclooxygenases in rheumatoid arthritis patients' synovium. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. (2013) 33:1416–9.
28. Antony T, Jose VM, Paul BJ, Thomas T. Efficacy and safety of leflunomide alone and in combination with methotrexate in the treatment of refractory rheumatoid arthritis. *Indian J Med Sci*. (2006) 60:318–26. doi: 10.4103/0019-5359.26608
29. Londono J, Santos AM, Santos PI, Cubidez MF, Guzman C, Valle-Onate R. Therapeutic efficacy and safety of methotrexate + leflunomide in Colombian patients with active rheumatoid arthritis refractory to conventional treatment. *Rev Bras Reumatol*. (2012) 52:837–45. doi: 10.1590/S0482-50042012000600003
30. Hodkinson B, Magomero KR, Tikly M. Combination leflunomide and methotrexate in refractory rheumatoid arthritis: a biologic sparing approach. *Ther Adv Musculoskelet Dis*. (2016) 8:172–9. doi: 10.1177/1759720X16664324
31. Fransen J, Van Riel PL. Outcome measures in inflammatory rheumatic diseases. *Arthritis Res Ther*. (2009) 11:244. doi: 10.1186/ar2745
32. Lee S, Cho Y, Kim J, Kang JW, Yoon GY, Lee JH, et al. The efficacy and safety of the herbal medicine geonchildan for patients with active rheumatoid arthritis: study protocol for a randomized, double-blind, placebo-controlled, parallel pilot trial. *Trials*. (2018) 19:471. doi: 10.1186/s13063-018-2849-3
33. Wang Z, Lin HH, Linghu K, Huang RY, Li G, Zuo H, et al. Novel compound-target interactions prediction for the herbal formula hua-yu-qiang-shen-tong-bi-fang. *Chem Pharm Bull*. (2019) 67:778–85. doi: 10.1248/cpb.c18-00808
34. Wang Z, Linghu KG, Hu Y, Zuo H, Yi H, Xiong SH, et al. Deciphering the pharmacological mechanisms of the huayu-qiangshen-tongbi formula through integrating network pharmacology and *in vitro* pharmacological investigation. *Front Pharmacol*. (2019) 10:1065. doi: 10.3389/fphar.2019.01065
35. Liu QS, Zhu XC, Li JA, Xing Y, Jiang H, Zhang J, et al. Effects of danshen injection on the proliferation of rheumatoid arthritis fibroblast-like synoviocytes cultured with human serum. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. (2013) 33:674–8.
36. Jie L, Du H, Huang Q, Wei S, Huang R, Sun W. Tanshinone IIA induces apoptosis in fibroblast-like synoviocytes in rheumatoid arthritis via blockade of the cell cycle in the G2/M phase and a mitochondrial pathway. *Biol Pharm Bull*. (2014) 37:1366–72. doi: 10.1248/bpb.b14-00301
37. Nicolin V, Dal Piaz F, Nori SL, Narducci P, De Tommasi N. Inhibition of bone resorption by Tanshinone VI isolated from Salvia miltiorrhiza Bunge. *Eur J Histochem*. (2010) 54:e21. doi: 10.4081/ejh.2010.e21
38. Liagre B, Vergne-Salle P, Corbiere C, Charissoux JL, Beneytout JL. Diosgenin, a plant steroid, induces apoptosis in human rheumatoid arthritis synoviocytes with cyclooxygenase-2 overexpression. *Arthritis Res Ther*. (2004) 6:R373–83. doi: 10.1186/ar1199
39. Liagre B, Leger DY, Vergne-Salle P, Beneytout JL. MAP kinase subtypes and Akt regulate diosgenin-induced apoptosis of rheumatoid synovial cells in association with COX-2 expression and prostanoid production. *Int J Mol Med*. (2007) 19:113–22. doi: 10.3892/ijmm.19.1.113
40. Jung HW, Jung JK, Son KH, Lee DH, Kang TM, Kim YS, et al. Inhibitory effects of the root extract of Dipsacus asperoides C.Y. Cheng et al. T.M.Ai on collagen-induced arthritis in mice. *J Ethnopharmacol*. (2012) 139:98–103. doi: 10.1016/j.jep.2011.10.020
41. Lin TH, Yang RS, Wang KC, Lu DH, Liou HC, Ma Y, et al. Ethanol extracts of fresh *Davallia formosana* (WL1101) inhibit osteoclast differentiation by suppressing RANKL-induced nuclear factorkappa B activation. *Evid Based Complement Alternat Med*. (2013) 2013:647189. doi: 10.1155/2013/647189
42. Wang JY, Yuan Y, Chen XJ, Fu SG, Zhang L, Hong YL, et al. Extract from Eucommia ulmoides Oliv. ameliorates arthritis via regulation of inflammation, synoviocyte proliferation and osteoclastogenesis in vitro and in vivo. *J Ethnopharmacol*. (2016) 194:609–16. doi: 10.1016/j.jep.2016.10.038
43. Jiang JB, Qiu JD, Yang LH, He JP, Smith GW, Li HQ. Therapeutic effects of astragalus polysaccharides on inflammation and synovial apoptosis in rats with adjuvant-induced arthritis. *Int J Rheum Dis*. (2010) 13:396–405. doi: 10.1111/j.1756-185X.2010.01555.x
44. Zhou J, Xu G, Yan J, Li K, Bai Z, Cheng W, et al. Rehmannia glutinosa (Gaertn.) DC polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice. *J Ethnopharmacol*. (2015) 164:229–38. doi: 10.1016/j.jep.2015.02.026
45. Xu M, Guo Q, Wang S, Wang N, Wei L, Wang J. Anti-rheumatoid arthritic effects of *Saussurea involucrata* on type II collagen-induced arthritis in rats. *Food Funct*. (2016) 7:763–70. doi: 10.1039/C5FO00603A
46. Luo J, Song WJ, Xu Y, Chen GY, Hu Q, Tao QW. Benefits and safety of tripterygium glycosides and total glucosides of paeony for rheumatoid arthritis: an overview of systematic reviews. *Chin J Integr Med*. (2019) 25:696–703. doi: 10.1007/s11655-019-3221-5
47. Zhai KF, Duan H, Cui CY, Cao YY, Si JL, Yang HJ, et al. Liquiritin from glycyrrhiza uralensis attenuating rheumatoid arthritis via reducing inflammation, suppressing angiogenesis, and inhibiting MAPK signaling pathway. *J Agric Food Chem*. (2019) 67:2856–64. doi: 10.1021/acs.jafc.9b00185
48. Curtis JR, Beukelman T, Onofrei A, Cassell S, Greenberg JD, Kavanaugh A, et al. Elevated liver enzyme tests among patients with rheumatoid arthritis or psoriatic arthritis treated with methotrexate and/or leflunomide. *Ann Rheum Dis*. (2010) 69:43–7. doi: 10.1136/ard.2008.101378
49. Shea B, Swinden MV, Ghogomu ET, Ortiz Z, Katchamart W, Rader T, et al. Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis. *J Rheumatol*. (2014) 41:1049–60. doi: 10.3899/jrheum.130738

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REVIEW
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The Link Between Autonomic Nervous System and Rheumatoid Arthritis: From Bench to Bedside

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Neuronal stimulation is an emerging field of research focused on the management and treatment of various diseases through the reestablishment of physiological homeostasis. Electrical vagus nerve stimulation has recently been proposed as a revolutionary therapeutic option for rheumatoid arthritis (RA) in combination with or even as a replacement for conventional and biological drugs. In the past few years, disruption of the autonomic system has been linked to RA onset and activity. Novel research on the link between the autonomic nervous system and the immune system (immune-autonomics) has paved the way for the development of innovative RA management strategies. Clinical evidence supports this approach. Cardiovascular involvement, in terms of reduced baroreflex sensitivity and heart rate variability-derived indices, and mood disorders, common comorbidities in patients with RA, have been linked to autonomic nervous system dysfunction, which in turn is influenced by increased levels of circulating pro-inflammatory cytokines. This narrative review provides an overview of the autonomic nervous system and RA connection, discussing most of the common cardiac and mental health-related RA comorbidities and their potential relationships to systemic and joint inflammation.

Keywords: vagus nerve, central nervous system, autonomic nervous system, mood disorder, rheumatoid arthritis, depression, therapy

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease leading to progressive joint damage and associated with vascular, metabolic, and psychological comorbidities. RA is considered to pose a major global public health challenge, as its overall prevalence and incidence rates are increasing worldwide (1). Fundamentally, it is of paramount importance to reduce the future burden of this disease through the pursuit of innovative RA treatments that are driven mainly by increasing knowledge of its pathophysiology. An emergent field in this context is the study of autonomic nervous system (ANS) imbalance observed in association with many immune-mediated inflammatory diseases, comprising RA, systemic lupus erythematosus, systemic sclerosis, and inflammatory bowel diseases (2–4). The immune system and ANS can express and respond to numerous common regulatory molecules (e.g., glucocorticoids, cytokines, neuropeptides, and neurotransmitters), which constitute the molecular basis of a complex bidirectional response

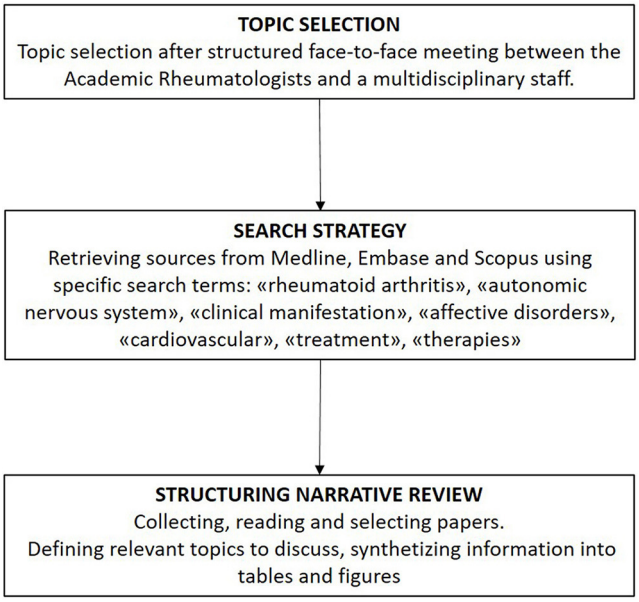

FIGURE 1 | The flow diagram of the narrative review process.

to homeostasis perturbations induced by infection or inflammation. These findings have led to an innovative research field in RA focused on the emerging concept of "immuno-autonomics," reflecting the anatomical and functional linkage of the immune system to the nervous system (5). With the understanding of the relevance of this connection, we may consider if and where the ANS is disrupted in RA and hence which therapeutic implications might be relevant. Whether ANS impairment is the result of chronic inflammation or a primitive alteration that affects immune system functioning, disease onset, and severity remains to be established. In this narrative review (**Figure 1**) (6), we discuss and review the ongoing progress in this field, focusing mainly on current knowledge of the potential connections between ANS and the pathogenesis and clinical manifestations of RA. We then consider the potential innovative therapeutic prospects that target this pathway.

RELEVANCE OF THE AUTONOMIC NERVOUS SYSTEM TO THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

The ANS operates through visceral reflex arcs mediated by cholinergic and catecholaminergic signaling (**Figure 2**). Communication between the ANS and the immune system occurs in two main ways: (i) via direct innervation of the lymphoid organs by the efferent sympathetic nervous system (SNS) [postganglionic noradrenergic fibers innervate the bone marrow, thymus, spleen, and lymph nodes by liberating noradrenaline (NA), neuropeptides (substance P, somatostatin, vasoactive intestinal peptide, and neuropeptide Y), neurokinins, and opioids whose receptors are expressed on immune cells] and (ii) by indirect humoral action mediated by NA (liberated into the bloodstream after medullary activation by the SNS) and steroids [due to activation of the hypothalamic–pituitary–adrenal (HPA) axis neuroendocrine response] (7, 8).

Via beta-2 adrenergic receptors, NA inhibits lymphocyte proliferation, the release of pro-inflammatory cytokines [interleukin (IL)-2, IL-12, interferon gamma, and tumor necrosis factor (TNF)-α], chemotaxis, the activity of natural killer cells and T lymphocytes, and the production of antibodies by B cells. It also stimulates anti-inflammatory cytokines (IL-4, IL-5, and IL-10). Not only postganglionic fibers but also lymphocytes can release NA and acetylcholine, thereby regulating some immune functions in autocrine and paracrine ways (7, 8).

Conversely, the immune system is capable of communicating with the nervous system, mainly via HPA axis activation by pro-inflammatory cytokines and afferent vagal fibers. Neurons express pattern recognition receptors, including toll-like and cytokine receptors (e.g., TNF-R and type 1 IL-1 receptors) (9, 10). This dialog extends beyond the HPA axis; cytokines also directly influence the cerebral cortex and primitive brain (the limbic system, brain stem, and hypothalamus). Cerebral manifestations such as fever, illness, attention deficit, anorexia, and disrupted interaction with external stimuli are examples of their effects during the acute phase of immune activation. In efforts to discern the physiology and pathology of RA, the identification of specific reflexes and pathways is fundamental. A "cholinergic anti-inflammatory pathway," named after its principal mediator acetylcholine, has been described; its identification has led to the understanding of the "inflammatory reflex" (11). This neural circuit, like other visceral reflex arcs, includes afferent and efferent arms. Vagus afferent fibers are activated peripherally by products of inflammation and then the signal travels to the nucleus tractus solitarius; once the stimulus has been relayed and integrated by other nuclei in the brain stem or hypothalamus (e.g., the rostral ventrolateral medulla and locus coeruleus) (12, 13), the efferent signal leaves the nucleus ambiguus and dorsal motor nucleus and travels back through the vagus nerve until it reaches the celiac ganglia (13, 14). There, preganglionic cholinergic fibers form synapses with adrenergic interneurons, whose splenic fibers end up in the white pulp of the spleen and interact, via NA release, with beta-2 adrenergic receptors of a specific T cell subset. The latter is distinguished by the expression of choline acetyltransferase and when activated induces acetylcholine biosynthesis and release, which in turn stimulates macrophages of the red pulp binding alpha-7 subunit of nicotinic acetylcholine receptors (α7nAChRs) toward an anti-inflammatory immune response (15, 16). The anti-inflammatory signal may bypass the vagus efferent fibers, traveling from the preganglionic fibers of the sympathetic chain directly to the interneurons of the celiac ganglia.

Thus, under basal conditions, the vagus nerve senses the precise location of nascent inflammation and rapidly tonically dampens the overactivation of the innate immune response (17). As the major regulator of the peripheral nervous system (PNS), the vagus modulates inflammation in many anatomical regions, comprising the liver (14), heart (18), pancreas (19), and gastrointestinal tract (20, 21).

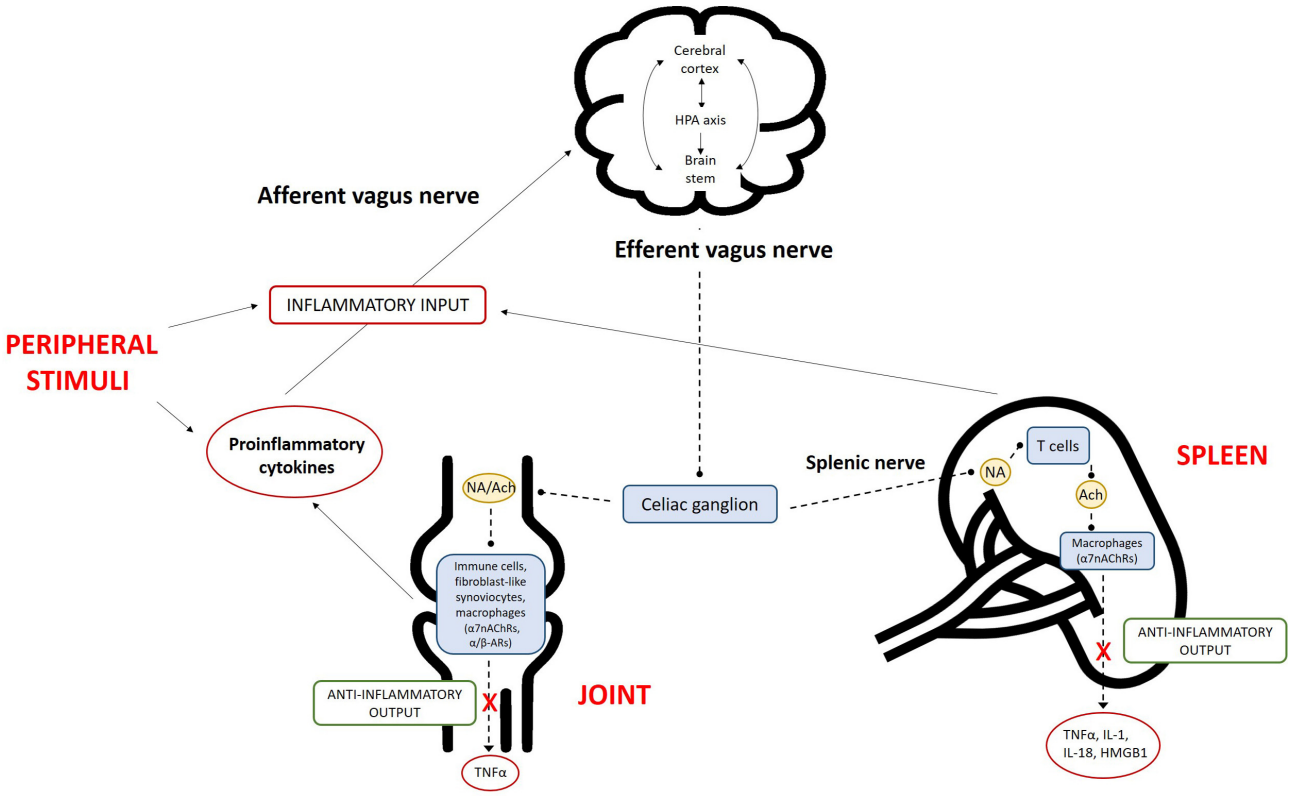

FIGURE 2 | Overview of the inflammatory reflex in rheumatoid arthritis (RA). The central nervous system (CNS) detects peripheral inflammation through the afferent vagus nerve and pro-inflammatory cytokines. The signals act at different levels via (i) activation of the hypothalamic–pituitary–adrenal (HPA) axis, which reduces inflammation; (ii) the cerebral cortex; and (iii) nuclei in the brain stem. The efferent branch of the vagus nerve is activated via the activation of alpha-7 subunit of nicotinic acetylcholine receptors (α7nAChRs) on macrophages, resulting in decreased cytokine production. This mechanism is called the "cholinergic anti-inflammatory pathway." The combination of inflammation in peripheral tissues, signaling of this information to the brain, and the subsequent efferent neuronal response comprises the "inflammatory reflex." The activation of α7nAChRs on macrophages can occur locally in the RA joint or in the spleen. The sympathetic fibers of the splenic nerve are activated in the celiac ganglion, leading to noradrenaline (NA) production in the spleen. NA binds to α/β-adrenergic receptors (α/β-ARs) on choline acetyltransferase-positive T cells, which then produce the anti-inflammatory parasympathetic neurotransmitter acetylcholine (Ach), in turn activating α7nAChRs and leading to the reduced production of pro-inflammatory cytokines. In the joint, the activation of a7nAChRs on fibroblast-like synoviocytes or other immune cells also reduces cytokine production. TNF, tumor necrosis factor; IL, interleukin; HMGB1, high mobility group box 1.

The establishment and maintenance of a chronic response involves humoral neuroendocrine mechanisms. This cooperation not only affects innate immunity but also modulates cell trafficking and the lymphoid architecture, ultimately leading to the regulation of humoral immunity and possibly the prevention of T cell-mediated tissue damage (22, 23).

Autonomic imbalance seems to be an early finding, rather than the result of chronic inflammation, in patients with RA. In a prospective cohort study, in which ANS activity was assessed using a validated method via the measurement of subjects' resting heart rate (HR) and heart rate variability (HRV), individuals at risk of RA who subsequently developed arthritis had significantly higher resting HRs than healthy subjects (24). This finding is in agreement with those from other studies, which reflect reduced PNS activity and hence an impaired inflammatory reflex, in patients with RA (25, 26). The early impairment of the PNS in RA, even before the fulfillment of the disease classification criteria (27), is consistent with the correlation between increased inflammatory status and decreased parasympathetic activity observed in healthy subjects in large observational studies (28–30). In detail, levels of inflammatory markers [C-reactive protein (CRP) and IL-6] are inversely related to HRV in young adults (29), and circulating TNF level is an independent predictor of depressed HRV (31), reinforcing the concept of a complex dialog between immunity and the ANS.

As the PNS and SNS work together, we would expect the SNS to be less active when the PNS is impaired. Contrary to this expectation, the SNS has been found to be overactive in patients with RA and high NA levels (25), rendering comprehension of its pathogenetic role difficult (32). One explanation could involve a difference in intracellular signaling (5). Splenic beta-2 adrenergic receptors usually promote a T helper (Th) 2 and T regulatory (Treg) response via G-coupled proteins and protein kinase A pathways. However, the scenario in which the SNS seems to be chronically activated may entail the downregulation of beta-2 adrenergic receptors and a shift toward Th1 and/or Th17 immune responses via mitogen-activated protein kinase pathways (5).

Collectively taken, these data support the critical role of ANS in RA pathogenic network.

AUTONOMIC NERVOUS SYSTEM AND AFFECTIVE DISORDERS IN RHEUMATOID ARTHRITIS

Peripheral cytokines profoundly influence neuronal function and brain circuitry. As briefly mentioned above, they reach the brain by different routes and, once there, affect brain function through several mechanisms. They may directly stimulate (i) the central nervous system (CNS) cell population (microglia, astrocytes, and neurons), producing additional cytokines (33); (ii) the HPA axis, resulting in the production of corticotropin-releasing factor and adrenocorticotropic hormone; and (iii) cortisol, influencing many other physiological processes in the CNS. Cytokines alter the metabolism of several neurotransmitters, including serotonin (34, 35), dopamine (35), and glutamate (36, 37), leading to the decreased production of norepinephrine and the trophic or growth factors that are essential for neurogenesis and neuroplasticity (38–40). Changes in all of these factors and amines may lead to the development of psychiatric disorders, further corroborating the link between cytokine increments and mental health. In many studies, the continuous elevation of IL levels has been correlated with impairments in structures profoundly affected in mood disorders, such as the hippocampal region (41, 42) and other areas of the brain (43–45), as well as changes in functional connectivity (46, 47).

In the CIA model, researchers observed exacerbated symptomatology in association with greater cytokine production in mice lacking α7nAChR, suggesting that nicotinic receptor expression is relevant in RA and that the activation of these receptors would have beneficial effects. Interestingly, cholinergic agonists suppressed inflammatory cytokine production in RA whole blood cultures (48). Choline acetyltransferase expression was observed in fibroblast-like synoviocytes and mononuclear cells in RA and osteoarthritis synovial biopsy samples, suggesting that local acetylcholine production contributes to the regulation of joint inflammation by the aforementioned "cholinergic anti-inflammatory pathway" (49). Although the activation of α7nAChR leads to control of the degree of inflammation, the effects of this receptor's action on central neurons, brain functionality, and related cognitive behaviors have not been examined. In general, results indicate that (i) muscarinic agonist administration, (ii) electrical vagus nerve stimulation (VNS) to activate preganglionic parasympathetic nerves, and (iii) treatment with nAChR agonists can all act systemically (although not necessarily identically) to reduce the production of inflammatory cytokines (presumably mostly by macrophages).

Abundant data support associations between autoimmune diseases and psychiatric disorders, with immune activation identified as the common core feature (50, 51). The prominence of psychiatric comorbidities, including major depressive disorder (MDD), bipolar disorder, and anxiety disorders (ADs), in the presence of autoimmune diseases such as RA supports the theory that affective disorders can be considered to be inflammatory conditions (52).

From an epidemiological point of view, concomitant RA and depression has been reported in 6.8–66.2% of patients (53–62). Risk factors for MDD onset in patients with RA include female sex (63, 64), unmarried status, the lack of sufficient social support (65), a high rate of disability (66), and chronic pain (67). Moreover, the presence of depressive symptoms increases the risk of suicide in subjects, and especially women, with RA (68).

In most described cases, depression appears secondary to RA development (69–72). Several pathogenetic mechanisms have been proposed to explain this association. From a neurobiological perspective, reduced expression of BDNF, but not serum levels of pro-inflammatory cytokines (59) or TNF-α (57), has been found in depressed patients with RA with respect to subjects without depression. In contrast, other studies have documented a relationship between MDD and the inflammatory state, defined by plasma CRP levels (73) or indirectly via clinical indices of RA activity (74). The implication of over-inflammation in the onset of mood symptoms in RA is also supported by the evidence of alterations in the micro-structure of brain white matter in subjects with RA and comorbid depression as a result of vasculitis, ischemic brain lesions, and dots of demyelination (75). In contrast, one study failed to identify shared genetic vulnerability to RA and MDD (76). From a psychological perspective, the RA self-schema construct seems to predict the onset of depression. Interpersonal conflicts seem to increase the risk of depression in patients with RA. Vulnerability to depressive symptoms in patients with RA appears to be related to functional disabilities (59, 77–79) and pain (78, 80–82) caused by the disease, although some researchers failed to find an association between RA activity or severity and depression (56, 81, 83).

Comorbid depression increases social impairment (84, 85) and disability (86) in patients with RA. Functional capacity seems to be more compromised in women than in men with concomitant RA and MDD (63). The occurrence of depression in patients with RA worsens the subjective sensation of pain (87–90), thereby encouraging the abuse of analgesic compounds (91, 92). After all, the association between depression and pain sensitivity is already known within the framework of fibromyalgia (93). Of note, the presence of depressive symptoms in musculoskeletal pathologies even reach 81.8% as reported in a recent study (94), where a correlation between the severity of depressive symptoms and fibromyalgia was also identified. Although most studies have focused on the effects of RA on mental health, some authors have hypothesized conversely that depression directly influences RA activity and the number of compromised joints (95–97). In addition, comorbid depression in patients with RA favors the onset of medical complications such as atherosclerosis (98) and myocardial infarction (99), resulting in increased lethality (100, 101). Depressive symptoms also may prevent patients' treatment adherence, thereby worsening the prognosis of RA (96, 102).

Taken as a whole, these data indicate that depression worsens the quality of life (90, 103) and general health status (104) of subjects with RA.

Depression is often underestimated in patients with RA, delaying its proper management (94, 105). The treatment of depressive symptoms seems to ameliorate the clinical symptoms of RA (106), although some data contradict the existence of this effect (107). Antidepressants demonstrated to be effective in cases of RA-MDD comorbidity include dothiepin (108) and sertraline (109), eventually combined with cognitive behavioral treatment (107). Non-pharmacological strategies, including yoga, mindfulness meditation, and emotion regulation therapy, have been shown to significantly ameliorate mood symptoms and inflammatory status in patients with RA (110, 111). Finally, immunomodulating drugs, such as anti-TNF-α drugs (105, 112), anti-IL-6 monoclonal antibodies (113), and other biologics (114), appear to ameliorate anxiety and depressive symptoms in patients with RA. Of note, in line with the autonomic dysfunctions already mentioned for RA, VNS was proposed as a treatment for depression in the light of its anti-inflammatory effect (115). This convergence of the efficacy of VNS both for RA and depression opens up extremely promising therapeutic prospective for the treatment of subjects suffering from RA and mood symptoms (116).

ADs have the common psychopathological nucleus of anxiety and partly shared neurobiological abnormalities. They include panic disorder, social phobia (SP) (also known as social anxiety disorder), and generalized anxiety disorder (GAD). Their prevalence in RA ranges from 13 to 70% (53, 55, 65, 78, 117–119) with higher incidence and prevalence compared to the general population (120). Anxious states often occur concomitantly with depressive symptoms (54, 65). SP seems to be more common in individuals with RA than in those with other autoimmune conditions (121, 122). ADs are considered to be a risk factor for the future development of RA (120) and appear to negatively affect its course, worsening functional disabilities (78, 90, 123) and quality of life (90, 103, 124, 125) and changing pain perceptions (82, 89, 126). Moreover, psychological stress and anxiety symptoms were reported to be the most frequent causes of joint symptom exacerbation (127). In support of these clinical data, patients with RA and anxiety were found to have higher serum levels of IL-17 than those without AD (128).

Some authors have hypothesized that ADs in patients with RA are triggered by psychosocial factors, such as pronounced neuroticism, a lower educational level, and poor social support (69). According to this explanation, RA-associated disability in case of AD comorbidity is attributable more to psychological factors implicated in all chronic diseases than to the worsening of symptoms as a result of an increased pro-inflammatory state (129).

Proper AD treatment in patients with RA is essential to prevent functional decline (130) and poor adherence to treatment (131). Some drugs prescribed for RA, such as anti-TNF-α medications, seem to have beneficial effects on anxiety symptoms (112), and their discontinuation is related to the worsening of anxiety (132).

AUTONOMIC NERVOUS SYSTEM AND CARDIOVASCULAR MANIFESTATIONS IN RHEUMATOID ARTHRITIS

Cardiovascular autonomic dysfunction in RA has been investigated by measuring heart rate (HR) and heart rate variability (HRV) (24). The link between the cardiovascular system and ANS imbalance in RA could partly explain the well-documented augmented cardiovascular disease and RA-related mortality, not fully justified by traditional risk factors.

It is well known how chronic inflammation influences the development of cardiovascular disease (CVD) by promoting atherosclerosis, myocardial remodeling, and insulin resistance and by modifying lipid levels and function and oxidative stress (133). Furthermore, multimodality imaging is useful to identify high-risk patients who benefit from preventive strategies or treatment intervention (134); the link between brain and heart damage in RA has been documented by magnetic resonance imaging (135).

Fundamentally, we have limited understanding of the mechanisms underpinning the connections between ANS and CVD. An extensive assessment of ANS by means of HR, cardiac/sympathetic baroreflex, and muscle sympathetic nerve activity (MSNA) was carried out in 30 RA patients (normo- and hypertensive) matched with a control group. Regardless the presence of hypertension, HR and sympathetic activity were increased and cardiac baroreflex sensitivity was reduced in RA patients, while sympathetic baroreflex sensitivity was preserved. Moreover, these findings were correlated with pain (VAS) and inflammation (CPR). Hence, a heightened sympathetic outflow was confirmed along with a reduced arterial baroreflex control of the heart both linked directly to RA symptoms, excluding the bias hypertension, a common cardiovascular risk factor (136).

Although data suggest that ANS imbalance is detrimental for cardiovascular disease, including cardiac arrhythmias, hypertension (137), and increased mortality (138, 139), breaking through the original trigger of this vicious circle with ultimate damage of the cardiovascular system is particularly difficult. Moreover, patients with RA without clinical cardiovascular disease have reduced left ventricular systolic function assessed by global longitudinal strain by speckle-tracking echocardiography, and it is related with disease activity (140).

Another study showed how a reduced coronary microvascular perfusion in RA patients, in terms of subendocardial viability ratio, associates with markers of disease activity and classical CVD risk factors, including heart rate. This reinforces the hypothesis that inflammation, by the stimulation of ANS, may impair myocardial perfusion (141).

Finally, according to previous literature, the evidence of cardiovascular autonomic dysfunction might be used to identify patients with RA who respond better to TNF inhibitors (25, 142). In particular, a low parasympathetic outflow and a high sympathetic outflow were associated with a poor anti-TNF response, making HRV a promising useful predictor of outcome, with great benefits for overall costs and quality-adjusted life-years (QALYs) (143).

TABLE 1 | Targeting vagus nerve by electrical stimulation in rheumatoid arthritis.

Clinical trial	Study population	Device and regimen	Primary endpoint	Safety data	Length
Koopman 2016 (151)	17 active RA (7 naïve to bDMARDs and 10 bDMARDs failure) Open label	Cyberonics device around the cervical vagus nerve; implantation under general anesthesia. Day 0–28 daily stimulation with escalating intensity	DAS28-CRP at week 6 (baseline 6.05 ± 0.18 at day −21 vs. 4.16 ± 0.39 at day 42, $p < 0.001$)	No serious adverse events, mild/moderate events associated with implanting device	12 weeks
Addorisio 2019 (152)	9 active RA Randomized cross-over	Non-invasive vibrotactile auricular stimulation Stimulation twice daily for 2 days	DAS28-CRP at week 1 (baseline 4.19 ± 0.33 vs. 2.79 ± 0.21 at day 7, $p < 0.01$)	No adverse events	1 week
Marsal 2020* (153)	30 active RA Open label	Non-invasive vibrotactile auricular stimulation Data on regimen stimulation not available	DAS28-CRP at week 12–1.40 ($p < 0.01$)	1 device superficial skin abrasion resolved without intervention	12 weeks
Genovese 2020 (154)	14 active RA with insufficient response ≧ 2 b/tsDMARDs Stage 1 open label; stage 2 randomized, multicentre, sham-controlled trial	Miniaturized VNS device implanted on the left cervical vagus nerve	Safety and tolerability of the implantation surgical procedure, device and the active treatment	Horner's syndrome, procedural pain, contact dermatitis, vocal cord paralysis. All adverse events were related to the surgical procedure and resolved without permanent clinically significant sequelae	12 weeks

*RA, rheumatoid arthritis; DAS, disease activity score; CRP, C-reactive protein; bDMARD, biological disease-modifying anti-rheumatic drug; tsDMARDS, targeted synthetic DMARDs; VNS, vagus nerve stimulation. *Results only available from the congress abstract.*

Furthermore, cardiovascular autonomic imbalance associates with comorbidities including depressive and anxiety disturbances compared to healthy controls (144). Finally, looking at a therapeutic prospective, non-pharmacological intervention such as exercise training, given its well-known benefits on cardiovascular autonomic function, might help restore ANS imbalance, increasing vagal-related indices in RA with consequent potential benefit on pain and inflammation (145). Likewise, ANS imbalance could be partially restored by optimizing pain management (146).

IMPLICATIONS FOR TREATMENT AND FUTURE PERSPECTIVES

Altered ANS parameters have been associated with increased Disease Activity Score-28 (DAS-28), CRP, and erythrocyte sedimentation rate (25, 147). Seropositive RA patients are more prone to ANS dysfunction and more likely to experience the amelioration of cardiovascular autonomic neuropathy when treated with synthetic and biologic disease-modifying antirheumatic drugs (DMARDS) than seronegative patients with RA (148, 149). Thus, seropositivity, along with disease activity and pro-inflammatory cytokine levels, is predictive of autonomic dysfunction (150). Moreover, in patients with RA and ankylosing spondylitis, HRV was shown to predict the clinical response to anti-TNF therapy; specifically, patients with more vagal activity responded better to this treatment (142). Biologic, and to a lesser extent synthetic, DMARDs significantly improved autonomic neuropathy, including all of its parasympathetic, sympathetic, and sudomotor components, in patients with RA and ankylosing spondylitis (148).

Multiple strategies can be used to achieve these goals. VNS can dampen inflammation early in a discrete and localized manner. Administration of the specific agonist of splenic α7nAChR acts on downstream of the pathway. Antagonization of the SNS is a possible alternative that should be explored. As shown in **Table 1**, four clinical trials examining VNS have been published (151–154), and one of these has been presented at the last EULAR e-congress (153). As they involved the use of different devices, modalities, timing, and stimulation sites, comparison of their results is (152) not feasible, but the researchers observed significant and rapid declines in the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1b after VNS in patients with epilepsy, those with RA, and healthy subjects (151, 152). In the first trial, VNS was performed directly via an electronic device (Cyberonics) implanted under general anesthesia. Three helical coiled cuffs around the vagus nerve and a lead were then tunneled subcutaneously from the neck and connected to a pulse generator placed in a subcutaneous pocket on the chest wall (151). The only adverse events reported were mild/moderate and related to the surgical implanting approach. In the most recent trial, a MicroRegulator device was implanted on the left cervical vagus nerve. The device was well tolerated without adverse events. The sample size was small, but VNS reduced disease activity in 50% of the highly drug refractory RA patients (154). In the other two trials, a non-invasive device consisting of a hand-held probe with a tip producing radial displacement in a circular pattern at the probe was used. Transcutaneous auricular VNS consisted of the application of electrical signals to the cutaneous territory supplied by the auricular branch of the vagus nerve at the cymba concha (152, 153). In all trials, disease activity was rapidly attenuated after VNS in patients with active RA

(biologic-naïve subjects and those for whom multiple treatments had failed).

Considering the consistent percentage of patients who do not respond at all to the available drugs or have unsatisfactory responses, have gradual loss of responsiveness over time, or have drug-related adverse events, a non-pharmacological approach such as bioelectronics might be a useful further tool in the successful expansion of the therapeutic armamentarium of RA.

Bioelectronic medicine, based on neuromodulation of the nervous system restoring organ and immune system function, is a new potential tantalizing and promising field. In particular, the use of non-invasive devices has lesser adverse effects than drugs and has greater treatment adherence. Naturally, the therapeutic potential of immuno-autonomics has been further demonstrated, and the optimal neurostimulation parameters to achieve and maintain significant clinical changes are still unknown.

CONCLUSION

The innovative view of the ANS and RA axis described here allows for the development of new strategies for RA management. The ANS influences key aspects of joint pathophysiology and may be a target of novel therapeutic approaches. The emergence of immuno-autonomics and encouraging results of clinical trials could have extraordinary implications for RA monitoring and treatment. Further research in this field will be useful to understand the full potential of therapeutic models based on the brain–joint axis, with the integration of different findings.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Safiri S, Kolahi AA, Hoy D, Smith E, Bettampadi D, Mansournia MA, et al. Global, regional and national burden of rheumatoid arthritis 1990-2017: a systematic analysis of the Global Burden of Disease study 2017. *Ann Rheum Dis.* (2019) 78:1463–71. doi: 10.1136/annrheumdis-2019-215920
2. Cao X, Aballay A. Neural inhibition of dopaminergic signaling enhances immunity in a cell-non-autonomous manner. *Curr Biol.* (2016) 26:2398. doi: 10.1016/j.cub.2016.08.046
3. Aydemir M, Yazisiz V, Basarici I, Avci AB, Erbasan F, Belgi A, et al. Cardiac autonomic profile in rheumatoid arthritis and systemic lupus erythematosus. *Lupus.* (2010) 19:255–61. doi: 10.1177/0961203309351540
4. Stojanovich L, Milovanovich B, de Luka SR, Popovich-Kuzmanovich D, Bisenich V, Djukanovich B, et al. Cardiovascular autonomic dysfunction in systemic lupus, rheumatoid arthritis, primary Sjogren syndrome and other autoimmune diseases. *Lupus.* (2007) 16:181–5. doi: 10.1177/0961203306076223
5. Taylor PC, Holman AJ. Rheumatoid arthritis and the emergence of immuno-autonomics. *Rheumatology.* (2019) 58:2079–80. doi: 10.1093/rheumatology/key225
6. Gasparyan AY, Ayvazyan L, Blackmore H, Kitas GD. Writing a narrative biomedical review: considerations for authors, peer reviewers, and editors. *Rheumatol Int.* (2011) 31:1409–17. doi: 10.1007/s00296-011-1999-3
7. Rosas-Ballina M, Olofsson PS, Ochani M, Valdes-Ferrer SI, Levine YA, Reardon C, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science.* (2011) 334:98–101. doi: 10.1126/science.1209985
8. Kawashima K, Fujii T, Moriwaki Y, Misawa H. Critical roles of acetylcholine and the muscarinic and nicotinic acetylcholine receptors in the regulation of immune function. *Life Sci.* (2012) 91:1027–32. doi: 10.1016/j.lfs.2012.05.006
9. Wong CH, Jenne CN, Lee WY, Leger C, Kubes P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science.* (2011) 334:101–5. doi: 10.1126/science.1210301
10. Li M, Shi J, Tang JR, Chen D, Ai B, Chen J, et al. Effects of complete Freund's adjuvant on immunohistochemical distribution of IL-1beta and IL-1R I in neurons and glia cells of dorsal root ganglion. *Acta Pharmacol Sin.* (2005) 26:192–8. doi: 10.1111/j.1745-7254.2005.00522.x
11. Tracey KJ. The inflammatory reflex. *Nature.* (2002) 420:853–9. doi: 10.1038/nature01321
12. Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol Med.* (2003) 9:125–34. doi: 10.1007/BF03402177
13. Berthoud HR, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci.* (2000) 85:1–17. doi: 10.1016/S1566-0702(00)00215-0
14. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature.* (2000) 405:458–62. doi: 10.1038/35013070
15. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature.* (2003) 421:384–8. doi: 10.1038/nature01339
16. Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med.* (2004) 10:1216–21. doi: 10.1038/nm1124
17. Rosas-Ballina M, Goldstein RS, Gallowitsch-Puerta M, Yang L, Valdes-Ferrer SI, Patel NB, et al. The selective alpha7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. *Mol Med.* (2009) 15:195–202. doi: 10.2119/molmed.2009.00039
18. Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa L, Yang H, et al. Pharmacological stimulation of the cholinergic antiinflammatory pathway. *J Exp Med.* (2002) 195:781–8. doi: 10.1084/jem.20011714
19. van Westerloo DJ, Giebelen IA, Florquin S, Bruno MJ, Larosa GJ, Ulloa L, et al. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. *Gastroenterology.* (2006) 130:1822–30. doi: 10.1053/j.gastro.2006.02.022
20. Ghia JE, Blennerhassett P, Collins SM. Vagus nerve integrity and experimental colitis. *Am J Physiol Gastrointest Liver Physiol.* (2007) 293:G560–7. doi: 10.1152/ajpgi.00098.2007
21. Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, Verdu EF, Collins SM. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology.* (2006) 131:1122–30. doi: 10.1053/j.gastro.2006.08.016
22. Nakai A, Hayano Y, Furuta F, Noda M, Suzuki K. Control of lymphocyte egress from lymph nodes through beta2-adrenergic receptors. *J Exp Med.* (2014) 211:2583–98. doi: 10.1084/jem.20141132

23. Mina-Osorio P, Rosas-Ballina M, Valdes-Ferrer SI, Al-Abed Y, Tracey KJ, Diamond B. Neural signaling in the spleen controls B-cell responses to blood-borne antigen. *Mol Med.* (2012) 18:618–27. doi: 10.2119/molmed.2012.00027
24. Koopman FA, Tang MW, Vermeij J, de Hair MJ, Choi IY, Vervoordeldonk MJ, et al. Autonomic dysfunction precedes development of rheumatoid arthritis: a prospective cohort study. *EBioMedicine.* (2016) 6:231–7. doi: 10.1016/j.ebiom.2016.02.029
25. Adlan AM, Lip GY, Paton JF, Kitas GD, Fisher JP. Autonomic function and rheumatoid arthritis: a systematic review. *Semin Arthrit Rheum.* (2014) 44:283–304. doi: 10.1016/j.semarthrit.2014.06.003
26. Koopman FA, Stoof SP, Straub RH, Van Maanen MA, Vervoordeldonk MJ, Tak PP. Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. *Mol Med.* (2011) 17:937–48. doi: 10.2119/molmed.2011.00065
27. Koopman FA, van Maanen MA, Vervoordeldonk MJ, Tak PP. Balancing the autonomic nervous system to reduce inflammation in rheumatoid arthritis. *J Intern Med.* (2017) 282:64–75. doi: 10.1111/joim.12626
28. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Abedini S, Hansen JF. Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent heart disease. *Eur Heart J.* (2004) 25:363–70. doi: 10.1016/j.ehj.2003.12.003
29. Sloan RP, McCreath H, Tracey KJ, Sidney S, Liu K, Seeman T. RR interval variability is inversely related to inflammatory markers: the CARDIA study. *Mol Med.* (2007) 13:178–84. doi: 10.2119/2006-00112.Sloan
30. Thayer JF, Fischer JE. Heart rate variability, overnight urinary norepinephrine and C-reactive protein: evidence for the cholinergic anti-inflammatory pathway in healthy human adults. *J Intern Med.* (2009) 265:439–47. doi: 10.1111/j.1365-2796.2008.02023.x
31. Malave HA, Taylor AA, Nattama J, Deswal A, Mann DL. Circulating levels of tumor necrosis factor correlate with indexes of depressed heart rate variability: a study in patients with mild-to-moderate heart failure. *Chest.* (2003) 123:716–24. doi: 10.1378/chest.123.3.716
32. Vlcek M, Rovensky J, Blazicek P, Radikova Z, Penesova A, Kerlik J, et al. Sympathetic nervous system response to orthostatic stress in female patients with rheumatoid arthritis. *Ann N Y Acad Sci.* (2008) 1148:556–61. doi: 10.1196/annals.1410.026
33. Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, et al. Evidence for brain glial activation in chronic pain patients. *Brain.* (2015) 138(Pt 3):604–15. doi: 10.1093/brain/awu377
34. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry.* (2002) 7:468–73. doi: 10.1038/sj.mp.4000995
35. Capuron L, Miller AH. Cytokines and psychopathology: lessons from interferon-alpha. *Biol Psychiatry.* (2004) 56:819–24. doi: 10.1016/j.biopsych.2004.02.009
36. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev.* (2006) 86:1009–31. doi: 10.1152/physrev.00049.2005
37. Yang X, Yang HB, Xie QJ, Liu XH, Hu XD. Peripheral inflammation increased the synaptic expression of NMDA receptors in spinal dorsal horn. *Pain.* (2009) 144:162–9. doi: 10.1016/j.pain.2009.04.005
38. Hayley S, Poulter MO, Merali Z, Anisman H. The pathogenesis of clinical depression: stressor- and cytokine-induced alterations of neuroplasticity. *Neuroscience.* (2005) 135:659–78. doi: 10.1016/j.neuroscience.2005.03.051
39. Barrientos RM, Sprunger DB, Campeau S, Watkins LR, Rudy JW, Maier SF. BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration. *J Neuroimmunol.* (2004) 155:119–26. doi: 10.1016/j.jneuroim.2004.06.009
40. Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry.* (2006) 59:1116–27. doi: 10.1016/j.biopsych.2006.02.013
41. Marsland AL, Gianaros PJ, Abramowitch SM, Manuck SB, Hariri AR. Interleukin-6 covaries inversely with hippocampal grey matter volume in middle-aged adults. *Biol Psychiatry.* (2008) 64:484–90. doi: 10.1016/j.biopsych.2008.04.016
42. Zimmerman G, Shaltiel G, Barbash S, Cohen J, Gasho CJ, Shenhar-Tsarfaty S, et al. Post-traumatic anxiety associates with failure of the innate immune receptor TLR9 to evade the pro-inflammatory NFkappaB pathway. *Transl Psychiatry.* (2012) 2:e78. doi: 10.1038/tp.2012.4
43. Wartolowska K, Hough MG, Jenkinson M, Andersson J, Wordsworth BP, Tracey I. Structural changes of the brain in rheumatoid arthritis. *Arthritis Rheum.* (2012) 64:371–9. doi: 10.1002/art.33326
44. Chang L, Shoptaw S, Normand J. Brain abnormalities in HIV and stimulant users: interventions and prevention. *J Food Drug Anal.* (2013) 21:S7–9. doi: 10.1016/j.jfda.2013.09.021
45. Li Q, Cheung C, Wei R, Cheung V, Hui ES, You Y, et al. Voxel-based analysis of postnatal white matter microstructure in mice exposed to immune challenge in early or late pregnancy. *Neuroimage.* (2010) 52:1–8. doi: 10.1016/j.neuroimage.2010.04.015
46. Baliki MN, Geha PY, Apkarian AV, Chialvo DR. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci.* (2008) 28:1398–403. doi: 10.1523/JNEUROSCI.4123-07.2008
47. Napadow V, Kim J, Clauw DJ, Harris RE. Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. *Arthritis Rheum.* (2012) 64:2398–403. doi: 10.1002/art.34412
48. Bruchfeld A, Goldstein RS, Chavan S, Patel NB, Rosas-Ballina M, Kohn N, et al. Whole blood cytokine attenuation by cholinergic agonists *ex vivo* and relationship to vagus nerve activity in rheumatoid arthritis. *J Intern Med.* (2010) 268:94–101. doi: 10.1111/j.1365-2796.2010.02226.x
49. Grimsholm O, Rantapaa-Dahlqvist S, Dalen T, Forsgren S. Unexpected finding of a marked non-neuronal cholinergic system in human knee joint synovial tissue. *Neurosci Lett.* (2008) 442:128–33. doi: 10.1016/j.neulet.2008.06.082
50. Bennett FC, Molofsky AV. The immune system and psychiatric disease: a basic science perspective. *Clin Exp Immunol.* (2019) 197:294–307. doi: 10.1111/cei.13334
51. Jeppesen R, Benros ME. Autoimmune diseases and psychotic disorders. *Front Psychiatry.* (2019) 10:131. doi: 10.3389/fpsyt.2019.00131
52. Bauer ME, Teixeira AL. Inflammation in psychiatric disorders: what comes first? *Ann N Y Acad Sci.* (2019) 1437:57–67. doi: 10.1111/nyas.13712
53. el-Miedany YM, el-Rasheed AH. Is anxiety a more common disorder than depression in rheumatoid arthritis? *Joint Bone Spine.* (2002) 69:300–6. doi: 10.1016/S1297-319X(02)00368-8
54. VanDyke MM, Parker JC, Smarr KL, Hewett JE, Johnson GE, Slaughter JR, et al. Anxiety in rheumatoid arthritis. *Arthritis Rheum.* (2004) 51:408–12. doi: 10.1002/art.20474
55. Covic T, Cumming SR, Pallant JF, Manolios N, Emery P, Conaghan PG, et al. Depression and anxiety in patients with rheumatoid arthritis: prevalence rates based on a comparison of the Depression, Anxiety and Stress Scale (DASS) and the hospital, Anxiety and Depression Scale (HADS). *BMC Psychiatry.* (2012) 12:6. doi: 10.1186/1471-244X-12-6
56. Sato E, Nishimura K, Nakajima A, Okamoto H, Shinozaki M, Inoue E, et al. Major depressive disorder in patients with rheumatoid arthritis. *Mod Rheumatol.* (2013) 23:237–44. doi: 10.3109/s10165-012-0643-8
57. Uguz F, Kucuk A, Aydogan S, Arslan S, Kurt HG, Toker A, et al. Is major depression associated with serum levels of tumor necrosis factor-alpha in patients with rheumatoid arthritis? *J Psychosom Res.* (2015) 79:530–2. doi: 10.1016/j.jpsychores.2015.09.011
58. Katz P, Margaretten M, Trupin L, Schmajuk G, Yazdany J, Yelin E. Role of sleep disturbance, depression, obesity, and physical inactivity in fatigue in rheumatoid arthritis. *Arthritis Care Res.* (2016) 68:81–90. doi: 10.1002/acr.22577
59. Cheon YH, Lee SG, Kim M, Kim HO, Sun Suh Y, Park KS, et al. The association of disease activity, pro-inflammatory cytokines, and neurotrophic factors with depression in patients with rheumatoid arthritis. *Brain Behav Immun.* (2018) 73:274–81. doi: 10.1016/j.bbi.2018.05.012
60. Kwiatkowska B, Klak A, Raciborski F, Maslinska M. The prevalence of depression and insomnia symptoms among patients with rheumatoid arthritis and osteoarthritis in Poland: a case control study. *Psychol Health Med.* (2019) 24:333–43. doi: 10.1080/13548506.2018.1529325
61. Milic V, Grujic M, Barisic J, Marinkovic-Eric J, Duisin D, Cirkovic A, et al. Personality, depression and anxiety in primary Sjogren's syndrome

