WOMEN IN SCIENCE - RHEUMATOLOGY 2021

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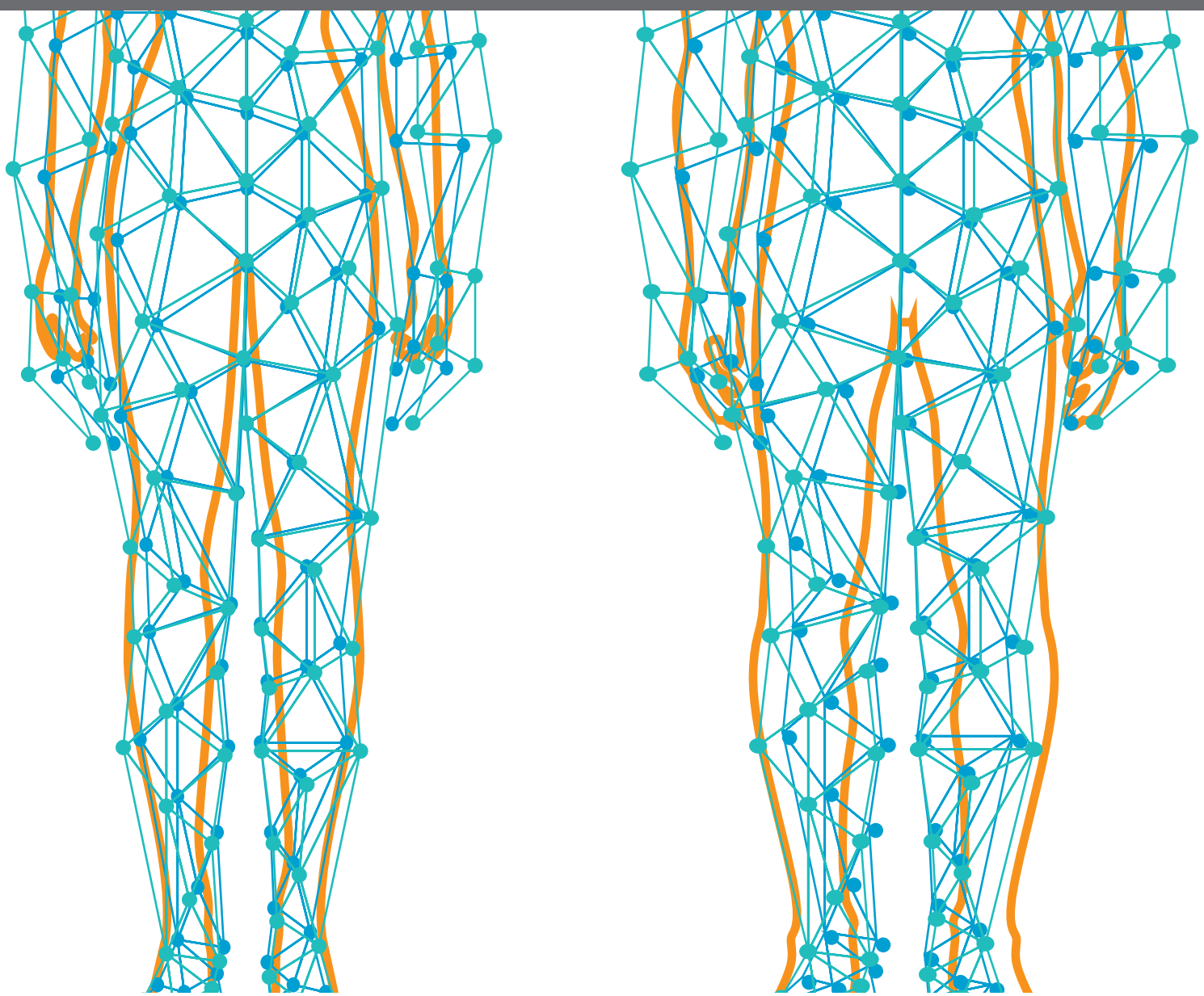

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WOMEN IN SCIENCE - RHEUMATOLOGY 2021

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Table of Contents

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Editorial: Women in science—Rheumatology 2021

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Editorial on the Research Topic
Women in science—Rheumatology 2021

Even though the proportion of women in science, technology, engineering, mathematics, and medicine has arisen in the last decades, a gender imbalance among conference speakers, editors, academic positions and hiring committees still exists throughout all of these disciplines (1, 2). In fact, it was recently demonstrated that in most scientific fields men comprise more than a half of the workforce, especially at senior levels (3). Rheumatology is a medical discipline that saw a great growth in the last years. Despite the significant contribution provided by women in the research in this field, as underlined in a recent publication (4), the gender imbalance in senior academic and authorship positions is no exception (2, 5). The increase of the number of women in rheumatology is an interesting data that comes also from Arab Countries, as underlined in the first publication of our collection. Ziade et al. described the situation of the women rheumatologists' workforce in the Arab League of Associations for Rheumatology countries, underlying their increasing presence, along with their lower proportion in leadership positions and suggesting that social media platform could help them to assert themselves. In line with this universal need, in the United States, an Association of Women in Rheumatology (AWIR) was founded with the mission to promote the science and practice of rheumatology, foster the advancement and education of women in the field, and advocate access to the highest quality health care, and management of patients with rheumatic diseases[1]. The same purpose is promoted by an Italian women association in rheumatology, "Reumatologhe Donne" (ReDO)[2].

Inspired by these principles in favor of gender equity in rheumatology, the aim of our Research Topic was to offer space to women, who are responsible of the manuscripts of this collection as first or senior authors, giving room to different scientific topics.

Women are often proactive in a multidisciplinary team, which is a fundamental need for the cure of multisystemic rheumatic conditions, as demonstrated by the paper of Schmoll et al., in which pneumologists and rheumatologists collaborated in order to cure the rheumatologic long-term complications of cystic fibrosis, or in the papers of Kramer et al., in which a multidisciplinary team studied sicca syndrome, related or not to other autoimmune symptoms, and Carmona-Fernandes et al. in which a collaboration with

1 https://rheumhighlights.com/2020/awir

2 https://www.reumatologhedonne.it/

vascular surgeons let rheumatologists to hypothesize the basis of the relationship between bones and vessels in the context of atherosclerotic disease and osteoporosis. Some rheumatologic conditions are very common, and a collaboration with epidemiologists is very welcome, in order to better understand relevant public health conditions, such as osteoarthritis, as reported by Costa et al. Furthermore, a collaboration with gynecologists is important for the process of counseling in view of a pregnancy, such as demonstrated by the paper of Triggianese et al.

In our collection, clinical research papers are well represented. Marinello et al. discussed the important theme of the involvement of patients in the cure of their disease in the form of shared decision making, which may be crucial for patients with rare diseases, such as Behçet's Syndrome. Mo et al. retrospectively analyzed the structural progression of the sacroiliac joint and clinical features in patients with axial spondylarthritis reducing TNF inhibitor's dose, discouraging the complete drug withdrawal. Büttner et al. proposed the use of the fluorescence optical imaging for the detection of early psoriatic arthritis.

Women are also involved in basic sciences' research. Nadali et al. explored the potential role of a soluble multiligand receptor as a new biomarker of metabolic failure developed during chronic inflammation and Kuret et al. discussed the application of the single cell RNA sequencing in the field of autoimmune diseases.

Those are examples of excellent manuscripts written under the supervision of women leaders, encompassing basic and clinical research and supporting the concept of multidisciplinary collaboration to achieve relevant results. As pointed out by a review (6), despite recent improvements in the number of women in rheumatology, further efforts must be spent to better understand and overcome causes of inequity between women and men, which still remain relevant in academia, considering that, according with a recent estimation, women are predicted to comprise the majority of the rheumatology workforce by 2025 (7, 8). A proposal of potential interventions for career advancement in academic rheumatology has just been published in order to inform an European Alliance of Associations for Rheumatology (EULAR) task force (9).

Author contributions

SP wrote the first draft. GS edited and reviewed the draft. All authors approved the final version of the manuscript.

Conflict of interest

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

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ORIGINAL RESEARCH
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Low Soluble Receptor for Advanced Glycation End Products Precedes and Predicts Cardiometabolic Events in Women With Rheumatoid Arthritis

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Background: Cardiovascular disease (CVD) causes premature mortality in rheumatoid arthritis (RA). Levels of soluble (s)RAGE change with aging, hypertension and hypercholesterolemia. We assessed whether sRAGE was associated with increased risk of CVD in RA patients.

Methods: Serum sRAGE was measured in 184 female RA patients and analyzed with respect to CVD risk estimated by the Framingham algorithm (eCVR), metabolic profile and inflammation. Levels of sRAGE in 13 patients with known cardio-metabolic morbidity defined the cut-off for low sRAGE. Prospective 5-year follow-up of new CV and metabolic events was completed.

Results: Low sRAGE was significantly associated with previous history and with new imminent cardiometabolic events in the prospective follow-up of RA patients. In both cases, low sRAGE reflected higher estimation of CVR in those patients. Low sRAGE was attributed to adverse metabolic parameters including high fasting plasma glucose and body fat content rather than inflammation. The association of sRAGE and poor metabolic profile was prominent in patients younger than 50 years.

Conclusions: This study points at low sRAGE as a marker of metabolic failure developed during chronic inflammation. It highlights the importance for monitoring metabolic health in female RA patients for timely prevention of CVD.

Trial registration: ClinicalTrials.gov with ID NCT03449589. Registered 28, February 2018.

Keywords: advanced glycation end product, cardiovascular disease, soluble RAGE, rheumatoid arthritis, cardiometabolic events

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INTRODUCTION

Glycation is the process of non-enzymatic binding of sugar molecules glucose and fructose with proteins, lipids and nucleic acids. Glycation directly depends on glucose concentration and occurs at random sites of a molecule. It leads to the loss of molecule's function and degradation into the advanced glycation end products (AGEs) (1). Excessive glycation may occur both in response

to the oxidative stress, hypoxia and inflammation. In turn, circulating AGEs in the extracellular compartment activate the proinflammatory receptor for advanced glycation end products (RAGE) and participate in perpetuation of inflammation (2). Under inflammatory conditions, other non-glycated RAGE ligands such as S100 proteins and HMGB1 are accumulated. RAGE ligands induce proinflammatory signaling through the membrane-bound RAGE causing nuclear translocation of NF-kappa B followed by cytokine production (2).

Broad range of harmful consequences of long lasting hyperglycosemia for health is well-documented (3), while cellular malfunction in response to high circulating glucose requires better understanding. Exposure of proteins to glucose enhances the process of unselective glycation (4–6). Measurement of glycated hemoglobin is clinically used to monitor DM (4). Ingestion of high glycated milk protein results in a rise of plasma glucose (7). However, there is a controversial view on levels of sRAGE in T2D. Several studies indicated decreased levels of sRAGE in T2D without complications (8, 9) and others reported high levels of sRAGE in T2D with cardiovascular or renal complications due to increased production of AGEs (10–12). AGEs have a key role in chronic inflammation and their accumulation has reported both in CVD, atherosclerosis and RA. Other factors as male gender, smoking and hyperglycemia have been reported to raise generation of AGEs independently to RA. Interestingly disease activity or erosivity of RA had no association with AGEs (13).

RAGE is a multiligand receptor, which belongs to the immunoglobulin superfamily of cell surface molecules and is physiologically expressed by cells involved in innate immune responses, including macrophages and granulocytes, and also on endothelial cells, vascular smooth muscle cells, and adipocytes (14). A soluble form of RAGE (sRAGE) is either generated via the proteolytic cleavage of extracellular domain of the membrane-bound RAGE or formed by endogenous splicing of RAGE mRNA transcripts. It acts as a decoy receptor by catching RAGE ligands and preventing them from binding to the membrane-bound RAGE and thereby modulating the pro-inflammatory effects of RAGE signaling (2, 15). Soluble RAGE is considered to protect against adverse effects of proinflammatory RAGE ligands. Low levels of sRAGE were suggested to be a very early marker of endothelial dysfunction (16), and were reported in coronary artery disease (17, 18), atherosclerosis (19), essential hypertension (20, 21), hypercholesterolemia (22), and in RA (23), where CVD remained to be the major cause of premature death. We have previously reported that chronic inflammation in RA is associated with significantly lower serum sRAGE compared to healthy controls and patients with non-inflammatory joint diseases (23). Furthermore, the presence of anti-RAGE antibodies locally in the joints of RA patients was related to a less destructive joint disease (24).

In the present prospective study, we assess an association between serum sRAGE and cardiometabolic health in female RA patients. We search for the CVD risk factors attributed to the low serum levels of sRAGE.

MATERIALS AND METHODS

Patients

One hundred eighty-four female patients with established RA were recruited into the study. All the patients fulfilled the American Rheumatism Association 1987 revised criteria for RA (25). Patients were randomly chosen from the methotrexate (MTX)-treated patient cohorts at two rheumatology units in Sweden, Sahlgrenska University Hospital in Gothenburg and the Northern Älvsborg Country Hospital in Uddevalla during the period from November 2011 until September 2013. Patients under the age of 18, patients with other rheumatologic diseases, and juvenile idiopathic arthritis were excluded. At inclusion, 93% ($n = 172$) of patients received MTX treatment. Fifty-one patients (28%) had treatment with biologics including infliximab ($n = 23$), etanercept ($n = 12$), golimumab ($n = 5$), adalimumab ($n = 3$), rituximab ($n = 3$), tocilizumab ($n = 4$), abatacept ($n = 1$). Twenty-five MTX-treated patients (16%) received concomitantly other disease modifying drugs (14 sulfasalazine, 6 hydroxychloroquine, 4 combination of sulfasalazine and hydroxychloroquine, and 1 cyclosporine A). Oral corticosteroids (median dose 5.0 mg/day) were regularly used by 20 patients (11%). All patients completed the questionnaire about their current medication, concomitant diseases and smoking habits. At inclusion, all patients were examined by experienced rheumatologists and the clinical (tenderness and swelling of 28 joints) and laboratory (erythrocyte sedimentation rate, C-reactive protein) disease activity variables were recorded. Disease activity score in 28 joints (DAS28) was calculated (http://www.4s-dawn.com/DAS28/). The clinical information with regard to patients' age, sex, body mass index (BMI), body fat content (26), and disease duration were collected.

Ethical Consideration

The study was approved by the Swedish Ethical Review Authority (Dnr. 659-2011) and performed in accordance with the Declaration of Helsinki. The informed written consent was obtained from all subjects prior to enrolment in the study. The trial is registered at ClinicalTrials.gov with ID NCT03449589.

Calculation of Estimated Cardiovascular Risk

A 10-year risk for development of CVD was estimated (eCVR) using a digital version of the Framingham algorithm (27) and included sex, age, systolic blood pressure, treatment for hypertension, current smoking, diabetes, HDL, and total cholesterol.

CVD Follow-Up at 5 Years

Five years after enrollment, the patients were contacted for a structured telephone interview and a questionnaire was sent to their home address. The questions were asked for any CV event, and about current medication with antihypertensive drugs, anticoagulants, anti-diabetic drugs, and use of statins. The reported CV events and changes in medications were then controlled in patients' medical records and the Swedish National Health Registry. We were able to reach all patients except 3

patients—two of them were diseased and one patient had moved out of Sweden.

Collection and Preparation of Blood Samples

The blood samples were obtained after overnight fast. Blood was collected from the peripheral cubital vein directly into the vacuum tubes containing serum clot activator (Vacuette, Greiner Bio-One, Kremsmunster, Austria), mixed thoroughly and left to coagulate for 3–4 h at room temperature. The tubes were then centrifuged at 2,000 × g for 10 min, the serum carefully collected, aliquoted, and stored at −80°C until use.

Measurement of sRAGE

The levels of sRAGE in serum were determined using a specific sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Serum was diluted 1/3 in assay buffer and introduced into the ELISA plates coated with mouse monoclonal antibody against RAGE. After 2 h of incubation with serum, polyclonal capture antibody against the extracellular portion of RAGE was used. The reaction was visualized by tetramethylbenzidine substrate. The minimum detectable concentration of sRAGE was 4 pg/ml. According to the manufacturer, no significant cross-reactivity to EN-RAGE, HMGB1, S100A10, or S100Baa was observed.

Other Serological Measures

The measurement of adipokines and cytokines were determined using specific sandwich ELISA kits according to the instructions from the manufacturers (R&D Systems, Minneapolis, MN, USA) as previously described (28). The inflammatory parameters, blood lipids and RF/ACPA antibodies were measured at the accredited Laboratory of Clinical Chemistry at the Sahlgrenska University Hospital according to clinical routines. Plasma glucose levels were measured using FreeStyle Lite kit (Abbott Diabetes Care Ltd., Oxon, UK) and insulin levels by sandwich ELISA kit (DY8056, R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

Descriptive statistics for continuous variables are presented as the median with interquartile range, and for categorical variables as the number and the percentage. Univariate correlation between variables was examined by the Spearman's correlation test. Any two factors with a correlation coefficient >0.3 were investigated for co-linearity. For continuous variables, the difference between groups was assessed by using the Mann-Whitney *U*-test. The difference in frequency, sensitivity and specificity of calculations were performed using Chi Square and Fisher's exact test. Analyses were performed using Graph Pad Prism 8 for Microsoft Windows. All tests were two tailed and $p < 0.05$ was considered statistically significant.

TABLE 1 | The baseline characteristic of the study cohort ($n = 171$), female patients with RA.

	RA study cohort $n = 171$	CMRG $n = 13$
sRAGE, pg/ml	1,417 [1,093–1738]	1,259 [1,114–1,504]
Age, years	53 [44–62]	60 [53–63]
Disease duration, years	7 [4–14]	8 [3.5–19]
BMI, kg/m^2	24.85 [22.3–28]	27 [25.7–29.4]
Body fat, %	36.32 [32.37–41.7]	39.4 [36.6–43.6]
TG/HDL, ratio	0.5 [0.3–0.7]	3.1 [2.4–4.0]
DAS-28	3.0 [2.3–3.9]	3.5 [3.0–4.6]
ESR, mm/h	9 [5–14]	11 [6–15]
RF and/or ACPA positive	157/170 (92%)	13/13 (100%)
Methotrexate dose, mg/week	17.5 [12.5–20]	17.5 [5.6–24]
Biological DMARD	51(30%)	4/13 (31%)
TNFα-inhibitors	43 (25.1%)	1/13 (7.6%)
Medication for hypertension	22 (13%)	6/13 (46%)
Medication for dyslipidemia	9 (5.2%)	1/13 (15%)
Current medication with NSAID	44 (26%)	2/13 (15%)
Current oral corticosteroids,	20/171 (12%)	1/13 (7.6%)
Current oral corticosteroids, mg/day	5 [5–5]	1/13 (7.6%)
eCVR, %	6.6 [3.8–11.0]	18 [7.5–29.2]
SBP, mmHg	130 [120–140]	135 [123–140]
Current smoker	23/170 (13%)	3/13 (23%)
Former smoker	96/170 (57%)	10/13 (77%)
IL6, pg/ml	2.26 [0.12–8.94]	2.53 [1.48–7.67]
IL1β pg/ml	0 [0–10.6]	0 [0–0]
Leptin/adiponectin ratio	4.0 [1.6–10]	7.7 [1.7–28]
Resistin, ng/ml	21 [13–37]	21 [7.0–42]
Visfatin, ng/ml	2.58 [1.05–4.58]	2.4 [1.0–7.0]
IGF1, μg/l	138 [109–176]	139 [104–188]

The RA patients with diagnosed cardiometabolic diseases/events (n = 13) are referred to as cardiometabolic reference group (CMRG). The data are expressed as median [interquartile range] and number (%).

ACPA, anti-citrullinated protein antibody; bDMARD, biological disease modifying anti rheumatic drug; BMI, body mass index; eCVR, estimated cardiovascular risk; ESR, estimated sedimentation rate; HDL, high density lipoprotein; IGF1, insulin-like growth factor 1; IL, interleukin; MTX, methotrexate; NSAID, none steroidal anti-inflammatory drug; RF, rheumatoid factor; SBP, systolic blood pressure; TG, triglyceride; RTX, rituximab; sRAGE, soluble receptor for advanced glycation end products.

RESULTS

Soluble RAGE and Clinical, Metabolic, and Inflammatory Features in RA

Out of 184 patients included at the baseline, we identified 7 patients with type 2 diabetes (T2D) and 6 patients with previous CV events. As T2D and CVD could affect sRAGE levels, these 13 patients with cardiometabolic diseases were extracted from the cohort, analyzed separately and comprised a cardiometabolic reference (CMR) group. The baseline characteristics of CMR group ($n = 13$) and the remaining study cohort ($n = 171$) are shown in **Table 1**.

Expectedly, CMR group had significantly higher eCVR compared to the remaining 171 patients (**Table 1**). This high

CVR was largely attributed to high fasting plasma glucose levels and adverse composition of blood lipids including TG and TG/HDL ratio, leptin/adiponectin ratio, and BMI (**Table 1**). CMR group had significantly higher disease activity estimated by DAS28 compared to the remaining 171 RA patients. Interestingly, sRAGE levels had significant strong positive correlation with insulin (r 0.643, $p = 0.028$), HOMA index (r 0.626, $p = 0.032$), and age (r 0.675, $p = 0.013$) within the CMR group.

To investigate whether sRAGE concentrations were associated with high CV risk, sRAGE values within the lower 75% of the CMRG were considered low and were used to dichotomize the CV event free RA patients into high sRAGE ($sRAGE^{hi}$; $n = 73$) and low sRAGE ($sRAGE^{lo}$; $n = 98$) groups (**Figure 1**). The median eCVR was comparable between the groups with high and low sRAGE levels (**Figure 1**). We found neither differences in cardiometabolic nor in RA-related disease activity parameters (**Figure 1**).

Next, we performed univariate correlation analysis between sRAGE and CV risk parameters in un-dichotomized RA cohort and observed bi-directional correlation profile between sRAGE and eCVR (**Supplementary Figure 1**). Thus, we analyzed correlation between sRAGE levels and cardio-metabolic and inflammatory parameters within respective group. The correlation pattern of sRAGE was remarkably different between the $sRAGE^{hi}$ and $sRAGE^{lo}$ patients (**Figure 2A**). This difference in correlation between $RAGE^{hi}$ and $sRAGE^{lo}$ groups was confirmed by the Fisher r-to-z test and was significant for eCVR-BMI, body fat index, age, IL-6, and IGF-1 (**Figure 2A**). Additionally, in patients within $sRAGE^{hi}$ group, sRAGE showed significant positive correlation with plasma glucose, eCVR and age. In relation to RA-related risk factors, sRAGE correlated positively with DAS28, tender and swollen joints, IL6, and resistin, whereas a negative correlation was seen between sRAGE and serum levels of IGF1 (**Figure 2A**). In contrast, in patients within $sRAGE^{lo}$ group, a positive correlation was seen between sRAGE level and IGF1, whereas eCVR-BMI and body fat content correlated negatively to sRAGE.

The analysis of traditional CVR factors such as hypertension, dyslipidemia, overweight, smoking, and age showed no significant difference between $sRAGE^{lo}$ and $sRAGE^{hi}$ groups (**Figure 2B**).

We thereafter studied RA-related CVR factors, which included higher ESR, the presence of RA-specific antibodies, long disease duration and active RA disease defined by DAS28. The comparison showed that none of the RA-related risk factors had significant difference between $sRAGE^{lo}$ and $sRAGE^{hi}$ groups (**Figure 2C**).

Since eCVR is age dependent and a decrease of sRAGE levels with increasing age has been reported in several studies (29–31), we performed the analysis separately for the patients of different age groups. We compared the traditional and RA-related CVR factors as well as serum levels of adipokines and cytokines for ages <50 years ($n = 66$), and ≥50 years ($n = 105$) in $sRAGE^{lo}$ and $sRAGE^{hi}$ group. We observed no significant differences in sRAGE levels between the patients <50 years compared to those ≥50 years within respective $sRAGE^{lo}$ and $sRAGE^{hi}$ groups.

We found that dominating CVR parameters in $sRAGE^{lo}$ group were age dependent. In RA patients <50 years, low sRAGE group ($n = 37$) was significantly different from high sRAGE group ($n = 29$) by having higher BMI ($p = 0.028$), IL-6 concentration ($p = 0.019$), and more tender points ($p = 0.042$) (**Figure 2D**). Thus, metabolic and inflammation-related factors dominated CVR in patients <50 years. In RA patients ≥50 years, low sRAGE group ($n = 61$) showed significant differences in the profile of blood lipids. We found significantly lower HDL ($p = 0.023$), lower total cholesterol ($p = 0.018$), and adiponectin ($p = 0.023$), compared to high sRAGE group ($n = 44$) (**Figure 2E**).

Prospective Follow-Up for Development of New Cardiometabolic Events

Within 5 years, 11 of 171 patients (6.4%) developed new cardiometabolic events (CME). In the $sRAGE^{lo}$ group, seven events were observed including 1 patient with new T2D diagnosis, 2 chronic atrial fibrillations (AF), 2 transitory ischemic attacks, 1 patient got deep venous thrombosis and one patient deceased due to aorta dissection. In the $sRAGE^{hi}$ group, CME occurred in 4 patients including 1 patient with new T2D combined with AF, and 1 AF, 1 stroke, and 1 incidental aortic aneurysm were reported. The prevalence of new CME was not different between the $sRAGE^{lo}$ and $sRAGE^{hi}$ groups (6.9 vs. 6.1%, respectively).

Next, we wanted to study whether the patients with new CME were different at inclusion with respect to inflammation and metabolic characteristics compared to patients in $sRAGE^{hi}$ and $sRAGE^{lo}$ groups that had no new CME. The new CME group had significantly lower sRAGE levels compared with the $sRAGE^{hi}$ group (**Figure 3A**). Importantly, new CME group had significantly higher eCVR compared to both the $sRAGE^{lo}$ and $sRAGE^{hi}$ groups. Patients in the new CME group were significantly older and had the adverse metabolic parameters such as higher plasma glucose levels and increased body fat compared with patients in the $sRAGE^{hi}$ and $sRAGE^{lo}$ groups. Inflammation, measured by ESR, IL6, IL1β, and DAS28, was not different between the new CME group and the remaining RA patients. Further, we compared the new CME group with CMR group, which accumulated CVR factors and had the highest eCVR (**Figure 1**). The baseline parameters of the patients with new CME were similar to the CMR group with respect to eCVR and also sRAGE (**Figure 3B**). We observed no differences in other cardiometabolic and inflammatory parameters between those groups.

DISCUSSION

In the present study we show that low serum levels of sRAGE are significantly associated with previous history and with new imminent cardiometabolic events in female RA patients. In both cases, this corresponded to higher estimation of CVR in the patients with low sRAGE. This low sRAGE was largely attributed to adverse metabolic parameters rather than signs of inflammation. We observed high fasting plasma glucose, and overweight to be the major contributors to CVD risk in younger

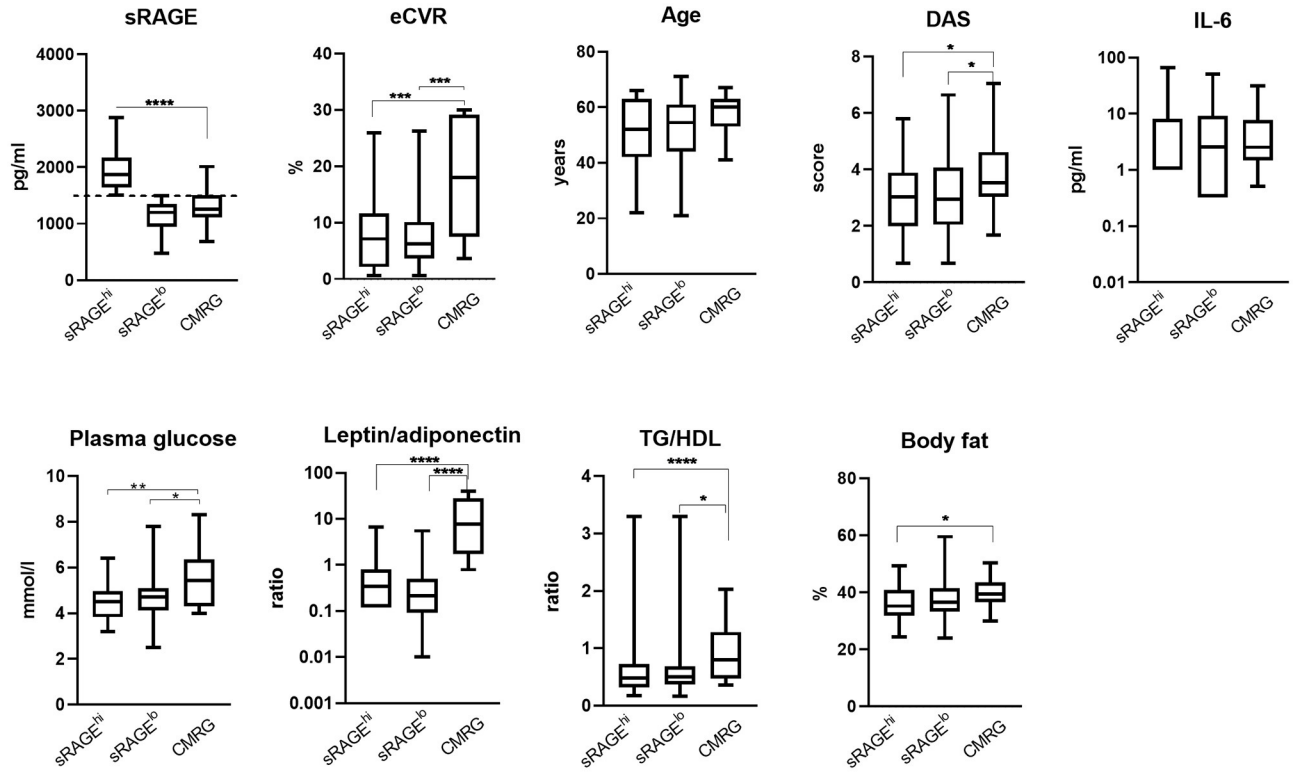

FIGURE 1 | Metabolic and inflammation-related characteristics of female patients with rheumatoid arthritis. Serum levels of soluble receptor for advanced glycation end products (sRAGE) were measured in 184 patients and the patients with no previous cardiovascular events were divided into the high (sRAGE^{hi}, $n = 73$) or low (sRAGE^{lo}, $n = 98$) groups, accordingly. Cardio-metabolic reference group (CMRG) consisted of patients with history of cardiovascular events and/or type II diabetes ($n = 13$). SRAGE in the upper quartile of CMR group (above 1,504 pg/ml, indicated with dotted line) defined the cut-point for dichotomization. Statistical evaluations were calculated using the Mann-Whitney *U*-test. The box plots indicate medians and interquartile ranges; whiskers show min to max. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$. TG/HDL, ratio between serum triglycerides and high-density lipoproteins; DAS28, disease activity score; IL, interleukin.

sRAGE^{lo} group. Also, 5-year follow up showed that the patients with new CME had remarkably low sRAGE levels and reach the level of CMR group in eCVR. The patients with new CME displayed significant accumulation of unfavorable metabolic factors combining high plasma glucose with overweight.

Recently, Dozio et al. suggested circulating sRAGE as an early marker of cardiometabolic disease. They showed that healthy obese women presented lower sRAGE levels than normal-weight women, and found inverse association between sRAGE levels with BMI, total fat mass, and visceral fat in the epicardial region (32). In another study, investigating healthy subjects from the general population with no T2D, CVD, hypertension, or treatment for hyperlipidemia, the authors found that BMI and waist circumference were inversely associated with sRAGE in women (33). Consistent with above findings in healthy women, we observed a significant association between low sRAGE levels with total body fat and eCVR in the patients below 50 years. Similarly, patients with new CME had low sRAGE and displayed significantly increased plasma glucose levels and body fat content compared to both sRAGE^{lo} in sRAGE^{hi} groups. These findings suggest that low sRAGE reflects a metabolic misbalance prior to imminent clinical CME.

In our cohort, we have analyzed the total levels of circulating sRAGE, which exists in two main isoforms—a soluble RAGE cleaved from the membrane-bound full-length RAGE by proteases (34–36), and an endogenously secreted RAGE produced by alternative splicing (15). While cleaved RAGE has a strong association with inflammation markers, endogenously secreted RAGE remains constant among age groups in the healthy population and reflects metabolic disturbances related to obesity and insulin resistance (29). In our sRAGE^{hi} group, sRAGE levels were primarily associated with inflammation including disease activity and IL6. In contrary, in sRAGE^{lo} group, sRAGE had a negative correlation with BMI, eCVR and age, reflecting metabolic disturbance rather than inflammation. What could explain these seemingly controversial associations?

Under physiological conditions, the cell surface RAGE has relatively low expression in non-inflamed tissues (37) whereas during inflammation, it is up-regulated responding to ligand exposure (1). In active RA, a plethora of inflammatory ligands for RAGE are present, both in the synovium (38–40) as well as in the circulation (41, 42) thereby modulating the expression of cell-bound RAGE. In our sRAGE^{hi} patient group, but not in sRAGE^{lo} group, we observed a positive correlation between

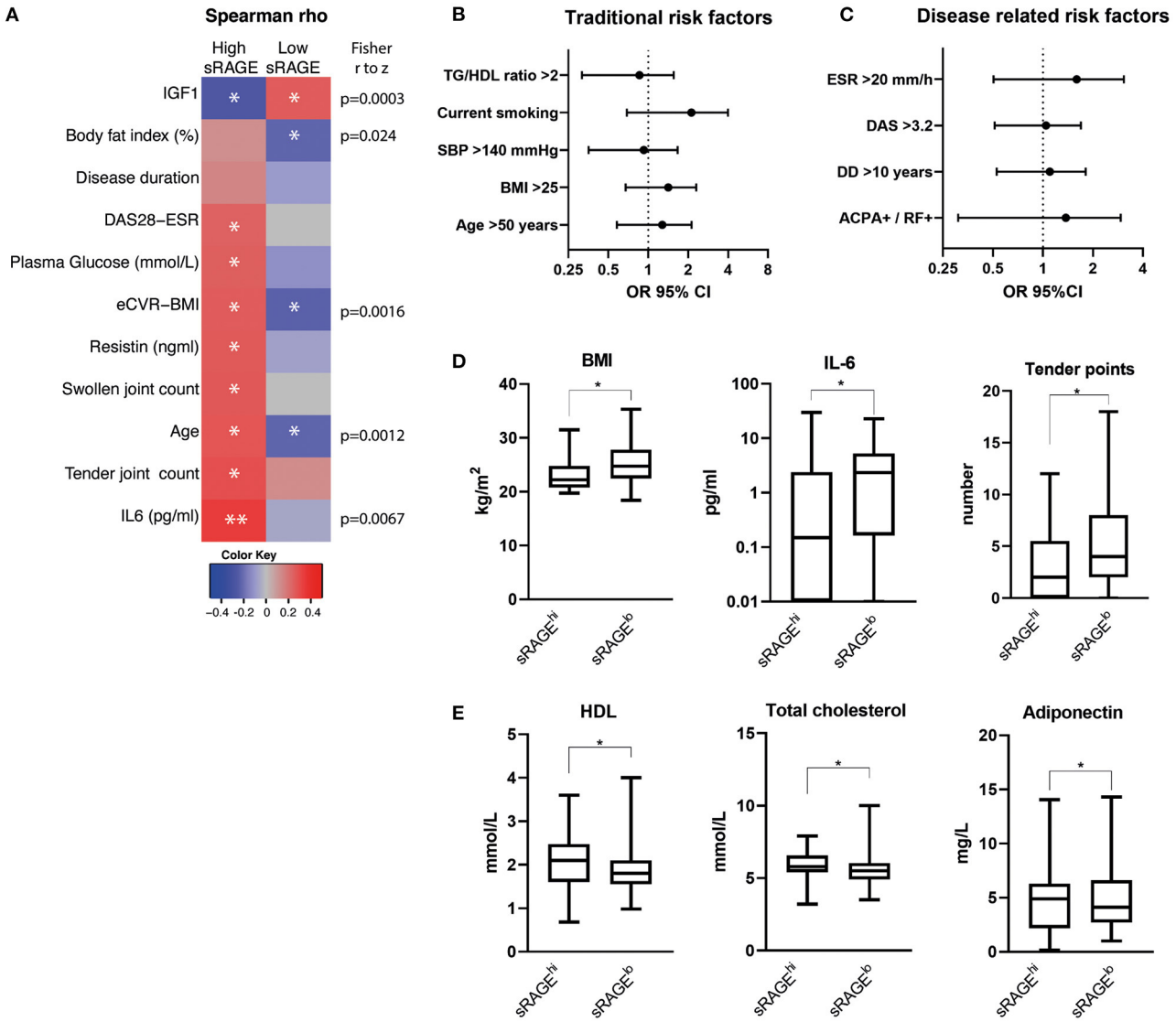

FIGURE 2 | Correlation between sRAGE levels and cardio-metabolic and inflammatory parameters within respective groups **(A)** and the differences in frequency of the traditional cardiovascular risk factors **(B)** and RA-related risk factors **(C)** between the patients with high ($n = 73$) and low ($n = 98$) serum levels of soluble RAGE. Correlation matrix in **(A)** shows Spearman's *R*-values in color as indicated by color key code. $^{*}P < 0.05$; $^{**}P < 0.01$. The differences as indicated by forest plots in **(B,C)** are calculated as odds ratio (OR) with 95% confidence interval (CI). The *p*-values are obtained by chi-square statistics. Comparison of the traditional, inflammation- and RA-related CVR factors for ages <50 years ($n = 66$) **(D)**, and ≥50 years ($n = 105$) **(E)**, in sRAGE^{lo} and sRAGE^{hi} group. The comparison is done pairwise using the Mann-Whitney *U*-test. The box plots indicate medians and interquartile ranges; whiskers show min to max. $^{*}P < 0.05$; $^{**}P < 0.01$. BMI, body mass index; DAS28, disease activity score with assessment of 28 joints; eCVR, estimated cardiovascular risk; IL, interleukin; RAGE, receptor for advanced glycation end products; HDL, high density lipoprotein. TG/HDL, ratio between triglycerides and high-density lipoprotein; TC, total cholesterol; ESR, erythrocyte sedimentation rate; ACPA/RF, presence of antibodies to citrullinated peptide antibodies and/or rheumatoid factor; DD, disease duration; SBP, systolic blood pressure.

markers of inflammation. In fact, the more inflammatory ligands for RAGE in the surrounding milieu, the higher expected density of the cell-bound receptor, which predisposes to increased production of sRAGE by cleavage and sRAGE levels are probably a simple reflection of RAGE production in tissues.