- Association with sociodemographic factors and comorbidity. *PLoS ONE.* (2019) 14:e0210466. doi: 10.1371/journal.pone.0210466
62. Nichter B, Norman S, Haller M, Pietrzak RH. Physical health burden of PTSD, depression, and their comorbidity in the U.S. veteran population: morbidity, functioning, and disability. *J Psychosom Res.* (2019) 124:109744. doi: 10.1016/j.jpsychores.2019.109744
63. Aurrecoechea E, Llorca Diaz J, Diez Lizuain ML, McGwin G Jr, Calvo-Alen J. Gender-associated comorbidities in rheumatoid arthritis and their impact on outcome: data from GENIRA. *Rheumatol Int.* (2017) 37:479–85. doi: 10.1007/s00296-016-3628-7
64. Hsieh MC, Hsu CW, Lu MC, Koo M. Increased risks of psychiatric disorders in patients with primary Sjogren's syndrome-a secondary cohort analysis of nationwide, population-based health claim data. *Clin Rheumatol.* (2019) 38:3195–203. doi: 10.1007/s10067-019-04705-z
65. Lok EY, Mok CC, Cheng CW, Cheung EF. Prevalence and determinants of psychiatric disorders in patients with rheumatoid arthritis. *Psychosomatics.* (2010) 51:338-e8. doi: 10.1176/appi.psy.51.4.338
66. Khongsaengdao B, Louthrenoo W, Srisurapanont M. Depression in Thai patients with rheumatoid arthritis. *J Med Assoc Thai.* (2000) 83:743–7.
67. Katon WJ, Buchwald DS, Simon GE, Russo JE, Mease PJ. Psychiatric illness in patients with chronic fatigue and those with rheumatoid arthritis. *J Gen Int Med.* (1991) 6:277–85. doi: 10.1007/BF02597420
68. Timonen M, Viilo K, Hakko H, Sarkioja T, Ylikulju M, Meyer-Rochow VB, et al. Suicides in persons suffering from rheumatoid arthritis. *Rheumatology.* (2003) 42:287–91. doi: 10.1093/rheumatology/keg082
69. Evers AW, Kraaimaat FW, Geenen R, Jacobs JW, Bijlsma JW. Longterm predictors of anxiety and depressed mood in early rheumatoid arthritis: a 3 and 5 year followup. *J Rheumatol.* (2002) 29:2327–36.
70. Covic T, Adamson B, Spencer D, Howe G. A biopsychosocial model of pain and depression in rheumatoid arthritis: a 12-month longitudinal study. *Rheumatology.* (2003) 42:1287–94. doi: 10.1093/rheumatology/keg369
71. Wang SL, Chang CH, Hu LY, Tsai SJ, Yang AC, You ZH. Risk of developing depressive disorders following rheumatoid arthritis: a nationwide population-based study. *PLoS ONE.* (2014) 9:e107791. doi: 10.1371/journal.pone.0107791
72. Ryu E, Chamberlain AM, Pendegraft RS, Petterson TM, Bobo WV, Pathak J. Quantifying the impact of chronic conditions on a diagnosis of major depressive disorder in adults: a cohort study using linked electronic medical records. *BMC Psychiatry.* (2016) 16:114. doi: 10.1186/s12888-016-0821-x
73. Kojima M, Kojima T, Suzuki S, Oguchi T, Oba M, Tsuchiya H, et al. Depression, inflammation, and pain in patients with rheumatoid arthritis. *Arthritis Rheum.* (2009) 61:1018–24. doi: 10.1002/art.24647
74. Kuriya B, Joshi R, Movahedi M, Rampakakis E, Sampalis JS, Bombardier C, et al. High disease activity is associated with self-reported depression and predicts persistent depression in early rheumatoid arthritis: results from the ontario best practices research initiative. *J Rheumatol.* (2018) 45:1101–8. doi: 10.3899/jrheum.171195
75. Hamed SA, Selim ZI, Elattar AM, Elserogy YM, Ahmed EA, Mohamed HO. Assessment of biocorrelates for brain involvement in female patients with rheumatoid arthritis. *Clin Rheumatol.* (2012) 31:123–32. doi: 10.1007/s10067-011-1795-1
76. Tylee DS, Sun J, Hess JL, Tahir MA, Sharma E, Malik R, et al. Genetic correlations among psychiatric and immune-related phenotypes based on genome-wide association data. *Am J Med Genet Part B Neuropsychiatr Genet.* (2018) 177:641–57. doi: 10.1002/ajmg.b.32652
77. Doeglas DM, Suurmeijer TP, van den Heuvel WJ, Krol B, van Rijswijk MH, van Leeuwen MA, et al. Functional ability, social support, and depression in rheumatoid arthritis. *Qual Life Res.* (2004) 13:1053–65. doi: 10.1023/B:QURE.0000031339.04589.63
78. Zyrianova Y, Kelly BD, Gallagher C, McCarthy C, Molloy MG, Sheehan J, et al. Depression and anxiety in rheumatoid arthritis: the role of perceived social support. *Ir J Med Sci.* (2006) 175:32–6. doi: 10.1007/BF03167946
79. Zagar I, Delimar V, Pap M, Peric D, Laktasic Zerjavic N, Peric P. Prevalence and correlation of depressive symptoms with functional scores, therapy and disease activity among croatian patients with rheumatoid arthritis: a preliminary study. *Psychiatr Danub.* (2018) 30:452–8. doi: 10.24869/psyd.2018.452
80. Cakirbay H, Bilici M, Kavakci O, Cebi A, Guler M, Tan U. Sleep quality and immune functions in rheumatoid arthritis patients with and without major depression. *Int J Neurosci.* (2004) 114:245–56. doi: 10.1080/00207450490269471
81. Melikoglu MA, Melikoglu M. The relationship between disease activity and depression in patients with Behcet disease and rheumatoid arthritis. *Rheumatol Int.* (2010) 30:941–6. doi: 10.1007/s00296-009-1080-7
82. Ryan S, McGuire B. Psychological predictors of pain severity, pain interference, depression, and anxiety in rheumatoid arthritis patients with chronic pain. *Br J Health Psychol.* (2016) 21:336–50. doi: 10.1111/bjhp.12171
83. Santos EF, Duarte CM, Ferreira RO, Pinto AM, Geenen R, da Silva JP. Multifactorial explanatory model of depression in patients with rheumatoid arthritis: a structural equation approach. *Clin Exp Rheumatol.* (2019) 37:641–8.
84. Dickens C, Jackson J, Tomenson B, Hay E, Creed F. Association of depression and rheumatoid arthritis. *Psychosomatics.* (2003) 44:209–15. doi: 10.1176/appi.psy.44.3.209
85. Mok CC, Lok EY, Cheung EF. Concurrent psychiatric disorders are associated with significantly poorer quality of life in patients with rheumatoid arthritis. *Scand J Rheumatol.* (2012) 41:253–9. doi: 10.3109/03009742.2012.664648
86. Soosova MS, Macejova Z, Zamboriova M, Dimunova L. Anxiety and depression in Slovak patients with rheumatoid arthritis. *J Ment Health.* (2017) 26:21–7. doi: 10.1080/09638237.2016.1244719
87. Garip Y, Eser F, Bodur H. Comorbidities in Turkish patients with rheumatoid arthritis: association with the health-related quality of life in terms of disease activity, functional and radiological status, severity of pain, and social and emotional functioning. *Acta Reumatol Port.* (2016) 41:344–9.
88. Zautra AJ, Parrish BP, Van Puymbroeck CM, Tennen H, Davis MC, Reich JW, et al. Depression history, stress, and pain in rheumatoid arthritis patients. *J Behav Med.* (2007) 30:187–97. doi: 10.1007/s10865-007-9097-4
89. Rogers HL, Brotherton HT, de Luis A, Olivera-Plaza SL, Cordoba-Patino AF, Pena-Altamar ML. Depressive symptoms are independently associated with pain perception in Colombians with rheumatoid arthritis. *Acta Reumatol Port.* (2015) 40:40–9.
90. Piccinni A, Maser JD, Bazzichi L, Rucci P, Vivarelli L, Del Debbio A, et al. Clinical significance of lifetime mood and panic-agoraphobic spectrum symptoms on quality of life of patients with rheumatoid arthritis. *Compr Psychiatry.* (2006) 47:201–8. doi: 10.1016/j.comppsych.2005.08.002
91. Jobski K, Kollhorst B, Garbe E, Schink T. The risk of ischemic cardio- and cerebrovascular events associated with oxycodone-naloxone and other extended-release high-potency opioids: a nested case-control study. *Drug Saf.* (2017) 40:505–15. doi: 10.1007/s40264-017-0511-8
92. Lee YC, Kremer J, Guan H, Greenberg J, Solomon DH. Chronic opioid use in rheumatoid arthritis: prevalence and predictors. *Arthritis Rheumatol.* (2019) 71:670–7. doi: 10.1002/art.40789
93. Dunne FJ, Dunne CA. Fibromyalgia syndrome and depression: common pathways. *Br J Hosp Med.* (2012) 73:211–7. doi: 10.12968/hmed.2012.73.4.211
94. Ingegnoli F, Schioppo T, Ubiali T, Ostuzzi S, Bollati V, Buoli M, et al. Patient perception of depressive symptoms in rheumatic diseases: a cross-sectional survey. *J Clin Rheumatol.* (2020). doi: 10.1097/RHU.0000000000001564. [Epub ahead of print].
95. Irwin MR, Olmstead R, Carrillo C, Sadeghi N, Fitzgerald JD, Ranganath VK, et al. Sleep loss exacerbates fatigue, depression, and pain in rheumatoid arthritis. *Sleep.* (2012) 35:537–43. doi: 10.5665/sleep.1742
96. Cabrera-Marroquin R, Contreras-Yanez I, Alcocer-Castillejos N, Pascual-Ramos V. Major depressive episodes are associated with poor concordance with therapy in rheumatoid arthritis patients: the impact on disease outcomes. *Clin Exp Rheumatol.* (2014) 32:904–13.
97. Euesden J, Matcham F, Hotopf M, Steer S, Cope AP, Lewis CM, et al. The relationship between mental health, disease severity, and genetic risk for depression in early rheumatoid arthritis. *Psychosom Med.* (2017) 79:638–45. doi: 10.1097/PSY.0000000000000462
98. Liu YL, Szklo M, Davidson KW, Bathon JM, Giles JT. Differential association of psychosocial comorbidities with subclinical atherosclerosis in rheumatoid arthritis. *Arthritis Care Res.* (2015) 67:1335–44. doi: 10.1002/acr.22635

99. Scherrer JF, Virgo KS, Zeringue A, Bucholz KK, Jacob T, Johnson RG, et al. Depression increases risk of incident myocardial infarction among Veterans Administration patients with rheumatoid arthritis. *Gen Hosp Psychiatry.* (2009) 31:353–9. doi: 10.1016/j.genhosppsych.2009.04.001
100. Marrie RA, Walld R, Bolton JM, Sareen J, Patten SB, Singer A, et al. Psychiatric comorbidity increases mortality in immune-mediated inflammatory diseases. *Gen Hosp Psychiatry.* (2018) 53:65–72. doi: 10.1016/j.genhosppsych.2018.06.001
101. Nikiphorou E, de Lusignan S, Mallen C, Roberts J, Khavandi K, Bedarida G, et al. Prognostic value of comorbidity indices and lung diseases in early rheumatoid arthritis: a UK population-based study. *Rheumatology.* (2020) 59:1296–305. doi: 10.1093/rheumatology/kez409
102. Brandstetter S, Riedelbeck G, Steinmann M, Loss J, Ehrenstein B, Apfelbacher C. Depression moderates the associations between beliefs about medicines and medication adherence in patients with rheumatoid arthritis: cross-sectional study. *J Health Psychol.* (2018) 23:1185–95. doi: 10.1177/1359105316646440
103. Savka S. Improved quality of life of patients with rheumatoid arthritis and nonpsychotic mental disorders. *Wiad Lek.* (2019) 72:47–51. doi: 10.36740/WLek201901109
104. Smarr KL, Parker JC, Kosciulek JF, Buchholz JL, Multon KD, Hewett JE, et al. Implications of depression in rheumatoid arthritis: do subtypes really matter? *Arthritis Care Res.* (2000) 13:23–32. doi: 10.1002/1529-0131(200002)13:1<23::AID-ART5>3.0.CO;2-W
105. Hider SL, Tanveer W, Brownfield A, Mattey DL, Packham JC. Depression in RA patients treated with anti-TNF is common and under-recognized in the rheumatology clinic. *Rheumatology.* (2009) 48:1152–4. doi: 10.1093/rheumatology/kep170
106. Hewlett S, Ambler N, Almeida C, Cliss A, Hammond A, Kitchen K, et al. Self-management of fatigue in rheumatoid arthritis: a randomised controlled trial of group cognitive-behavioural therapy. *Ann. Rheum Dis.* (2011) 70:1060–7. doi: 10.1136/ard.2010.144691
107. Parker JC, Smarr KL, Slaughter JR, Johnston SK, Priesmeyer ML, Hanson KD, et al. Management of depression in rheumatoid arthritis: a combined pharmacologic and cognitive-behavioral approach. *Arthritis Rheum.* (2003) 49:766–77. doi: 10.1002/art.11459
108. Dhavale HS, Gawande S, Bhagat V, Durge V, Londhe V, Kini S, et al. Evaluation of efficacy and tolerability of dothiepin hydrochloride in the management of major depression in patients suffering from rheumatoid arthritis. *J Indian Med Assoc.* (2005) 103:291–4.
109. Slaughter JR, Parker JC, Martens MP, Smarr KL, Hewett JE. Clinical outcomes following a trial of sertraline in rheumatoid arthritis. *Psychosomatics.* (2002) 43:36–41. doi: 10.1176/appi.psy.43.1.36
110. Zautra AJ, Davis MC, Reich JW, Nicassario P, Tennen H, Finan P, et al. Comparison of cognitive behavioral and mindfulness meditation interventions on adaptation to rheumatoid arthritis for patients with and without history of recurrent depression. *J Consult Clin Psychol.* (2008) 76:408–21. doi: 10.1037/0022-006X.76.3.408
111. Gautam S, Tolahunase M, Kumar U, Dada R. Impact of yoga based mind-body intervention on systemic inflammatory markers and co-morbid depression in active Rheumatoid arthritis patients: a randomized controlled trial. *Restor Neurol Neurosci.* (2019) 37:41–59. doi: 10.3233/RNN-180875
112. Uguz F, Akman C, Kucuksarac S, Tufekci O. Anti-tumor necrosis factor-alpha therapy is associated with less frequent mood and anxiety disorders in patients with rheumatoid arthritis. *Psychiatry Clin Neurosci.* (2009) 63:50–5. doi: 10.1111/j.1440-1819.2008.01905.x
113. Sun Y, Wang D, Salvadore G, Hsu B, Curran M, Casper C, et al. The effects of interleukin-6 neutralizing antibodies on symptoms of depressed mood and anhedonia in patients with rheumatoid arthritis and multicentric Castleman's disease. *Brain Behav Immun.* (2017) 66:156–64. doi: 10.1016/j.bbi.2017.06.014
114. Genty M, Combe B, Kostine M, Ardouin E, Morel J, Lukas C. Improvement of fatigue in patients with rheumatoid arthritis treated with biologics: relationship with sleep disorders, depression and clinical efficacy. A prospective, multicentre study. *Clin Exp Rheumatol.* (2017) 35:85–92.
115. Bottomley JM, LeReun C, Diamantopoulos A, Mitchell S, Gaynes BN. Vagus nerve stimulation (VNS) therapy in patients with treatment resistant depression: a systematic review and meta-analysis. *Compr Psychiatry.* (2019) 98:152156. doi: 10.1016/j.comppsych.2019.152156
116. Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. *J Inflamm Res.* (2018) 11:203–13. doi: 10.2147/JIR.S163248
117. Isik A, Koca SS, Ozturk A, Mermi O. Anxiety and depression in patients with rheumatoid arthritis. *Clin Rheumatol.* (2007) 26:872–8. doi: 10.1007/s10067-006-0407-y
118. Lisitsyna TA, Veltishchev DY, Seravina OF, Kovalevskaya OB, Starovoytova MN, Desinova OV, et al. Comparative analysis of anxiety-depressive spectrum disorders in patients with rheumatic diseases. *Ter Arkh.* (2018) 90:30–7. doi: 10.26442/terarkh201890530-37
119. Hassan AA, Nasr MH, Mohamed AL, Kamal AM, Elmoghazy AD. Psychological affection in rheumatoid arthritis patients in relation to disease activity. *Medicine.* (2019) 98:e15373. doi: 10.1097/MD.0000000000015373
120. Marrie RA, Walld R, Bolton JM, Sareen J, Walker JR, Patten SB, et al. Rising incidence of psychiatric disorders before diagnosis of immune-mediated inflammatory disease. *Epidemiol Psychiatr Sci.* (2019) 28:333–42. doi: 10.1017/S2045796017000579
121. Watad A, Bragazzi NL, Adawi M, Aljadeff G, Amital H, Comaneshter D, et al. Anxiety disorder among rheumatoid arthritis patients: insights from real-life data. *J Affect Disord.* (2017) 213:30–4. doi: 10.1016/j.jad.2017.02.007
122. Reinhorn IM, Bernstein CN, Graff LA, Patten SB, Sareen J, Fisk JD, et al. Social phobia in immune-mediated inflammatory diseases. *J Psychosom Res.* (2020) 128:109890. doi: 10.1016/j.jpsychores.2019.109890
123. Stebbings S, Herbison P, Doyle TC, Treharne GJ, Highton J. A comparison of fatigue correlates in rheumatoid arthritis and osteoarthritis: disparity in associations with disability, anxiety and sleep disturbance. *Rheumatology.* (2010) 49:361–7. doi: 10.1093/rheumatology/kep367
124. Mangelli L, Gribbin N, Buchi S, Allard S, Sensky T. Psychological well-being in rheumatoid arthritis: relationship to 'disease' variables and affective disturbance. *Psychother Psychosom.* (2002) 71:112–6. doi: 10.1159/000049354
125. Karimi S, Yarmohammadian MH, Shokri A, Mottaghi P, Qolipour K, Kordi A, et al. Predictors and effective factors on quality of life among Iranian patients with rheumatoid arthritis. *Mater Sociomed.* (2013) 25:158–62. doi: 10.5455/msm.2013.25.158-162
126. Esen SA, Karabulut Y, Esen I, Atmis V. Effects of the Disease Characteristics and the treatment on psychological status in patients with rheumatoid arthritis and ankylosing spondylitis. *Curr Rheumatol Rev.* (2018) 14:271–8. doi: 10.2174/1573397113666170728123518
127. Yilmaz V, Umay E, Gundogdu I, Karaahmet ZO, Ozturk AE. Rheumatoid arthritis: are psychological factors effective in disease flare? *Eur J Rheumatol.* (2017) 4:127–32. doi: 10.5152/eurjrheum.2017.16100
128. Liu Y, Ho RC, Mak A. The role of interleukin (IL)-17 in anxiety and depression of patients with rheumatoid arthritis. *Int J Rheum Dis.* (2012) 15:183–7. doi: 10.1111/j.1756-185X.2011.01673.x
129. Murphy H, Dickens C, Creed F, Bernstein R. Depression, illness perception and coping in rheumatoid arthritis. *J Psychosom Res.* (1999) 46:155–64. doi: 10.1016/S0022-3999(98)00073-7
130. Whitehouse CE, Fisk JD, Bernstein CN, Berrigan LI, Bolton JM, Graff LA, et al. Comorbid anxiety, depression, and cognition in MS and other immune-mediated disorders. *Neurology.* (2019) 93:1081–2. doi: 10.1212/WNL.0000000000006854
131. Adina TS, Mihaela-Simona S, Lucia CP, Stefan Cristian D, Maria B, Lili BA, et al. The influence of socio-demographic factors, lifestyle and psychiatric indicators on adherence to treatment of patients with rheumatoid arthritis: a cross-sectional study. *Medicina.* (2020) 56:178–192. doi: 10.3390/medicina56040178
132. Mattey DL, Dawes PT, Hassell AB, Brownfield A, Packham JC. Effect of psychological distress on continuation of anti-tumor necrosis factor therapy in patients with rheumatoid arthritis. *J Rheumatol.* (2010) 37:2021–4. doi: 10.3899/jrheum.100050
133. Urman A, Taklalsingh N, Sorrento C, McFarlane IM. Inflammation beyond the joints: rheumatoid arthritis and cardiovascular disease. *Scifed J Cardiol.* (2018) 2:1000019.
134. Mavrogeni S, Dimitroulas T, Sfikakis PP, Kitas GD. Heart involvement in rheumatoid arthritis: multimodality imaging and the emerging role of cardiac magnetic resonance. *Semin Arthritis Rheum.* (2013) 43:314–24. doi: 10.1016/j.semarthrit.2013.05.001

135. Markousis-Mavrogenis G, Koutsogeorgopoulou L, Dimitroulas T, Katsifis G, Vartela V, Mitsikostas D, et al. Is there a brain/heart interaction in rheumatoid arthritis and seronegative spondyloartropathies? A combined brain/heart magnetic resonance imaging reveals the answer. *Curr Rheumatol Rep.* (2020) 22:39. doi: 10.1007/s11926-020-00922-7
136. Adlan AM, Paton JF, Lip GY, Kitas GD, Fisher JP. Increased sympathetic nerve activity and reduced cardiac baroreflex sensitivity in rheumatoid arthritis. *J Physiol.* (2017) 595:967–81. doi: 10.1113/JP272944
137. Fisher JP, Paton JF. The sympathetic nervous system and blood pressure in humans: implications for hypertension. *J Hum Hypertens.* (2012) 26:463–75. doi: 10.1038/jhh.2011.66
138. Barretto AC, Santos AC, Munhoz R, Rondon MU, Franco FG, Trombetta IC, et al. Increased muscle sympathetic nerve activity predicts mortality in heart failure patients. *Int J Cardiol.* (2009) 135:302–7. doi: 10.1016/j.ijcard.2008.03.056
139. Panoulas VF, Toms TE, Douglas KM, Sandoo A, Metsios GS, Stavropoulos-Kalinoglou A, et al. Prolonged QTc interval predicts all-cause mortality in patients with rheumatoid arthritis: an association driven by high inflammatory burden. *Rheumatology.* (2014) 53:131–7. doi: 10.1093/rheumatology/ket338
140. Hanvivadhanakul P, Buakhamsri A. Disease activity is associated with LV dysfunction in rheumatoid arthritis patients without clinical cardiovascular disease. *Adv Rheumatol.* (2019) 59:56. doi: 10.1186/s42358-019-0100-x
141. Sandoo A, Protogerou AD, Hodson J, Smith JP, Zampeli E, Sfikakis PP, et al. The role of inflammation, the autonomic nervous system and classical cardiovascular disease risk factors on subendocardial viability ratio in patients with RA: a cross-sectional and longitudinal study. *Arthritis Res Ther.* (2012) 14:R258. doi: 10.1186/ar4103
142. Holman AJ, Ng E. Heart rate variability predicts anti-tumor necrosis factor therapy response for inflammatory arthritis. *Auton Neurosci.* (2008) 143:58–67. doi: 10.1016/j.autneu.2008.05.005
143. Zimmermann M, Vodicka E, Holman AJ, Garrison LP, Jr. Heart rate variability testing: could it change spending for rheumatoid arthritis patients in the United States? An exploratory economic analysis. *J Med Econ.* (2018) 21:712–20. doi: 10.1080/13696998.2018.1470519
144. Hu MX, Milaneschi Y, Lamers F, Nolte IM, Snieder H, Dolan CV, et al. The association of depression and anxiety with cardiac autonomic activity: the role of confounding effects of antidepressants. *Depress Anxiety.* (2019) 36:1163–72. doi: 10.1002/da.22966
145. Pecanha T, Lima AH. Inflammation and cardiovascular autonomic dysfunction in rheumatoid arthritis: a bidirectional pathway leading to cardiovascular disease. *J Physiol.* (2017) 595:1025–6. doi: 10.1113/JP273649
146. Fazalbhoy A, Birznieks I, Macefield VG. Individual differences in the cardiovascular responses to tonic muscle pain: parallel increases or decreases in muscle sympathetic nerve activity, blood pressure and heart rate. *Exp Physiol.* (2012) 97:1084–92. doi: 10.1113/expphysiol.2012.066191
147. Lazzerini PE, Acampa M, Capecchi PL, Hammoud M, Maffei S, Bisogno S, et al. Association between high sensitivity C-reactive protein, heart rate variability and corrected QT interval in patients with chronic inflammatory arthritis. *Eur J Intern Med.* (2013) 24:368–74. doi: 10.1016/j.ejim.2013.02.009
148. Syngle A, Verma I, Krishan P, Garg N, Syngle V. Disease-modifying anti-rheumatic drugs improve autonomic neuropathy in arthritis: DIANA study. *Clin Rheumatol.* (2015) 34:1233–41. doi: 10.1007/s10067-014-2716-x
149. Sandhu V, Allen SC. The effects of age, seropositivity and disease duration on autonomic cardiovascular reflexes in patients with rheumatoid arthritis. *Int J Clin Pract.* (2004) 58:740–5. doi: 10.1111/j.1368-5031.2004.00210.x
150. Syngle V, Syngle A, Garg N, Krishan P, Verma I. Predictors of autonomic neuropathy in rheumatoid arthritis. *Auton Neurosci.* (2016) 201:54–9. doi: 10.1016/j.autneu.2016.07.008
151. Koopman FA, Chavan SS, Miljko S, Grazio S, Sokolovic S, Schuurman PR, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc Natl Acad Sci USA.* (2016) 113:8284–9. doi: 10.1073/pnas.1605635113
152. Addorisio ME, Imperato GH, de Vos AF, Forti S, Goldstein RS, Pavlov VA, et al. Investigational treatment of rheumatoid arthritis with a vibrotactile device applied to the external ear. *Bioelectron Med.* (2019) 5:4. doi: 10.1186/s42234-019-0020-4
153. Marsal S, Corominas H, Lopez Lasanta M, Reina-Sanz D, Perez-Garcia C, Borrell Paños H, et al. Pilot clinical study of a non-invasive auricular vagus nerve stimulation device in patients with rheumatoid arthritis. *Ann Rheum Dis.* (2020) 79:999. doi: 10.1136/annrheumdis-2020-eular.3315
154. Genovese MC, Gaylis NB, Sikes D, Kivitz A, Horowitz DL, Peterfy C, et al. Safety and efficacy of neurostimulation with a miniaturised vagus nerve stimulation device in patients with multidrug-refractory rheumatoid arthritis: a two-stage multicentre, randomised pilot study. *Lancet Rheumatol.* (2020) 2:e527–38. doi: 10.1016/S2665-9913(20)30172-7

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Fast-Track Ultrasound Clinic for the Diagnosis of Giant Cell Arteritis Changes the Prognosis of the Disease but Not the Risk of Future Relapse

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Background: Color Duplex sonography (CDS) of temporal arteries and large vessels (LV) is a recently validated diagnostic methodology for Giant Cell Arteritis (GCA). CDS combined with a fast-track approach (FTA) has improved the early diagnosis of the disease.

Objectives: To assess FTA effects on the prevention of permanent visual loss (PVL), relapse and late complications of GCA compared to conventional practice. To assess the impact of COVID-19 pandemic on outcomes of GCA patients assessed with FTA.

Methods: GCA patients diagnosed up to June 2020 at the Rheumatology Department, University of Pavia, were included. FTA was implemented since October 2016. FTA consists in the referral within 1 working day of a suspected GCA case to an expert rheumatologist who performs clinical evaluation and CDS.

Results: One hundred sixty patients were recruited [female 120 (75%), mean age 72.4 ± 8.2 years]. Sixty-three (39.4%) evaluated with FTA, 97 (60.6%) with conventional approach. FTA patients were older (75.1 ± 7.6 vs. 70.6 ± 8.2 years old; $p < 0.001$). Median follow-up duration was shorter in the FTA group compared to the conventional one (0.9 vs. 5.0 years; $p < 0.001$). There was no difference between the two cohorts regarding major vessel district involvement (LV-GCA 17.5% vs. 22.7%; $p = 0.4$). PVL occurred in 8 (12.7%) FTA patients and 26 (26.8%) conventional ones ($p = 0.03$). The relative risk of blindness in the conventional group was 2.11 (95% C.I. 1.02–4.36; $P = 0.04$) as compared to FTA. Median symptom latency of patients experiencing PVL was higher in the conventional group (23 days IQR 12–96 vs. 7 days IQR 4–10, $p = 0.02$). During COVID-19 there was a significant increase in the occurrence of PVL (40%) including bilateral blindness despite a regularly operating FTA clinic. Cumulative incidence of relapses and time to first relapse did not change after FTA introduction ($P = 0.2$). No difference in late complications (stenosis/aneurysms) was detected.

Conclusions: FTA including CDS evaluation contributed to a substantial reduction of PVL in GCA by shortening the time to diagnosis and treatment initiation. Relapse rate did not change upon FTA introduction, highlighting the need for better disease activity monitoring and treatment strategies optimization based on risk stratification that would predict the occurrence of relapse during glucocorticoid de-escalation.

Keywords: giant cell arteritis, sonography, ultrasoud, fast-track, permanent visual loss, large vessel vasculitis

INTRODUCTION

Giant Cell Arteritis (GCA) is the most prevalent primary systemic vasculitis (1). GCA typically affects the aorta and its major branches, even though medium and small-sized arteries may be targeted as well (2). Indeed, permanent visual loss (PVL), one of the most feared complications of GCA, results from damage to ophthalmic, retinal or ciliary arteries (3). Other clinical manifestations include headache, jaw or tongue claudication, scalp tenderness and polymyalgia rheumatica (PMR). Sometimes the picture is protean with increased inflammatory markers, fever and weight loss and it may get underdiagnosed. The heavy morbidity burden of GCA is due to its rather late onset (usually occurring over the age of 50 years old) and the occurrence of PVL in approximately 20% patients, usually at the very beginning of the disease. Treatment with high-dose glucocorticoids (GC) should be promptly initiated when the disease is suspected to prevent PVL. The clinical spectrum of GCA also comprises large vessel complications such as thoracic and abdominal aortic aneurysm or dissection, which can increase the risk of mortality (2, 4).

Strategies for early GCA diagnosis have been implemented in the last decade in order to reduce the occurrence of blindness. Color-duplex sonography (CDS) of temporal arteries (TAs) and large extra-cranial vessels (LVs) has been recently recognized as a first-line diagnostic tool for patients with suspected GCA in centers with the adequate expertise in the technique. A fast track approach (FTA), incorporating CDS has been associated with a significant reduction of PVL in two retrospective studies (5, 6). However, some patients still manifest with PVL as the very first symptom of this disease.

The role of imaging, including CDS, as a prognostic tool and in the follow-up of patients with GCA including the detection of relapses is still controversial (7).

OBJECTIVES

The aim of this study is to assess the role of FTA on PVL prevention and long-term relapse risk and outcome of patients with GCA, comparing the FTA outcomes with conventional practice while taking into-account the influence of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic on the FTA activity.

METHODS

The present study included patients who were diagnosed with GCA between January 1st, 2005 and June 30th, 2020 at the Rheumatology Department of IRCCS Fondazione Policlinico San Matteo hospital in Pavia, Italy.

The study has been approved by the Institutional Review Board of the University of Pavia and patients consented to their data use.

FTA was implemented since October 1st, 2016. FTA consists in the referral within one working day of a suspected GCA case to an expert rheumatologist, who performs the clinical examination and CDS of temporal and axillary arteries. Other anatomical sites (e.g., facial arteries, occipital arteries, carotid arteries) could be assessed according to the patient's clinical picture. Patients can be referred to the FTA from the general practitioner or other Departments. Patients enrolled after October 2016 were considered FTA if they received a rheumatologist's evaluation within one working day after the first medical contact. Patients assessed as part of the FTA between March 1st and June 30th 2020 during the coronavirus 2019 (COVID-19) pandemic were analyzed separately. Patients assessed prior to October 2016 were included in the conventional practice group (no-FTA).

The diagnosis of GCA was confirmed by the expert rheumatologist on the basis of typical symptoms, data on inflammatory markers, and imaging (CDS, ^{18}FDG PET-CT) or temporal artery biopsy (TAB) findings. Since the FTA implementation, the same rheumatologist (S.M.) performed the clinical and ultrasonographic assessment.

Clinical manifestations at disease onset, laboratory tests, date of symptoms onset, date of diagnosis, comorbidities, information on treatment, and follow-up, including relapses were extracted from the clinical records and recorded on an electronic database. Data were extracted retrospectively for the conventional cohort. FTA patients were assessed prospectively since diagnosis. Treatment followed current recommendations for both cohorts including high-dose GC (1 mg/kg/day; maximum 60 mg/day) maintained for 1 month and then tapered while monitoring disease activity. In patients with recent onset of ischaemic visual symptoms i.v. methylprednisolone was prescribed for the first 3 days (1 g/day). Adjunctive immunosuppressive treatment (most frequently Methotrexate or Tocilizumab) were prescribed to relapsing patients (8, 9). PVL was defined by the complete or partial visual impairment caused by vasculitic damage of the visual pathway [e.g., anterior ischaemic optic neuropathy (AION); posterior ischaemic optic neuropathy

(PION)] confirmed after ophthalmologic assessment. Symptom latency was calculated as the time interval between the onset of the first symptom attributable to GCA (e.g., date of headache or other cranial symptoms onset, girdle inflammatory pain onset, systemic symptoms onset) and the diagnosis. Large-vessel GCA (LV-GCA) was defined as the presence of extra-cranial LV involvement (e.g., aortic, axillary arteries) detected by imaging (most frequently CDS and/or ^{18}FDG PET-CT) with or without temporal artery involvement. Relapse was defined as the reoccurrence of symptoms and signs of GCA, with or without elevation of the inflammatory markers, with the need to increase the dosage of GC or to add or increase the dose of another immunosuppressive drug. Disease complications during follow-up are defined by the occurrence of stenosis or aneurysms/dissections and/or ischaemic complications.

Ultrasonographic Assessment

CDS was performed by the same rheumatologist experienced in vascular ultrasound for the assessment of large vessel vasculitis (S.M.). Patients were assessed with the MyLab Seven Esaote ultrasound machine with a high-frequency (18–6 MHz) linear transducer. Focus was set at 5 mm for temporal arteries and 3 cm for axillary arteries. A Doppler frequency of 10 MHz was applied. Pulse repetition frequency (PRF) was set at 2–3 KHz for temporal artery, and at 3–4 KHz for axillary arteries. A low wall filter was selected to allow the identification of low velocity flow. The color box was adjusted to obtain an angle steer correction ≤ 60 degrees.

The common, parietal, and frontal branches of each temporal artery and the axillary arteries in longitudinal and transverse plans were assessed at each visit. The presence of a halo was defined according to the accepted definitions as a homogenous, hypoechoic wall thickening, well-delineated toward the luminal side, visible both in longitudinal and transverse planes, most commonly concentric in transverse scans (10). The compression sign was applied in transverse views to confirm the findings (11). CDS was defined as positive if displaying a halo at the level of at least one branch of the temporal artery or at least one axillary artery. Intima-media thickness was measured at the site of maximum thickness on longitudinal plans.

Statistical Analysis

Categorical variables were presented as numbers and percentages. Continuous variables were displayed as means with standard deviation (S.D.) if normally distributed or as medians with interquartile range (IQR) in case of non-normal distribution. Ratios were presented with 95% confidence intervals (C.I.). For comparison between groups, the independent-samples *t*-test for continuous variables and the chi-squared test for categorical variables were applied. For non-parametric numerical variables, the Wilcoxon signed-rank test was used. Statistical analysis was performed the software RStudio (R version 3.6.3 2020-02-29, Copyright © 2020 The R Foundation for Statistical Computing). $P < 0.05$ were considered to be significant.

RESULTS

Baseline Assessment

One-hundred and sixty patients were recruited, 120 were females (75%), the mean age at diagnosis was 72.4 ± 8.2 years. Sixty-three (39.4%) were evaluated with FTA, while 97 (60.6%) underwent the conventional approach.

FTA patients were older (75.1 ± 7.6 vs. 70.6 ± 8.2 years old; $p < 0.001$). At the time of diagnosis, a larger percentage of comorbidity-free patients was observed in the non-FTA group (16.7 vs. 3.8%; $P = 0.02$). Nevertheless, major cardiovascular comorbidities like coronary artery disease, dyslipidaemia, diabetes, stroke, peripheral artery disease and hypertension were similar between the two cohorts ($P > 0.05$) except for heart failure (1.0% conventional vs. 7.5% FTA; $P = 0.03$). Overall, 19.4% (ranging 15.5–25%) of patients with newly diagnosed GCA had a previous diagnosis of polymyalgia rheumatica.

FTA contributed to shorten the time to diagnosis (62 days IQR 20–122 in the conventional cohort vs. 33 days IQR 15–83 in the FTA cohort; $P = 0.035$) (**Table 1**), especially for cranial GCA patients (61 days IQR 15–107 vs. 30 days IQR 13–53; $P = 0.007$). Of note, among patients with PVL the duration of symptoms (since the onset of the first symptom attributable to GCA) before receiving a diagnosis of GCA was higher in the conventional group (23 days IQR 12–96 vs. 7 days IQR 4–10; $P = 0.02$).

There was no difference between FTA and the conventional group regarding major vessel district involvement (cranial GCA 82.5 vs. 77.3%, respectively; $P = 0.4$). The clinical presentation was similar between the two groups: the frequency of PMR, headache, amaurosis fugax, jaw claudication and fever did not change between the two cohorts ($P > 0.05$). Some symptoms were more frequently recorded in the FTA group compared to the conventional practice: weight loss (30.2 vs. 13.4%; $P = 0.01$), tongue claudication (11.1 vs. 1.0%; $P = 0.004$) and scalp tenderness (20.6 vs. 5.2%; $P = 0.003$). PVL occurred in 8 (12.7%) patients in the FTA group compared to 26 (26.8%) in the conventional one ($P = 0.03$). The relative risk of blindness in the conventional group was 2.11 (95% C.I. 1.02–4.36; $P = 0.04$) as compared to FTA. The overall occurrence of ischaemic manifestations of GCA (AION, PION and cerebrovascular accidents) was higher in the non-FTA cohort (28.9 vs. 12.7; $P = 0.02$) (**Table 2**). The relationship between symptoms latency in the whole cohort and visual outcomes, and visual survival over time are shown in **Supplementary Figures 1**, **2**.

Forty-four (45.4%) patients of the conventional cohort underwent TAB, with 25 (56.8%) samples were compatible with GCA at the histological examination. TAB was performed in 3 patients of the new cohort, two of them without signs of active vasculitis and one with a sampling error. TAB sensitivity was 53.2% (95% C.I. 38.1–67.9%).

Within the FTA group, all 63 (100.0%) were evaluated with CDS at the time of diagnosis, 52 of them having specific ultrasound findings of GCA (**Figures 1**, **2**), yielding a sensitivity of 82.5% (95% C.I. 70.9–91.0%). Thirty-one (49.2%) had bilateral halo at CDS. Forty-three presented with halo sign exclusively at temporal arteries, 4 had only axillary artery halos, 5 had both districts involved. Temporal artery abnormalities at the

TABLE 1 | Clinical features of the cohort at baseline.

	Total (*N* = 160)	Conventional (*N* = 97)	FTA (*n* = 63)	*P*-value
Age at diagnosis, mean (S.D.), years	72.4 (8.2)	70.6 (8.2)	75.1 (7.6)	<0.001
Female, *n* (%)	120 (75)	77 (79.4)	43 (68.3)	0.1
Previous PMR, *n* (%)	31 (19.4)	15 (15.5)	16 (25.4)	0.1
Symptom latency, median (IQR), days	46 (15–101)	62 (20–122)	33 (15–83)	0.04
Symptoms latency of patients with PVL, median (IQR), days	14 (8–65)	23 (12–96)	7 (4–10)	0.02
Comorbidities				
Coronary heart disease	15 (10.1)	9 (9.4)	6 (11.3)	0.7
Dyslipidaemia	30 (20.1)	18 (18.8)	12 (22.6)	0.6
Diabetes mellitus	18 (12.1)	14 (14.6)	4 (7.5)	0.2
Stroke	14 (9.4)	11 (11.5)	3 (5.7)	0.2
Peripheral artery disease	7 (4.7)	5 (5.2)	2 (3.8)	0.2
Hypertension	85 (57.0)	53 (55.2)	32 (60.4)	0.5
Heart failure	5 (3.4)	1 (1.0)	4 (7.5)	0.03
Other comorbidities	102 (68.5)	73 (76.0)	39 (73.6)	0.7
No comorbidities	18 (12.1)	16 (16.7)	2 (3.8)	0.02

TABLE 2 | Clinical picture at the time of diagnosis in the two cohorts: conventional approach (No FTA) vs. FTA.

	No FTA (*N* = 97)	FTA (*N* = 63)	*P*-value
Symptoms at diagnosis			
Constitutional (fever or weight loss), *n* (%)	36 (37.1)	28 (44.4)	0.4
Fever ≥ 38°C (≥100.4 F), *n* (%)	32 (33.0)	17 (27.0)	0.4
Weight loss ≥2 kg, *n* (%)	13 (13.4)	19 (30.2)	0.01
Any cranial symptom, *n* (%)	88 (90.7)	53 (84.1)	0.2
Any ocular symptom, *n* (%)	37 (38.1)	17 (27.0)	0.1
Permanent visual loss, *n* (%)	26 (26.8)	8 (12.7)	0.03
Amaurosis fugax, *n* (%)	14 (14.4)	7 (11.1)	0.5
Diplopia, *n* (%)	4 (4.1)	1 (1.6)	0.4
Jaw claudication, *n* (%)	33 (34.0)	29 (46.0)	0.1
Tongue claudication, *n* (%)	1 (1.0)	7 (11.1)	0.004
Scalp tenderness, *n* (%)	5 (5.2)	13 (20.6)	0.003
Headache, *n* (%)	77 (79.4)	45 (71.4)	0.3
PMR, *n* (%)	48 (49.5)	32 (50.7)	0.9
Increased ESR/CRP, *n* (%)	79 (90.8)	59 (96.7)	0.2
ESR (mm/h), median (IQR)	82 (48–102)	78 (63–96)	0.5
CRP (mg/L), median (IQR)	35 (20–99)	59 (24–98)	0.5
Anemia (<12 g/dL in females, <13 g/dL in males), *n* (%)	38 (65.5)	17 (77.2)	0.3
Hemoglobin, mean (S.D.), g/dL	11.4 (1.6)	11.5 (1.4)	1.0
Cranial GCA	75 (77.3)	52 (82.5)	0.4
LV-GCA	22 (22.7)	11 (17.5)	0.4
Ischaemic GCA	28 (28.9)	8 (12.7)	0.02

time of referral was a predictor of positive CDS (43.8 vs. 10.0%; $P = 0.046$), and it was strongly correlated to a positive halo sign at the level of the temporal arteries (47.7 vs. 7.1%; $P = 0.006$), as well as to bilateral halo sign (51.7 vs. 24.1%; $P = 0.03$).

Forty-two patients who underwent CDS (66.7%) had previously received GC for a median time of 12 days (IQR 3–101). Twenty-two patients (34.9%) had been treated with high-dose GC therapy (≥30 mg/day) for a median time of 4 days (IQR 3–8). The median prednisone-equivalent dosage on the day of scan was 37.5 mg/day (IQR 10–50).

CDS sensitivity was lower in patients with high-dose GC doses protracted for >5 days ($N = 10$), reducing to 60.0% (95% C.I. 26.2–87.8%).

Follow-Up and Relapses

The median follow-up duration was 3.2 years (IQR 1.0–5.9 years), shorter in the FTA group compared with the conventional one [0.9 years (IQR 0.2–2.0) vs. 5.0 years (IQR 3.5–8.7); $P < 0.001$].

During the follow-up, 82 patients relapsed (51.3%). The median time to disease reoccurrence was 8 months (IQR 3–20 months). The calculated relapse rate per 10 person-years was 3.0 (95% C.I. 2.6–3.4 years). Fifty (31.3%) patients experienced more than one relapse. In the conventional group there were 64 (66%) relapses overall, over a longer follow-up duration; in the FTA 18 (28.6%) relapses were observed. The calculated relapse rate per 10 person-years did not differ between the two cohorts: [2.9 (95% C.I. 2.5–3.4) in the conventional group vs. 3.6 (95% C.I. 2.3–5.2) in the FTA (**Table 3**).

The cumulative incidence of relapse and time to first relapse did not change after FTA was implemented ($P = 0.23$) (**Figure 3**).

During follow-up there were no new cases of PVL related to disease relapses. Symptoms at the time of first relapse were different between the two groups. In the FTA cohort more patients were presenting with PMR-like symptoms (50.0 vs. 23.4%; $P = 0.03$), whereas cranial symptoms (22.2 vs. 64.1%; $P = 0.002$), especially headache (16.7 vs. 48.4%; $P = 0.02$), were more prominent in the historical cohort. Interestingly, none of the FTA patients with ischaemic GCA relapsed during follow-up (0.0% with ischaemic disease vs. 26.6% without ischaemic GCA; $P = 0.01$).

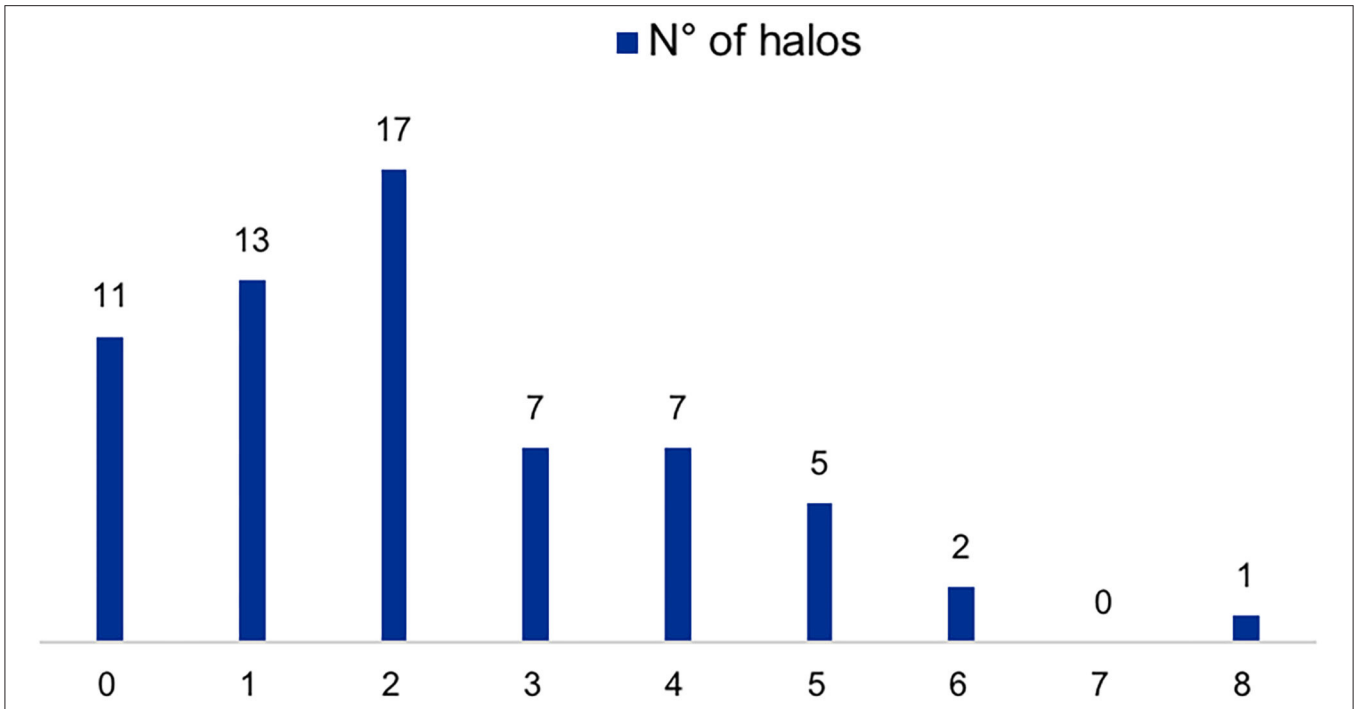

FIGURE 1 | CDS findings of patients evaluated with the FTA. Number of patients according to number of sites with halos (0–8 sites including the different branches of the temporal artery and the axillary arteries).