Besides inflammation, another molecular explanation for decreased sRAGE is conceivable. Hyperglycemia leads to increased non-enzymatic glycation of proteins, i.e., production of AGEs. Soluble RAGE binds AGEs without activating cellular pathways and functions as a decoy to AGEs, increasing its consumption and decreasing detectable circulating level. On the other hand, binding up AGEs leads to blocking of AGE-RAGE signaling, reduces the positive feedback loop for RAGE up-regulation and thereby potentially limits enzymatic cleavage of RAGE. This explanation is well-applicable for the patients of

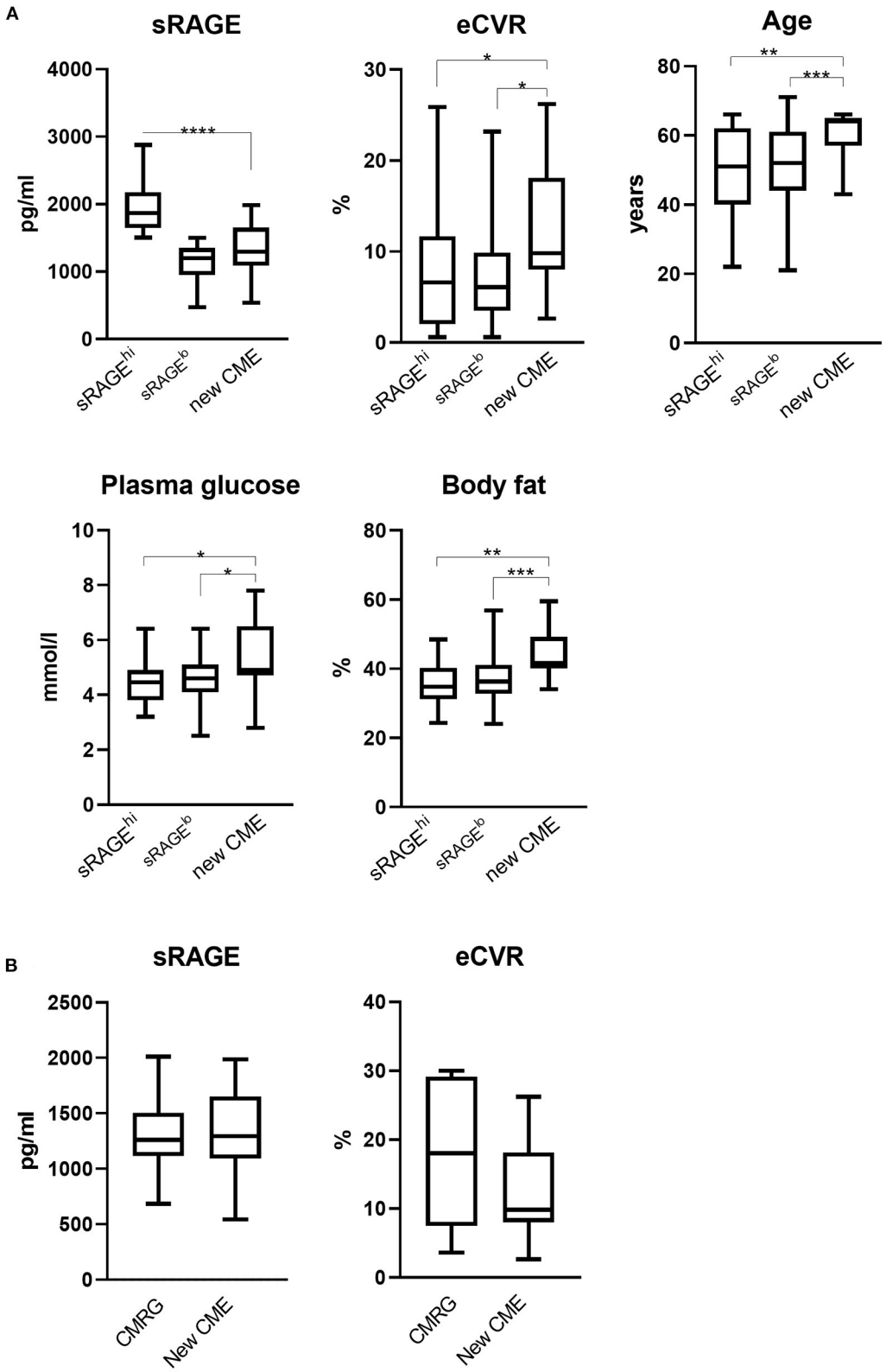

FIGURE 3 | (A) Comparison of metabolic and inflammation-related characteristics of RA patients with new cardiometabolic events. During the prospective follow up for 5 years, 11 of 171 patients developed new cardiometabolic events (CME). This group was compared to the patients with high (sRAGE^{hi}, $n = 69$) and low (sRAGE^{lo}, $n = 91$) serum levels of soluble RAGE. eCVR, estimated cardiovascular risk; RAGE, receptor for advanced glycation end products. **(B)** Comparison of new CME group ($n = 13$) with CMR ($n = 11$) group with respect to eCVR and sRAGE. *The comparison is done pairwise using the Mann-Whitney *U*-test. The box plots indicate medians and interquartile ranges; whiskers show min to max. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$.

CMRG and new CME groups, both recognized by low sRAGE and high plasma glucose level.

Of importance, the levels of circulating sRAGE could be affected by several drugs. The effect of treatment for hypertension and hyperlipidemia (22, 43) as well as DMARD treatment with methotrexate (23) has been shown to modulate sRAGE levels in several studies. However, in our cohort, we did not find any differences in the level of sRAGE between groups either treated or not with statins, DMARDs or antihypertensive drugs **Supplementary Table 1**.

Our study has certain limitations, which need to be taken into account. Firstly, the study had a cross-sectional design, although the patients were clinically followed up for 5 years with respect to cardiometabolic events. A structured consecutive blood sampling during the follow up period would have probably rendered more clear-cut results. However, data from the community-based atherosclerosis risk study ARIC, which measured sRAGE levels with 3 years apart, suggested that sRAGE concentrations within individual subjects are relatively stable. Thus, a single measure could be valuable to evaluate the long-term CV risk (44). Secondly, as discussed above, we have analyzed the total amount of sRAGE and therefore the study does not permit any conclusions with regards to sRAGE isoforms and their relations to CVR in RA women. This question needs to be addressed in future studies.

Taken together, this study shows that low sRAGE reflected higher CV risk in female RA patients. It was associated with previous history and with new forthcoming cardiometabolic events. The study also emphasizes metabolic misbalance behind low sRAGE in female RA patients and puts it forward as a useful biomarker to monitor cardiometabolic health in RA patients.

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 the Ethical Review Board of Gothenburg with permission code 659-2011. All methods used in this study were carried out in accordance with relevant Swedish guidelines and regulations and following the Good Clinical Practice. The informed written consent was obtained from all subjects prior to enrolment in the study.

AUTHOR CONTRIBUTIONS

MB and RP: conceptualization, funding acquisition, and resources. MN, LL, SS, and ME: data curation. MN, ME, MB, and RP: formal analysis. MN, LL, SS, ME, and KA: investigation. MN and MB: methodology. SS and MB: project administration. ME: software. RP: supervision. MN, MB, and RP: validation. MN, ME, and RP: visualization. MN: writing—original draft. MN, LL, SS, ME, KA, MB, and RP: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.594622/full#supplementary-material

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ORIGINAL RESEARCH
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Atherosclerosis and Bone Loss in Humans–Results From Deceased Donors and From Patients Submitted to Carotid Endarterectomy

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Background and Aims: Atherosclerosis and osteoporosis share common risk factors, as well as inflammatory mechanisms. Our aim was to understand how atherosclerotic lesions are related with disturbances in bone.

Methods: Gene expression of pro-inflammatory and bone metabolism related proteins (*IL-1β, IL-6, IL-17A, TNF, RANKL, OPG, COL1, CTSK, OCL, TRAP, CBFA1, DKK1, SOST, ADIPOQ,* and *ADIPOR1*) were analyzed in arteries and bones from 45 deceased donors and adipose tissue was used as control. Additionally, in 139 patients with advanced atherosclerosis submitted to carotid endarterectomy we compared calcium content (Alizarin red) and plaque inflammatory scores ($CD3^+$, $CD68^+$, and adiponectin) of patients with normal bone mineral density (BMD) with those with low BMD and explored the associations between gene expression in atherosclerotic plaques and BMD. Serum levels of pro-inflammatory and bone related proteins were measured both in donors and patients. Associations were investigated by the Pearson or Spearman correlation tests, and multivariate regression analyzes were performed when justified.

Results: Gene expression of bone remodeling and pro-inflammatory proteins correlated positively in bone and aorta, independently of age and sex of donors, but not in adipose tissue. The expression of bone formation genes was significantly higher in atheroma plaques from endarterectomized patients with normal vs. low BMD as well as inflammatory $CD68^+$ scores, regardless of patients' age and sex, but not of body mass index. No relationship was observed between serum levels and gene expression levels of pro-inflammatory or bone remodeling proteins.

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Conclusions: Our results suggest that the relationship between bones and vessels in the context of atherosclerotic disease and osteoporosis may rely on the intrinsic connection between the tissues involved, independently of disease stage. Serum measurements of pro-inflammatory and bone-remodeling proteins do not accurately translate tissue pathologic processes.

Keywords: atherosclerosis, osteoporosis, pro inflammatory cytokines, bone remodeling biomarkers, tissue expression analysis

INTRODUCTION

Atherosclerosis and osteoporosis are among the most prevalent diseases, frequently occurring in the same individual, and their prevalence increases with aging (1, 2).

Atherosclerosis is a chronic inflammatory process that evolves from fatty streaks to atheroma plaques and causes progressive stenosis of large and medium-sized arteries (3), as a consequence of accumulation of lipids, inflammatory cells, fibrous elements, cellular waste products and calcium (1).

Inflammation, as a key mechanism of atherosclerosis (2), affects its progression throughout all phases (4). Endothelial dysfunction and inflammatory lesions are mediated by several pro-inflammatory cytokines, present in atherosclerotic plaques produced by monocytes and macrophages (5). Infiltrates of CD68-positive macrophages and CD3- and CD8-positive T cells have been associated with plaque ruptures (6). Moreover, high serum levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) (3) are associated with an increase of cardiovascular (CV) risk, as demonstrated by epidemiological studies (7).

Osteoporosis (OP) is a skeletal bone disorder characterized by a decline in bone mineral density (BMD) and microarchitectural deterioration of bone tissue, which causes a reduction in bone strength and, consequently, leads to an increased risk of fracture (8–10). BMD can be determined by dual x-ray absorptiometry (DXA).

Bone is an active tissue, which is self-remodeled in a coupled action of bone-resorbing cells, osteoclasts, and bone forming cells, osteoblasts (11).

The Receptor Activator of NF-kB (RANK)/RANK Ligand (RANKL)/Osteoprotegerin (OPG) system, essential to the regulation of bone remodeling and to the physiopathology of OP (12), is closely related to inflammation. Not only inflammatory cells produce RANKL, but the interaction between RANK and RANKL leads to the release of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF, which increase bone resorption (13). Interestingly, the RANK/RANKL/OPG system and the Wnt pathway have also been implicated in the development of atherosclerosis and could be contributing pathways in the regulation of vascular calcification mechanisms (14).

These two diseases share common risk factors (1), as well as molecular and pathophysiological mechanisms (13), although conceivable common underlying mechanisms are not yet fully understood.

Our aim was to understand the relationship between atherosclerotic lesions and bone disturbances. Using samples from deceased donors, we aimed to analyze if a link between bone and vessel exists regarding gene expression patterns of pro-inflammatory cytokines and bone remodeling markers. Additionally, in a group of patients with advanced atherosclerosis submitted to carotid endarterectomy, we aimed to understand whether gene expression patterns of pro-inflammatory cytokines and bone remodeling markers in atherosclerotic plaques and plaque morphology are related to bone mineral density.

MATERIALS AND METHODS

Patients

Deceased Donors' Samples

A sample of bone from the iliac crest and a section of the abdominal aorta were collected from 45 deceased donors at the time of organ collection for transplantation, immediately preserved at 4°C and processed on average in <24 h. A blood sample was also obtained. From a subgroup of seven patients (four men and three women), an additional sample of subcutaneous adipose tissue was collected. Due to confidentiality aspects, no clinical information beyond age and gender could be retrieved, but all of them had clearance to be organ donors, which means that they did not present major health issues at the time of death.

Endarterectomized Patients/Advanced Atherosclerosis Samples

Atherosclerotic plaques and fasting blood samples were collected from 139 patients submitted to carotid endarterectomy surgery. A structured protocol was applied to all patients for recording demographic data, CV risk factors, history of previous fractures, personal and family history of OP, other comorbidities, lifestyle, and past and current medication.

All patients performed a dual X-ray absorptiometry (DXA) and were classified with osteoporosis, osteopenia or normal BMD according to the WHO classification criteria (15).

This study was approved by the Ethics Committee of Centro Académico de Medicina de Lisboa and patients signed written informed consent prior to any protocol-specific procedure. All proceedings were conducted in accordance with the regulations governing biomedical investigation such as the Declaration of Helsinki, as amended in Fortaleza, Brazil (2013) (16).

Biologic Samples Collection and Storage

Endarterectomy samples–the central and visually more developed plaque was sectioned crosswise over the longitudinal

axis in two sections: one for RNA extraction that was fragmented in smaller pieces and immediately frozen in liquid nitrogen (snap-frozen) and stored at −80°C, and the other for histology and immunohistochemistry that was frozen in optimal cutting temperature compound (OCT) and stored at −80°C.

Deceased organ donor samples—Bone biopsies and aorta sections were processed the same way as the atheroma plaques.

Blood samples–were centrifuged upon arrival to the lab and the serum was collected and stored at −80°C for later analysis.

RNA Isolation From Bones and Aortas

Samples were reduced to a fine powder with a pestle and mortar cold with liquid nitrogen and the RNA was extracted with TRIzol® (Invitrogen™), according to a modified version of Hughes et al. protocol (17). Briefly, the powder was placed in TRIzol® and homogenized, and chloroform was added to solubilize the lipids. A digestion with proteinase K was performed at 55°C, and it was subsequently treated with isopropyl alcohol to precipitate the RNA. The RNA pellet was cleaned with ethanol and then dissolved in RNase/DNase-free water.

The RNA quantification and quality were obtained by absorbance measured using the NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, USA).

RNA Expression–Quantitative RT-PCR

Total RNA was reverse-transcribed to cDNA according to the manufacturer's instructions (DyNAmo cDNA Synthesis Kit, Thermo Fisher Scientific Inc., Waltham, MA, USA).

The quantitative PCR was performed using DyNAmo Flash SYBR® Green qPCR Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the results measured with Rotor Gene 6000 (Qiagen, Germany) for deceased donors' samples and with 7500 Fast Real-Time PCR System (Applied Biosystems®, Foster City, CA, USA) for advanced atherosclerosis patients' samples. The sequences of the primers used (*IL-1β, IL-6, IL-17A, TNF, RANKL, OPG,* Collagen type I *(COL1)*, Cathepsin K *(CTSK)*, Osteocalcin (*OCL),* Tartrate resistant acid phosphatase *(TRAP),* Core-Binding Factor Alpha I *(CBFA1)*, Dickkopf-related protein 1 *(DKK1)*, Sclerostin *(SOST)*, Adiponectin (*ADIPOQ)*, Adiponectin receptor 1 (*ADIPOR1*) are listed in **Supplementary Tables 1, 2**. The efficiency of qPCR was analyzed using the standard curve method, as described previously (18). The values obtained were normalized with the housekeeping gene 18S rRNA.

Histological Evaluation

Frozen plaques were sectioned crosswise over their longitudinal axis using a cryostat, and the major segment was used for histological analysis.

Alizarin Red S (Sigma, Missouri, USA) histological staining was used for calcium determination. The protocol was adapted (no de-paraffinization needed, slides were slowly immersed in distilled water) from IHC World website (19).

Immunohistochemical staining of the plaques to identify a proinflammatory/unstable profile was also performed with CD3 (eBioscience, San Diego, USA), CD68 (eBioscience, San Diego, USA) and Adiponectin (Boster Biological Technology, Pleasanton, USA) antibodies. Tissue sections were incubated with the primary antibody and with EnVision+ (Dako, Glostrup, Denmark). Color was developed in solution containing diaminobenzidine-tetrahydrochloride (Sigma, Missouri, USA), 0.5% H_2O_2 in phosphate-buffered saline buffer (pH 7.6). Slides were counterstained with hematoxylin and mounted (20).

Histological and immunohistochemical evaluations were performed using a semi-quantitative score of 0 to 3 (0–0 to 10% staining; 1–10 to 50% staining; 2–50 to 75% staining; 3–more than 75% staining). Slides were observed in a ZEISS Primo Star (ZEISS, Oberkochen, Germany) microscope.

Serum Cytokine and Bone Markers Quantification

RANKL and OPG serum levels were determined using Biomedica ELISA (Enzyme-Linked Immunosorbent Assay) (Cat. No. BI-20462 and BI-20403, respectively), and C-terminal telopeptide of type 1 collagen (CTX) and procollagen type 1 N propeptide (P1NP) with SunRed Biological Technology (Cat. No. 201-12-1350 and 201-12-2130, respectively). Data were acquired in the microplate reader Infinite® M200 (Tecan).

Cytometric Bead Array (CBA) determination was performed for pro-inflammatory cytokines, IL-1β, IL-6, IL-17A, and TNF [BD™ CBA Enhanced Sensitivity Flex Set; Cat. No. 561509 (for IL-1β), Cat. No. 561512 (for IL-6), Cat. No. 562143 (for IL-17A), and Cat. No. 561516 (for TNF)] and data were collected in the Accuri™ C6 flow cytometer from BD™ Biosciences.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 20. Quantitative variables are described as means and standard deviation and qualitative variables as percentages and absolute frequencies.

Statistical significance was considered for a two-tailed $p < 0.05$ and the confidence interval for all statistical analyses was 95%. The normality of the distribution for continuous variables was evaluated using Kolmogorov–Smirnov test. Comparisons between groups and correlations were performed using parametric and non-parametric tests, as appropriate. When justified, multivariable linear regression analyses with backward selection of covariates was performed.

RESULTS

Deceased Donors

Population Description

A total of 45 donors were included in this study with ages ranging between 15 and 80 years old: 23 men with 49.6 ± 17.8 years old and 22 women with 61.1 ± 12.9 years old. The aortas were evaluated macroscopically and in five of them (11.1%) calcifications were visible. These patients corresponded to a 78-year-old man and four women with a mean age of 66.5 ± 9.9 years old.

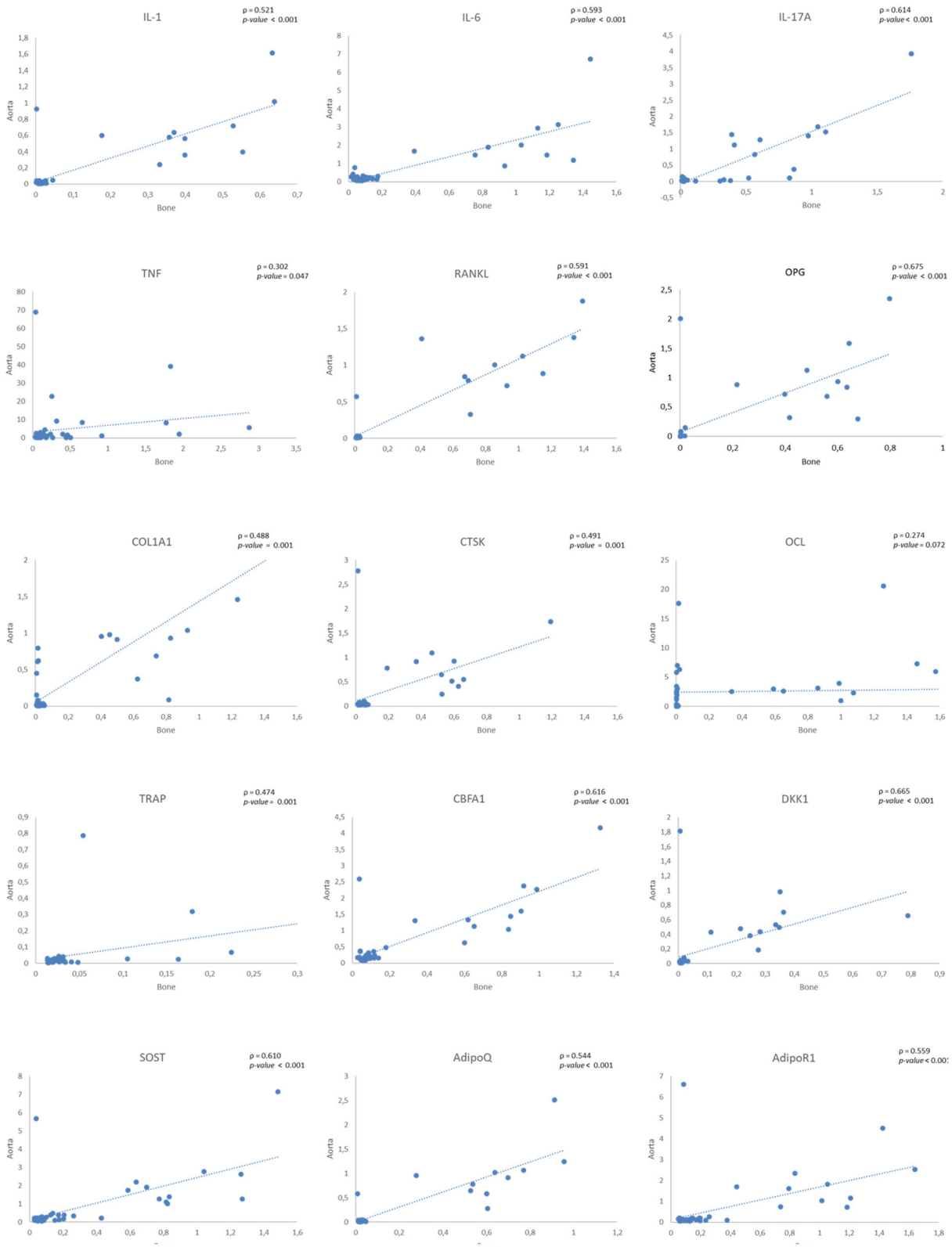

FIGURE 1 | Correlations of gene expression levels between bone and aorta samples. IL, Interleukin; TNF, Tumor necrosis factor; RANKL, Receptor Activator of NF-kB Ligand; OPG, Osteoprotegerin; COL1A1, Collagen type I; CTSK, Cathepsin K; OCL, Osteocalcin; TRAP, Tartrate resistant acid phosphatase; CBFA1, Core-Binding Factor Alpha I; DKK1, Dickkopf-related protein 1; SOST, Sclerostin; AdipoQ, Adiponectin; AdipoR1, Adiponectin receptor 1.

RNA Expression–Quantitative RT-PCR

A positive correlation between gene expression levels in bone and aorta samples was observed for all studied genes, except *OCL* gene (see **Figure 1**). The correlations found were classified, as described by Evans (21), as weak for the *TNF* gene; moderate for the *IL-1β, IL-6, RANKL, COL1A1, CTSK, TRAP, ADIPOQ,* and *ADIPOR1* genes; and strong for the IL-*17A, OPG, CBFA1, DKK1,* and *SOST* genes.

No association was found between gene expression levels of bone and aorta samples individually with adipose tissues samples.

Donors with macroscopic aortic calcifications had higher expression of *RANKL* and *TRAP* in the aorta and of *IL-17A* and *SOST* in bone—**Supplementary Table 3**.

No significant differences in the gene expression of inflammatory and bone remodeling markers in aorta, bone or adipose tissue samples were found between men and women—**Supplementary Table 4** neither in association with age.

Serum Cytokines and Bone Markers Analysis

We found that IL-1β levels were significantly higher in men than in women [442.7 fg/mL (range 179.3–4569) vs. 89.4 fg/mL (range 38.9–504.9); $p = 0.011$]. Age did not influence serum cytokine or bone markers levels.

No significant correlations were found between serum levels and gene expression levels, either from bone, aorta or adipose tissue samples, for any of the proteins studied.

Atherosclerosis Patients

Population Description

A total of 139 patients with advanced atherosclerosis were included in the study, where 95 (68.3%) were men, 70.3 ± 8.7 years old, and 44 (31.7%) were women, 71.5 ± 9.6 years old.

We have further compared the clinical characteristics, co-morbidities and therapies between patients with normal BMD (t-score > -1) and patients with low BMD (t-score ≤ -1). Details are listed in **Table 1**. Patients with low BMD were older ($p = 0.001$) and had lower BMI levels ($p < 0.001$). They did not differ significantly in any of the other variables evaluated.

TABLE 1 | Clinical characteristics, co-morbidities and therapies of atherosclerosis patients with normal and low BMD.

Characteristic	Normal BMD (*N* = 69)	Low BMD (*N* = 64)	*p*-value
Age (years)	67.9 ± 8.6	73.0 ± 8.6	0.001
Gender	49 M/20 F	41 M/23 F	0.392
Alcohol (above 3U/day)	19 (27.5%)	15 (23.4%)	0.554
Calcium intake (mg/day)	848.6 ± 498.27	896.8 ± 468.9	0.372
Current/Previous smokers	9 (13.0%)/28 (40.6%)	8 (12.5%)/29 (45.3%)	0.850
Active lifestyle	7 (10.1%)	5 (7.8%)	0.724
BMI (Kg/m^2)	28.5 ± 4.4	25.5 ± 4.0	<0.001
Hypertension	59 (85.5%)	49 (76.6%)	0.187
Previous CV event	44 (63.8%)	37 (57.8%)	0.482
Dyslipidemia	59 (85.5%)	53 (82.8%)	0.833
Type 2 Diabetes	20 (28.9%)	17 (26.6%)	0.716
Glucocorticoids (> 3 months)	4 (5.8%)	8 (12.5%)	0.167
Statins therapy	52 (75.4%)	46 (71.9%)	0.651

Quantitative variables are presented as means ± SD and qualitative variables as absolute values and proportions of total (%). BMD, bone mineral density; BMI, body mass index; CV, cardiovascular.

RNA Expression–Quantitative RT-PCR

Regarding bone remodeling markers in the atheroma plaques, we found that genes associated with bone formation were expressed at higher levels in patients with normal BMD than in patients with low BMD (**Table 2**). The difference was statistically significant for, specifically, *CBFA1* (0.98 ± 0.08 vs. 0.71 ± 0.06, $p = 0.009$) and *OCL* (1.27 ± 0.12 vs. 0.83 ± 0.08, $p = 0.003$). The association between BMD and *CBFA1* and *OCL* expression remained significant in a linear regression model adjusted for gender, age, and BMI.

Additionally, we found that *OCL* gene expression level in the plaques was higher in patients with dyslipidemia (1.12 ± 0.73 vs. 0.74 ± 0.69, $p = 0.007$). However, in multivariate analysis *OCL* gene expression levels remained significantly associated only with BMD.

The gene expression of inflammatory markers (*IL-1β, IL-6, IL-17A,* and *TNF*) in the plaques was similar in patients with normal and low BMD and was not related to other patient's characteristics (demographic, BMI, lifestyle habits, co-morbidities or medication).

Histological Evaluation

Plaque CD3 and CD68 immunohistochemistry scores were higher in patients with normal BMD than in patients with low BMD (**Table 3**). Additionally, CD3 (0.82 ± 0.97 vs. 0.34 ± 0.53, $p = 0.006$) and CD68 (0.89 ± 0.89 vs. 0.45 ± 0.63, $p = 0.005$) immunohistochemistry scores were higher in male patients. In the independent analysis, CD3 ($\rho = -0.221$, $p = 0.009$) and CD68 ($\rho = -0.181$, $p = 0.033$) immunohistochemistry scores were inversely related to patients age.

In a linear regression model adjusted for sex and age, CD68 scores were significantly associated with BMD ($\beta = -0.203$, $p = 0.016$). In a model with further adjustment to BMI, only gender and BMI maintained a significant association with CD68 scores.

No significant correlations were found between any of the histological studies performed (alizarin red S, CD3, CD68, or adiponectin) and the results of the ELISAs or gene expression quantifications.

Serum Cytokines and Bone Markers Analysis

Regarding serum levels analyzed, we did not find differences between patients with normal or low BMD (**Table 4**).

No significant associations were found between gene expression in the plaque and serum levels of bone remodeling markers or inflammatory markers.

TABLE 2 | Gene expression levels in the atheroma plaques of atherosclerosis patients with normal and low BMD.

Gene	Normal BMD (*N* = 69)	Low BMD (*N* = 64)	*p*-value
IL-1β	1.86 ± 1.57	1.59 ± 1.18	0.617
IL-6	0.65 ± 0.35	0.60 ± 0.31	0.527
IL-17A	1.62 ± 1.56	1.93 ± 1.95	0.360
TNF	137.2 ± 172.2	112.2 ± 175.6	0.262
RANKL	1.46 ± 0.95	1.23 ± 0.91	0.137
OPG	2.66 ± 1.99	2.29 ± 1.82	0.288
RANKL/OPG	0.46 ± 0.26	0.44 ± 0.28	0.767
COL1A1	0.89 ± 0.54	0.72 ± 0.48	0.065
CTSK	0.81 ± 0.49	0.77 ± 0.50	0.477
OCL	1.27 ± 0.84	0.84 ± 0.54	0.009
TRAP	6.93 ± 10.51	5.69 ± 7.54	0.442
CBFA1	0.98 ± 0.63	0.71 ± 0.47	0.015
DKK1	3.63 ± 2.67	3.04 ± 2.66	0.116
SOST	2.89 ± 1.89	2.89 ± 2.17	0.647
AdipoQ	34.4 ± 47.1	27.2 ± 35.6	0.671
AdipoR1	1.91 ± 1.16	1.62 ± 1.01	0.223

Variables are presented as means ± SD. BMD, bone mineral density; IL, Interleukin; TNF, Tumor Necrosis Factor; RANKL, Receptor Activator of NF-kB Ligand; OPG, Osteoprotegerin; COL1A1, Collagen type I; CTSK, Cathepsin K; OCL, Osteocalcin; TRAP, Acid phosphatase tartrate resistant; CBFA1, Core-Binding Factor Alpha I; DKK1, Dickkopf-related protein 1; SOST, Sclerostin; AdipoQ, Adiponectin; AdipoR1, Adiponectin receptor 1.

TABLE 3 | Histological scores of atherosclerosis patients with normal and low BMD.

Molecule	Normal BMD (*N* = 69)	Low BMD (*N* = 64)	*p*-value
Alizarin Red S	1.94 ± 0.95	1.92 ± 1.04	0.991
$CD3^+$	0.78 ± 0.91	0.47 ± 0.76	0.020
$CD68^+$	0.91 ± 0.84	0.55 ± 0.79	0.004
Adiponectin	1.16 ± 0.90	1.13 ± 0.90	0.780

Variables are presented as means ± SD. BMD, bone mineral density; CD, Cluster of differentiation.

TABLE 4 | Serum levels of atherosclerosis patients with normal and low BMD.

Protein	Normal BMD (*N* = 69)	Low BMD (*N* = 64)	*p*-value
IL-1β (fg/mL)	14.8 ± 49.1	12.9 ± 35.8	0.780
IL-6 (fg/mL)	2139.5 ± 1730.7	1982.3 ± 1953.7	0.235
IL-17A (fg/mL)	17.9 ± 50.2	15.3 ± 30.8	0.380
TNF (fg/mL)	11.8 ± 47.7	8.02 ± 37.2	0.352
RANKL (pmol/L)	0.022 ± 0.019	0.019 ± 0.015	0.389
OPG (pmol/L)	8.11 ± 5.45	7.97 ± 5.44	0.885
RANKL/OPG	0.0033 ± 0.0040	0.0034 ± 0.0040	0.990
CTX (ng/mL)	30.8 ± 6.9	30.8 ± 8.3	0.865
P1NP (ng/mL)	130.5 ± 75.2	135.6 ± 75.4	0.678
CTX/P1NP	0.21 ± 0.09	0.23 ± 0.08	0.288
Adiponectin (ng/mL)	114909.4 ± 93591.1	130745.4 ± 99158.3	0.403

Variables are presented as means ± SD. BMD, bone mineral density; IL, Interleukin; TNF, Tumor Necrosis Factor; RANKL, Receptor Activator of NF-kB Ligand; OPG, Osteoprotegerin; CTX, C-terminal telopeptide of type 1 collagen; P1NP, procollagen type 1 N propeptide.

DISCUSSION

With our work we aimed to understand how atherosclerotic lesions are related with disturbances in bone and specifically if there is a role for inflammation in this relationship.

Regarding several genes related to inflammation and bone remodeling we found that, at the gene expression level, there was a positive correlation between bones and vessels, specifically with the aorta, suggesting a link between these two systems. In addition, the observed correlation did not extend to adipose tissue, which supports that this is not a widespread finding.

No differences on the gene expression pattern with age or sex were found in deceased donors' samples, suggesting that the relationships described above do not vary significantly throughout life, neither between women and men, pointing that, in pathologic processes, bone and vessel disturbances are possibly linked due to an intrinsic connection between the tissues involved. To the best of our knowledge there are no previous reports of any relation between the expression of these genes, either in the vessels or in bones, with age or gender.

We could not find an association between gene expression and the circulating levels of the same proteins. As reviewed by Vogel et al. (22) 60% of the variation in protein concentration that cannot be explained by measuring mRNAs alone is at least partially due to translation and protein degradation. Other works have also observed the lack of association between OPG, PTH, and RANKL gene expression in bone and serum levels (23, 24).

In the second part of our work we have used atherosclerotic plaques from patients with advanced atherosclerosis that were submitted to endarterectomy surgery and where bone mineral density was evaluated by DXA. We found that more than 45% of the enrolled patients had low BMD, which is in accordance with the age range of this population and with previous studies where bones and vessels were both evaluated by imaging methods (25, 26).

We found that the expression of bone formation genes (*CBFA1* and *OCL*) on atherosclerotic plaques was lower in those patients who have decreased BMD. Yet, our results do not suggest lower risk of plaque calcification, as we have not found any differences in the calcium content between patients with normal or low BMD.

The presence of these proteins in plaques is in accordance with previous studies showing that calcified plaques composition share some features of bone structure (27), but these results seem contradictory to other studies (28–30) that reported an association between low BMD and vascular calcification. These differences might be related to different methodologies used to quantify vascular calcification (microscopic vs. macroscopic) and also to different stages of plaque development, as calcification can begin at any point of plaque formation and progression (27).

When analyzing the results of immunohistochemical staining performed on the atherosclerotic plaques, our results

support that atheroma plaques from men have higher levels of inflammation, as previously proposed (31, 32). BMI also influences plaques inflammatory score, highlighting the link between obesity and atherosclerosis progression (33). Accordingly, in deceased donors, serum levels of pro-inflammatory cytokine IL-1β were also higher in men.

Patients with low BMD had plaques with lower inflammatory profile. Low BMD has been associated with echogenic (more calcified) plaques (34, 35) and calcification and inflammation are thought to be active at different stages of disease progression (36), with inflammation at early phases and calcification predominantly later (32, 37), which might explain our findings.

Despite the limitations related to the cross-sectional design, correlative nature of the work with lack of functional data, and the lack of clinical information of deceased donors, our study has some strengths. Samples from deceased donors allowed us to directly evaluate gene expression on bone and aortas from the same subjects and in a wide range of ages, going behind indirect diagnostic methods used *in vivo*. Regarding atherosclerosis patients, we did not have access to bone tissue of these patients to perform the study directly on the affected tissue. However, contrary to most studies, we had access to carotid atheroma plaques, and not only to ultrasound evaluation of atherosclerosis, and all patients had BMD evaluated by DXA.

CONCLUSIONS

We have described a positive correlation between bone and aorta concerning the expression of inflammation and bone remodeling genes, independent of age and sex. Bone-remodeling genes are not only expressed on atherosclerotic plaques, but also on vessel walls, regardless of the presence of plaques.

Additionally, the atheroma plaques of patients with low BMD present lower levels of bone formation markers (*CBFA1* and *OCL*) and a lower score CD68 immunostaining than those with normal BMD.

Our results suggest that the relationship between the changes observed in vessels and bones in the context of atherosclerotic disease and OP may rely on the intrinsic connection between the tissues involved, independently of the disease stage.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available upon request. This data can be found here: https://zenodo.org/record/1403777#.YD0JGi0qK9Y.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Centro Académico de Medicina de Lisboa. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DC-F, MS, JF, and HC contributed to conception and design of the study. SB, AC, PS, AF, FC-F, CG, RCr, MF, MI, and LP collected clinical data and biologic samples. NL, RCa, and DC-F performed laboratory work. DC-F organized the database and performed the statistical analysis. DC-F and SB wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.672496/full#supplementary-material

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Factors Associated With Clinical and Radiographic Severity in People With Osteoarthritis: A Cross-Sectional Population-Based Study

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Background: Hip/knee osteoarthritis (HKOA) is a leading cause of disability and imposes a major socioeconomic burden. The aim of this study is to estimate the prevalence of HKOA in Portugal, characterised the clinical severity of HKOA in the population, and identified sociodemographic, lifestyle, and clinical factors associated with higher clinical and radiographic severity.

Methods: Participants with a diagnosis of HKOA from the EpiReumaPt study (2011–2013) were included (n = 1,087). Hip/knee osteoarthritis diagnosis was made through a structured evaluation by rheumatologists according to American College of Rheumatology criteria. Clinical severity was classified based on Hip Disability and Osteoarthritis Outcome Scale (HOOS) and Knee Injury and Osteoarthritis Outcome Scale (KOOS) score tertiles. Radiographic severity was classified based on the Kellgren-Lawrence grades as mild, moderate, or severe. Sociodemographic lifestyle and clinical variables, including the presence of anxiety and depression symptoms, were analysed. Factors associated with higher clinical and radiographic severity were identified using ordinal logistic regression models.

Results: Hip/knee osteoarthritis diagnosis was present in 14.1% of the Portuguese population [12.4% with knee osteoarthritis (OA) and 2.9% with hip OA]. Clinical severity was similar between people with hip (HOOS = 55.79 ± 20.88) and knee (KOOS = 55.33 ± 20.641) OA. People in the high HOOS/KOOS tertile tended to be older (64.39 ± 0.70 years), female (75.2%), overweight (39.0%) or obese (45.9%), and had multimorbidity (86.1%). Factors significantly associated with higher clinical severity tertile were age [55–64 years: odds ratio (OR) = 3.18; 65–74 years: OR = 3.25; ≥75 years: OR = 4.24], female sex (OR = 1.60), multimorbidity (OR = 1.75), being overweight (OR = 2.01) or obese (OR = 2.82), and having anxiety symptoms (OR = 1.83). Years of education was inversely associated with higher clinical severity. Factors significantly associated with higher radiographic severity were age (65–74 years: OR = 3.59; ≥75 years: OR = 3.05)

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and being in the high HOOS/KOOS tertile (OR = 4.91). Being a female and live in Lisbon or in the Centre region were inversely associated with the higher radiographic severity.

Conclusion: Hip/knee osteoarthritis is present in ∼1.1 million of Portuguese people. Age, educational level, and obesity are independently associated with HKOA clinical severity, whereas age, sex, geographic location, and clinical severity are independently associated with radiographic severity.

Keywords: prevalence, hip osteoarthritis, knee osteoarthritis, clinical severity, radiographic severity

INTRODUCTION

Osteoarthritis (OA), which is the most common articular disease, is a leading cause of chronic disability and a major public health problem (1). Globally, more than 300 million people have hip and/or knee osteoarthritis (HKOA), which is responsible for 9.6 million years lived with disability, and its incidence and prevalence continue to rise (2).

Worldwide, the direct annual mean cost per patient with HKOA is estimated to be 6.7 k€, reaching 10.8 k€ if patients undergo total joint replacement (3). Moreover, the annual indirect cost per patient may surpass the direct cost and is estimated to range from 0.2 k€ to 12.3 k€ (3). Portugal has the highest growth rate of total joint replacement among Organisation for Economic Co-operation and Development countries, with a 20% increase in incidence between 2005 and 2011 (4). Additionally, indirect costs due to premature exit from work represent 0.4% of the Portuguese gross domestic product (5).

Overweight and high body mass index (BMI), physical inactivity, previous joint injuries, and ageing are the main risk factors for the onset and severity of HKOA (6). Data from the EpiReumaPt study reveals that, in the Portuguese population, female sex, higher age, multimorbidity, low levels of physical activity, and physical disability are associated with the diagnosis of OA among adults with ≥50 years old (7). Similar to other middle- and high-income countries (8), Portugal is an ageing country, in which 80% of older adults are overweight and 75% of the adult population is physically inactive (9). Therefore, the prevalence of HKOA and its associated socioeconomic burden is expected to increase exponentially over the next decades (10).

People with HKOA often experience chronic pain, fatigue, sleep problems, disability, impaired quality of life, with a consequent negative impact on mental health, which progressively limits their participation in social, leisure, and occupational activities (1, 11). People with HKOA have heterogeneous presentations and disease severity depending on factors such as structural joint damage, the presence of non-communicable diseases (e.g., diabetes, obesity), risk factor exposure, age of symptom onset, and psychosocial factors (12). Therefore, radiographic and clinical severity are important predictors of individual burden and healthcare service utilisation (13). Although there is no consensus on the gold standard for evaluating HKOA severity, the general recommendation is to use a combination of radiographic and clinical severity measures (14).

Internationally, much attention has been paid to suboptimal outcomes of HKOA at the patient and system levels, as epidemiological data raise concerns about the severity of HKOA and the escalating burden of this disease (15). However, in Portugal, there are little available epidemiological data on the severity of HKOA and its associated factors. Thus, we aimed to estimate the prevalence of HKOA in Portugal, characterise the clinical severity of HKOA in the population, and identify sociodemographic, clinical, and lifestyle factors associated with clinical and radiographic severity. This information is crucial for obtaining a better understanding of the individual burden of HKOA, estimating future increases in health resource demands, and identifying needs for implementing prevention and management strategies for people with HKOA.

MATERIALS AND METHODS

Data Source

This study was developed under the scope of EpireumaPt, a national cross-sectional population-based study that aims to comprehensively assess the burden of rheumatic and musculoskeletal diseases (RMDs) in Portugal. EpiReumaPt includes a representative sample of the Portuguese population that was assessed to identify and characterise the population with RMD in Portugal (16). The study included non-institutionalised adults (≥18 years old) who lived in private households in Portugal Mainland and Islands (Madeira and Azores). Recruitment was conducted between September 2011 and December 2013 through a process of multistage random sampling of private households in Portugal stratified according to administrative territorial units (NUTS II: Norte, Centro, Lisboa and Vale do Tejo, Alentejo, Algarve, Azores, and Madeira) and the size of the population within each locality. In total, 28,502 households were contacted, 8,041 individuals refused to participate, and 10,661 individuals completed the interviews (**Figure 1**).

The EpireumaPt methodology consisted of a three-stage approach (**Figures 2**) (16). In the first stage, participants completed a face-to-face interview to collect sociodemographic and health-related information and to screen for RMDs. A person was considered to have a positive screening if they mentioned a previously known RMD, if any algorithm in the screening questionnaire was positive, or if they reported muscle, vertebral, or peripheral joint pain in the previous 4 weeks. Interviews were conducted by a team of non-medical healthcare professionals

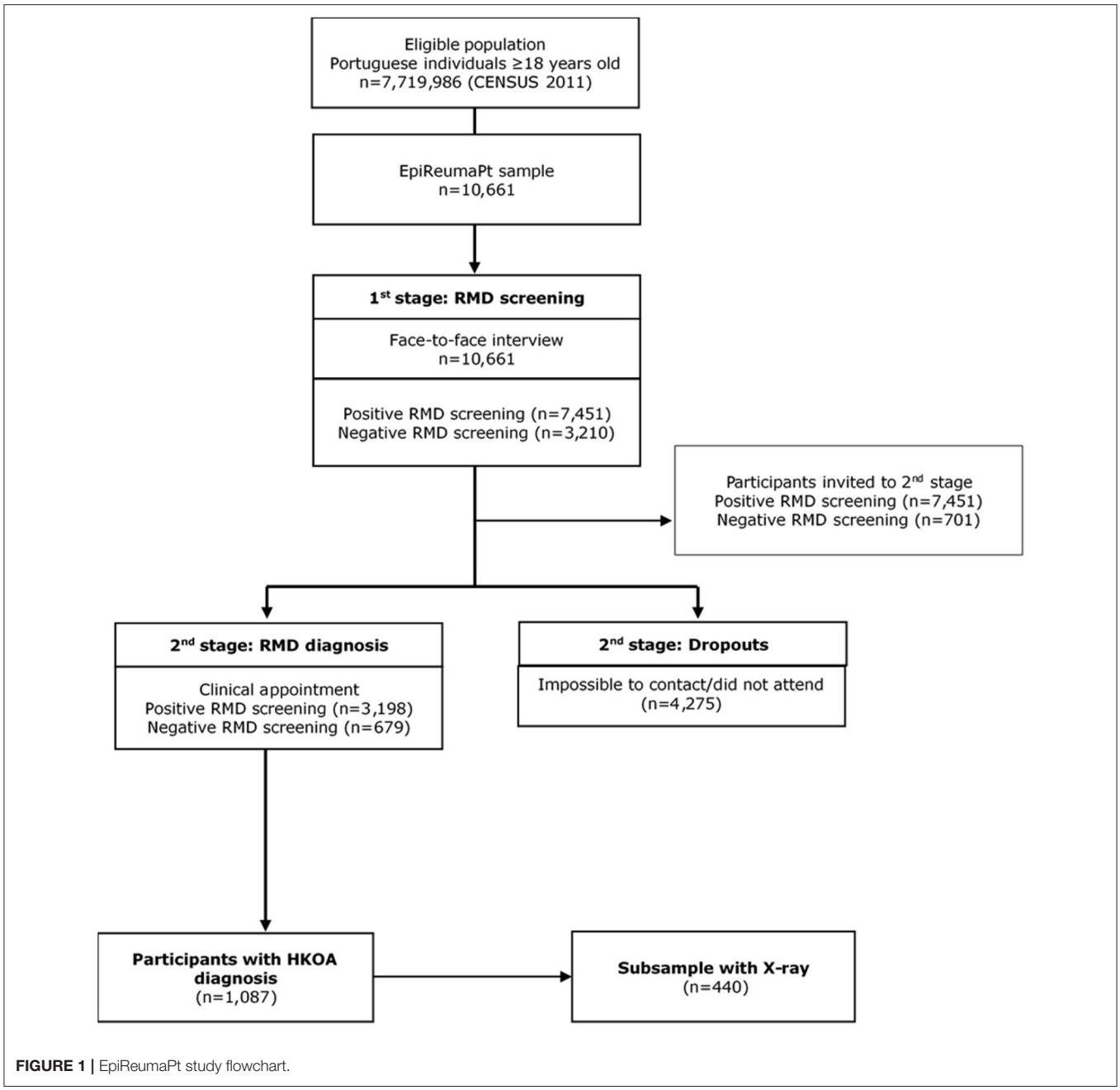

FIGURE 1 | EpiReumaPt study flowchart.

who were trained for this purpose, and data were collected using a computer-assisted personal interview system.

Participants who screened positive for at least one RMD ($n = 7{,}451$) and ∼20% ($n = 701$) of participants with negative screening for RMDs were invited for the second stage, which consisted of a clinical appointment with a rheumatologist. Of these participants, 4,275 did not attend the clinical appointment. Therefore, at the end of stage two, there were 3,877 clinical observations; 3,198 participants received a confirmation of RMD, and 679 did not have an RMD. Clinical assessments were performed at the primary care centre within the participant's neighbourhood by a multidisciplinary team consisting of a rheumatologist, X-ray technician, and nurse. Clinical appointments consisted of a structured evaluation, laboratory, and imaging exams, if needed, to establish the diagnosis and evaluate disease-related information. Simple X-rays were performed for 122 hips and 479 knees, among other joints, according to participants' musculoskeletal complaints. The rheumatologists involved were blind to prior health-related data.

In the third stage, three rheumatologists reviewed all data and validated the diagnoses. Diagnostic agreement among the three rheumatologists was 98.3% with a Cohen's K coefficient of 0.87 [95% confidence interval (CI): 0.83, 0.91] (16). When data were

insufficient to fulfil international classification criteria for each RMD, an additional meeting with the rheumatologists took place in order to reach agreement on the final diagnosis. When doubts persisted, the opinion of the rheumatologist who performed the clinical assessment in the second stage prevailed.

As the EpiReumaPt population is similar to the Portuguese population (CENSUS 2011), weights were calculated and calibrated according to age strata, sex, size of locality, and NUTS II in the first stage of the study to reproduce the known population totals for the crossing margins of these four variables. In the second stage, weights were recalibrated taking into account the inclusion probabilities considering the results of screening, and adjusted for non-response, as described in detail elsewhere (16).

Study Population

This study included participants in the EpiReumaPt study with a validated diagnosis of HKOA according to American College of Rheumatology diagnosis criteria (17, 18).

Outcomes

The outcomes of this study were measures of HKOA clinical and radiographic severity, which were assessed in the second stage of EpireumaPt, during clinical appointments. Clinical severity was evaluated with Portuguese versions of the Knee Injury and Osteoarthritis Outcome Scale (KOOS) (19) and Hip Disability and Osteoarthritis Outcome Scale (HOOS) (20). These self-reported outcome measures evaluate short-term and long-term consequences of HKOA in five dimensions: pain, symptoms, activities of daily living, sports and leisure, and quality of life. Scores for each dimension are transformed into a 0–100 scale, with 0 representing extreme hip/knee problems and 100 representing no hip/knee problems (19, 20). A final composite score (HOOS/KOOS) was calculated with the mean score of each dimension as previously recommended (21). Relationships between the core OA domains of pain, function, and quality of life are complex, fluctuate over time, and are intimately related to each other. Therefore, a composite score is considered optimal for capturing the multidimensional features of OA (22). We computed the tertile of the sample score distribution to categorise participants into low (65.00–100), middle (45.2–64.80), and high (0.00–45.00) tertiles of clinical severity, because there are no validated cut-offs for this measurement tool (23).

For radiographic severity, the Kellgren-Lawrence (K-L) system was used to classify joint structural deterioration using antero-posterior X-rays into four severity grades: grade 0 (normal), grade 1 [doubtful joint space narrowing (JSN) and possible osteophytic lipping], grade 2 (definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph), grade 3 (multiple osteophytes, definite JSN, sclerosis, possible bony deformity), and grade 4 (large osteophytes, marked JSN, severe sclerosis, and definite bony deformity) (24). We considered the radiographic severity of HKOA as mild if K-L $\leq$ 2, moderate if K-L = 3, and severe if K-L = 4 (25).

For both outcome measures, if more than one joint was affected, the joint with the worse score/classification was considered.

Sociodemographic, Clinical, and Lifestyle Factors

Sociodemographic, clinical, and lifestyle variables were collected during the first and second phase of EpiReumaPt.

Sociodemographic and Anthropometric Factors

Sociodemographic variables were age, sex, geographic location according to NUTS II territorial units, marital status, and education level. Madeira and Azores were merged in the analysis as the Islands region. Marital status was dichotomized as "with partner" (married or lived in consensual union) or "no partner" (single, widowed, or divorced). Education level was categorised according to years of education completed: <4 years (less than primary education), 4–9 years (primary or secondary education), or ≥10 years (secondary or superior education). Body mass index was categorised as underweight (≤18.49 kg/m^2), healthy weight (≥18.5 and ≤24.99 kg/m^2), overweight (≥25 and ≤29.99 kg/m^2), or obese (≥30 kg/m^2).

Clinical and Lifestyle Factors

Lifestyle variables included alcohol intake ("never or occasionally" or "daily" intake), smoking ("never" or "occasionally or daily"), and regular exercise/sports ("yes" or "no").

The number of chronic non-communicable diseases was calculated as the numeric count of the following self-reported conditions: high blood pressure, high cholesterol, cardiac disease, diabetes mellitus, chronic lung disease, problems in the digestive tract, renal colic, neurological disease, allergies, mental or psychiatric illness, cancer, thyroid or parathyroid problems, hypogonadism, and hyperuricemia. Multimorbidity was defined as having ≥2 chronic non-communicable diseases (26).

The presence of anxiety and depression symptoms was evaluated using depression (HADS-D) and anxiety (HADS-A) subscales of the Hospital Anxiety and Depression Scale. Both subscales have a range from 0 to 21, with higher values representing more symptoms. Final HADS-A and HADS-D scores were categorised with validated cut-offs as "anxiety symptoms" (HADS-A ≥11) or "without anxiety symptoms" (HADS-A <11) and as "depression symptoms" (HADS-D ≥11) or "without depression symptoms" (HADS-D <11) (27).

Data Analysis

Prevalence estimates were computed as weighted proportions for hip OA, knee OA, and HKOA. The logit transformation method was used to calculate 95% CIs.

Using descriptive statistics, participants with HKOA in each HOOS/KOOS tertile were characterised based on their sociodemographic, clinical, and lifestyle features. Mean scores on HOOS/KOOS subscales (symptoms, pain, activities of daily living, sports and leisure, and quality of life) for people with HKOA were plotted by age, sex, and radiographic severity (**Supplementary Material**, **Supplementary Figure 1**). The independency between HOOS/KOOS tertile and K-L classification was analysed using chi-square independency tests with a significance level based on adjusted F ($p < 0.005$).

Differences between HOOS/KOOS tertiles were analysed using *t*-tests for continuous variables and chi-square independency tests ($p < 0.05$) for categorical variables. As only a subsample of participants received X-rays ($n = 440$), we performed tests for independency of sociodemographic, clinical, and lifestyle characteristics between participants with and without X-rays to better interpret the final results (**Supplementary Material**, **Supplementary Table 1**).

To analyse variables associated with HOOS/KOOS tertile and K-L classification, two separate ordinal regression models were computed. During this stage of the analysis, age classes were merged as <55 years old, 55–64 years old, 65–74 years old, and ≥75 years old, and BMI categories were merged as normal or underweight (<25.00 kg/m^2), overweight (25–29.99 kg/m^2), and obese (≥30.00 kg/m^2), due to low frequencies in some categories.

For each ordinal regression model, during univariate analysis, a level of significance of 0.25 for relationships between each independent variable and the outcome was considered as the cut-off to enter in the multivariable ordinal regression model, to avoid early exclusion of potentially important variables (**Supplementary Material**, **Supplementary Table 2**) (28). Sociodemographic and anthropometric, clinical, and lifestyle variables were tested in the univariate analysis. Before running the models, the assumption of proportional odds and multicollinearity were validated, and independent variables with bivariate correlations above $r > 0.75$ were excluded (29).

The logit link function (29) was used because it improved the performed of both multivariable ordinal regression models according to their classification properties and McFadden Pseudo-R^2. This function computed proportional odds ratios (ORs) with 95% CIs. We used a stepwise procedure to construct the final models. Thus, in the first step, all socio-demographic and anthropometric, clinical and lifestyle variables that reached a significance level $p < 0.25$ in the univariate analysis were included. In the following steps, the variables less associated with the outcome were removed one by one, until we reached the final models, where only significant variables remained. The ordinal regression models were adjusted for sex, age, presence of multimorbidity, and BMI, which are known factors associated with HKOA severity, with potential counfounding effect. Therefore, we forced the entry of these variables in all steps. As participants with missing data were automatically excluded from this analysis, K-L classification was not included in the HOOS/KOOS model. Model fit was evaluated using McFadden Pseudo-R^2. All analyses were weighted and performed with SPPS Complex Samples 26 for MacOS (IBM Corp., Armonk, NY, USA).

RESULTS

The weighted prevalence estimate of HKOA in Portugal was 14.1% (95% CI: 12.6, 15.7, weighted $n = 1{,}138{,}264$), with the knee being the most affected joint [knee: 12.4% (95% CI: 11.1, 13.9, weighted $n = 1\ 002\ 192$), hip: 2.9% (95% CI: 2.3, 3.7, weighted $n = 238\ 038$)] (**Table 1**). The prevalence of HKOA increased across each age class, being present in 40.9% (95% CI: 34.3, 47.8) of people ≥75 years old, and was more prevalent in women (17.5%, 95% CI: 15.3, 19.9). Clinical severity, according to HOOS/KOOS ($n = 996$), was similar between participants with hip OA and knee OA (HOOS = 55.79 ± 20.88, KOOS = 55.33 ± 20.641).

TABLE 1 | Estimated prevalence of HKOA by sex, age, and severity.

	Hip and/or Knee OA *n* = 1,087	Knee OA *n* = 981	Hip OA *n* = 199
Total prevalence % (95% CI)	14.1 (12.6–15.7)	12.4 (11.1–13.9)	2.9 (2.3–3.7)
Total prevalence weighted counts (*n*)	1,138,264	1,002,192	238,038
Prevalence by sex % (95% CI)			
Male	10.4 (8.5–12.7)	8.5 (7.0–10.4)	2.9 (1.9–4.3)
Female	17.5 (15.3–19.9)	16.0 (13.9–18.2)	3.0 (2.4–3.8)
Prevalence by age % (95% CI)			
<45 years old	1.8 (1.1–2.8)	1.5 (0.4–0.9)	0.4 (0.2–0.9)
45–54 years old	14.5 (11.3–18.6)	12.0 (9.3–15.3)	3.2 (1.5–6.5)
55–64 years old	24.2 (20.0–28.9)	21.5 (17.9–25.7)	4.8 (2.6–8.6)
65–74 years old	35.5 (30.1–41.4)	31.5 (26.5–36.9)	7.4 (5.7–9.6)
≥75 years old	40.9 (34.3–47.8)	37.1 (30.8–43.8)	8.6 (11.8–23.2)
Clinical severity			
HOOS/KOOS score, mean ± SD	59.6 ± 21.80	55.33 ± 20.641	55.79 ± 20.88
Radiographic severity *n* (%)			
Mild (K-L ≤ 2)	197 (48.3)	177 (48.7)	36 (62.1)
Moderate (K-L = 3)	153 (34.0)	140 (32.1)	14 (35.8)
Severe (K-L = 4)	90 (17.8)	87 (19.2)	3 (3.2)

All percentages and means ± standard deviations (SDs) are weighted.
Radiographic severity non-weighted sub-sample (n = 440): mild n = 197, moderate n = 153; severe n = 90.

Characterisation of Population by Clinical Severity

The mean age of the HKOA population was 64.39 ± 12.90 years old and increased from the lowest (57.82 ± 1.67 years old) to the high (64.39 ± 0.70) HOOS/KOOS tertile (**Table 2**). The largest proportion of the population with HKOA lived in the north of Portugal ($n = 271$, 35.8%), but clinical severity did not differ across Portugal regions. There was an unequal distribution of education levels across HOOS/KOOS tertiles; the high tertile contained the largest proportion of people with <4 years of education ($n = 138$, 38.1%) and the smallest proportion of people with ≥10 years of education ($n = 18$, 6.1%). More than 80% of people with HKOA were overweight ($n = 387$, 43.6%) or obese ($n = 369$, 35.8%) and were mostly in the middle and high HOOS/KOOS tertiles.

Regarding lifestyle variables, 10.7% ($n = 71$) of Portuguese adults with HKOA smoked, and 27.8% ($n = 211$) drank alcohol

TABLE 2 | Sociodemographic and anthropometric characteristics of participants with HKOA by clinical severity.

	Total	HOOS/KOOS low tertile	HOOS/KOOS middle tertile	HOOS/KOOS high tertile	*p*-Value[a]
Sample size	*n* = 996	*n* = 281	*n* = 361	*n* = 354	
Age (mean ± SD)	64.39 ± 12.90	57.82 ± 1.67	66.24 ± 0.77	64.39 ± 0.70	<0.001
<45 years old, *n* (%)	37 (6.2)	32 (93.8)	3 (3.2)	2 (3.0)	<0.001
45–54 years old, *n* (%)	129 (15.6)	59 (48.1)	47 (34.3)	23 (17.6)	
55–64 years old, *n* (%)	268 (23.4)	74 (32.5)	110 (34.9)	84 (22.9)	
65–74 years old, *n* (%)	340 (31.4)	79 (26.3)	125 (35.4)	136 (38.3)	
≥75 years old, *n* (%)	222 (23.3)	37 (19.6)	76 (34.1)	109 (46.2)	
Female, *n* (%)	720 (65.8)	180 (51.3)	265 (71.1)	275 (75.2)	<0.001
Geographic location, *n* (%)					0.240
North	271 (35.8)	82 (38.6)	88 (32.2)	101 (36.4)	
Centre	243 (27.7)	58 (25.5)	86 (27.7)	99 (30)	
Lisbon	163 (23.5)	56 (25.9)	58 (24.8)	49 (19.9)	
Alentejo	67 (6.5)	9 (3.0)	32 (9.1)	26 (7.6)	
Algarve	21 (1.8)	8 (2.2)	4 (1.0)	9 (2.2)	
Islands	231 (4.7)	68 (4.8)	93 (5.3)	70 (3.9)	
Marital status (partner), *n* (%)	639 (64.4)	191 (61.8)	228 (64.9)	220 (66.4)	0.664
Years of education, *n* (%)					<0.001
<4 years	246 (22.6)	35 (9.7)	73 (20.1)	138 (38.1)	
4–9 years	631 (62.9)	187 (67.9)	247 (65.0)	197 (55.8)	
≥10 years	117 (14.4)	58 (22.3)	41 (14.8)	18 (6.1)	
BMI, *n* (%)					0.003
Underweight	3 (0.2)	1 (0.4)	1 (0.1)	1 (0.3)	
Normal weight	162 (20.5)	66 (28.9)	58 (16.4)	38 (15.1)	
Overweight	387 (43.6)	134 (43.8)	139 (47.6)	114 (39.0)	
Obese	369 (35.8)	72 (26.9)	143 (35.9)	154 (45.9)	

Categorical variables are presented as n (%); continuous variables are presented as mean ± SD. All percentages and means ± SDs are weighted.
[a]*p-value from independency tests: complex samples t-tests for continuous variables and Chi-square tests for categorical variables. Significance level is based on adjusted F.*

daily (**Table 3**). Few people with HKOA performed regular physical exercise ($n = 209$, 21.3%), particularly those in the high HOOS/KOOS tertile ($n = 53$, 14.4%). The overall proportion of people with HKOA who also had multimorbidity was 74.1% ($n = 756$), which was most pronounced in the high HOOS/KOOS tertile ($n = 305$, 86.1%). The proportions of people with anxiety ($n = 95$, 26.0%) and depression ($n = 85$, 23.6%) symptoms also increased in the high HOOS/KOOS tertile.

HOOS/KOOS tertiles were independent of K-L classification [$F_{(3.26;3542.35)} = 33.69$, p=0.002]. Across increasing HOOS/KOOS tertiles, the proportion of people with mild K-L classification decreased and the proportion of people with severe K-L classification increased (**Figure 2**). However, the highest clinical severity tertile was heterogeneous, consisting of 30.75% of people with mild, 39.94% of people with moderate, and 29.31% of people with severe K-L classification. Results regarding the characterisation of the population with hip and the population with knee OA are presented in **Supplementary Material**, **Supplementary Table 2**.

Factors Associated With Clinical Severity

In the final ordinal regression model for clinical severity, the following factors were significantly associated with a higher HOOS/KOOS tertile: being 55–64 years old (OR = 3.18; 95% CI 1.80, 5.62; $p < 0.001$), 65–74 years old (OR = 3.25; 95% CI: 1.87, 5.67; $p < 0.001$), or ≥75 years old (OR = 4.24; 95% CI: 2.26, 8.00; $p < 0.001$) compared with <55 years old; being female (OR = 1.60; 95% CI: 1.09, 2.35; $p = 0.017$); having multimorbidity (OR = 1.75; 95% CI: 1.13, 2.71; $p = 0.013$); being overweight (OR = 2.01; 95% CI 1.16, 3.48; $p = 0.013$) or obese (OR = 2.82; 95% CI: 1.62, 4.90; $p < 0.001$) compared with normal weight; and having anxiety symptoms (OR = 1.83; 95% CI: 1.20, 2.81; $p = 0.005$) (**Table 4**). On the other hand, having 4–9 years of education (OR = 0.50; 95% CI: 0.32, 0.77; $p = 0.002$) or ≥10 years of education (OR = 0.30; 95% CI: 0.17, 0.52; $p < 0.001$) were significantly and inversely associated with a higher HOOS/KOOS tertile.

Compared with the results of univariate analysis, multimorbidity (OR = 2.90; 95% CI: 1.90; 4.42; $p < 0.001$) and anxiety symptoms (OR = 1.96; 95% CI: 1.33, 2.80; $p = 0.001$), and the age stratas 55–64 (OR = 3.27; 95% CI: 1.81, 5.95; $p < 0.001$) and ≥75 years old (OR = 6.06; 95% CI: 4.33, 1.00; $p < 0.001$) were less strongly associated with clinical severity in the multivariable ordinal regression model (**Supplementary Material**, **Supplementary Table 3**). By contrast, the age strata 65–74 years old (OR = 2.31; 95% CI: 2.56, 7.29) and BMI (overweight:

TABLE 3 | Lifestyle and clinical characteristics of participants with HKOA by clinical severity.

	Total	HOOS/KOOS low tertile	HOOS/KOOS middle tertile	HOOS/KOOS high tertile	*p*-Value[a]
Sample size	*n* = 996	*n* = 281	*n* = 361	*n* = 354	
Lifestyle variables, *n* (%)					
Smoker	71 (10.7)	31 (17.3)	24 (9.0)	16 (5.6)	0.007
Alcohol intake (daily)	211 (27.8)	70 (33.9)	74 (26.1)	67 (23.3)	0.117
Regular exercise	209 (21.3)	81 (28.5)	75 (20.6)	53 (14.4)	0.007
Clinical variables, mean ± SD					
HOOS/KOOS	55.79 ± 20.88	79.50 ± 9.60	54.35 ± 5.91	33.21 ± 9.87	<0.001
(min–max)	(0.00–100)	(65.00–100)	(45.20–64.80)	(0.00–45.00)	
Multimorbidity (yes), *n* (%)	756 (74.1)	175 (60.0)	276 (76.4)	305 (86.1)	<0.001
Anxiety symptoms (HADS-A), mean ± SD	6.70 ± 4.21	6.0 ± 4.05	6.39 ± 4.18	7.72 ± 4.19	<0.001
HADS-A ≥ 11, *n* (%)	193 (18.5)	40 (12.9)	58 (16.6)	95 (26.0)	0.002
Depression symptoms (HADS-D), mean ± SD	6.04 ± 4.49	4.54 ± 4.07	5.97 ± 4.15	7.63 ± 4.68	<0.001
HADS-D ≥ 11, *n* (%)	159 (16.8)	25(11.5)	49 (15.1)	85 (23.6)	0.028

Categorical variables are presented as n (%); continuous variables are presented as mean ± SD. All percentages and means ± SDs are weighted.
[a]*p-value from independency tests: complex samples t-tests for continuous variables and Chi-square tests for categorical variables. Significance level is based on adjusted F.*

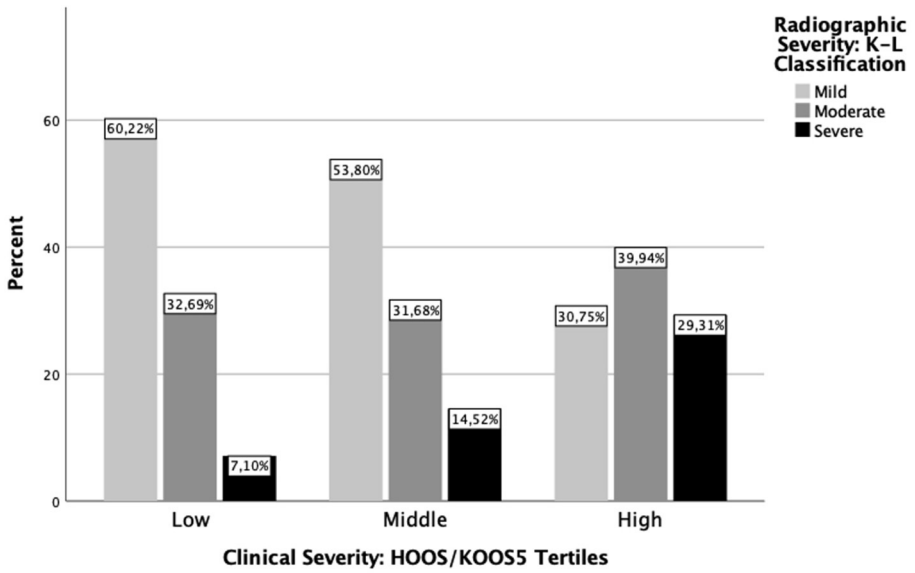

FIGURE 2 | Distribution of radiographic severity classification according to clinical severity tertile. The y-axis indicates the proportion of K-L classification grades within each HOOS/KOOS tertile, and the x-axis indicates HOOS/KOOS tertile. Bars represent K-L classification grade: mild ≥2, moderate = 3, or severe = 4.

OR = 1.87; 95% CI: 1.06, 3.30; $p = 0.003$; obese: OR = 2.72; 95% CI: 1.53, 4.85; $p = 0.001$) were more strongly associated with clinical severity in the multivariable ordinal regression model.

Factors Associated With Radiographic Severity

Regarding the subpopulation of participants who received an X-ray ($n = 440$), the final ordinal regression model for radiographic severity showed that a severe K-L classification was associated with being 65–74 years old (OR = 3.59; 95% CI: 1.43, 9.02; $p = 0.007$) or ≥75 years old (OR = 3.05; 95% CI: 1.13, 8.21; $p = 0.028$) compared with <55 years old and being in a high HOOS/KOOS tertile (OR = 4.91; 95% CI: 2.57, 9.40; $p < 0.001$) compared with a low HOOS/KOOS tertile (**Table 5**). By contrast, a less severe K-L classification was associated with being female (OR = 0.41; 95% CI: 0.24, 0.69; $p = 0.001$) and living in the Lisbon (OR = 0.23; 95% CI: 0.11, 0.48; $p < 0.001$) or Centre region (OR = 0.35; 95% CI: 0.20, 0.61; $p < 0.001$).

Compared with the results of univariate analysis, the following variables were more strongly associated with radiographic severity in the multivariable ordinal regression model: age (65–74 years old: OR = 2.60; 95% CI: 1.18, 5.71; $p = 0.018$; ≥75

TABLE 4 | Factors associated with clinical severity in the final multivariable ordinal regression model.

	OR (95% CI)	*p*-Value
Age		
<55 years old[a]	–	–
55–64 years old	3.18 (1.80, 5.62)	<0.001
65–74 years old	3.25 (1.87, 5.67)	<0.001
≥75 years old	4.24 (2.26, 8.00)	<0.001
Sex		
Male[a]	–	–
Female	1.60 (1.09, 2.35)	0.017
Number of non-communicable diseases		
No multimorbidity[a]	–	–
Multimorbidity	1.75 (1.13, 2.71)	0.013
Education level		
<4 years[a]	–	–
4–9 years	0.50 (0.32, 0.77)	0.002
≥10 years	0.30 (0.17, 0.52)	<0.001
BMI (kg/m^2)		
Normal or underweight (<25 kg/m^2)[a]	–	–
Overweight (25–29.99 kg/m^2)	2.01 (1.16, 3.48)	0.013
Obese (≥30 kg/m^2)	2.82 (1.62, 4.90)	<0.001
Anxiety (HADS-A)		
No anxiety symptoms (HADS-A<11)[a]	–	–
Anxiety symptoms (HADS-A≥11)	1.83 (1.20, 2.81)	0.005

Total sample included in the analysis: n = 968. Test of parallel lines: Wald $F_{(10)} = 0.630$; $p = 0.789$ (assumption of proportional odds validated); McFadden Psuedo-$R^2 = 0.115$. This model correctly classified 51.4% of cases. All analysis were weighted.
[a]Reference classes.

TABLE 5 | Factors associated with radiographic severity in the final multivariable ordinal regression model.

	OR (95% CI)	*p*-Value
Age		
<55 years old[a]	–	–
55–64 years old	1.43 (0.54, 3.79)	0.470
65–74 years old	3.59 (1.43, 9.02)	0.007
≥75 years old	3.05 (1.13, 8.21)	0.028
Sex		
Male[a]	–	–
Female	0.41 (0.24, 0.69)	0.001
Chronic non-communicable diseases		
No multimorbidity		
Multimorbidity	0.71 (0.37, 1.36)	0.300
Geographic location		
North[a]	–	–
Centre	0.35 (0.20, 0.61)	<0.001
Lisbon	0.23 (0.11, 0.48)	<0.001
Alentejo	0.64 (0.30, 1.35)	0.237
Algarve	1.40 (0.27, 7.34)	0.689
Islands	0.80 (0.24, 2.65)	0.717
BMI (kg/m^2)		
Normal or underweight (<25 kg/m^2)[a]	–	–
Overweight (25–29.99 kg/m^2)	1.61 (0.75, 3.44)	0.222
Obese (≥30 kg/m^2)	1.67 (0.77, 3.58)	0.191
HOOS/KOOS		
Low tertile[a]	–	–
Middle tertile	1.69 (0.85, 3.40)	0.137
High tertile	4.91 (2.57, 9.40)	<0.001

Total sample included in the analysis: n = 376. Test of parallel lines: Wald $F_{(14)} = 0.789$, $p = 0.681$ (assumption of proportional odds validated); McFadden Pseudo-$R^2 = 0.132$. This model correctly classified 54.1% of cases. All analyses were weighted.
[a]Reference classes.

years old: OR = 2.71; 95% CI: 1.16, 6.35; $p = 0.022$), sex (female: OR = 0.65; 95% CI: 0.42, 1.00; $p = 0.052$), and high HOOS/KOOS tertile (OR = 3.68; 95% CI: 1.82, 7.43; $p < 0.001$) (**Supplementary Material**, **Supplementary Table 4**). By contrast, geographic location (Centre: OR = 0.49; 95% CI: 0.29, 0.83, $p = 0.008$; Lisbon: OR = 0.30; 95% CI: 0.16, 0.57; $p < 0.001$) was less strongly associated with radiographic severity in the multivariable ordinal regression model.

DISCUSSION

This study shows that 14.1% of people in Portugal have HKOA, mostly involving the knee, which corresponds to the ∼1.1 million people with this disease in Portugal, as previously reported (11). The Portuguese dataset used in Global Burden of Diseases report (GBD) to estimate global prevalence of OA was EpireumaPt, but no data were published on HKOA together in Portugal (11). The prevalence of OA, globally, ranges from 5.4 to 24.2% for the knee and 0.9 to 7.4% for the hip (30). According to the GBD report, Portugal, the United Kingdom, and Finland have the highest age-standardised prevalence of HKOA in Europe (4,000–4,400 per 1,000,000 individuals) (2).

Different from the present study, the cohorts in several previous studies were limited to radiographic-only diagnoses or older age classes (2, 31, 32), which may not encompass people in the early stages of the disease or with early-onset HKOA. For example, the GBD report only included people with HKOA confirmed radiologically with grades 2–4 K-L and, as such, likely underestimates the true prevalence (2, 32). Our data show that the prevalence of HKOA is higher in females and increases across each age class, being present in up to 40% of people who are ≥75 years old, consistent with previous reports (2, 31, 33, 34). Although sex differences in the incidence and prevalence of HKOA have been previously studied, they are not yet fully understood. However, early exposure to oestrogen (i.e., menarche at a younger age), parity, and menopause may provide hormonal and biomechanical explanations for the greater prevalence of OA in females (35). Furthermore, we found that risk factors for the onset and severity of OA are highly prevalent in the Portuguese population, particularly the lack of regular exercise and the presence of overweight or obesity, and are even higher than those previously reported for the overall Portuguese population (9), and compared with international HKOA cohorts (36).

Although the proportion of people with moderate and severe radiographic OA increased with greater clinical severity,

we found that people in the high clinical severity tertile had heterogeneous radiographic severity classifications. This finding is supported by previous studies showing that HKOA radiographic severity is correlated with clinical severity but in a non-linear fashion (14) and is an imprecise guide for predicting clinical severity (37).

Regarding radiographic severity, >80% of people in our study had mild or moderate severity, similar to other studies (38). The Chingford Women's study, a 15-year longitudinal cohort study, concluded that 41.5% of knees worsened by at least one K-L grade over this time span, with a 3.9% annual rate of disease worsening (39). Thus, if the prevalence of HKOA is increasing (10), a higher proportion of people with mild and moderate HKOA will progress to moderate or severe HKOA at a considerable rate. People with severe HKOA are 5.3 times more likely to have surgery (13), thus increasing the demand for healthcare resources and the socio-economic impact of this disease (3).

Regarding the factors associated with HKOA severity, age was associated with both clinical and radiographic severity. All older age strata were associated with a high clinical severity tertile compared with the <55 years old stratum, whereas only age strata above 65 years old were associated with high radiographic severity. These results are consistent with the observed impact of pain and functional impairment due to HKOA on social and work activities, which can lead to absenteeism, presentism, or premature work withdrawal and negatively affect the quality of life of adults at younger ages living with HKOA (5, 40). The association of clinical and radiographic OA severity with age is well-documented in the literature (2, 31, 33, 34) and is explained by both cumulative exposure to risk factors including chronic non-communicable diseases and by biological age-related structural joint changes (41). However, we found contradictory results pertaining to the association between sex and HKOA severity. Being female was simultaneously associated with higher clinical severity and milder radiographic severity. Previous literature also reports conflicting associations between sex and the clinical severity of OA (42). Data from the European SHARE cohort reveal that women have overall disadvantages in terms of activity limitations, pain, depression, and self-reported health status compared with men (43). On the other hand, a systematic review by Bastick et al. found strong evidence that sex is not associated with radiographic severity (44). However, male sex is often linked with physically demanding jobs (e.g., firefighting, construction, mining, carpentry) and contact sports, which increases the probability of previous trauma, injuries, and structural joint damage and represents a major joint-level risk factor for OA (22).

We also found that multimorbidity was associated with greater clinical severity. Non-communicable diseases are prevalent in the HKOA population and are associated with greater utilisation of healthcare services (45). A systematic review and meta-analysis by Calders et al. likewise found that a higher non-communicable disease count is associated with the worsening of pain and performance-based physical function. Specifically, the presence of cardiac disease, hypertension, and back pain are important predictors of the deterioration of physical functioning, and diabetes is associated with worse pain (46). In addition, being overweight or obese was associated with greater clinical severity but not radiographic severity in the present study. Similarly, previous research indicates that BMI is a dose-responsive risk factor for OA clinical severity (47). However, other studies report that BMI is associated with both clinical and radiographic severity (48) and that greater mechanical load (49) and systemic inflammation due to a high BMI may play a role in OA onset and clinical and radiographic severity (50).

In addition, we found that anxiety symptoms and socioeconomic factors, such as low education, were associated with clinical severity but not radiographic severity. As previously described, anxiety is associated with worse pain and physical function trajectories (51). Moreover, previous research indicates that education is an important social determinant for several chronic diseases, including OA, and is related to lifestyle factors, a lack of preventive measures, low access to healthcare services, and low literacy levels (42, 52). Psychosocial variables such as a low level of self-efficacy, catastrophising, and pain sensitisation are also associated with poorer clinical outcomes (41). Thus, the lived experience of people with HKOA and its multifactorial influences, such as psychosocial factors, may be distinct from its structural changes, suggesting that HKOA is best framed with a biopsychosocial approach (41).