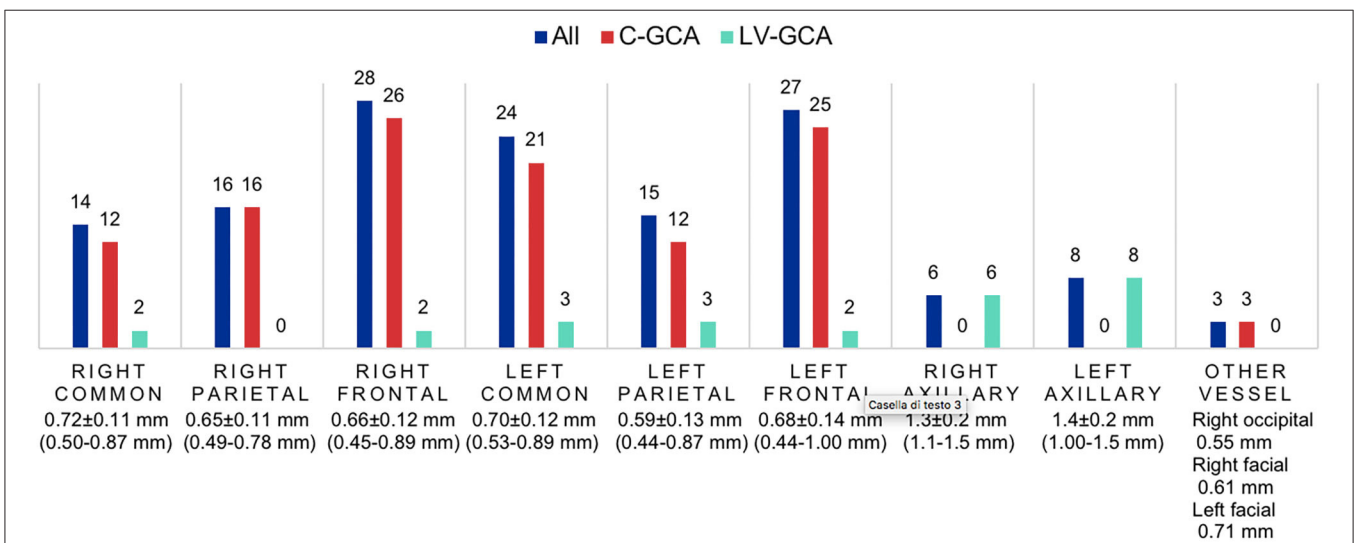

FIGURE 2 | CDS findings distribution and intima-media thickness of patients evaluated with the FTA. Number of patients with positive CDS in each vascular district, the average halo thickness (± S.D., range) is reported below. C-GCA, cranial giant cell arteritis; LV-GCA, large-vessel giant cell arteritis.

The ongoing therapy at the time of relapse was similar between the two groups. The median dose of prednisone was 12.5 vs. 13.75 mg/day between the two cohorts ($P = 0.4$).

Out of 18 patients of the FTA group with a confirmed GCA relapse, 14 CDS scans were performed (77.8%). Six scans had signs of active disease (42.9%) in patients with confirmed relapses. Bilateral halo was found in 2 patients with relapse (33.3%).

Regarding the non-FTA group, 13 patients were evaluated with CDS for relapse assessment. The total number of CDS was 22, with 10 of them showing a halo (45.5%) of which four were bilateral halos (40.0%).

TABLE 3 | Relapse characteristics between the conventional (No FTA) and the FTA cohorts.

	No FTA (*N* = 64)	FTA (*N* = 18)	*P*-value
Age at relapse, mean (S.D.), years	70.3 (8.1)	72.7 (7.7)	0.3
Females, *n* (%)	50 (80.0)	12 (66.7)	0.2
Previous PMR, *n* (%)	12 (18.8)	5 (27.7)	0.4
Time to 1st relapse (if any), median (IQR), months	8.5 (3.8–20.3)	7.5 (3.3–10.8)	0.4
Relapse rate per 10 person-years, median (95% C.I.)	2.9 (2.5–3.4)	3.6 (2.3–5.2)	0.3
Any cranial symptom, *n* (%)	41 (64.1)	4 (22.2)	0.002
Any ocular symptom, *n* (%)	4 (6.3)	0 (0.0)	0.3
Permanent visual loss, *n* (%)	0 (0.0)	0 (0.0)	nd
Amaurosis fugax, *n* (%)	4 (6.3)	0 (0.0)	0.3
Jaw claudication, *n* (%)	4 (6.3)	1 (5.6)	0.9
Tongue claudication, *n* (%)	0 (0.0)	0 (0.0)	nd
Scalp tenderness, *n* (%)	3 (4.7)	0 (0.0)	0.4
Headache, *n* (%)	31 (48.4)	3 (16.7)	0.02
PMR, *n* (%)	15 (23.4)	9 (50.0)	0.03
Increased ESR/CRP, *n* (%)	41 (67.2)	12 (66.6)	0.9
ESR, median (IQR), mm/h	36 (25–53)	32 (22–47)	0.2
CRP, median (IQR), mg/L	11.0 (5.4–18.6)	12.5 (5.2–26)	0.9
GC dose, median (IQR), mg/day	12.5 (7.5–25)	13.75 (10–25)	0.4
Immunosuppressive drug, *n* (%)	9 (14.1)	0 (0.0)	0.09
Cranial GCA	48 (75.0)	16 (88.9)	0.2
LV-GCA	16 (25.0)	2 (11.1)	0.2
Ischaemic GCA	17 (26.6)	0 (0.0)	0.01

Anemia at the time of diagnosis (Hb <11 g/dL) was associated with a higher relapse risk (HR 1.96 95% C.I. 1.04–3.69; $P = 0.04$). Microcytosis (MCV <80 fL) at the time of diagnosis was found to be a good predictor of early disease relapse (HR 2.80 95% C.I. 2.33–3.26; $P = 0.03$). A platelet count above 450,000/mm^3 was another predictor of relapse (HR 2.67 95% C.I. 1.24–5.7; $P = 0.01$).

The risk of multiple disease relapses was associated to LV-GCA (HR 2.02 95% C.I. 1.07–3.81; $P = 0.03$).

At the end of the follow-up, 38 patients (23.7%) were taking an adjunctive immunosuppressive (IS) agent (34 methotrexate, 3 Tocilizumab, 1 azathioprine) initiated after a median time of 10 months (IQR 5-29 months). There were no significant differences in the requirements for adjunctive IS between the two cohorts. LV-GCA patients were at higher risk of requiring IS (HR 2.42 95% C.I. 1.16–5.04; $P = 0.02$).

Chronic Complications

Large vessel complications (aneurysm, stenosis, vessel ectasia or dissection) were found to be more frequent in patients with LV-GCA (HR 4.58 95% C.I. 1.92–10.92; $P = 0.0006$) (**Figure 4**). There were no differences between the FTA and the conventional cohort.

FAST-TRACK CLINIC AT THE TIME OF COVID-19

During the lockdown period due the SARS-CoV-2 pandemic outbreak in Italy (March-April 2020) there was a significant decrease in the number of patients referred to the clinic compared to the preceding months (9 visits compared to 4) and to the corresponding period in the previous year (16 visits compared to 4) despite a regularly operating service (**Figure 5**). Since the end of the lockdown period the number of new referrals has increased again (15 new visits in the period May-June 2020), nevertheless, a delay in referral has been recorded since the pandemic outbreak. Overall there have been 10 confirmed diagnoses of GCA during this period, female (80%), mean age 76 ± 5 years. The rate of PVL due to GCA has significantly increased during the pandemic, and especially in the rate of bilateral AION which occurred in two patients during the period March-April 2020 compared to one case over the previous 4 years (October 2016-February 2020) of the fast-track clinic activity. PVL occurred in 4 (40%) of GCA patients assessed since March 2020 (vs. 12.7% in the previous FTA period; $P = 0.03$). The duration of symptoms prior to diagnosis since the COVID-19 outbreak in patients developing PVL has increased to 23 days (IQR 15–56) compared to 7 days (IQR 4–10) of the normal FTA activity; the two patients developing bilateral blindness were referred after a mean of 31 days since the symptoms onset.

DISCUSSION

Our data confirm that FTA including CDS assessment is an innovative disease-modifying strategy leading to a significant reduction of PVL and related disability. The effectiveness of the FTA approach has been further highlighted in our cohort by the significant increase in diagnostic delay and occurrence of PVL, including bilateral blindness, observed during the COVID-19 pandemic due to the patients', and possibly care givers' fear of seeking medical attention and attending the hospital. The use of CDS for the diagnosis of GCA has been first described in 1997 (12), however only in recent years there has been a formal validation and consensus of the role of ultrasound in the management of LVV (9, 13). The recognition of CDS as the first imaging modality in patients with cranial GCA was only included in International recommendations 2 years ago (9, 13). In Centers with the adequate expertise and machine equipment and settings, ultrasound has replaced TAB for the diagnosis of patients with suspected GCA and a highly suggestive clinical picture (7, 13, 14) As demonstrated by the change of practice in our two cohorts, the need for TAB reduced by 93% since the use of CDS as part of the FTA clinic. CDS has the advantage of being a quick, repeatable, low-cost procedure that allows to assess the whole length of the temporal artery (reducing false negatives related to the skipped nature of GCA inflammation) and to extent the examination to other cranial or extra-cranial arteries, optimizing the diagnostic yield (15, 16). CDS has been demonstrated to be more sensitive compared to TAB in the diagnosis of GCA (14), as confirmed by our study. The implementation of CDS into FTA clinics

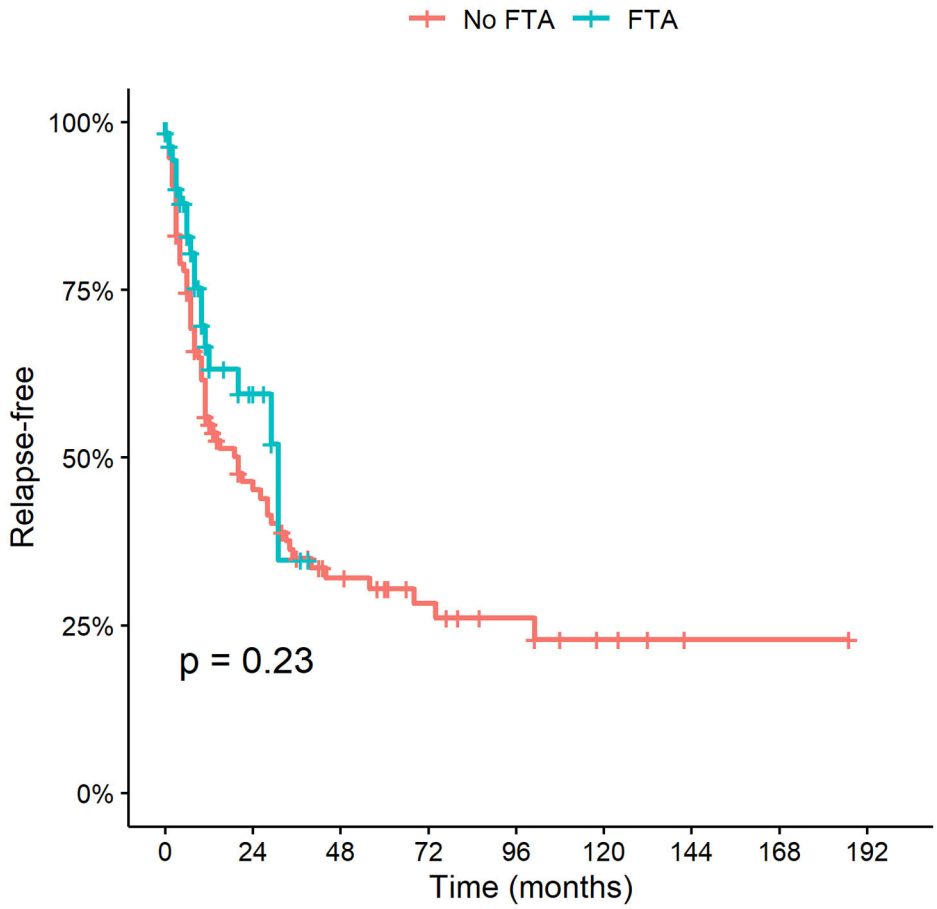

FIGURE 3 | Time to first relapse before and after FTA introduction.

allowing for a prompt and direct assessment and interpretation of imaging findings by the treating physician has led to the demonstration that very early diagnosis with the aid of CDS can significantly reduce the risk of PVL in patients with GCA (6, 17). Our data highlight the importance of early diagnostic assessment and treatment initiation through FTA, but also of early referral through the prompt recognition of prodrome symptoms possibly preceding the occurrence of ischaemic complications of GCA. In our cohort, patients assessed with FTA had less frequent comorbidities albeit being generally older. This characteristic might have contributed to reduce the confounding effect in case of presentation with unspecific symptoms and could have led to a more rapid referral compared to the conventional practice group. Blindness has been described in 15–30% of patients with GCA and is a complication that mostly occurs at the early stages of the disease, often being the presenting symptom leading to the diagnosis (18). Nevertheless, up to 28% of patients with PVL report premonitory reversible transient visual symptoms that can prompt the urgent referral if properly investigated and recognized (18). Other factors associated with PVL include jaw claudication, absence of constitutional symptoms or milder elevation of inflammatory markers (19, 20). Visual loss is usually irreversible in GCA, however, early initiation of GC, particularly if initiated within the 1st day of visual symptoms has been reported as the sole prognostic factor for a partial improvement of visual loss (9, 19). Diagnostic delay is known to be associated with the occurrence of bilateral PVL as confirmed by our data observed during COVID-19 outbreak (19). Clinical suspicion avoiding over-reliance on temporal headache alone is key to the early recognition of GCA. In our FTA cohort some symptoms were recorded as more frequent such as tongue claudication, scalp tenderness or weight loss. It is possible that patients with a clinical presentation more easily resembling GCA would be referred more rapidly, however, increased attention to rarer or less specific symptoms of GCA is pivotal to optimize the clinical suspicion. To further improve the outcome of LVV, the expedited process allowing for early access to specialist evaluation and confirmatory investigational tests obtained with FTA should be paralleled by educational programmes and clear recommendations for fast-track referral offered to primary care and other relevant specialists to further reduced the symptom latency period (21, 22). An improvement of the general public awareness of GCA could also be beneficial to reduce the diagnostic delay. During the COVID-19 pandemic several

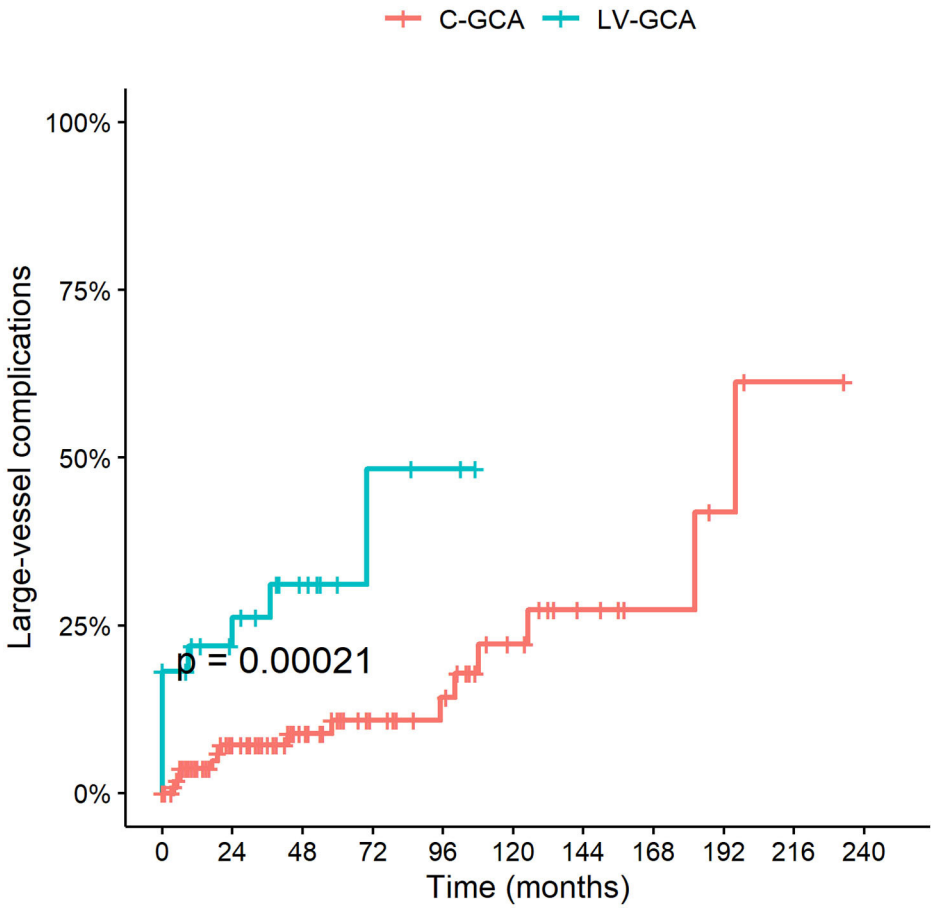

FIGURE 4 | Cumulative incidence of LV complications among LV-GCA compared to C-GCA. LV complications include thoracic/abdominal aortic aneurysm or dissection and stenosis, aneurysm or ectasia of other large arteries.

medical emergencies, including acute coronary syndromes, have recorded an unusual decrease in hospitalization and increased out-of-hospital mortality as a result of avoidance or delay in seeking medical attention (23, 24). Similarly, we have observed a significant increase in the rate of ischaemic complications (with 40% of PVL), including bilateral blindness with two new cases over a period of 2 months, compared to only one case over the 4-year activity of the FTA (25). The duration of symptom prior to the first evaluation for suspected GCA has increased during the COVID-19 outbreak despite a regularly operating fast-track clinic. The negative outcome of newly diagnosed patients with GCA observed during SARS-CoV-2 outbreak confirms once more that urgent referral and FTA are key in the management of LVV with a significant change in visual prognosis.

Nonetheless, our data suggest that FTA does not impact the long-term outcomes and relapse-risk. Our findings are in line with previous reports suggesting that early diagnosis of GCA does not seem to significantly improve the future risk of relapse (26). Predictors of relapse in LVV are poorly understood. Our data confirm that markers of inflammation, baseline anemia, and LV-GCA are associated with a higher risk of relapse (27–30). Increasing interest is emerging to find different predictors of relapse or future disease complications as part of the FTA approach, including quantitative analysis of CDS findings suggestive of more extensive or more severe vascular involvement at disease onset (31). A recently proposed ultrasonographic score combining information on the number of sites with halo and the halo thickness was suggested to be associated with ocular ischaemia (32). Nevertheless, to date, no reliable imaging biomarker has been identified to predict the risk of relapse or future ischaemic complications during follow-up (33, 34).

Our study has some limitations, including the retrospective collection of some of the data. Moreover, comparisons between the two cohorts regarding some of the rarer complications of GCA might have been limited by the small sample size.

The unmodified disease course despite the FTA and early diagnosis strategies in GCA suggest that a change in therapeutic strategy should be applied since the early stages of the disease to significantly modify the long-term outcome. Available evidence demonstrated a glucocorticoid-sparing effect and efficacy in reducing the risk of relapse in newly diagnosed or relapsing

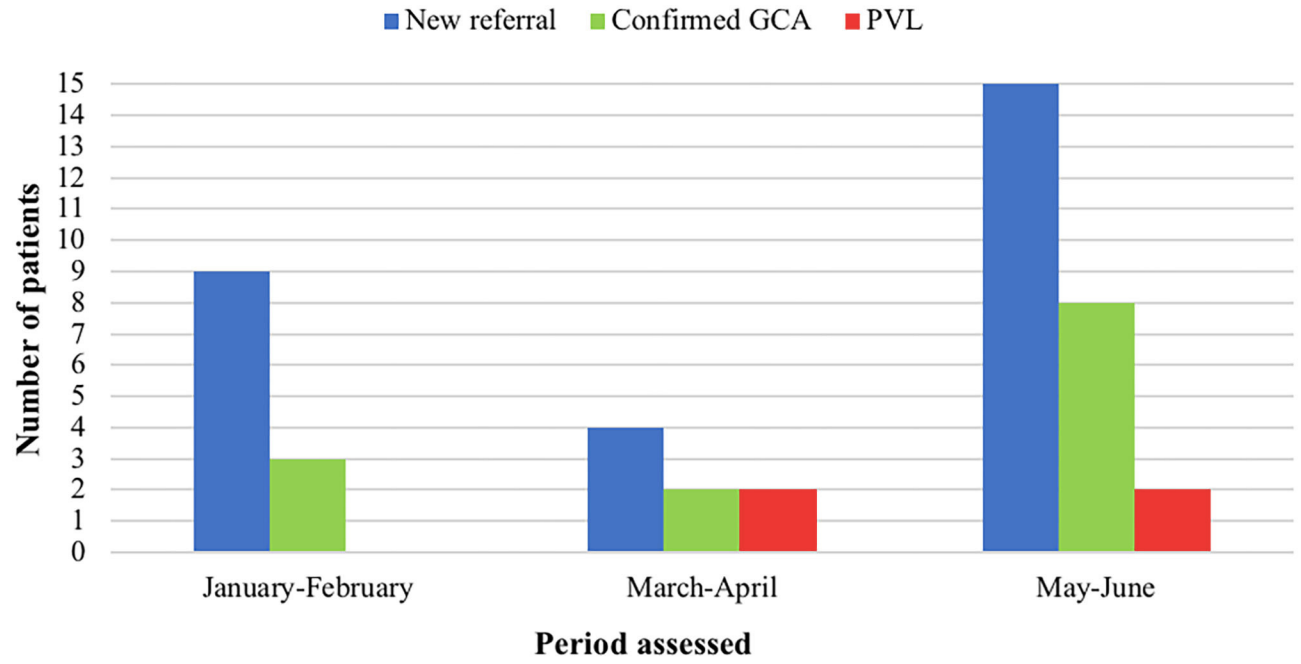

FIGURE 5 | Comparison of the fast-track clinic activity amongst different periods of 2020, including the lockdown months due to COVID-19 pandemic (March-April). GCA, giant cell arteritis; PVL, permanent visual loss.

patients with GCA treated with methotrexate or tocilizumab (35, 36). Nevertheless, in clinical practice patient-tailored evaluations of safety and cost-effectiveness issues are often taken into consideration in this elderly population group treated with concomitant high-dose glucocorticoids. Current recommendations suggest to reserve adjunctive therapy to selected patients with refractory or relapsing disease or with the presence or increased risk of glucocorticoid related adverse events and complications. A risk stratification process to select patients with poor prognosis to be treated more intensively upfront still requires further research. Data obtained from the FTA clinics suggest that the window of opportunity to obtain long-term modifications of the disease process in GCA is exceedingly short and should probably be associated with an optimization of the disease awareness to further accelerate referral and with a tailored therapeutic approach to potentiate treatment in patients at higher risk of relapse and complications.

CONCLUSION

FTA including CDS evaluation contributed to a substantial reduction of the PVL risk in GCA by shortening the time to diagnosis and treatment initiation. Relapse rate and LV-complications did not change upon FTA introduction, highlighting the need for better disease activity monitoring strategies and risk stratification at disease onset that would predict the occurrence of relapse during glucocorticoid de-escalation.

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 University of Pavia Ethical Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SM, AB, EB, PD, and CM contributed equally to the ideation and realization of the research project and critically reviewed the manuscript. SM and AB wrote the manuscript. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.589794/full#supplementary-material

Supplementary Figure 1 | Cumulative risk of blindness according to symptom latency in the whole cohort of patients (convenational approach, fast-track, and fast-track during COVID-19 pandemic).

Supplementary Figure 2 | Kaplan-Meyer curves of visual survival in the conventional group compared to the fast-track approach.

REFERENCES

1. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 Revised international chapel hill consensus conference nomenclature of vasculitides. *Arthritis Rheum.* (2013) 65:1–11. doi: 10.1002/art.37715
2. Salvarani C, Boiardi L. Polymyalgia rheumatica and giant-cell arteritis. *N Engl J Med.* (2002) 347:261–71. doi: 10.1056/NEJMra011913
3. Salvarani C, Cantini F, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. *Lancet.* (2008) 372:234–45. doi: 10.1016/S0140-6736(08)61077-6
4. Nuenninghoff DM, Hunder GG, Christianson TJH, McClelland RL, Matteson EL. Incidence and predictors of large-artery complication (aortic aneurysm, aortic dissection, and/or large-artery stenosis) in patients with giant cell arteritis: a population-based study over 50 years. *Arthritis Rheum.* (2003) 48:3522–31. doi: 10.1002/art.11353
5. Patil P, Williams M, Maw WW, Achilleos K, Elsideeg S, Dejaco C, et al. Fast track pathway reduces sight loss in giant cell arteritis: results of a longitudinal observational cohort study. *Clin Exp Rheumatol.* (2015) 33(2 Suppl. 89):S103–6.
6. Diamantopoulos AP, Haugeberg G, Lindland A, Myklebust G. The fast-track ultrasound clinic for early diagnosis of giant cell arteritis significantly reduces permanent visual impairment: towards a more effective strategy to improve clinical outcome in giant cell arteritis? *Rheumatology.* (2016) 55:66–70. doi: 10.1093/rheumatology/kev289
7. Monti S, Floris A, Ponte CB, Schmidt WA, Diamantopoulos AP, Pereira C, et al. The proposed role of ultrasound in the management of giant cell arteritis in routine clinical practice. *Rheumatology.* (2018) 57:112–9. doi: 10.1093/rheumatology/kex341
8. Mukhtyar C, Guillevin L, Cid MC, Dasgupta B, Groot K de, Gross W, et al. EULAR recommendations for the management of large vessel vasculitis. *Ann Rheum Dis.* (2009) 68:318–23. doi: 10.1136/ard.2008.088351
9. Hellmich B, Agueda A, Monti S, Buttgereit F, de Boysson H, Brouwer E, et al. 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. *Ann Rheum Dis.* (2019) 79:19–30. doi: 10.1136/annrheumdis-2019-215672
10. Chrysidis S, Duftner C, Dejaco C, Schäfer VS, Ramiro S, Carrara G, et al. Definitions and reliability assessment of elementary ultrasound lesions in giant cell arteritis: a study from the OMERACT large vessel vasculitis ultrasound working Group. *RMD Open.* (2018) 4:e000598. doi: 10.1136/rmdopen-2017-000598
11. Aschwanden M, Daikeler T, Kesten F, Baldi T, Benz D, Tyndall A, et al. Temporal artery compression sign – a novel ultrasound finding for the diagnosis of giant cell arteritis. *Ultraschall Med.* (2013) 34:47–50. doi: 10.1055/s-0032-1312821
12. Schmidt WA, Kraft HE, Vorpahl K, Völker L, Gromnica-Ihle EJ. Color duplex ultrasonography in the diagnosis of temporal arteritis. *N Engl J Med.* (1997) 337:1336–42. doi: 10.1056/NEJM199711063371902
13. Dejaco C, Ramiro S, Duftner C, Besson FL, Bley TA, Blockmans D, et al. EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice. *Ann Rheum Dis.* (2018) 77:636–43. doi: 10.1136/annrheumdis-2017-212649
14. Luqmani R, Lee E, Singh S, Gillett M, Schmidt WA, Bradburn M, et al. The role of ultrasound compared to biopsy of temporal arteries in the diagnosis and Treatment of Giant Cell Arteritis (TABUL): a diagnostic accuracy and cost-effectiveness study. *Health Technol Assess.* (2016) 20:1–238. doi: 10.3310/hta20900
15. Germano G, Monti S, Ponte C, Possemato N, Caporali R, Salvarani C, et al. The role of ultrasound in the diagnosis and follow-up of large-vessel vasculitis: an update. *Clin Exp Rheumatol.* (2017) 35:S194–8.
16. Monti S, Agueda AF, Luqmani R. The use of ultrasound in the management of large-vessel vasculitis: an evolving concept. *Clin Exp Rheumatol.* (2018) 36:S96–102.
17. Patil P, Williams M, Maw WW, Achilleos K, Elsideeg S, Dejaco C, et al. Fast track pathway reduces sight loss in giant cell arteritis: results of a longitudinal observational cohort study. *Clin Exp Rheumatol.* (2015) 33(2 Suppl 89):S103–6.
18. Vodopivec I, Rizzo JF. Ophthalmic manifestations of giant cell arteritis. *Rheumatology.* (2018) 57:ii63–72. doi: 10.1093/rheumatology/kex428
19. González-Gay MA, Blanco R, Rodríguez-Valverde V, Martínez-Taboada VM, Delgado-Rodriguez M, Figueroa M, et al. Permanent visual loss and cerebrovascular accidents in giant cell arteritis: predictors and response to treatment. *Arthritis Rheum.* (1998) 41:1497–504. doi: 10.1002/1529-0131(199808)41:8<1497::AID-ART22>3.0.CO;2-Z
20. Singh AG, Kermani TA, Crowson CS, Weyand CM, Matteson EL, Warrington KJ. Visual manifestations in giant cell arteritis: trend over 5 decades in a population-based cohort. *J Rheumatol.* (2015) 42:309–15. doi: 10.3899/jrheum.140188
21. Mollan SP, Paemeleire K, Versijpt J, Luqmani R, Sinclair AJ. European headache federation recommendations for neurologists managing giant cell arteritis. *J Headache Pain.* (2020) 21:28. doi: 10.1186/s10194-020-01093-7
22. Helliwell T, Muller S, Hider SL, Prior JA, Richardson JC, Mallen CD. Challenges of diagnosis and management of giant cell arteritis in general practice: a multimethods study. *BMJ Open.* (2018) 8:e019320 doi: 10.1136/bmjopen-2017-019320
23. De Filippo O, D'Ascenzo F, Angelini F, Bocchino PP, Conrotto F, Saglietto A, et al. Reduced rate of hospital admissions for ACS during covid-19 outbreak in Northern Italy. *N Engl J Med.* (2020) 383:88–9. doi: 10.1056/NEJMc2009166
24. Baldi E, Sechi GM, Mare C, Canevari F, Brancaglione A, Primi R, et al. Out-of-hospital cardiac arrest during the Covid-19 outbreak in Italy. *N Engl J Med.* (2020) 383:496–8. doi: 10.1056/NEJMc2010418
25. Monti S, Delvino P, Bellis E, Milanesi A, Brandolino F, Montecucco C. Impact of delayed diagnoses at the time of COVID-19: increased rate of preventable bilateral blindness in giant cell arteritis. *Ann Rheum Dis.* (2020) 79:1658–9. doi: 10.1136/annrheumdis-2020-217915
26. Hocevar A, Rotar Z, Jese R, Semrl SS, Pizem J, Hawlina M, et al. Do early diagnosis and glucocorticoid treatment decrease the risk of permanent visual loss and early relapses in giant cell arteritis: a prospective longitudinal study. *Medicine.* (2016) 95:e3210. doi: 10.1097/MD.0000000000003210
27. Alba MA, García-Martínez A, Prieto-González S, Tavera-Bahillo I, Corbera-Bellalta M, Planas-Rigol E, et al. Relapses in patients with giant cell arteritis: prevalence, characteristics, and associated clinical findings in a longitudinally followed cohort of 106 patients. *Medicine.* (2014) 93:194–201. doi: 10.1097/MD.0000000000000033
28. Dumont A, Parienti J-J, Delmas C, Boutemy J, Maigné G, Martin Silva N, et al. Factors associated with relapse and dependence on glucocorticoids in giant cell arteritis. *J Rheumatol.* (2020) 47:108–16. doi: 10.3899/jrheum.181127
29. Kermani TA, Warrington KJ, Cuthbertson D, Carette S, Hoffman GS, Khalidi NA, et al. Disease relapses among patients with giant cell arteritis: a prospective, longitudinal cohort study. *J Rheumatol.* (2015) 42:1213–7. doi: 10.3899/jrheum.141347
30. Hernández-Rodríguez J, García-Martínez A, Casademont J, Filella X, Esteban M-J, López-Soto A, et al. A strong initial systemic inflammatory response is associated with higher corticosteroid requirements and longer duration of therapy in patients with giant-cell arteritis. *Arthritis Rheum.* (2002) 47:29–35. doi: 10.1002/art1.10161
31. Monti S, Ponte C, Pereira C, Manzoni F, Klersy C, Rumi F, et al. The impact of disease extent and severity detected by quantitative ultrasound analysis in the diagnosis and outcome of giant cell arteritis. *Rheumatology.* (2019) 59:2299–307. doi: 10.1093/rheumatology/kez554
32. van der Geest KSM, Borg F, Kayani A, Paap D, Gondo P, Schmidt W, et al. Novel ultrasonographic Halo Score for giant cell arteritis: assessment of diagnostic accuracy and association with ocular ischaemia. *Ann Rheum Dis.* (2020) 79:393–9. doi: 10.1136/annrheumdis-2019-216343
33. Ponte C, Serafim AS, Monti S, Fernandes E, Lee E, Singh S, et al. Early variation of ultrasound halo sign with treatment and relation with clinical features in patients with giant cell arteritis. *Rheumatology.* (2020) Keaa196. doi: 10.1093/rheumatology/keaa196

34. Schmidt WA, Krause A, Schicke B, Kuchenbecker J, Gromnica-Ihle E. Do temporal artery duplex ultrasound findings correlate with ophthalmic complications in giant cell arteritis? *Rheumatology.* (2009) 48:383–5. doi: 10.1093/rheumatology/ken515
35. Mahr AD, Jover JA, Spiera RF, Hernández-García C, Fernández-Gutiérrez B, Lavalley MP, et al. Adjunctive methotrexate for treatment of giant cell arteritis: an individual patient data meta-analysis. *Arthritis Rheum.* (2007) 56:2789–97. doi: 10.1002/art.22754
36. Stone JH, Tuckwell K, Dimonaco S, Klearman M, Aringer M, Blockmans D, et al. Trial of tocilizumab in giant-cell arteritis. *N Engl J Med.* (2017) 377:317–28. doi: 10.1056/NEJMoa1613849

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ORIGINAL RESEARCH
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OrthoRehab: Development of a New Methodology for the Comparison Study Between Different Types of Ankle–Foot Orthoses in Foot Dysfunction

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Foot dysfunction is one of the most likely consequences of rheumatoid arthritis and stroke. It is characterized by severe changes in the gait pattern due to a significant increase in the plantar flexion. Some of these dysfunctions can be compensated by using an ankle–foot orthosis. However, the clinical decision about which orthosis best suits the patient creates a real problem for physicians/therapists.

Purpose: The main goal of this paper is to present a quantitative support tool that can assist the physicians/therapists in deciding which orthosis is most suitable for each subject.

Methodology: In order to achieve such goal, a platform named OrthoRehab was developed, and it was tested in three conditions: without any orthosis and with two different ankle–foot orthoses. The data were acquired in the Gait Laboratory of Rehabilitation Medicine Center of Alcoitão using a VICON NEXUS 1.8.5® motion capture system that allows the capturing of kinematic and kinetic data.

Results: The results reveal that OrthoRehab is a user-friendly, easy to apply tool that analyzes very relevant data for the clinical staff.

Conclusion: The developed decision support tool, OrthoRehab, offers a quantitative analysis and provides insight to which orthosis achieves the best performance in comparison with the patient's gait pattern with no orthosis.

Keywords: gait analysis, device, orthoses, rehabilitation, software, evaluation

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INTRODUCTION

Major chronic diseases enumerated by the World Health Organization (WHO), for example, cancers, mental disorders, cardiovascular diseases, chronic respiratory diseases, and rheumatological diseases, have a huge impact on the quality of life of the individuals and represent the predominant health problems of the century (1, 2). Foot impairment is a major adverse condition in rheumatoid arthritis (RA) and stroke. Taking the total number of adult stroke patients into account, 10–20% have equinus foot as a severe consequence and >90% of patients with

RA have reported foot complaints during the course of the disease such as hallux valgus (65%), longitudinal arch (42%) flattening, and claw toe (39%) (3–7). This is one of the most dysfunctional deformities with a significant impact on gait and quality of life of individuals who have suffered from stroke or RA (5, 6, 8–12). The possible treatment for these dysfunctions/deformities is the use of ankle–foot orthoses (AFOs) or, in severe cases, surgical intervention. AFOs are external biomechanical devices capable of improving the gait and physical functioning of the affected lower limb.

Traditionally, the AFOs are chosen based on the therapist's knowledge and clinical experience, patient's needs, or the qualitative analysis of the patient's gait (13). Moreover, the guidelines for AFO prescription provide a general recommendation and are not specific for each type of orthosis. For that reason, current clinical criteria for choosing a particular AFO are limited and subjective (14). This has a huge impact on a patient's gait performance while using the device (13).

Currently, there is a considerable research gap regarding the complete analysis of gait pattern, including the quantitative assessment of spatiotemporal, kinematic, and kinetic parameters, at the same time, for each patient during the use of AFO. Moreover, research is also missing about which orthosis best fits the functional needs of each subject (9, 15). Efficacy studies of AFOs to promote walking ability should be developed, and they will support physicians/therapists to make more precise and reliable decisions on the rehabilitation process (13). Therefore, this paper presents the OrthoRehab—a clinical decision tool that supports physicians/therapists to define AFO for a patient with foot dysfunctions during the rehabilitation process. Thus, the main goals of this paper are (1) to describe the development process of the OrthoRehab and (2) to present the results of its application in the clinical environment.

MATERIALS AND METHODS

OrthoRehab was designed and developed for a multidisciplinary team, composed of physicians, therapists, and biomedical engineers from Rehabilitation Medicine Center of Alcoitão (CMRA) and NOVA University of Lisbon. This study was approved by the Portuguese Ethics Committees of this Center.

Regarding the methodology used for the development of the OrthoRehab, the following steps were performed with the contribution of all team members:

1. Choice of the requirements of the platform
2. A decision on what gait parameters should be analyzed
3. Division of these parameters by categories: spatiotemporal, kinematic, and kinetic
4. Structuring the platform according to the categories defined in the previous step
5. Defining the calculation of the parameters under analysis
6. Programming the graphical interface.

In order to define the requirements, we asked potential end users, including the physicians and therapists who participated in this project, what facilities they thought would be important for the platform to have (16). The results of this consultation allowed identifying the following specific needs:

- easy to use: the tool should be quick to learn, and physicians/therapists should be able to use it
- should be in digital format
- facilitate the analysis of the parameters
- provide reports
- be compatible with VICON NEXUS gait analysis files.

A platform (OrthoRehab) was developed to meet these requirements. The graphical user interface was developed using MATLAB 2014b® software. This allowed the introduction of VICON NEXUS 1.8.5® (software used for gait acquisitions in this study) files and performing the analysis of the spatiotemporal, kinematic, and kinetic parameters.

Thus, the platform OrthoRehab was organized in the following sections:

1. Import/export files (file compatibility)
2. Calculation of parameters
3. Visualization of results
4. Report creation.

Import/Export Files (File Compatibility)

The data acquisition is carried out using the software VICON NEXUS 1.8.5® adapted to a computer. Since this new platform was designed to be compatible with this system, it does not increase any workload on clinicians other than importing the generated files into the developed graphical interface. After the introduction of the files in the software, the data are studied and analyzed. Finally, the outputs were saved in a database.

Although the VICON NEXUS 1.8.5® software already analyzes all gait parameters, it considers only one gait cycle, in one condition (with or without orthoses), therefore being too general and unspecific.

Calculated Parameters

The analyzed parameters, divided into spatiotemporal, kinematic, and kinetic (**Table 1**), were chosen in partnership with the team (physicians and therapists) from of the CMRA and according to the methodology used by Boudarham (7), Kinsella (17), and Manca (18).

First of all, each condition (with and without orthosis) is verified whether the gait tests have adequate dynamic data of both lower limbs, that is, if there is at least a gait cycle without artifacts on the force platforms. Then, all the parameters listed below (**Table 1**) are calculated for each condition and for each gait cycle, and the average value of the parameters is determined for each condition. Finally, all values and curves are shown in the graphical interface corresponding to the average of all running cycles for the lower limb under analysis.

Spatiotemporal Analysis

The spatiotemporal parameters analyzed in each gait cycle are those related to time intervals, distances, velocity, cadence, and respective asymmetries. These asymmetries correspond to the differences in the values of each lower limb.

TABLE 1 | Analyzed spatiotemporal, kinematic, and kinetic parameters.

Spatiotemporal parameters	Step time and their asymmetry (s)	
	Step length and their asymmetry (m)	
	Step width and their asymmetry (m)	
	Velocity and global velocity (average velocity between both lower limbs) (m/s)	
	Cadence and their asymmetry (number of steps per minute)	
	Stance phase and their asymmetry (% gait cycle)	
	Swing phase (% gait cycle)	
	Single support phase and their asymmetry (% gait cycle)	
Kinematic parameters	Ankle joint's sagittal plane	Foot strike (°)
		Dorsiflexion's maximum (°)
		Plantar flexion's maximum (°)
	Ankle joint's coronal plane	Foot strike (°)
		Contralateral limb's foot off (°)
	Vertical extension between maximum and minimum of pelvis's sagittal plane (°)	
Kinetic parameters	Vertical ground reaction force	First maximum (N/kg)
		Second maximum (N/kg)
	Maximum of ankle joint's power (W/kg)	

For all conditions under analysis, each spatiotemporal parameter is calculated, and its values are firstly compared with normative ones. Afterward, the condition in which the patients show the best performance (i.e., higher velocities and lower asymmetries, is highlighted).

Kinematic Analysis

The kinematic analysis curves correspond to the average value over the gait cycles of the angles of movement by the percentage of each cycle.

The kinematic parameters analyzed by the OrthoRehab are:

- Sagittal plane of the tibiotarsal joint—defined by the points: initial contact, maximum dorsiflexion, and maximum plantar flexion
- Frontal plane of the tibiotarsal joint—defined by the points: initial contact and the release of the fingers of the contralateral lower limb
- Sagittal plane of the pelvis—defined by the vertical extension between the maximum and minimum points.

For ankle joint and ground reaction force, the developed interface shows charts with angle and force curves, respectively, for at most three conditions (without orthosis and with two different types of orthoses, generically denominated A and B). The kinematic analysis identifies which condition (without orthosis, with orthosis A or orthosis B) allows an increase in dorsiflexion amplitude, a decrease in the varus, and a lower energy expenditure of the gait of the individuals under analysis.

Kinetic Analysis

The kinetic parameters analyzed by the program are those related to force and energy, namely, vertical ground reaction force and a maximum of ankle joint's power.

The dynamic parameters analyzed by the program are:

- The vertical reaction force of the soil—the analyzed points are the first and second maxima
- Strength of the tibiotarsal joint—the point analyzed is the maximum of the curve that occurs just before the foot leaves the ground (of the lower limb under analysis). The purpose of studying the vertical force of the ground reaction is to assess the individual's ability to exert force on himself.

The dynamic analysis curves correspond to the mean value of the force per percentage of support phase and power and per cycle percentage. The vertical extension of the pelvis and the ankle joint force values are calculated for the three conditions and the lowest and highest values, respectively, are selected and highlighted. The values are presented in tables.

Graphical User Interface Visualization of Results

Since the visualization of the results is an essential issue for the usability of the interface, the graphical interface architecture and the functionalities were defined with physicians and therapists using a co-creation methodology.

Interface Design Requirements

The graphical interface was developed to fulfill the following requirements:

- Utility and functionality—should perform the calculations on the parameters of interest and functionally presents them
- Sequential and intuitional—all relevant information should be distributed in a simple, sequential, and intuitive way
- Innovative—an original tool that allows a comparative analysis between clinical results obtained for different types of orthoses
- Without additional work for physicians and therapists.

Graphical Interface Architecture

The graphical interface developed has an architecture that provides the following main sections: Main Menu, Import Files, and three tabs for data analysis: Space–Time Analysis; Kinematic Analysis; Dynamic Analysis.

The parameters under analysis are shown in tables and graphs. The program starts when the Main Menu window (**Figure 1**) opens. This window allows the user to introduce the data of each patient and import the files to be analyzed. After the files are imported, it is possible to access the three available types of analysis. Also, a support manual was also prepared to understand the design of the program, as well as all its functionalities.

Functionalities

The graphic interface developed has the following functionalities:

- Ability to read files in CSV format produced by VICON NEXUS 1.8.5® software

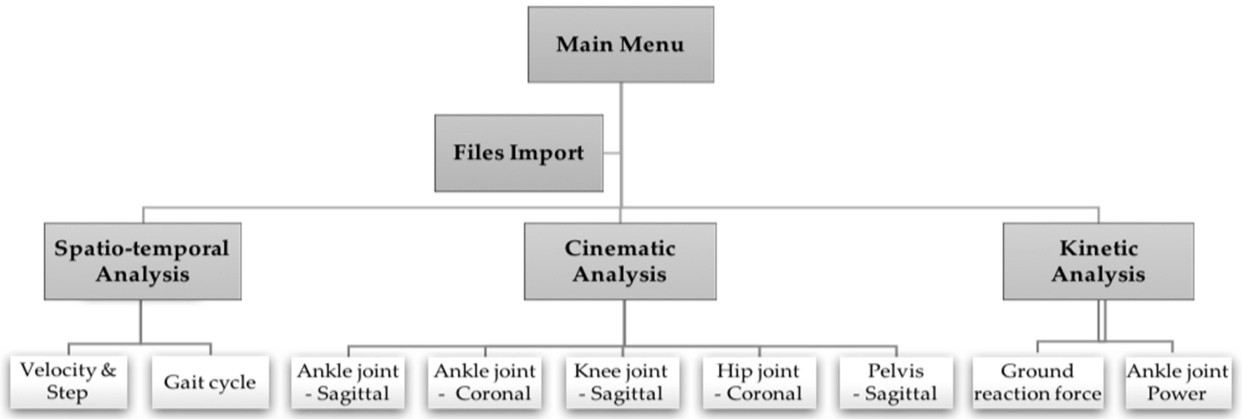

FIGURE 1 | Program structure.

- Perform spatiotemporal, kinematic, and kinetic analyses in three possible conditions: without orthosis, with orthosis A and with orthosis B—although the program is idealized to analyze three conditions, it is possible to perform the analyses with only two conditions under study
- Possibility to analyze one or both lower limbs
- Show the mean value of the parameters under analysis
- Present in the form of a table or graph the parameters analyzed for both lower limbs in the different conditions
- Highlight values that are closer to the normative standard (for example, higher speed) whenever possible
- Selection of the name of the dynamic orthoses under study, with no restriction for the type of the orthoses
- The option of activation of the non-pathological pattern in the graphs
- Graphical interactivity—zoom in and zoom out of the graphs, as well as display coordinates of points of the curves by placing the cursor
- Data recording capability
- Creation of a database (XLSX format) organized with all the parameters of interest
- Automatic addition of data from individuals to the database.

PILOT STUDY

The OrthoRehab tool was tested in three participants (two females and one male) with equinus foot dysfunction caused by a stroke (left hemiplegia). None of the subjects had previous experience with any of the orthoses under test (**Table 2**).

The inclusion criteria are the diagnosis of stroke with injury only in the right hemisphere (ability to understand and speech not compromised); equine foot, with dorsiflexion of the tibiotarsal joint up to 0° passively and modified Ashworth scale with scores: 0, 1, 1+, or 2; age between 55 and 65 years (excluding young strokes and degenerative motor disorders characteristic of older ages); ability to carry out independent walking by third parties in the minimum distance of 10 m, being allowed the use of a walking aid; ability to walk with shoes but without orthosis; the initial phase of training with orthosis: five training sessions or <1 week of use.

TABLE 2 | Participant's data.

Subject	1	2	3
Gender	Male	Female	Female
Age (years)	47	40	67
Height (±0.05 cm)	179.50	163.00	149.00
Body mass (±0.1 kg)	70.4	67.0	65.0
Time since stroke (days)	95	420	62
Ankle joint tone (1–4)	2	1	1
Passive joint range (Modified Ashword Scale)	Ankle joint dorsiflexion: +- 0"	No limitations	Ankle joint dorsiflexion: +- 0"
FAC - functional ambulatory category (1–6)	1	3	3
Dynamic balance in standing position (0–56) (Berg Balance Scale)	15	48	45

Procedure

Each subject was previously informed about the procedures and the objectives of the study and signed an informed consent.