This study also revealed geographic associations, with people living in the North region having higher radiographic severity than people living in the Lisbon and Centre regions. In the northern region of Portugal, industrial employment is higher than the national average, and agriculture, forestry, construction, and manufacturing industries are important sources of employment (53). A recent systematic review concludes that people in the agriculture, construction, and metal industries have a higher probability of developing knee OA (54). Additionally, due to current sociodemographic characteristics of this region and the projected prevalence of chronic diseases and long-term disability, the northern region of Portugal is expected have a higher proportion of individuals with at least one chronic disease and long-term disability in 2031, mainly due to lower education levels (55).

Limitations

This study has some limitations that should be considered. Its cross-sectional design does not allow the establishment of a temporal relationship between associated factors and HKOA severity, thus it is not possible to establish cause-and-effect relationships between modifiable variables, such as BMI, and HKOA symptoms and physical function. The estimation of prevalence using sample weights is not free from error, although it is considered that weights should be used in all statistics analysis when dealing with complex survey data (56).

Although hip and knee OA may impose similar burdens on the domains of one's life (2, 31), some studies show that people with hip OA have greater disease severity and an earlier requirement for joint replacement (13). However, we did not thoroughly investigate differences in factors associated with hip vs. knee OA.

As HOOS and KOOS scores do not have validated cut-off values, we categorised our sample by tertile distribution. Thus, it should be noted that the low, middle, and high HOOS/KOOS tertiles do not directly correspond to mild, moderate, and severe radiographic severity. Moreover, we did not use any imputation method, given the amount of missing X-ray data, for the overall sample. The subgroup of participants who received X-rays was older, presented higher clinical severity, and had a larger number of non-communicable diseases (**Supplementary Material**, **Supplementary Table 1**); thus, this should be considered when interpreting the results.

"Regular exercise" was self-reported by participants and did not consider the precise amount and intensity of weekly physical activity. Hence, our data may overestimate the proportion of people who performed exercise and misrepresent the association between exercise and disease severity. Moreover, as HKOA onset and severity is multifactorial (1), several important factors may have not been included in this analysis, namely other psychosocial factors and previous injuries.

Strengths and Implications

This is the first study with a representative sample of the Portuguese population with HKOA that characterised this population and analysed factors associated with disease severity. Unlike most epidemiologic research on HKOA, the radiographic and clinical severity analyses in this study allow a deeper understanding of people with HKOA from their lived experience of the disease as well as from a structural perspective. Moreover, our case definition of people with HKOA also included people with early-onset ($\geq$18 years old) and early stages of the disease (0–4 grade K-L), allowing more accurate results.

Given the risk factors for HKOA onset and greater severity present in this study's sample and also in the overall Portuguese population (9), our study raises concerns regarding the need for preventive measures and political strategies to improve lifestyle factors and specific interventions directed at people with HKOA. Furthermore, sociodemographic and health-related data from our sample of Portuguese individuals suggest that the socioeconomic and individual burden of this disease may increase over the next decades. Clearly, there is a gap between international recommendations for physical activity and weight management in OA and current care given the small proportion of people who exercise and the large proportion of people who are overweight or obese. Adherence to behavioural strategies and access to care have already been identified as barriers to the optimization of health management among people with HKOA. Nonetheless, health professionals' lack of awareness of OA as a serious disease and lack of knowledge of current recommendations should also be taken into account by health politicians and managers to promote a collective approach to preventing and treating this disease (57).

CONCLUSIONS

Hip and/or Knee OA is present in 14.1% of the Portuguese adults. Age, female sex, multimorbidity, lower education, higher BMI, and anxiety symptoms are associated with higher clinical severity of HKOA, whereas age, geographic location, male sex, and clinical severity are associated with higher radiographic severity of HKOA. Given the cross-sectional design of this study, these factors should be interpreted as an association with higher severity, and not a cause of higher severity. Known risk factors for OA severity, such as decreased physical activity, obesity, and multimorbidity, are highly present among the population of people with HKOA in Portugal. Our findings highlight the need for effective prevention and management strategies focused on identified risk factors, namely weight management and exercise programs and control of chronic non-communicable diseases.

DATA AVAILABILITY STATEMENT

The data underlying this article were provided by the EpiDoc Unit - CEDOC by permission. Data will be shared upon request to the corresponding author with the permission of EpiDoc Unit group leaders.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of NOVA Medical School and Portuguese Data Protection Authority (Comissão Nacional de Proteção de Dados). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DC and CS contributed to the drafting of the manuscript. DC, EC, CN, and AR contributed to the analysis and interpretation of the data and statistics. HC, JB, and AR contributed to the conception and design of the main project (EpiReumaPt), provision of study materials, obtaining funding for the main project, administrative/logistic support, and collection of data. All authors critically revised and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.773417/full#supplementary-material

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Structural Lesion Progression of the Sacroiliac Joint and Clinical Features in axSpA During TNFi Reduction: A Retrospective Cohort Study

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Objective: In the clinic, some patients with axial spondyloarthritis (axSpA) have to reduce tumor necrosis factor inhibitor (TNFi) for various reasons. However, there are few studies about how to balance the relapse and TNFi reduction. Here we retrospectively analyzed the structural progression of the sacroiliac joint (SIJ) and clinical features in axSpA during TNFi reduction.

Methods: A total of 108 patients with axSpA who followed up for 2 years and completed at least baseline, 12-month, and 24-month MRI scans of SIJ were divided into the tapering group ($n = 63$) and withdrawal group ($n = 45$) according to whether TNFi was stopped. We divided 2 years into five intervals, calculating the average dose quotient (DQ) for each of 540 intervals from 108 patients. By using generalized estimation equations with inverse probability of treatment weighting, we investigated the unbiased effects of average DQ on structural progression and treatment response.

Results: The disease activity (such as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), Ankylosing Spondylitis Disease Activity Score (ASDAS)-CRP, and ASDAS-ESR) and relapse rate were lower in the tapering group at 12 and 24 months ($p < 0.05$). Δerosion ($\beta = -0.0100$, $p = 0.00026$) and $\Delta$the Spondyloarthritis Research Consortium of Canada (SPARCC; $\beta = -0.0959$, $p < 0.0001$) were negatively correlated with average DQ. The average DQ 30 (74.8%, 80.0%) or 41.6 (76.5%, 83%) was best to discriminate the status of treatment response or the status of bone marrow edema, but considering operability, the average DQ 25 (78.0%, 63.3%) was also acceptable especially for patients with HLA-B27 negative and non-severe fat metaplasia.

Conclusion: Complete TNFi withdrawal was not recommended. Our study provided a referable strategy (tapering then maintained the average DQ over 30 or even 25) for patients who need TNFi reduction. Higher dose usage of TNFi was associated with a slower erosion progression of SIJ.

Keywords: axial spondyloarthritis, tumor necrosis factor inhibitor, tapering, sacroiliac joint, magnetic resonance imaging

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that mainly affects the spine and sacroiliac joints (SIJ) (1–3). And the inflammatory response and structural damage can cause serious impairment in physical flexibility, work efficiency, and life quality (4–6). According to the radiologic sacroiliitis manifestations, it can be divided into non-radiographic axial spondyloarthritis (nr-axSpA) and radiographic axSpA, and the latter mainly includes ankylosing spondylitis (AS) (7–9). Although divided into two categories, patients with nr-axSpA or AS are similar in symptom burdens, clinical features, comorbidity, and tumor necrosis factor inhibitor (TNFi) response.

A series of randomized controlled trials and large cohort studies demonstrated that patients with axSpA responded well to the full dose of the TNFi treatment and most of these studies focused on the radiographic progression of the spine in patients with AS (10–20). Although the radiographic improvement effect on the spine is controversial, results from a meta-analysis published in 2020 indicated that >4 years of TNFi usage was associated with delayed structural progression of the spine (21). However, in the real world, it is inescapable that some patients have to opt for dose reduction or withdrawal because of intolerance to full-standard TNFi, risk of potential infection, unaffordability to high expense, intolerability for long-term subcutaneous injection, or multiple reasons (22, 23), while complete withdrawal often leads to relapse (24, 25). Several studies have begun to use tapering strategy for patients with axSpA and have shown that tapering TNFi to the extent of 75% and 50% full dose has comparable efficacy in maintaining low disease activity (26–32). The European Alliance of Associations for Rheumatology (EULAR) recently recommended that tapering TNFi can be considered for patients who have achieved sustained remission (2). Although the tapering strategy is recommended, there are limited studies focused on the referable strategy of tapering. In addition, previous studies mainly focused on the radiographic changes of the spine, while the impact of tapering TNFi on structural lesion progression of the SIJ was rarely studied. In this study, we retrospectively observed the effect of the TNFi tapering strategy on disease activity and the structural progression of the SIJ.

METHODS

Study Population and Clinical Assessment

We retrospectively reviewed the medical data of 2,272 patients with axSpA who were admitted to Tongji Hospital from January 2017 to January 2021. The diagnosis was made according to the Assessment of Spondyloarthritis international Society (ASAS) classification criteria for axSpA or modified 1984 New York criteria for AS (7, 8). Among them, patients who had been treated with TNFi or other biological agents before the first visit or combined continuous treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) or disease-modifying antirheumatic drugs (DMARDs) were excluded. Finally, a total of 108 patients underwent TNFi reduction and then followed maintaining therapy or a complete withdrawal, finished follow-up visit for at least 2 years (every 3 months in the first year and every 6 months in the second year), and completed at least baseline, 12-, and 24-month MRI scans of SIJ. Disease activity, such as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Ankylosing Spondylitis Disease Activity Score (ASDAS), clinical and laboratory parameters, and MRI of SIJ were collected and analyzed. Of the 108 patients, 63 patients who adopted a strategy of gradual reduction (reduce TNFi dose every 3 months) after achieving clinical remission or low disease activity and followed maintaining therapy of TNFi were defined as the tapering group. The rest 45 patients who completely stopped TNFi therapy after achieving clinical remission or low disease activity were referred to as the withdrawal group. The primary aim of our study was to investigate the relationship between the TNFi dose reduction and the disease relapse rate or the structural lesion progression of the SIJ, to obtain a reasonable and acceptable treatment strategy for some patients with axSpA who had to reduce or even stop TNFi for various reasons.

This study was approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science & Technology (project identification code: 2020-S275). Clinical trial registration and ID number is ChiCTR2100043491. The retrieved data are de-identified and the requirement for informed consent was therefore waived.

Interval Calculation

Each patient enrolled in the study was divided into five intervals, namely, 0–3, 3–6, 6–9, 9–12, and 12–24 months, based on the characteristics of the data available. Of the 108 patients, there were 540 intervals. We defined the response and relapse status in therapeutic intervals based on ASDAS-CRP (**Supplementary Figure 1**). The response status included two conditions, one of which was that the patients remained in remission (ASDAS-CRP < 1.3) or low disease activity (ASDAS-CRP < 2.1) for the entire interval, and the other of which was that the ASDAS-CRP score of the patients was changed from a higher level (ASDAS-CRP ≥ 2.1) to a lower level within the interval. Relapse status also included two conditions, one of which was that ASDAS-CRP score of the patients was changed from a lower level to a higher level (ASDAS-CRP ≥ 2.1) within the interval, the other of which was that patients remained high disease activity (ASDAS-CRP ≥ 2.1) unchanged for the entire interval. All 108 patients completed at least baseline, 12-, and 24-month MRI scans of SIJ. However, not all patients had MRI data at 3, 6, and 18 months. For the missing values, we used the linear interpolation method to impute based on the measured values before and after each follow-up point. Then we calculated the average dose quotient (DQ) (30), changes in fat metaplasia, erosion, backfill and the Spondyloarthritis Research Consortium of Canada (SPARCC) score, the status of bone marrow edema (SPARCC score increased or not), and the status of treatment response (response or relapse) for each interval.

TNFi Reduction

In the tapering group, the TNFi dose was mainly characterized as followed: after the full dose of TNFi for 3–6 months to achieve disease remission or low disease activity, intervals of TNFi treatment were gradually prolonged, and each phase lasted for 3 months. In the first tapering phase, the dose was reduced to 66.7% or 50% of the full dose, in the second to 35% or 25%, and in the third to 16.7% or 12.5%. To quantify the dose reduction of TNFi, we used the term "DQ", calculated as (actual dose/standard dose) × (standard dosing interval/actual dosing interval) × 100 according to Závadain et al. (30). For example, when etanercept is administered at 25 mg once a week instead of the full dose of twice a week at 25 mg, DQ = 25/25 × 3.5/7 × 100 = 50, which is 50% of the standard dosing regimen. In other words, a standard dose of etanercept (25 mg twice a week) is described as DQ100. Then during the first reduction, the dose of etanercept will be changed to 25 mg once a week, described as DQ50. During the second reduction, the dose of etanercept will be further changed to 25 mg once every 2 weeks, described as DQ25, and withdrawal described as DQ0. Other TNFis, such as adalimumab, infliximab, and golimumab, are reduced similarly (**Supplementary Figure 3**). The characteristics of the withdrawal group were mainly as follows: after the full dose of TNFi for 3–6 months to achieve low disease activity or disease remission, all patients in this group had rapidly reduced and stopped TNFi within 12 months. The average DQ of 3 months interval was calculated by adding the DQ at the beginning of the interval to the DQ at the end of the interval and then dividing by 2. The average DQ of 12–24 months interval was calculated as [(DQ at 12 months + DQ at 18 months)/2 + (DQ at 18 months + DQ at 24 months)/2]/2.

Pelvic MRI

MRI-SIJ were obtained at least at baseline, 12, and 24 months and evaluated independently by two trained readers who were blinded for all clinical information and time order. The inflammation of SIJ was scored by the SPARCC scoring system using STIR images (33). Structural abnormalities, namely, erosion, fat metaplasia, and backfill, were evaluated by Sacroiliac Joint Structural Score (SSS) using T1-weighted images (34). The average of scores from the two readers was used as the final score.

Data Collection and Statistical Analysis

The data of patients regarding demographics, age, gender, smoking status (ever vs. never), HLA-B27 positivity, disease duration, serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), disease activity indices, such as BASDAI, BASFI, and ASDAS, were recorded at baseline. Disease activity (BASDAI, BASFI, and ASDAS), inflammatory biomarkers (CPR and ESR), and pelvic MRI data (SPARCC, fat metaplasia, erosion, and backfill) were collected at every follow-up visit from the electronic medical record system.

Numeric data were presented as mean (SE) or median [interquartile range, IQR], and categorical data were presented as percentages. The Student's *t*-test or Mann-Whitney U-test was used to compare the continuous variables and the Chi-square test or Fisher's exact test was used to compare the categorical data. All these descriptive data were analyzed with the R package "tableone". To obtain an unbiased estimate of the effect of the average DQ value, we used inverse-probability-of-treatment weighting (IPTW) fitted to a marginal structural model (MSM) considering its ability to yield causal inference between the treatment exposure and outcome in the presence of time-dependent covariates that are also intermediate variables (35). Stabilized weights were calculated in IPTW with R package "ipw" (parameter "family" was set as "Gaussian" in this process), and the dominator as the estimated probability of treatment with different average DQ values was based on baseline covariates (gender, age, diagnosis, disease duration, smoking history, and positivity of HLA-B27), ASDAS-CRP value at the start of the follow-up interval. The numerator of weight was given by the estimated probability of different average DQ values treatment based on the baseline covariates aforementioned only. The correlation between IPTW weighted (or not) average DQ value and disease activity status or radiologic indices was realized with generalized estimating equation (GEE) via geeglm function in R package "geepack". A receiver operator characteristic (ROC) curves were plotted with R package "pROC" and "WeightedROC", and forest plots were realized with R package "forestplot". All statistical analyses were performed with R version 4.0.3.

RESULTS

Baseline Characteristics Between Two Groups

A total of 108 patients who underwent a dose reduction or complete withdrawal and followed up for at least 2 years were included in this study. Among them, there were 63 patients in the tapering group, 45 patients in the withdrawal group, and the proportions of patients with AS in the two groups were 57.1 and 60%, respectively, ($p = 0.844$). Baseline characteristics were similar between the two groups. All the patients had a high disease activity (BASDAI > 4 or ASDAS ≥ 2.1) at baseline, with the median (IQR) ASDAS-CRP of 3.45 (2.81, 4.21) in the tapering group and 3.14 (2.63, 3.96) in the withdrawal group. There was no difference in the disease activity and severity of structural damage at baseline (**Table 1**).

Disease Activity and MRI Features at 12- and 24 Months Between Two Groups

At 12 months, BASDAI (0.40[0.00, 1.00] vs. 1.50[0.60, 2.10]; $p < 0.001$), BASFI (0.00[0.00, 0.40] vs. 0.50[0.00, 1.00]; $p = 0.004$), ASDAS-CRP (0.88[0.55, 1.62] vs. 1.83[1.29, 2.53]; $p < 0.001$), ASDAS-ESR (0.98[0.72, 1.52] vs. 1.77[1.23, 2.58]; $p < 0.001$), CRP (2.30[0.75, 5.25] vs. 5.21[1.70, 10.20]; $p = 0.012$), ESR (6.00[4.00, 14.50] vs. 10.00[6.00, 23.00]; $p = 0.031$), and SPARCC scores (1.00[0.00, 3.00] vs. 5.00[3.00, 7.00]; $p < 0.001$) in the tapering group were significantly lower than those in the withdrawal group. While the fat metaplasia, erosion, and backfill of SIJ were not statistically different between two groups. At 24 months, disease activity (BASDAI, BASFI, ASDAS-CRP, and ASDAS-ESR), inflammatory indicators (CRP and ESR), and SPARCC

TABLE 1 | Demographic and clinical characteristics of patients with axSpA at baseline.

		Group		*p* value
		Tapering (*n* = 63)	**Withdrawal (*n* = 45)**	
Diagnosis (%)	nr-axSpA	27 (42.9)	18 (40.0)	0.844
	AS	36 (57.1)	27(60.0)	
Gender (%)	Female	21 (33.3)	14 (31.1)	0.838
	Male	42(66.7)	31 (68.9)	
Age (median [IQR])		28.00 [23.00, 33.00]	30.00 [25.00, 35.00]	0.145
Duration (median [IQR])		12.00 [3.00, 24.00]	13.00 [5.00, 24.00]	0.312
HLA-B27 (%)	−	10 (15.9)	11 (24.4)	0.327
	+	53 (84.1)	34 (75.6)	
Smoking (%)	−	38 (60.3)	26 (57.8)	0.844
	+	25 (39.7)	19 (42.2)	
SPARCC (median [IQR])		20.00 [13.00, 24.00]	18.00 [13.00, 25.00]	0.717
Fat metaplasia (median [IQR])		12.00 [7.00, 18.00]	12.00 [8.00, 16.00]	0.810
Erosion (median [IQR])		11.00 [7.00, 16.00]	11.00 [8.00, 17.00]	0.415
Backfill (median [IQR])		0.00 [0.00, 3.00]	0.00 [0.00, 3.00]	0.282
BASDAI (median [IQR])		4.00 [3.42, 4.50]	3.80 [3.05, 4.20]	0.124
BASFI (median [IQR])		1.70 [1.00, 2.70]	1.60 [0.75, 2.40]	0.373
ASDAS-CRP (median [IQR])		3.45 [2.81, 4.21]	3.14 [2.63, 3.96]	0.386
ASDAS-ESR (median [IQR])		3.45 [2.75, 4.17]	3.15 [2.67, 4.16]	0.477
CRP (median [IQR])		14.70 [8.40, 25.20]	18.90 [8.35, 29.35]	0.331
ESR (median [IQR])		24.00 [15.00, 40.00]	25.00 [13.50, 43.50]	0.935

AS, ankylosing spondylitis; HLA, human leukocyte antigen; SPARCC, Spondyloarthritis Research consortium of Canada; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

TABLE 2 | Characteristic of disease activity and MRI features at months 12 and 24.

	Group		*p* value
	Tapering (n = 63)	**Withdrawal (n = 45)**	
Month 12			
BASDAI (median [IQR])	0.40 [0.00, 1.00]	1.50 [0.60, 2.10]	**<0.001**
BASFI (median [IQR])	0.00 [0.00, 0.40]	0.50 [0.00, 1.00]	**0.004**
ASDAS-CRP (median [IQR])	0.88 [0.55, 1.62]	1.83 [1.29, 2.53]	**<0.001**
ASDAS-ESR (median [IQR])	0.98 [0.72, 1.52]	1.77 [1.23, 2.58]	**<0.001**
CRP (median [IQR])	2.30 [0.75, 5.25]	5.21 [1.70, 10.20]	**0.012**
ESR (median [IQR])	6.00 [4.00, 14.50]	10.00 [6.00, 23.00]	**0.031**
SPARCC (median [IQR])	1.00 [0.00, 3.00]	5.00 [3.00, 7.00]	**<0.001**
Fat metaplasia (median [IQR])	20.00 [14.00, 25.00]	20.00 [14.00, 24.00]	0.827
Erosion (median [IQR])	17.00 [11.50, 22.00]	18.00 [16.00, 24.00]	0.182
Backfill (median [IQR])	3.00 [0.00, 6.00]	4.00 [2.00, 6.00]	0.083
Relapse rate (%)	17.5	75.6	**<0.0001**
Month 24			
BASDAI (median [IQR])	1.20 [0.30, 1.80]	2.50 [1.50, 3.50]	**<0.001**
BASFI (median [IQR])	0.10 [0.00, 0.65]	0.90 [0.10, 1.50]	**0.003**
ASDAS-CRP (median [IQR])	1.57 [1.02, 2.38]	2.36 [1.77, 3.33]	**<0.001**
ASDAS-ESR (median [IQR])	1.48 [1.02, 2.00]	2.46 [1.82, 3.39]	**<0.001**
CRP (median [IQR])	5.20 [1.88, 9.29]	9.70 [3.47, 16.90]	**0.045**
ESR (median [IQR])	11.00 [5.50, 19.50]	20.00 [8.00, 38.00]	**0.007**
SPARCC (median [IQR])	3.00 [2.00, 5.00]	9.00 [8.00, 12.00]	**<0.001**
Fat metaplasia (median [IQR])	21.00 [15.00, 26.00]	22.00 [16.00, 25.00]	0.644
Erosion (median [IQR])	19.00 [13.50, 24.50]	22.00 [16.00, 30.00]	**0.041**
Backfill (median [IQR])	4.00 [3.00, 6.00]	7.00 [4.00, 8.00]	**<0.001**
Relapse rate (%)	69.8%	97.8%	**<0.001**

SPARCC, Spondyloarthritis Research consortium of Canada; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range. Bolded values are statistically significant.

scores in the tapering group were still significantly lower than those in the withdrawal group. For the structural lesion, except fat metaplasia (21.00[15.00, 26.00] vs. 22.00[16.00, 25.00], $p = 0.644$), erosion (19.00[13.50, 24.50] vs. 22.00[16.00, 30.00]; $p = 0.041$), and backfill (4.00[3.00, 6.00] vs. 7.00[4.00, 8.00]; $p < 0.001$) were much lower in the tapering group (**Table 2**). These results suggested that patients in the tapering group had better control of disease activity and slower structural lesion progression of SIJ, especially at 24 months.

We then analyzed the relapse rate and the DQ value of TNFi during 24 months. Only 11 patients experienced a relapse within the first year in the tapering group. The relapse rates in the tapering group and withdrawal group were 17.5% and 75.6% at 12 months ($p < 0.001$), and 69.8% and 97.8% at 24 months ($p < 0.001$). For the DQ values, at 0 and 3 months, both groups were 100 [100, 100]. At 6, 9, 12, 18, and 24 months, the tapering groups were 50 [50, 100], 50 [35, 50], 33.3 [25, 35], 25 [16.7, 33.3], and 16.7 [12.5, 33.3], respectively, while the withdrawal groups were 50 [0, 66.7], 25 [0, 25], 0 [0, 0], 0 [0, 0], and 0 [0, 0], respectively (**Figure 1A**). There were 19 patients (such as, 11 patients with AS) in the tapering group who did not experience a relapse throughout 24 months. Furthermore, the median time to relapse was much later in the tapering group [24 months (95% CI: 21–26)], compared with that in the withdrawal group [12 months (95% CI: 10–13)] ($p < 0.0001$, log-rank test; **Figure 1B**).

We further explored whether the response of patients with AS and non-axSpA to the TNFi reduction treatment was similar in each group. The results showed that there were no significant differences in disease activity, SPARCC score, and structural damage between the patients with AS and non-axSpA in each group at 12 months. There was also no significant difference in the tapering group at 24 months, while in the withdrawal group,

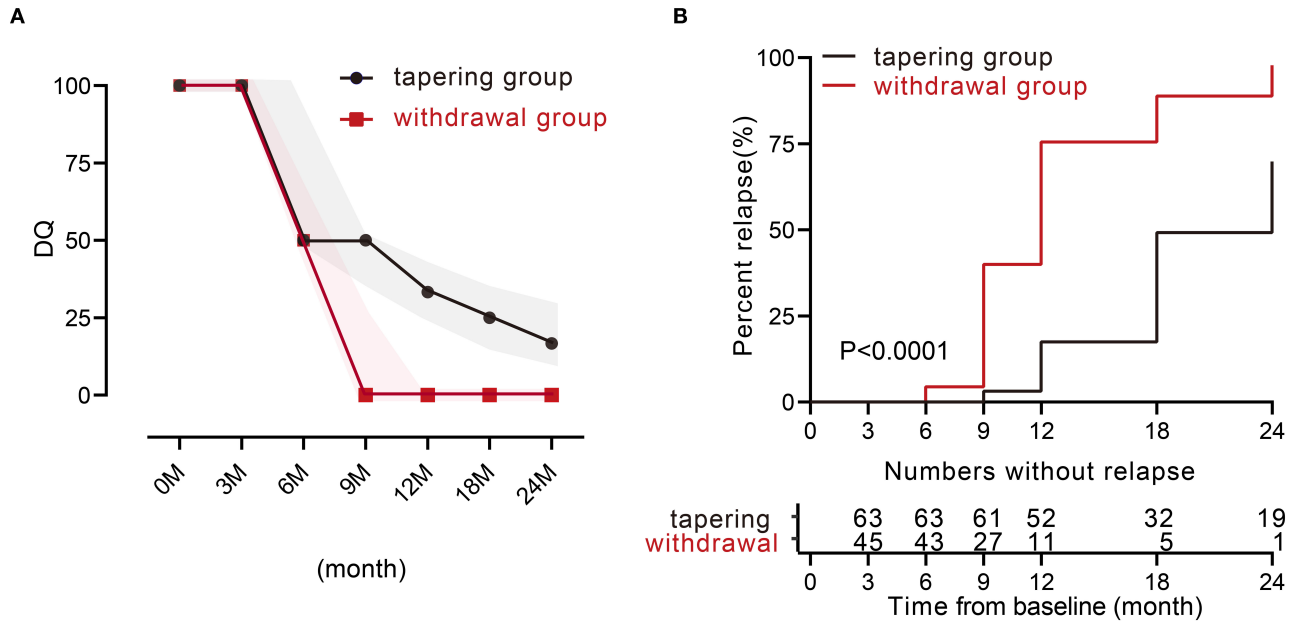

FIGURE 1 | TNFi levels and characteristics of relapse during 24 months between the two groups. **(A)** Medians of dose quotient in the two groups during the 24 months. The shadow represents the interquartile range. **(B)** Relapse rate during the 24 months between the two groups ($p < 0.0001$). TNFi, tumor necrosis factor inhibitor.

patients with AS had more severe fat metaplasia at 24 months (data not shown).

Relationship Between Average DQ and Structural Lesion Progression, Bone Marrow Edema, and Treatment Response

We have shown that backfill and erosion progress is slower in the tapering group at 24 months. To further investigate the unbiased effects between TNFi dose and structural progression, treatment response, or disease activity, we divided 2 years into five intervals and obtained 540 intervals from 108 patients. Average DQ, ΔSPARCC, Δfat metaplasia, Δerosion, Δbackfill, and the status of treatment were calculated for each interval. By using the GEE univariate model, we got the beta estimate of average DQ for ΔSPARCC was −0.0989 [−0.1107, −0.0872] ($p < 0.0001$), Δfat metaplasia was 0.0032 [−0.0038, 0.0102] ($p = 0.373$), Δerosion was −0.0094 [−0.0145, −0.0042] ($p = 0.00034$), Δbackfill was −0.0064 [−0.0106, −0.0023] ($p = 0.0025$), and the occurrence of relapse was −0.0428 [−0.0559, −0.0297] ($p < 0.0001$). We further adopted IPTW to adjust confounding factors. The adjusted beta estimates of average DQ for ΔSPARCC, Δfat metaplasia, Δerosion, Δbackfill, and the occurrence of relapse were −0.0959 [−0.1077, −0.0842] ($p < 0.0001$), −0.0045 [−0.0232, 0.0142] ($p = 0.635$), −0.0100 [−0.0153, −0.0046] ($p = 0.00026$), −0.0034 [−0.0109, 0.0041] ($p = 0.375$), and −0.0344 [−0.0490, −0.0198] ($p < 0.0001$) respectively (**Figure 2A**). These results suggested that Δerosion, ΔSPARCC, and the occurrence of relapse were negatively correlated with average DQ.

ROC Curves to Evaluate Therapeutic Effects

Tapering or withdrawal of the TNFi could lead to relapse. To discriminate the treatment status of patients (response or relapse), we performed the ROC curve with two statistical models (GEE and GEE and IPTW). The area under the curve (AUC) was 83.9% (GEE) and 80.5% (GEE and IPTW). The optimal cut-off value was when the average DQ was 29.1 (80.5%, 78.1%) (GEE) and 30.0 (74.8%, 80%) (GEE and IPTW). Similarly, we also examined the treatment status of bone marrow edema (SPARCC score increased or not). The AUC was 84.2% (GEE) and 84.3% (GEE and IPTW). The optimal cut-off value was when the average DQ was 40.2 (77.5%, 81.8%) (GEE) and 41.6 (76.5%, 83%) (GEE and IPTW). These results indicated that at the average DQ 30, theoretically speaking, 80% of patients still achieved a good TNFi response, while at the average DQ 41.6, 83% of patients still obtained no progress of bone marrow edema (**Figure 2B**). However, considering operability and the convenience of clinical administration, we found that at average DQ 25, the status of treatment response (78%, 63.3%) and the status of bone marrow edema (88.5%, 61.9%) were also acceptable.

In addition, based on the feature of maintained DQ value, we found no patients relapsed among 16 patients who maintained DQ value over 25 (DQ > 25) in the tapering group at 24 months, while 44 of 47 (93.6%) patients who maintained DQ ≤25 in the tapering group relapsed during 24 months (**Supplementary Figure 2**).

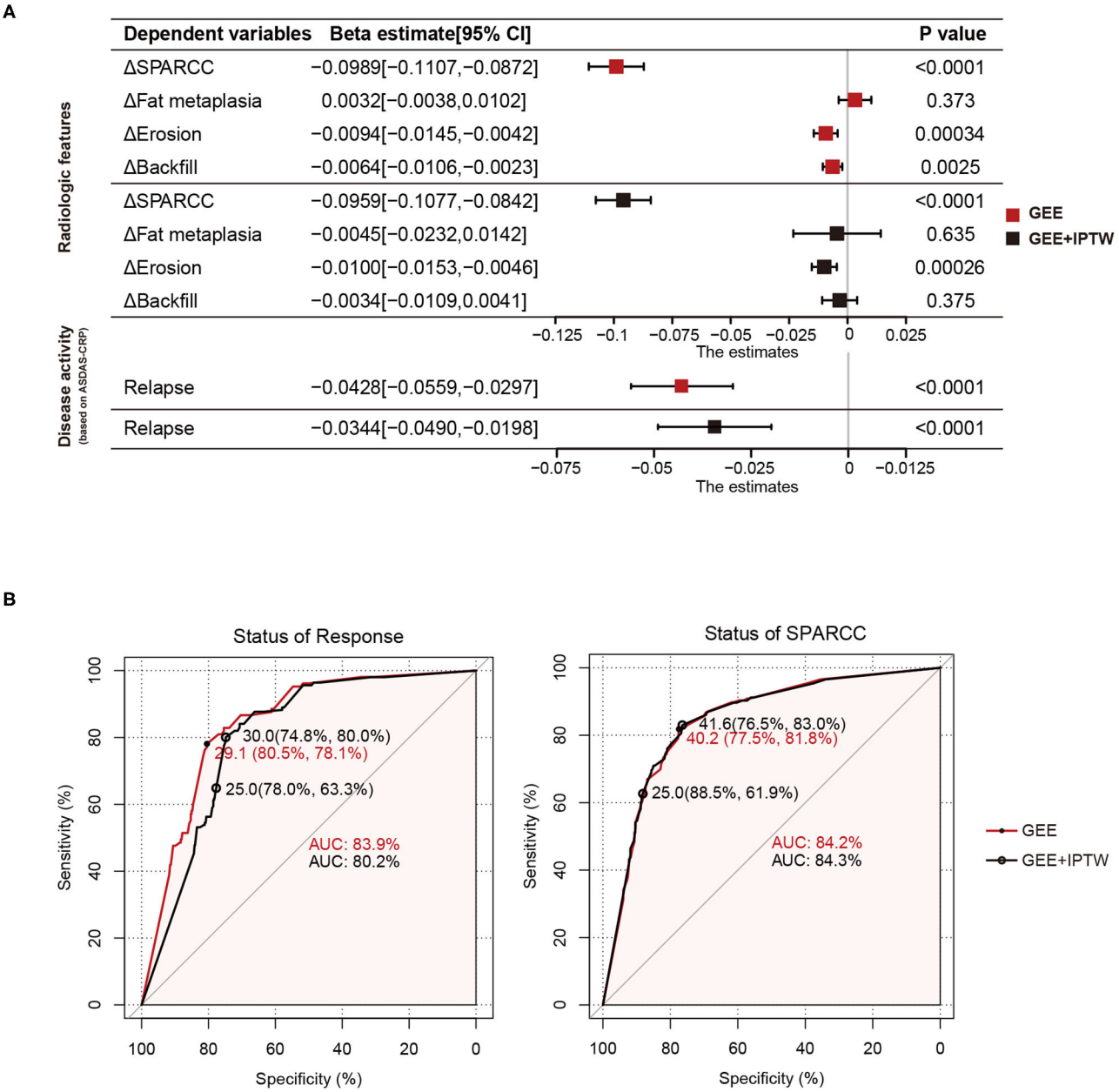

FIGURE 2 | Average DQ values of 540 intervals from 108 patients with axSpA were fitted to relapse status and MRI features (change of SPARCC, fat metaplasia, erosion, and backfill score between the end and the start of the corresponding intervals) with GEE or GEE+IPTW methods. **(A)** Forest plots. **(B)** ROC curves of average DQ value in discriminating the status of treatment response (response or relapse) and status of SPARCC (SPARCC score increased or not). DQ, dose quotient; axSpA, axial spondyloarthritis; SPARCC, the Spondyloarthritis Research Consortium of Canada; GEE, generalized estimating equation; IPTW, inverse-probability-of-treatment weighting.

The Risk Factors for Relapse Before Average DQ Less Than 25

To further explore which factors were related to relapse before average DQ <25. We compared the baseline data for these 24 patients (relapse group) with the remaining 39 patients (response group) in the tapering group. Interestingly, we found that the positive rate of HLA-B27 was 100% in the relapse group and 74.4% in the response group ($p = 0.010$). In addition, the baseline fat metaplasia in the relapse group was 19.42 ± 1.92, significantly higher than that in the response group 9.62 ± 0.88 ($p < 0.001$). Other factors, such as gender, age, AS or not, smoking rate, SPARCC, erosion, backfill, BASDAI, BASFI, ASDAS-CRP, ASDAS-ESR, CRP, and ESR, were not statistically significant between these two groups (**Table 3**).

TABLE 3 | Baseline data between patients relapsed or not before the average DQ <25 in the tapering group.

		Group		*p* value
		Relapse (*n* = 24)	**Response (*n* = 39)**	
Diagnosis (%)	nr-axSpA	10 (41.7)	17 (43.6)	0.881
	AS	14 (59.3)	22 (56.4)	
Gender (%)	Female	8 (33.3)	13 (33.3)	1.000
	Male	16 (66.7)	26 (66.7)	
Age (median [IQR])		29.50 [23.00, 37.]	27.00 [23.00, 32.00]	0.220
Duration (median [IQR])		8.00 [3.00, 36.00]	12.00 [4.00, 24.00]	0.645
HLA.B27 (%)	−	0 (0.0)	10 (25.6)	**0.010**
	+	24 (100.0)	29 (74.4)	
Smoking (%)	−	15 (62.5)	23 (59.0)	0.781
	+	9 (37.5)	16 (41.0)	
SPARCC (median [IQR])		18.00 [11.25, 21.00]	22.00 [15.00, 25.00]	0.126
Fat metaplasia (mean ± SE)		19.42 ± 1.92	9.62 ± 0.88	**<0.001**
Erosion (mean ± SE)		10.00 ± 1.42	12.21 ± 0.89	0.171
Backfill (median [IQR])		0.00 [0.00, 2.75]	0.00 [0.00, 3.00]	0.853
BASDAI (median [IQR])		3.95 [3.22, 4.87]	4.00 [3.50, 4.50]	0.921
BASFI (median [IQR])		1.80 [1.05, 3.25]	1.70 [0.90, 2.50]	0.859
ASDAS-CRP (median [IQR])		3.48 [2.38, 3.99]	3.45 [2.85, 4.23]	0.436
ASDAS-ESR (median [IQR])		3.24 [2.46, 4.00]	3.46 [2.77, 4.46]	0.315
CRP (median [IQR])		13.65 [5.39, 28.94]	14.70 [9.26, 23.67]	0.921
ESR (median [IQR])		21.50 [10.50, 44.25]	25.00 [17.00, 38.00]	0.552

AS, ankylosing spondylitis; HLA, human leukocyte antigen; SPARCC, Spondyloarthritis Research consortium of Canada; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; ASDAS, Ankylosing Spondylitis Disease Activity Score; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IQR, interquartile range; SE, standard error. Bolded values are statistically significant.

DISCUSSION

In the clinic, some patients have to opt for dose reduction or withdrawal because of intolerance to full-standard TNFi, risk of potential infection, unaffordability to high expense, intolerability for long-term subcutaneous injection, or multiple reasons. EULAR recently recommended that tapering TNFi can be considered for patients who have achieved sustained remission. However, there is no unified standard yet. To explore a referable dose reduction strategy, we retrospectively examined the effects of different TNFi dose-reduction strategies on patients with axSpA. Firstly, rapid dose reduction then complete withdrawal after clinical symptom relief was not recommended. Secondly, our study provided a reference strategy that gradually tapering followed by maintaining the average DQ over 30 for patients who need TNFi reduction, or over 25 for patients with HLA-B27 negative and non-severe fat metaplasia at baseline were acceptable.

Several studies have explored the efficacy of tapering strategies in patients with axSpA (26–32). Landewé et al. found that after achieving sustained remission at 48 weeks, a half dose of certolizumab pegol could maintain 79% of patients flare-free for the next 48 weeks in patients with early axSpA, which was comparable to 83.7% in the full-dose group. Gratacós et al. claimed that after achieving clinical remission for ≥6 months, 81.3% of patients in the reduced dose arm maintained low disease activity in the next year, which is comparable to that of patients in the full-dose arm (83.8%). In our study, the disease activity was much lower in the tapering group compared with the withdrawal group at months 12 and 24. At month 12, 95.2% (60/63) of the patients in the tapering group reduced the TNFi dose to 50% (12 patients) or <50% (48 patients). At this time point, 82.5% (52/63) of the patients were still without relapse in this group. However, with further reduction, the relapse rate and the relapse risk increased significantly. In addition, we also found that the cut-off value of the average DQ was 30 for maintaining a relatively good treatment response. Therefore, TNFi tapering is an alternative option for those who cannot use full-dose TNFi due to various reasons, but the maintaining dose of TNFi should not be reduced to a level below average DQ 30. In addition, rapid dose reduction then complete withdrawal after clinical symptom relief was not recommended. However, in the clinic, the average DQ 30 is not operable and convenient for clinical administration, so we further analyzed the average DQ 25 and found the treatment effects were acceptable. In addition, the relapse rate of patients with DQ >25 was significantly lower than patients with DQ ≤25 at 24 months.

Many studies have focused on the efficacy of TNFi reduction therapy, but few studies paid attention to the risk factors of disease relapse during the reduction process. Almirall et al. found that a shorter duration of remission before dose reduction, shorter duration of TNFi treatment, and shorter disease duration were the risk factors of relapse (36). In our study, we found that patients with HLA-B27 positive or more severe fat metaplasia at baseline were more likely to relapse before the average DQ was <25. Previous studies have reported that the presence of HLA-B27 in patients with AS is correlated with higher disease activity and poor functional status (37–39). In addition, evidence showed that HLA-B27 influences disease activity by a pathway not involving TNF-α (40). This may be the reason why patients with HLA-B27 positive had a higher risk of relapse during dose reduction of TNFi. Studies showed that fat metaplasia appeared to be a feature of tissue response after inflammation resolution, and it was a risk factor for the development of syndesmophytes and ankylosis (41–43). Therefore, reducing the average DQ to <25 was not recommended especially for those patients with HLA-B27 positive or severe fat metaplasia.

Only a few studies investigated the effect of TNFi on SIJ of patients with axSpA. Almirall et al. found no significant progress in the SIJ in a cohort of patients with nr-axSpA treated with

TNF-α blockers for 2 years (44). Another study of the RAPID-axSpA phase III randomized trial found that patients with axSpA treated with TNFi did not show significant SIJ progression after 4 years (45). However, these two studies lacked matched controls. Dougados et al. assessed the changes of SIJ in patients with recent onset axSpA receiving etanercept for 2 years and found a slower progression of SIJ compared with patients not receiving TNFi (20). In our study, we also found that the tapering strategy had a slower progression of erosion in SIJ. Another retrospective cohort study observed the effects of TNFi reduction on hip arthritis, showing that the acute inflammatory changes in the full-dose group and tapering group were equivalent (28). In our research, we used MRI to observe inflammation for SIJ and suggested that tapering TNFi could better control bone marrow edema compared with the complete withdrawal of TNFi. What is more, higher doses of TNFi treatment resulted in less severe bone marrow edema and slower erosion progression of the SIJ.

This study has some limitations to be considered. Firstly, the sample size of each group was relatively small. Secondly, because it was a retrospective study, it was impossible to ensure that the tapering strategy for each patient is strictly uniform, but we used DQ and average DQ to minimize this bias, according to a study published by Závadain et al. (30). Our study firstly explored the correlation between the average DQ and structural lesion progression of SIJ in the tapering therapy and explored a referable reduction strategy in the clinic for those patients who need TNFi reduction because of various reasons. In conclusion, we suggest that gradually tapering followed by maintaining the average DQ over 30 or even 25 is an acceptable strategy for patients who need TNFi reduction. However, we also indicate that a higher dose of TNFi is associated with a slower erosion progression of SIJ. Therefore, in clinical practice, we need to adopt appropriate treatment strategies according to the actual situation of the patients. Although future randomized controlled investigations with a larger sample size are needed to provide more information, this retrospective cohort study provided a referable value on the therapeutic efficacy of this TNFi tapering strategy for patients with axSpA.

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 Tongji Medical College of Huazhong University of Science and Technology (project identification code: 2020-S275). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LD and MW designed the study. QM and YD analyzed the data and wrote the paper. CY, JZ, and SC contributed to the interpretation of the data. All authors revised the paper and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.781088/full#supplementary-material

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Empowering Patients in the Therapeutic Decision-Making Process: A Glance Into Behçet's Syndrome

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Behçet's syndrome (BS) represents a challenging condition, characterized by a variable spectrum of disease profile and associated with a significant limitation of the daily activities as well as a potential negative impact on relationships and psychological status. Considering also the complexity of the therapeutic management of BS, that often includes biological off-label treatments, the participation in the therapeutic decision-making process of the BS patients is essential to ensure the integration of the care process into the life of the patient. For this reason, the empowerment of BS patients represents a crucial need and the present work is aimed at fully exploring all the potential variables implicated in the BS patient empowerment, also highlighting major points to consider and concrete actions to be planned in the immediate future in order to implement a pragmatic facilitation of the patients' empowerment.

Keywords: Behçet disease, patient empowerment, patient education, decision making process (DMP), rare disease (RD)

HIGHLIGHTS

- The process of patients' empowerment needs to be addressed as a systematic approach and should ensure the involvement of multiple stakeholders in order to be really efficient and effective.
- Considering the rarity and complexity of BS, patients' empowerment can highly contribute to improve the lives of patients, caregivers and families living with the disease and it foresees to work for the optimization of patient-clinician communication, self-management, patient education, sharing of the therapeutic decision-making process, partnership in research and policy making.

- BS patients' organizations, BS healthcare professionals and policy-makers can play a crucial role in co-designing and co-creating new initiatives and projects aimed at joining forces and promoting patients' empowerment across the BS community.

INTRODUCTION

According to the World Health Organization, patient empowerment is defined as "a process through which people gain greater control over decisions and actions affecting their health" (1) and for obvious reasons this process can be considered of certain efficacy only when approached at both individual and community level. Several elements have been reported so far as being fundamental to the patient empowerment and among them, patients' awareness of their role in the care process, the need for adequate knowledge enabling the engagement with the healthcare provider, the acquisition of specific skills and the existence of a facilitating environment definitely represent the mainstay of the empowerment process. Patients' empowerment means above all enablement, therefore patients and caregivers have to right to know, to be motivated, responsible and enabled to be part of the care process, also when the process involves research activities and medicine development (2–4).

Independently from its specific field of application, health policy makers should support and address this essential need, both if patient empowerment represents a goal to be reached and when it is adopted as a specific approach. At the same time, healthcare professionals have the duty to encourage and contribute to patients' empowerment also in clinical practice. This is particularly crucial in people living with rare diseases, in which knowledge related to diagnosis, treatment and complications is often limited and expertise is scattered (5, 6); however, in spite of the several challenges that rare diseases patients experience, the existing rare diseases patients' organizations facilitate the establishment or joining communities that play an essential role in providing the often-lacking information (7).

In the clinical environment of rare systemic autoimmune diseases, Behçet's disease (BS) represents a challenging condition, characterized by a variable spectrum of disease profile (8); while prevalent muco-cutaneous involvement and arthritis represent the main clinical features in patients with a benign disease subset, there are other patients who potentially develop sight or life-threatening manifestations, due to ocular, neurological or major vascular involvement (9). The relapsing nature of the disease can determine exacerbations and remission of symptoms over time and various demographic factors are considered predictable of poor outcome in the short and long-term, such as age at disease onset, duration of disease or gender. In fact, younger male BS patients are generally more suitable to have a more severe disease, due to an increased frequency both of morbidity and mortality, related to ocular, vascular and neurological involvement (8). Moreover, the chronic characteristics of the disease are strongly associated with a significant limitation of the daily activities as well as a potential negative impact on relationships with other people and psychological status (10, 11). Taking into account all these elements, it is clear that in order to manage in the most appropriate way the therapeutical approach according to disease activity, a very careful and tight control is strongly recommended in BS patients (12). Considering also the complexity of the therapeutic management of BS, that often includes biological off-label treatments, the participation in the therapeutic decision-making process of the BS patients is essential to ensure the integration of the care process into the life of the patient. For this reason, the empowerment of BS patients represents a crucial need to enable both this kind of integration and participation and to date, only few initiatives are ongoing to promote patients' empowerment in BS. Besides the fact that all patients living with BS could highly benefit in being included in the empowerment processes, it is also important to highlight that the different profiles of BS patients (such as age, type of organ involvement, severity of disease, etc.) may require targeted initiatives aimed at addressing the specific needs of the patient. Therefore, the present work is aimed at fully exploring all the potential variables implicated in the BS patient empowerment, also highlighting major points to consider and concrete actions to be planned in the immediate future in order to make real a pragmatic facilitation of the patients' empowerment.

DOMAINS TO ADDRESS PATIENTS' EMPOWERMENT IN BS

Sharing of the Therapeutic Decision-Making Process

Current evidence shows that adherence to therapy is usually higher in patients directly involved in the therapeutic decision-making process and, consequently, so is their outcome. In particular, lack of information concerning potential risks and benefits of the therapeutic options and inadequate communication between physicians and patients are some of the main risk factors for patients' discontinuation of treatment (13, 14).

Such observations revealed the need for new therapeutic decision-making processes, taking distances from the old paternalistic model which focused on simply informing patients about their treatment options rather than sharing them together to reach a common decision.

In facts the shared-decision making (SDM) process considers both the physician and the patient as its essential components: the former contributes with experience and the expertise on clinical guidelines and treatment's targets, while the latter expresses personal preferences and goals, as well as expectations from the treatment (15).

As reported in **Table 1**, the process comprises the following steps:

1. To describe the therapeutic options to patients and encourage them to actively participate into the conversation;
2. To explain the potential risks and benefits of each option;
3. To consider and evaluate patients' doubts and preferences;
4. To reach a shared decision on the best option;

TABLE 1 | Points to consider to promote patients' empowerment in BS.

Domains	Points to consider for patients' empowerment in BS
Sharing of the therapeutic decision-making process	- Specific SDM models should be developed for BS. - Behçet's disease healthcare professionals should be trained in adopting shared decision-making models in BS.
The role of healthcare professionals in improving patients' empowerment in clinical practice	- Ensure the organization of training activities for healthcare professionals on how to communicate with patients and for patients to ensure an appropriate level of health literacy. - Co-create Patient Decision Aids dedicated to BS. - At hospital level, it is crucial to ensure a patient-centered approach during all phases of care dedicated to patients with BS (access to patient-clinician direct communication, to information on the disease, on the clinic, etc.).
Patient-clinician communication	- It is essential to promote training and education of healthcare professionals dealing with BS not only on the clinical aspects of BS, but also on how to communicate in general with the patient and on how to ensure a patient-centered and disease-specific communication related on BS. - Educational activities for patients should be focused on health literacy and on the specificities of BS. - Supporting the use of communication tools among BS clinics and patients' organizations including web-based ones, such as brochures, media platforms, workshops, open conferences could contribute to ensure an adequate access to information.
Self-management	- By joining efforts of the whole BS community of patients and healthcare professionals, it is desirable to identify a core set of areas of intervention in order to launch specific initiatives promoting BS self-management at local, national and international level.
The role of caregivers	- The empowerment of caregivers should also be ensured in order to improve the quality of life of both patients and caregivers. - While co-designing patient education programmes, dedicated initiatives should be specifically organized also for caregivers. - It is important to ensure access to information on BS also to caregivers.
Partnership in research	- In order to enable and encourage partnership among patients in the research process, support the creation of digital platforms dedicated to this aim. - Support the validation and standardization of co-designed outcome measures for BS research.
Patient education	- Specific patients' education programmes need to be developed for BS. - Any educational programme should be developed in co-design with BS patients' and BS patients' representatives in order to address their educational needs and priorities. - Caregivers and family members should also receive specific training and should participate in the co-design process.
Patients' empowerment and policy maker	- Promoting the creation of patients' organizations and federations dedicated to BS can support the empowerment of patients at different level and ensure the active participation of BS patients' representatives in policy making and other relevant initiatives.

5. To critically evaluate the decision and express any concern on the beginning of that specific medication (16).

The benefits gained by this approach include the beginning of a tailored therapy which the patient can actually benefit from the reduction of unwarranted health practice variations and, most importantly, the acknowledgment of the patients' right to participate in decisions involving their health. In addition, principles and strategies of SDM process in the therapeutic decisions may contribute to improve patients' adherence, as highlighted by the International Patient Decision Aid Standards Collaboration (17)[1]. However, currently, models of SDM tailored to BS are still lacking, probably also because the application of therapeutic SDM process in BS appears even more challenging as it implies different therapeutical approach, both traditional and new molecules which bring different clinical outcome according to the main disease manifestations (e.g., anti-IL-1 against muco-cutaneous disease, anti-IL-6 against neurologic disease etc.).

[1]http://ipdas.ohri.ca/index.html

Points to Consider

- Specific SDM models should be developed for BS.
- BS healthcare professionals should be trained in adopting SDM models in BS.

The Role of the Healthcare Professionals in Improving Patients' Empowerment in Clinical Practice

The processes of patient empowerment in clinical practice are strictly related to the concepts of patient-centered care and participation in the decision-making process (18). These two concepts can be achieved also with the contribution of the healthcare professionals by ensuring an appropriate efficient communication and open dialogue among patients and healthcare professionals and encouraging an active participation of patients in their care. Healthcare professionals can in fact play a crucial role in promoting the empowerment of patients, not only by providing detailed information on the disease and on the treatment options available, but also involving patients

in the decisions that affect their quality of life. Establishing a robust relationship among healthcare professionals and patients and involving patients in a shared decision process are also essential to promote patient's empowerment and have proved to contribute to a better clinical outcome (19).

One of the tools that are currently available to support patients in actively participating to the decision-making process is the Patient Decision Aids (PDA) (20, 21), that are defined as "tools designed to help people participate in decision making about health care options. They provide information on the options and help patients clarify and communicate the personal value they associate with different features of the options." Thanks to the development of PDA, patients and healthcare professionals can actively share their point of view regarding the best treatment options and thus representing a tangible and powerful tool of patients' empowerment. Even if some PDA are available for some rheumatic diseases (22), so far, no PDA are available in BS and in order to promote and support the participation of patients in decision-making process, co-creating PDA specific for BS should be a priority of the BS community.

Another important aspect related to the role of the healthcare professionals in improving patients' empowerment in clinical practice, is the organization of specific initiatives at hospital level. These include ensuring access to specific information on BS and on the BS clinic to patients (for example on the website of the hospital, providing printed leaflets at the clinic, etc.) and organizing on-line communication channels among patients and healthcare professionals (such as dedicated email addresses, FAQs, etc.), as well as ensuring patients' access to their medical records. In addition, the provision of telemedicine services for rare diseases such as BS is also particularly important and can highly contribute to empowering patients, especially considering the scattered knowledge existing worldwide.

It is important to highlight that the healthcare professionals that can contribute to patients' empowerment are not only clinicians, but also nurses, occupational therapists, physiotherapists, social workers, psychologists, etc. For this reason, specific training on communication and on how to ensure the active participation of patients in the shared-decision making process should be dedicated to all these different professionals, that can highly contribute to the empowerment of patients.

Points to Consider

- Ensure the organization of training activities for healthcare professionals on how to communicate with patients and for patients to guarantee an appropriate level of health literacy.
- Co-create Patient Decision Aids dedicated to BS.
- At hospital level, it is crucial to ensure a patient-centered approach during all phases of care dedicated to BS patients (access to patient-clinician direct communication, to information on the disease, on the clinic, etc).

Patient-Clinician Communication

Communication between the healthcare professional and the patient is a complex and interactive process. Thanks to an interactive communication, doctors and patients can share precious details regarding a medical history, co-identify signs and symptoms necessary for a correct diagnosis and share decisions on treatments based on the assessment of the risk/benefit of the therapy.

The main factors correlated to an appropriate patient-clinician communication include patient participation, efficient SDM, treatment satisfaction and building a mutual trust relationship (23). Evidence from the literature seems to suggest that a good patient-clinician communication means better global health, less organ damage, lower disease activity and fewer medication side effects (24–29). Other works focused on communication in chronic diseases, assessing that doctor's beliefs, attitudes and style impact on the relationship built with the patient and, as a result, doctors who are more informative, show more sensitivity to patients' concerns and offer more reassurance and support, tend to have patients satisfied with care and committed to treatment recommendations (30, 31). Moreover, the use of a simple, understandable and non-technical language appears essential for an efficient patient-clinician communication, as well as non-verbal communication seems to be predictive for patients' satisfaction: in fact, patients' poor understanding, in some cases due to the use of medical terminology during consultations, can cause anxiety, fear and disappointment (32).

Communication between patients and their doctors has been greatly revolutionized by the narrative medicine, a branch of health humanities that employs narratives of patients in clinical practice, research and education as a way to promote healing process (33, 34). In addition, it is of great interest the narrative reciprocity: the narrative and potentially reciprocal nature of attention in health care. "Narrative reciprocity might enable not only so-called shared decision-making and patient autonomy. It might open the door to mutual acknowledgment of the value of each participant's beliefs and habits" (35).

Moreover, the widespread use of web-sites, forums and social networks provides patients with direct access to the medical and scientific information available online. Patients use these channels as a tool of personal participation, even directly interacting with health structures and professionals by digital platforms. In this scenario where patients increasingly pursue to play an active role in their care process, patient-reported outcomes (PROs) represent a remarkable tool for clinicians to learn and understand patients' experiences and needs. Indeed, PROs quantify health outcomes reported directly by patients, without external interpretation or inputs (36, 37). A recent study evaluated the use of PROs in rheumatoid arthritis consultations and showed that PROs were feasible, increased a shared understanding of how disease affects patients' function in daily life, encouraged communication and shared decision-making and eventually resulted in high patient satisfaction and treatment confidence. Moreover, PROs helped clinicians to identify new symptoms and adjust treatment as needed (38). Therefore, an effective patient-clinician communication potentially improves three aspects of empowerment:

1. Level of knowledge of disease and its implications;

2. Ability to control and monitor treatment progress, treatment adherence and disease-related lifestyle adjustments;
3. Active participation in interviews and better level of preparation for consultations with HCPs.

Points to Consider

- It is essential to promote training and education of healthcare professionals dealing with BS not only on the clinical aspects of BS, but also on how to communicate in general with the patient and on how to ensure a patient-centered and disease-specific communication related on BS.
- Educational activities for patients should be focused on health literacy and on the specificities of BS.
- Supporting the use of communication tools among BS clinics and patients' organizations including web-based ones, such as brochures, media platforms, workshops and open conferences could contribute to ensure an adequate access to information.

Self-Management

The concept of self-management in the process of empowering patients arises from two main unmet needs: the health care system's difficulty in sustaining the efforts and costs of dealing with chronic conditions and rare diseases, and patients' need to develop a higher self-awareness of their condition.

A successful process of self-management is composed by different coexisting factors:

- Predisposing factors include motivation, self-efficacy and self-confidence showed by patients playing an active role in their health decision-making process;
- Reinforcing factors comprise family, patient organizations and health care professionals;
- Enabling factors are mainly represented by problem solving skills and access to healthcare information, both digital and not digital (39–41).

Self-management can be defined as the knowledge and skills that patients can acquire to better live with their condition, including the confidence in dealing with treatments management (e.g., for BS, suspension of immunosuppressive therapy in case of infection) and the be able to identify symptoms or signs needing immediate medical attention, emotional management, etc. In addition, health care professionals could strongly support the self-management by providing education and adequate information related to the disease and its management, in order to practically increase patients' skills in co-managing their health issues (42).

The recently published EULAR recommendations for the implementation of self-management strategies in inflammatory arthritis also offered a definition of self-management, that is the ability of the individual to deal with symptoms, treatment, lifestyle changes and psychosocial and cultural consequences of their condition. According to this work, self-management is inspired by two key themes: to achieve independence of the patient, the former, and the idea that self-management should be supported by others (e.g., family, patients organizations, healthcare professionals), the latter. Therefore, it becomes clear how EULAR found an integration between self-management and self-management support (39).

Other skills that patients should learn and improve for an efficient self-management approach were also previously identified (39, 40); some examples are offered by patient education, active involvement in problem solving/goal setting/decision-making, active interaction with significant others (healthcare professionals, family, patients' organizations), medication management, enhancing resource utilization (community, digital healthcare etc.), improving stress management (also cognitive behavior therapy, if needed), healthy behaviors (e.g., regular physical activity, diet, body weight control, quitting smoking) and managing work duties.

On the contrary, little evidence is currently available on the potential self-management strategies in BS patients. An important starting point is the identification of the factors mostly affecting people suffering from BS. In these regards, three works described BS patients' specific needs dividing them into four main domains (43, 44):

1. Sign and symptoms: mucocutaneous manifestations (especially oral and genital ulcers), pain, vision issues, fatigue and sleep disturbances;
2. Functioning: impact of the disease on speech and vision, lack of energy for daily activities, adaptation skills and self-care;
3. Psychological profile: impact on emotions and emotional management techniques;
4. Social impact: ability to socialize, impact on familial relationships.

Taking into account the evidence already available, it appears clear that more attention should be dedicated to the self-management of BS and to do that, it would be crucial to identify, together with BS patients, a core set of areas of intervention that can concretely stimulate the cultural change needed to join efforts and work at multi-stakeholder level on this goal.

Points to Consider

- By joining efforts of the whole BS community of patients and healthcare professionals, it is desirable to identify a core set of areas of intervention in order to launch specific initiatives promoting BS self-management at local, national and international level.

The Role of Caregivers

Caregivers can be defined as "a person who gives care to people who need help taking care of themselves" (45), thus including parents, partners, friends, members of the family or healthcare professionals. To date, little attention has been given to the role that caregivers play in rare diseases and even less attention to their empowerment. Besides the launch of a survey dedicated to BS caregivers, very few data are available on the role played by BS caregivers in empowering patients and on the actual need of devoting more efforts toward the empowerment of BS caregivers.

Caregivers have an essential role in the life of BS patients, as they support the care of patients in the wider and most complex aspects, ranging from the management of treatments on a daily basis to supporting the wellbeing of patients. In its

systemic and rare nature, BS caregivers can range from parents of a child affected by BS to partners, friends or family members of adult/elderly BS patients. Despite the importance of the role of caregivers, the burden of BS caregivers is still not fully explored and BS caregivers are not always appropriately informed and supported.

Therefore, it is important to mention that one of the most urgent needs in BS is the organization of educational activities dedicated to BS caregivers, in order to empower them in providing appropriate care to their patient, in knowing better the disease and the treatment options available, as well as in taking care of their own quality of life (46).

The introduction of healthcare professionals that can also support patient and caregiver information and education, such as specialized nurses, can also highly contribute to empowering caregivers.

In clinical practice, the point of view of caregivers should be also considered, especially in terms of quality of life and of disease burden and dedicated information should be made available also to BS caregivers (information on BS, on how to access the clinic, patient/caregiver-clinician communication channel, etc.).

Points to Consider

- The empowerment of BS caregivers should also be ensured in order to improve the quality of life of both patients and caregivers.
- While co-designing patient education programmes, dedicated initiatives should be specifically organized also for BS caregivers.
- It is important to ensure access to information on BS also to caregivers.

Partnership in Research

Health research landscape is increasingly changing. Researchers historically considered themselves as "gatekeepers" as they decide the objectives of their works and how to measure the reliability of their data; moreover, usually the results are shown and discussed on scientific papers and during conferences exclusively dedicated to healthcare professionals. Therefore, without patients' involvement in research, the paradigm researcher-gatekeeper fails to capture what is really important from the patients' point of view and at catching many key points like the evaluation of health outcomes from the patient's point of view.

In this regard, two conditions should be met for research to qualify as patient-oriented:

1. Patients are involved as research partners with multidisciplinary and transdisciplinary team members along a continuum in addressing patient priorities and/or planning research (e.g., data collection and analysis, interpretations, diffusion, dissemination and application of results).
2. Studies aim to (i) address outcomes deemed important by patients; (ii) have a direct impact on at least 1 of the following targets: patient health and experience, health professionals' practice, health care services and policies; or (iii) achieve both (i) and (ii) (47, 48).

As far as BS is concerned, a systematic review assessed that outcome measures used so far still need to be validated and standardized (49), suggesting they might benefit from an active partnership between researchers and patients in the research process. Although some steps forward have been covered by promoting patients' involvement in core set outcome definitions and outcome measurements development (50, 51), there is still a long way to go.

Points to Consider

- In order to enable and encourage partnership among patients in the research process, support the creation of digital platforms dedicated to this aim.
- Support the validation and standardization of co-design co-designed outcome measures for BS research.

Patient Education

Patients' education can be considered as one of main principles behind patients' empowerment, both in terms of knowledge and awareness on the disease and in terms of rights and responsibility that the patient has in the care process (52).

Besides the many initiatives that are already ongoing at European and National level (53), the high need of patient education for rare diseases is continuously highlighted in many different contexts. With regards to BS, the initiatives currently in place to support patient education are very limited. Among those, BehçeTalk (54) was recently launched in Italy by the Behçet Clinic of Pisa and National Association of Behçet disease and Behçet-like-Odv (Simba) as a patient education programme for patients, families and caregivers living with BS. The programme is offering educational webinars on the different aspects of the disease, as well a parallel programme that foresees support groups coordinated by a psychologist with specific expertise in BS. Ever since the very beginning of the programme, patients and clinicians dealing with BS have expressed the need to extend this programme also outside the Italian borders and for this reason, the programme will soon be launched worldwide also in English and in other languages. This experience has demonstrated that the BS patient community has identified an emerging unmet need in patient's education.

Considering the specificities of BS, it is easy to imagine how important it is to provide a specific education on the disease to BS patients and how this will inevitably lead to empower them not only in participating in the decision-making processes related to their care, but also in the self-management and in improving their daily life. Knowing the therapeutic options available, understanding how to be adherent to treatment, learning how to manage the disease in the daily life are in fact, concepts that could provide a considerable added value in the life of the patients, especially since many treatments adopted in BS are prescribed off-label and require specific adherence protocol that the patient needs to know in order to ensure their efficacy. An important principle needed in patients' education, is related to ensuring that any educational programme or activity is developed in co-design with patients and patients' representatives. As confirmed in the BehçeTalk programme, involving patients and patients' representatives in the development of patient education

programmes ensures that the needs and the priorities of patients are being addressed and the educational activity are tailored to what patients consider important.

Parallelly, the need for education is focused not only on training patients on their disease, but also, as highlighted in other disease areas (55), on providing similar knowledge also to the ones that live and support the patients during their journeys: their family caregivers. The burden of the disease and quality of life are often not properly addressed in BS caregivers and also for this reason, they should be included in the design of educational programmes and specific educational activities should be also addressed to them.

Points to Consider

- Specific patients' education programmes need to be developed for BS.
- Any educational programme should be developed in co-design with BS patients' and BS patients' representatives in order to address their educational needs and priorities.
- Caregivers and family members should also receive specific training and should participate in the co-design process.

Patient's Empowerment and Policy Maker

The process of patients' empowerment is part of a holistic approach that involves patients and caregivers/healthcare professionals at both individual and community level (56). Patients' empowerment has, in fact, two different but complementary dimensions: individual empowerment and collective empowerment. Collective patients' empowerment enables a community to express their needs and most of all, can facilitate the involvement of patients' representatives in policy-making aimed at shaping healthcare systems while addressing those needs. Ensuring patients' empowerment and involvement at policy-making level can in fact support the co-design of healthcare systems and services that are more patient-centered and that can be more effective and efficient.

The inclusion of patients in policy making and in the co-design of care services is usually ensured thanks to the representation of patients from patients' organizations (POs). POs can represent a community expressing their needs and priorities and act as stakeholder in the co-design of health policies, while advocating at political and social level to address those needs. With regards to BS, some BS POs are currently active at different levels [such as Simba Odv in Italy (57), Behçet UK (58), ABSA in America (59), etc.] and are often participating in important initiatives. In addition, through federations of rare disease POs, such as EURORDIS (60), BS POs are also (directly or indirectly) involved in policy making. However, many geographical areas are still lacking a dedicated PO for BS, and it is desirable, that more national PO and federations can be founded around the world in order to build a stronger community of patients, families, healthcare professionals and policy-makers that can join forces and co-create new knowledge and new policies to better address the needs of the BS community.

Points to Consider

- Promoting the creation of patients' organizations and federations dedicated to BS can support the empowerment of patients at different level and ensure the active participation of BS patients' representatives in policy making and other relevant initiatives.

CASE SCENARIO

Rebecca is 35 years old and she received a diagnosis of BS when she was 19 years old. After the diagnosis, she searched "Behçet's syndrome" on the internet and she felt overwhelmed by all the information found. At this stage, she found out about the national patients' organization dealing with BS and she had the opportunity of discussing her diagnosis, her symptoms, her daily life with other BS patients. Thanks to the patients' organization, she was referred to an expert center in her country. The healthcare professionals in the center provided her with informative material on the disease and on the treatment she was prescribed. However, many issues were still not completing clear to her, such as how to self-manage the treatment and when to suspend it, which were the possible side effects, etc. After discussing her concerns with the patients' organization, Rebecca was encouraged to discuss this further with her clinician and to participate in an educational program dedicated to her disease. Rebecca attended different sessions of the educational program on how to self-manage her treatments; thanks to her efforts, to the educational program and to the role played by the patients' organization, Rebecca felt more empowered and addressed her concerns during the next consultation, discussing how to self-manage her treatment. The joined efforts of the different stakeholders involved in the empowerment of Rebecca, have contributed to improving her knowledge of the disease and the treatment, as well as in enabling her in being actively involved in her therapeutic decision-making processes.

CONCLUSIONS

The process of patients' empowerment needs to be addressed as a systematic approach and should ensure the involvement of multiple stakeholders in order to be really efficient and effective. Considering the rarity and complexity of BS, patients' empowerment can highly contribute to improve the lives of patients, caregivers and families living with the disease. Specific domains to be addressed in order to promote patients' empowerment in BS include patient-clinician communication, self-management, patient education, sharing of the therapeutic decision-making process, partnership in research and policy making, in which not only the individual patients, but also healthcare professionals and caregivers can strongly contribute.

In this scenario, BS patients' organizations, BS healthcare professionals and policy-makers can play a crucial role in co-designing and co-creating new initiatives and projects aimed at promoting patients' empowerment across the BS community. Joining forces across the whole community is, in fact a condition sine qua non for the implementation of a cultural change toward

a new multi-dimensional and multi-stakeholder approach in the management of BS.

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.

AUTHOR CONTRIBUTIONS

RT, DM, and MM conceived the paper. RT, DM, and FDC wrote the manuscript. All authors were involved in the discussion and in the agreement procedure that provided the points to consider. All authors repeatedly edited the manuscript and approved the final version.

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BRIEF RESEARCH REPORT
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Multimodal Assessment and Characterization of Sicca Syndrome

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Background: Sicca syndrome represents a heterogeneous group of conditions, such as Sjögren syndrome, causing xerophthalmiaand xerostomia. This study characterizes in depth patients with Sicca syndrome and evaluates salivary gland ultrasound (SGUS).

Methods: Principal component analysis and hierarchical clustering of clinical parameters, such as ESSPRI, ESSDAI and laboratory data, were performed on all referrals for assessment of Sicca symptoms between October 2018 and March 2021. SGUS and labial gland biopsies were compared across groups.

Results: A total of 583 patients were assessed. Objective dryness was confirmed in 73% of the patients. Cluster analysis identified 3 groups with *post-hoc* analysis confirming distinct phenotypes: *Somatic Group* (283/583; 49%) with more frequent symptoms but limited objective dryness; *Dry Without Autoimmune Features* (DAF_{neg}, 206/584; 35%), and *Dry With Autoimmune Features* (DAF_{pos}, 94/584;16%). DAF_{pos} patients had highest autoantibody titers (anti-SSA(Ro) 240 vs. 3.6 vs. 3.8; $p < 0.001$), most extra-glandular manifestations ($p < 0.001$), and highest median SGUS Score (DAF_{pos}: 8 [IQR 4–10] vs. SG: 2 [1–4] vs. DAF_{neg} 4 [2–5]; $p < 0.001$). No tangible correlation with primary Sjögren syndrome criteria was observed.

Discussion: SGUS score correlated with a subset of patients with Sjögren syndrome, identified in the DAF_{pos} cluster. This study highlights heterogeneity within sicca and, indeed, Sjögren syndrome, highlighting the need for further studies.

Keywords: sicca syndrome, Sjögren syndrome, lip biopsy, sicca symptoms, salivary gland ultrasonography (SGUS)

KEY NOTES

- Patients exhibiting sicca symptoms, including those fulfilling criteria for primary Sjögren syndrome, are inherently heterogeneous, with inconsistent findings on salivary ultrasound.
- Novel clustering of patients with sicca symptoms incorporating principal component analysis of numerous relevant factors revealed 3 distinct phenotypes with distinct patterns of salivary gland involvement on ultrasound.
- Correlation between these clustered phenotypes and traditional definitions was limited, but suggested that clinically distinct subgroups among patients with Sjögren syndrome exist.
- Refinement of distinct disease entities within primary Sjögren syndrome appears feasible, and salivary gland ultrasound may assist in discrimination. Implications for future studies and, ultimately, tailored therapies require further evaluation.

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BACKGROUND

Sicca syndrome can be considered an overarching term for symptomatic ocular (xerophthalmia) and oral dryness (xerostomia). Dryness is common, particularly with increasing age, affecting up to 30% of the population over 65 years (1). Current diagnostic approaches focus on determining if an autoimmune etiology exists while excluding drug side effects or manifestations of other systemic diseases that can either induce hyposecretion or lacrimal gland destruction (2). Sjögren syndrome encapsulates the autoimmune sicca syndrome and may be considered as primary Sjögren syndrome (pSS) when occurring in apparent isolation or secondary in the presence of another recognizable autoimmune condition. In practical terms, diagnosis centers on clinical history, objective measurements of xerophthalmia and xerostomia, and auto-antibody profiling. pSS requires evidence of autoimmune inflammation of salivary or lacrimal glands, as outlined in the joint American College of Rheumatology (ACR) and European League against Rheumatism (EULAR) classification criteria (3). These are deliberately broad, affording some heterogeneity in clinical features, but require either the presence of anti-SSA antibodies or evidence of lymphocytic sialadenitis on labial gland biopsy (LBx). Imaging is not required for the diagnosis of Sicca syndrome or the classification of pSS, but data for various modalities exist. Punctate calcification of parotid glands on computer tomography has demonstrated high diagnostic specificity, but utility is limited because of radiation exposure (4). Magnetic resonance imaging of the same glands has shown changes in both T1- and T2-weighted signal intensities (5). Salivary gland ultrasound (SGUS) has been purported as a low-cost and radiation-free alternative for many years, with hypoechoic lesions correlating with more severe disease in a variety of scoring systems (6–8). Furthermore, SGUS has proven to be an easily acquired diagnostic tool (9). Although specificity has been favorable, reported sensitivity has been moderate (10).

Given the inherent heterogeneity of such cohorts, such as the heterogeneous group of patients with pSS and varying manifestations, this finding is perhaps unsurprising (11, 12). The aim of this study is to independently assess the role of SGUS in a large, unselected Sicca syndrome cohort that has undergone extended-criteria phenotypic clustering.

METHODS

All patients referred for rheumatological assessment of suspected Sicca syndrome at our Institution between October 2018 and March 2021 were prospectively included. Structured clinical data, assessing symptoms, and ESSPRI and ESSDAI scores were collected. All the patients were tested for antinuclear and anti-SSA(Ro)/anti-SSB(La) antibodies, rheumatoid factor along with differential blood count, and standard biochemistry indices. Xerophthalmia was assessed by Schirmer test and xerostomia by Saxon test in all the patients, and < 3.5 g in 2 min (stimulated saliva flow) and < 5 mm in 5 min (lacrimal flow) were considered as reduced. LBx was performed in the patients with suspected pSS as indicated and graded according to Chisholm Mason Score, with grade ≥ 3 being considered diagnostic (13). SGUS consisted of bilateral assessment of both parotid and submandibular glands and was performed by 2 blinded sonographers experienced in the procedure. Image interpretation adhered to criteria defined by a score from 0 to 3 depending on homogeneity, and both cumulative totals and DeVita scores were considered in the analysis (6, 14). All scans were independently re-scored by the non-performing sonographer, and a consensus score derived from both scores was used in the analysis. In case of disagreement, the images were reviewed, and the lower scores were employed. All the participants provided informed written consent prior to inclusion, and the study was approved by the Institutional Review Board at Hannover Medical School (8179_BO_S_2018).

With the exception of SGUS and LBx scores, all collected continuous variables were included in a principal component analysis. Missing values were estimated by multiple imputation, and the calculated dimensions were evaluated by hierarchical clustering. The sensitivity and specificity of both SGUS and LBx within the identified clusters were then evaluated. All the statistical analyses were performed using R v4.0.3 (R Foundation, Vienna, Austria). Multiple imputations were performed using the missMDA package, and the FactoMineR package for principal component analysis and hierarchical clustering. Graphs were created using ggplot2, scatterplot3d, and rgl packages where appropriate. In cases where three or more groups were compared, Kruskal Wallis test was performed for categorical variables and ANOVA tests for comparing quantitative variables, where appropriate. The Mann-Whitney test was performed for two variables.

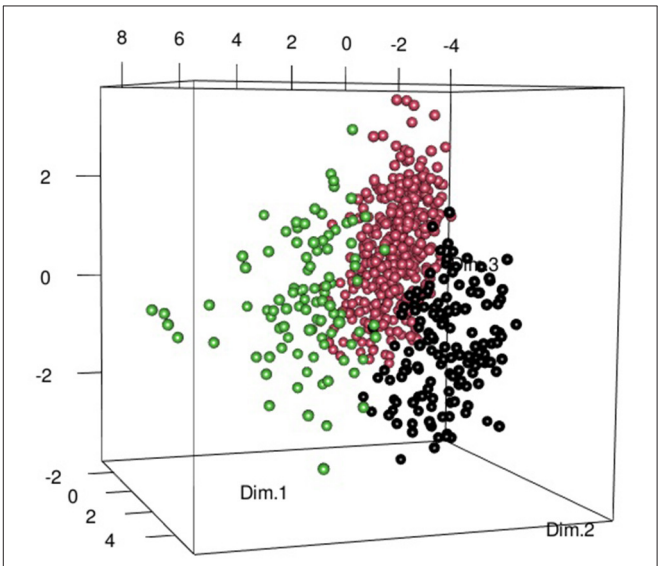

FIGURE 1 | Three-dimensional (3D) scatter plot composed of the first 3 dimensions of the principal component analysis, identified as providing greatest inertia gain on hierarchical clustering. The Somatic Group (SG) is represented by the black points. Patients in the Dryness without autoimmune features (DAF_{neg}) group are represented by pink points and Dryness with autoimmune features (DAF_{pos}) patients with green dots. This Figure was produced using the scatterplot3d and rgl packages.

TABLE 1 | Comparing and contrasting the clinical demographics and attributes of the entire cohort subdivided into the three groups identified through principal component analysis and subsequent hierarchical clustering.