The gait acquisitions were performed in the Biomechanics Laboratory of CMRA with controlled conditions of temperature and light and a regular floor. The acquisition procedure was performed in four steps:

1. Measurement of anthropometric parameters such as weight, height, distance between iliac crests, leg length, knee width, and ankle width
2. The software requires the placement of 16 reflective markers (eight on each lower limb) in specific anatomical places according to standard protocols for a lower body motion

analysis using the Vicon Plug-In Gait Model. In total, 16 reflective markers are placed, eight on each lower limb. These are placed in medial and lateral locations of the joints that are considered anatomical landmarks (19, 20).
3. The participants, wearing shoes, walked along the force platforms at a speed that they considered comfortable in the three conditions: (I) with no orthosis; (II) with orthosis A (posterior support); (III) with orthosis B (anterior support).
4. Point 3 was repeated two more times for each condition.

Kinetic data were collected with four force platforms from Advanced Mechanical Technology, Inc., AMTI OR6-7-2000 (50.8 × 46.4 cm) with four analogical amplifiers AMTI that were longitudinally oriented and embedded flush with the ground. Kinematic data were collected by six infrared cameras VICON T-Series T10 (1 megapixel). Additionally, there were two digital video cameras Basler piA1000-48gc GigE. All the equipment was connected to VICON NEXUS 1.8.5® software that allowed the simultaneous collection of kinematic and kinetic data of the gait of the subjects under analysis at a frequency of 100 Hz for infrared cameras and 1,000 Hz for force platforms.

RESULTS

In order to demonstrate the feasibility of OrthoRehab, as mentioned above, it was applied to three patients by a physician and a therapist. In this section, we present the end users' comments and the results from the pilot study.

End Users' Comments

The physician and the therapist who applied the OrthoRehab tool, during the proof-of-concept process, indicated that the tool is easy to be applied and it can support them to choose the most suitable type of orthosis according to the patient's needs. Additionally, the users mentioned that OrthoRehab can be used without workload and provide an evaluation that they cannot have in a conventional approach. Moreover, a high level of consensus was found with regard to whether there was a clinical need for the proposed new tool.

There was, also, a high level of agreement that the key to the success of the OrthoRehab was including physicians and therapists in the co-creation of this tool.

OrthoRehab Application

The analysis was performed only in the gait cycles of the left lower limb (affected limb) in the three conditions using OrthoRehab tool.

The average values, taking into account the three participants and the three trials performed for each one of them, were calculated.

Spatiotemporal Parameters

For velocity analysis, the highest value was selected. All participants presented values that were much lower than those referred to as non-pathological: 1.3 m/s (19). However, on average, the highest velocity was observed when the patients were using orthosis B.

Regarding the asymmetry of the cadence, the results show that A is the orthosis that causes the lowest values, while orthosis B reduced the asymmetry of time and step width. For step length, none of the orthoses proved to be efficient.

To reduce stance phase asymmetry, B is the orthosis that shows more benefits. To decrease the single support phase asymmetry, the best choice is A. In all participants, an increase of stance phase and a decrease of single support phase were also verified for both orthoses.

Kinematic Parameters

Maximum dorsiflexion and maximum plantar flexion of the sagittal plane of the ankle joint are taken into account to evaluate the increase of dorsiflexion. The gait performance was considered improved when the values of interest taken from the curves with orthosis are higher when compared with the values taken from the curves without orthosis.

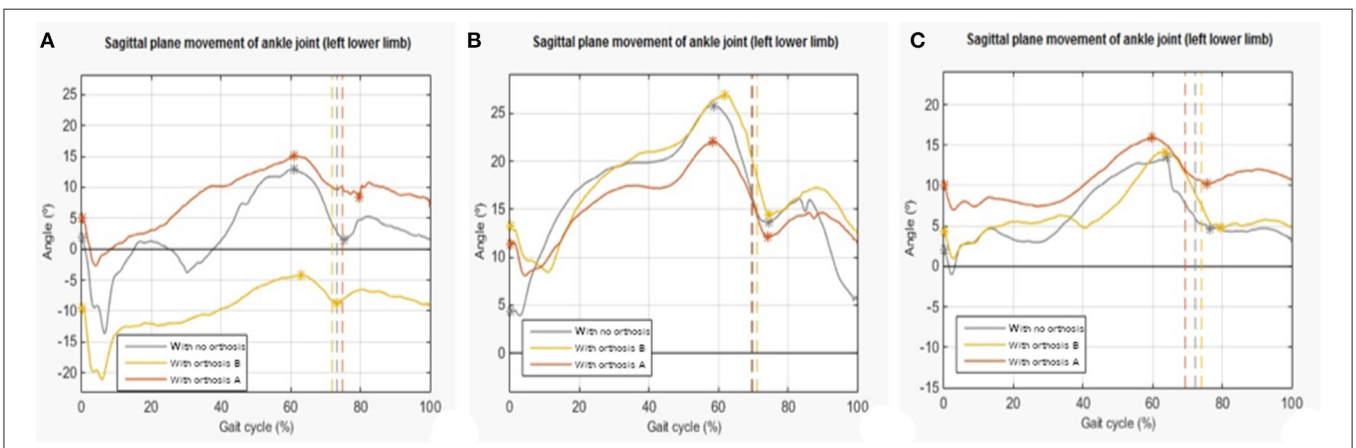

FIGURE 2 | Examples of plots representing the sagittal plane movement of the ankle joint of the pathological lower limb in the three conditions in the analysis. **(A)** Trial 1 of subject 1. **(B)** Trial 1 of subject 2. **(C)** Trial 1 of subject 3. The vertical line presents the contact phase, and the * is the higher value.

The angular results show that, on average, A is the orthosis that increases dorsiflexion [an increase of 6.133° (±0.001°) in foot impact, 0.614° (±0.001°) in maximum dorsiflexion, and 3.805° (±0.001°) in maximum plantar flexion]. Only subject 2 shows different results (**Figure 2**).

The increase in the stance phase was evaluated in order to analyze temporal behavior. The results obtained show that, on average, the use of orthoses does not improve temporal variation.

For angular analysis of foot impact and contralateral raised foot in the coronal plane of the ankle joint, we evaluated the increase in the *varus*.

The angular results show that, on average, both orthoses can decrease the characteristic *varus* of equinus foot dysfunction. However, the orthosis that shows quantitatively more significant improvement is B [an increase of 0.768° (±0.001°) in foot impact and 0.394° (±0.001°) on raised foot of contralateral limb]. Once again, subject 2 shows different results (**Figure 3**).

About the temporal variation, the methodology used is the same as the one used for the sagittal plane. Once again, the results show that, on average, the use of orthosis does not improve temporal variation.

The vertical extension of the angular pelvis in the sagittal plane is directly related to the gait's energy expenditure. We look for the lowest values of extension, since they mean lower energy expenditure and consequently more efficient gait. Although the data show that both orthoses impart a beneficial effect, on average, the A orthosis produces better results.

Kinetic Parameters

Concerning the ground reaction force, the higher force produced better performance. When the first and the last peaks were analyzed, on average, only A shows positive results in both peaks.

Regarding temporal variation, we can say that there is an improvement in the first peak of the curve if it is on the left of the corresponding peak without orthosis, and there is an improvement in the second peak if it is on the right. This criterion is adopted because it is verified, in the analyzed cases, that the first peak occurs later and the second peak occurs earlier relative to non-pathological behavior. The results show that, on average, no orthosis improves the first peak temporally and A shows benefits in the second peak (**Table 3**). It is also observed that the temporal relationship between the first and the second peak is much lower than the standard value of reference: 60% (21).

For the maximum force of the ankle joint, which occurs immediately before the raised foot, the results show that, on average, none of the orthoses can improve this parameter.

DISCUSSION/CONCLUSIONS

In this paper, we present the development of a decision support tool called OrthoRehab, as well as the steps involved in this process. Performance of the tool was evaluated in a clinical environment. Regarding the usability of the tool, the clinical team considered that OrthoRehab was easier to use without workload as well as user-friendly.

TABLE 3 | Mean values of the affected lower limb at the moment when the two peaks of reaction force of the soil occur.

		Average value (±0.01% support phase)		Normative reference value [17]
		Subject 1	Subject 2	
1st Peak	With no orthosis	48.01	42.37	20%
	Orthosis B	50.28	47.47	
	Orthosis A	49.79	50.25	
2nd Peak	With no orthosis	64.74	58.25	90%
	Orthosis B	67.59	56.00	
	Orthosis A	66.64	58.00	
Difference between the 1st and 2nd peak	With no orthosis	16.73	15.88	60%
	Orthosis B	17.31	8.52	
	Orthosis A	16.85	7.75	

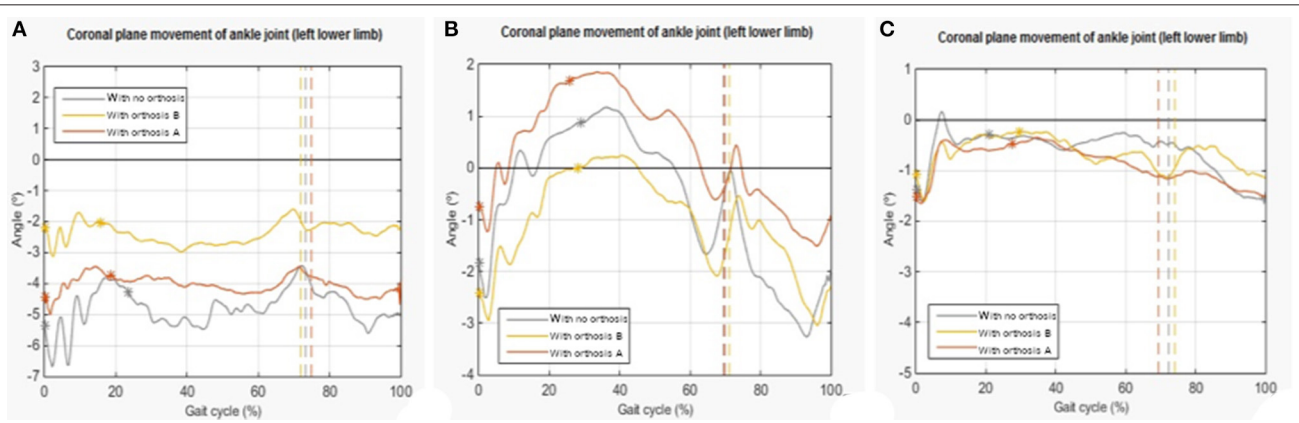

FIGURE 3 | Examples of plots representing the ankle joint's coronal plane of the pathological lower limb in the three conditions in the analysis. **(A)** Trial 1 of subject 1. **(B)** Trial 1 of subject 2. **(C)** Trial 1 of subject 3. The vertical line presents the contact phase, and the * is the higher value.

OrthoRehab provides simultaneous analysis for each subject in three conditions: with no orthosis and with two different AFOs. This tool gives a comparative and quantitative analysis of the most relevant gait parameters for foot dysfunction. Therefore, OrthoRehab contributes to the development of clinical plans for each subject with this dysfunction and, more specifically, it helps with the correct dynamic AFOs prescription.

The tool development methodology began with the decision about what were the most valuable kinematic and kinetic parameters for analysis of foot dysfunction and their distribution categories. This allowed developing a software that analyzes these parameters and plots them using an appropriate graphical interface. These two items (parameters and plots) provide physicians and therapists a global, integrated, and innovative evaluation of patient's gait in relation to the orthoses under study.

OrthoRehab was applied in a real clinical context, and it proved to be a reliable and suitable tool, fulfilling the objectives that were established.

OrthoRehab will help physicians and therapists in making a better, personalized, and reasoned decision about the AFO prescription. This tool can be useful in different clinical areas such as rheumatology, ortho-traumatologic, and also in populations of varying ages, since it can be applied to any person who has been prescribed a lower limb orthosis. Additionally, the OrthoRehab can be adapted to be applied to other dysfunctions/deformities and/or with different orthoses.

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 Rehabilitation Medicine Center of Alcoitão. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CQua, BL, JJ, and CQui contributed to the conception, design of the study, and participated in drafting the final manuscript. BL performed the tool and analyzed the data. All authors contributed to the conception, design of the review, contributed to manuscript revision, read, and approved the submitted.

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REFERENCES

1. World Health Organization. 2008–2013 action plan for the global strategy for the prevention and control of non-communicable diseases. Prevent and control cardiovascular diseases, cancers, chronic respiratory diseases diabetes. (2008). Available online at: https://apps.who.int/iris/bitstream/handle/10665/44009/9789241597418_eng.pdf?sequence=1 (accessed May 22, 2020).
2. Daïen D, Tubery A, Beurai-Weber M, Caila G, Picot M, Jaussent A, et al. Relevance and feasibility of a systematic screening of multimorbidities in patients with chronic inflammatory rheumatic diseases. *Joint Bone Spine*. (2019) 86:49–54. doi: 10.1016/j.jbspin.2018.03.016
3. Trieb K. Management of the foot in rheumatoid arthritis. *J Bone Joint Surg Br*. (2005) 87:1171e1177. doi: 10.1302/0301-620X.87B9.16288
4. Yano K, Ikari K, Inoue E, Sakuma Y, Mochizuki T, Koenuma N, et al. Features of patients with rheumatoid arthritis whose debut joint is a foot or ankle joint: a 5,479-case study from the IORRA cohort. *PLoS ONE*. (2018) 13:e0202427. doi: 10.1371/journal.pone.0202427
5. Mochizuki T, Yano K, Ikari K, Okazaki K. Effect of an orthosis on foot center of pressure translation for treatment of hallux valgus in patients with rheumatoid arthritis: a report of 17 cases. *Asia-Pacific J Sports Med Arthroscopy Rehabil Technol*. (2020) 19:7e10. doi: 10.1016/j.asmart.2019.10.003
6. Son SM, Park IS, Yoo JSJ. Short-term effect of botulinum toxin an injection on spastic equinovarus foot in cerebral palsy patients: a study using the foot pressure measurement system. *Ann Rehabil Med*. (2015) 39:1. doi: 10.5535/arm.2015.39.1.1
7. Boudarham J, Pradon D, Roche N, Bensmail D, Zory R, et al. Effects of a dynamic-ankle-foot orthosis (Liberte®) on kinematics and electromyographic activity during gait in hemiplegic patients with spastic foot equinus. *NeuroRehabilitation*. (2014) 35:369–79. doi: 10.3233/NRE-141128
8. de Quervain IAK, Simon SR, Leurgans S, Pease WS, McAllister D, et al. Gait pattern in the early recovery period after stroke. *J Bone Joint Surg Am*. (1996) 78:1506–14. doi: 10.2106/00004623-199610000-00008
9. Bollens B, Gustin T, Stoquart G, Detrembleur C, Lejeune T, Deltombe T, et al. A randomized controlled trial of selective neurotomy vs. botulinum toxin for spastic equinovarus foot after stroke. *Neurorehabil Neural Repair*. (2013) 27:695–703. doi: 10.1177/1545968313491002
10. Cinone N, Letizia S, Santoro L, Facciorusso S, Armiento R, Picelli A, et al. Combined effects of isokinetic training and botulinum toxin type A on spastic equinus foot in patients with chronic stroke: a pilot, single-blind, randomized controlled trial. *Toxins*. (2019) 11:210. doi: 10.3390/toxins11040210
11. Sung, K, Chung C, Lee K, Kwon K, Lee JH, Park MS, et al. Discrepancy between true ankle dorsiflexion and gait kinematics and its association with severity of planovalgus foot deformity. *BMC Musculoskeletal Disord*. (2020) 21:250. doi: 10.1186/s12891-020-03285-3
12. Jafarian F, Payehdar S, Forghany S. Effects of ankle foot orthoses on restricted ankle joint dorsiflexion due to plantarflexors stiffness in people with stroke: a scoping review. *J Rehabil Sci Res*. (2019) 6:59–62. doi: 10.30476/jrsr.2019.75366
13. Totah D, Menon M, Jones-Hershinow C, Barton K, Gates DH. The impact of ankle-foot orthosis stiffness on gait: a systematic literature review. *Gait Posture*. (2019) 69:101–11. doi: 10.1016/j.gaitpost.2019.01.020
14. Chisholm A, Perry S. Ankle-foot orthotic management in neuromuscular disorders: recommendations for future research. *Disabil Rehabil*. (2012) 7:437–49. doi: 10.3109/17483107.2012.680940
15. Kobayashi T, Leung AK, Akazawa Y, Hutchins SW. Correlations between Berg balance scale and gait speed in individuals with stroke wearing

ankle–foot orthoses–a pilot study. *Disabil Rehabil Assist Technol*. (2016) 11:219–22. doi: 10.3109/17483107.2014.932019
16. Barrett LF, Barrett DJJSSCR. An introduction to computerized experience sampling in psychology. *Soc Sci Comput Rev*. (2001) 19:175–85. doi: 10.1177/089443930101900204
17. Kinsella S, Moran KJG. Gait pattern categorization of stroke participants with equinus deformity of the foot. *Gait Posture*. (2008) 27:144–51. doi: 10.1016/j.gaitpost.2007.03.008
18. Manca M, Ferraresi G, Cosma M, Cavazzuti L, Morelli M, Benedetti MG. Gait patterns in hemiplegic patients with equinus foot deformity. *Adv Neuromotor Stroke Rehabil*. (2014) 2014:939316. doi: 10.1155/2014/939316
19. Richards J. *Biomechanics in Clinic and Research*. London: Churchill Livingstone (2008).
20. Vicon Motion Systems Limited. *Plug-inGait: ProductGuide—Foundation Notes*. (2010). Available online at: https://www.vicon.com/downloads/documen-tation/vicon-documentation/plug-in-gait-product-guide (accessed June 30, 2016).
21. Ferreira LAB, Neto HP, Christovão TCL, Duarte NAC, Lazzari RD, Galli M, et al. Effect of ankle-foot orthosis on gait velocity and cadence of stroke patients: a systematic review. *J Phys Therapy Sci*. (2013) 25:1503–8. doi: 10.1589/jpts.25.1503

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ORIGINAL RESEARCH
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Establishing Classification Tree Models in Rheumatoid Arthritis Using Combination of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and Magnetic Beads

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Background: There is no simple method for early diagnosis and evaluation of rheumatoid arthritis (RA). This study aimed to determine potential biomarkers and establish diagnostic patterns for RA using proteomic fingerprint technology combined with magnetic beads.

Methods: The serum protein profiles of 97 RA patients and 76 healthy controls (HCs) were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) with weak cationic exchange (WCX) magnetic beads. Samples were randomly divided into training (83 RA patients and 56 HCs) and test sets (14 RA patients and 20 HCs). Patients were classified according to their Disease Activity Score: in remission, $n = 28$; with low disease activity, $n = 17$; with moderate disease activity, $n = 21$; with high disease activity, $n = 31$. There are 44 RA patients alone, 22 RA patients with interstitial lung disease (RA-ILD), 18 RA patients with secondary Sjögren's syndrome (RA-sSS), 6 RA patients with osteonecrosis of the femoral head (RA-ONFH), and 7 RA patients with other complications. Eleven patients were treated with etanercept only for half a year, after which their serum protein profiles were detected. The proteomic pattern was identified by Biomarker Patterns Software, and the potential biomarkers for RA diagnosis were further identified and quantified by enzyme-linked immunosorbent assay.

Results: The diagnostic pattern with four potential protein biomarkers, mass-to-charge (m/z) 3,448.85, 4,716.71, 8,214.29, and 10,645.10, could accurately recognize RA patients from HCs (specificity, 91.57%; sensitivity, 92.86%). The test set were correctly classified by this model (sensitivity, 95%; specificity, 100%). The components containing the four biomarkers were preliminarily retrieved through the ExPasy database, including the C-C motif chemokine 24 (CCL24), putative metallothionein (MT1DP), sarcolipin (SLN), and C-X-C motif chemokine 11 (CCXL11). Only the CCL24 level was detected to have a significant decrease in the serum of RA patients as compared with HCs ($p < 0.05$). No significant difference was found in others, but a decreasing trend

consistent with the down-regulation of the four biomarkers detected by MALDI-TOF-MS was observed. The diagnostic models could effectively discriminate between RA alone and RA with complications (RA-ILD: m/z 10,645.10 and 12,595.86; RA-sSS: m/z 6,635.62 and 33,897.72; RA-ONFH: m/z 2,071.689). The classification model, including m/z 1,130.776, 1,501.065, 2,091.198, and 11,381.87, could distinguish between RA patients with disease activity and those in remission. RA with low disease activity could be efficiently discriminated from other disease activity patients by specific protein biomarkers (m/z 2,032.31, 2,506.214, and Z9286.495). Two biomarkers (m/z 2,032.31 and 4,716.71) were applied to build the classification model for RA patients with moderate and high disease activities. Biological markers for etanercept (m/z 2,671.604064, 5,801.840579, 8,130.195641, and 9,286.49499) were observed between the responder ($n = 7$) and non-responder groups ($n = 4$) ($p < 0.05$).

Conclusion: We successfully established a series of diagnostic models involving RA and RA with complications as well as assessed disease activity. Furthermore, we found that CCL24 may be a valuable auxiliary diagnostic indicator for RA. These results provide reference values for clinical practice in the future.

Keywords: rheumatoid arthritis, MALDI-TOF-MS, weak cationic exchange magnetic beads, biomarkers, classification tree model

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune and inflammatory disease characterized by synovitis and vasculitis, which lead to the destruction of cartilage, joint deformation, loss of joints function, and systemic organ damages, and it affects approximately 0.5–1% of population (1). Pulmonary involvement is a common extraarticular manifestation of RA (2), particularly interstitial lung disease (ILD), which occurs in 1–58% of RA patients and has significant effect on morbidity and mortality (3, 4). In addition, both secondary Sjögren's syndrome (sSS) (5, 6) and osteonecrosis of the femoral head (ONFH) in RA have a pooled prevalence of 19.5 and 5.75–53.8%, respectively (7, 8).

Diagnosis of RA according to the American College of Rheumatology (ACR) criteria in 1987 is based on clinical symptoms, presence of rheumatoid factor (RF), and imaging tests; however, such criteria are not suitable for early-stage RA patients. Compared with the 1987 ACR diagnosis guideline, the sensitivity of the ACR/2010 European League Against Rheumatism (EULAR) classification standard is higher mainly due to the addition of anti-citrullinated protein antibodies. However, the specificity of anti-citrullinated protein antibodies in RA is only 60–75%, which suggests low diagnostic efficacy (9). A population-based incidence study revealed that the incidence of RF-negative RA has significantly increased and RF-positive RA has significant decreases (10). These can lead to difficulty in early diagnosis. Furthermore, disease activity is evaluated by the Disease Activity Score (DAS) tool and others, which are complex, inconvenient, or maybe not suitable for general clinical practice. High-resolution computer tomography, which is used for diagnosing early possible complications such as ILD (11), is expensive and has radiation risk (12). Although magnetic resonance has been demonstrated to be a useful imaging test in diagnosing ONFH, it is also expensive (13, 14). Meanwhile, due to low sensitivity and specificity of anti-Ro and anti-La antibodies and considering the invasiveness of lip biopsy, diagnosis of early RA-sSS is difficult (15, 16). Therefore, there has been increasing interest in identifying specific and powerful biomarkers for both the diagnosis of RA, RA-ILD, RA-sSS, and RA-ONFH and the evaluation of disease activity in order to increase the diagnostic efficiency and early treatment.

Early intervention ($\leq$3 months) using biologic agents was the strongest predictor of successful remission, as confirmed in clinical practice. However, clinical applications of biological agents have been limited by their expensive cost (17). Therefore, it is important to explore promising biomarkers to evaluate the potential clinical effects of etanercept and determine which patient would benefit the most, with the possibility of modulating treatment for RA patients who are not responding to the drug in order to reduce their economic burden.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (18) is currently one of the most important and key proteomic technologies (19). It can be used to make high-throughput protein analysis of large samples, and it has high sensitivity, resolving capability, and reproducibility (20). Weak cation exchange (WCX) magnetic beads (21) use their large surface to capture proteins and small molecular peptides of interest (22). The use of MALDI-TOF-MS and WCX magnetic beads, especially combined with bioinformatics tools, is appropriate for setting up the classification tree model to assist in preliminary biomarker discovery (23).

Many researchers have already applied MALDI-TOF-MS to generate protein fingerprints and build serological classification tree models in certain diseases, including rheumatic diseases [early RA (24), RA (25), systemic lupus erythematosus (26), and SS (27)] and other diseases (28, 29), and these were highly effective in discriminating patients and controls (30). However, these studies only built the diagnostic model for RA patients, but they did not have database retrieval, and some studies lacked further validation for the classification tree models. This study aimed to detect a series of specific proteomic diagnostic model for RA, RA-ILD, RA-sSS, and RA-ONFH as well as the potential biomarkers to distinguish the RA disease activity and to identify etanercept's clinical effect using proteomic fingerprint technology (MALDI-TOF-MS) combined with WCX magnetic beads. For the potential protein for diagnosis of RA, we made a further preliminary retrieval through the ExPasy database and verified these biomarkers by enzyme-linked immunosorbent assay (ELISA).

METHODS

Patients and Healthy Controls

A total of 173 serum samples were collected in our study from May 2015 to July 2017 at Shanxi Bethune Hospital. The study population included 97 RA patients (RA alone, $n = 44$; RA-ILD, $n = 22$; RA-sSS, $n = 18$; RA-ONFH, $n = 6$; RA with other complications, $n = 7$) and 76 healthy controls (HCs). All patients were diagnosed using the 1987 ACR or the 2010 ACR/EULAR criteria. All patients were classified according to their DAS, as calculated from the online Disease Activity Score-28 (DAS28) for Rheumatoid Arthritis with ESR tool based on 28 joints: in remission (DAS28 < 2.6, $n = 28$), with low disease activity ($2.6 <$ DAS28 ≤ 3.2, $n = 17$), with moderate disease activity ($3.2 <$ DAS28 ≤ 5.1, $n = 21$), and with high disease activity (DAS28 > 5.1, $n = 31$). All ILD cases were diagnosed by high-resolution computer tomography of the chest. Meanwhile, all sSS patients were diagnosed based on the American-European Consensus Group classification criteria (2002) and the 2012 ACR classification criteria. ONFH diagnosis was based on the detection of marrow foci with decreased signals on T1-weighted images and the characteristic "double-line sign" on T2-weighted images. None of the patients had any active or latent bacterial, fungal, or viral infection at the time of enrolment. Eleven patients only received the same etanercept treatment after inclusion, and their clinical outcome was assessed at week 24. They never received anti-tumor necrosis factor therapy before then. Their ACR 20/70% improvement criteria (ACR20/70), which were used to determine the therapeutic effects of etanercept, C-reactive protein level, erythrocyte sedimentation rate, and presence of RF were evaluated. Finally, four non-responders (as defined by ACR20 negative) and seven responders (ACR70 positive) to etanercept at week 24 were evaluated in our research.

We use two cohorts to build and test the RA diagnostic model. Cohort 1 (training set) included 83 RA patients and 56 HCs to establish a serological classification tree mode to distinguish them. Cohort 2 (blinded testing set) included 14 RA patients and 20 healthy individuals to test the classification efficiency of this RA diagnosis model. The detailed clinical and demographic features of the study subjects are provided in **Table 1**. Furthermore, we used serum samples from RA patients ($n = 22$) and HCs ($n = 22$) for ELISA to verify the results of MALDI-TOF-MS.

TABLE 1 | Clinical and demographic characteristics of patients and healthy controls.

Characteristic	RA	HCs
Age (years,'x ± s)	56 ± 13	53 ± 12
Sex (male/female)	68\29	57\19
Disease duration (M (Q1, Q3), months)	84 (7,120)	—
Tender joint counts (M (Q1, Q3), numbers)	4 (0,24)	—
Swollen joint counts (M (Q1, Q3), numbers)	2 (0,24)	—
Erythrocyte sedimentation rate (M (Q1, Q3), mm/h)	42 (5,108)	—
C-reactive protein (M (Q1, Q3), mg/dL)	42 (5,108)	—
Rheumatoid factor, positive (%)	59 (71.1%)	—
Anti-CCP antibody, positive (%)	51 (61.4%)	—
DAS28ESR ('x ± s)	4.93 ± 1.30	—
Only Treatment of Recombinant Human Tumor Necrosis Factor-α ReceptorII:IgG Fc Fusion Protein for Injection(%)	11 (11%)	—

TABLE 2 | Biomarker statistics for RA vs. HCs spectra in the decision tree classification.

M/Z	RA (Mean ± SD)	HCs (Mean ± SD)	*P*
10,645.10	10.39 ± 5.81	7.44 ± 5.06	0.002
4,716.71	2.27 ± 1.49	5.92 ± 4.25	0.000
3,448.85	2.37 ± 2.02	3.76 ± 2.10	0.000
8,214.28	1.39 ± 0.83	2.76 ± 1.66	0.000

Sera Collection and Preparation

Blood samples (4 mL) were collected and centrifuged at 3,000 rpm for 5 min at 4°C. All serum samples was divided and immediately stored at 80°C. The collection and analysis interval was within 3 months.

Magnetic Bead–Based Sample Preparation for MALDI-TOF-MS

Serum samples were pretreated with magnetic beads. In brief, 10 μL of each serum sample was mixed with 20 μL of U9 (9 mol urea, 2% CHAPS) in a 0.5-mL EP tube. After incubating for 30 min at 4°C, the sample was diluted 1:40 by adding 370 μL of buffer (containing 50 mmol NaAC, pH 4.0). Then, 50 μL of WCX magnetic beads (50 mg/mL) was added to a polymerase chain reaction tube, which was placed in a magnet separator for 1 min, and the supernatant was carefully removed using a pipette. The magnetic beads were then washed twice with 100 μL buffer. A 100-μL diluted serum sample was carefully added and mixed with the activated magnetic beads by pipetting up and down several times; this was, incubated for 1 h at 4°C and washed twice with 100 μL buffer. After binding and washing,

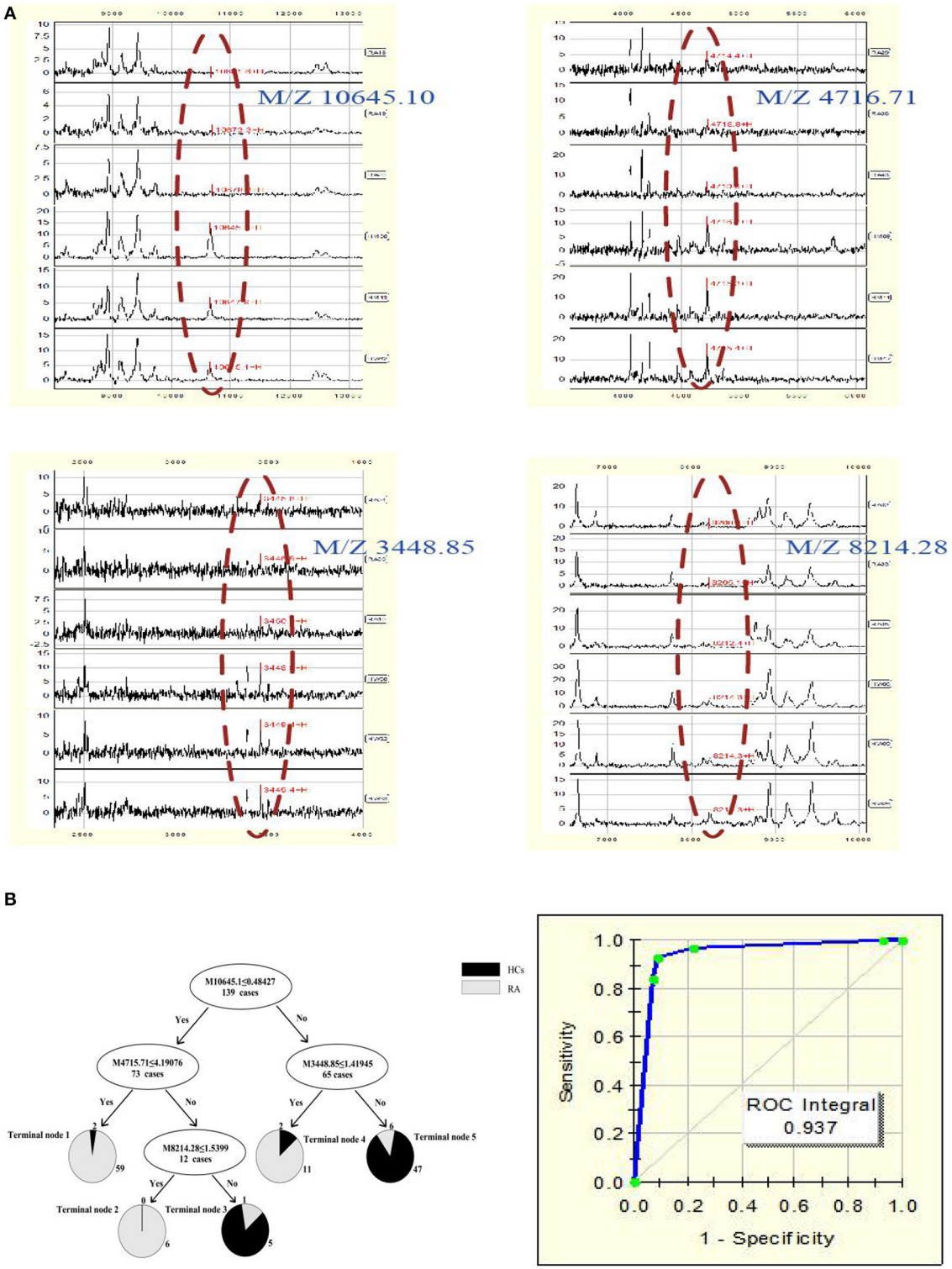

FIGURE 1 | (A) Serum protein/peptide spectrum of RA patients and HCs. **(B)** Dot plots and ROC curve of selected biomarker candidates in patients with RA and healthy controls. The mass-to-charge value in the nodes was followed by lower or equal to intensity value. If the answer to the question in a node of the tree was yes, it proceeded down to the left node, otherwise it proceeded down to the right node. When samples allocated to terminal node 1, 2, and 4 were assigned as RA, whereas those classified into terminal node 3 and 5 were assigned as HCs.

the bound proteins were eluted from the magnetic beads using 10 μL of 0.5% trifluoroacetic acid. Then, 5 μL of the eluted sample was diluted 1:2 in 5 μL of sinapic acid (50% acetonitrile + 0.5% trifluoroacetic acid), and 1 μL of the resulting mixture was aspirated and spotted onto 8 spots of prestructured sample support (Au-chip). After air-drying for approximately 5 min at room temperature, the protein crystal on the chip was detected by MALDI-TOF-MS (PBS IIc; Ciphergen Biosystems, Fremont, CA, USA).

Enzyme-Linked Immunosorbent Assay

The serum concentrations were measured by ELISA using an ELISA kit provided by Xinqaun Company (Taiyuan, China) in accordance with the manufacturer's instructions.

Data Analysis

The data analysis involved three stages: (i) peak detection and alignment; (ii) selection of differently expressed peaks among groups that may represent potential biomarkers of RA, RA-ILD, RA-SS, and RA-ONPH; (iii) data analysis using a decision tree algorithm.

Peak detection was performed using Ciphergen ProteinChip version 3.0.2 (Ciphergen Biosystems). The protein peaks with mass-to-charge (m/z) ranging from 2,000 to 50,000 were selected for analysis, whereas those with m/z ranging between 0 and 2,000 were eliminated from the analysis to avoid interference from adducts, artifacts from energy-absorbing molecules, and other possible chemical contaminants. Peak detection involved (i) baseline subtraction, (ii) mass accuracy calibration, and (iii) automatic peak detection. Using Biomarker Wizard version 3.1.0 (Ciphergen Biosystems), biomarkers that represent consistent protein peak sets across multiple spectra were generated. Baseline subtraction was performed on all spectra. The peak m/z of 4,901 was selected to normalize dimension. The settings for auto-detect peaks to cluster were as follows: first pass: signal-to-noise ratio, 5; minimum peak threshold, 10%; cluster completion: cluster mass window, 0.3%; second pass: signal-to-noise ratio, 2.

Statistical analysis was conducted using SPSS version 18 (IBM Corp., Armonk, NY, USA). Both parametric Student's t test and non-parametric Mann–Whitney U test were applied. Results were considered statistically significant if $p < 0.05$.

RESULTS

Discriminating m/z Peaks Between RA and Control Subjects

Among the m/z 1,000–20,000 peak range, there were a total of 115 differential protein peaks between the RA and HCs groups. Of these peaks, 58 protein peaks statistically differed ($p < 0.05$), including 22 that were overexpressed in RA samples ($p < 0.05$).

Establishment of the Serological Classification Tree Model for RA

RA Patients and HCs

A total of 22 protein peaks were used by the Biomarker Patterns Software (BPS) version 5.0 (Ciphergen Biosystems) to establish the most optimal classification tree based on the lowest error cost of misclassification (represented as relative cost of 0.462). The most optimal tree model consisted of m/z peaks 3,448.85, 4,716.71, 8,214.28, and 10,645.10 (**Table 2**), all of which were down-regulated in patients with RA compared with HCs (**Figure 1A**). The classical protein/peptide spectra of serum sample from RA patients and HCs are shown in **Figure 1A**. All 139 spectra of the training set were differentiated into five terminal nodes (**Figure 1B**). This classification tree had a specificity of 91.57% and a sensitivity of 92.86% for distinguishing between RA patients and HCs (**Table 3**). All 14 RA spectra and 20 HCs in the test set were correctly classified by this model, yielding a sensitivity of 95.00% and a specificity of 100% (**Table 3**). The integral of receiver operating characteristic curve (ROC) of this decision tree supplied by BPS version 5.0 was 0.937 (**Figure 1B**).

TABLE 3 | Prediction success of classification tree model for RA.

Actual class (according decision tree classification class to clinical diagnosis)	Decision tree classification class	
	RA, *n* (%)	HCs, *n* (%)
In the training set		
RA ($n = 83$)	76 (91.57)	7 (8.43)
HCs ($n = 56$)	4 (7.143)	52 (92.86)
In the blinded testing set		
RA ($n = 14$)	14 (100)	0 (0)
HCs ($n = 20$)	1 (5)	19 (95)

ELISA Validation

The components containing four targeted protein peaks of the RA diagnostic model were preliminarily retrieved through the ExPasy database. The proteins of m/z 10,645.10, 4,716.71, 3,448.85, and 8,214.28 might correspond to C-C motif chemokine 24 (CCL24; code: O00175), putative metallothionein (MT1DP; code: A1L3X4), sarcolipin (SLN; code: O00631), and C-X-C motif chemokine 11 (CCXL-11; code: O14625), respectively.

Therefore, the above targeted biomarkers were further identified and quantified through ELISA. A significant decrease was observed in the CCL24 level in the serum of RA patients (75.12 ± 69.59 ng/mL) as compared with HCs (125.3 ± 41.9 ng/mL) ($p < 0.05$) (**Figure 2A**). No significant difference was found in MT1DP, SLN, and CXCL-11 between RA patients and HCs (**Figures 2B–D**). Remarkably, the ELISA results, i.e., the four biomarkers had lower levels in RA patients than HCs, were consistent with the MALDI-TOF-MS results.

Establishment of the Classification Tree Model for Assessing Disease Activity in RA Patients

RA Patients With Remission and Disease Activity

This classification tree model was established from 28 RA patients with remission and 69 disease activity patients and had m/z 1,130.776, 1,501.065, 2,091.198, and 11,381.87 (**Table 4**) and six terminal nodes (**Figure 3A**). It had a sensitivity of 94.12% and a

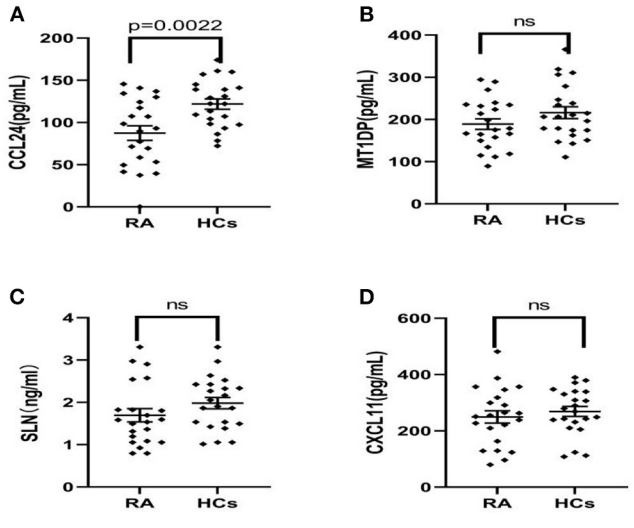

FIGURE 2 | (A–D) ELISA results of CCL24, MT1DP, SLN, and CXCL11 in the serum of RA patients ($n = 22$) and health controls ($n = 22$), indicated the CCL24 level has a significantly decreased expression in the RA serum and other biomarkers had not a difference. Plots indicate individual protein level of each group. Data are presented as mean±SEM. ns, no significant.

TABLE 4 | Biomarker statistics for assessing the activity of disease in RA patients.

M/Z	Remission (Mean ± SD)	Disease activity (Mean ± SD)	*P*
1,130.78	1.01 ± 1.75	1.18 ± 1.54	0.013
1,501.70	1.56 ± 1.79	−0.19 ± 1.26	0.003
2,091.20	3.08 ± 1.34	4.52 ± 2.01	0.025
11,381.87	0.02 ± 0.16	0.16 ± 0.13	0.014
M/Z	**Low disease activity (Mean ± SD)**	**Other disease activity (Mean ± SD)**	***p***
2,013.49	3.71 ± 1.80	5.52 ± 2.59	0.019
8,765.23	4.25 ± 4.48	2.09 ± 1.91	0.026
M/Z	**Moderate disease activity (Mean ± SD)**	**High disease activity (Mean ± SD)**	***P***
2,032.31	1.57 ± 1.44	2.59 ± 1.95	0.047
8,214.28	2.65 ± 1.43	1.83 ± 1.35	0.042

specificity of 93.33% (**Table 5**). The integral of ROC was 0.990 (**Figure 4**).

RA Patients With Low Disease Activity and Other Disease Activity

This classification tree model was built from the serum samples of 17 RA patients with low disease activity and 52 patients with other disease activity and had m/z 2,032.31, 2,506.21, and 9,286.50 (**Table 4**) and five terminal nodes (**Figure 3B**). It had a sensitivity of 86.67% and a specificity of 80.65% (**Table 5**). The integral of ROC was 0.898 (**Figure 4**).

RA Patients With Moderate Disease Activity and High Disease Activity

This classification tree model was established to detect a distinction between RA patients with moderate disease activity and those with high disease activity and had m/z 2,032.31 and 4,716.71 (**Table 4**) and three terminal nodes (**Figure 3C**) It had a sensitivity of 74.19% and a specificity of 76.19% (**Table 5**). The integral of ROC was 0.786 (**Figure 4**).

Discriminating m/z Peaks Between RA and RA With Complications (RA-ILD, RA-sSS, and RA-ONFH)

RA and RA-ILD

A total of 13 protein peaks from serum samples including 22 RA-ILD and 44 RA patients without complications were detected ($p < 0.05$) by MALDI-TOF-MS combined with WCX magnetic beads, which were down-regulated in patients with RA-ILD. We also used BPM5.0 to establish the most optimal tree model to distinguish between RA-ILD and RA without complications, which had m/z 10,645.10 and 12,595.86 (**Table 6**) and included three terminal nodes (**Figure 3D**). It has a sensitivity of 86.36% and a specificity of 84.09%, respectively (**Table 7**). The integral of ROC was 0.856 (**Figure 5**).

RA and RA-sSS

Thirteen proteins from serum samples of 18 RA-sSS and 44 RA patients without complications were detected ($p < 0.05$). All were down-regulated in patients with RA-sSS. We also established the classification tree model by BPM5.0, which comprised m/z 6,635.62 and 33,897.72 (**Table 6**). The RA-sSS classification tree model included 3 terminal nodes (**Figure 3E**). After identification using the model, the patients were classified as having RA and RA-sSS with a specificity of 77.78% and a sensitivity of 79.55% (**Table 7**). The integral of ROC of this RA-sSS decision tree was 0.794 (**Figure 5**).

RA and RA-ONFH

There were two differential protein peaks/spot detected between 6 RA-ONFH and 44 RA patients without complications ($p < 0.05$). They were down-regulated in patients with RA-ONPH and were added into BPM5.0 to generate the classification tree model, which had m/z 2,071.69 (**Table 6**). The classification tree model included two terminal nodes (**Figure 3F**), which had a sensitivity of 66.67% and a specificity of 95.45% (**Table 7**). The integral of ROC was 0.811 (**Figure 5**).

Developing Biological Markers for Etanercept Used in RA Patients

A total of four protein peaks were detected in RA patients between the responder ($n = 7$) and non-responder groups ($n = 4$) ($p < 0.05$), which included m/z 2,671.60, 5,801.84, 8,130.19, and

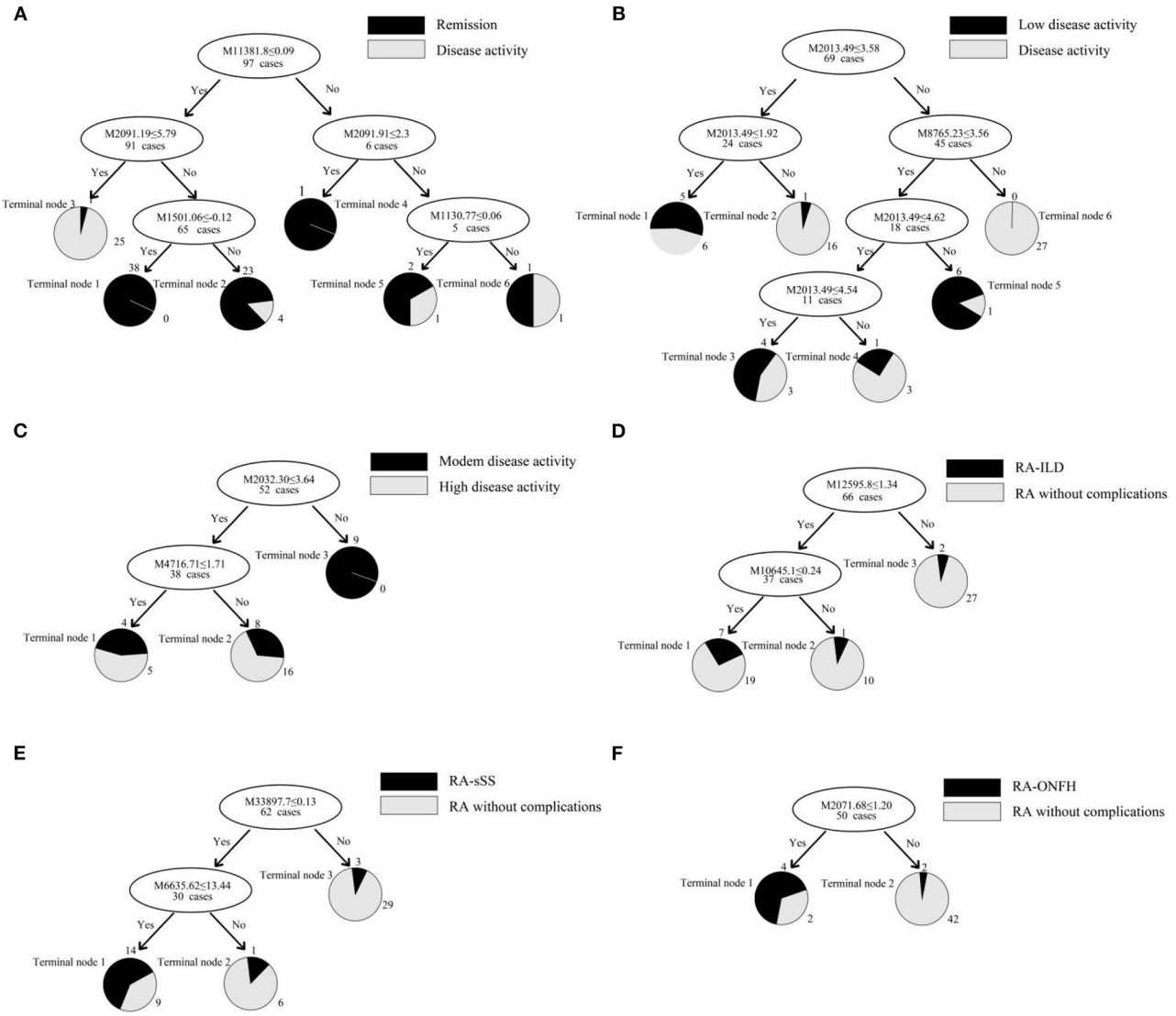

FIGURE 3 | (A–F) Classification tree model for assessing disease activity in RA patients and distinguishing RA patients with complications. The mass-to-charge value in the nodes was followed by lower or equal to intensity value. If the answer to the question in a node of the tree was yes, it proceeded down to the left node, otherwise it proceeded down to the right node. When proceeding to the terminal nodes, the decision tree assigned to samples to different groups. **(A)**: patients with remission (terminal node 2, 4, and 5) and disease activity (terminal node 1, 3, and 6); **(B)**: RA patients with low disease activity (terminal node 1, 3, and 5) and other disease activity (terminal node 2, 4, and 6); **(C)**: RA patients with moderate disease activity (terminal node 2) and high disease activity (terminal node 1 and 3); **(D)**: RA-ILD (terminal node 1); **(E)**: RA-sSS (terminal node 1); **(F)**: RA-ONFH (terminal node 1).