	Somatic		**DAF_{neg}**		**DAF_{pos}**		***p*-value**
N (%)	283	(49)	206	(35)	94	(16)	
Female, *n* (%)	239	(84)	142	(69)	81	(86%)	<0.001
Age at Onset, years	47.3	[36.6–55.9]	60.2	[51.1–67.3]	50.1	[35.5–59.4]	<0.001
BMI, kgm^{-2}	26.1	[23.0–31.0]	24.7	[21.7–27.7]	24.6	[22.4–28.0]	0.003
Smoker, *n* (%)	50	(18)	15	(7)	4	(4)	0.06
ESSPRI Scores							
-Dryness	6	[3–7]	2	[1–3]	4	[2–4]	<0.001
-Limb Pain	7	[5–8]	5	[2–6]	6	[4–8]	
-Fatigue	8	[6–9]	3	[2–5]	5	[3–8]	
Reported Symptoms							
Raynaud, *n* (%)	86	(30)	51	(25)	44	(47)	0.006
Arthralgia, *n* (%)	222	(78)	118	(57)	61	(65)	0.002
Myalgia, *n* (%)	197	(70)	87	(42)	40	(43)	0.003
Stiffness, *n* (%)	98	(35)	37	(18)	23	(25)	0.001
Parotitis, *n* (%)	62	(22)	24	(12)	33	(35)	0.001
Sand corn*, *n* (%)	168	(59)	62	(30)	44	(47)	0.001
Ocular Inflammation, *n* (%)	120	(42)	45	(22)	26	(27)	0.005
ESSDAI							
-Score	5	[2–12]	5	[0–11]	11	[4–17]	<0.001
ESSDAI Constitutional, *n* (%)							0.02
-None	194	(69)	173	(84)	74	(79)	
-Low	79	(28)	26	(13)	16	(17)	
-Moderate	10	(3)	7	(3)	4	(4)	
ESSDAI Lymphadenopathy, *n* (%)							0.09
-None	232	(82)	182	(88)	88	(94)	
-Low	50	(18)	24	(12)	4	(4)	
-Moderate	1	(<1)	-	-	1	(1)	
-High	-	-	-	-	1	(1)	
ESSDAI Glandular Involvement, *n* (%)							0.21
-None	263	(93)	197	(96)	85	(90)	
-Low	19	(7)	8	(4)	8	(9)	
-Moderate	1	(<1)	1	(<1)	1	(1)	
ESSDAI Articular Involvement, *n* (%)							0.002
-None	241	(85)	199	(97)	73	(78)	
-Low	37	(13)	3	(1)	14	(15)	
-Moderate	4	(1)	4	(2)	7	(7)	
-High	1	(<1)	-	-	-	-	
ESSDAI Cutaneous Involvement, *n* (%)							0.16
-None	262	(93)	195	(95)	83	(89)	
-Low	14	(5)	6	(3)	5	(5)	
-Moderate	7	(2)	4	(2)	6	(6)	
-High	-	-	1	(<1)	-	-	
ESSDAI Pulmonary Involvement, *n* (%)							0.03
-None	251	(89)	177	(86)	73	(78)	
-Low	16	(6)	8	(4)	4	(4)	
-Moderate	12	(4)	11	(5)	11	(12)	
-High	4	(1)	10	(5)	6	(6)	
ESSDAI Renal Involvement, *n* (%)							0.26
-None	279	(98)	206	(100)	89	(95)	

(Continued)

TABLE 1 | Continued

	Somatic		DAF_{neg}		DAF_{pos}		*p*-value
-Low	2	(1)	-	-	3	(3)	
-Moderate	-	-	-	-	2	(2)	
-High	2	(1)	-	-	-	-	
ESSDAI Muscular Involvement, *n* (%)							0.38
-None	272	(96)	197	(96)	87	(93)	
-Low	5	(2)	6	(3)	1	(1)	
-Moderate	4	(1)	3	(1)	5	(5)	
-High	2	(<1)	-	-	1	(1)	
ESSDAI Peripheral Nerve Involvement, *n* (%)							0.77
-None	206	(73)	154	(75)	65	(69)	
-Low	35	(12)	15	(7)	7	(7)	
-Moderate	32	(11)	22	(11)	17	(18)	
-High	10	(4)	15	(7)	5	(5)	
ESSDAI Central Nerve Involvement, *n* (%)							0.13
None	263	(93)	200	(97)	88	(94)	
Moderate	9	(3)	1	(<1)	2	(2)	
High	11	(4)	5	(2)	4	(4)	
ESSDAI Hematological Involvement, *n* (%)							0.012
-None	223	(79)	163	(79)	51	(54)	
-Low	51	(18)	36	(18)	32	(34)	
-Moderate	8	(3)	6	(3)	11	(12)	
-High	1	(<1)	1	(<1)	-	-	
ESSDAI Biological Involvement, *n* (%)							<0.001
-None	237	(84)	167	(81)	49	(52)	
-Low	36	(13)	33	(16)	21	(22)	
-Moderate	10	(3)	6	(3)	24	(26)	
Antibody Titres							
-ANA ≥ 1:160	178	(63)	152	(74)	44	(47)	<0.001
-RhF U/ml	10.0	[10.0–10.9]	10.0	[10.0–11.3]	23.3	[11.7–71.0]	<0.001
-Alpha-Fodrin U/ml	9	[5–22]	9	[6–19]	12	[6–25]	0.05
-anti-SSA(Ro) U/ml	3.6	[0.3–101.3]	3.8	[0.3–102.3]	240.0	[192.8–240.0]	<0.001
-anti-SSB(La) U/ml	0.4	[0.3–3.4]	0.3	[0.3–1.9]	73.1	[3.8–312.5]	<0.001
Measurable Dryness							
Saxon, g	3.5	[2.4–4.9]	4.2	[3.3–5.3]	2.3	[0.6–3.7]	<0.001
Schirmer, mm	7.0	[2.0–17.9]	3.0	[0.5–12.0]	2.5	[0.0–7.1]	<0.001
Labial Gland Biopsy, *n* (%)							
-Biopsy performed	150	(53)	120	(58)	18	(19)	
-Chisholm grade ≥3	66	(44)	64	(53)	9	(50)	
-Median Score	2	[1–3]	3	[2–3]	3	[3–4]	
Salivary Gland Ultrasound, *n* (%)							
-SGUS = 0	39	(14)	39	(19)	1	(1)	
-SGUS ≥ 6	73	(26)	55	(27)	38	(41)	
SGUS Score	2	[1–4]	4	[2–5]	8	[4–10]	<0.001

*The results are shown as mean and interquartile range unless stated otherwise. * Foreign body or grain of sand feeling in the eye.*

RESULTS

A total of 583 patients were included; the majority of whom were female (462/583, 79%). Median age at symptom onset was 56 [interquartile range (IQR) 49.5–68] years. After subjective dryness, the most common symptoms were arthralgia ($n = 401$, 69%) and myalgia ($n = 324$, 56%). Objective dryness, defined as positive Schirmer and/or Saxon test, was observed in 425 (73%)

of the patients. None of those included had previously undergone radiotherapy or were receiving tricyclic antidepressants at the time of inclusion. Applying the ACR/EULAR pSS criteria across the cohort, in total, 231 (40%) fulfilled the classification criteria and 85 (15%) possessed none of required features (**Supplementary Figure 1**). A comprehensive summary of the entire cohort is included in the supplementary data. Following principal component analysis, hierarchical clustering identified three clearly demarcated groups (**Figure 1**), which were then phenotypically characterized in detail (**Table 1**).

Almost half (283/583; 49%) of the patients largely lacked objective abnormalities. Interestingly, these patients reported most subjective dryness, as well as highest pain and fatigue scores, and were referred to as the somatic group (SG). Just over a third of the patients (206/583; 35%) exhibited objective dryness mainly in the absence of autoimmune features (DAF_{neg}). Xerophthalmia was particularly prevalent, with patients being older and less likely to be female. Anti-SSA(Ro) antibodies were generally negative or of low titers in these patients. Ninety-four (16%) patients displayed objective dryness with autoimmune features (DAF_{pos}). These patients had the most severe xerophthalmia and xerostomia, most prevalent and markedly higher anti-SSA(Ro) antibody titers, and tended to be younger at the time of disease onset.

LBx was performed on only 288 (49%) of the patients. Given the high probability of selection bias, no statistical analysis was performed within the subgroups. A Chisholm grade ≥ 3 was observed in 139 (48%) of the biopsies performed. Of these, 101 (73%) were associated with a pathologic Saxon and/or Schirmer test, whereas only 34 (24%) corroborated a positive anti-SSA(Ro) antibody. The latter point may be at least partially explained by lower referrals for biopsy in anti-SSA(Ro)-positive patients. This can be seen within the clustered phenotypes, where only 19% of the DAF_{pos} patients underwent biopsy, compared to the 58% of DAF_{neg} and 53% of the SG. Although histologic grading tended to be slightly higher among the DAF_{pos} patients, the proportion of biopsies considered positive was similar across all the groups.

Salivary gland ultrasound (SGUS) was performed on all the patients, with cumulative scores ranging from 0 to 12 and a median score of 4 [IQR 2–6] across the entire cohort. Sixty patients (10%) had no detectable SGUS abnormality, and all but 2 were from the DAF_{neg} group (**Supplementary Figure 2**). Only 7 (7%) of the DAF_{pos} patients exhibited an SGUS total of < 5 (**Supplementary Figure 2**). Overall, the median SGUS score was lowest in the SGat 2 [IQR 1–4], with the DAF_{neg} group returning a median score of 4 [IQR 2–5]. Although the DAF_{pos} group scored much higher with a median of 8 [IQR 4–10], differences among all the groups proved highly significant (**Figure 2**). Furthermore, distinct patterns of glandular involvement were observed, with parotid involvement being almost exclusively occurring in the DAF_{pos} group (**Supplementary Figure 3**). With regard to fulfillment of pSS criteria in somatic and DAF_{neg} patients, a much higher proportion was observed in those with SGUS ≥ 6 vs. SGUS < 6 (84 vs. 55%). A full description of the relationship between pSS criteria and cumulative SGUS score is included in the supplementary data (**Supplementary Figure 2**).

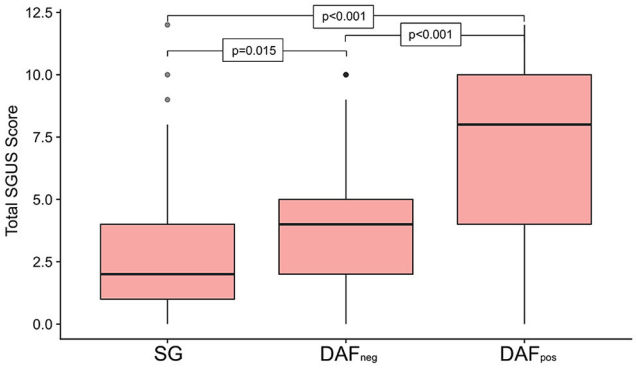

FIGURE 2 | Boxplot summarizing the cumulative ultrasound score for all four sites investigated, subdivided by phenotype group. Significant differences were seen across all the 3 groups, with patients in the Dryness with autoimmune features (DAF_{pos}) group returning the highest scores. Upper and lower box margins represent the interquartile range, with the central dark horizontal line representing the median score. Key: DAF_{neg}, dryness without autoimmune features; DAF_{pos}, dryness with autoimmune features; SG, somatic group.

DISCUSSION

This study evaluated the utility of SGUS in unselected patients referred for evaluation of Sjögren syndrome, and explored the relationship between Sicca syndrome and Sjögren syndrome beyond the ACR/EULAR criteria. Reported dryness proved an unreliable feature, with over a quarter of the patients lacking measurable xerophthalmia and xerostomia. Forty percent of the patients fulfilled the pSS criteria. Close scrutiny revealed significant heterogeneity among the patients, and indeed SGUS could not reliably characterize patients in this group. Given these observations and cohort size, we instead considered all available data to develop a more granular, inclusive characterization of sicca syndrome, better reflecting everyday clinical decision-making. This identified three distinct groups that were compatible with everyday clinical experiences. Clearly the DAF_{pos} group is small and represents a subgroup of more active pSS, given the higher ESSDAI scores and that all but one patient fulfilled the pSS criteria. However, sizable minorities in both the DAF_{neg} and SG groups also fulfilled the pSS criteria. Further interpretation comparing the calculated phenotypes and pSS should be discouraged, given the limited number of LBx performed. While ethically difficult to justify without a clear clinical indication, it is likely that fulfillment of the pSS criteria is underreported. Interestingly, positivity rates of labial gland biopsies were almost identical across all the groups.

The most significant finding was the exceptional correlation between the SGUS and DAF_{pos} patients, particularly with regard to parotid gland abnormalities. There has been some debate about the utility of SGUS as an additional criterion for pSS (15, 16), and our data would support its utility in identifying the most active patients. Indirectly, this corroborates a recent pSS study in which 29% of patients with pSS and low SGUS scores were considered to have milder disease (17).

Despite the large sample size, the retrospective nature of this analysis and the limited availability of LBx limit the interpretation of outcomes. Although both sonographers were blinded during data collection, further limitations arise from using only two observers. No adjustments were made for symptom duration before imaging. This would require serial rescanning and longitudinal analysis, which are beyond the scope of this study.

In conclusion, sicca syndrome comprises a heterogeneous group of conditions in which Sjögren syndrome remains an awkward fit. Performing novel statistical analysis on a broader range of parameters, three distinct phenotypes were identified. Parotid gland involvement in SGUS occurred almost exclusively in patients with autoimmune features. More research is needed to explore this relationship over time and further refine models evaluating sicca syndrome, and in turn assist treatment study design with a view to tailored therapy strategies.

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 at Hannover Medical School (8179_BO_S_2018). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EK: data collection, writing of the manuscript, database work, and patient flow. TSe: data collection and critical review. TSk, SB, AJ, and VG: critical review. TW: critical review and study design. DE: study design, organization, analyses, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.777599/full#supplementary-material

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REVIEW
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Single Cell RNA Sequencing in Autoimmune Inflammatory Rheumatic Diseases: Current Applications, Challenges and a Step Toward Precision Medicine

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Single cell RNA sequencing (scRNA-seq) represents a new large scale and high throughput technique allowing analysis of the whole transcriptome at the resolution of an individual cell. It has emerged as an imperative method in life science research, uncovering complex cellular networks and providing indices that will eventually lead to the development of more targeted and personalized therapies. The importance of scRNA-seq has been particularly highlighted through the analysis of complex biological systems, in which cellular heterogeneity is a key aspect, such as the immune system. Autoimmune inflammatory rheumatic diseases represent a group of disorders, associated with a dysregulated immune system and high patient heterogeneity in both pathophysiological and clinical aspects. This complicates the complete understanding of underlying pathological mechanisms, associated with limited therapeutic options available and their long-term inefficiency and even toxicity. There is an unmet need to investigate, in depth, the cellular and molecular mechanisms driving the pathogenesis of rheumatic diseases and drug resistance, identify novel therapeutic targets, as well as make a step forward in using stratified and informed therapeutic decisions, which could now be achieved with the use of single cell approaches. This review summarizes the current use of scRNA-seq in studying different rheumatic diseases, based on recent findings from published *in vitro, in vivo*, and clinical studies, as well as discusses the potential implementation of scRNA-seq in the development of precision medicine in rheumatology.

Keywords: single cell, RNA sequencing, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, precision medicine

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INTRODUCTION

The beginning of the 21st century was marked by the introduction of new generation sequencing (NGS) technology, leading the way toward a new chapter in genomic and transcriptomic research. NGS technology enabled routine sequencing, quantifying and analyzing millions of transcripts simultaneously in different cell mixtures and tissues (1), while RNA sequencing (RNA-seq) has

become a fundamental tool for performing transcriptome-wide analysis of differential transcript expression and mRNA splicing in physiological and pathological states (2). However, with conventional or bulk RNA-seq the average gene expression level for each transcript in a sample is determined, consisting of a large and heterogeneous population of cells (3). These results reflect the gene expression signatures of predominant cell types in the sample, while the transcriptomic information of rare cell subpopulations and cell-to-cell variability are lost (4). This hampers the precise characterization of a tissue composition in health and disease and thus limits our understanding of disease development and pathology, to the majority of cells present in the tissue (5).

To overcome this problem, a revolutionizing new technology allowing analysis of the whole transcriptome at a resolution of an individual cell, was introduced in 2009 (6) and gained widespread popularity in 2014, with the development of microdroplet method and subsequent lower cost and higher throughput (7). Single cell RNA sequencing (scRNA-seq) now represents an indispensable tool to study complex biological systems (e.g., the immune system) at a single cell level, allowing the discovery of rare and novel cell types, simultaneous characterization of multiple different cell states, and more accurate and integrated understanding of their roles in tissue homeostasis (8). The ability of scRNA-seq to determine the gene expression patterns and molecular events within an individual cell, in contrast to a pooled sample of cells, is transforming our understanding of disease pathology, as well as mechanisms of drug resistance that will eventually lead to development of more targeted therapies (9). The enormous power of scRNA-seq technology has been proven especially in biological systems where cellular heterogeneity is a key aspect, such as immunology and autoimmunity, stem cell biology, and tumor cell biology (4).

In this review, we briefly introduce the development of scRNA-seq, and outline its main concepts, workflows, advantages and challenges. We also provide a detailed description of the current applications of scRNA-seq in autoimmune inflammatory rheumatic diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (SSc). Finally, we discuss the potential implementation of scRNA-seq to facilitate the development of precision medicine in rheumatology.

OVERVIEW OF SCRNA-SEQ TECHNOLOGY

The first single cell transcriptome analysis based on the NGS platform was described in 2009, when Tang et al. (6) combined high throughput RNA-seq technology with single cell cDNA amplification for studying early developmental stages of a mouse embryo. They discovered that scRNA-seq technology can determine the expression of 75% (5,270) more genes in a mouse blastomere than a microarray and identified 1,753 previously unknown splice junctions (6). Since then, the scRNA-seq technology has developed immensely with substantial improvements in protocols, resolution, throughput and precision (10, 11). Now, we have the possibility to analyse the transcriptome of thousands of single cells simultaneously with greater depth and accuracy. In addition to the transcriptome, other single cell molecular technologies that enable unbiased screening of the genome, DNA methylation, chromatin accessibility, and spatial resolution of gene expression are significantly expanding as well (12).

The rapid development and increased popularity of scRNA-seq methodology also led to a large number of existing protocols and commercially available scRNA-seq platforms, each with their own advantages and limitations. The variety of available scRNA-seq methods makes the selection of the most appropriate platform for a study challenging, despite being one of the crucial steps for achieving desired research results. The selection greatly depends on the research question, biological sample, as well as available funding. Various protocols and scRNA-seq methods/platforms with their sensitivity, throughput and cost have been extensively reviewed elsewhere (13–16). It is beyond the scope of this article to describe the technology in detail, but it is necessary to mention some of its main aspects, workflows, and limitations in order to allow further discussion of its application in studying rheumatological disorders.

Typical scRNA-seq Workflow

The typical workflow of scRNA-seq consists of the following major steps: isolation of single cells, messenger RNA (mRNA) capture, reverse transcription and cDNA amplification, library preparation, high throughput sequencing, and bioinformatic data analysis. Different scRNA-seq platforms utilize different technologies to capture and physically separate single cells into individual compartments (reaction units), as well as different chemistry to amplify and create libraries for sequencing (13, 17, 18).

Single Cell Isolation

The isolation of viable and intact single cells from the tissue of interest is a crucial and limiting step, often achieved by enzymatic treatment and/or mechanical agitation (13). Previously, scRNA-seq analysis was thought to require cells, isolated from fresh tissue samples, which substantially limited the human tissue collection. Recently, however, standardized cryopreservation protocols ensuring preserved integrity of the specimen were implemented. This is especially beneficial for performing a larger, multi-center study across multiple physically distant institutions in order to obtain a higher number of cells and samples for scRNA-seq. For example, a standardized cryopreservation protocol for skin samples was established by Mirizio et al. (19). The authors compared transcriptomes of cells obtained from skin tissues, preserved either in CryoStor® CS10 cell preservation medium (frozen) or placed in Roswell Park Memorial Institute (RPMI) medium (fresh). The cryopreserved skin cells had comparable cell viability and yield to freshly prepared single cell solutions. More importantly, gene expression signatures were correlated and conserved across all 18 identified cell clusters. However, cryopreservation negatively affected the keratinocyte populations (19). The quality of preservation observed in the skin samples is in line with that observed by Donolin et al. (20) for synovial tissues. The CryoStor® CS10 preserved

synovial tissue-derived cells retained intact transcriptomes and cell surface phenotypes (20). CryoStor® CS10 preservation offers an acceptable alternative to fresh tissue for single cell isolation and sequencing,

Once a suspension of single cells is obtained, there are several methods that can be used to deliver each cell into an individual reaction compartment or unit. The traditional methods include limiting dilution, micromanipulation, laser capture microdissection (LCM), and flow-activated cell sorting (FACS) (21). Although the traditional single cell isolation methods can be used to separate individual cells into compartments, the downstream analytical processes, such as cell lysis, reverse transcription, and library construction cannot be performed directly in these compartments, requiring an extra step or equipment, which is prone to introducing errors and may lead to significant material loss (4, 11).

Microfluidic devices, on the other hand, can be used for both: to capture each individual cell into one compartment/reaction unit, either a microwell or a microdroplet, as well as to perform downstream, highly standardized and automated reactions directly in every unit (22, 23). Microdroplet technology (i.e., 10x Genomics Chromium) encapsulates each aqueous droplet in a continuous oil phase which contains an individual cell mixed with gel beads with uniquely barcoded set of oligonucleotides, called unique molecular identifiers (UMIs). The mRNAs from the lysed cell bind to the bead oligonucleotides, and with reverse transcription, the bead-specific barcode integrates into the cDNA, allowing subsequent identification of the cell origin after pooling (24–26). Microwell-based technology (i.e., the BD Rhapsody system and Fluidigm C1) has a similar concept with microwells instead of microdroplets. Single cells in suspension are captured into microwell arrays that contain barcoded magnetic beads, comparable to the gel beads used in microdroplet technology (27). Microfluidic technology has gained widespread popularity due to the low sample volume required and low analysis cost (5), however its major limitations are the introduction of empty compartments, and/or the inclusion of two (doublet) or more cells in one compartment (resulting in two different transcriptome profiles assigned to a single cell), leading to systematic error in the scRNA-seq analysis (28). Single cell isolation and the droplet-based microfluidic platform are illustrated in **Figure 1**.

Reverse Transcription and cDNA Amplification

Isolated individual cells are subsequently lysed to release as many intact RNA molecules as possible, followed by mRNA enrichment, usually by oligodT priming. The mRNAs originating from the same cell are tagged with the same UMIs in order to differentiate between transcripts from different cells (29) (**Figure 1**). Reverse transcriptase with low RNase H activity and increased thermostability is frequently used for reverse transcription of mRNA and first strand cDNA synthesis, while the second strand can be generated using different strategies (5). One approach includes a template-switching mechanism at the 5' end of the RNA template (e.g., SMART technology) (29, 30), while another strategy uses either poly(A) or poly(C) tails to ligate the 5' end of cDNA and generate common adaptors for downstream PCR amplification (6, 31).

PCR is typically used to amplify cDNA from small amounts of input material. However, the exponential amplification can potentially bias the representation of gene expression profiles toward shorter amplicons with a lower amount of G-C paired bases (13, 32). To avoid this, UMIs have been introduced. Using UMIs, each transcript can be assigned to its cell of origin, which eliminates PCR bias and improves accuracy (26, 29, 33). As an alternative, *in vitro* transcription that uses T7 RNA polymerase and ensures linear amplification can be performed instead of PCR, but it is time consuming as it requires an additional reverse transcription and may lead to 3′ coverage biases (5, 34, 35).

Library Preparation and High Throughput Sequencing

Amplified and tagged cDNAs from every cell are pooled and sequenced by NGS, using library preparation techniques and sequencing platforms similar to those used for bulk RNA-seq. In general, the methods for cDNA sequencing library preparation can be categorized into two groups: i) full-length and ii) molecular tag-based (13). Full-length methods (e.g., SMART-seq, SMART-seq2, SUPeR-seq, and MATQ-seq) cover the entire transcriptome and are thus suitable for cell-type and isoform discovery, as well as allelic gene expression analysis. However, by using the full-length sequencing method, the samples cannot be combined with UMIs, multiplexed and pooled into a single tube for library generation, thus increasing overall cost and labor intensity (17, 33, 36). On the other hand, molecular tag-based methods (e.g., MARS-seq, STRT-seq, CEL-seq, CEL-seq2, Drop-seq, inDrops) can be combined with UMIs, since they sequence either the 5′ or 3′ end of the transcript and thus enable multiplexing and sample pooling, and consequently reduce cost as well as allow high throughput (17, 25, 37).

Bioinformatic Data Analysis

Although scRNA-seq is now becoming more accessible to many laboratories through commercially available reagents and platforms, computational protocols are still very limited and data analysis requires experienced personnel that can adapt fast with appropriate bioinformatics tools and pipelines required for specific scRNA-seq usage (38). ScRNA-seq data processing usually includes two main procedures: i) pre-processing, including quality control of raw reads, batch effect correction, normalization, read alignment, and gene expression quantification, and ii) downstream analysis consisting of differential expression and gene set enrichment analysis, subpopulation clustering, cell cycle phase assignment, reconstruction of cell trajectory and pseudo time (12, 39–41). Once reads are obtained from a sequencing platform, several quality control steps are necessary before further analyses (14). For example, inviable cells and doublets can contaminate the raw data and therefore need to be identified and removed. Batch effect must be corrected as it can introduce systematic error and confound the technical and biological variability, leading to significant differences in gene expression profile and misinterpretation of the data. The next essential step is normalization of the data, to adjust for several factors, such as

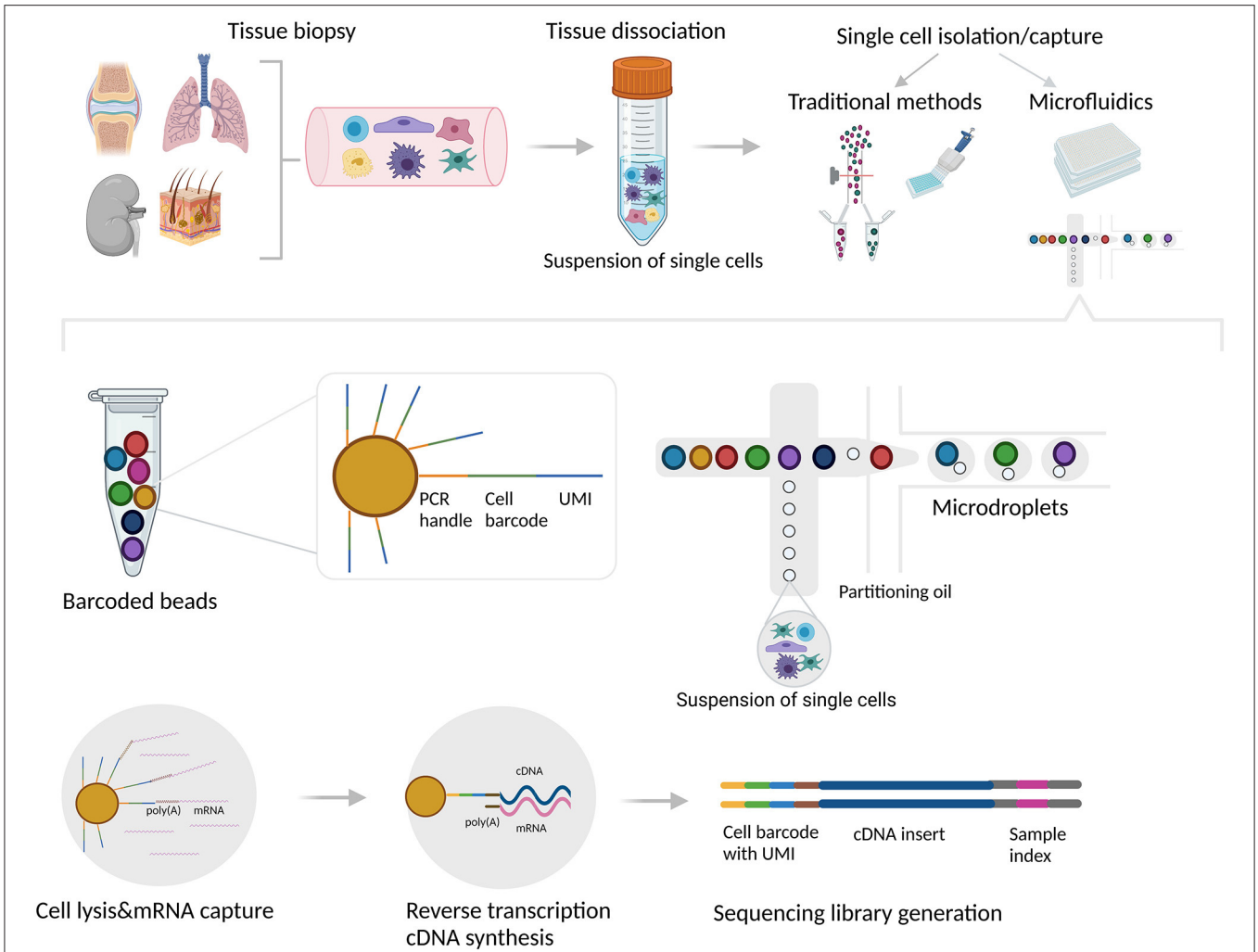

FIGURE 1 | Single cell isolation, capture and library generation using droplet-based microfluidic system. Tissue biopsies are usually dissociated mechanically and/or enzymatically. Single cells can be subsequently isolated and captured into individual reaction units using various methods/platforms. The traditional methods include limiting dilution, micromanipulation, laser capture microdissection (LCM), and flow-activated cell sorting (FACS). Although these methods can be used to separate individual cells into compartments, the downstream analytical processes (cell lysis, reverse transcription, and library construction) cannot be performed directly in these compartments. In contrast, microdroplet technology can be used for both: to capture each individual cell into one compartment/reaction unit, as well as to perform downstream reactions directly in every unit. In the droplet-based microfluidic system (i.e., 10x Genomics Chromium), aqueous droplets are formed in a continuous oil phase. Each droplet contains individual cell mixed with gel beads containing oligo sequences for a bead-specific barcode, unique molecular identifier (UMI) and poly-dT sequence which hybridizes with poly(A) tails of each mRNA. With reverse transcription, the bead-specific barcode integrates into the cDNA, allowing subsequent identification of the cell origin. The figure was created with BioRender.com.

sequencing depth, gene length, dropouts, and other technical effects (5, 11, 40). After the trimming, mapping the reads to a reference genome, and gene expression quantification, subsequent analyses of feature selection, dimensionality reduction, and visualization can be performed using several different bioinformatic tools, extensively reviewed in (41) and listed in the publicly available database (https://www.scrna-tools.org/), such as Scanpy, Seurat, Cell Ranger, t-SNE, UMAP, and SPRING. Single cells can be either clustered into subpopulations, based on their shared transcriptional signature, or trajectory analysis with pseudo-temporal ordering can be performed to phenotypically identify cellular states (5, 11, 40). A scRNA-seq bioinformatics platform that will cover complete bioinformatic pipeline from raw data generation, and storage, as well as virtualized bioinformatic data analysis with state-of-the-art tools on Linux and R is currently being established as part of the ELIXIR research infrastructure for life science information (https://elixir-europe.org).

Challenges and Limitations

The first and most challenging step in scRNA-seq is obtaining a high yield of high quality and viable single cells without causing significant alteration in their transcriptional profile. To achieve this, several optimization steps might be required, depending

on the type of tissue in question. High cell-to-cell variability is a common problem in scRNA-seq data due to the technical (e.g., RNA capture efficiency, limited amount of RNA present in single cells) and biological noise (e.g., stochastic gene expression, a variety of cellular states, cell sizes and cell cycle phases). We can overcome some of these issues by increasing the number of sequenced single cells and by subgrouping cell populations into clusters. Once the clusters are formed, the data from each cluster can be pooled to give a more sensitive and complete representation of the gene expression pattern (13). Furthermore, batch effects and systematic errors commonly occur in scRNA-seq. Batch effects can be a consequence of sample handling (i.e., cells being sequenced separately at different sequencing depth), using different lots of reagents and several biological specimens. They can be corrected by different computational tools, such as ComBat (42), but should preferably be avoided by adequate experimental design, multiple biological replicates (43, 44) or by pooling cells across experimental conditions and samples with subsequent demultiplexing using cell tagging strategy (45), or via genetic variation (46). Other limitations mostly include high cost of the method, challenges related to bioinformatic data analysis, difficulties in the interpretation of the results and their meaningful translation into a clinical setting. The balance between capturing sufficient amounts of RNA and at the same time, obtaining the fidelity of information, is critical when using scRNA-seq technologies. The results should therefore be carefully analyzed and interpreted, also having in mind various limitations of the technology.

APPLICATION OF SCRNA-SEQ IN AUTOIMMUNE INFLAMMATORY RHEUMATIC DISEASES

Autoimmune rheumatic diseases are chronic, debilitating, and painful conditions associated with considerable morbidity and mortality that affect more than five million individuals worldwide (47). They comprise more than 150 different disorders and predominantly affect the connective tissue, joints, skin and the musculoskeletal apparatus (48). The majority of rheumatic disorders develop as a consequence of an abnormal systemic immune response leading to immune cell activation and differentiation, instructed also by the affected tissue microenvironment (e.g., tissue resident stromal cells) which initiates and perpetuates the inflammatory reaction (49, 50). Considerable heterogeneity within and between the affected tissues complicates the understanding of the underlying mechanisms of disease development, and more importantly, contributes to treatment resistance and failure (4, 51). Therefore, using scRNA-seq to uncover the functional status and exact molecular phenotype of individual cells will be paramount in identifying novel therapeutic targets and specific predictors of treatment responses, leading to a more informed and stratified treatment decision-making process. To successfully implement scRNA-seq and other single cell studies in RA and SLE, the NIH Accelerating Medicines Partnership (AMP) RA/SLE Network was established. The AMP RA/SLE network has already made several important discoveries, as well as protocol optimizations and standardizations, that will facilitate future inter-institutional collaborations (20, 51, 52).

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic disease that predominantly affects the joints with recurrent and persistent inflammation that can, if left untreated, eventually lead to joint destruction and disability. Various cell types in the joint are importantly implicated in RA-associated inflammation and tissue destruction, which include synovial fibroblasts, macrophages, lymphocytes, osteoclasts, and vascular endothelial cells. Synovial fibroblasts and macrophages have emerged as the key cells mediating local inflammatory response and destruction in the affected joints and they are currently considered as important therapeutic targets (4, 51, 53, 54).

Synovial Tissue-Derived Fibroblasts

The first study, to our knowledge, using scRNA-seq to decipher the heterogeneity of synovial tissue-derived cells in RA joint was published in 2018 by Stephenson et al. (55) (**Table 1**). By sequencing 20,387 single cells, isolated from joints of five RA patients, they identified two major fibroblast subpopulations (DAF^+ and $THY1^+$), and determined their anatomical positions in the synovial tissue. DAF^+ fibroblasts were predominantly located in the synovial lining, while THY^+ fibroblasts populated the sublining region. Distinct anatomical location also indicated different functions, since DAF^+ fibroblasts had upregulated expression of several genes, particularly important for endothelial cell proliferation and regulation of reactive oxygen species responses, while THY^+ fibroblasts were enriched in metallopeptidase activity and the organization of the extracellular matrix (55). These findings were confirmed by Mizoguchi et al. (56) and Zhang et al. (52) who discovered that the sublining $THY1^+$ fibroblast subset was significantly enriched in patients with RA compared to patients with osteoarthritis (OA). These fibroblasts abundantly secreted pro-inflammatory cytokines, had proliferative and invasive properties, as well as reflected RA disease activity, and correlated with immune cell infiltration in the synovium (56). The exploration of heterogeneity of synovial fibroblasts in RA continued further and it was generally confirmed that RA progression might be driven by the two types of fibroblasts. The sublining $THY1^+$ fibroblasts promote severe and persistent inflammatory arthritis, while $THY1^-$ fibroblasts, restricted to the synovial lining layer, mediate bone and cartilage damage with little effect on inflammation. It was also shown that deleting or removing both subpopulations of fibroblasts suppressed inflammation and bone erosion in murine models of arthritis (57). A subsequent study showed that the lining and sublining fibroblasts do not separate entirely into two clusters, but are instead overlapped, along a gradient corresponding to their location. The expression of surface fibroblast markers changes based on their proximity to endothelial cells, e.g., the expression of CD90:PRG4 decreases gradually with greater distance from endothelial cells, and this is regulated by endothelium-derived NOTCH signaling. Furthermore, genetic

deletion of NOTCH3 or blocking NOTCH3 signaling with a monoclonal antibody inhibited inflammation and prevented joint damage in mice models, proposing NOTCH3 signaling as an important therapeutic target (58).

Synovial Tissue-Derived Macrophages

A specific macrophage subset, defined as HBEGF$^+$ inflammatory macrophages is the dominant CD14$^+$ subset found in RA synovial tissues. These macrophages produce inflammatory cytokines, such as IL1β and growth factors, such as heparin binding epidermal growth factor (HBEGF) and epiregulin, that are formed under the influence of resident fibroblasts and the tumor necrosis factor α (TNFα). They also promote fibroblast invasiveness and thus contribute to fibroblast-mediated joint destruction. Moreover, *ex vivo* experiments showed that non-steroidal anti-inflammatory drugs (NSAIDs), like naproxen, inefficiently block TNFα-induced responses of HBEGF$^+$ macrophages, suggesting that NSAID therapy promotes a classic pro-inflammatory macrophage phenotype, perpetuating inflammation instead of resolution (59). Alivernini et al. (60) later discovered that there are distinct subsets of synovial macrophages that regulate inflammation and remission in RA. By profiling 32,000 synovial macrophages, they identified four distinct macrophage subpopulations with gene expression patterns that differ between patients with early/active RA, treatment-refractory/active RA, and RA in sustained remission. Two macrophage subpopulations (MerTK^{pos}) had gene expression signatures enriched in negative regulators of inflammation. These macrophages abundantly produced inflammation-resolving lipid mediators and induced the tissue repair mechanism in synovial fibroblasts *in vitro*. RA patients in remission with a low percentage of MerTK^{pos} macrophages had an increased risk of disease relapse, indicating that MerTK^{pos} subpopulations could represent a potential treatment strategy for RA (60). In another study, a specific subset of mice synovial macrophages (CX3CR1$^+$), different from monocyte-derived macrophages, was found to display characteristics more common to epithelial cells and formed an internal immunological barrier at the synovial lining. CX3CR1$^+$ lining macrophages limited the inflammatory reaction in the joint by generating a tight-junction-mediated physical and functional barrier that could protect the joint from destruction (61).

Other Immune Cell Types

Focusing on the resolution of inflammation in RA, Andreev et al. (62) discovered that induction of asthma can cause resolution of arthritis following the occurrence of a specific subset of regulatory eosinophils (rEos) in the joints of arthritis-induced mice. These cells have proresolving features and proliferate upon exposure to IL5. RA patients in remission were reported to have rEos in the blood and synovium, while treatment with mepolizumab (anti-IL5 antibody) caused arthritis relapse in RA patients with concomitant asthma (62).