9,286.49. Three proteins at m/z 5,801.84, 8,130.19, and 9,286.49 were decreased in the responder group, whereas one at m/z 2,671.60 was overexpressed in the responder population.

DISCUSSION

We developed a classification tree model for early RA diagnosis by MALDI-TOF-MS with WCX magnetic beads, which included m/z 3,448.85, 4,716.71, 8,214.28, and 10,645.10. This diagnostic model was applied for RA and had a much higher specificity (91.566%) and sensitivity (92.857%). In addition, the classification tree model was tested among 14 RA patients and 20 HCs in blinded test set and had a sensitivity of 95.0% and a specificity of 100%. The four peaks might be critical peptides or proteins involved in the pathogenesis. Similar to the study by Zhang et al. (31), clinically useful biomarkers for RA were identified with this approach to discriminate patients with RA from HCs. Zhang et al. (32) reported that m/z 15,715.5, 7,771.4, 8,959.4, 8,469.8, and 8,710.8 in serum are of certain value for differential diagnosis of RA from osteoarthritis (OA) and HCs. Compared with these prior studies, we not only completed the building of the RA diagnostic model but also determined the origin and full identity of the biomarkers for RA diagnosis.

We preliminarily retrieved components comprising these four protein peaks of diagnostic model from the ExPasy database, which was used to discriminate RA patients from the HCs. The four biomarkers may probably be CCL24, MT1DP, SLN, and CXCL-11, which were further identified and quantified through ELISA. Only the CCL24 level was detected to have a significant decrease in the serum of RA patients as compared with HCs ($p < 0.05$). No significant difference was found in others, but a decreasing trend consistent with the down-regulation of the four biomarkers detected by MALDI-TOF-MS was observed. The results agreed with previous reports. In Aloush et al. study, a significant decrease was observed in the level of CCR3 receptor in the serum of RA patients compared with HCs (33). CCL24/eotaxin-2 acts via highly specific activation of the CCR3 receptor. Another study observed that anti-eotaxin-2 antibody has a significant protective effect in adjuvant-induced arthritis in rats (34). Noticeably, a specific pattern was observed for mRNA expression in CCL24/eotaxin-2, which was higher among RA patients with lower X-ray scores than those with severe X-ray scores. The same results were also found in the comparison between the two groups with RA with low and medium disease activity and the subgroup with high activity (35). Combined with the above results, CCL24 may play a vital role in the pathogenesis of RA. The lower level of CCL24 in our result may be due to the reaction of eotaxin-2 to therapeutic manipulation in RA. However, in study by Uchida et al. (36), who detected discriminatory biomarkers to differentiate RA from OA using synovial fluid, they purified the protein based the detected specific peaks and the result revealed that m/z 10,850 was the clearest signal found specifically in RA and that it is myeloid-related protein 8, which might be related to the disease activity in RA. Despite the different results among research, these results highlighted that the CCL24, as a biomarker of this model, may be vital proteins or peptides that participate in the pathogenesis of RA and may be a promising potential therapeutic target for arthritis (37).

Although other biomarkers for the RA diagnostic model did not significantly differ in our result, the ELISA results, i.e.,

TABLE 5 | Establishment of the classification tree models for assessing the activity of disease in RA patients.

	Classification tree model	Accuracy (%)	Sensitivity (%)	Specificity (%)
Remission/ Disease activity(28/69)	M/Z1130.78, 1,501.07, 2,091.20, 11,381.87	92.78	94.12	93.33
Low disease activity/Other disease activity (17/52)	M2013.49, 8,765.23	82.61	86.67	80.65
Moderate disease activity/High disease activity (21/31)	M/Z2032.31, 4,716.71	75.00	74.19	76.19

TABLE 6 | Biomarker statistics for RA vs. RA with complications spectra in the decision tree classification.

M/Z	RA + ILD (Mean ± SD)	RA (Mean ± SD)	*P*
10,645.10	0.12 ± 0.36	0.47 ± 0.70	0.003
12,595.86	0.84 ± 0.44	1.55 ± 0.84	0
M/Z	**RA+sSS (Mean ± SD)**	**RA (Mean ± SD)**	***p***
6,635.62	11.38 ± 6.10	14.79 ± 5.89	0.045
33,897.72	0.11 ± 0.06	0.15 ± 0.05	0.037
M/Z	**RA+ONFH (Mean ± SD)**	**RA (Mean ± SD)**	***P***
2,071.69	1.73 ± 2.11	3.43 ± 1.63	0.025

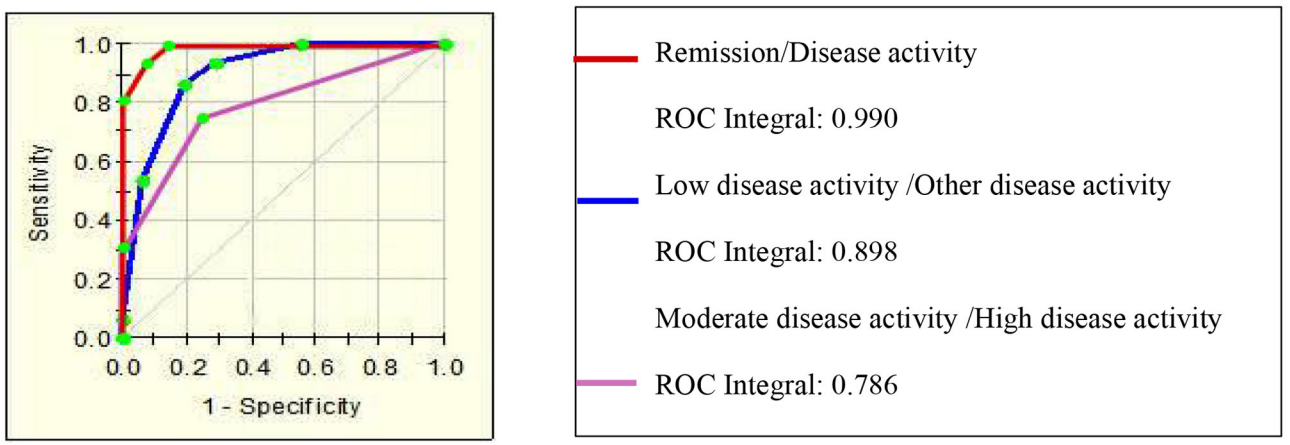

FIGURE 4 | The ROC of the diagnostic models for assessing the activity of disease in RA patients.

TABLE 7 | The classification tree models for RA and RA with complications.

	Classification tree model	Accuracy	Sensitivity	Specificity
RA/RA- ILD(44/22)	M/Z10645.10, 12,595.86	84.85%	86.36%	84.09%
RA/RA-sSS(44/18)	M/Z6635.62, 33,897.72	79.03%	77.78%	79.55%
RA/RA-ONFH(44/6)	M/Z2071.69	92.00%	66.67%	95.45%

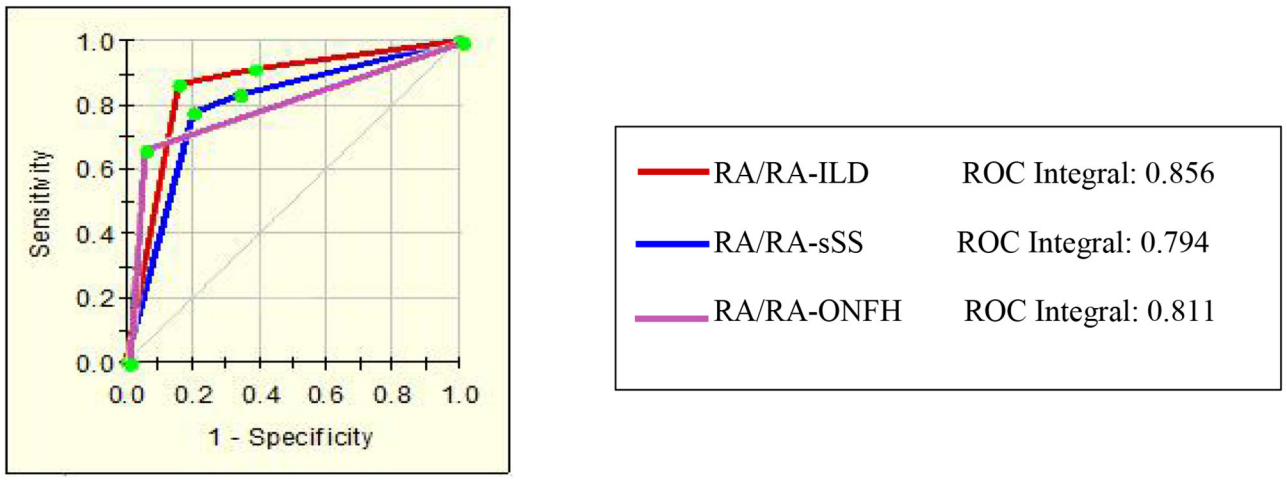

FIGURE 5 | The ROC of the diagnostic models of RA with complications.

the four biomarkers had lower levels in RA patients than in HCs, were consistent with the MALDI-TOF-MS results. Thus far, MT1DP and SLN as the possible components, are not reported in studies about autoimmune disease. According to previous studies, the fourth protein, CXCL11, is believed to be the dominant CXCR3 agonist because it is more potent (38), and it has been believed to play a vital role in directing Th1 cells to sites of inflammation (39). Recent studies strongly concluded that the CXCR3 receptor is a potential therapeutic target for treating autoimmune diseases, such as RA and multiple sclerosis (40). Although we were not able to identify other biomarkers, possibly due to the relatively small sample of patients in this study, the possibility remains that this chemokine plays a vital role in RA.

We also aimed to construct a series of clinical models for an accurate assessment model of disease activity. Thus far, there are no studies about it, and we did not find relevant conclusions for the assessment model of RA in the present research. Consequently, we established the classification tree model for assessing the disease activity in RA patients. At first, we can use the classification model including m/z 1,130.78, 1,501.07, 2,091.20, and 11,381.87 to distinguish patients with disease activity from RA patients with remission. RA patients with low disease activity could be efficiently discriminated from other disease activity patients with the combined use of specific protein biomarkers (m/z 2,032.31, 2,506.21, and 9,286.50). Finally, m/z 2,032.31 and 4,716.71 were applied to build the classification model for RA patients with moderate disease activity and high disease activity. These models are possibly the most convenient and efficient clinical methods for RA patients.

After assessing and diagnosing RA patients using the established model, they should be comprehensively assessed to determine other complications, which can prompt early treatment. Therefore, we built the classification tree model for to accurately recognize RA-ILD, RA-sSS, and RA-ONFH from RA. The most optimal tree model to distinguish between RA-ILD and RA without complication had m/z 10,645.10, and 12,595.86. In the present research, the diagnostic model was applicable for RA-sSS and/or SS-ILD (41), but no biomarkers discriminating for RA-ILD were found. The RA-sSS model had m/z 6,635.62 and 33,897.72 and had a specificity of 77.78% and a sensitivity of 79.55%. In the study of Li et al. (42), m/z peaks at 8,133.85, 11,972.8, 2,220.81, and 4,837.66 were used to establish a diagnostic model for Primary Sjögren's syndrome from systemic lupus erythematosus, RA, and HCs. The m/z peaks of RA-sSS model were different among these studies. We believe that the difference might be due to the different characteristics of the subjects. Our study detected that m/z 2,071.68 has potential in discriminating between RA and RA-ONFH. A previous study (43) showed that seven proteins were found based on two-dimensional electrophoresis patterns from the sera of 10 patients with ONFH and 10 normal subjects. ELISA revealed that the levels of tissue plasminogen activator, plasminogen activator inhibitor 1, crosslaps, and anti-p53 antibody in patients with ONFH were always significantly different among patients with OA, RA, and fracture. In the diagnostic models of RA with complications, all had different protein peaks; thus, further research is needed to clarify its specific components.

The biological markers for etanercept used in RA patients were also generated in our study. A total of 4 protein peaks, including m/z 2,671.60, 5,801.84, 8,130.20, and 9,286.49, were detected in RA patients between the responder ($n = 7$) and non-responder groups ($n = 4$). The study of Trocme et al. (44) revealed factors predictive of infliximab therapeutic response. Five proteins at 3.86, 7.77, 7.97, 8.14, and 74.07 kDa were overexpressed in the non-responder group ($n = 28$), whereas one at 28 kDa was increased in the responder group ($n = 32$). Biomarker characterization showed that apolipoprotein A-1 was predictive of a good response to infliximab, whereas platelet factor four was associated with non-response, thereby providing some thoughts to the scientific research in our future study.

These data suggested that MALDI-TOF-MS combined with WCX magnetic beads could be helpful in clinical applications for efficient diagnosis and accurate assessment of disease activity, and it is useful for differentiating RA patients with complications, which plays an important role in providing early treatment to RA patients, thereby preventing worse symptoms in the future. Nevertheless, the identification of these biomarkers is essential in understanding the pathogenesis of RA, on which they may play a vital role. Therefore, our future study will complete the identification of other potential diagnostic biomarkers for RA, and their identification will further improve the usefulness of the model, shed further light on the pathogenesis of RA, and promote the study on the diagnostic biomarkers for RA.

In many health-care systems, the medical treatment of patients comprises three phases (clinical diagnosis, assessment, and treatment), to which the proposed models we established above are applied. Unlike findings about biomarkers discovered in previous studies, this study not only explored the diagnostic criteria established by proteomic technology to accurately recognize RA patients from HCs but also assessed the disease activity and developed classification tree models for comprehensive assessment of patient's condition and to determine whether they have other complications. Our study detected the potential biomarkers for etanercept used in RA patients, which can help solve future clinical problems. We further identified and quantified the biomarkers for RA and found that the CCL24 level was lower in the RA group than in HCs. In future study, we will complete the identification of other potential diagnostic biomarkers to explore their role in the pathogenesis and to detect the precise therapeutic target.

However, this study has some limitations. The tested sample size is small and should be increased. To identify the discovered protein peak, the sample size should be increased. Furthermore, no pathological controls with undifferentiated arthritis, OA, or inflammatory joint diseases were assayed in this study. Thus, these should be considered in future research. More effort in future studies should be done, including increasing the sample size and completing other molecular identification of the potential specific biomarkers reported in this study for a better understanding of RA.

CONCLUSION

Our study successfully established a series of specific proteomic diagnostic models for RA, RA-ILD, RA-sSS, and RA-ONFH and detected the potential biomarkers to distinguish RA disease activity and to identify etanercept's clinical effect using proteomic fingerprint technology (MALDI-TOF-MS) combined with WCX magnetic beads. Furthermore, we have also demonstrated the differential expression of CCL24 in RA serum and HCs, which appears to have a pathogenetic role in RA and may serve as therapeutic targets in the future. Further exploration of these findings requires a larger sample size.

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 Ethics Committee of the Shanxi Bethune Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LZ: study concept and design. DM, NL, and LZ: acquisition, analysis and interpretation of data, statistical analysis, drafting of the manuscript, and critical revision of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. McInnes I, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med.* (2011) 365:2205–19. doi: 10.1056/NEJMra1004965
2. Turesson C, O'Fallon W, Crowson C, Gabriel S, Matteson E. Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. *Ann Rheum Dis.* (2003) 62:722–7. doi: 10.1136/ard.62.8.722
3. Spagnolo P, Lee J, Sverzellati N, Rossi G, Cottin V. The Lung in rheumatoid arthritis: focus on interstitial lung disease. *Arthritis Rheumatol.* (2018) 70:1544–54. doi: 10.1002/art.40574
4. Gochuico B, Avila N, Chow C, Novero L, Wu H, Ren P, et al. Progressive preclinical interstitial lung disease in rheumatoid arthritis. *Arch Intern Med.* (2008) 168:159–66. doi: 10.1001/archinternmed.2007.59
5. Hajiabbasi A, Shenavar Masooleh I, Alizadeh Y, Banikarimi A, Ghavidel Parsa P. Secondary Sjogren's syndrome in 83 patients with rheumatoid arthritis. *Acta Med Iran.* (2016) 54:448–53.

6. Gilboe I, Kvien T, Uhlig T, Husby G. Sicca symptoms and secondary Sjögren's syndrome in systemic lupus erythematosus: comparison with rheumatoid arthritis and correlation with disease variables. *Ann Rheum Dis.* (2001) 60:1103–9. doi: 10.1136/ard.60.12.1103
7. Weinstein R. Clinical practice. Glucocorticoid-induced bone disease. *N Engl J Med.* (2011) 365:62–70. doi: 10.1056/NEJMcp1012926
8. Watanabe Y, Kawai K, Hirohata K. Histopathology of femoral head osteonecrosis in rheumatoid arthritis: the relationship between steroid therapy and lipid degeneration in the osteocyte. *Rheumatol Int.* (1989) 9:25–31.doi: 10.1007/bf00270286
9. Mun S, Lee J, Park A, Kim H, Lee Y, Son H, et al. Proteomics approach for the discovery of rheumatoid arthritis biomarkers using mass spectrometry. *Int J Mol Sci.* (2019) 20:4368. doi: 10.3390/ijms20184368
10. Myasoedova E, Davis J, Matteson E, Crowson C. Is the epidemiology of rheumatoid arthritis changing? Results from a population-based incidence study, 1985-2014. *Ann Rheum Dis.* (2020) 79:440–4. doi: 10.1136/annrheumdis-2019-216694
11. Travis W, Costabel U, Hansell D, King T, Lynch D, Nicholson A, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med.* (2013) 188:733–48. doi: 10.1164/rccm.201308-1483ST
12. Assayag D, Elicker B, Urbania T, Colby T, Kang B, Ryu J, et al. Rheumatoid arthritis-associated interstitial lung disease: radiologic identification of usual interstitial pneumonia pattern. *Radiology.* (2014) 270:583–8. doi: 10.1148/radiol.13130187
13. Saini A, Saifuddin A. MRI of osteonecrosis. *Clin Radiol.* (2004) 59:1079–93. doi: 10.1016/j.crad.2004.04.014
14. Jones L, Hungerford D. Osteonecrosis: etiology, diagnosis, and treatment. *Curr Opin Rheumatol.* (2004) 16:443–9.doi: 10.1097/01.moo.0000127829.34643.fd
15. Baldini C, Talarico R, Tzioufas A, Bombardieri S. Classification criteria for Sjogren's syndrome: a critical review. *J Autoimmun.* (2012) 39:9–14.doi: 10.1016/j.jaut.2011.12.006
16. Kyriakidis N, Kapsogeorgou E, Tzioufas A. A comprehensive review of autoantibodies in primary Sjögren's syndrome: clinical phenotypes and regulatory mechanisms. *J Autoimmun.* (2014) 51:67–74. doi: 10.1016/j.jaut.2013.11.001
17. Gremese E, Salaffi F, Bosello S, Ciapetti A, Bobbio-Pallavicini F, Caporali R, et al. Very early rheumatoid arthritis as a predictor of remission: a multicentre real life prospective study. *Ann Rheum Dis.* (2013) 72:858–62.doi: 10.1136/annrheumdis-2012-201456
18. Tsuchida S, Umemura H, Nakayama T. Current status of Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology. *Molecules.* (2020) 25:4775. doi: 10.3390/molecules25204775
19. Angeletti S, Ciccozzi M. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry in clinical microbiology: an updating review. *Infect Genet Evol.* (2019) 76:104063.doi: 10.1016/j.meegid.2019.104063
20. Baumann S, Ceglarek U, Fiedler G, Lembcke J, Leichtle A, Thiery J. Standardized approach to proteome profiling of human serum based on magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Chem.* (2005) 51:973–80. doi: 10.1373/clinchem.2004.047308
21. Whiteaker J, Zhao L, Zhang H, Feng L, Piening B, Anderson L, et al. Antibody-based enrichment of peptides on magnetic beads for mass-spectrometry-based quantification of serum biomarkers. *Anal Biochem.* (2007) 362:44–54.doi: 10.1016/j.ab.2006.12.023
22. Peter J, Otto A. Magnetic particles as powerful purification tool for high sensitive mass spectrometric screening procedures. *Proteomics.* (2010) 10:628–33.doi: 10.1002/pmic.200900535
23. Li Y, Hu C, Leng X, Zhao G, Li N, Xu Y. Promising diagnostic biomarkers for primary biliary cirrhosis identified with magnetic beads and MALDI-TOF-MS. *Anat Rec.* (2009) 292:455–60. doi: 10.1002/ar.20870
24. Goëb V, Thomas-L'Otellier M, Daveau R, Charlionet R, Fardellone P, Le Loët X, et al. Candidate autoantigens identified by mass spectrometry in early rheumatoid arthritis are chaperones and citrullinated glycolytic enzymes. *Arthritis Res Ther.* (2009) 11:R38.doi: 10.1186/ar2644
25. de Seny D, Fillet M, Meuwis M, Geurts P, Lutteri L, Ribbens C, et al. Discovery of new rheumatoid arthritis biomarkers using the surface-enhanced laser desorption/ionization time-of-flight mass spectrometry ProteinChip approach. *Arthritis Rheum.* (2005) 52:3801–12. doi: 10.1002/art.21607
26. Huang Z, Shi Y, Cai B, Wang L, Wu Y, Ying B, et al. MALDI-TOF MS combined with magnetic beads for detecting serum protein biomarkers and establishment of boosting decision tree model for diagnosis of systemic lupus erythematosus. *Rheumatology (Oxford).* (2009) 48:626–31. doi: 10.1093/rheumatology/kep058
27. Giusti L, Baldini C, Bazzichi L, Bombardieri S, Lucacchini A. Proteomic diagnosis of Sjögren's syndrome. *Expert Rev Proteomics.* (2007) 4:757–67. doi: 10.1586/14789450.4.6.757
28. Lin X, Yang S, Du J, Tian Y, Bu L, Huo S, et al. Detection of lung adenocarcinoma using magnetic beads based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry serum protein profiling. *Chin Med J.* (2010) 123:34–9. doi: 10.3760/cma.j.issn.0366-6999.2010.01.006
29. Li J, Zhang Z, Rosenzweig J, Wang Y, Chan D. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clin Chem.* (2002) 48:1296–304. doi: 10.1016/S0009-8981(02)00170-5
30. Cheng A, Chen L, Chien K, Chen Y, Chang J, Wang H, et al. Oral cancer plasma tumor marker identified with bead-based affinity-fractionated proteomic technology. *Clin Chem.* (2005) 51:2236–44. doi: 10.1373/clinchem.2005.052324
31. Yan Z, Chaojun H, Chuiwen D, Xiaomei L, Xin Z, Yongzhe L, et al. Establishing serological classification tree model in rheumatoid arthritis using combination of MALDI-TOF-MS and magnetic beads. *Clin Exp Med.* (2015) 15:19–23.doi: 10.1007/s10238-013-0265-2
32. Zhang X, Yuan Z, Shen B, Zhu M, Liu C, Xu W. Discovery of serum protein biomarkers in rheumatoid arthritis using MALDI-TOF-MS combined with magnetic beads. *Clin Exp Med.* (2012) 12:145–51. doi: 10.1007/s10238-011-0154-5
33. Aloush V, George J, Elkayam O, Wigler I, Oren S, Entin-Meer M, et al. Decreased levels of CCR3 in CD4+ lymphocytes of rheumatoid arthritis patients. *Clin Exp Rheumatol.* (2010) 28:462–7. doi: 10.1186/1471-2474-11-143
34. Ablin J, Entin-Meer M, Aloush V, Oren S, Elkayam O, George J, et al. Protective effect of eotaxin-2inhibition in adjuvant-induced arthritis. *Clin Exp Immunol.* (2010) 161:276–83. doi: 10.1111/j.1365-2249.2010.04172.x
35. Zhebrun D, Totolyan A, Maslyanskii A, Titov A, Patrukhin A, Kostareva A, et al. Synthesis of some CC chemokines and their receptors in the synovium in rheumatoid arthritis. *Bull Exp Biol Med.* (2014) 158:192–6.doi: 10.1007/s10517-014-2720-9
36. Uchida T, Fukawa A, Uchida M, Fujita K, Saito K. Application of a novel protein biochip technology for detection and identification of rheumatoid arthritis biomarkers in synovial fluid. *J Proteome Res.* (2002) 1:495–9. doi: 10.1021/pr025531w
37. Cole K, Strick C, Paradis T, Ogborne K, Loetscher M, Gladue R, et al. Interferon-inducible T cell alpha chemoattractant (I-TAC): a novel non-ELR CXC chemokine with potent activity on activated T cells through selective high affinity binding to CXCR3. *J Exp Med.* (1998) 187:2009–21. doi: 10.1084/jem.187.12.2009
38. Karin N, Razon H. Chemokines beyond chemo-attraction: CXCL10 and its significant role in cancer and autoimmunity. *Cytokine.* (2018) 109: 24–8. doi: 10.1016/j.cyto.2018.02.012
39. C. Jenh, M. Cox, L. Cui, E. Reich, L. Sullivan, S. Chen, et al. A selective and potent CXCR3 antagonist SCH 546738 attenuates the development of autoimmune diseases and delays graft rejection. *BMC immunology.* (2012) 13:2.doi: 10.1186/1471-2172-13-2
40. Korniejewska A, McKnight A, Johnson Z, Watson M, Ward S. Expression and agonist responsiveness of CXCR3 variants in human T lymphocytes. *Immunology.* (2011) 132:503–15.doi: 10.1111/j.1365-2567.2010.03384.x
41. Li Y, Sun X, He J, Jia R, Yang D, Zhang X, et al. Screening for serum specific biomarkers in patients with primary Sjögren's syndrome and interstitial lung disease using proteomic fingerprint techniques. *Beijing Da Xue Xue Bao.* (2012) 44:240–3. doi: 10.3969/j.issn.1671-167X.2012.02.017

42. Li Y, Sun X, Zhang X, Yang Y, Jia R, Liu X, et al. Establishment of a novel diagnostic model for Sjögren's syndrome by proteomic fingerprinting. *Clin Rheumatol.* (2014) 33:1745–50. doi: 10.1007/s10067-014-2762-4
43. Tan X, Cai D, Wu Y, Liu B, Rong L, Chen Z, et al. Comparative analysis of serum proteomes: discovery of proteins associated with osteonecrosis of the femoral head. *Transl Res.* (2006) 148:114–9. doi: 10.1016/j.trsl.2006.05.001
44. Trocmé C, Marotte H, Baillet A, Pallot-Prades B, Garin J, Grange L, et al. Apolipoprotein A-I and platelet factor 4 are biomarkers for infliximab response in rheumatoid arthritis. *Ann Rheum Dis.* (2009) 68:1328–33. doi: 10.1136/ard.2008.093153

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Artificial Intelligence for Ultrasound Informative Image Selection of Metacarpal Head Cartilage. A Pilot Study

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Objectives: This study aims to develop an automatic deep-learning algorithm, which is based on Convolutional Neural Networks (CNNs), for ultrasound informative-image selection of hyaline cartilage at metacarpal head level. The algorithm performance and that of three beginner sonographers were compared with an expert assessment, which was considered the gold standard.

Methods: The study was divided into two steps. In the first one, an automatic deep-learning algorithm for image selection was developed using 1,600 ultrasound (US) images of the metacarpal head cartilage (MHC) acquired in 40 healthy subjects using a very high-frequency probe (up to 22 MHz). The algorithm task was to identify US images defined informative as they show enough information to fulfill the Outcome Measure in Rheumatology US definition of healthy hyaline cartilage. The algorithm relied on VGG16 CNN, which was fine-tuned to classify US images in informative and non-informative ones. A repeated leave-four-subject out cross-validation was performed using the expert sonographer assessment as gold-standard. In the second step, the expert assessed the algorithm and the beginner sonographers' ability to obtain US informative images of the MHC.

Results: The VGG16 CNN showed excellent performance in the first step, with a mean area (AUC) under the receiver operating characteristic curve, computed among the 10 models obtained from cross-validation, of 0.99 ± 0.01. The model that reached the best AUC on the testing set, which we named "MHC identifier 1," was then evaluated by the expert sonographer. The agreement between the algorithm, and the expert sonographer was almost perfect [Cohen's kappa: 0.84 (95% confidence interval: 0.71–0.98)], whereas the agreement between the expert and the beginner sonographers using conventional assessment was moderate [Cohen's kappa: 0.63 (95% confidence interval: 0.49–0.76)]. The conventional obtainment of US images by beginner sonographers required 6.0 ± 1.0 min, whereas US videoclip acquisition by a beginner sonographer lasted only 2.0 ± 0.8 min.

Conclusion: This study paves the way for the automatic identification of informative US images for assessing MHC. This may redefine the US reliability in the evaluation of MHC integrity, especially in terms of intrareader reliability and may support beginner sonographers during US training.

Keywords: hyaline cartilage, ultrasonography, metacarpal head, artificial intelligence, deep learning, convolutional neural network, rheumatoid arthritis, osteoarthritis

INTRODUCTION

Hyaline cartilage is a highly specialized connective tissue characteristic of synovial joints. Its principal function is to provide low-friction articular surfaces and to act as a shock absorber during the joint movement (1). Hyaline cartilage lacks blood vessels, thus it has a limited capacity for intrinsic healing and repair. In this regard, the integrity of this noble tissue is essential to joint health. The chondrocyte, the unique cell type in adult hyaline cartilage, maintains a stable equilibrium between the synthesis and the degradation of extracellular matrix components. With age and/or in the presence of various rheumatic diseases, such as rheumatoid arthritis and osteoarthritis, this balance is undermined, and the catabolic activity exceeds the anabolic one, thus leading to dehydration, degeneration, and thinning of the cartilage layer (2).

Although conventional radiography is the most adopted imaging method for the assessment of joint damage in daily clinical practice, it provides only an indirect visualization of the hyaline cartilage through the evaluation of the joint space narrowing. The accuracy of conventional radiography has been questioned in non-weight-bearing joints such as the ones of hands and wrists, which are commonly involved in different rheumatic diseases (3, 4). Moreover, in several studies, conventional radiography was found to be less sensitive than ultrasonography (US) in the detection of joint damage (5–11).

Recently, US has been suggested as a reliable and reproducible tool for the assessment of the hyaline cartilage of the small joints of the hand (4, 11–17). One of the main drawbacks of US is its subjectivity in the interpretation of US findings and the consequent variable inter- and intraobserver reliability (18–20). This issue is particularly relevant for the beginner musculoskeletal sonographer (18–20). Thus, the development of a tool that can enhance the US learning process is noteworthy (21).

In the last few years, artificial intelligence has been gaining importance in US, and a number of advantages have been claimed for this alternative method over the conventional acquisition and interpretation of US images, including faster performance, higher reliability and better standardization of image acquisition (22–27). Only a few studies have applied artificial intelligence in the field of musculoskeletal diseases (28–32), and no studies explored the artificial intelligence (AI) in the US assessment of hyaline cartilage, except our previous preliminary work (33).

To date, deep learning (DL) has shown its value in the healthcare domain for computer-assisted medical image analysis (24). DL is a branch of AI, and its algorithms are inspired by human brain, being able to learn from a large amount of data by itself. In fact, DL has the advantage of directly learning image features from raw data, avoiding the need to design hand-crafted features as for traditional machine learning approaches (23). In particular, Convolutional Neural Networks (CNNs), one of the most popular DL algorithms, are widely used for medical image analysis tasks such as classification, segmentation, detection, and biometric measurements, with applications in diagnosis and image-guided interventions and therapy (23). CNNs are able to recognize complex structures in an image by applying convolutional filters (each defined by a kernel matrix) whose weights are iteratively learned during CNN training. Training a CNN relies on labeled data, which are used to learn the mapping from the input to the desired output. Training a CNN from scratch requires a large amount of labeled data, which may not always be available, especially in the medical image analysis domain where image labeling by expert clinicians is a resource-expensive procedure. Moreover, training a CNN from scratch may often lead to overfitting issues (i.e., CNN inability to generalize on new sets of data). A feasible alternative is to exploit transfer learning. Transfer learning consists of extracting knowledge from one task (where large annotated datasets are available) and using the extracted knowledge for a second one. It has been demonstrated that using transfer learning is particularly useful for medical image analysis since limited training data are usually available (34, 35).

Driven by this last consideration, we decided to use transfer learning for training a CNN for US informative-frame selection. We investigated CNNs pretrained on ImageNet, a large image database that includes more than 1 million of annotated natural images (e.g., cats, dogs, cars).

The main aim of this study was to develop an automatic DL algorithm for US informative-image selection of hyaline cartilage at metacarpal head level. An image was defined informative when it shows enough information to fulfill the Outcome Measure in Rheumatology US definition of healthy hyaline cartilage (36). The algorithm performance and that of three beginner sonographers were compared with an expert assessment, which was considered the gold standard.

MATERIALS AND METHODS

Study Design

The study was conducted from January 2019 to March 2020. The study was divided in two steps. In the first one, a CNN algorithm for informative image selection was developed and trained using 1,600 static US images. The US images were acquired by an expert

(E.F.) in musculoskeletal US who evaluated the metacarpal head cartilage (MHC) from the 2nd to the 5th digit bilaterally in 40 healthy subjects.

In the second step of the study, the CNN output was compared with the conventional assessment of the MHC carried out independently by three beginner (E.M., F.F., and J.D.B.) and the expert (E.F.) sonographers. A beginner sonographer was defined as a sonographer with limited experience (<1 year) in the US assessment of hyaline cartilage. MHC from the 2nd to the 5th digit of both hands of eight healthy subjects was independently evaluated on the same day by the beginner and the expert sonographers. The beginner sonographers were asked to provide a set of eight US images per subject (one US image per each metacarpal head) for a total of 192 static images and a set of eight 10-s videoclips on the same healthy subjects for a total of 192 videoclips. A random sample of 128 static images and 64 videoclips were used in the reliability analysis. The US images evaluated by the beginners (each beginner judged a third of the US images) and the videoclips assessed by the algorithm were tested against the expert opinion who had to state whether the US images were informative or not. Expert was blinded to the US images authorship (i.e., beginner sonographer or artificial intelligence algorithm). The time required for each US examination was registered.

Subjects

Healthy subjects were selected from relatives visiting or accompanying in- and out-patients, friends of the authors, and medical students attending the "Carlo Urbani" Hospital (Jesi, Ancona, Italy). Healthy subjects were enrolled because they had the lowest probability to present US abnormalities indicative of cartilage damage. In fact, pathologic findings may generate a bias in the interpretation of US images by both the beginner sonographers and the algorithm.

Exclusion criteria were as follows: (i) previous diagnosis of inflammatory/degenerative arthropathies; (ii) joint pain [visual analog scale (VAS) ≥10/100] and/or analgesic or non-steroidal anti-inflammatory drugs' intake in the 4 weeks preceding the visit; (iii) age <18 years old; and (iv) hard tissue enlargement or deformity of the metacarpophalangeal, proximal, or distal interphalangeal joints suggestive of hand osteoarthritis and/or joint inflammation at physical examination. The following demographic data were recorded: sex, age, handedness, height, weight, and body mass index.

US Image Acquisition and Interpretation

US examinations were carried out using a MyLab Class C (Esaote SpA, Genoa, Italy), equipped with a very high-frequency broadband linear probe (10–22 MHz). A grayscale standard setting was adopted (B-mode frequency: 22 MHz, master gain: 70%, mechanical index: 0.3, dynamic range: 12, depth 15 mm, focus position at the area of interest).

The hands were placed on the table, with the metacarpophalangeal joints in maximal flexion (>60°) (14, 36, 37). The metacarpal head from the 2nd to the 5th digit of both hands was scanned on the dorsal aspect from radial to ulnar and from proximal to distal sides to ensure the maximal exploration of the hyaline cartilage using the EULAR standard scans (38). Particular attention was paid to ensure a perpendicular insonation of the cartilage surface (14, 36, 37).

The normal appearance of the hyaline cartilage is characterized by a homogenous hypo-anechoic layer delimited by two regular, sharp, and bright margins where insonated orthogonally (36, 37). An US image displaying such characteristics was defined informative. The detection in the videoclip by the algorithm or by the expert of at least a frame showing US features of healthy hyaline cartilage was a sufficient criterion to classify it as informative. **Figure 1** provides a pictorial evidence of a healthy MHC.

CNN Algorithm for Informative Frame Selection

A VGG16 CNN, pre-trained on the ImageNet dataset, was used for transfer learning. The VGG16 architecture was chosen for two main reasons: (1) its sequential architecture results to be particularly suitable for small-size training set and low image variability (2, 39) its shallow architecture (only 3 × 3 convolutional layers stacked on top of each other in increasing depth) is associated with low computation cost.

In the original VGG16 architecture implementation, the ImageNet images are processed through 13 convolutional (conv) layers to perform feature extraction. The filters used in each conv layer have very small receptive field (3 × 3) (the smallest size to capture notion about left/right, up/down and center),

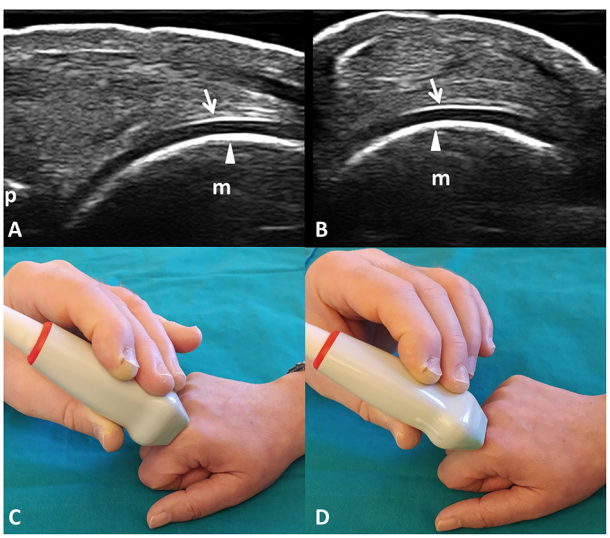

FIGURE 1 | Healthy hyaline cartilage. Dorsal longitudinal **(A)** and transverse **(B)** scans of the hyaline cartilage of the 2nd metacarpal head in a healthy subject. Hyaline cartilage appears as a homogeneously hypo-anechoic layer delimited by two regular, sharp, continuous, and hyperechoic interfaces where insonated orthogonally. Arrows indicate the outer margin (i.e., the chondrosynovial interface); arrowheads indicate the inner margin (i.e., the osteochondral interface). **(C,D)** The position of the probe in the dorsal longitudinal **(C)** and transverse **(D)** scans. m, metacarpal head; p, base of the proximal phalanx.

followed by a rectified linear unit (ReLU) activation function. After the convolutional layers, three fully connected (FC) layers (4,096 neurons in the first two layers and 1,000 neurons in the last one) followed by a softmax layer are used to predict class probability.

In this work, the ImageNet pretrained weights were used as a starting point for the CNN training process. We modified the three FC layers using 1,024, 512, and 2 neurons in the first, second, and third FC layers, respectively, to adapt the architecture to our binary classification problem (informative—non-informative image classification) (**Figure 2**). The architecture was trained freezing the first 10 conv layers and tuning the remaining ones. In this way, we managed to exploit the knowledge encoded in the VGG16 trained on the large ImageNet dataset.

Training Strategy

Prior training the VGG16, US images were resized to 224 × 224 pixels and converted to RGB images, repeating the grayscale channel for three times, to match the image input dimension required by the pre-trained VGG16. Intensity mean was removed from each image.

The mini-batch gradient descent, with a momentum of 0.9, was used as optimizer using the categorical cross-entropy as loss function. The batch size was set to 64 as a balance between training speed and gradient convergence. Training was performed for 100 epochs with a learning rate of 0.0001.

A leave-four-subject out cross-validation was performed for testing the classification. The dataset was divided into 10 subsets of subjects. In turn, one of the 10 subsets (containing four subjects) was used as the test set while the other nine subsets were used as training set. The validation was performed selecting randomly four subjects from the training set, obtaining in such a way 10 models.

The analyses were performed using Keras with TensorFlow library as backend on Google Colaboratory (https://colab.research.google.com/).

Performance Metrics

To measure the performance of our approach, we computed the mean area under the curve (AUC) of the operating characteristic curve (ROC) and the mean classification Precision (*Prec*), Recall (*Rec*), and f1-score (*f1*) for the *i*th class, with i ∈ C (informative, non-informative), where TP_i,FP_i,FN_i were the true positives, false positives, and the false negatives, respectively.

$$Prec_i = \frac{TP_i}{TP_i + FP_i} \quad (1)$$

$$Rec_i = \frac{TP_i}{TP_i + FN_i} \quad (2)$$

$$f1_i = \frac{2\ x\ Prec_i\ x\ Rec_i}{Prec_i + Rec_i} \quad (3)$$

Statistical Analysis

Results are expressed as number and/or corresponding percentage for qualitative variables and as mean and standard deviation (SD) for quantitative variables. The Chi-square test and the Mann-Whitney test were used to compare the qualitative and quantitative variables, respectively. The agreement in the informative image selection between the expert (i.e., the gold standard) and the algorithm, and between the expert and the conventional assessment of the beginners was calculated using an unweighted Cohen's kappa and interpreted according to Landis and Koch (40).

Two-tailed *p*-values <0.05 were considered significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 26.0 for Windows, Chicago, Illinois, USA).

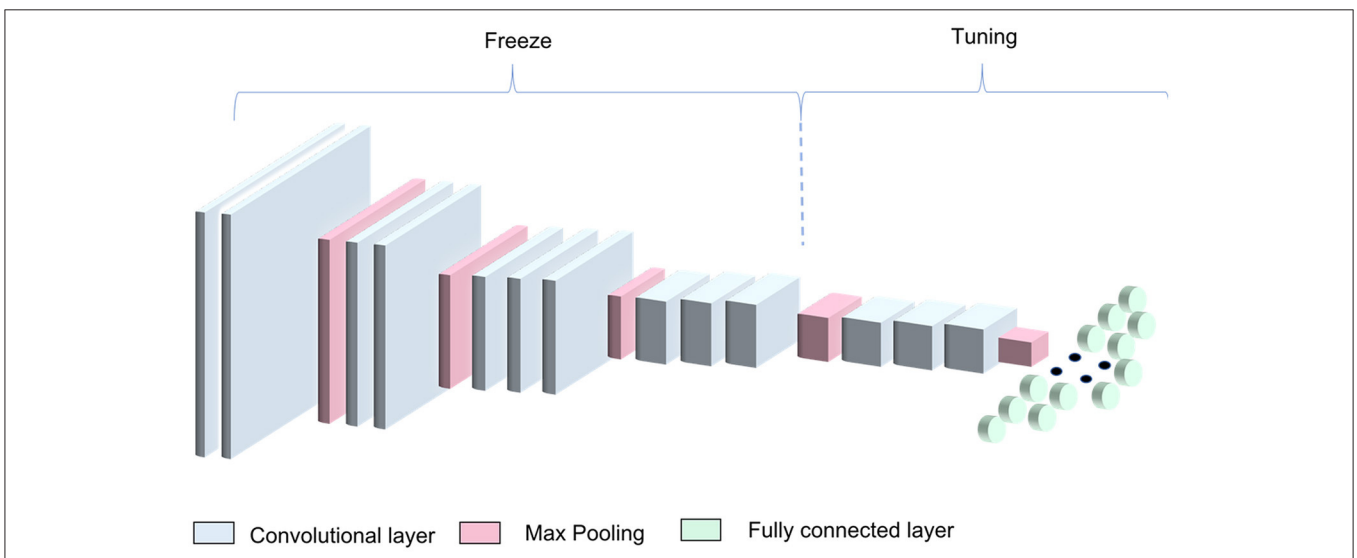

FIGURE 2 | VGG16 transfer-learning strategy. The algorithm was obtained by freezing the first four blocks of the VGG16 pretrained on the ImageNet dataset and training the remaining blocks along with the dense layers.

RESULTS

Subjects

A total of 48 healthy subjects were included in this monocentric and cross-sectional study: 40 in the training and testing of the algorithm (first step) and 8 in the reliability analysis (second step). **Table 1** shows the main demographic characteristics of the participants.

Artificial-Intelligence Algorithm: Training and Testing

In the first step of the study, the VGG16 CNN showed excellent performance in the informative image selection task, with an AUC of 0.99 ± 0.01 (**Figure 3**) computed among the 10 models.

Table 2 shows the classification results for both informative and non-informative frame obtained from the cross-validation procedure.

Figure 4 shows an example of informative and non-informative frames selected by the model that reached the best AUC, which we named "MHC identifier 1."

TABLE 1 | Demographic characteristics of the healthy participants.

Sex (female/male)	33/15
Age (years, mean ± SD)	54.6 ± 5.6
Handedness (right/left)	40/8
Height (cm, mean ± SD)	170.7 ± 9.5
Weight (kg, mean ± SD)	72.4 ± 6.4
Body mass index (kg/m^2, mean ± SD)	24.8

SD, standard deviation.

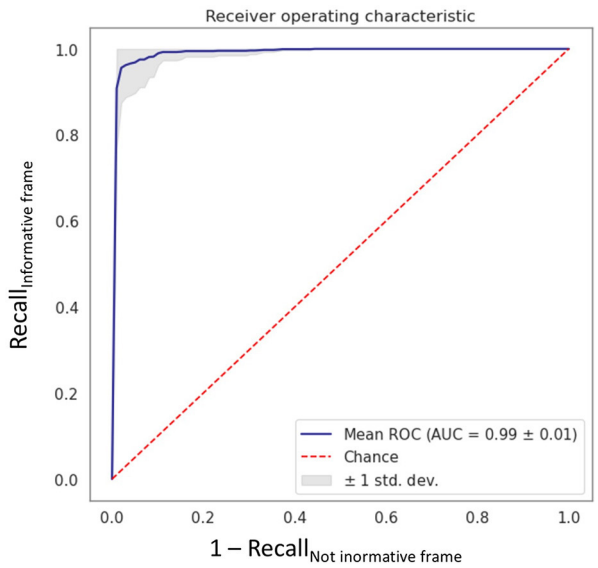

FIGURE 3 | Receiving operating characteristic curve obtained with the fine-tuned VGG16 CNN algorithm "MHC identifier 1." AUC, area under the curve; ROC, receiving operating characteristics; std dev, standard deviation.

Feasibility

The average time required to complete the conventional US assessment was 6.0 ± 1.0 and 4.0 ± 0.5 min for beginners and expert sonographer, respectively ($p < 0.01$). On the other hand, the time spent to acquire the videoclips was 2.0 ± 0.8.

Reliability Analysis

The agreement between the automatic algorithm and the expert sonographer was almost perfect (Cohen's kappa: 0.84, 95% confidence interval: 0.71–0.98); whereas, the agreement between the expert and the beginners using conventional assessment was moderate (Cohen's kappa: 0.63, 95% confidence interval: 0.49–0.76) ($p < 0.01$) (**Table 3**) without significant difference in the interobserver agreement between the expert and each beginner sonographer ($p = 0.14$).

TABLE 2 | Classification metrics for precision, recall, and f1-score, with i ∈ C (informative, non-informative).

	i	Precision	Recall	f1-score
VGG16 CNN algorithm "MHC identifier 1"	Informative	0.94 (0.07)	0.98 (0.02)	0.97 (0.05)
	Non-informative	0.98 (0.02)	0.96 (0.06)	0.97 (0.04)

Values in brackets are the standard deviation.
MHC, metacarpal head cartilage.

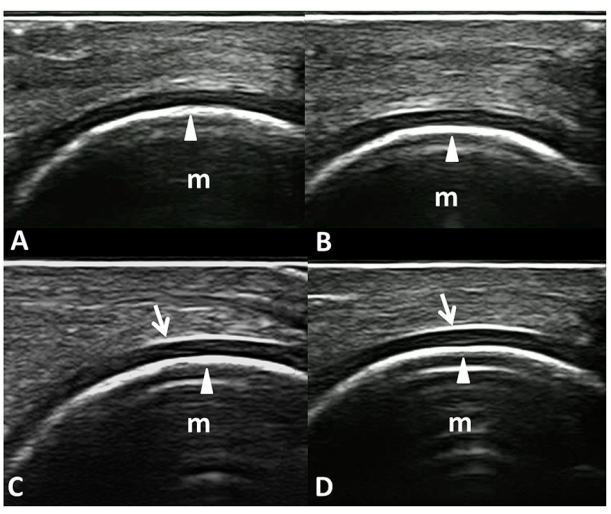

FIGURE 4 | Informative and non-informative US frame selected by the VGG16 CNN algorithm "MHC identifier 1." Dorsal longitudinal scans acquired at the metacarpal head level in healthy subjects. **(A,B)** Examples of non-informative frames; **(C,D)** Examples of informative frames. In fact, while the inner margin (arrowheads) is evident in all the panels, the chondrosynovial (arrows) interface is clearly visible only in the lower images **(C,D)**. m, metacarpal head; arrows, chondrosynovial interface; arrowheads, osteochondral interface.

TABLE 3 | Interobserver agreement.

		Beginners	
US static images ($n = 128$)		**Informative**	**Non-informative**
Expert	Informative	56 (43.8)	8 (6.3)
	Non-informative	16 (12.5)	48 (37.5)
Total agreement: 81.3%			
MHC Identifier 1			
10-s videoclips ($n = 64$)		**Informative**	**Non-informative**
Expert	Informative	31 (48.4)	2 (3.1)
	Non-informative	3 (4.7)	28 (43.8)
Total agreement: 92.2%			

Values in brackets are percentages.
MHC, metacarpal head cartilage; US, ultrasound.
The beginner sonographers and the VGG16 CNN algorithm "MHC identifier 1" agreement with the expert sonographer considered the gold standard.