RA is also characterized by production of autoantibodies, such as anti-citrullinated antibodies (ACPA) and rheumatoid factor (RF) by autoreactive B cells. Recently, a published analysis of the B cell frequencies discovered that RF and ACPA B cells are rare in RA blood, but undetectable in healthy controls. ACPA B cells displayed more somatic hypermutations than RF B cells, accompanied by the upregulation of genes that promote class-switching and T cell-dependent responses. On the other hand, RF B cells expressed transcriptional signatures that stimulate rapid memory reactivation through various innate immune pathways. ACPA and RF B cell-enriched transcripts belong to distinct regulatory pathways, indicating that different molecular mechanisms drive ACPA and RF production in RA (63). A subset of RA patients may develop an especially aggressive ACPA-negative destructive RA phenotype that mostly affects larger joints (66). To find biological markers to differentiate this rare type of RA from ACPA-positive, and other subgroups of ACPA-negative RA, whole-exome sequencing and subsequent scRNA-seq were performed to study somatic mutations in CD8$^+$ T cells of an index RA patient. A stable mutation in the clonally expanded CD8$^+$ T cells was discovered and these cells were characterized by upregulated expression of cytotoxic gene products and molecules associated with pro-inflammatory signaling, suggesting that this particular clone might be important for promoting chronic inflammation. Patients with this type of RA might be more effectively treated with therapeutic approaches that target CD8$^+$ T cell-mediated signaling (64).

A Dichotomy Between Local and Systemic Inflammatory Signature

Several novel biomarkers for diagnosis and predicting treatment response in RA have been identified by studies using unbiased high throughput approaches, however, translation into a clinical setting has proven to be challenging. This might be associated with differential inflammatory responses observed systemically in peripheral blood and locally in the synovial tissue of arthritic joints, as recently shown by Lee et al. (65). ScRNA-seq data from matched blood- and synovial tissue-derived cells from a mouse model of arthritis revealed that the inflammatory response in peripheral blood does not reflect the local inflammatory reaction in arthritic joints. Since this kind of dichotomy exists between gene expression signatures and pathways in synovial tissue as compared to peripheral blood, the identification of reliable novel biomarkers in RA may require simultaneous analyses of peripheral blood and disease-associated tissue, as well as standardized pipelines and protocols to generate relevant multidimensional data using scRNA-seq (65) (**Table 1**). A step forward has been made by Donlin et al. (20), who established a robust protocol to obtain a high yield of viable synovial cells, derived from cryopreserved synovial tissues with intact transcriptomes and cell surface phenotypes, which has been adopted and utilized by the AMP RA network (20).

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a prototypical chronic autoimmune disease that can affect multiple organs, including the skin, joints, lungs, and kidneys. The most common and serious manifestation of SLE is lupus nephritis (LN), that affects roughly 50% of patients with SLE and, in 10% of these patients, progresses to end-stage renal disease (67). Due

TABLE 1 | Application of scRNA-seq technology in rheumatoid arthritis.

Sample type	Subjects or mouse model (number)	Cells sequenced (number)	Single cell platform	Main findings	Reference
Human synovial tissue	RA patients ($n = 5$)	Synovial cells ($n = 20,387$)	Drop-seq	13 hematopoietic and fibroblast populations, $CD55^+$ synovial lining fibroblast and $THY1^+$ sublining fibroblasts.	Stephenson et al. (55)
Human synovial tissue	RA patients ($n = 2$), OA patients ($n = 2$)	FACS sorted fibroblasts ($n = 384$)	Smart-seq2	Expanded $PDPN^+CD34^-THY1^+$ sublining fibroblast subset in RA vs. OA, secrete pro-inflammatory cytokines, are proliferative, and invasive, reflect RA disease activity, and correlate with immune cell infiltration.	Mizoguchi et al. (56)
Mice synovial tissue	STIA mice ($n = 3$)	FACS sorted fibroblasts ($n = 2,814$)	10X Genomics Chromium	Sublining $FAP\alpha^+THY1^+$ fibroblasts drive severe and persistent inflammation, lining $FAP\alpha^+THY1^-$ fibroblasts mediate bone and cartilage damage.	Croft et al. (57)
Human synovial tissue and organoids	RA patients ($n = 6$) OA patients ($n = 6$)	Stromal cells ($n = 35,153$), synovial organoid cells ($n = 6,412$)	10X Genomics Chromium	Fibroblasts display positional identity, regulated by endothelium-derived Notch signaling, blocking Notch3 and/or Notch signaling prevents joint damage.	Wei et al. (58)
Human synovial tissue	RA patients ($n = 36$), OA patients ($n = 15$)	Synovial cells ($n = 5,262$)	CEL-Seq2	Expanded sublining $CD34^-THY1^+$fibroblasts, $IL1B^+$ pro-inflammatory monocytes, $ITGAX^+TBX21^+$ B cells and $PDCD1^+$ Tph and Tfh cells in RA vs OA.	Zhang et al. (52)
Human synovial tissue	RA patients ($n = 10$), OA patients ($n = 2$)	FACS sorted $CD14^+$ cells ($n = 940$)	CEL-Seq2	$HBEGF^+$ inflammatory macrophages are the dominant $CD14^+$ subset in RA, promote fibroblast invasiveness and contribute to fibroblast-mediated joint destruction.	Kuo et al. (59)
Human synovial tissue	Active RA ($n = 5$), treatment-refractory RA ($n = 6$), remission RA ($n = 6$), UPA ($n = 4$), healthy ctrl ($n = 4$)	FACS sorted synovial macrophages ($n = 32,000$)	10X Genomics Chromium	$MerTK^{pos}TREM2^{high}$ and $MerTK^{pos}LYVE1^{pos}$ macrophages had gene expression signature enriched in negative regulators of inflammation, abundantly produced inflammation-resolving lipid mediators and induced the repair response of synovial fibroblasts *in vitro*.	Alivernini et al. (60)
Mice synovial tissue	K/BxN serum-induced arthritis mice (ND)	FACS sorted $CD45^+CD11b^+Ly6G^-$ macrophages ($n = 7,362$)	10X Genomics Chromium	CX3CR1+ lining macrophages display features common to epithelial cells, form an internal immunological barrier, limit the inflammatory reaction and protect the joint.	Culeman et al. (61)
Mice synovial and lung tissue	K/BxN serum-induced arthritis mice ($n = 15$); ovalbumin-induced asthmatic mice ($n = 8$)	FASC sorted $CD45^+$ $CD11b^+$ Siglec-F^+ granulocytes (ND)	10X Genomics Chromium	Induction of asthma can cause resolution of arthritis following the occurrence of a specific subset of rEos in the joints, with proresolving features. They are found in blood and synovium of RA patients in remission.	Andreev et al. (62)
Human peripheral blood	RA patients ($n = 6$)	FACS sorted $CD19^+$ B cells ($n = 2,349$)	Modified STRT-Seq	ACPA B cells displayed more somatic hypermutations, and upregulated genes promoting class-switching and T cell-dependent response, RF B cells upregulated genes stimulating memory reactivation through innate immune pathways.	Lu et al. (63)
Human peripheral blood	Index RA patient ($n = 1$)	Magnetic bead separated $CD8^+$ T cells (ND)	10X Genomics Chromium	A stable mutation in the clonally expanded $CD8^+$ T cells, characterized by upregulated expression of cytotoxic gene products and molecules associated with pro-inflammatory signaling in a patient with ACPA-negative destructive RA.	Kelkka et al. (64)
Mice synovial tissue and peripheral blood	antigen-induced arthritis mice ($n = 1$)	Synovial cells ($n = 8,426$), blood cells ($n = 4,310$)	Seq-Well	Shared pathways and upstream regulators (TNF and IFNy) between mice and human synovial cells, no significant overlaps in transcriptional signatures between mice synovial tissue and peripheral blood.	Lee et al. (65)

ACPA, anti-citrullinated antibodies; ctrl, control; FACS, flow-activated cell sorting; IL, interleukin; IFN, interferon; ND, not defined; OA, osteoarthritis; RA, rheumatoid arthritis; rEos, regulatory eosinophils; RF, rheumatoid factor; STIA, serum transfer-induced arthritis; UPA, undifferentiated arthritis; TNF, tumor necrosis factor; Tfh, T folicular helper cells; Tph, T peripheral helper cells.

to the highly diverse clinical manifestations (characterized by flares and remission) and unpredictable disease course, clinical management of SLE remains challenging, calling for further multidimensional studies to improve treatment and prognostic decisions (68, 69).

Renal Tissue-Derived Cells

The first study utilizing scRNA-seq in SLE was published in 2017 (70), and confirmed the upregulated interferon (IFN) response in renal tubular cells of SLE patients, as well as correlation between IFN-response scores and chronicity index, IgG deposition, and proteinuria. SLE patients who responded to therapy had significantly lower IFN scores compared to nonresponders. Interestingly, the gene expression profiles from keratinocytes, isolated from nonlesional skin of patients with LN, also revealed upregulated IFN response and IFN-inducible genes compared to healthy controls. Skin tissue, which is more accessible compared to a renal biopsy, might therefore be exploited to reveal renal injury and damage (70) (**Table 2**). A subsequent study from the same group confirmed these findings using a higher throughput method (the Fluidigm 800-well platform instead of 96-well), with increased cell capture, also allowing for the identification of mesangial cell profiles from patients with LN. A high IFN response score and gene signature associated with fibrosis in tubular cells were associated with treatment failure. scRNA-seq of renal tissues determined molecular signatures clinically relevant to prognosis, which could be used to stratify patients into subgroups to provide a more personalized therapeutic procedure according to the molecular phenotype of the patients (71). The IFN signature was further explored by Arazi et al. (72) who found a clear IFN response in most infiltrating leukocytes from renal biopsies that correlated with the IFN signature found in peripheral blood. The study also revealed upregulated expression of chemokine receptors CXCR4 and CX3CR1 in renal tissues of patients with LN, suggesting their potential as therapeutic targets. Gene expression signatures of leukocytes found in urine and renal biopsies significantly correlated, implying that less invasively obtained urine samples might be used instead of renal biopsies (72). A subsequent study confirmed that proteins found in urine can predict the cell composition of the renal immune infiltrate in LN patients after integrating the urine proteomics with the scRNA-seq of renal biopsies. The urine chemokine gradient significantly correlated with the number of renal-infiltrating $CD8^+$ T cells. The authors concluded that patient-specific pathways could be noninvasively determined in the urine samples, potentially enabling personalized treatment (73).

Peripheral Blood Immune Cells

A number of studies have already shown that the IFN type I cytokine family, involved in the immune response against viral infections, is importantly implicated in the pathogenesis of SLE. IFN I activates JAK/STAT signaling cascade leading to the induction of a variety of IFN-stimulated genes (ISGs), not only in the renal tissue of patients with LN, but also in leukocytes, obtained from peripheral blood of SLE patients (80, 81). Recently, a large scale study found that increased expression of ISGs in different immune cell subpopulations can distinguish pediatric SLE from healthy controls. Expansion of specific subpopulations enriched in ISGs was especially pronounced in pediatric, as well as adult SLE patients with the highest disease activity (74). In addition to type I IFNs, Hjorton et al. (75) demonstrated that RNA-containing immune complexes (RNA-ICs), found in patients with SLE, have the capacity to induce type III IFN (e.g., IFNλ1–3) production, which increased in the presence of GM-CSF, IL3, IL6 and IFNα2b, indicating that both type I and type III IFNs have a contributing role in SLE. Using scRNA-seq, they found that type III IFNs, dominated by IFNλ1, were exclusively expressed in a specific minor cluster of plasmacytoid dendritic cells (pDC), within a subset of the type I IFN expressing pDCs (75). The important role of IFNλ in immune dysregulation and tissue inflammation was later confirmed in a mouse model of TLR7-induced lupus. IFNλ receptor deletion resulted in significantly lower immune cell activation, and reduced skin and kidney damage of lupus mice. ScRNA-seq analysis of mice spleen and human peripheral blood revealed that only mice neutrophils and human B cells upregulate ISGs in response to stimulation with IFNλ. However, IFNλ was able to activate keratinocytes and mesangial cells to produce chemokines and promote inflammation in the skin and kidneys. IFNλ may thus exert its effects at barrier sites, rather than through peripheral immune cells (76).

Type I IFN signature is particularly enhanced in low density granulocytes (LDG), a subgroup of neutrophils, which have been shown to be implicated in the pathogenesis of SLE (82). In addition to upregulated IFN production, LDGs have increased neutrophil extracellular trap (NET) formation activity, pro-inflammatory effects and lower apoptosis rates compared to normal density granulocytes (83). Differential gene expression analysis revealed multiple upregulated ISGs in the LDG cluster compared with other cell clusters in SLE patients, indicating that LDGs significantly drive the type I IFN signature. There are two types of LDGs present in SLE patients: immature and intermediate mature LDGs with distinct transcriptional and epigenetic profiles, as well as functional properties. The presence of intermediate mature LDGs was associated with SLE organ damage, as well as the presence and severity of coronary artery disease (78). Analyzing several deposited scRNA-seq datasets, as well as their own experimental dataset, Deng et al. (79) confirmed that among SLE peripheral blood immune cells, LDGs exhibited the highest ISG activity, further pointing toward their prominent role in SLE pathogenesis. Their hypothesis that LDG and neutrophils infiltrate the kidneys during LN was confirmed in a lupus model of MRL/lpr mice. A prominent granulocyte infiltration was observed in the affected kidneys, decreasing significantly when mice were treated with avacopan (a selective inhibitor of the C5a receptor), which blocked C5a-mediated chemotaxis and granulocyte infiltration, as well as improved renal function (79).

Systemic Sclerosis (SSc)

Systemic sclerosis is an incurable, chronic orphan disease characterized by inflammation, vascular changes, autoimmunity and fibrosis of the skin and internal organs, including the heart, kidneys and lungs (84). Interstitial lung disease (ILD)

TABLE 2 | Application of scRNA-seq technology in systemic lupus erythematosus.

Sample type	Subjects or mouse model (number)	Cells sequenced (number)	Single cell platform	Main findings	Reference
Human renal and skin tissue, peripheral blood	SLE patients with LN ($n = 24$), healthy controls ($n = 5$)	Renal cells ($n = 361$), skin keratinocytes ($n = 329$), $CD4^+CD14^+$ PBMC ($n = 209$)	Fluidigm C1 (96-well)	Upregulated IFN response score in renal tubular cells correlated with chronicity index, IgG deposition, and proteinuria, upregulated IFN-response and ISGs also in keratinocytes from skin of LN patients.	Der et al. (70)
Human renal and skin tissue	SLE patients with LN ($n = 21$), healthy controls ($n = 3$)	Tubular cells ($n = 1,221$), skin keratinocytes ($n = 1,939$)	Fluidigm C1/SMART-Seq (800-well)	A high IFN response score and fibrotic signature in tubular cells were associated with treatment failure.	Der et al. (71)
Human renal tissue and urine	SLE patients with LN ($n = 24$), healthy controls ($n = 10$)	FACS sorted $CD45^+$ renal leukocytes ($n = 2,736$), $CD45^-CD10^+$ epithelial cells ($n = 145$), $CD45^+$ urine leukocytes ($n = 577$)	CEL-Seq2	21 leukocyte subsets found in renal biopsies, IFN signature found in most leukocytes, correlated with that in peripheral blood, upregulated expression of chemokine receptors CXCR4 and CX3CR1 in renal tissues of patients with LN, correlation between gene expression signatures of leukocytes from urine and kidneys.	Arazi et al. (72)
Human renal tissue	SLE patients with LN ($n = 6$)	FACS sorted $CD45^+$ renal leukocytes, $CD45^-CD10^+$ epithelial cells (ND)	CEL-Seq2	Proteins found in urine can predict the cell composition of the renal immune infiltrate, the urine chemokine gradient significantly correlated with the number of kidney-infiltrating $CD8^+$ cells.	Fava et al. (73)
Human peripheral blood	SLE children ($n = 33$), healthy children ($n = 11$), adult SLE patients ($n = 8$), adult healthy controls ($n = 6$)	PBMCs from children ($n = 276,000$), PBMCs from adults (82,000)	10X Genomics Chromium	Increased expression of ISGs in children with SLE vs healthy controls, ISG^{hi} derived mostly from 8/20 PBMC subpopulations (especially plasma cells), ISG enriched subpopulations were associated with high disease activity in children and adult SLE patients.	Nehar-Belaid et al. (74)
Human peripheral blood	Healthy blood donors ($n = 2$)	Isolated pDCs ($n = 1,413$), stimulated with RNA-IC, IL-3, and IFN-α2b	ddSEQ (Biorad)	RNA-ICs induced type III IFN (e.g., IFN-λ1–3) production in pDCs in a TLR-MyD88-dependent manner, type III IFNs, dominated by IFN-λ1, were exclusively expressed in a specific minor cluster of pDCs, within a subset of the type I IFN expressing pDC, also enriched in genes coding for CCL4, CCL3, TNF, CCL3L3, and IL12A.	Hjorton et al. (75)
Human peripheral blood, mouse spleen	Healthy blood donors (ND), wild type mice (ND)	Human whole blood cells ($n = 19,266$), mice spleen cells ($n = 18,520$), stimulated with IFN-λ2 or IFN-α	10X Genomics Chromium	IFN-λ receptor deletion resulted in significantly lower immune cell activation, and reduced damage of skin and kidneys in lupus mice, only mice neutrophils and human B cells upregulated ISGs in response to stimulation with IFN-λ, IFN-λ activated keratinocytes and mesangial cells to produce chemokines.	Goel et al. (76)
Human peripheral blood	SLE patients ($n = 3$), healthy controls ($n = 2$)	Isolated B cells ($n = 15,039$)	10X Genomics Chromium	IFN signature determined in a subset of switched memory B cells, increased expression of ISGs in multiple B cell clusters from SLE patients, upregulated expression of CD52 in B cells.	Bhamidipati et al. (77)
Human peripheral blood	SLE patients ($n = 3$)	Isolated PBMCs ($n = 26,925$)	10X Genomics Chromium	LDGs significantly drive the type I IFN signature in SLE patients, two subpopulations of LDGs identified in SLE: immature and intermediate mature LDGs, the latter are associated with SLE organ damage and the presence and severity of coronary artery disease.	Mistry et al. (78)
Human peripheral blood	SLE patients ($n = 2$), healthy controls ($n = 1$)	Isolated PBMCs ($n = 26,925$ together with deposited databases)	10X Genomics Chromium	LDGs exhibited the highest ISG activity in SLE PBMCs, ISG expression was associated with PLSCR1, TCF4, IRF9 and STAT1, prominent granulocyte infiltration was observed in kidneys of a murine lupus model (MRL/lpr mice), decreasing significantly after treatment with avacopan (a selective inhibitor of the C5a receptor).	Deng et al. (79)

IFN, interferon; IL, interleukin; ISG, interferon inducible genes; LDG, low density granulocytes; LN, lupus nephritis; ND, not defined; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid dendritic cells; RNA-IC, RNA-immune complex; SLE, systemic lupus erythematosus.

is a frequent complication of SSc (in up to 80% of patients), which can result in respiratory failure and death in about a third of patients (85, 86). The striking patient-to-patient variability and a lack of knowledge in understanding the mechanisms underlying inflammation and fibrosis in ILD are associated with very limited therapeutic options currently available for SSc-ILD patients and consequently with high mortality rates (87). Novel studies exploiting scRNA-seq data of SSc-ILD lung and skin tissues (**Table 3**) might provide a valuable insight into previously unknown subsets of cells that drive the pathogenesis of SSc and identify novel therapeutic targets.

Lung Tissue-Derived Cells

In 2019, Valenzi et al. (88) analyzed lung tissue-derived cells from SSc-ILD patients and healthy controls, focusing on fibroblasts and myofibroblasts, which play a key role in fibrosis due to their capacity of extracellular matrix depositing and remodeling. A great expansion of myofibroblasts (expressing high levels of ACTA2) appeared in SSc-ILD samples, including a subpopulation of actively proliferating myofibroblasts. Myofibroblasts phenotypically changed in SSc-ILD and significantly upregulated expression of collagens and other profibrotic genes. While myofibroblasts can derive from multiple sources in pathological states, it was anticipated that MFAP5^{hi} fibroblasts may act as their progenitors in SSc-ILD (88). In 2020, Tsukui et al. (89) utilized scRNA-seq to characterize all collagen-producing cells in normal and SSc-ILD lungs. Using a murine model with bleomycin-induced SSc, they identified a specific cluster of fibroblasts, mostly found in fibrotic murine lungs. This cluster expressed collagen triple helix repeat containing protein 1 (CTHRC1), previously shown to be increased in the lungs of patients with fibrosis. ScRNA-seq of human normal and fibrotic lungs revealed that CTHRC1-expressing fibroblasts were uniquely present in fibrotic lungs and localized within fibroblastic foci. This fibroblast subpopulation exhibited enhanced migratory properties and could be importantly implicated in the development of fibrosis in mice and humans (89).

Idiopathic pulmonary fibrosis (IPF) is another form of ILD of unknown etiology, sharing some similar aspects and mechanisms of development with SSc-related ILD. The significant role of inflammation is more established in the pathogenesis of SSc-ILD, while investigating the shared and distinct mechanisms between SSc-ILD and IPF, may yield important new insights influencing therapeutic development for both diseases (96). Sequencing single cells from IPF, SSc-ILD and healthy lung tissue, Valenzi et al. (90) identified three main subsets of macrophages: SPP1^{hi}, FABP4^{hi}, and FCN1^{hi} monocyte-derived macrophages. Type I IFN signaling together with increased expression of ISGs were significantly upregulated in macrophages obtained from SSc-ILD patients compared to IPF, while IFNγ signaling was upregulated in macrophages, cytotoxic T cells, and natural killer cells of IPF. Alveolar type 1 (AT1) cells were decreased in both diseases compared to healthy tissue and exhibited the most distinct expression patterns between IPF and SSc-ILD. SSc-ILD lung tissue-derived cells showed deregulated expression of genes involved in protein ubiquitination and catabolism, as well as cellular response to oxygen levels, suggesting that cell stress leads to death of AT1 cells. The authors identified for the first time an aberrant subset of KRT5^{-}KRT17^{+} basaloid cells with high expression of markers of cellular senescence and epithelial mesenchymal transition in SSc-ILD. Compared to IPF, SSc-ILD patients had upregulated genes involved in vasculogenesis, prostaglandin biosynthesis, and platelet-derived growth factor receptor signaling, implicating a significant expansion of the endothelium (90).

Immune Complexes

Various studies demonstrated the presence of immune complexes (ICs) in the sera, lungs, and bronchoalveolar lavage (BAL) fluid of SSc patients, indicating their potential role in disease pathogenesis, possibly through myeloid cell activation (97–99). Gao et al. (91) confirmed this hypothesis, showing evidence that ICs activate monocytes to promote lung fibroblast migration through secretion of osteopontin (OPN), which was potentiated by monocyte colony stimulating factor (MCSF) and IL6. The levels of OPN were increased in the serum of SSc-ILD patients and its expression was significantly enriched in lung tissue macrophages, as demonstrated by scRNA-seq. OPN has a potential to be used as a systemic biomarker to predict future SSc-ILD progression, as well as a novel therapeutic target (91).

Skin Tissue-Derived Cells

The majority of SSc patients develop skin fibrosis in earlier stages of the disease. As in lung fibrosis, the development of skin fibrosis is also driven mainly by fibroblasts and myofibroblasts (100). A largescale study by Tabib et al. (92) showed that the transcriptional profile of SSc dermal fibroblasts changed significantly compared to healthy dermal fibroblasts. They identified a new fibroblast subcluster from SSc patients that expressed PRSS23 and had upregulated expression of genes involved in extracellular matrix and collagen fibril organization, wound healing, and skeletal system development. Within the PRSS23^{+} cluster, a second population (SFRP2^{+}, SFRP4^{+}), identified as myofibroblasts, was found exclusively in SSc skin. The pseudo-time analysis showed that the SFRP2^{hi} fibroblasts were the immediate progenitors of myofibroblasts, however only a fraction of SFRP2^{hi} SSc fibroblasts differentiated into myofibroblasts, which is driven by upstream transcription factors, including FOSL2, RUNX1, STAT1, FOXP1, IRF7, CREB3L1, and SMAD3 (92). A subsequent study by the same group focused on skin myeloid cell populations and revealed 12 myeloid cell clusters, three of which were specifically identified in SSc skin. One SSc-associated cluster consisted of macrophages that expressed high levels of FCGR3A, while the second SSc-associated myeloid cluster expressed various monocyte markers. The presence of the latter cluster was was associated with more severe skin disease. Proliferating macrophages and dendritic cells were determined almost uniquely in SSc skin. Gene expression profiles in these and other myeloid subclusters revealed high expression of chemokines and enrichment in processes associated with innate immune activation, possibly through toll-like receptors (TLRs). However, there was significant variability in the appearance and activation status of myeloid cells observed

TABLE 3 | Application of scRNA-seq technology in systemic sclerosis.

Sample type	Subjects or mouse model (number)	Cells sequenced (number)	Single cell platform	Main findings	Reference
Human lung tissue	SSc-ILD patients ($n = 4$), healthy controls ($n = 4$)	Lung tissue cells ($n = 56,196$)	10X Genomics Chromium	Three fibroblast subpopulations: SPINT2^{hi}, MFAP5^{hi}, and WIF1^{hi}, expanded myofibroblasts in SSc-ILD, with upregulated expression of collagens and other profibrotic genes.	Valenzi et al. (88)
Human and mice lung tissue	SSc-ILD patients ($n = 2$), IPF patients ($n = 3$), bleomycine induced SSc mice ($n = 2$), control mice ($n = 2$)	Human lung tissue cells ($n = 83,316$), murine lung tissue cells ($n = 25,953$)	10X Genomics Chromium	A specific cluster of fibroblasts present uniquely in murine and human fibrotic lungs with high expression of CTHRC1, localized within fibroblastic foci.	Tsukui et al. (89)
Human lung tissue	SSc-ILD patients ($n = 8$), IPF patients ($n = 8$), healthy controls ($n = 5$)	Lung tissue cells ($n = 85,756$)	10X Genomics Chromium	3 main subsets of macrophages: SPP1^{hi}, FABP4^{hi} and FCN1^{hi}. Type I IFN signaling was upregulated in SSc-ILD, IFNγ signaling was upregulated in IPF. AT1 exhibited the most distinct expression patterns between IPF and SSc-ILD, KRT5-/KRT17+ aberrant basaloid cells were identified in SSc-ILD.	Valenzi et al. (90)
Human lung tissue	SSc-ILD patients ($n = 3$)	FACS sorted CD45+, EPCAM+ and CD31+ lung tissue cells ($n = 29,000$)	10X Genomics Chromium	ICs activate monocytes to promote fibroblast migration through secretion of OPN, further amplified by MCSF and IL6. The levels of OPN are increased in the serum of SSc-ILD patients and its expression is significantly enriched in lung tissue macrophages.	Gao et al. (91)
Human skin tissue	SSc patients ($n = 12$), healthy controls ($n = 10$)	Skin tissue cells ($n = 65,199$)	10X Genomics Chromium	A new subcluster of fibroblasts in SSc, expressing PRSS23, SSc skin myofibroblasts co-expressed SFRP2 and SFRP4, SFRP2^{hi}WIF1^{-} fibroblasts are the progenitors of myofibroblasts.	Tabib et al. (92)
Human skin tissue	SSc patient ($n = 1$), healthy control ($n = 1$)	Skin tissue cells ($n = 184$)	SmartSeq2	Endothelial cells in SSc were enriched in extracellular matrix generation, negative regulation of angiogenesis and EMT, the most upregulated genes in SSc were HSPG2, and APLNR.	Apostolidis et al. (93)
Human skin tissue	SSc patients ($n = 12$), healthy controls ($n = 10$)	Skin tissue cells ($n = 65,199$); myeloid cells ($n = 2,465$)	10X Genomics Chromium	Myeloid subpopulation in SSc skin that expressed monocyte markers (FCN1, EREG, S100A8 and S100A9) was associated with more severe skin disease. Proliferating macrophages and pDCs were determined almost uniquely in SSc skin.	Xue et al. (94)
Human skin tissue	SSc patients ($n = 27$), healthy controls ($n = 10$)	FACS sorted skin CD3+ T-cells ($n = 3,729$)	10X Genomics Chromium	Identified a cluster of recirculating CXCL13^{+} T cells uniquely detected in SSc skin, with gene expression profile similar to Tfh cells, adjacent to CD20^{+} B cells within inflammatory infiltrates in the skin, lower frequency of CD3^{+}CXCL13^{+} cells in SSc patients treated with immunosuppressive drugs.	Gaydosik et al. (95)

AT1, alveolar type 1; EMT, epithelial-to-mesenchymal transition; FACS, flow-activated cell sorting: IC, immune complex; IFN, interferon; IL, interleukin; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; MCSF, macrophage colony-stimulating factor; OPN, osteopontin; pDC, plasmacytoid dendritic cells; SSc, systemic sclerosis.

between patients, suggesting different underlying mechanisms of pathogenesis and/or temporal disease activity (94).

Some of the most evident clinical characteristics of SSc, including Raynaud's phenomenon, telangiectasias, and pulmonary arterial hypertension, develop as a consequence of vascular injury and the underlying endothelial dysfunction (101, 102). The endothelial cell gene expression profile in the skin of SSc patients was found to be enriched in processes associated with extracellular matrix generation, negative regulation of angiogenesis and epithelial-to-mesenchymal transition. Among the most upregulated genes in SSc skin vs healthy controls were HSPG2, an extracellular matrix protein, induced by TGFβ, and APLNR, that plays a role in angiogenesis. These two proteins have not been previously associated with SSc pathogenesis, however, they have been linked to vascular dysfunction and fibrosis in different settings, making them of interest in further SSc studies (93).

Several T-lymphocyte subsets and associated cytokines have been implicated in the inflammatory and fibrotic processes of SSc, however their heterogeneity in SSc skin has only recently been revealed by Gaydosik et al. (95). They detected several subsets of infiltrating, as well as tissue-resident T-lymphocytes in healthy and SSc skin that were enriched in different signaling pathways. A cluster of recirculating $CXCL13^+$ T cells was uniquely detected in SSc skin. These cells had a gene expression profile similar to that of Tfh cells, although they lack the canonical Tfh expression of CXCR5 and BCL6. They were found adjacent to $CD20^+$ B cells within inflammatory infiltrates in the skin, indicating they might promote B cell responses. A significantly lower frequency of $CD3^+CXCL13^+$ cells was detected in SSc patients treated with immunosuppressive drugs, compared with untreated patients. This indicates that a more targeted T cell-based therapy might be used in SSc patients, resulting in an improved efficacy and lower toxicity (95).

Other Rheumatic Diseases

Psoriatic Arthritis

Psoriatic arthritis (PsA) is an inflammatory arthritis of the joints, affecting approximately one third of patients suffering from skin psoriasis. The pathogenesis of PsA is complex and involves multiple cell types, such as osteoblasts and osteoclasts, as well as immune cells within the joint synovial lining tissue and/or fluid (103). ScRNA-seq of synovial fluid from affected joints of PsA patients revealed that the predominant cell types identified belong to a monocyte/macrophage cluster, representing classical, non-classical and intermediate cells. Compared to RA and OA, the frequency of $CD14^+CD6^-$ classical monocytes/macrophages was reduced, while the frequency of $CD14^+CD16^+$ intermediate monocytes/macrophages was increased in the synovial fluid of PsA patients (**Table 4**). Protease-activated receptor 2 (PAR2) expression was found within monocytes/macrophage clusters and consistent with this, serine proteinases that bind to PAR2, were detected in PsA synovial fluid. Monocyte/macrophage PAR2 activation by tryptase-6 resulted in secretion of high levels of monocyte chemoattractant protein-1 (MCP-1). The invading macrophages can thus both produce and respond to tryptase-6 via PAR activation, and mediate further recruitment of peripheral monocytes/macrophages into the inflamed joint, leading to sustained inflammation and disease progression. Typtase-6-PAR2 signaling pathway may thus represent a novel therapeutic target in PsA (104).

A large scale study used complementary single cell approaches to study leukocytes from the affected joints and peripheral blood of PsA patients. They reported on 16 clusters of memory $CD4^+$ and $CD8^+$ T cells in synovial fluid of affected joints. A specific cluster of synovial $CD8^+$ T cells exhibited higher expression of the proliferation markers, indicating their active proliferation within inflamed joints. Increased expression of the MHCII genes HLA-DRB1 and HLA-DRA, as well as the effector molecules granzyme A (GZMA) and granzyme B (GZMB) was observed in synovial fluid-derived $CD8^+$ T cells, as compared to peripheral blood-derived $CD8^+$ T cells. Furthermore, the T cell receptor alpha chain gene TRAV27 was also significantly upregulated in the synovial fluid $CD8^+$ T cell cluster, indicating a clonal expansion of this subpopulation in the synovium that was confirmed by scRNA-seq. The expanded $CD8^+$ T cell population was characterized by increased expression of chemokine receptor CXCR3, while two CXCR3 ligands, CXCL9 and CXCL10, were elevated in PsA synovial fluid (105).

Axial Spondyloarthritis

Axial spondyloarthritis (axSpA) is an inflammatory disease of the axial skeleton associated with significant pain and disability. AxSpA frequently occurs together with inflammatory bowel diseases (IBD) and the overlapping characteristics present significant challenges in the diagnosis and treatment approaches in individuals afflicted simultaneously with both diseases (110). Performing scRNA-seq in PBMCs from patients with axSpA, Crohn's disease (CD) and a combination of both, Lefferts et al. (106) discovered an expansion of mature $GZMB^+$ T cells of both $CD4^+$ and $CD8^+$ lineages in the peripheral blood of CD-axSpA patients compared to patients with either CD or axSpA (**Table 4**). Furthermore, a prominent IFN signature was observed in all T cell populations from CD-axSpA patients, together with elevated plasma levels of IFNγ and IL6. These data demonstrated that fundamental immunological differences exist between CD-axSpA, axSpA and CD, indicating that CD-axSpA is a distinct disease entity, requiring distinct therapeutic approaches for effective treatment (106) (**Table 4**).

Sjögren's Syndrome

Patients with Sjögren's syndrome (SjS), an autoimmune disease of the exocrine glands, are characterized with oral and ocular dryness, resulting from extensive lymphocytic infiltration of the salivary and lacrimal glands (111). Approximately 30–40% of patients develop systemic manifestations that involve the kidneys, lungs, and nervous system (112). The disease is very heterogeneous, which limits the complete understanding of the pathogenic mechanisms, and complicates the discovery of novel therapeutic targets. Furthermore, in certain cases, it may progress to non-Hodgkin's lymphoma. Hong et al. (107) identified two $CD4^+$ T cell subpopulations that were significantly expanded in peripheral blood of patients with SjS (**Table 4**). The first subpopulation was characterized by high expression

TABLE 4 | Application of scRNA-seq technology in PsA, AxSpA, SjS and KD.

Sample type	Subjects or mouse model (number)	Cells sequenced (number)	Single cell platform	Main findings	Reference
Human synovial fluid	PsA patients ($n = 3$)	Synovial fluid cells (ND)	10X Genomics Chromium	Reduced CD14+CD6- classical and increased CD14+CD16+ intermediate monocytes/macrophages in PsA, Monocyte/macrophage PAR2 activation by tryptase-6 resulted in increased secretion of MCP-1.	Abji et al. (104)
Human synovial tissue, synovial fluid and peripheral blood	PsA patients ($n = 9$)	FACS sorted $CD4^+CD8^+$ T cells ($n = 41,202$ from PBMC and synovial fluid; $n = 251$ from synovial tissue)	10X Genomics Chromium, SmartSeq2	16 clusters of memory CD4 and CD8 T cells in synovial fluid, a specific cluster of synovial CD8 T cells with higher expression of MKI67 and STMN111. T-cell receptor alpha-chain gene TRAV27 was significantly upregulated in the CD8 T cell cluster. The expanded CD8 T cell population was characterized by increased expression of CXCR3, while CXCL9 and CXCL10, were elevated in PsA synovial fluid.	Penkava et al. (105)
Human peripheral blood	AxSpA ($n = 2$), CD (n = 2), CD-axSpA ($n = 2$) patients, healthy controls ($n = 2$)	FACS sorted $CD45^+$ leukocytes ($n = 50,580$)	10X Genomics Chromium	CD-axSpA patients showed an expansion of mature GZMB+ T cells f both CD4+ and CD8+ lineages in the peripheral blood, a prominent IFN activation signature and elevated plasma levels of IFN-y and IL-6.	Lefferts et al. (106)
Human peripheral blood	SjS patients ($n = 5$), healthy controls ($n = 5$)	Isolated PBMC ($n = 57,288$)	10X Genomics Chromium	Significant expansion of CD4+ cytotoxic T-lymphocytes and CD4+ TRAV13-2+ T cell in SjS, upregulated type I and II IFN signaling, and increased expression of ISGs (IFITM3, IFITM2, IFITM1, and XAF1).	Hong et al. (107)
Human peripheral blood	KD child ($n = 1$), healthy child ($n = 1$)	Isolated PBMC ($n = 19,197$)	10X Genomics Chromium	Identified 14 cell clusters in KD samples, expanded populations of NKT cells and plasmacytoid dendritic cell, lower frequency of naïve CD8+ T cells, T helper cell, B cells, multilymphoid progenitor cells in KD child. Major limitations: small samples size and lack of a validation cohort.	Fan et al. (108)
Human peripheral blood	KD children ($n = 2$), healthy children ($n = 2$)	Enriched monocytes ($n = 8,880$)	BD Rhapsody	$CD14^+CD16^-$ classical, $CD14^+CD16^+$ intermediate and $CD14^-CD16^+$ nonclassical monocytes were found in KD, classical monocytes were significantly expanded and expressed higher levels of SELL and MALAT, and lower levels of CXCL8 and JUN, classical monocytes in KD are less differentiated compared to their counterparts in healthy children.	Geng et al. (109)

AxSpa, axial spondyloarthritis; CD, Chron's disease; FACS, flow-activated cell sorting; GZMB, granzyme B; IFN, interferon; IL, interleukin; KD, Kawasaki disease; ND, not defined; NKT, natural killer T cells; PBMC, peripheral blood mononuclear cells; PsA, psoriatic arthritis; SjS, Sjogren's syndrome.

of cytotoxicity genes ($CD4^+$ cytotoxic T-lymphocytes), and the second had increased expression of T cell receptor (TCR) variable genes ($CD4^+$ $TRAV13\text{-}2^+$ T cells). Upregulated type I and II IFN signaling, together with increased expression of ISGs was found in most immune cells in SjS patients. The specific expansion of $CD4^+$ cytotoxic T-lymphocytes may be important for disease development and its depletion might be a promising treatment strategy (107).

Kawasaki Disease

Kawasaki disease (KD) is an acute systemic vasculitis, predominantly affecting vessel walls of medium-sized arteries and typically occurs in children. The disease is more frequent in children of Asian origin and is the most common cause of acquired heart disease in children in developed countries (113). Although the etiology of KD remains unknown, several studies have shown that it might develop as a consequence of an aberrant immune response (114). This was confirmed by two different studies performing scRNA-seq in PBMCs of KD patients (108, 109) (**Table 4**). The first one identified 14 cell clusters in KD samples, with expanded populations of natural killer T (NKT) cells and pDC, and lower frequency of naïve $CD8^+$ T cells, T helper cells, B cells, and lymphoid progenitor cells. Although the study provided evidence of immune dysregulation in KD patients, there is a significant limitation of including only one KD patient and one healthy control, as well as the lack of a larger validation cohort (108). The second study focused on monocyte subsets, and discovered three monocyte subpopulations in KD children, including $CD14^+CD16^-$ classical, $CD14^+CD16^+$ intermediate and $CD14^-CD16^+$ nonclassical monocytes. In KD patients, classical monocytes were significantly expanded compared to healthy children. Trajectory analysis showed that classical monocytes in KD eventually differentiated into nonclassical monocytes. This transition occurred through intermediate monocytes, as well as through a phenotype more similar to classical monocytes found in healthy children. Classical monocytes in KD might thus have a less differentiated phenotype compared to their healthy counterparts. Altered monocyte subpopulations might be considered as biomarkers for KD diagnosis and treatment (109).