DISCUSSION

Last-generation US systems allow the real-time identification of otherwise undetectable musculoskeletal abnormalities, which have a growing impact in the management of many rheumatic diseases (41, 42). However, US is a highly operator-dependent technique, and sonographer skills and experience may affect both acquisition and interpretation processes (19, 20). Several international initiatives were undertaken to ensure the standardization of US assessment and to increase its reproducibility in rheumatological setting (36, 38, 43, 44). The use of artificial intelligence in musculoskeletal US may further increase its reproducibility and may save sonographers time as shown in cardiological setting (45).

The correct acquisition of an US image is the essential step to ensure an accurate and reliable assessment of the image itself (31, 39, 46). Thus, we believe that the availability of an algorithm facilitating the identification of the region of interest during the acquisition process of US images represents a further step toward the standardization of US examination.

This study describes the first steps taken to develop an algorithm that can identify informative US images for the assessment of the MHC. The application of such an algorithm, that we called "MHC identifier 1," may redefine the US reliability in the evaluation of the MHC integrity, especially in terms of intrareader reproducibility. MHC identifier 1 showed an almost perfect agreement with the expert sonographer. Here are some possible explanations of disagreement between the algorithm and the expert: while processing an US videoclip, the algorithm may detect even just one frame to define it as informative, which may be missed or considered not relevant by the expert assessment. Conversely, the expert may consider sufficiently assessable US images even if rejected by the algorithm for not strictly fulfilling all the morphostructural criteria. The choice to use both videoclips and static images could be considered a limit of the present study. However, we decided to test the performance of the algorithm using videoclips instead of pictures, since its task will be to support the sonographer during a real-time US examination and not only in the interpretation of static images. Conversely, the sonographer selects a representative US image which conveys the message of a part of the US examination. Finally, according to the data we recorded, the use of this algorithm may shorten the time required to obtain informative US images up to one-third. However, it should be borne in mind that, to date, MHC identifier 1 cannot be routinely applied to clinical practice. In fact, the images must be manually exported from the US system and transferred in a computer where the algorithm can analyze them. A future development may include the incorporation of MHC identifier1 into an US machine to test the clinical value and the feasibility of this method in real-life setting. Thus, the feasibility of this method, including easiness of its use, costs, and availability are yet to be determined.

Our study presents some limitations. First, the same expert sonographer, who served as the imaging "gold standard," was also the teacher and tutor of the beginner sonographers. This fact may imply a possible risk of systematic bias. Second, only a relatively low number of US images were used in the reliability exercise. However, this limit should be read in light of the fact that it is a pilot study. Third, its monocentric design may limit the generalizability of our results. Fourth, the impact of machine-assisted acquisition of US images was not evaluated. Furthermore, the intrareader reliability of both the sonographers and the algorithm was not tested. Finally, the impact of using different US systems needs to be tested, as images in this study were all obtained with the same US system.

The assessment of MHC status is progressively gaining a relevant role in the management of patients with different chronic arthropathies. In fact, in 2019, the Outcome Measure in Rheumatology US Working Group proposed the US definitions of cartilage damage in patients with rheumatoid arthritis and osteoarthritis (36, 44). The same group of experts is currently carrying out a Delphi exercise to define and quantify the structural joint damage (including cartilage lesion) in rheumatoid arthritis by US.

Although preliminary, our results open up new horizons in the use of artificial intelligence in the US evaluation of hyaline cartilage. In fact, the algorithm MHC identifier 1 could enhance the learning process improving the awareness of the beginner sonographer regarding the probe positioning required to obtain images conveying essential information to assess the MHC. In addition, the ability of this algorithm to identify informative frame on videoclips may suggest its use as a tool that could assist the sonographer during the real time US examination.

Further implementation may allow to measure the MHC thickness and to evaluate the hyaline cartilage of other sites commonly involved by rheumatic diseases such as the knee and the hip (16, 17, 47).

The possibility of identifying, evaluating, and measuring the hyaline cartilage in a reliable and faster way may reduce the US examination time, shorten the learning curve of beginner sonographers by taking advantage

of AI feedbacks, and promote new studies in this field (e.g., aimed to compare the semiquantitative scoring system of cartilage damage and the quantitative assessment, and to follow-up the progression of cartilage damage).

In conclusion, this study describes the first steps in the development of an algorithm identifying informative US images for assessing the MHC. The automatic selection of US images acquired by beginner sonographers resulted reliable and feasible, as shown by the comparison with an expert sonographer. The application of such an algorithm may redefine the US reliability in the evaluation of the MHC integrity, especially in terms of intrareader reliability and may support beginner sonographers during US training. However, this algorithm needs further validation before its use in clinical practice.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EC, EFi, MF, and SM substantially contributed to study conception and design, acquisition, analysis, and interpretation of data. IG substantially contributed to acquisition, analysis, and interpretation of data. EFr and WG substantially contributed to analysis and interpretation of data. All the authors revised the paper and approved the final version of the article to be published.

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REFERENCES

1. Fox SAJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. *Sports Health*. (2009) 1:461–8. doi: 10.1177/1941738109350438
2. Pap T, Korb-Pap A. Cartilage damage in osteoarthritis and rheumatoid arthritis-two unequal siblings. *Nat Rev Rheumatol*. (2015) 11:606–15. doi: 10.1038/nrrheum.201595
3. Navarro-compán V, Landewé R, Provan SA, Ødegård S, Uhlig T, Kvien TK, et al. Relationship between types of radiographic damage and disability in patients with rheumatoid arthritis in the EURIDISS cohort: a longitudinal study. *Rheumatology*. (2014) 54:83–90. doi: 10.1093/rheumatology/keu284
4. Mandl P, Supp G, Baksa G, Radner H, Studenic P, Gyebnar J, et al. Relationship between radiographic joint space narrowing, sonographic cartilage thickness and anatomy in rheumatoid arthritis and control joints. *Ann Rheum Dis*. (2015) 74:2022–7. doi: 10.1136/annrheumdis-2014-205585
5. Døhn UM, Ejbjerg BJ, Court-Payen M, Hasselquist M, Narvestad E, Szkudlarek M, et al. Are bone erosions detected by magnetic resonance imaging and ultrasonography true erosions? A comparison with computed tomography in rheumatoid arthritis metacarpophalangeal joints. *Arthritis Res Ther*. (2006) 8:R110. doi: 10.1186/ar1995
6. Wakefield RJ, Gibbon WW, Conaghan PG., O'Connor P, McGonagle D, Pease C, et al. The value of sonography in the detection of bone erosions in patients with rheumatoid arthritis: a comparison with conventional radiography. *Arthritis Rheum*. (2000) 43:2762–70. doi: 10.1002/1529-0131(200012)43:12<2762::AID-ANR16>3.0.CO;2
7. Szkudlarek M, Klarlund M, Narvestad E., Court-Payen M, Strandberg C, Jensen KE, et al. Ultrasonography of the metacarpophalangeal and proximal interphalangeal joints in rheumatoid arthritis: a comparison with magnetic resonance imaging, conventional radiography and clinical examination. *Arthritis Res Ther*. (2006) 8:R52. doi: 10.1186/ar1904
8. Scheel AK, Hermann KGA, Ohrndorf S, Werner C, Schirmer C, Detert J, et al. Prospective 7 year follow up imaging study comparing radiography, ultrasonography, and magnetic resonance imaging in rheumatoid arthritis finger joints. *Ann Rheum Dis*. (2006) 65:595–600. doi: 10.1136/ard.2005041814
9. Funck-Brentano T, Etchepare F, Joulin SJ, Gandjbakch F, Pensec VD, Cyteval C, et al. Benefits of ultrasonography in the management of early arthritis: a cross-sectional study of baseline data from the ESPOIR cohort. *Rheumatology*. (2009) 48:1515–9. doi: 10.1093/rheumatology/kep279
10. Wiell C, Szkudlarek M, Hasselquist M., Møller JM, Vestergaard A, Nørregaard J, et al. Ultrasonography, magnetic resonance imaging, radiography, and clinical assessment of inflammatory and destructive changes in fingers and toes of patients with psoriatic arthritis. *Arthritis Res Ther*. (2007) 9:R119. doi: 10.1186/ar2327
11. Hurnakova J, Filippucci E, Cipolletta E, Matteo Di. A, Salaffi F, Carotti M, et al. Prevalence and distribution of cartilage damage at the metacarpal head level in rheumatoid arthritis and osteoarthritis: an ultrasound study. *Rheumatology*. (2019) 58:1206–13. doi: 10.1093/rheumatology/key443
12. Möller B, Bonel H, Rotzetter M, Villiger PM, Ziswiler H. Measuring finger joint cartilage by ultrasound as a promising alternative to conventional radiograph imaging. *Arthritis Rheum*. (2009) 61:435–41. doi: 10.1002/art24424
13. Filippucci E, da Luz KR, Di Geso L, Salaffi F, Tardella M, Carotti M, et al. Interobserver reliability of ultrasonography in the assessment of cartilage damage in rheumatoid arthritis. *Ann Rheum Dis*. (2010) 69:1845–8. doi: 10.1136/ard.2009125179
14. Cipolletta E, Filippucci E, Matteo Di. A, Tesei G, Cosatti M, Di Carlo M, et al. The reliability of ultrasound in the assessment of hyaline cartilage in rheumatoid and healthy metacarpal heads. *Ultraschall Med*. (2020). doi: 10.1055/a-1285-4602. [Epub ahead of print].
15. Iagnocco A, Conaghan PG, Aegerter P, Moller I, Bruyn GAW, Chary-Valckenaere I, et al. The reliability of musculoskeletal ultrasound in the detection of cartilage abnormalities at the metacarpo-phalangeal joints. *Osteoarthritis Cartilage*. (2012) 20:1142–6. doi: 10.1016/j.joca.2012.07003
16. Cipolletta E, Filippucci E, Matteo Di A, Hurnakova J, Di Carlo M, Pavelka K, et al. FRI0634 standard reference values of metacarpal head cartilage thickness measurement by ultrasound in healthy subjects. *Ann Rheum Dis*. (2019) 78:1014–5. doi: 10.1136/annrheumdis-2019-eular5807
17. Cipolletta E, Hurnakova J, Matteo Di A, Di Carlo M, Pavelka K, Grassi W, et al. Prevalence and distribution of cartilage and bone damage at metacarpal head in healthy subjects. *Clin Exp Rheumatol* (2020). [Epub ahead of print].
18. Hammer HB, Iagnocco A, Mathiessen A, Filippucci E, Gandjbakhch F, Kortekaas MC, et al. Global ultrasound assessment of structural lesions in osteoarthritis: a reliability study by the OMERACT ultrasonography group

on scoring cartilage and osteophytes in finger joints. *Ann Rheum Dis*. (2016) 75:402–7. doi: 10.1136/annrheumdis-2014-206289
19. Gutiérrez M, Di Geso L, Rovisco J, Di Carlo M, Ariani A, Filippucci E, et al. Ultrasound learning curve in gout: a disease-oriented training program. *Arthritis Care Res*. (2013) 65:1265–74. doi: 10.1002/acr22009
20. Gutierrez M, Filippucci E, Ruta S, Salaffi F, Blasetti P, di Geso L, et al. Inter-observer reliability of high-resolution ultrasonography in the assessment of bone erosions in patients with rheumatoid arthritis: experience of an intensive dedicated training programme. *Rheumatology*. (2011) 50:373–80. doi: 10.1093/rheumatology/keq320
21. Filippucci E, Meenagh G, Ciapetti A, Iagnocco A, Taggart A, Grassi W. E-learning in ultrasonography: a web-based approach. *Ann Rheum Dis*. (2007) 66:962–5. doi: 10.1136/ard.2006064568
22. Akkus Z, Cai J, Boonrod A, Zeinoddini A, Weston AD, Philbrick KA., et al. A survey of deep-learning applications in ultrasound: artificial intelligence–powered ultrasound for improving clinical workflow. *J Am Coll Radiol*. (2019) 16:1318–28. doi: 10.1016/j.jacr.2019.06004
23. Liu S, Wang Y, Yang X, Lei B, Liu L, SX Li, et al. Deep learning in medical ultrasound analysis: a review. *Engineering*. (2019) 5:261–75. doi: 10.1016/j.eng.2018.11020
24. Litjens G, Kooi T, Bejnordi BE, Setio AAA, Ciompi F, Ghafoorian M, et al. A survey on deep learning in medical image analysis. *Med Image Anal*. (2017) 42:60–88. doi: 10.1016/j.media.2017.07005
25. Stoel B. Use of artificial intelligence in imaging in rheumatology – current status and future perspectives. *RMD Open*. (2020) 6:e001063. doi: 10.1136/rmdopen-2019-001063
26. Zaffino P, Moccia S, De Momi E, Spadea MF. A review on advances in intra-operative imaging for surgery and therapy: imagining the operating room of the future. *Ann Biomed Eng*. (2020) 48:2171–91. doi: 10.1007/s10439-020-02553-6
27. Moccia S, De Momi E, El Hadji S, Mattos LS. Blood vessel segmentation algorithms - review of methods, datasets and evaluation metrics. *Comput Methods Programs Biomed*. (2018) 158:71–91. doi: 10.1016/j.cmpb.2018.02001
28. Chang RF, Lee CC, Lo CM. Computer-aided diagnosis of different rotator cuff lesions using shoulder musculoskeletal ultrasound. *Ultrasound Med Biol*. (2016) 42:2315–22. doi: 10.1016/j.ultrasmedbio.2016.05016
29. Klauser AS, Franz M, Bellmann WR, Gruber J, Hartig F, Mur E, et al. Contrast-enhanced ultrasonography for the detection of joint vascularity in arthritis - subjective grading versus computer-aided objective quantification. *Ultraschall der Medizin*. (2011). 32:E31–7. doi: 10.1055/s-0031-1281671
30. Chang RF, Lee CC, Lo CM. Quantitative diagnosis of rotator cuff tears based on sonographic pattern recognition. *PLoS ONE*. (2019) 14:e0212741. doi: 10.1371/journal.pone0212741
31. Andersen JKH, Pedersen JS, Laursen MS, Holtz K, Grauslund J, Savarimuthu TR, et al. Neural networks for automatic scoring of arthritis disease activity on ultrasound images. *RMD Open*. (2019) 5:e000891. doi: 10.1136/rmdopen-2018-000891
32. Christensen ABH, Just SA, Andersen JKH, Savarimuthu TR. Applying cascaded convolutional neural network design further enhances automatic scoring of arthritis disease activity on ultrasound images from rheumatoid arthritis patients. *Ann Rheum Dis*. (2020) 79:1189–93. doi: 10.1136/annrheumdis-2019-216636
33. Fiorentino MC, Moccia S, Cipolletta E, Filippucci E, Frontoni E. A learning approach for informative-frame selection in US rheumatology images. In: Autexier S, Campbell J, Rubio J, Sorge V, Suzuki M, Wiedijk F, editors. *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*. New York, NY: Springer Verlag (2019). p. 228–36. doi: 10.1007/978-3-030-30754-7_23
34. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, et al. Dermatologist-level classification of skin cancer with deep neural networks. *Nature*. (2017) 542:115–8. doi: 10.1038/nature21056
35. Tajbakhsh N, Shin JY, Gurudu SR, Hurst RT, Kendall CB, Gotway MB, et al. Convolutional neural networks for medical image analysis: full training or fine tuning? *IEEE Trans Med Imaging*. (2016) 35:1299–312. doi: 10.1109/TMI.2016253 5302
36. Mandl P, Studenic P, Filippucci E, Bachta A, Backhaus M, Bong D, et al. Development of semiquantitative ultrasound scoring system to assess cartilage in rheumatoid arthritis. *Rheumatology*. (2019) 58:1802–11. doi: 10.1093/rheumatology/ke z153
37. Torp-Pedersen S, Bartels E, Wilhjelm J, Bliddal H. Articular Cartilage thickness measured with US is not as easy as it appears: a systematic review of measurement techniques and image interpretation. *Ultraschall der Medizin - Eur J Ultrasound*. (2010) 32:54–61. doi: 10.1055/s-0029-1245386
38. Möller I, Jan I, Backhaus M, Ohrndorf S, Bong DA, Martinoli C, et al. The 2017 EULAR standardised procedures for ultrasound imaging in rheumatology. *Ann Rheum Dis*. (2017) 76:1974–9. doi: 10.1136/annrheumdis-2017-211585
39. Patrini I, Ruperti M, Moccia S, Mattos LS, Frontoni E, De Momi E. Transfer learning for informative-frame selection in laryngoscopic videos through learned features. *Med Biol Eng Comput*. (2020) 58:1225–38. doi: 10.1007/s11517-020-02127-7
40. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. (1977) 33:159–74. doi: 10.2307/2529310
41. Filippucci E, Cipolletta E, Mashadi Mirza R, Carotti M, Giovagnoni A, Salaffi F, et al. Ultrasound imaging in rheumatoid arthritis. *Radiol Med*. (2019) 124:1087–100. doi: 10.1007/s11547-019-01002-2
42. Ruta S, Reginato AM, Pineda C, Gutierrez M. Pan-American League Against Rheumatisms (PANLAR) Ultrasound Study Group. General applications of ultrasound in rheumatology: why we need it in our daily practice. *J Clin Rheumatol*. (2015) 21:133–43. doi: 10.1097/RHU0000000000000230
43. Naredo E, D'Agostino MA, Conaghan PG, Backhaus M, Balint P, Bruyn GAW, et al. Current state of musculoskeletal ultrasound training and implementation in Europe: results of a survey of experts and scientific societies. *Rheumatology*. (2010) 49:2438–43. doi: 10.1093/rheumatology/keq243
44. Bruyn GA, Iagnocco A, Naredo E, Balint PV, Gutierrez M, Hammer HB, et al. OMERACT definitions for ultrasonographic pathologies and elementary lesions of rheumatic disorders 15 years on. *J Rheumatol*. (2019) 46:1388–93. doi: 10.3899/jrheum181095
45. Davis A, Billick K, Horton K, Jankowski M, Knoll P, Marshall JE, et al. Artificial intelligence and echocardiography: a primer for cardiac sonographers. *J Am Soc Echocardiogr*. (2020) 33:1061–6. doi: 10.1016/j.echo.2020. 04025
46. Moccia S, Vanone GO, Momi E, Laborai A, Guastini L, Peretti G, et al. Learning-based classification of informative laryngoscopic frames. *Comput Methods Programs Biomed*. (2018) 158:21–30. doi: 10.1016/j.cmpb.2018.01030
47. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, et al. Osteoarthritis. *Nat Rev Dis Primers*. (2016) 2:16072. doi: 10.1038/nrdp.201672

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frontiers in Medicine

BRIEF RESEARCH REPORT
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Innovations Developed by Patients and Informal Caregivers for Needs Associated to Rheumatic Diseases

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Until recently, innovation in healthcare was mainly achieved through the development of new drugs, therapies, and medical devices by big pharma and medtech companies; however, the innovative potential for this field is much broader. The patients and caregivers' role in healthcare is often associated with disease management, demand for their own illness data, and its exchange with other patients. However, the patients and caregivers' capacity to innovate to cope with limitations associated with their health condition is a growing phenomenon and starting to be supported by healthcare stakeholders to achieve a truly patient-centric system. Our previous research has shown that these uncommon innovators can develop a wide range of solutions, from simple adaptations and products to highly technological biomedical devices. In this paper, we present novel solutions developed by rheumatic patients, their caregivers, and collaborators, published on the "Patient Innovation" platform (https://patient-innovation.com/), with a focus on the innovator profile, the need that triggers the innovative process, the type of motivation behind the product, and the products developed. The most significant needs that motivate innovation are the will to increase the level of independence (71%) and to be able to perform daily routine activities (65%). In over 80% of cases, the fact that the market does not fully fulfill the needs felt during daily activities is the main motivation to innovate. It is thus concluded that there is room for innovation in rheumatic diseases with solutions developed by patients and informal caregivers that intend to solve needs that the healthcare market is not covering.

Keywords: patient innovation, user innovation, health, rheumatic diseases, disability

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INTRODUCTION

Rheumatic diseases are in the group of diseases recognized earliest in the world (1), and although the most common rheumatic diseases present a high prevalence in the elderly population, rheumatic diseases affect people of all ages and currently have a great impact on patients' daily living as well as on society, as it is commonly reflected in high economic costs through a great consumption of healthcare and social resources.

Despite the critical importance of the healthcare sector for the economy and society, where average health spending reached 8.8% of gross domestic product (GDP) across the Organization for Economic Co-operation and Development (OECD) (2), current healthcare provision does not

always meet patient's real and daily needs. To solve this, some patients and informal caregivers do not wait for a solution to come up in the market, and thus start developing innovative solutions to overcome limitations associated with the health condition that they face (3, 4). Von Hippel started to introduce the concept of user innovation as individuals or firms who expect to directly benefit from a product that they have created (5, 6); this concept differs from producer innovators—firms or individuals who develop novel products to benefit from selling it as a better or new product or service. Thus, when translating the concept of user innovation to the healthcare domain, patient innovators are citizens who develop useful and innovative solutions to cope with their health disorders. These patient innovations range from simple products for everyday use to unknown therapies and high-tech solutions (7). These findings suggested that patients and informal caregivers worldwide may contribute with a high number of innovative solutions with a valid need-based motivation and, if shared and adopted, can improve not only their quality of life but also the life of others with similar health conditions (8). This was the motivation for the creation of the website www.patient-innovation.com, a non-profit international, multilingual, and open platform which currently presents over 1,500 solutions developed by patients, informal caregivers, and collaborators which have been submitted or collected, medically validated, and shared.

In this paper, it is our intention to present and describe relevant innovative solutions developed by rheumatic patients, their informal caregivers, and collaborators, who decided to solve some of their everyday needs—originated by the health condition that they face—but could not find any solution in the market.

METHODS

In order to analyze innovative solutions developed by patients and informal caregivers, the '*Patient Innovation*' platform (www.patient-innovation.com) was used as its presents itself as a centralized inventory of patient-developed solutions.

The '*Patient Innovation*' platform provides the opportunity to patients and caregivers to actively engage in sharing solutions within the *Patient Innovation* community. In order to share a novel solution, the user must register on the website (the connection to *Patient Innovation* is always encrypted and personal data is only stored in high security data centers located in the European Union) and, once logged in, the user can write down his/her solutions for dealing with a condition in his/her daily life (information required: solution title, description, about the innovator, and optional images and videos). Before being published, the *Patient Innovation*'s medical team evaluates and validates (or not) the submitted solutions, by only approving the ones that are not drugs, chemicals, intake/topical substances, invasive devices, or other visibly and intrinsically dangerous proposals. Once these are online, they are available to everyone who may be interested, or can benefit from the published solutions.

The medically validated and published solutions in the '*Patient Innovation*' platform were screened, and solutions that showed features to be possible solutions for rheumatologic diseases were selected. In a first stage analysis, disease-based motivation was screened and rheumatology-based solutions were distinguished from solutions that cover rheumatologic patients' needs/symptoms but were based on other medical conditions. Through the innovation description and information available about the innovator, the type of innovator (patient, caregiver, or collaborator), sex, country, product category (the exact role/goal of the solution developed, divided into the following categories: activities of daily living, pain/therapy, hobbies, and movement), motivation (why the innovators had to develop a novel solution to cope with a limitation/need related with the health condition that they face, divided into the following categories: "the products in the market were not useful to satisfy the need," "there was not any alternative in the market," "cost of the alternatives in the market," and "other") and need to innovate (the need that made the patient, caregiver, or collaborator to innovate and create a novel product, divided into the following categories: "to be able to perform personal hygiene tasks," "to be able to perform a daily routine activity," "to increase the level of independency," "to be able to move," "to reduce pain," "to replace a lost function," "to improve therapy," and "other") were studied. The criteria used for categorization was developed by the authors based on the information available in the solutions description fields (solution title, description, about the innovator, optional images, and videos) posted on the '*Patient Innovation*' platform.

RESULTS

The '*Patient Innovation*' platform presented 1,399 medically screened and published solutions when consulted on July 8th 2020 for the purpose of this research work. From those, 101 published innovations showed features that marked them as possible solutions for rheumatologic patients; most of those solutions were developed for activities of daily living (54%), whereas for hobbies (24%), pain/therapy (15%), and movement (9%) are other significant types of products described in the platform.

Thirty-four percent ($n = 34$, **Table 1**) of those solutions were developed as a response to rheumatologic-based needs by patients, informal caregivers, and collaborators who face difficulties associated with this disease category. The great majority of rheumatologic-based solutions (82%) were developed in the same proportion by patients (those who develop solutions for themselves) and informal caregivers (those who develop solutions for loved ones and/or family); eighteen percent ($n = 6$) of the solutions were developed by collaborators (those who develop solutions for someone out of his/her family/friends circle). Fifty-four percent ($n = 19$) of those solutions were developed by men (46% by women) and by citizens from countries of five continents (e.g., USA, Portugal, China, Australia, and Kenya).

When analyzing the need behind the innovation process for the rheumatologic-based solutions (**Figure 1**), the most significant ones rely on efforts to increase the level of independency (71%) and to be able to perform a daily routine

TABLE 1 | Innovative solutions to cope with rheumatic diseases limitations developed by patients, informal caregivers, and collaborators published in https://patient-innovation.com/.

Product	Type of innovator	Country of origin	Rheumatologic Disease
Hair dryer adaptation for stiff upper limbs[1]	Patient	Portugal	Fibromyalgia
Home-made orthotic for foot pain[2]		USA	Arthritis
Home-made pouches for hot-cold therapy to reduce pain[3]		Canada	Arthritis
ViEx, a string-based device to reduce hand joints pain[4]		USA	Rheumatoid arthritis
Handmade toys to cope with Arthritis[5]		USA	Juvenile Arthritis
Food cutting board and knives to overcome poor hand grip[6]		New Zealand	Rheumatoid Arthritis
Tool to help with gardening without having to bend down[7]		Belgium	Arthritis
Adapted transport container for disabled people scooters[8]		Belgium	Ehlers-Danlos Syndrome
Nose-pad, a device to work in a computer/tablet using nose and lips[9]		Sweden	Arthritis
Gehrad, a walking aid device[10]		Germany	Osteoarthritis
Protective capes for wheelchair users[11]		Belgium	Ehlers-Danlos Syndrome
Sandi Gloves, an easier way to sand[12]		Australia	Arthritis
Chronically Simple, an app for chronic diseases management[13]		Canada	Ehlers-Danlos Syndrome
Fashionable walking aids[14]		UK	Fibromyalgia
Adaptor for car key turning aid to avoid wrist twisting motion[15]	Caregiver	USA	Arthritis
Accessible zipper pull[16]		USA	Arthritis
Assistive door locker[17]		USA	Arthritis
Blanket support to avoid overweighting joints and still not get cold[18]		Portugal	Arthritis
Light intensity regulator for car traveling [19]		Portugal	Arthritis
Exoskeleton glove to enhance hand movements[20]		Greece	Arthritis
Wheelchair tank to ease beach and countryside visits[21]		UK	Arthritis
Egg collector to avoid having to bend over[22]		Belgium	Myopathy
Stair-climbing wheelchair[23]		China	Arthritis
Adapted clothes to enable minimum body movement while dressing[24]		China	Osteoarthritis
Stair-climbing booster[25]		China	Arthritis
Toothpaste squeezer for people with poor grip[26]		USA	Arthritis
Orthorod, an adapted fishing pole[27]		USA	Arthritis
Swachchta Suvidha Brush, a simple slip-on floor cleaner[28]		India	Spondylosis, Arthritis
Book page turning device[29]	Collaborator	USA	Arthritis
Kitchen safety handle for limited hand strength and flexibility[30]		Taiwan	Rheumatoid Arthritis
Obi, a robotic device for autonomous feeding[31]		USA	Arthrogryposis
3D printing device to help to dress[32]		USA	Arthrogryposis Multiplex Congenita
E-Con, an all-terrain wheelchair[33]		Kenya	Arthritis
Finger support to ease finger deformities[34]		USA	Arthritis

Patient Innovation platform location (https://patient-innovation.com/xxxx/zzzz): [1]/node/502, [2]/post/654, [3]/post/920, [4]/post/1203, [5]/post/1234, [6]/post/1345, [7]/post/1365, [8]/post/1372, [9]/post/1376, [10]/post/1642, [11]/post/1648, [12]/post/1944, [13]/post/2416, [14]/post/2473, [15]/post/587, [16]/post/669, [17]/post/670, [18]/post/880, [19]/post/881, [20]/post/1204, [21]/post/1266, [22]/post/1362, [23]/post/1843, [24]/post/1845, [25]/post/1849, [26]/post/1901, [27]/post/2088, [28]/post/2355, [29]/post/861, [30]/post/1304, [31]/post/1537, [32]/post/2022, [33]/post/2039, [34]/post/2209.

activity (65%). Also, pain reduction (24%), replacing a lost function (12%), and mobility (12%) are other relevant needs found in the innovations analyzed.

Following the World Health Organization's definition for medical device (9), 32% of the solutions developed as a response to rheumatologic-based needs under analysis are framed as Class I medical devices (10), which meets the amount of pain reduction and replace a lost function as described above; still, 68% of the analyzed solutions are not framed as medical devices. However, only 15% of these rheumatologic-based solutions are approved by regulatory authorities and are currently being commercialized.

Consequently, the type of products developed by these uncommon innovators mainly rely on activities of daily living (41%), pain/therapy (24%), movement (18%), and hobbies (18%). These innovators found as their main motivation to innovate the fact that the products in the market did not fully meet a need they had (82%). Furthermore, the cost of alternative products (27%) and the inexistence of useful alternatives (9%) available in the market are also important motivations to these innovators to start developing novel solutions to cope with limitations that they face every day.

DISCUSSION

Although "innovation" is a broad term and it can be applicable in different disease's aspects, we can highlight that in the recent years "innovation" in rheumatology has widely expanded in digital health, namely through electronic health records, virtual visits, wearable technologies, and digital therapeutics which improve access, outcomes, adherence, and research (11, 12) or new

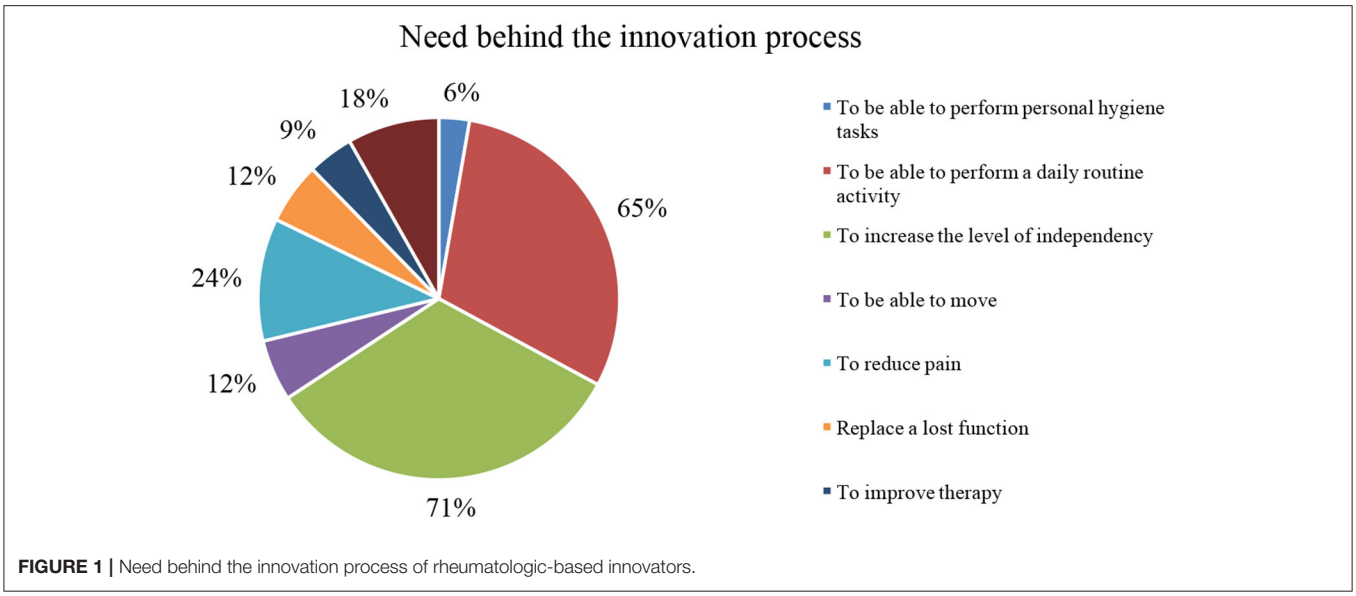

FIGURE 1 | Need behind the innovation process of rheumatologic-based innovators.

therapeutic production approaches such as biosimilars (13). Still, this paper intends to expose that there is still some room for innovation through solutions developed by patients and informal caregivers who intend to solve needs that the healthcare market is not yet covering. To do that, the authors have analyzed 34 rheumatologic-based solutions published on *Patient Innovation*'s database, 14 developed by patients and 14 by informal caregivers, which reinforces that one major source of innovation are the needs that these citizens feel in their daily lives. The fact that 54% of those solutions were developed by men (46% by women) and by citizens from countries of five continents (e.g., USA, Portugal, China, Australia, and Kenya) reflects the patient innovation process to be as global and diverse as the medical diseases themselves. The products analyzed vary from simple devices (e.g., food cutting board and knives to overcome poor hand grip) to high-tech solutions (e.g., exoskeleton glove to enhance hand movements).

Thirty-two percent of the solutions developed under analysis are framed as Class I medical devices; however, only 15% are approved by regulatory authorities and are currently being commercialized, as most of the solutions here described were developed to solve a daily life need felt by patients and informal caregivers and were not manufactured on a large scale for commercialization (at least at the analysis moment). This is a common approach for patient innovators, as this kind of uncommon inventors have only developed their novel solutions because they have a validated need that motivated them to innovate, and thus their main goal was to fulfill that need, not to profit from it. Still, there has been an increasing number of patients and informal caregivers who have been establishing their own solutions in the market as an effective way to distribute their solutions to others with similar needs (14, 15).

The authors would like to highlight the potential of platforms such as '*Patient Innovation*' and other information and communication technologies to promote an easy interaction between common citizens and healthcare professionals. Mainly, healthcare professionals and other stakeholders can use these resources to learn more about patients' real needs and the solutions that they have developed to cope with them, as well as to start including patients as consultants and their innovation process into the producer innovation processes. As innovation by patients and caregivers is still a new topic that has only started to be explored in recent years, it is important that healthcare industry representatives increase their awareness of this phenomenon and support this innovation process to achieve a more effective and cheaper product development process.

Although this work only shows a brief sample of novel solutions developed by common citizens for needs associated with rheumatic diseases, as the authors are sure that many more solutions exist developed by these uncommon inventors than the ones published in '*Patient Innovation*' platform, it is thus concluded that solutions developed by patients and informal caregivers can significantly contribute to rheumatic diseases innovations as it aims to solve needs that the healthcare market is not yet covering.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://patient-innovation.com/.

AUTHOR CONTRIBUTIONS

MJ was responsible for conceptualization, information extraction, formal analysis, methodology, writing, and edition. PO and HC were responsible for funding acquisition, project administration, resources, supervision, validation, writing, review, and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Sangha O. Epidemiology of rheumatic diseases. *Rheumatology (Oxford).* (2000) 39(Suppl 2):3–12. doi: 10.1093/rheumatology/39.suppl_2.3
2. Development OfEC-oa. *OECD Health Statistics*. (2020). Available online at: http://www.oecd.org/els/health-systems/health-data.htm2020
3. Oliveira P, Zejnilovic L, Canhão H, von Hippel E. Innovation by patients with rare diseases and chronic needs. *Orphanet J Rare Dis.* (2015) 10:41. doi: 10.1186/s13023-015-0257-2
4. Zejnilovic L, Oliveira P, Canhão H. Innovations by and for the patients: and how can we integrate them into the future health care system. In: Albach H, Meffert H, Pinkwart A, Reichwald R, von Eiff W, editors. *Boundaryless Hospital: Rethink and Redefine Health Care Management*. Berlin: Springer-Verlag (2016). p. 341–57.
5. von Hippel E. The dominant role of users in the scientific instrument innovation process. *Res Policy.* (1976) 5:212–39. doi: 10.1016/0048-7333(76)90028-7
6. Von Hippel E. Lead Users: a source of novel product concepts. *Manag Sci.* (1986) 32:791–805. doi: 10.1287/mnsc.32.7.791
7. Oliveira P, Canhão H. Users as service innovators: evidence from banking to healthcare. In: Lakhani K, Harhoff D, editors. *Revolutionizing Innovation*. Cambridge, MA: MIT Press (2014). 32 p.
8. Oliveira P, Zejnilovic L, Azevedo S, Rodrigues AM, Canhão H. Peer adoption and development of health innovations by patients: National Representative Study of 6204 Citizens. *J Med Internet Res.* (2019) 21:e11726. doi: 10.2196/11726
9. Organization WH. *Medical Device - Full Definition.* Available online at: https://www.who.int/medical_devices/full_deffinition/en/
10. Strålin M. *Classification of Medical Devices and Their Routes to CE Marking.* CE Check Support (2020). Available online at: https://support.ce-check.eu/hc/en-us/articles/360008712879-Classification-Of-Medical-Devices-And-Their-Routes-To-CE-Marking
11. Solomon DH, Rudin RS. Digital health technologies: opportunities and challenges in rheumatology. *Nat Rev Rheumatol.* (2020) 16:525–35. doi: 10.1038/s41584-020-0461-x
12. Rodrigues, Canhão. Innovation and digital transformation to support clinical care and prevent osteoporosis related fractures. *Acta Reumatol Port*. (2019) 44:171–2. Available online at: http://actareumatologica.pt/article_download.php?id=1408
13. Dörner T, Strand V, Cornes P, Gonçalves J, Gulácsi L, Kay J, et al. The changing landscape of biosimilars in rheumatology. *Ann Rheum Dis.* (2016) 75:974–82. doi: 10.1136/annrheumdis-2016-209166
14. Shcherbatiuk V. *Users as Developers and Entrepreneurs of Medical Treatments/Devices: The Case of Patients and their Families and Friends*. Catholic University of Portugal (2012).
15. Habicht H, Oliveira P, Shcherbatiuk V. User innovators: when patients set out to help themselves and end up helping many. *Die Unternehmung.* (2013) 66:277–94. doi: 10.5771/0042-059X-2012-3-277

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ORIGINAL RESEARCH
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The Impact of Serum Anti-neutrophil Cytoplasmic Antibody on Clinical Characteristics and Outcomes in Pediatric-Onset Systemic Lupus Erythematosus Patients

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Background: Systemic lupus erythematosus (SLE), an autoimmune disease, is characterized by the overproduction of autoantibodies. Anti-neutrophil cytoplasmic antibodies (ANCAs) have been recognized in SLE for decades. To date, their association with SLE disease activity, especially in pediatric-onset SLE (pSLE) patients, is limited.

Methods: We conducted a retrospective case-control study of pSLE patients with ANCAs from 2010 to 2020. Clinical characteristics, laboratory data, renal histological features, treatment and outcomes were analyzed.

Results: A total of 70 pediatric-onset SLE patients (9 ANCA-positive vs. 61 ANCA-negative) with a median age of 12.23 years (age ranging from 4 years to 18 years) at diagnosis were enrolled. Among patients with ANCAs, MPO-ANCA was found in seven and PR3-ANCA in two of those cases. Patients with ANCAs had a tendency to have hematuria compared with those without ANCAs (66 vs. 24.6%, respectively; $p = 0.026$). Of the 70 SLE patients, 8 with ANCAs and 44 without ANCAs underwent renal biopsies. Patients with ANCAs (25%, 2/8) were more likely to lack the typical full-house pattern in their renal immunofluorescence (IF) staining.

Conclusion: pSLE patients with ANCAs tend to have hematuria and an absence of typical IF histology. However, patients with and without ANCAs showed no difference in their clinical presentations and treatment outcomes.

Keywords: anti-neutrophil cytoplasmic antibody, pediatric-onset systemic lupus erythematosus, lupus nephritis, hematuria, children

BACKGROUND

Systemic lupus erythematosus (SLE), a prototype of autoimmune disease with systemic involvement, is characterized by the breakdown of self-tolerance and various autoantibody production. Pediatric-onset SLE (pSLE) accounts for 15–20% of all cases and usually presents with a more aggressive clinical phenotype compared with adult-onset SLE. Although pediatric and adult lupus patients share similar clinical manifestations, lupus nephritis (LN) among the pediatric-onset population has been suggested to be different from the adult-onset form in its abrupt onset and relatively poor response to treatment (1).

Anti-neutrophil cytoplasmic antibodies (ANCAs) are a group of autoantibodies directed against cytoplasmic antigens within human neutrophils and monocytes and have been detected in patients with small vessel vasculitis and inflammatory disease (2–4). In fact, vasculitis is a common manifestation in lupus patients, and the presence of ANCAs related to clinical presentation, including interstitial lung disease, chronicity index and histopathology of lupus nephritis, has been flourishingly reported in adult lupus patients in this 5-year study (5–9). However, the association of ANCAs with pediatric-onset SLE has not been well-reviewed and has limited the discussion on the association between ANCA presence and clinical characteristics (10, 11). To date, the impact of ANCAs on renal manifestations, disease severity and clinical outcome among pediatric SLE patients remains to be elucidated.

The aim of this study was to investigate the impact of ANCAs on clinical manifestations, organ involvement and outcomes in pediatric-onset lupus patients.

METHODS

This retrospective case-control study was approved by the Ethics Committee on Human Studies at Chang Gung Memorial Hospital in Taiwan, R.O.C. (IRB 201601678A3C501). Informed consent was obtained from the patients and their parents, and all methods including chart review were performed in accordance with the relevant regulations.

Subjects

One hundred and eleven SLE patients diagnosed according to the 1997 SLE American College of Rheumatology criteria from 2010 to 2020 were recruited from a tertiary hospital (12). Patients with disease onset before the age of 18 who received regular clinical follow-up every 1–3 months for more than 6 months were enrolled (**Figure 1**). Nine patients with ANCAs were identified. Sixty-one patients without ANCAs were consecutively enrolled in the control group after adjustment for age, sex, and SLE disease activity index (SLEDAI) score (13).

Clinical and Laboratory Characteristics and Outcome

The SLE-related laboratory data, including renal function (creatinine data and urinary analysis), hematology including lymphopenia (white blood count $<4{,}000/mm^3$ more than twice), leukopenia (lymphocyte count $<4{,}000/mm^3$ more than two times), hemolytic anemia with reticulocytosis and thrombocytopenia (platelet count $<1{,}000.00/mm^3$ in the absence of drug use), complement C3 and C4, anti-nuclear antibody (ANA), and anti-double stranded DNA antibody (anti-dsDNA Ab), were collected, and clinical manifestations noted within the previous 10 years, including mucocutaneous presentation (malar rash, photosensitivity, discoid rash or oral ulcer), cardiovascular systems (myocarditis, pericardial effusion or pericarditis), non-erosive arthritis, pulmonary manifestations (hemoptysis or abnormality on image by radiologists) and neurological disorder (neuritis, seizure or psychosis), at presentation and follow up were recorded. Proteinuria was defined as daily protein excretion more than 4 $mg/m^2/h$, 500 mg per 24 h or more than 3+ on urinalysis based on the 1997 American College of Rheumatology revised criteria (12). Hematuria was diagnosed as more than five red blood cells per high-power field. The demographic data, medications and outcomes, including acute kidney injury, chronic renal failure and all-cause mortality were also analyzed during the follow-up. Hypocomplementemia was defined as a C3 level less than the lower limit (90 mg/dl) or a C4 level less than the lower limit (10 mg/dl). Disease activity, quantified by the SLEDAI-2K score, was also calculated (13). Acute kidney injury was defined as a serum creatinine level more than 0.3 mg/ dl within 48 h or serum creatine more than 1.5 times that of the baseline value within the prior seven days (14). Furthermore, we utilized the bedside Schwartz equation to estimate the measured glomerular filtration rate (mGFR, ml/min/1.73 m^2) status, and chronic kidney disease (CKD) was assessed at 6 months after the administration of proper treatment: stage 1, mGFR>90; stage 2, 89>mGFR>60; stage 3, 59>mGFR>30; stage 4, 29> mGFR>15 and stage 5, mGFR<15 (15, 16).

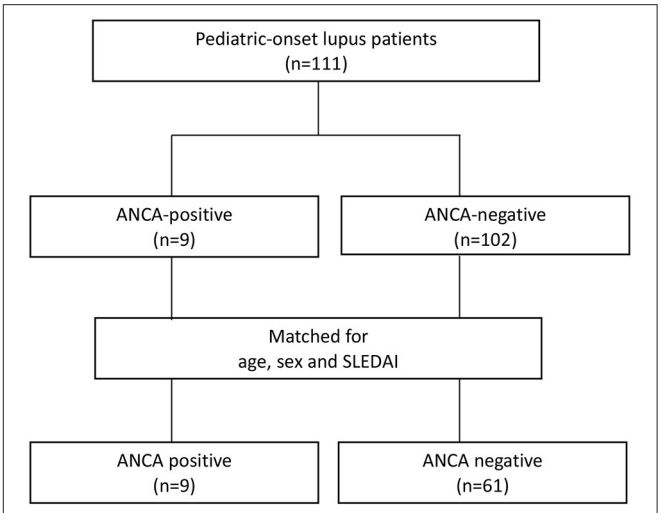

FIGURE 1 | Flow chart of patient collection and categorization.

Renal Histopathology

Fifty-two of the patients (8 positive and 44 negative for ANCAs) underwent renal biopsy due to nephritis. The histological studies, including light microscopy, immunofluorescence (IF)

and electron microcopy studies, were reviewed by a pathologist who was an expert in renal diseases. The full-house pattern, which is the histological hallmark of lupus nephritis as described in the 2012 Systemic Lupus International Collaborating Clinics criteria based on their proposed classification of systemic lupus erythematosus, was defined as the presence of IgG, IgA, IgM, C1q and C3 on IF staining (12). Lupus nephritis, activity and chronicity were classified according to the International Society of Nephrology/Renal Pathology Society (17).

TABLE 1 | Clinical Data of Lupus patients with Anti-neutrophil cytoplasmic antibodies (ANCA) at the first diagnosis.

	1	2	3	4	5	6	7	8	9
Onset age (year)	14	11	13	13	14	15	14	10	12
Follow-up duration (months)	195	124	124	19	81	53	21	99	228
Sex	Female	Female	Female	Male	Female	Female	Female	Female	Female
Laboratory data at presentation									
ANA (Normal range < 1:80)	-	+	+	+	+	+	+	+	+
Anti-dsDNA antibodies*(Normal range <130 WHO unit/mL)	83.5	35.6	490.1	49.1	195.3	47	351.6	40.5	221.3
ANCA, p-ANCA (Normal range < 5 IU/ml)	6.1	11.8	11	134.7	134	146	146	-	-
ANCA, c-ANCA (Normal range <3 IU/ml)	-	-	-	-	-	-	-	5.7	3.3
Hypocomplementemia/ (Normal range C3 >90 or C4>10 mg/dl)	+	+	+	+	-	+	+	+	+
Urine protein	Negative	18.2mg/m^2/h	12.8 mg/m^2/h	79.8 mg/m^2/h	30mg/dl	77.6 mg/m^2/h	718 24 mg/m^2/h	118.7 mg/m^2/h	1+
Hematuria	-	+	+	+	+	-	+	+	-
Serum albumin (Normal range 2.8–5.4 g/dl)	-	4.6	2.86	3.09	3.79	3.86	2.9	3.25	4.8
Serum creatinine** (Normal range 0.2–1.0 mg/dl)	-	0.47	0.34	0.91	0.65	0.49	5.39	0.36	0.51
Renal pathology									
Light microscopy	-	LN III	Cast nephropathy	LN IV	LN V	LN V	LN V	LN IV	LN IV
Full house	-	+	(IgG 1+)	+	(IgG 3+)	+	+	+	+
Organ involvement at presentation									
Kidney	-	+	+	+	+	+	+	+	+
Hematology	+	-	+	+	+	-	+	+	+
Dermatology	-	+	-	+	-	-	-	+	+
Joints	+	+	-	-	-	-	-	-	-
Heart	-	-	+	-	-	-	-	-	-
Neurology	-	-	-	-	-	+	-	-	-
Pulmonary	-	-	-	+	-	-	-	-	-
Treatment									
Steroid	+	+	+	+	+	+	+	+	+
Azathioprine	+	-	+	-	-	+	-	_	+
HCQ	+	+	+	-	-	-	-	+	+
MMF	-	+	-	+	+	+	+	-	-
Others	-	Cyclosporine, Cyclophosphamide	-	Plasma exchange	Cyclosporine	-	Plasma exchange	Cyclophosphamide	-
Outcome									
Persistent hematuria	-	-	-	+	+	-	-		-
Chronic kidney disease (stage)	-	-	-	2	1	1	3	1	-

LN, lupus nephritis; HCQ, hydroxychloroquine; MMF, mycophenolic acid.

**Anti-double stranded DNA (dsDNA) antibodies (WHO unit/mL), **serum creatinine (mg/dl) at biopsy.*

+Case 1 did not have the indication for renal biopsy.

Measurement of Serum ANCAs

Serum ANCA levels were routinely checked at presentation, every clinic visit and follow-up for all enrolled patients during the follow-up. Serum ANCA levels were measured by an enzyme-linked immunosorbent assay (ELISA), and an anti-MPO antibody level >5.0 U/ml and anti-PR3 antibody level >3.0 U/ml were defined as positive findings according to the manufacturer's instructions (Pharmacia Diagnostic AB, Uppsala, Sweden). In addition, defining ANCA positivity according to the ELISA method for MPO and PR3 (not as immunofluorescence assay testing positive) is an important issue, while it has been suggested that the p-ANCA pattern in the immunofluorescence test could be misinterpreted due to the interference of ANA (6, 18).

Statistical Analyses

Data were analyzed using Student's *t* test to compare the means between two groups of continuous data. The chi-square test or Fisher's exact test (if any value was <5 in all comparisons) was used for categorical data to assess differences in clinical characteristics and evaluate the result of renal biopsy, medication and outcomes. Moreover, 95% confidence intervals were applied. Statistical analysis was performed with SPSS Statistics version 22.0.0 (SPSS Inc., Chicago, United States), and a p-value <0.05 was considered indicative of statistical significance.

RESULTS

Demographic and Clinical Manifestations of pSLE Patients With ANCAs

As shown in **Table 1**, nine patients (one male and eight females) with a mean age of 12.6 ± 1.5 years (ranging from 10 to 15 years) and ANCA positivity were enrolled over 10 years. Seven and two patients had anti-MPO antibody and anti-PR3 antibody, respectively. Eight patients had proteinuria or hematuria at the initial presentation and underwent renal biopsy. Two patients had biopsy-proven membranous glomerulopathy and cast nephropathy without full-house deposition on IF staining. Seven, four, two and one patient had hematological presentations, mucocutaneous lesions, arthritis and serositis, respectively. The cases with high ANCA levels (ANCA level >100) had severe extra-renal presentations; specifically, case 4 had pulmonary hemorrhage and case 6 had vestibulocochlear neuritis. Four patients (cases 4, 5, 6, and 7) with high serum ANCA levels of more than 100 IU/ml had acute kidney injury at presentation and reached chronic kidney disease during follow up. After 6 month treatment, two patients had persistent hematuria.

Comparison of pSLE Patients With and Without ANCAs

Demographic Data

Sixty-one pSLE patients were enrolled in the control group. As shown in **Table 2**, there were no differences between patients with and without ANCAs with respect to sex (88.9 vs. 90.2% were female), age at diagnosis (12.93 ± 3.28 vs. 13.02 ± 1.63 years old) or follow-up duration (98.73 ± 68.43 vs. 132.16 ± 73.14 months).

TABLE 2 | Clinical characteristics of pediatric-onset SLE patients with and without anti-neutrophil cytoplasmic antibodies (ANCAs).

	ANCA-positive (n = 9)	ANCA-negative (n = 61)	P-value
Female (%)	8 (88.9%)	55 (90.2%)	0.905
Onset age (year)	12.93 ± 3.28	13.02 ± 1.63	0.937
Follow-up (months)	98.73 ± 68.43	132.16 ± 73.14	0.368
SLEDAI$^+$ at presentation	12.11 ± 6.92	12.95 ± 7.11	0.314
Laboratory data at presentation			
C3 (g/L)	66.49 ± 29.67	54.31 ± 35.51	0.334
C4 (g/L)	11.86 ± 7.32	9.75 ± 7.54	0.449
Anti-dsDNA antibodies (unit/mL)	178.68 ± 156.60	240.76 ± 150.78	0.948
Creatinine (mg/dL)	1.16 ± 1.62	0.68 ± 0.43	0.059
WBC (10^6/L)	6255.56 ± 4143.40	5417.54 ± 2930.88	0.455
Platelets (10^6/L)	189.78 ± 93.03	180.51 ± 113.32	0.817
Hemoglobin (g/L)	9.86 ± 2.75	11.30 ± 2.91	0.168
Proteinuria (%)	4 (50%)	41 (67.2%)	0.435
Hematuria (%)	6 (66.6%)	15 (24.6%)	0.026*
Renal histology			
Activity	8.25 ± 2.63	7.28 ± 4.39	0.64
Chronicity	0.5 ± 1.00	2.30 ± 2.48	0.11
Treatment at presentation and during follow-up, numbers			
Prednisolone (%)	9 (100%)	61(100%)	N/A
Azathioprine (%)	5 (55.6%)	44 (72.1%)	0.128
Cyclophosphamide (%)	3 (33.3%)	23 (37.7%)	1.000
Mycophenolic acid (%)	3 (33.3%)	31 (50.8%)	0.479
Hydroxychloroquine (%)	6 (66.7%)	43 (70.5%)	1.000
Cyclosporine (%)	1 (11.1%)	12 (19.7%)	1.000
Rituximab (%)	0 (0%)	3 (4.9%)	1.000
Outcome, numbers			
Acute kidney injury (%)	1 (11.1%)	3 (4.9%)	0.538
Chronic renal disease (%)	5 (55.6%)	29 (47.5%)	0.970
End-stage renal disease (%)	0 (0%)	4 (6.6%)	0.143
Death (%)	0 (0%)	1 (1.7%)**	0.696

$^+$ *SLEDAI, SLE disease activity index; **Died of sepsis, *p < 0.05.*

Laboratory Characteristics

There were no significant differences in serological characteristics, including complement, anti-dsDNA antibody, serum creatinine, white blood cell count, platelet count and hemoglobin, between patients with and without ANCAs at the time of diagnosis. However, patients with ANCAs had a higher rate of initial hematuria than those without ANCAs (62.5 vs. 24.6%, respectively; $p = 0.026$), as shown in **Table 2**.

Organs Involved

As shown in **Figure 2**, skin, cardiovascular and joint involvements were the most common manifestations in patient with ANCA. Only patients without ANCAs had gastrointestinal organ damage. However, there was no significant difference in organ involvement between the two groups.

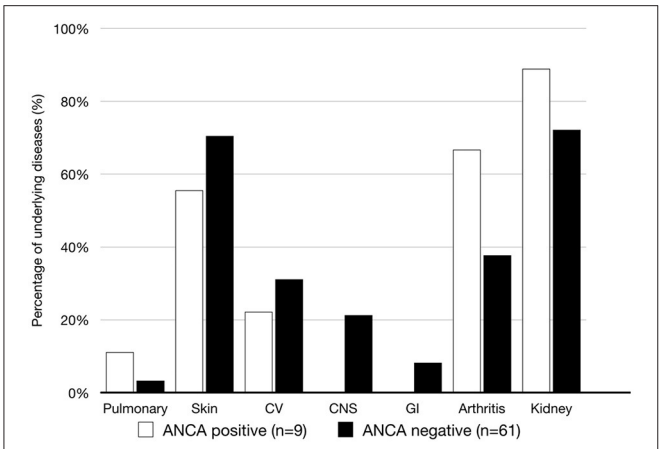

FIGURE 2 | Organ systems involved in the patients with and w ithout ANCAs during the follow-up were investigated. CV, cardiovascular; GI, gastrointestinal.

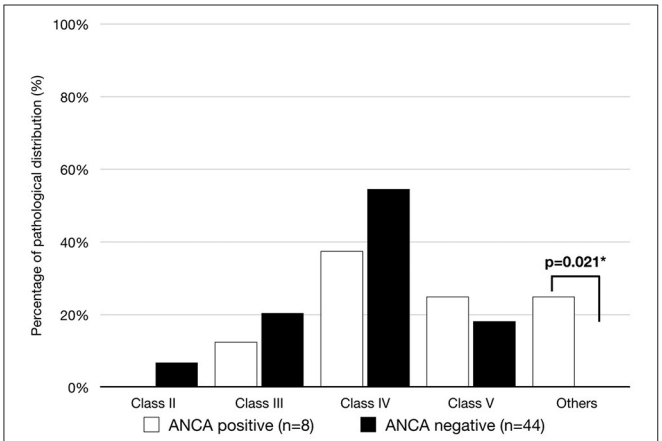

FIGURE 3 | Patients with biopsy with ISN classes of lupus nephritis in the ANCA-positive and ANCA-negative groups. A significant difference was observed in the ANCA-positive group, but the classic full-house deposition was absent (p-value <0.05).

Renal Histology

The lack of typical full-house IF staining was revealed in two patients with ANCAs but not in patients without ANCAs (25 vs. 0%, $p = 0.021$). In patients with ANCAs, the histological features were class IV glomerulonephritis (3, 37.5%), class V glomerulonephritis (2, 25%), class I glomerulonephritis (1, 12.5%) and nephritis with an absence of glomerulonephritis (2, 25%). As shown in **Figure 3**, in the 44 lupus patients without ANCAs, class IV was also the most prevalent histopathology (24, 54.5%), followed by class II, class III and class IV glomerulonephritis (3, 6.8%; 9, 20.4%; 9,18.2%; respectively).

Treatment

As shown in **Table 2**, all of the patients received steroid treatment. Immunosuppressants, including azathioprine and hydroxychloroquine, were used if there was hematological or mucocutaneous involvement. For the patients with biopsy-proven proliferative lupus nephritis, intravenous cyclophosphamide or mycophenolic acid (MMF) was administered. In addition, rituximab and cyclosporin were administered in cases of poor response to each of these drugs. There were no obvious differences between patients with or without ANCAs.

Outcomes

During the observation period, in the ANCA group, one patient had acute kidney injury and five had chronic kidney disease after 6 months of treatment, while three patients had acute kidney injury and twenty-nine had chronic kidney disease in the non-ANCA group, which did not correspond to a significant difference. Of the patients without ANCAs, four needed hemodialysis, and one died due to sepsis during follow up. In contrast to the patients without ANCAs, none of the nine patients with ANCAs progressed to renal failure or death during the follow-up period. However, no significant difference in mortality between patients with and without ANCAs was found.

DISCUSSION

This was the first longitudinal study that analyzed the impact of ANCAs on pSLE patients clinical implications. Our results indicate that the positive ANCA titers in pSLE cases are significantly associated with hematuria. In cases with positive ANCAs, two distinct histopathologies and atypical presentation without the full-house pattern of deposition in IF staining were discovered. Furthermore, in the case analysis, obvious renal manifestation as hematuria was noted in positive ANCA cases, and some patients with a high ANCA level also had pulmonary hemorrhage and neurological manifestations. Therefore, we focused on determining the renal association of hematuria and IF staining.

During the Hill et al. clinical follow-up, patients with ANCA-associated vasculitis had persistent hematuria (17). In addition, Mahoney et al. noted that persistent hematuria may be observed in ANCA vasculitis in adults, but it is not related to recurrence (19). In adult lupus patients with ANCAs, however, Pyo et al. reported no increased incidence of hematuria (5). To date, only two studies have mentioned the clinical presentation in pSLE with ANCAs, but neither studied the clinical manifestations of hematuria (10, 20). As indicated by Wang et al., the ANCA was thought to relate to neutrophil extracellular trap (NET) formation, which caused cell death and provided a novel source of autoantigens that resulted in poor renal outcome of lupus patients (21). Additionally, in our study, we found that pediatric-onset SLE patients may be prone to renal damage, which is considered to be related to ANCAs with hematuria.

The typical renal histopathological presentation of patients with ANCA-associated systemic vasculitis includes necrotizing vasculitis. According to previous reports, crescentic glomerulonephritis and global sclerosing were the most common renal histopathologies among patients with ANCA-associated renal vasculitis (22). In addition, Turner-Stokes et al. found a high frequency of lupus class IV-S lupus nephritis in ANCA-positive adult patients (8). Furthermore, recent studies

confirmed that ANCA-associated vasculitis is related to the chronicity index in adults (5). However, there was no significant difference in treatment outcome or disease severity (8). After reviewing these 8 cases with ANCA-associated LN, we did not find evidence of differences in outcomes, treatment, crescent formation, classification or chronicity index in pSLE patients in the presence or absence of ANCAs. However, while examining the deposition of immunofluorescence, the renal biopsy samples lacked the full-house pattern present in the two pSLE patients with ANCAs. Although the relationship between the mechanism of ANCA vasculitis and lupus needs further investigation, the early recognition of ANCAs in pSLE patients may result in favorable and appropriate outcomes in contrast to the results of a previous study. However, this may contribute to a faster and more intensive treatment for patients with than without ANCAs.

In a review of laboratory data, Su et al. found that ANCA-positive patients with new-onset adult SLE may have higher serum creatinine levels than ANCA-negative patients (9). However, the laboratory data or renal histopathological presentation were never reviewed in pSLE patients with ANCA. In our study, although not statistically significant, the serum creatinine of patients with ANCAs was higher than that of patients without ANCAs in our study. However, the lack of significance may be due to the small number of subjects in this case or the quicker intervention than that received by the adult lupus nephritis patients.

Furthermore, as is already known, a positive ANCA titer may be related to interstitial lung disease (7). Yu et al. conducted a study on lupus patients with ANCAs that indicated no obvious difference in mucocutaneous, gastrointestinal and arthritis aspects but indicated a propensity for lupus myocarditis (23). An evidence study indicated that lupus patients with ANCAs are more likely to have severe neurological presentations, including psychosomatic manifestations (24). In a Faure-Fontenla study, pSLE patients with positive ANCA did not have evidence of vasculitis-associated features or specific organ involvement (10). This is also in accordance with research reporting no obvious extra-renal characteristics.

These were some limitations of our study. One is the small population due to the low prevalence [16% prevalence of ANCA in patients with lupus nephritis (21)] in pSLE patients, which may mask the possibility of complications, and we had to select appropriate control cases with the same disease severity for analysis. Furthermore, although the treatment may have selection bias, we treated our patients according to the current standard protocol (25).

CONCLUSION

Currently, hematuria and the lack of a full house were found in pediatric lupus patients with ANCA, and patients with or without ANCAs showed no difference in their clinical presentations and treatment outcomes. Further study is warranted to verify our findings.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by This retrospective case-control study was approved by the Ethics Committee on Human Studies at Chang Gung Memorial Hospital in Taiwan, R.O.C. (IRB 201601678A3C501). Informed consent was obtained from the patients and their parents. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

C-CG, M-HT, C-YW, H-YY, and J-LH: study conception and design. C-CG: acquisition of data and drafting the article. C-CG, M-HT, C-YW, and H-YY: analysis and interpretation of data. M-HT and C-YW: revising the article. All authors have finally approved the submitted version to be published.

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REFERENCES

1. Aggarwal A, Srivastava P. Childhood onset systemic lupus erythematosus: how is it different from adult SLE? *Int J Rheum Dis.* (2015) 18:182–91. doi: 10.1111/1756-185x.12419
2. Kirito Y, Yamamoto D, Uchiyama T. Proteinase 3-antineutrophil cytoplasmic antibody-positive ulcerative colitis presenting with abducens neuropathy. *BMJ Case Rep.* (2017) 2017:bcr2016218353. doi: 10.1136/bcr-2016-218353
3. Mahler M, Damoiseaux J, Ballet V, Dillaerts D, Bentow C, Cohen Tervaert JW, et al. PR3-anti-neutrophil cytoplasmic antibodies (ANCA) in ulcerative colitis. *Clin Chem Lab Med.* (2017) 56:e27–e30. doi: 10.1515/cclm-2017-0346
4. Olbjorn C, Cvancarova Smastuen M, Thiis-Evensen E, Nakstad B, Vatn MH, Perminow G. Serological markers in diagnosis of pediatric inflammatory bowel disease and as predictors for early tumor necrosis factor blocker therapy. *Scand J Gastroenterol.* (2017) 52:414–9. doi: 10.1080/00365521.2016.1259653

5. Pyo JY, Jung SM, Song JJ, Park YB, Lee SW. ANCA positivity at the time of renal biopsy is associated with chronicity index of lupus nephritis. *Rheumatol Int.* (2019) 39:879–84. doi: 10.1007/s00296-019-04263-2
6. Weinstein A. ANCA in lupus nephritis. *Lupus.* (2018) 27:522. doi: 10.1177/0961203317734925
7. Alba MA, Flores-Suarez LF, Henderson AG, Xiao H, Hu P, Nachman PH, et al. Interstital lung disease in ANCA vasculitis. *Autoimmun Rev.* (2017) 16:722–9. doi: 10.1016/j.autrev.2017.05.008
8. Turner-Stokes T, Wilson HR, Morreale M, Nunes A, Cairns T, Cook HT, et al. Positive antineutrophil cytoplasmic antibody serology in patients with lupus nephritis is associated with distinct histopathologic features on renal biopsy. *Kidney Int.* (2017) 92:1223–31. doi: 10.1016/j.kint.2017.04.029
9. Su F, Xiao W, Yang P, Chen Q, Sun X, Li T. Anti-neutrophil cytoplasmic antibodies in new-onset systemic lupus erythematosus. *An Bras Dermatol.* (2017) 92:466–9. doi: 10.1590/abd1806-4841.20175476
10. Faure-Fontenla MA, Rodriguez-Suarez RS, Arias-Velasquez R, Garcia-Gonzalez JE. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus in childhood. *J Rheumatol.* (1999) 26:2480–1.
11. Bakkaloglu A, Topaloglu R, Saatci U, Ozdemir S, Ozen S, Bassoy Y, et al. Antineutrophil cytoplasmic antibodies in childhood systemic lupus erythematosus. *Clin Rheumatol.* (1998) 17:265–7.
12. Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum.* (2012) 64:2677-86. doi: 10.1002/art.34473
13. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum.* (1992) 35:630–40.
14. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract.* (2012) 120:c179–84. doi: 10.1159/000339789
15. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. *Pediatrics.* (1976) 58:259–63.
16. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis.* (2002) 39(2 Suppl 1):S1–266.
17. Hill GS, Delahousse M, Nochy D, Tomkiewicz E, Remy P, Mignon F, et al. A new morphologic index for the evaluation of renal biopsies in lupus nephritis. *Kidney Int.* (2000) 58:1160–73. doi: 10.1046/j.1523-1755.2000.00272.x
18. Sobral S, Ramassur K, Apsley E, Isenberg D. Do anti-neutrophil cytoplasmic antibodies play a role in systemic lupus erythematosus (SLE) patients? Analysis of the University College Hospital SLE cohort. *Lupus.* (2018) 27:343–4. doi: 10.1177/0961203317724218
19. Mahoney SL, Nachman PH. Persistent hematuria in ANCA vasculitis: ominous or innocuous? *Clin J Am Soc Nephrol.* (2018) 13:201–2. doi: 10.2215/cjn.14101217
20. Bobek D, Vukovic J, Malenica B, Bojanic K, Rukavina I, Jelusic M. Anti-neutrophil cytoplasmic antibody positivity in five children with systemic lupus erythematosus–what is the importance of this finding? *Acta Dermatovenerol Croat.* (2014) 22:264–70.
21. Wang Y, Huang X, Cai J, Xie L, Wang W, Tang S, et al. Clinicopathologic characteristics and outcomes of lupus nephritis with antineutrophil cytoplasmic antibody: a retrospective study. *Medicine.* (2016) 95:e2580. doi: 10.1097/MD.0000000000002580
22. Cordova-Sanchez BM, Mejia-Vilet JM, Morales-Buenrostro LE, Loyola-Rodriguez G, Uribe-Uribe NO, Correa-Rotter R. Clinical presentation and outcome prediction of clinical, serological, and histopathological classification schemes in ANCA-associated vasculitis with renal involvement. *Clin Rheumatol.* (2016) 35:1805–16. doi: 10.1007/s10067-016-3195-z
23. Yu YW, Liu ZR, Xie D, Chen SX, Li HY. [Clinical significance of antineutrophil cytoplasmic antibodies in patients with lupus nephritis]. *Nan fang yi ke da xue xue bao = J South Med Univ.* (2006) 26:833–6.
24. Martinez-Morillo M, Lopez R, Ibernon M, Olive A. [Renal p-ANCA vasculitis in patients with systemic lupus erythematosus]. *Med Clin.* (2011) 137:379–80. doi: 10.1016/j.medcli.2010.10.004
25. Bertsias G, Ioannidis JP, Boletis J, Bombardieri S, Cervera R, Dostal C, et al. EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. *Ann Rheum Dis.* (2008) 67:195–205. doi: 10.1136/ard.2007.070367

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Risk of Systemic Lupus Erythematosus in Patients With Anti-phospholipid Syndrome: A Population-Based Study

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Objective: To investigate the association between anti-phospholipid syndrome (APS) and the risk of newly diagnosed systemic lupus erythematosus (SLE).

Methods: We used 2003–2013 data derived from Taiwan's National Health Insurance Research Database to conduct this nationwide, population-based. We identified AS patients newly diagnosed between 2005 to 2013 as the study group and applied age-sex matched (1:20) and propensity score-matched (PSM) (1:2) non-SLE individuals as controls. The association between APS and risk of incident SLE was determined by calculating hazard ratios (HRs) with 95% confidence intervals (CIs) using Cox proportional hazard regression analysis.

Results: We identified 1,245 patients with APS as well as 24,900 age- and sex-matched non-APS controls and 727 APS patients as well as 1,454 PSM non-APS controls. We found that the risk for incident SLE in the APS group was 80.70 times higher than the non-APS group, and the association remained robust after PSM (HR, 28.55; 95% CI, 11.49–70.91). The increased risk for SLE in patients with APS mainly existed within 5 years after the diagnosis of APS. The sensitivity analyses found that the risk for SLE in patients with APS was consistent excluding patients with ITP/AIHA and using distinct definitions of SLE.

Conclusion: The present population-based study revealed a robust association between SLE risk and recent APS and highlights the need for vigilance of SLE-associated symptoms in patients who had been diagnosed with APS.

Keywords: anti-phospholipid syndrome, systemic lupus erythematosus, autoimmune diseases, risk, propensity matching

INTRODUCTION

Systemic lupus erythematosus (SLE) can manifest with haematological and vascular abnormalities prior to the diagnosis of SLE, and recent studies including our study have found an increased risk for SLE in patients with haematological abnormalities, including immune thrombocytopenia (ITP) and autoimmune hemolytic anaemia (AIHA) (1, 2). However, clinical evidence with regards to the association between vascular abnormalities and SLE remains sparse.

Anti-phospholipid syndrome (APS), characterised by obstetric morbidities and/or arterial/venous thrombosis, has been highly implicated with SLE. APS and SLE are two closely correlated autoimmune diseases with overlapped clinical and biological characteristics (3–5). Approximately 30% of APS has been reported to be associated with SLE (6, 7). Cervera et al. conducting a 10-year-follow-up study among 1,000 patients with APS in 13 European countries, reported that 36.2% of APS was associated with SLE (7). Furthermore, the anti-phospholipid antibodies were found in ∼40% of patients with SLE (6, 8). Additionally, SLE-associated APS was found to be more likely to have thrombocytopenia than patients with primary APS (7, 9). Therefore, APS appears to be associated with the development of SLE, and we hence aim to investigate the association between APS and incident SLE. In the present study, we used a nationwide population-based database and propensity matching to address the strength and time-course of association between APS and the development of SLE.

MATERIALS AND METHODS

Ethics Statement

The Institutional Review Board of Taichung Veterans General Hospital in Taiwan approved the present study (approval number: CE17100B). The requirement for informed consent was waived due to that the data in the present study were anonymised before analyses.

Data Sources

The data used in this study were derived from the National Health Insurance Database (NHID). The Taiwan National Health Research Institutes (NHRI) collected and maintained the enrollment files and original reimbursement claims data from the National Health Insurance (NHI) administration, and then released them to the NHID. The database includes the stored medical claims from 1997 to 2013 for nearly 99% of the 23.74 million Taiwanese residents. Diagnosis of inpatients and outpatients based on the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM), clinical examinations, prescriptions, and medical expenses are recorded in this database, which can be used to study the incidence and correlation of diseases. However, the NHIRD lacked laboratory data and some personal information such as smoking, drinking, body weight, and body length.

Study Design and Participants

This retrospective study was designed to address the association between anti-phospholipid syndrome (APS) and incident systemic lupus erythematosus (SLE), and the flow-chart is illustrated in **Figure 1**. The study population was a nationwide population from the period between 2003 and 2013 (n = 23,280,949), and we identified patients who had one inpatient or three outpatient visits with a diagnosis of APS between 2003 and 2012 as APS cases (n = 2,976). The index date was defined as the date on which APS was first diagnosed in the hospital. The exclusion criteria included the following: (1) index date before 2005 (n = 295); (2) diagnosis of SLE before the index date (n = 1,361); (3) death during hospitalisation (n = 3); and (4) missing data regarding region of residence or insured amount (n = 21). A total of 1,296 APS patients met the aforementioned inclusion criteria and were defined as the APS cases in this study. As for the control group, we enrolled individuals who had at least one ambulatory visit during 2005–2012 and applied the following exclusion criteria. (1) an APS diagnosis from 2003 to 2013 (n = 267), (2) an SLE diagnosis before the index date (n = 2,978), and (3) missing data about region of residence or insured amount (n = 13,104). A total of 874,316 individuals were eligible for analyses as the control group. We used age-sex matching and propensity matching in this study. We matched the APS group and the control group at a ratio of 1:20 for sex, age, and year of the index date. After matching, there were 1,245 patients in the APS group and 24,900 individuals in the non-APS group. Moreover, we aimed to reduce the impact of bias and confounding variables on the incidence of SLE through propensity-score matching (PSM), which was conducted at a ratio of 1:2 for sex, age, index date, and selected comorbidities. In the propensity-matched subjects, we identified 727 APS patients and 1,454 control individuals without APS.

Outcome and Relevant Variables

The ICD-9-CM code of 710.0 was used to identify SLE patients, and ICD-9-CM code of 289.8 was applied for APS. The main outcome of this study was a diagnosis of the above code and at least one hospitalisation or three outpatient visits in a year. We considered baseline comorbidities associated with the risk of developing SLE, including human immunodeficiency virus infection (ICD-9-CM codes 042–044, V08), hyperthyroidism (ICD-9-CM code 242), thyroiditis (ICD-9-CM code 245), diabetes mellitus (ICD-9-CM code 250), hyperlipidemia (ICD-9-CM codes 272.0–272.4), affective psychosis (ICD-9-CM code 296), hypertension (ICD-9-CM codes 401–405), coronary artery disease (ICD-9-CM codes 410–414), vasculitis (ICD-9-CM code 443.0), cerebral vascular accident (ICD-9-CM codes 430–438), chronic obstructive pulmonary disease (ICD-9-CM codes 490–496), asthma (ICD-9-CM code 493), inflammatory bowel disease (ICD-9-CM codes 555–556), pancreatitis (ICD-9-CM codes 577.0 and 577.1), chronic liver diseases (ICD-9-CM codes 571 and 573), chronic kidney disease (ICD-9-CM code 585), rheumatoid arthritis (ICD-9-CM code 714.0), systemic sclerosis (ICD-9-CM code 710.1), Sjogren's syndrome (ICD-9-CM code 710.2), ankylosing spondylitis (ICD-9-CM code 720.0), and osteoporosis (ICD-9-CM code 733). Comorbidities

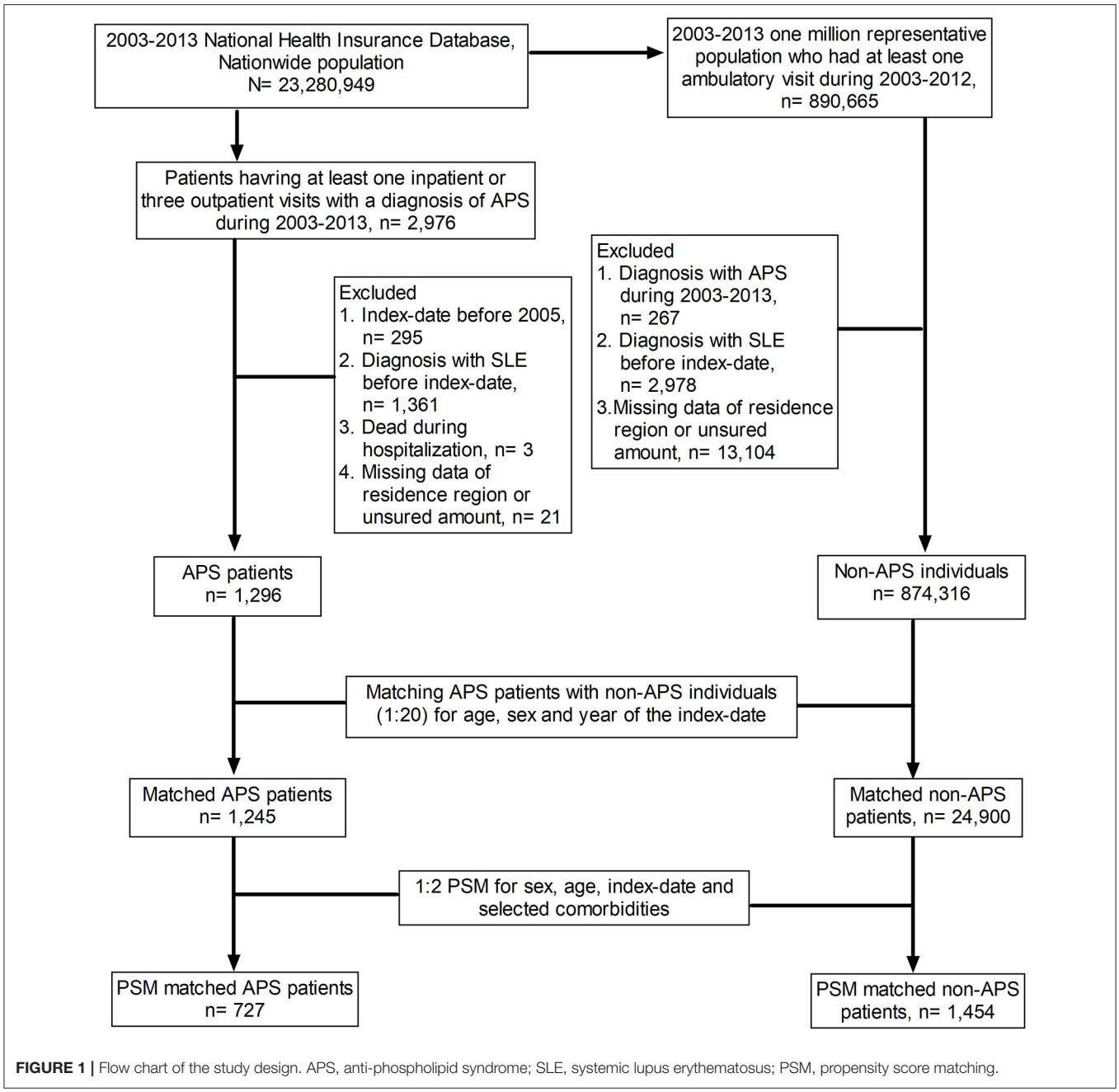

FIGURE 1 | Flow chart of the study design. APS, anti-phospholipid syndrome; SLE, systemic lupus erythematosus; PSM, propensity score matching.

were identified as diseases diagnosed within 1 year before the index date.

Statistical Analysis

Following matching, we assessed the balance of baseline characteristics in the populations using the absolute standardised difference (ASD). A high degree of balance was reflected by an ASD < 0.1. We counted follow-up person-months and the number of persons diagnosed with SLE, calculated the incidence of SLE (per 100,000 person-months), and estimated the crude relative risk with its 95% confidence interval using Poisson regression. Multivariate Cox proportional hazard regression analysis was then used to estimate the adjusted HR (aHR) for SLE. Four different models were used to investigate the effects of APS exposure and covariates on aHR of SLE, including APS exposure alone, demographic variables, medical utilisation and comorbidities, and a conditional Cox model with APS exposure alone was performed in propensity score-matched populations. Sensitivity analysis was used to estimate the risk of SLE in APS exposure patients who were in age-matched and sex-matched populations under different SLE definitions. Kaplan-Meier curves were generated on the cumulative incidence of SLE in the APS and non-APS groups. The differences between the curves were evaluated using the Log-rank test. In all our studies,

$p < 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Analysis Software Version 9.4 (SAS Institute Inc., NC, USA).

RESULTS

Characteristics of the Study Population

We used the 2003–2013 Taiwanese National Health Insurance Database (NHIRD) to identify 1,296 patients with newly diagnosed APS between 2005 and 2012. We then selected age- and sex-matched (1:20) non-APS subjects, and 1,245 patients with APS as well as 24,900 non-APS controls were eligible for analyses. Furthermore, we selected a comparison group through propensity-score matching (PSM) (1:2) for age, sex, comorbidities, and potential confounders including ITP and AIHA. In the propensity-matched subjects, we enrolled 727 APS patients and 1,454 PSM-matched non-APS controls to address the risk for SLE in patients with APS (**Figure 1**). **Table 1** summaries the baseline characteristics of enrolled subjects with APS and control individuals selected by age-sex matching (1:20) as well as propensity score matching (1:2) (See **Supplementary Dataset** for details).

Comparison of the Risk for SLE in Subjects With and Without APS

We compared the incidence rate of SLE in patients with and without APS. In the age- and sex-matched subjects, we found a higher incidence rate of SLE (289.79 per 100,000 person-months) in patients with APS compared with the incidence rate of SLE (2.742 per 100,000 person-months) in patients without APS. Similar distinct incident SLE between APS and non-APS controls was found in the propensity-matched subjects, and the incidence rate of SLE in patients with and without APS was 254.15 per 100,000 person-months and 8.19 per 100,000 person-months, respectively (**Table 2**). After adjustment of the potential confounders, including comorbidities, urbanisation level, history of thromboembolism, pregnancy morbidities, autoimmune hemolytic anaemia, and immune thrombocytopenia, we found that APS was independently associated with incident SLE (HR 80.70; 95% CI 51.37–126.77) (**Table 2**, **Supplementary Table 1**). In the propensity-matched subjects, the association between APS and incident SLE remained robust using the conditional Cox regression model (HR 28.55; 95% CI 11.19–70.91). Furthermore, we used Kaplan-Meier plot to demonstrate the time-course of newly diagnosed SLE in patients with and without APS. We found an increased risk for SLE in patients with APS than those without APS and SLE mainly diagnosed within 5 years after the diagnosis of APS (**Figure 2**).

Sensitivity Analyses

We performed sensitivity analyses through using distinct definitions of SLE based on SLE treatment and excluding patients who might have secondary APS from non-APS controls (**Table 3**). We defined SLE by stringent criteria by management with systemic corticosteroid and disease-modifying antirheumatic drugs (DMARDs), and adjHR was 81.12 (95% CI 51.27–128.37) in incident SLE underwent systemic corticosteroid and 82.46 (95% CI 48.82–139.28) in incident SLE underwent DMARDs. The strength of association was consistent with the association between APS and incident SLE without stringent criteria by management (adjHR 80.70, 95% CI 51.37–126.77). Moreover, to mitigate the potential confounding effect of secondary APS on the association between APS and SLE, we hence excluded patients with autoimmune diseases, inflammatory bowel disease, autoimmune hemolytic anaemia and idiopathic thrombocytopenia from the non-APS controls. We found that the association between APS and incident SLE remained consistent after exclusion patient who might has secondary APS (adjHR 88.90; 95% CI 55.21–143.12). Collectively, using a population-based database and propensity matching, we found that patients with APS had a significantly higher risk for SLE compared with those in non-APS controls.

DISCUSSION

In the present nationwide population-based study, we aimed to address the association between APS and incident SLE using age-sex matching and propensity matching. We identified that diagnosis with APS was highly associated with an increased risk for SLE, and the SLE mainly diagnosed within 5 years after the diagnosis of APS. These findings highlight the essential need of vigilance for SLE in patients diagnosed with APS.

APS is highly associated with SLE and may affect the outcome in patients with SLE (10–12). Unlike SLE, which is the prototypical autoimmune disease with a wide range of anti-nuclear antibodies and clinical presentations, APS mainly manifested with thrombotic events and a positive anti-phospholipid antibody (13). Therefore, there is a crucial need to explore the risk of incident SLE in patients with APS. In line with our findings, Freire et al., using an APS cohort with 80 APS patients, reported that 17.5% (14/80) of patients with primary APS evolved into SLE within 5.2 ± 4 years (14). Given that thrombotic event mainly managed by non-rheumatologists, the aforementioned evidence highlight the need for collaboration with the rheumatologist for subtle signs of SLE, particularly in the first few years after the diagnosis of APS.

Anti-phospholipid syndrome (APS) is characterised by vascular thrombosis, including venous and arterial thrombosis (6). Intriguingly, Zuily et al. recently conducted a hierarchical cluster analysis using 30 data points among 497 anti-phospholipid antibody-positive patients in the Alliance for Clinical Trials and International Networking (APS ACTION) registry and reported three main phenotypes, including female patients without autoimmune diseases but with venous thrombosis (36.0%, 179/497), female patients with SLE, thrombocytopaenia and haemolytic anaemia (36.2%, 180/497), and older men with arterial thrombosis and cardiovascular manifestations (27.8%, 138/497) (15). The aforementioned finding highlights the high correlation between SLE and hametological abnormalitie as shown in recent studies including our previous study and the present study (1, 2), and we further specified the risk and time-course of incident SLE in APS patients. Additionally, in line with the finding of Zuily et al. we

TABLE 1 | Baseline characteristics in the APS and non-APS groups.

	Before PSM (1:20 age–sex matching)			1:2 PSM		
	Non-APS ***n* = 24,900**	**APS** ***n* = 1,245**	**ASD**	**Non-APS** ***n* = 1,454**	**APS** ***n* = 727**	**ASD**
Sex			0.000			0.000
Female	19,400 (77.9)	970 (77.9)		1,094 (75.2)	547 (75.2)	
Male	5,500 (22.1)	275 (22.1)		360 (24.8)	180 (24.8)	
Age (years)			0.000			0.079
<30	3,960 (15.9)	198 (15.9)		262 (18.0)	140 (19.3)	
30–45	11,680 (46.9)	584 (46.9)		570 (39.2)	299 (41.1)	
45–65	6,720 (27.0)	336 (27.0)		438 (30.1)	216 (29.7)	
≥65	2,540 (10.2)	127 (10.2)		184 (12.7)	72 (9.9)	
Urbanisation			0.204			0.070
Urban	8,036 (32.3)	531 (42.7)		647 (44.5)	303 (41.7)	
Suburban	12,041 (48.4)	524 (42.1)		626 (43.1)	319 (43.9)	
Rural	4,823 (19.4)	190 (15.3)		181 (12.4)	105 (14.4)	
Low income[#]	15,463 (62.1)	741 (59.5)	0.053	935 (64.3)	466 (64.1)	0.004
Length of hospital stay*			0.598			0.054
0 day	21,448 (86.1)	774 (62.2)		983 (67.6)	503 (69.2)	
1–6 days	2,191 (8.8)	197 (15.8)		210 (14.4)	112 (15.4)	
≥7 days	1,261 (5.1)	274 (22.0)		261 (18)	112 (15.4)	
Comorbidities[†]						
No comorbidities	19,314 (77.6)	373 (30.0)	<0.001	775 (53.3)	373 (51.3)	0.131
Comorbidities 1–2	4,190 (16.8)	585 (47)		470 (32.3)	264 (36.3)	
Comorbidities ≥3	1,396 (5.6)	287 (23.1)		209 (14.4)	90 (12.4)	
Rheumatoid arthritis	86 (0.3)	82 (6.6)	0.346	54 (3.7)	32 (4.4)	0.035
Sjogren's syndrome	56 (0.2)	236 (19.0)	0.671	6 (0.4)	10 (1.4)	0.102
Systemic sclerosis	0 (0.0)	18 (1.4)	NA	0 (0.0)	0 (0.0)	
Vasculitis	5 (0.02)	25 (2.0)	0.199	4 (0.3)	2 (0.3)	0.000
Hypertension	2,973 (11.9)	205 (16.5)	0.130	266 (18.3)	118 (16.2)	0.055
Diabetes mellitus	1,431 (5.7)	82 (6.6)	0.035	103 (7.1)	45 (6.2)	0.036
Hyperlipidemia	1,429 (5.7)	136 (10.9)	0.188	191 (13.1)	85 (11.7)	0.044
Thromboembolism	1,129 (4.5)	234 (18.8)		207 (14.2)	97 (13.3)	
Coronary artery disease	717 (2.9)	76 (6.1)	0.156	89 (6.1)	45 (6.2)	0.003
Cerebral vascular accident	467 (1.9)	112 (9.0)	0.318	148 (10.2)	52 (7.2)	0.108
Pulmonary embolism	5 (0.02)	34 (2.7)	0.234	0 (0)	1 (0.1)	NA
Venous thromboembolism	19 (0.1)	45 (3.6)	0.265	6 (0.4)	5 (0.7)	0.037
Portal vein thrombosis	1 (0.004)	4 (0.3)	0.079	1 (0.1)	0 (0)	NA
Arterial embolism and thrombosis	25 (0.1)	16 (1.3)	0.143	6 (0.4)	3 (0.4)	0.000
Pregnancy morbidity	40 (0.2)	90 (7.2)		19 (1.3)	5 (0.7)	
Spontaneous abortion	3 (0.01)	11 (0.9)	0.131	0 (0.0)	0 (0.0)	
Habitual abortion	9 (0.04)	71 (5.7)	0.344	0 (0.0)	0 (0.0)	
Pre-eclampsia/eclampsia	23 (0.1)	11 (0.9)	0.114	15 (1.0)	4 (0.6)	0.054
Abortion	5 (0.02)	4 (0.3)	0.073	4 (0.3)	1 (0.1)	0.030
Infertility	155 (0.6)	178 (14.3)	0.539	93 (6.4)	51 (7.0)	0.025
Raynaud's syndrome	5 (0.02)	25 (2.0)	0.199	4 (0.3)	2 (0.3)	0.000
Thromboangiitis obliterans	2 (0.01)	6 (0.5)	0.096	0 (0.0)	1 (0.1)	NA
Osteoporosis	299 (1.2)	57 (4.6)	0.203	34 (2.3)	21 (2.9)	0.034
Asthma	438 (1.8)	44 (3.5)	0.111	50 (3.4)	25 (3.4)	0.000
Chronic obstructive pulmonary disease	968 (3.9)	90 (7.2)	0.146	109 (7.5)	49 (6.7)	0.029
Chronic kidney disease	147 (0.6)	18 (1.4)	0.085	25 (1.7)	12 (1.7)	0.005
Chronic liver diseases	812 (3.3)	87 (7.0)	0.170	82 (5.6)	44 (6.1)	0.018

(Continued)

TABLE 1 | Continued

	Before PSM (1:20 age–sex matching)			1:2 PSM		
	Non-APS *n* = 24,900	**APS** *n* = 1,245	**ASD**	**Non-APS** *n* = 1,454	**APS** *n* = 727	**ASD**
Hyperthyroidism	215 (0.9)	35 (2.8)	0.145	29 (2.0)	18 (2.5)	0.033
Thyroiditis	35 (0.1)	80 (6.4)	0.358	17 (1.2)	13 (1.8)	0.051
Pancreatitis	32 (0.1)	18 (1.4)	0.149	19 (1.3)	8 (1.1)	0.019
Affective psychosis	181 (0.7)	38 (3.1)	0.171	36 (2.5)	16 (2.2)	0.018
Ankylosing spondylitis	22 (0.1)	46 (3.7)	0.267	2 (0.1)	4 (0.6)	0.071
Inflammatory bowel disease	58 (0.2)	7 (0.6)	0.052	9 (0.6)	4 (0.6)	0.009
Human immunodeficiency virus	13 (0.1)	0 (0.0)	NA	0 (0.0)	0 (0.0)	
Autoimmune hemolytic anaemia	2 (0.01)	24 (1.9)	0.197	0 (0.0)	0 (0.0)	
Immune thrombocytopenia	1 (0.004)	35 (2.8)	0.240	0 (0.0)	0 (0.0)	
APS treatment at baseline						
No drug administration		165 (13.3)			143 (19.7)	
Antiplatelet/anticoagulant only		91 (7.3)			55 (7.6)	
Hydroxychloroquine/corticosteroid +/– Antiplatelet/anticoagulant		989 (79.4)			529 (72.8)	

[#] *Insured income lower than median income (21,900 New Taiwan dollars).*
[*] *Length of hospital stay is defined by hospitalisation days within 2 years of the index date.*
[†] *Comorbidities are comorbidities identified within 2 years before the index date.*
APS treatment is treatment received within 6 months after diagnosis with APS.
PSM, propensity score-matching; APS, anti-phospholipid syndrome; ASD, absolute standardised difference.

TABLE 2 | Incidence of SLE in the study groups before and after PSM.

	Before PSM (1:20 age–sex matching)		1:2 PSM	
	Non-APS	**APS**	**Non-APS**	**APS**
n	24,900	1,245	1,454	727
Follow-up person-months	1,057,555	43,480	61,051	25,576
SLE	29	126	5	65
Incidence rate* (95% CI)	2.742 (2.739–2.745)	289.79 (289.63–289.95)	8.19 (8.17–8.21)	254.15 (253.95–254.34)
Crude relative risk (95% CI)	Reference	105.68 (70.58–158.24)	Reference	31.03 (12.50–77.06)
Adjusted hazard ratio (95% CI)	Reference	80.70 (51.37–126.77)[†]	Reference	28.55 (11.49–70.91)[‡]

[*] *Incidence rate, per 10,000 person-months.*
SLE, systemic lupus erythematosus; PSM, propensity score-matching; APS, anti-phospholipid syndrome; CI, confidence interval.
[†,‡] *Definition of length of hospital day and comorbidities window.*

also found that venous thromboembolism (adjHR 2.67, 95% CI 1.34–5.32) and portal vein thrombosis (adjHR 4.78, 95% CI 1.06–21.53), instead of arterial and cardiovascular thrombosis, was independently associated with incident SLE in patients with APS (**Supplementary Table 1**).

Indeed, mechanisms underlie evolution into over SLE in patients with APS remain a research niche. The development of SLE involves a gradual loss of tolerance to self-antigens, followed by an autoantibodies production (16). Genetic susceptibility and environmental exposure, have been implicated with the development of SLE (17–20). Distinct Human leukocyte antigen-DRB1 and -DQB1 allele was reported to be associated with APS with and without SLE, and more studies are required to elucidate the genetic basis of APS-SLE (17, 21, 22). A number of environmental factors, including diet, medication, pollutant, vaccination and microbial infection, and complex gene-environment interaction have been implicated with the development of autoimmunity (20). Currently, hypercoagulation has been increasingly reported in patients with coronavirus COVID-19 infection (23). Given that COVID-19 infection and SLE are both implicated with dysregulated immune responses, such as type I interferon pathway, there are increasing studies to address the correlation among autoimmune disease, hypercoagulation and COVID-19 infection (24–27). These evidence highlight the substantial needs for studies to clarify the underlying biological mechanism for the development of SLE in patients with APS.

SLE is associated with ITP and APS; however, it is somehow difficult to delineate APS and ITP among patients

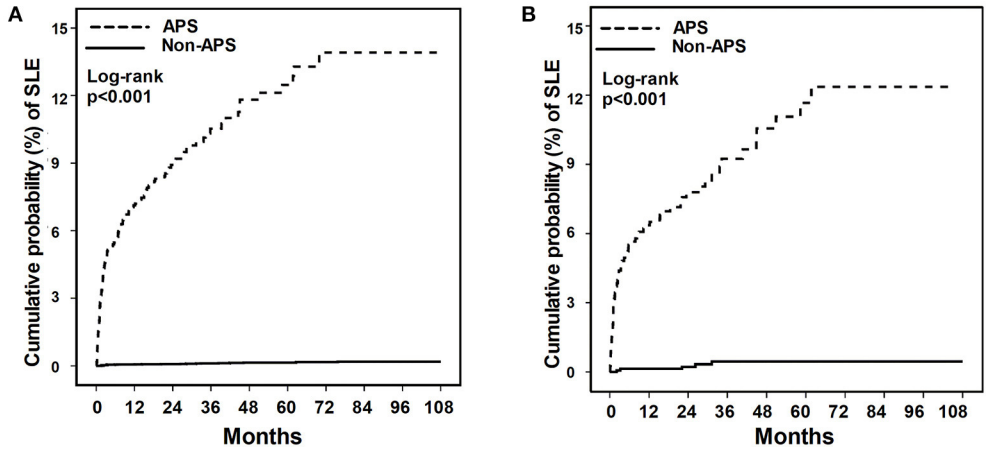

FIGURE 2 | The cumulative incidence of SLE for patients with and without APS. **(A)** age- and sex-matched population. **(B)** Propensity score-matched population. APS, anti-phospholipid syndrome; SLE, systemic lupus erythematosus.

TABLE 3 | Sensitivity analysis in the estimation of the SLE risk for APS exposure in age-matched and sex-matched populations.

Scenario	Definition of SLE event	aHR* (95%CI)
1	At least three outpatient visits or 1 admission within 1 year by rheumatologist (main finding)	80.70 (51.37–126.77)
2	Scenario 1+ treated with systemic corticosteroids or DMARDs (including HCQ or azathioprine)	81.12 (51.27–128.37)
3	Scenario 1+ treated with DMARDs (including HCQ or azathioprine)†	82.46 (48.82–139.28)
4	Exclusion of patients with rheumatoid arthritis, Sjogren's syndrome, systemic sclerosis, vasculitis, thyroiditis, ankylosing spondylitis, inflammatory bowel disease, human immunodeficiency virus, AIHA, ITP at baseline (excluding secondary APS)	88.90 (55.21–143.12)

aHR of AIHA exposure on the risk of SLE, the covariates including age group, sex, urbanisation, low income, length of hospital stay, and comorbidities listed in **Table 1.*
†The treatment of SLE was identified within 6 months after first diagnosis of SLE.
SLE, systemic lupus erythematosus; APS, anti-phospholipid syndrome; aHR, adjusted HR; DMARD, disease modifying antirheumatic drugs; HCQ, hydroxychloroquine; AIHA, autoimmune hemolytic anemias; ITP, immune thrombocytopenia.

with thrombocytopenia (28). In one population-based study aiming to address the association between ITP and SLE, Zhu et al. reported that patients with ITP were more likely to have APS compared with those without ITP (2.77 vs. 0.02%, $p < 0.05$) (1). Thrombocytopenia is a cardinal haematological manifestation of APS, but the presence of thrombocytopenia, the main manifestation in patients with ITP, does not exclude the risk for the development of thrombosis (28). In the present study, we found a consistent association between APS and incident SLE using regression adjusted with ITP diagnosis and excluded patients with ITP. Therefore, APS should be an independent risk factor for incident SLE.

There are limitations in this study. First, the lack of laboratory data to validate the diagnosis of SLE in the claim database. However, we have conducted sensitivity analyses and used stringent criteria with concomitant management with systemic corticosteroid and DMARDs, and the strength of association between APS and incident is consistent. Therefore, the concern regarding the diagnosis of SLE should be at least partly mitigated. Second, the diagnosis of APS without laboratory data should also be a concern. Given that APS is relatively a specific diagnosis in patients with thromboembolism, we think that the diagnosis of APS with one inpatient or three outpatient visits could mainly be underestimated, instead of overestimated, in the present study. The strength of the study was the use of nationwide, population-based database to minimise the risk of selection and participation bias although clinical features and laboratory data, including anti-nuclear antibody and APS profiles, are not available in NHIRD. Third, the undiagnosed SLE in the diagnosis of APS could also be a concern, and we believe this concern somehow indicates the needs for collaboration with the rheumatologist in the management of patients with APS. Fourth, the finding of the present study should be further validated in another independent cohort.

CONCLUSION

In conclusion, using a population-based study, we demonstrate a high risk of developing SLE among APS patients, in particular during the first 5 years after APS diagnosis. These findings highlight the substantial need for close monitoring for SLE among patients with APS. More studies are warranted to explore factors including genetic

and environmental factors leading to SLE in patients with APS.

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/s.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Taichung Veterans General Hospital in Taiwan (approval number: CE17100B). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

H-HC and W-CC conceptualised the research and drafted the manuscripts. H-HC, C-HL, and W-CC acquired and interpreted the data. All authors have read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.654791/full#supplementary-material

REFERENCES

1. Zhu FX, Huang JY, Ye Z, Wen QQ, Wei JC. Risk of systemic lupus erythematosus in patients with idiopathic thrombocytopenic purpura: a population-based cohort study. *Ann Rheum Dis.* (2020) 79:793–99. doi: 10.1136/annrheumdis-2020-217013
2. Mo HY, Wei JCC, Chen XH, Chen HH. Increased risk of systemic lupus erythematosus in patients with autoimmune haemolytic anaemia: a nationwide population-based cohort study. *Ann Rheum Dis.* (2020) 80:403–4. doi: 10.1136/annrheumdis-2020-219328
3. Tincani A, Andreoli L, Chighizola C, Meroni PL. The interplay between the antiphospholipid syndrome and systemic lupus erythematosus. *Autoimmunity.* (2009) 42:257–9. doi: 10.1080/08916930902827918
4. Belizna C, Stojanovich L, Cohen-Tervaert JW, Fassot C, Henrion D, Loufrani L, et al. Primary antiphospholipid syndrome and antiphospholipid syndrome associated to systemic lupus: are they different entities? *Autoimmun Rev.* (2018) 17:739–45. doi: 10.1016/j.autrev.2018.01.027
5. Shoenfeld Y, Meroni PL, Toubi E. Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentations on the same scale? *Curr Opin Rheumatol.* (2009) 21:495–500. doi: 10.1097/BOR.0b013e32832effdd
6. Giannakopoulos B, Krilis SA. The pathogenesis of the antiphospholipid syndrome. *N Engl J Med.* (2013) 368:1033–44. doi: 10.1056/NEJMra1112830
7. Cervera R, Serrano R, Pons-Estel GJ, Ceberio-Hualde L, Shoenfeld Y, de Ramon E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 10-year period: a multicentre prospective study of 1000 patients. *Ann Rheum Dis.* (2015) 74:1011–8. doi: 10.1136/annrheumdis-2013-204838
8. Tarr T, Lakos G, Bhattoa HP, Szegedi G, Shoenfeld Y, Kiss E. Primary antiphospholipid syndrome as the forerunner of systemic lupus erythematosus. *Lupus.* (2007) 16:324–8. doi: 10.1177/0961203307077993
9. Unlu O, Erkan D, Barbhaiya M, Andrade D, Nascimento I, Rosa R, et al. The impact of systemic lupus erythematosus on the clinical phenotype of antiphospholipid antibody-positive patients: results from the antiphospholipid syndrome alliance for clinical trials and international clinical database and repository. *Arthritis Care Res.* (2019) 71:134–41. doi: 10.1002/acr.23584
10. Meroni PL, Tsokos GC. Editorial: systemic lupus erythematosus and antiphospholipid syndrome. *Front Immunol.* (2019) 10:199. doi: 10.3389/fimmu.2019.00199
11. Ruiz-Irastorza G, Egurbide MV, Ugalde J, Aguirre C. High impact of antiphospholipid syndrome on irreversible organ damage and survival of patients with systemic lupus erythematosus. *Arch Intern Med.* (2004) 164:77–82. doi: 10.1001/archinte.164.1.77
12. Torricelli AK, Ugolini-Lopes MR, Bonfa E, Andrade D. Antiphospholipid syndrome damage index (DIAPS): distinct long-term kinetic in primary antiphospholipid syndrome and antiphospholipid syndrome related to systemic lupus erythematosus. *Lupus.* (2020) 29:256–62. doi: 10.1177/0961203320901598
13. Agmon-Levin N, Shoenfeld Y. The spectrum between antiphospholipid syndrome and systemic lupus erythematosus. *Clin Rheumatol.* (2014) 33:293–5. doi: 10.1007/s10067-014-2486-5
14. Freire PV, Watanabe E, dos Santos NR, Bueno C, Bonfa E, de Carvalho JF. Distinct antibody profile: a clue to primary antiphospholipid syndrome evolving into systemic lupus erythematosus? *Clin Rheumatol.* (2014) 33:349–53. doi: 10.1007/s10067-013-2472-3
15. Zuily S, Clerc-Urmes I, Bauman C, Andrade D, Sciascia S, Pengo V, et al. Cluster analysis for the identification of clinical phenotypes among antiphospholipid antibody-positive patients from the APS ACTION registry. *Lupus.* (2020) 29:961203320940776. doi: 10.1177/0961203320940776
16. Leffers HCB, Lange T, Collins C, Ulff-Moller CJ, Jacobsen S. The study of interactions between genome and exposome in the development of systemic lupus erythematosus. *Autoimmun Rev.* (2019) 18:382–92. doi: 10.1016/j.autrev.2018.11.005
17. Rullo OJ, Tsao BP. Recent insights into the genetic basis of systemic lupus erythematosus. *Ann Rheum Dis.* (2013) 72 (Suppl. 2):ii56–61. doi: 10.1136/annrheumdis-2012-202351
18. Armstrong DL, Zidovetzki R, Alarcon-Riquelme ME, Tsao BP, Criswell LA, Kimberly RP, et al. GWAS identifies novel SLE susceptibility genes and explains the association of the HLA region. *Genes Immun.* (2014) 15:347–54. doi: 10.1038/gene.2014.23
19. Ceccarelli F, Perricone C, Borgiani P, Ciccacci C, Rufini S, Cipriano E, et al. Genetic factors in systemic lupus erythematosus: contribution to disease phenotype. *J Immunol Res.* (2015) 2015:745647. doi: 10.1155/2015/745647
20. Kamen DL. Environmental influences on systemic lupus erythematosus expression. *Rheum Dis Clin North Am.* (2014) 40:401–12, vii. doi: 10.1016/j.rdc.2014.05.003
21. Kapitany A, Tarr T, Gyetvai A, Szodoray P, Tumpek J, Poor G, et al. Human leukocyte antigen-DRB1 and -DQB1 genotyping in lupus patients with and without antiphospholipid syndrome. *Ann N Y Acad Sci.* (2009) 1173:545–51. doi: 10.1111/j.1749-6632.2009.04642.x
22. Ortiz-Fernandez L, Sawalha AH. Genetics of antiphospholipid syndrome. *Curr Rheumatol Rep.* (2019) 21:65. doi: 10.1007/s11926-019-0869-y
23. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* (2020) 46:1089–98. doi: 10.1007/s00134-020-06062-x
24. Novelli L, Motta F, De Santis M, Ansari AA, Gershwin ME, Selmi C. The JANUS of chronic inflammatory and autoimmune diseases onset during COVID-19 - a systematic review of the literature. *J Autoimmun.* (2020) 117:102592. doi: 10.1016/j.jaut.2020.102592
25. Xourgia E, Tektonidou MG. Type I interferon gene expression in antiphospholipid syndrome: pathogenetic,

clinical and therapeutic implications. *J Autoimmun.* (2019) 104:102311. doi: 10.1016/j.jaut.2019.102311
26. Kamel MH, Yin W, Zavaro C, Francis JM, Chitalia VC. Hyperthrombotic milieu in COVID-19 patients. *Cells.* (2020) 9:2392. doi: 10.3390/cells9112392
27. Talarico R, Aguilera S, Alexander T, Amoura Z, Antunes AM, Arnaud L, et al. The impact of COVID-19 on rare and complex connective tissue diseases: the experience of ERN ReCONNET. *Nat Rev Rheumatol.* (2021) 17:177–84. doi: 10.1038/s41584-020-00565-z
28. Artim-Esen B, Diz-Kucukkaya R, Inanc M. The significance and management of thrombocytopenia in antiphospholipid syndrome. *Curr Rheumatol Rep.* (2015) 17:14. doi: 10.1007/s11926-014-0494-8

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Effect of Chinese Herbal Medicines on Hearing Loss Risk in Rheumatoid Arthritis Patients: Retrospective Claims Analysis

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Objectives: Patients with rheumatoid arthritis (RA) are at a higher risk of extra-articular manifestations, especially hearing loss (HL). Although Chinese herbal medicines (CHM) are proven safe and effective treatments for inflammatory conditions, the effect of CHM use on HL in RA patients is unknown. This cohort study aims to determine the relationship between CHM use and the subsequent risk of HL among RA patients.

Methods: From health insurance claims data in Taiwan, a total of 6,905 persons aged 20–80 years with newly-diagnosed RA in 2000–2009 were identified. Of these, we recruited 2,765 CHM users and randomly selected 2,765 non-CHM users who matched with the users by the propensity score. Both cohorts were followed up until the end of 2012 to estimate the incidence of HL. Cox proportional hazards regression was used to estimate the adjusted hazard ratio (HR) for HL.

Results: The incidence of HL was lower in the CHM users than in the comparison cohort (8.06 vs. 10.54 per 1,000 person-years) (adjusted HR, 0.77; 95% CI, 0.63–0.94). Those who received CHM for more than 2 years had the greatest benefit against the onset of HL, with over 50% risk reduction. Prescriptions of Hai Piao Xiao, Yan Hu Suo, San-Qi, Huang Qin, Dang Shen, Jia-Wei-Xiao-Yao-San, Shu-Jing-Huo-Xue-Tang, and Dang-Gui-Nian-Tong-Tang were found to be associated with a reduced risk of HL.

Conclusions: Our findings suggest that adding CHM to conventional therapy may reduce the subsequent risk of HL in RA patients. Prospective randomized trials are recommended to further clarify whether the association revealed in this study supports such a causal relationship.

Keywords: rheumatoid arthritis, hearing loss, Chinese herbal medicines, risk, cohort study

KEY POINTS

- Despite recent improvements in rheumatology treatment, those with rheumatoid arthritis (RA) are found to experience a higher risk of extra-articular manifestations, such as hearing loss (HL), which has been postulated to affect cognitive function.
- Recently, Chinese herbal medicines (CHM) are regarded as a popular adjunctive treatment for patients with RA; nevertheless, the association between CHM and risk of HL among them is still unknown.
- This study utilized nationally representative longitudinal data and revealed the therapeutic effect of CHM on the subsequent risk of HL among RA patients.
- The most prominent effect is further observed among those receiving CHM for more than 2 years, with an over 50% reduction in HL incidence.

INTRODUCTION

Rheumatoid arthritis (RA) is a common autoimmune disease that attacks multiple joints throughout the body. The clinical hallmarks of RA include swelling, tenderness, and damage in synovial joints, that ultimately cause progressive functional limitations and physical disability in the affected patients. According to an analysis by the National Health Interview Survey 2011–2013, arthritis/rheumatism ranked among the top three conditions reported to cause work disability, regardless of age or sex, consequently imposing a tremendous socioeconomic burden (1). As estimated by a comprehensive nationwide database, the annual direct medical costs for one RA patient in the USA were approximately three times greater than those for a person without RA ($20,919 vs. $7,197) (2).

The chronic inflammation associated with RA can also affect other parts of the body, including the skin, eyes, and lungs. Recent studies have investigated extra-articular manifestations of RA, including hearing loss (HL) (3). One meta-analysis of 12 studies demonstrated that individuals with RA had more than double the likelihood of developing HL than did the general public (4). The elevated expression of pro-inflammatory mediators such as interleukin-6 (IL-6) and matrix metalloproteinase-3 was presumed to link these two disorders (5). HL can have social psychological consequences, as the resulting deficits in speech comprehension and difficulty communicating can cause the individual to withdraw from social activities (6). A recent longitudinal follow-up study using a national population sample showed that individuals with HL were over four times more likely to die than the general population (7). Thus, when managing patients with RA, preventing or treating HL is critical.

Over the past decade, Chinese herbal medicines (CHM) have become a commonly-used complementary therapy for patients with HL (8). Several herbs were proven to improve auditory function by normalizing the blood supply to the cochlea, decreasing the accumulation of toxic and inflammatory substances, and increasing the antioxidant defense in sensory hair cells (9), all of which play distinct roles in otoprotective mechanisms. For example, a compound from the root of Dan-Shen promotes hair cell survival and antioxidant protection by decreasing nuclear factor kappa beta (NF-κB) signaling (10–12). The results of these studies suggested that the integration of CHM into conventional treatments may be a useful measure to prevent the development of HL.

To date, the studies of the otoprotective effects of CHM have been conducted in relatively small patient cohorts, thus placing the broader applicability of the study results in question (8). Most importantly, no study has investigated the long-term effect of CHM use on HL prevention in RA patients. The range of potential medical resources available to the management of HL in RA patients may be limited by the paucity of available evidence. Therefore, using a nationwide medical claims database, this study aims to determine the effect of CHM use on the development of HL among RA patients.

METHODS

Data Source

The data used in this retrospective cohort study were obtained from the Longitudinal Health Insurance Database (LHID), a sub-dataset of the National Health Insurance Research Database (NHIRD) of Taiwan made up of 1 million randomly-sampled people who registered with the National Health Insurance (NHI) program in 2000. Constructed using a multistage stratified systematic sampling method, LHID has also been demonstrated that patients registered in it have similar age and sex distributions to the whole Taiwanese population (13). This cohort database includes (i) personal information; (ii) health insurance claims data; (iii) diagnostic codes; (iv) prescription drug registry; (v) socioeconomic data; and (vi) medical examination information on persons under the NHI program. This study was conducted in accordance with the Helsinki Declaration and was approved by the local Institutional Review Board and Ethics Committee of Buddhist Dalin Tzu Chi Hospital (No. B10004021-2).

Definition of Participants

To be included in this study, patients had to have a relevant code designated by the International Classification of Disease, Ninth Revision, Clinical Modification (ICD-9-CM). As shown in the **Figure 1**, we initially identified those who sought ambulatory health care services between 2000 and 2009 for newly diagnosed RA (ICD-9-CM code: 714.0). These patients were further linked to the catastrophic illness registry to ensure a valid diagnosis. The catastrophic illness certificate is granted based on formal diagnoses issued by physicians for conditions such as schizophrenia, mood disorders, autoimmune disorders, and cancer. The date when the patient gained approval for catastrophic illness registration was considered the index date. Only those aged 20–80 years were included. We then excluded those who had a previous diagnosis of HL before the first RA diagnosis ($n = 309$) and those who were not followed up for one complete year after RA diagnosis ($n = 58$), allowing temporal information to be reviewed. Patients were recognized as having HL if they had at least three ambulatory visits for treatment or if they had been hospitalized due to HL, as reflected in the use

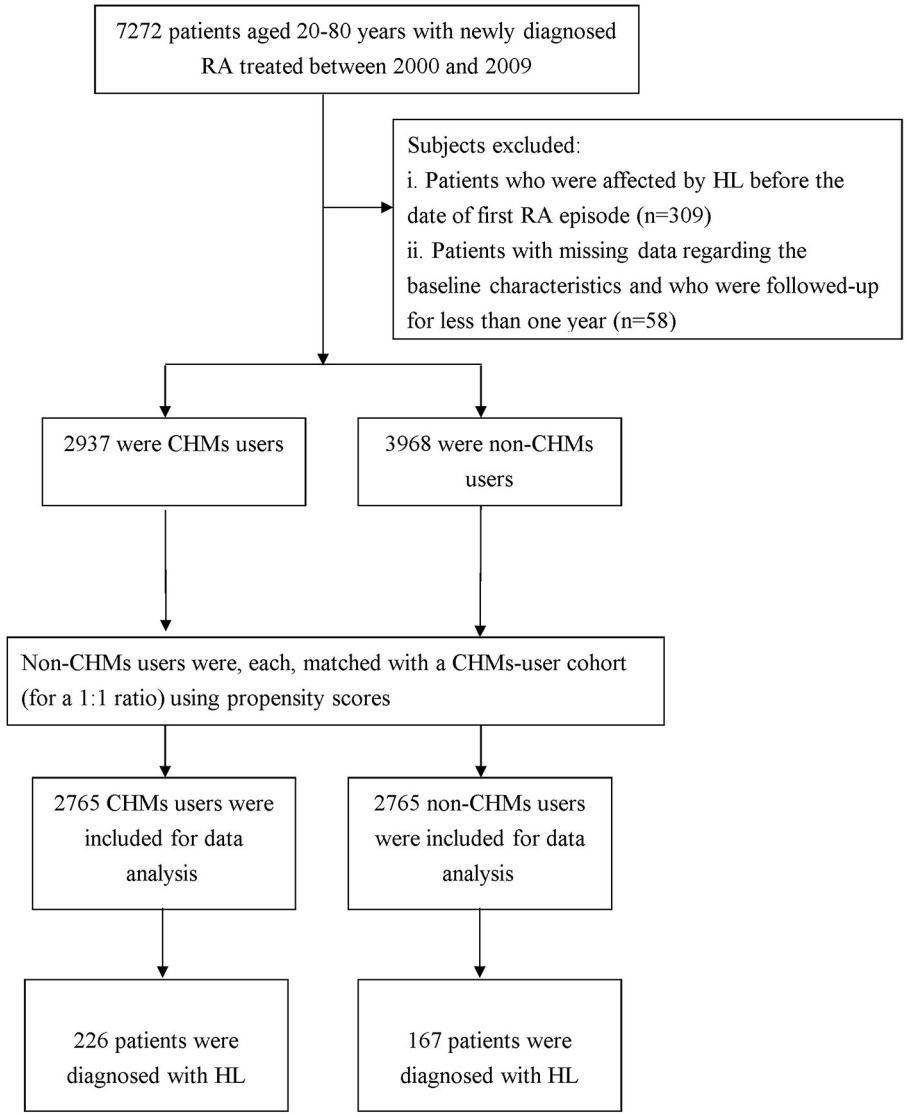

FIGURE 1 | Flowchart showing the method of selecting and following study subjects.

of ICD-9-CM codes 388.2, 388.4, 389.2, 389.9, 389.00, 389.10, or 389.12. The final cohort comprised 6,905 RA patients.

We used the frequency of visits to Chinese medicine physicians to determine whether the enrollee ever used CHM treatment. Those who used CHM to treat RA for more than 30 days were deemed CHM users (n = 2,937), and those treated for 30 days or less were considered non-CHM users. Because enrollees with a history of frequent CHM use would have a greater tendency to receive CHM than the general population, we matched each CHM user to one without CHM treatment by propensity scores to reduce potential biases. The propensity score was calculated using logistic regression based on patient demographics and baseline comorbidities at enrollment. The index date for the follow-up period was the date of CHM treatment initiation or, for non-CHM users, the date of the initial RA onset, corrected by immortal time bias (14). The follow-up time, in person-years, was calculated until the date of HL diagnosis or until censoring due to death, withdrawal from the insurance system, or loss to follow-up.

Measured Covariates

The covariates considered in this survey included sociodemographic characteristics and comorbidities. Basic sociodemographic factors included age, sex, income (estimated using insurance premium), and urbanization level. Monthly income was subdivided into 3 levels, as follows: $\leq$ 17,880 New Taiwan Dollar (NTD); 17,881–43,900 NTD; and $\geq$ 43,901 NTD. Urbanization levels were divided into the following 3 strata: urban (levels 1–2), suburban (levels 3–4), and rural (levels 5–7), with a lower level indicating greater urbanization (15).

Because previous illnesses may affect susceptibility to HL (8), the Charlson Comorbidity Index (CCI) score was used to summarize the influence of baseline comorbidities (16). To account for the use of conventional therapy, we defined patients who were prescribed with corticosteroids or disease-modifying anti-rheumatic drugs for 6 months or more as receiving conventional therapy. These data were obtained from both ambulatory care and inpatient hospitalization claims during the year preceding cohort entry. To avoid double counting and possible over-adjustment in the regression model, RA had been excluded from the CCI score estimation.

Statistical Analysis

All analyses were performed using SAS Version 9.3 software (SAS Institute Inc, Cary, NC, USA) with a two-tailed significance level set at 0.05. We first compared the distributions of sociodemographic data and comorbidities between two groups using the chi-square test and Student's *t*-test. Cox proportional hazards regression analysis was then used to evaluate the risk of developing HL based on the exposure of CHM use, expressed as the hazard ratio (HR). An unadjusted model and a fully adjusted model (with all baseline covariates included) were developed. To further test the robustness of the relationship between CHM use and sequent HL risk, we divided the CHM users into three subgroups: CHM use duration, 31–365 days; CHM use duration, 366–730 days; and CHM use duration >730 days. The Kaplan–Meier method was used to estimate the cumulative risk of HL in each group, and the difference between groups was assessed using the log-rank test. The proportional hazards assumption was examined by plotting the log [–log (survival function)] vs. the log (survival time).

We also performed two sensitivity analyses using separate procedures. First, we included only the RA patients who reported no comorbidities. Second, we added the prescription of biological agents for 6 months or longer to be a surrogate for RA severity into the regression model.

RESULTS

Among the included study participants, following the propensity score matching as aforesaid, 2,765 participants were classified into the CHM users and 2,765 were thus selected into the non-CHM users. After matching, no significant differences were observed between the two groups with respect to age, sex, monthly income, urbanization level of the residential area, receiving conventional therapy or comorbidities (**Table 1**).

A total of 393 first episodes of HL occurred in the RA cohort over an observation period of 42,191.95 person-year (PY) in 5,530 patients. We observed that CHM users had a lower incidence of HL (8.06/1,000 PY) compared to the non-CHM users (10.52/1,000 PY), with an adjusted HR of 0.77 (95% CI: 0.63–0.92) (**Table 2**). In addition, those receiving CHM for more than 730 days were at an even lower risk of HL (54%) than those without CHM use. The Kaplan–Meier analysis of survival by days of CHM use revealed a statistically significant difference in the survival rate free from HL across the three groups of CHM users ($p < 0.001$) (**Figure 2**).

TABLE 1 | Demographic data and selected comorbidities of study participants.

Variables	Non-CHM users *n* = 2,765 (%)	CHM users *n* = 2,765 (%)	*p*
Age (yr)			0.44
≤50	1,116 (40.4)	1,113 (40.3)	
>50	1,649 (59.6)	1,652 (59.7)	
Mean (SD)	54.08 ± 14.5	54.02 ± 13.5	
Sex			0.74
Female	2,035 (73.6)	2,046 (74.0)	
Male	730 (26.4)	719 (26.0)	
Monthly income			0.81
Low	1,204 (43.5)	1,186 (42.9)	
Medium	1,462 (52.9)	1,473 (53.3)	
High	99 (3.6)	106 (3.8)	
Residential area			0.75
Urban	1,574 (56.9)	1,551 (56.1)	
Suburban	425 (15.4)	423 (15.3)	
Rural	766 (27.7)	791 (28.6)	
Conventional therapy			0.13
Yes	2,027 (73.3)	2,076 (75.1)	
No	738 (26.7)	689 (24.9)	
CCI			0.43
Mean ± SD	6.03 ± 8.1	6.02 ± 8.5	

CHM, Chinese herbal medicines; SD, standard deviation; CCI, Charlson-Deyo Comorbidity Index.

Collectively, a more beneficial effect of CHM on HL was observed among younger patients (**Table 3**). Multivariable stratified analysis showed that the benefit of CHM therapy in reducing the incidence of HL was more predominant among females, with an adjusted HR of 0.68 (95% CI, 0.55–0.86) (**Table 3**). The 10 most commonly prescribed herbal formulas for RA treatment are shown in **Table 4**. Of these, Hai-Piao-Xiao, Yan Hu-Suo, San-Qi, Huang-Qin, Dang-Shen, Jia-Wei-Xiao-Yao-San, Shu-Jing-Huo-Xue-Tang, and Dang-Gui-Nian-Tong-Tang were found to be associated with a significant reduction in the HL risk (**Table 4**).

In the first sensitivity analysis, when only the RA patients who reported no comorbidities were included in the analysis, we found that CHM use was still associated with a lower incidence rate of HL, with an adjusted HR of 0.81 (95% CI, 0.75–0.97). In the second sensitivity analysis, 52.3% (1,446/2,765) of the CHM users and 52.7% (1,458/2,765) of the non-CHM users had received biological agents for 6 months or longer. When this factor was added to the model, we found CHM use was still associated with a lower incidence rate of HL, with an adjusted HR of 0.79 (95% CI, 0.68–0.96).

DISCUSSION

Hearing impairment in RA patients can decrease social interactions and compliance with healthcare treatment, thus

TABLE 2 | Risk of HL for RA patients with and without CHM use.

Patient group	Event	PY	Incidence (/1000 PY)	Crude HR (95% CI)	Adjusted HR* (95% CI)
Non-CHM users	226	21,472.89	10.52	1	1
CHM users	167	20,719.06	8.06	0.76 (0.64–0.94)	0.77 (0.63–0.92)
CHM use within 31–365 days	144	16,593.81	8.68	0.82 (0.67–0.98)	0.83 (0.67–0.99)
CHM use within 366–730 days	15	2,459.31	6.10	0.57 (0.34–0.96)	0.57 (0.34–0.96)
CHM use >730 days	8	1,665.93	4.80	0.45 (0.23–0.93)	0.46 (0.23–0.92)

**Model adjusted for age, sex, urbanization level, monthly income, conventional therapy, and Charlson-Deyo Comorbidity Index.*

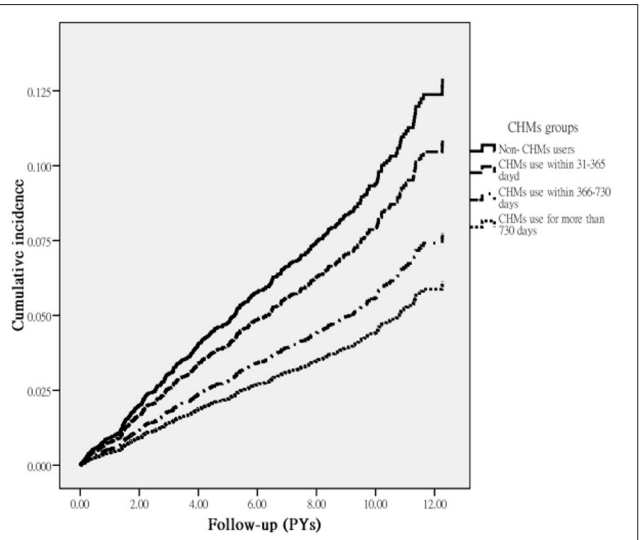

FIGURE 2 | Cumulative incidence of HL in RA patients with and without receiving CHM treatment during the study period (Log-rank test, $p < 0.001$).

worsening the manifestations of RA. Because HL is more common among RA patients than in the general population, more effective treatments are needed to prevent HL. In this evidence-based cohort study, we observed that those receiving CHM treatment had a 23% lower risk of HL than did those not using CHM. In those receiving CHM for more than 2 years, the risk of HL decreased by over 50%. The dose–response relationship observed between the intensity of CHM use and the decrease in the risk of HL supports a causal association between CHM use and HL prevention. No previous study has investigated the long-term impact of CHM on the HL risk in RA patients. The positive correlation between CHM use and decreased risk of HL observed herein is consistent with previous reports and bolsters a growing body of evidence on this topic (8, 9). The proposed mechanisms by which the prescribed herbal products protect against the onset of HL include reduction in blood viscosity, regulation of the inflammatory response, improvement of lymph circulation, and enhancement of the excitability and conductivity of the auditory nerve (5, 8), all of which exert a beneficial effect on cochlear microcirculation.

We found that younger patients benefited more from CHM use in lowering the HL risk. Compared to older patients, younger patients often have a more positive attitude toward their medical condition as well as more and better psychosocial and coping resources to rely upon (17), all of which might enhance the protective effect of CHM on the auditory system. We found that female patients also benefited more from CHM use. Compared to males, females often display better health literacy, more willingness to adhere to strict medical procedures, and more positive attitudes about self-care (18). In addition, besides suppressing the inflammatory cytokines that play decisive roles in the generation of neurodegenerative disorders (19), the female hormone estrogen was further found to protect the inner ear by amplifying the expression of insulin-like growth factor 1 in the cochlea, which have been shown to be responsible for cell proliferation, metabolism, and anti-apoptotic cellular responses (20).

Another focal aim of this study was to identify the specific CHM constituents that were likely related to the reduced risk of HL in RA patients. Of the commonly used single-product CHM to treat RA, a total of five herbs were found to be associated with a lower subsequent risk of HL. Studies in animal models indicate that extracts of Yan-Hu-Suo and Dan-Shen modulate the induction of IL-6 or tumor necrosis factor-α by abating NF-κB signaling (21, 22). Recent evidence implicates these inflammatory markers in the pathogenesis of central auditory disorders (8, 23).

We observed that the use of Huang-Qin was associated with a decreased risk of HL. Baicalin and baicalein, major flavonoids present in this formula, have attracted considerable attention because of their anti-inflammatory activity and antioxidant effects (9). Zhang et al. reported that baicalin use ameliorated the degeneration of brain and auditory nerve in rats with noise-induced HL by targeting the toll-like receptor 4/NF-κB signaling pathway (24). Activation of this signaling pathway may induce sensory cell degeneration and cochlear dysfunction (25), thus promoting the onset of HL.

Our study results indicate that the use of Hai-Piao-Xiao might lessen the subsequent susceptibility to HL among RA patients. A previous *in vitro* study showed that Hai-Piao-Xiao extract profoundly normalized the blood supply to the cochlea and increased the antioxidant defense in sensory hair cells (26), consequently modifying susceptibility to hearing disorders. A strong association between San-Qi and reduced HL risk was also observed in the present study. This herb is a species of the genus Panax and is commonly used to stimulate blood circulation to promote physiological function in

TABLE 3 | Incidence and risk of HL among RA patients with and without CHM use by sex and age.

Variables	Non-CHM users			CHM users			Crude HR (95% CI)	Adjusted HR (95% CI)
	Case	PY	Incidence†	Case	PY	Incidence		
Sex								
Female	179	15,449.44	11.59	119	15,149.27	7.86	0.68 (0.54–0.85)	0.68¥ (0.55–0.86)
Male	47	6,023.45	7.80	48	5,569.79	8.62	1.07 (0.75–1.49)	1.06¥ (0.71–1.52)
Age (year)								
≤50	70	8,844.38	7.91	49	8,622.35	5.68	0.72 (0.49–0.99)	0.69* (0.48–0.98)
>50	156	12,628.51	12.35	118	12,096.71	9.75	0.80 (0.63–1.01)	0.79* (0.63–1.01)

¥ Model adjusted for age, urbanization level, monthly income, conventional therapy, and Charlson-Deyo Comorbidity Index.

† Per 1000 PY.

**Model adjusted for sex, urbanization level, monthly income, conventional therapy, and Charlson-Deyo Comorbidity Index.*

TABLE 4 | Risk of HL associated with use of the 10 most-used single-herb and multi-herb CHM products for RA patients.

Chinese herbal medicine	Number of prescriptions	Crude HR (95% CI)	Adjusted HR* (95% CI)
Single-herb products			
Yan-Hu-Suo	6,527	0.72 (0.53–0.98)	0.71 (0.52–0.96)
Dan-Shen	4,828	0.69 (0.50–0.95)	0.67 (0.48–0.92)
Ji-Xue-Teng	4,596	0.89 (0.65–1.20)	0.81 (0.59–1.12)
Huang-Qin	4,235	0.67 (0.50–0.93)	0.62 (0.45–0.86)
Bei-Mu	4,181	0.95 (0.71–1.34)	0.94 (0.67–1.29)
Da-Huang	3,758	0.76 (0.53–1.09)	0.72 (0.51–1.03)
Hai-Piao-Xiao	2,783	0.56 (0.40–0.79)	0.53 (0.37–0.74)
Mu-Xiang	2,115	0.95 (0.65–1.40)	0.92 (0.63–1.37)
San-Qi	2,082	0.63 (0.44–0.90)	0.60 (0.42–0.86)
Chuan-Niu-Xi	1,650	0.78 (0.52–1.19)	0.76 (0.50–1.15)
Multi-herb products			
Shu-Jing-Huo-Xue-Tang	7,303	0.79 (0.58–1.01)	0.77 (0.59–1.04)
Jia-Wei-Xiao-Yao-San	6,968	0.74 (0.54–0.98)	0.63 (0.46–0.86)
Du-Huo-Ji-Sheng-Tang	4,962	0.88 (0.63–1.23)	0.88 (0.62–1.20)
Shao-Yao-Gan-Cao-Tang	4,625	0.71 (0.53–0.97)	0.67 (0.49–0.92)
Ge-Gen-Tang	4,596	0.89 (0.65–1.19)	0.88 (0.59–1.11)
Dang-Gui-Nian-Tong-Tang	3,972	0.75 (0.54–0.99)	0.71 (0.51–0.98)
Chuan-Xiong-Cha-Tiao-San	3,939	0.89 (0.64–1.24)	0.83 (0.60–1.15)
Xue-Fu-Zhu-Yu-Tang	3,583	0.95 (0.68–1.34)	0.93 (0.66–1.30)
Ping-Wei-San	3,323	0.84 (0.60–1.19)	0.83 (0.59–1.17)
Gan-Lu-Yin	3,188	0.73 (0.52–1.01)	0.72 (0.52–1.00)

**Model adjusted for age, sex, urbanization level, monthly income, conventional therapy, and Charlson-Deyo Comorbidity Index.*

the nervous or immune system. San-Qi activates the AKT/Nrf2-mediated redox pathway (27, 28), thus resulting in more efficient neurotransmitter function.

Of the commonly prescribed multi-herb products, Jia-Wei-Xiao-Yao-San may lower the risk of HL. In a rodent model, this formula was observed to increase synaptic plasticity and decrease the levels of inflammatory markers by activating intracellular signaling pathways, especially the brain-derived neurotrophic factor (29). This neurotrophic factor has been found in many areas of the brain and plays an important role in supporting the formation of memories and auditory processing (30). These phenomena may underlie the positive effect of Jia-Wei-Xiao-Yao-San observed in this study.

Consistent with the findings of earlier research (31), this study also revealed that Dang-Gui-Nian-Tong-Tang was the most frequently used Chinese herbal formula for treating rheumatic

disorders. The positive association between Dang-Gui-Nian-Tong-Tang use and a lower incidence of HL may result from its reported effects of increasing blood circulation and inhibiting inflammatory cytokines (32), processes that have been implicated in the development of hearing disorders. Regarding Shao-Yao-Gan-Cao-Tang, we speculate that this natural product may mitigate the onset of HL through its proven abilities of inhibit the development of inflammatory conditions and slow the degeneration of neurons via a variety of pharmacological effects, including antioxidant, anti-inflammatory, and anti-apoptotic activities (33).

While our study is the first to investigate the relationship between CHM use and subsequent risk of HL among RA patients, some limitations should be considered. First, although a positive association was observed between CHM use and a lower risk of HL, potential misclassification may have influenced the results because of the use of ICD-9-CM diagnostic codes alone. To minimize this, we enrolled only patients with new-onset RA or HL, and only after the patients had at least 3 outpatient visits with consistent diagnoses or at least one inpatient admission due to RA or HL. It should also be noted that the NHI of Taiwan randomly reviews the charts and audits medical charges to verify the accuracy of claims (13). Additionally, the coding approach and data availability were similar between the two groups, and any misclassification, if exists, was likely to have been non-differential, thus resulting in an underestimate (dilution) of the true strength of an association between exposure and disease. Second, a reliable index of RA severity was unavailable from the LHID, and failure to control for this factor may bias the findings. To address this potential problem, we performed two sensitivity analyses: one limited the analysis to the patients without comorbidity, and the other added the prescription of biological agents for 6 months or longer, a common surrogate used for RA severity (34, 35), to the analyses. These sensitivity analyses support that disease severity was not likely to introduce a remarkable effect on our conclusion—adding CHM to conventional therapy may reduce the subsequent risk of HL in RA patients. Third, the LHID lacks information on certain potential confounders such as social network relationships, smoking, alcohol intake, personality attributes, laboratory data, and education level. Research addressing these variables is needed to shed further light on these preliminary findings. Fourth, although our study revealed a substantial beneficial effect of CHM use on reducing HL among RA patients, it must be recognized that participants were not initially randomly categorized into users and non-users and were only recruited from a single country. Therefore, caution should be taken when interpreting the findings. Randomized controlled trials of cohorts including patients from additional countries are recommended to corroborate the present findings and to clarify the mechanisms underlying the clinical benefits of CHMs. Fifth, the NHI program in Taiwan only pays for Chinese herbal products prescribed by Chinese medicine physicians, and not for over-the-counter medicine (36). These limitations notwithstanding, this study also possessed several strengths, including the completeness of the records of hospital diagnoses and prescription claims, the minimal risk of selection bias and loss to follow-up, and the large population of both men and women. These factors provide sufficient power to conduct detailed analyses, which is unique given the relatively low incidence of RA in the general population. As to the safety of CHM, following a careful review of the Taiwan National Adverse Drug Reactions Reporting System (37), we found that there were no severe drug interaction effects occurring among Taiwanese RA patients who used the concomitant treatments of Western medicines and Chinese herbal products so far.

CONCLUSION

This study demonstrated that RA patients who used CHM may have a 23% lower risk of developing HL than did those without CHM use. Although a beneficial effect of CHM use on the risk of HL was found in this survey, future prospective randomized trials that overcome the limitations of this study are needed to provide more definite evidence of the association suggested herein. If the effect can be confirmed, efforts should be made to identify other groups of patients who may also benefit from it. Further studies exploring additional benefits of CHM on rheumatoid joint symptoms are also warranted.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board and Ethics Committee of Buddhist Dalin Tzu Chi Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

H-HL, HL, and T-YT: study concept and design. M-CL and T-YT: acquisition of data. HL, T-YT, and H-RG: data analysis. M-CL, T-YT, and W-LF: project management. W-JC, H-HL, HL, H-RG, T-YT, M-CL, and W-LF: writing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Centers for Disease Control and Prevention. *National Center for Health Statistics*. Available online at: https://www.cdc.gov/nchs/fastats/arthritis.htm (accessed February 17, 2021).
2. Chen CI, Wang L, Wei W, Yuce H, Phillips K. Burden of rheumatoid arthritis among US Medicare population: co-morbidities, health-care resource utilization and costs. *Rheumatol Adv Pract.* (2018) 2:rky005. doi: 10.1093/rap/rky005
3. Brekke M, Hjortdahl P, Kvien TK. Self-efficacy and health status in rheumatoid arthritis: a two-year longitudinal observational study. *Rheumatology.* (2001) 40:387–92. doi: 10.1093/rheumatology/40.4.387
4. Chaitidis N, Theocharis P, Festas C, Aritzi I. Association of rheumatoid arthritis with hearing loss: a systematic review and meta-analysis. *Rheumatol Int.* (2020) 40:1771–9. doi: 10.1007/s00296-020-04609-1
5. Nasution MES, Haryuna TSH. Elevated matrix metalloproteinase-3 level may affect hearing function in patients with rheumatoid arthritis. *J Chin Med Assoc.* (2019) 82:272–6. doi: 10.1097/JCMA.0000000000000036
6. Abrams H. Hearing loss and associated comorbidities. *Hearing Review.* (2017) 24:32–5.
7. Kim SY, Min C, Kim HJ, Lee CH, Sim S, Park B, et al. Mortality and cause of death in hearing loss participants: a longitudinal follow-up study using a national sample cohort. *Otol Neurotol.* (2020) 41:25–32. doi: 10.1097/MAO.0000000000002429
8. Castañeda R, Natarajan S, Jeong SY, Hong BN, Kang TH. Traditional oriental medicine for sensorineural hearing loss: can ethnopharmacology contribute to potential drug discovery? *J Ethnopharmacol.* (2019) 231:409–28. doi: 10.1016/j.jep.2018.11.016
9. Kang TH, Hong BN, Park C, Kim SY, Park R. Effect of baicalein from *Scutellaria baicalensis* on prevention of noise-induced hearing loss. *Neurosci Lett.* (2010) 469:298–302. doi: 10.1016/j.neulet.2009.12.009
10. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther.* (2017) 2:17023. doi: 10.1038/sigtrans.2017.23
11. Du S, Yao Q, Tan P, Xie G, Ren C, Sun Q, et al. Protective effect of tanshinone IIA against radiation-induced ototoxicity in HEI-OC1 cells. *Oncol Lett.* (2013) 6:901–6. doi: 10.3892/ol.2013.1486
12. Li GH, Li YR, Jiao P, Zhao Y, Hu HX, Lou HX, et al. Therapeutic potential of salviae miltiorrhizae radix et rhizoma against human diseases based on activation of nrf2-mediated antioxidant defense system: bioactive constituents and mechanism of action. *Oxid Med Cell Longev.* (2018) 2018:7309073. doi: 10.1155/2018/7309073
13. National Health Insurance Database. *LHID.* (2000). Available online at: https://nhird.nhri.org.tw/en/Data_Subsets.html (accessed February 17, 2021).
14. Shariff SZ, Cuerden MS, Jain AK, Garg AX. The secret of immortal time bias in epidemiologic studies. *J Am Soc Nephrol.* (2008) 19:841–3. doi: 10.1681/ASN.2007121354
15. Liu CY, Hung YT, Chuang YL, Chen YJ, Weng WS, Liu JS, et al. Incorporating development stratification of Taiwan townships into sampling design of large scale health interview survey. *J Health Manag.* (2006) 4:1–22. doi: 10.29805/JHM.200606.0001
16. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. *J Clin Epidemiol.* (1992) 45:613–9. doi: 10.1016/0895-4356(92)90133-8
17. Shih CC, Liao CC, Su YC, Tsai CC, Lin JG. Gender differences in traditional Chinese medicine use among adults in Taiwan. *PLoS ONE.* (2012) 7:e32540. doi: 10.1371/journal.pone.0032540
18. Clouston SAP, Manganello JA, Richards M. A life course approach to health literacy: the role of gender, educational attainment and lifetime cognitive capability. *Age Ageing.* (2017) 46:493–9. doi: 10.1093/ageing/afw229
19. April A, Feher A, McPhee L, Jessa A, Oh S, Einstein G. Estrogens, inflammation and cognition. *Front Neuroendocrinol.* (2016) 40:87–100. doi: 10.1016/j.yfrne.2016.01.002
20. Williamson TT, Ding B, Zhu X, Frisina RD. Hormone replacement therapy attenuates hearing loss: mechanisms involving estrogen and the IGF-1 pathway. *Aging Cell.* (2019) 18:e12939. doi: 10.1111/acel.12939
21. Li W, Huang H, Zhang Y, Fan T, Liu X, Xing W, et al. Anti-inflammatory effect of tetrahydrocoptisine from Corydalis impatiens is a function of possible inhibition of TNF-α, IL-6 and NO production in lipopolysaccharide-stimulated peritoneal macrophages through inhibiting NF-κB activation and MAPK pathway. *Eur J Pharmacol.* (2013) 715:62–71. doi: 10.1016/j.ejphar.2013.06.017
22. Shi L, An Y, Wang A, Gao Q, Yang Y. The protective effect of Salvia miltiorrhiza on gentamicin-induced ototoxicity. *Am J Otolaryngol.* (2014) 35:171–9. doi: 10.1016/j.amjoto.2013.08.022
23. Eisenhut M. Evidence supporting the hypothesis that inflammation-induced vasospasm is involved in the pathogenesis of acquired sensorineural hearing loss. *Int J Otolaryngol.* (2019) 2019:4367240. doi: 10.1155/2019/4367240
24. Wang CX, Xie GB, Zhou CH, Zhang XS, Li T, Xu JG, et al. Baincalein alleviates early brain injury after experimental subarachnoid hemorrhage in rats: possible involvement of TLR4/NF-κB-mediated inflammatory pathway. *Brain Res.* (2015) 1594:245–55. doi: 10.1016/j.brainres.2014.10.014
25. Zhang G, Zheng H, Pyykko I, Zou J. The TLR-4/NF-κB signaling pathway activation in cochlear inflammation of rats with noise-induced hearing loss. *Hear Res.* (2019) 379:59–68. doi: 10.1016/j.heares.2019.04.012
26. Vino AB, Ramasamy P, Shanmugam V, Shanmugam A. Extraction, characterization and in vitro antioxidative potential of chitosan and sulfated chitosan from cuttlebone of sepia aculeata orbigny, 1848. *Asian Pac J Trop Biomed.* (2012) 2:S334–41. doi: 10.1016/S2221-1691(12)60184-1
27. Pang HH, Li MY, Wang Y, Tang MK, Ma CH, Huang JM. Effect of compatible herbs on the pharmacokinetics of effective components of panax notoginseng in fufang xueshuantong capsule. *J Zhejiang Univ Sci B.* (2017) 18:343–52. doi: 10.1631/jzus.B1600235
28. Fei B, Liu Z, Xie L, Lv L, Zhu W, Liu J, et al. Panax notoginseng saponins protect auditory cells against cisplatin-induced ototoxicity by inducing the AKT/Nrf2 signaling-mediated redox pathway. *Mol Med Rep.* (2020) 22:3533–40. doi: 10.3892/mmr.2020.11390
29. Liu L, Ge F, Yang H, Shi H, Lu W, Sun Z, et al. Xiao-Yao-San formula improves cognitive ability by protecting the hippocampal neurons in ovariectomized rats. *Evid Based Complement Alternat Med.* (2020) 2020:4156145. doi: 10.1155/2020/4156145
30. Khalin I, Alyautdin R, Kocherga G, Bakar MA. Targeted delivery of brain-derived neurotrophic factor for the treatment of blindness and deafness. *Int J Nanomed.* (2015) 10:3245–67. doi: 10.2147/IJN.S77480
31. Xia X, May BH, Zhang AL, Guo X, Lu C, Xue CC, et al. Chinese herbal medicines for rheumatoid arthritis: text-mining the classical literature for potentially effective natural products. *Evid Based Comp Alternat Med.* (2020) 2020:7531967. doi: 10.1155/2020/7531967
32. Xu CJ, Rong C. Research progress of danggui niantong tang. *Chin J Exp Tradit Med Formulae.* (2010) 16:281–3.
33. Chen IC, Lin TH, Hsieh YH, Chao CY, Wu YR, Chang KH, et al. Formulated Chinese medicine shaoyao gancao tang reduces tau aggregation and exerts neuroprotection through anti-oxidation and anti-inflammation. *Oxid Med Cell Longev.* (2018) 2018:9595741. doi: 10.1155/2018/9595741
34. Lai NS, Livneh H, Fan YH, Lu MC, Liao HH, Tsai TY. Association of Chinese herbal medicines use with development of chronic obstructive pulmonary disease among patients with rheumatoid arthritis: a population-based cohort study. *Int J Chron Obstruct Pulmon Dis.* (2020) 15:691–700. doi: 10.2147/COPD.S267017
35. Lu MC, Livneh H, Yen CT, Huang HL, Lin MC, Yen SW, et al. Association of use of rehabilitation services with development of dementia among patients with rheumatoid arthritis: analysis of domestic data in Taiwan. *Front Med.* (2020) 7:446. doi: 10.3389/fmed.2020.00446
36. Ministry of Health Welfare. *Quality Control of TCM*. Available online at: https://www.mohw.gov.tw/cp-3701-58121-2.html (accessed May 01, 2021).
37. Taiwan Food and Dug Admiistrtion. *Taiwan National Adverse Drug Reactions Reporting System*. Available online at: https://www.fda.gov.tw/tc/siteContent.aspx?sid=4240 (accessed May 11, 2021).

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