A Step Toward Precision Medicine in Rheumatic Diseases

An important unmet need in rheumatic autoimmune inflammatory diseases is the identification of cell and/or molecular biomarkers that would predict which drug would be most appropriate for an individual or a group of patients to achieve disease remission sooner. The frequently utilized trial and error, "one- size- fits-all" therapeutic approach often leads to drug failure and is associated with reduced patients' quality of life, as well as presents a substantial financial burden. Hence, it should be replaced with a more targeted approach, that could ensure the best possible management of the disease for individual patients (115), which is also the ultimate goal of precision medicine. A prerequisite for successful implementation of precision medicine into the clinical setting is identification of biomarkers, that would stratify patients into responder/nonresponder groups. The clinical utility of scRNA-seq for the purposes of precision medicine have already been demonstrated in many biomedical fields, especially in oncology, and such studies provide valuable and helpful information, especially regarding experimental design and data analysis (38, 116). Notably, existing scRNA-seq studies, described in this review, have already enabled discoveries of novel subsets of cells with distinct anatomical positions, transcriptional profile and function in humans, as well as in mouse models of rheumatic diseases (**Tables 1–4**). Moreover, enrichment of certain cell types with specific transcriptional profiles in the affected tissue (e.g., specific clusters of synovial macrophages) has been associated with disease remission or relapse after using a conventional therapeutic approach in RA (60), while ISG-enriched subpopulations of PBMCs were associated with high disease activity in SLE patients (74). Although this abundance of data already revealed some promising biomarkers, additional studies with molecular characterization of the disease on both systemic and local, tissue levels are required. Furthermore, tissue-based *in vitro* models, coupled with scRNA-seq will enable better understanding of the complex mechanism of drug failure or response, as well as help identify novel therapeutic targets. For example, *ex vivo* tissue models, 3D models, or organoids could be used to identify novel drug candidates or serve as screening platforms (117). A 3D organoid co-culture of synovial fibroblasts and endothelial cells has yielded results (58) of the NOTCH3 signaling cascade, that could be an important novel therapeutic target in RA. Another possibility is to use patient-derived cells *ex vivo* (i.e., in organoids) to predict individual patient drug responses (118). This was demonstrated by Kuo et al. (59) who used dissociated synovial tissue-derived cells from RA patients, for testing currently used anti-inflammatory therapies. The response of synovial macrophages to each drug resulted in a distinct gene expression pattern, which may ultimately affect the patient's outcome. This type of patient-oriented and molecular-driven approach could inform and guide the design and patient selection for future more narrowly tailored clinical trials. To achieve the ultimate goal of controlling the disease activity more rapidly and reduce the economic, as well as personal burden of rheumatic diseases, innovative patient-centric, molecular pathology-driven clinical trial approaches are needed (119).

CONCLUSION

ScRNA-seq has enabled us to analyse in depth the complex cellular and molecular networks at the resolution of an individual cell in the heterogeneous tissue microenvironment. Additionally, computational tools analyzing scRNA-seq data offered us the opportunity to predict trajectories of different cell states, transitions and differentiation in the tissue during physiological and pathological processes. With the possibility to obtain massive amounts of data from individual cells, scRNA-seq has exceeded other traditional methods, such as flow cytometry, bulk RNA-seq and immunohistochemistry. The technique is particularly important for studying diseases with high patient heterogeneity, including autoimmune rheumatic disorders. However, the

translation of novel findings from single cell approaches into advanced diagnostic tools and treatments in rheumatic and other diseases has yet to be realized. There are still several challenges to overcome in order to provide valuable information for support of clinical decision-making. One challenge is the complexity of high dimensional single cell multiomics data which, for a successful and meaningful clinical interpretation, requires the interdisciplinary collaboration of bioinformatics, computational scientists, biologists and clinicians. Another challenge is the high cost of reagents and equipment, limiting the ability to profile large cohorts of patients with different clinical presentations and demographic backgrounds, as well as the integration of scRNA-seq and other single cell omics and high throughput data into a user-friendly interface that is easily accessible and available to the entire research community. To overcome these challenges, multicenter collaborations, such as in ELIXIR (https://elixir-europe.org/) an intergovernmental organization that brings together life science infrastructural resources from across Europe, are needed, coupled with practical and standardized protocols for wet-lab personnel, as well as bioinformatic pipelines.

Despite several challenges, which will likely be resolved with further development of the technology, as well as with tighter collaborations between wet-lab, dry-lab and clinical experts, the translational potential of scRNA-seq in rheumatic inflammatory diseases is obvious. Better understanding of the composition and functional states of tissue resident stromal and immune cells, together with their complex networks and interactions will provide further insights into disease development and progression, determine underlying mechanisms of drug resistance, as well as identify candidate therapeutic targets with low rates of off-target effects. With the recent availability of spatial transcriptomics, it will now be possible to explore gene expression signatures within the positional context of those cells in a tissue (120, 121). Furthermore, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), a method initially utilized for the analyses of cord blood and PBMCs, could easily be adapted to study the immune systems in patients with rheumatic diseases. Here, oligonucleotide-labeled antibodies that contain poly(A) tails are used to simultaneously determine cell surface proteins and transcriptomes. Several commercially available DNA-barcoded antibodies and fully validated sample processing platforms are already available for this purpose (51, 122). The application of several single cell omics, spatial transcriptomics and data integration from patients, animal and experimental models is likely to optimize the path toward precision medicine in autoimmune and inflammatory rheumatic diseases in the near future.

AUTHOR CONTRIBUTIONS

TK wrote the manuscript. SS-Š, BL, and PF contributed to specific parts of the review and revised and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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frontiers in Medicine

BRIEF RESEARCH REPORT
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Prevalence and Impact of Rheumatologic Pain in Cystic Fibrosis Adult Patients

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Background: With the improvement of cystic fibrosis (CF) patient survival, the prevalence of long-term complications increased, among them rheumatologic disorders.

Methods: The aim of this prospective study was to evaluate the prevalence of spinal and joint pain, and their impact on disability, anxiety, depression, and quality of life in CF adult patients.

Results: Forty-seven patients were analyzed, 72% of men, mean aged 28 years, with a mean body mass index of 22 kg/m² and a mean FEV_1% of 63%. Twenty-two patients (47%) described rheumatologic pain either spinal ($n = 15$, 32%) and/or joint pain ($n = 14$, 30%). Patients with spinal and/or joint pain were shorter ($p = 0.023$), more frequently colonized with *Staphylococcus aureus* ($p < 0.008$), had more frequent ΔF508 homozygous mutations ($p = 0.014$), and a trend for more impairment of the 6-min walking distance ($p = 0.050$). The presence of rheumatologic pain tended to be associated with disability according to the Health Assessment Questionnaire (HAQ) and anxiety. Compared with patients with no pain patients with both spinal and joint pain exhibited a more pronounced impact on the St George's Respiratory Questionnaire (SGRQ).

Conclusion: Rheumatologic pain is frequent in CF adult patients, and may affect daily living, anxiety and quality of life. Systematic assessment of rheumatologic pain should be included in the management of CF patients.

Keywords: cystic fibrosis, pain, joint, spinal, rheumatologic, quality of life

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BACKGROUND

Cystic fibrosis (CF) is the most common life-threatening genetic disease in Caucasian populations. Morbidity and mortality depend mainly on chronic respiratory failure and malnutrition. Beside usual clinical and spirometrics parameters and sweat chloride concentrations, emergent tools including biomarkers measured in blood, sputum or bronchoalveolar lavage (1), rheologic tests and low field nuclear magnetic resonance (2) may help to evaluate disease prognosis and efficacy of new pharmological treatments in the future.

With improving survival, the prevalence of long-term complications increased (3). In CF adults, rheumatologic disorders are frequently encountered (4, 5) including CF arthropathy

(CFA) (2–29%) (6–9), CF related bone disease (CFBD) including osteoporosis, fractures, and musculoskeletal manifestations (13 to 35%) (10–13), hypertrophic pulmonary osteoarthropathy (2 to 7%) and quinolone-induced arthropathy (14).

The definition of CFA is not fully accepted. Clinical manifestations are heterogeneous, usually characterized by recurrent transient episodes of painful mono- or polyarthritis lasting 1 day to several weeks and not classifiable as any of other rheumatic diseases (4, 7, 8, 14, 15). Joints of hands, feet, and knees are the most frequent sites (6, 8). The pathogenesis is unknown and its associations with respiratory exacerbations appear uncertain (4, 15). Intermittent arthritis may become chronic over time (8).

Pain is a common symptom in CF that impacts the quality of life (16–18), mood, work (17), and clinical outcomes (17). In adults, one of the most frequent pain locations is rheumatologic sites including back (15–70%), bone or muscles (44%), and joints (5–41% depending on the sites) (16–19). So far, no study specifically assessed the impact of rheumatologic pain on the quality of life in CF.

The aim of our study was to determine the prevalence of rheumatologic pain (spinal and/or joint) in a cohort of CF adult patients. In addition, we evaluated the impact of pain on patient's disability (from the Health Assessment Questionnaire widely used in rheumatic diseases for evaluating dependence), anxiety and depression (from the Hospital Anxiety and Depression Scale), and quality of life by Cystic Fibrosis Questionnaire for teenagers and adults (CFQ 14+), St George's Respiratory Questionnaire (SGRQ) and Medical Outcome Study Short Form 36 health survey (MOS SF-36) usually used in CF studies.

MATERIALS AND METHODS

Study Design

This monocentric study was prospectively conducted in the Department of Respiratory Diseases (University Hospital of Reims) between November 2016 and December 2019. Patients were included in the RINNOPARI study (Recherche et INNOvation en PAthologie Respiratoire Inflammatoire), an observational cohort of inflammatory chronic lung diseases. The study was approved by the Ethics Committee of Dijon EST I on 31st May 2016 (N°2016-A00242-49) and by the French National Agency for Medicines and Health Products (ANSM) on 25th April 2016. The protocol was registered on ClinicalTrials.gov (NCT02924818) on 5th October 2016. Each patient signed a written informed consent form.

CF patients were included if they were at least 18 years of age. Exclusion criteria were previous or planned lung transplantation and patients requiring an urgent visit. Anonymized data including demography, clinical characteristics, pulmonary function tests, and sputum microbiology were collected and registered on an electronic medical record.

Chronic infection by *Pseudomonas aeruginosa* and by extension chronic infection by *Staphylococcus aureus* was defined according to Leeds criteria (20).

Rheumatologic Assessment

Patients were asked to answer 4 questions regarding rheumatologic symptoms. Two questions assessed rheumatologic pain: one question assessed spinal pain ("Did you have spinal pain?"), and one question assessed joint pain ("Did you have joint pain?"). Patients were classified as either "no pain" (answering "no" to the two questions) or "pain" (answering "yes" to at least one question). Patients with "pain" were classified as "both pain" (spinal and joint pain) or "isolated pain" (spinal pain or joint pain).

Two additional questions assessed arthritis ("Did you have swelling joints? Did you have morning joint stiffness more than 30 minutes?"). Arthralgia associated with swelling joints and/or morning joint stiffness suggested inflammatory joint pain.

Patients' functional disability in the past week was assessed by the Health Assessment Questionnaire (HAQ), a validated scale consisting of eight sections: dressing, arising, eating, walking, hygiene, reach, grip and activities. The final score ranged between 0 (no assistance) and 3 (patient usually needs both a special device and help from another person) (21, 22).

Symptoms Score and Quality of Life Scales

Anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HAD) (23, 24).

The quality of life of CF patients was evaluated using dedicated questionnaires: (1) the Cystic Fibrosis Questionnaire for teenagers and adults (CFQ 14+) assessing the quality of life, symptoms, and disease effects. The score ranges from 0 to 100, the highest score corresponding to a better quality of life (25); (2) the St George's Respiratory Questionnaire (SGRQ) assessing symptoms and their impact on everyday activities. The total score includes the sum of 3 domains: impact, activity, and symptoms. A score of 100 indicates maximum impairment of quality of life (26); (3) the Medical Outcome Study Short Form 36 health survey (MOS SF-36) a multifaceted and generic scale assessing health status regardless of causal disease, sex, age, and treatment. A score of 100 indicates no impairment of quality of life (27).

Study Endpoints

The primary endpoint was the prevalence of rheumatologic pain (spinal and/or joint). Secondary endpoints were to evaluate the impact of rheumatologic pain on the patient's functional disability, mental health, and quality of life (HAQ, HAD, CFQ 14+, MOS SF-36, and SGRQ).

Statistical Analysis

Data were described as numbers (percentages), mean ± standard deviation. Given the limited number of patients, differences in all variables were assessed using Fisher's exact tests for qualitative variables and Mann–Whitney U-tests for quantitative variables. A correction was applied for multiple comparisons

Abbreviations: BMI, Body mass index; CF, Cystic fibrosis; CFA, Cystic fibrosis arthropathy; CFBD, Cystic fibrosis related bone disease; CFTR, Cystic fibrosis transmembrane conductance regulator; CFQ 14+, Cystic Fibrosis Questionnaire for teenagers and adults; FEV_1, Forced expiratory volume at first second; HAQ, Health assessment questionnaire; MOS SF-36, Medical outcome study short form 36 health survey; SGRQ, St George's Respiratory Questionnaire; TLC: Total lung capacity.

according to the Benjamini Hochberg procedure. A p-value $<$ 0.05 was considered statistically significant. Results were analyzed with SPSSv27.

The Cronbach's alpha value for each quality of life scores (HAQ, HAD, CFQ 14+, SGRQ and MOS SF-36) was calculated. A value $>$ 0.7 was considered as a high level of consistency.

RESULTS

Fifty-one consecutive CF patients were included in the study. Four patients were excluded ($n = 3$ for clinical rheumatologic missing data; $n = 1$ for previous lung transplantation), 47 patients were analyzed.

Patient Characteristics and Rheumatologic Assessment

Demographic, clinical, bacteriological characteristics, and rheumatologic assessment of patients are detailed in **Table 1**. Seventy-two percent of patients were men. The mean age was 28 years with a mean body mass index of 22 kg/m². Mean FEV_1% was 63% of the predicted value. The mean distance on the 6-min walking test was 80 ± 11% of the predicted value. Main treatments are detailed in **Supplementary Table S1**. Painkillers included paracetamol ($n = 4$, 8%), tramadol ($n = 1$, 2%) and non-steroidal anti-inflammatory drugs ($n = 2$, 4%).

Twenty-two patients described rheumatologic pain (47%), including spinal ($n = 15$, 32%) and/or joint pain ($n = 14$, 30%); seven patients (15%) reported both spinal and joint pain. Inflammatory joint pain appeared uncommon ($n = 3$, 6%). Of note, there was no difference in terms of painkillers treatment in patients with pain or no pain (**Supplementary Table S1**).

Patients suffering from spinal and/or joint pain were significantly shorter (167 ± 7 vs. 172 ± 9 cm, $p = 0.022$), had more frequent ΔF508 homozygous mutations (59 vs. 24%, $p = 0.014$), more frequent colonization by *Staphylococcus aureus* (91 vs. 56%, $p < 0.008$), and had a trend to an impaired 6-min walking distance (74 ± 10% vs. 84 ± 10%, $p = 0.050$). The prevalence of rheumatologic symptoms didn't increase with age (data not shown).

TABLE 1 | Clinical and rheumatologic characteristics, lung function, and microbiology data.

	Total	No pain	Pain	*p*-value
n	47	25 (53)	22 (47)	
Male	34 (72)	20 (80)	14 (64)	0.211
Age, years	28 ± 9	30 ± 10	26 ± 8	0.121
Height, cm	170 ± 8	172 ± 9	167 ± 7	0.023
Weight, kg	63 ± 12	65 ± 14	60 ± 11	0.228
BMI, kg/m²	22 ± 3	22 ± 3	22 ± 3	0.773
CFTR mutation				
ΔF508 homozygous	19 (40)	6 (24)	13 (59)	0.014
ΔF508 heterozygous	24 (51)	16 (64)	8 (36)	0.059
Other	4 (8)	3 (12)	1 (4)	0.611
Pancreatic insufficiency	37 (79)	18 (72)	19 (86)	0.230
Diabetes	16 (34)	7 (28)	9 (41)	0.351
Depression	7 (15)	4 (16)	3 (14)	0.820
Osteoporosis	9 (22)	4 (20)	5 (28)	0.573
Rheumatologic symptoms				
Spinal pain	15 (32)	0	15 (68)	–
Joint's pain	14 (30)	0	14 (64)	–
Swelling joints	1 (2)	1 (4)	0	0.343
Morning joint stiffness > 30 min	4 (8)	1 (4)	3 (14)	0.237
Exacerbation in the last year	31 (66)	16 (64)	15 (68)	0.682
Number of episodes per patient	2.3 ± 1.4	2.5 ± 1.5	2.2 ± 1.3	0.561
Chronic bacterial colonization				
Pseudomonas aeruginosa	19 (40)	12 (48)	7 (32)	0.241
Staphylococcus aureus	34 (72)	14 (56)	20 (91)	0.008
Spirometry				
FEV_1, %	63 ± 30	58 ± 28	68 ± 32	0.245
TLC, %	111 ± 13	109 ± 13	115 ± 12	0.255
6-min walking test				
Distance, %	80 ± 11	84 ± 10	74 ± 10	0.050

Data are expressed as frequency (percentage) or mean ± standard deviation.
BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; FEV_1, forced expiratory volume at first second; TLC, total lung capacity.

Impact of Rheumatologic Symptoms

The Cronbach's alpha value for HAQ, HAD, SGRQ, CFQ 14+ and MOS SF-36 was of 0.830, 0.816, 0.913, 0.602, and 0.288, respectively. Because of a low internal reliability, the CFQ 14+ and MOS SF-36 were not considered for the final analysis. Measures of rheumatologic HAQ, HAD and SGRQ are shown in **Table 2**. HAQ tended to be more impaired in patients with spinal and/or joint pain (0.18 ± 0.23 vs. 0.07 ± 0.16, $p = 0.061$). HAQ was impaired in patients with both spinal and joint pain when compared with patients with no pain (0.36 ± 0.28 vs. 0.07 ± 0.16, $p < 0.001$) (**Supplementary Table S2**).

The mean anxiety score assessed by the HAD questionnaire trended to be higher in patients with pain (7 ± 3 vs. 5 ± 3, $p = 0.058$) whereas no difference was observed for depression (**Table 2**). No difference was observed between patients with both spinal and joint pain, and either no pain, isolated spinal and isolated joint pain for anxiety and depression (**Supplementary Table S2**).

No impact on quality of life assessed by the SGRQ was found in patients with spinal and/or joint pain when compared with patients with no pain (**Table 2**).

We next analyzed the symptoms scores and SGRQ in patients with both spinal and joint pain (**Supplementary Table S2**). Compared with patients with no pain, they had a marked impaired quality of life identified in SGRQ (total score, impact and activity domains). Compared with patients with either spinal or joint pain, no difference was observed.

DISCUSSION

Our study confirmed that rheumatologic pain is frequent, concerning near half of the adult patients with cystic fibrosis (47%). It included spinal pain (32%), joint pain (30%), with 15% of patients suffering from both spinal and joint pain.

TABLE 2 | Symptoms score and disability, anxiety and depression, and quality of life scales.

	Total	No pain	Pain	*p*-value
n	44[#]	24 (55)[†]	20 (45)[†]	
HAQ	0.11 ± 0.20	0.07 ± 0.16	0.18 ± 0.23	0.061
HAD				
Anxiety (mean)	6 ± 4	5 ± 3	7 ± 3	0.058
Depression (mean)	3 ± 4	4 ± 3	5 ± 4	0.292
SGRQ				
Impact	20 ± 16	16 ± 14	24 ± 17	0.075
Activity	34 ± 21	29 ± 19	39 ± 22	0.116
Symptoms	45 ± 21	45 ± 22	44 ± 20	0.846
Total	25 ± 17	22 ± 15	29 ± 19	0.125

Data are expressed as frequency (percentage) or mean ± standard deviation.
[#]*Data are not available for 3 patients.*
[†]*Data are not available for 1 patient with "no pain" and for 2 patients with "pain".*
HAD, Hospital Anxiety and Depression Scale; HAQ, Health Assessment Questionnaire; SGRQ, St George's Respiratory Questionnaire.

Inflammatory joint pain appeared uncommon (6%). For the first time, our study focused on the impact of rheumatologic pain on disability, anxiety and depression, and quality of life.

During the past few years, many studies investigated pain in adult CF patients. The prevalence of painful symptoms varies between 89% in the past week (28), 82–89% during the previous month (16, 17) and 94.1% in the past 2 months (19). Painful episodes concern up to three (16.8%) or four (38.4%) different locations (19). Rheumatologic pain is one of the most frequent sites after headache, sinuses, or chest pain (18, 29, 30). In our study, the prevalence of spinal pain was 32%, in line with previous studies reporting between 10 and 28.4% for cervical pain (16, 19) and 50% for dorsal or back pain (16, 17, 19). The prevalence of back pain in the general young population is about 20% (31). However, no comparative study between young adults with or without CF is available. The causes of back pain are not fully established (30, 32). CF adult patients have a low bone mineral density and a high prevalence of osteoporosis (11, 12). Many studies described orthopedic complications of CFBD such as vertebral deformity (33) and scoliosis (13), rib and vertebral fractures (34). Postural abnormalities have been also reported (35). However, none of these studies described the relation between CFBD or postural abnormalities and pain. Lastly, it might be difficult to differentiate musculoskeletal pain from thoracic pain related to the use of accessory respiratory muscles in dyspneic patients with severe disease (16, 32).

Joint pain concerned 30% of our cohort, similar to the previous reviews reporting arthralgia between 20 and 41.4% (16, 19). In Hayes et al. study, the main arthralgia sites were knee (29.7%), wrist (18.9%), and finger (5%) (17). In our study, signs of inflammatory joint pain were unusual (6%). The comparison with other studies is difficult in the absence of an accepted definition of CFA and with various study designs. These studies described a prevalence of inflammatory joint included between 2 and 29% of the patients (6–9, 28). In Koch et al. study there was no significant difference regarding inflammatory signs such as joint swelling or warming between CF patients and controls (6).

Interestingly in our study, patients with spinal and/or joint pain were significantly shorter (167 ± 7 vs. 172 ± 9 cm, $p = 0.023$) and more frequently colonized with *Staphylococcus aureus* (91% vs. 56%, $p < 0.008$). The comparison is difficult with other specific studies concerning musculoskeletal and arthropathy in CF (6, 13, 36, 37). In Roehmel et al. study involving 186 CF children and adults (mean age: 27 years), patients with CFA (defined as at least one symptom out of the following: joint pain, joint swelling, joint reddening or limitation of movement) were more likely to be older, female gender, and to have a higher rate of total IgG, chronic colonization with *Aspergillus* spp. and pulmonary exacerbations (7). In Grehn et al. study from the German CF registry, CFA including arthropathy and arthritis was associated with increasing age, female gender, number of hospitalizations, chronic *Pseudomonas aeruginosa* infection, CF-related diabetes, pancreatic insufficiency and sinusitis/polyps (9). These results also support a correlation between pulmonary inflammation/infection and CFA. We also reported that patients suffering from spinal and/or joint pain had significantly more frequent ΔF508 homozygous mutations (59 vs. 24%, $p = 0.014$). By contrast, two previous studies didn't find an association between cystic fibrosis transmembrane conductance regulator (CFTR) mutations and CFA (7, 9).

From our results, rheumatologic pain may impact daily life activities. First, the 6-min walking distance of painful patients tended to be lower (74 ± 10% vs. 84 ± 10%, $p = 0.050$). To our knowledge, no previous rheumatologic CF study assessed the 6-min walk distance. Second, our results suggest that patients with both spinal and joint pain had a more important impairment on HAQ scale. Their functional disability is probably more important to those of the young general population (38). Some investigators have suggested that the Minimal Clinical Important Difference is 0.1 (39). Only one previous study showed that CF patients reported an impairment in everyday life functions assessed by the HAQ (6). Lastly, the results highlight that patients with both spinal and joint pain had a significant impairment of quality of life according to the SGRQ scores compared with patients with no rheumatologic pain. The majority of chronic diseases worsen health and affect the quality of life (40). Then it is not surprising that rheumatologic pain in CF, a disease including multimorbidity, impacts quality of life. The negative effects of back pain had been previously reported especially on the respiratory and emotion subscale (17). In our study, rheumatologic pain tended to be associated with anxiety but not with depression. Of note, in Hayes et al. study, back pain was also associated with anxiety but not with depression (17). Surprisingly, despite frequent rheumatologic pain in CF patients in our study, very few patients used painkillers, suggesting that rheumatologic pain treatment is overlooked.

There are several limitations to our study. First, our sample size is relatively small which could limit the identification of differences between different groups (painful and not painful patients, patients with spinal, joint and both pain). Second, we have not assessed the consequences of rheumatologic pain on asthenia, sleeping disorders, family life and study or work absenteeism. Third, the four questions used to detect

rheumatologic pain do not fully cover the characteristics of pain (acute or chronic, sites, intensity, duration). Lastly, the absence of additional tests for this study carried out in current practice not allowed to identify the origin of pain. A larger study should be conducted to elucidate the potential mechanisms of rheumatologic pain and its therapeutic management.

CONCLUSION

Our study confirms that rheumatologic pain is frequent concerning near half of cystic fibrosis adult patients. Patients with spinal and/or joint pain were more frequently colonized with *Staphylococcus aureus* and had more frequent ΔF508 homozygous mutations. The prevalence of rheumatologic symptoms didn't increase with age. No study had previously specifically assessed the impact of rheumatologic pain on patient's disability, anxiety and depression, and quality of life. The impact of both spinal and joint pain seems to be more important, in particular on disability and on quality of life, in comparison with patients with no pain. However, there is no evidence for more painkillers rescue. Our results highlight that the health care team should carefully assess patients and undertake additional tests in collaboration with rheumatologists to identify the cause of pain and therapeutic management.

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 RINNOPARI (Recherche et INNOvation en PAthologie Respiratoire Inflammatoire) study was approved by the Ethics Committee of Dijon EST I on 31st May 2016 (No. 2016-A00242-49) and by the French National Agency for Medicines and Health Products (ANSM) on 25th April 2016, and declared on ClinicalTrials.gov (NCT02924818) on 5th October 2016. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AS, CL, BR, GD, J-HS, and SD: substantial contributions to the conception. AS, CL, BR, FL, GD, J-HS, and SD: design of the work. AS, J-MP, BR, MG, SC, PM, JA, JH, J-HS, and SD: acquisition and analysis of the data. J-MP: software. AS, CL, J-MP, BR, MG, SC, PM, JA, JH, FL, GD, J-HS, and SD: drafting the work or substantively revising the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.804892/full#supplementary-material

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Follow-Up Comparison of Fluorescence Optical Imaging With Musculoskeletal Ultrasound for Early Detection of Psoriatic Arthritis

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Objectives: Early diagnosis of psoriatic arthritis (PsA) is crucial for a patient outcome but hampered by heterogenous manifestation and a lack of specific biomarkers. We recently showed that fluorescence optical imaging (FOI) can differentiate between patients with confirmed and suspected PsA. This study aims to follow-up (FU) patients with confirmed and suspected PsA focusing on patients with a change from suspected to confirmed PsA by the use of FOI in comparison with musculoskeletal ultrasound (MSUS).

Methods: Follow-up examination of patients included in the study performed by Erdmann-Keding et al. in which FOI of both hands was performed in a standardized manner using three predefined phases (p1–p3) and PrimaVista Mode (PVM). The comparison was drawn to grayscale–power Doppler (GS/PD) MSUS of the clinically dominant hand (wrist, MCP, PIP, DIP 2–5) from dorsal or palmar.

Results: Patients with a change from suspected to diagnosed PsA showed an increased prevalence of joints with pathological enhancement in FOI (p = 0.046) with an unchanged joint distribution pattern, especially with a dominant involvement of DIP joints. Compared to the baseline, these patients were three times more common to show enhancement in FOI p3 at FU. Newly detected pathologic joints by FOI (PVM, p2) and MSUS at FU were positively associated with the change of diagnosis from suspected to confirmed PsA (FOI: AUC 0.78; GSUS: AUC 0.77).

Conclusion: Fluorescence optical imaging appears to be a helpful tool to detect early PsA and to distinguish between acute and chronic disease stages. It could thereby become a suitable tool as a screening method to select psoriasis patients with an indication for further rheumatological evaluation.

Keywords: psoriatic arthritis, fluorescence optical imaging, ultrasound imaging, follow-up studies (MeSH), hand, inflammation

INTRODUCTION

Psoriatic arthritis (PsA) affects up to 30% of patients suffering from psoriasis (1, 2) and is characterized by systemic inflammation and extensive synovitis, which results in erosions of articular cartilage and bone that leads to joint destruction (3). As PsA is destructive and progressive as rheumatoid arthritis (RA), delay in diagnosis of 6 and 12 months impacts long-term joint damage and functional disability (4, 5). About 20% of the patients develop very destructive disabling arthritis (6). Hence, not only an early diagnosis of PsA but also methods to identify "at risk" patients for developing PsA are decisive.

Nonetheless, diagnosing PsA remains challenging since there are no specific biomarkers (7).

In addition, ClASsification criteria for Psoriatic Arthritis (CASPAR) was found to have exceptional specificity for PsA but is inappropriate to screen patients with PsO for PsA development due to low sensitivity (8). Several imaging modalities are used in the diagnostic process for PsA that includes magnetic resonance imaging (MRI) and musculoskeletal ultrasound (MSUS) in grayscale (GS) and in power Doppler (PD) mode. FOI with the Xiralite® system is a novel technology, which is sensitive for detecting inflammatory joint processes of the hands. It uses near-infrared light to visualize altered microcirculation such as hyperperfusion, neoangiogenesis, and capillary leakage after the application of a fluorescence dye (9).

The previous studies have already provided evidence that FOI is suitable for therapy monitoring in early rheumatoid or inflammatory arthritis (10, 11) and also for the detection of inflammatory skin changes in the hands and PsA-typical signal patterns (12, 13).

The high prevalence (10.1–15.5%) of undiagnosed PsA in patients with psoriasis requires a sensitive screening tool to select patients with an indication for further rheumatological evaluation (14). Erdmann-Keding et al. compared FOI and MSUS in detecting joint inflammation in patients with confirmed and suspected PsA (15). In this study, we performed follow-up (FU) examinations of the same cohort of patients to further investigate signal enhancement in different FOI phases (p1–p3), which represents acute or chronic inflammation.

Abbreviations: AUC, area under the curve; bDMARDs, biologic disease modifying antirheumatic drugs; BL, baseline data, baseline study; BMI, body mass index; BSA, body surface area; CASPAR, ClASsification criteria for Psoriatic Arthritis; CE, clinical examination; csDMARDs, conventional synthetical disease modifying antirheumatic drugs; DAS28, Disease Activity Score of 28 joints; DIP, distal interphalangeal joints II–V of the fingers; DMARD, Disease modifying antirheumatic Drugs; EULAR, European League Against Rheumatism; FOI, fluorescence optical imaging; FOIAS, fluorescence optical imaging activity score; FU, follow-up study; GS(US), grayscale mode, grayscale ultrasound; ICG, indocyanine green; MCP, metacarpophalangeal joints I–V of the fingers; MRI, magnetic resonance imaging; MSUS, musculoskeletal ultrasound; MTX, methotrexate; NAPSI, nail psoriasis severity index; OA, osteoarthritis; OMERACT, outcome measures in rheumatology; PASI, Psoriasis Area and Severity Index (PASI); PD(US), power Doppler mode, power Doppler ultrasound; PIP, proximal interphalangeal joints II–V of the fingers; PsA, psoriatic arthritis; PsO, psoriasis; PVM, PrimaVista Mode in FOI; p1, phase 1 in FOI; p2, phase 2 in FOI; p3, phase 3 in FOI; RA, rheumatoid arthritis; SJC, swollen joint count; TJC, tender joint count; T1, date of baseline examination; T2, date of follow-up examination; VAS, visual analog scale.

The aim of this study was to explore the potential of FOI for making an early diagnosis of PsA concentrating on those patients who underwent a change of diagnosis from suspected to diagnosed PsA between the two studies and to examine the possible application of FOI as a screening tool to identify exactly these patients.

MATERIALS AND METHODS

The study was conducted as a cooperation between the departments of Dermatology and Rheumatology of the Charité – Universitätsmedizin Berlin, Germany – and approved by its local ethic committee (EA1/025/10). Patients were included after written informed consent to participate in the study.

The study was designed as FU study to Erdmann-Keding et al. (11) which compared FOI with MSUS and clinical examination (CE) in 60 patients suffering from confirmed (n = 26) or suspected PsA (n = 34).

Patients were contacted between May 2014 and January 2015 *via* post or telephone to participate in the present FU study. Baseline data were collected between March 2010 to November 2011 and FU data between May 2014 and January 2015.

The recruited patients were assigned to three different groups:

- Diagnosed PsA after baseline assessment (group I).
- Still suspected PsA (group II).
- (Unchanged) Diagnosed PsA (group III).

The diagnosis of PsA was confirmed by the treating dermatologist or rheumatologist based on the medical history and clinical evaluation before the FU examination (**Supplementary Figure 1**).

To ensure good comparability to the baseline (BL) data from 2011, patients underwent the same assessments including a CE, MSUS in GS/PD of the clinically dominant hand (wrist, MCP, PIP, DIP), and FOI of both hands.

Clinical Examination

For CE, the Disease Activity Score 28 (DAS28) was used (16).

Skin involvement was evaluated by body surface area (BSA), Nail Psoriasis Severity Index (NAPSI), and the Psoriasis Area Severity Index (PASI). A visual analog scale (VAS 0–10 mm) was used to examine the patient's global assessment of joint pain, skin involvement, and pruritus (17–19).

Musculoskeletal Ultrasound

Musculoskeletal ultrasound examination (Esaote Mylab Twice, Genova; Italy) of the clinically dominant hand was performed by grayscale (GS) and power Doppler (PD) MSUS from dorsal and palmar using a linear transducer with 10–18 MHz. GSUS and PDUS were performed by following the EULAR recommendations and OMERACT definitions (20, 21).

To avoid a possible variance between different sonography devices and examiners, all patients were examined on the same ultrasound machine and examiner (SO) at BL and FU.

The sonographer is a EULAR-certified Teacher (Level II) with a relatively long ultrasound experience of about 10 years at the time of FU examination.

Settings for PDUS were as follows: pulse repetition frequency 0.75 kHz, power Doppler frequency 11.1 MHz, wall filter 3.

The wrist, metacarpophalangeal (MCP) joints 2–5, proximal interphalangeal joints (PIP) 2–5, and distal interphalangeal joints (DIP) 2–5 were evaluated semiquantitatively for synovitis [0 = absent, 1 = mild, 2 = moderate, 3 = severe; (22, 23)] and for tenosynovitis (0–1) in both GSUS and PDUS modes. Superficial erosions were scored for the presence and absence (0–1). For each patient, a sum score of all joints was calculated.

Fluorescence Optical Imaging

Fluorescence optical imaging (FOI) was performed with the Xiralite X4 device (Xiralite GmbH, Berlin, Germany) following a standardized procedure. The total examination time lasted 360 s including intravenously administration of indocyanine green (ICG) bolus (ICG-Pulsion, 0.1 mg/kg/body weight) 10 s after the beginning. By recording one image per second, the system provided 360 images in total. Alteration of the dye concentrations as signal intensity was presented by false color scale. For evaluation, a film modus with three predefined phases based on signal intensity in the fingertips (p1–p3) and an automatically generated composite image (PrimaVista Mode, PVM) were considered.

Phase 1 refers to the period between the start of the examination, the injection of ICG, and the beginning of the increased signal intensity in the fingertips whereas phase 2 includes remaining increased signal intensities in the fingertips recognizable by the red color. Phase 3 is defined by missing high signals in the fingertips until the end of the image stack (9, 12, 24).

To analyze joint activity, a semiquantitative grading system for wrist, MCP 2–5, PIP 2–5, and DIP 2–5 of each hand from grade 0 to 3 [0 = no signal enhancement, 1 = low signal enhancement $\leq$ 25%, 2 = moderate signal enhancement (>25%, $\leq$50%), 3 = strong signal enhancement (>50% of affected joint area)] was used (9, 12). All FOI findings were evaluated blinded to the patient group by three readers (AMG, SO, and JB) on consensus agreement. To create an optimal comparability of the FOI results of the baseline and FU study, the FOI data of the baseline study were again evaluated according to the mentioned definition.

Statistical Analysis

Statistical analysis and data management were performed using STATA 12 (StataCorp LLC, TX, United States). The analysis of joint involvement based on CE, GS/PDUS, and FOI at the BL and FU separately was performed for the three different groups defined above.

A joint was considered to be affected if the grading was at least one (grade $\geq$1). GSUS was used as the reference method to determine the absolute consistency, sensitivity, and specificity with PDUS, swollen joints, and FOI (PVM, p1–p3). Further analysis included the assessment whether a joint was newly affected in FU (date of the baseline examination: T1−, date of the FU: T2+), presented with no change or was not affected anymore (T1+, T2−). The association of newly detected affected joints regarding the change of diagnosis was evaluated by calculating the area under the curve (AUC) (25) to assess the strength of association. The *post hoc* test by Sidak was used to determine whether there were significant differences in the mean number of joints detected by the different examination methods GSUS and FOI. Agreement rates were calculated by absolute agreement (in%) and prevalence-adjusted bias-adjusted kappa for FOI, GSUS, and CE for BL and FU examination. $p < 0.05$ were considered significant.

RESULTS

From the 60 patients examined by Erdmann-Keding et al. at BL (15), six patients could not be contacted due to loss of contact data. A total of 30 of 54 patients contacted consented to participate in this FU study resulting in 50% successful rerecruitment rate.

They were then assigned to the three different groups:

- Diagnosed PsA after baseline assessment (group I, $n = 10$).
- Still suspected PsA (group II, $n = 6$).
- Diagnosed PsA (group III, $n = 14$).

Fluorescence optical imaging could be completed in 29 patients. One examination had to be interrupted due to orthostatic dysregulation.

Demographic Data

Results of the demographic and clinical features of the study population are shown in **Table 1**. At the time of FU, systemic therapy was administered to 80% of patients from group I, 67% from group II, and 93% from group III.

Comparison of Clinical Examination, Ultrasound, and Fluorescence Optical Imaging

Group I–Diagnosed Psoriatic Arthritis After Baseline Assessment

Compared to BL, patients with a change from suspected to diagnosed PsA showed an increased prevalence of joints with pathological enhancement in FOI ($p = 0.046$), especially in p2 ($p = 0.037$), and an unchanged joint distribution pattern, that is, with a dominant involvement of the DIP joints (**Tables 2**, **3** and **Figure 1**).

Patients of this group were three times more common to show enhanced signal in p3 in FOI at FU – compared to BL (p = n.s.) (**Figure 1**).

In FOI, the largest number of increased signal intensity was found in FOI p2–both in 2011 and 2014 (46.7% in BL, 47.8% in FU, **Table 2**).

In 64.4% of pathologic joints across all examination methods, the PIP and DIP joints were affected, which increased to 78.7% in FU examination.

Significantly, more joints were affected in GSUS at FU compared to baseline examination ($p = 0.005$).

TABLE 1 | Demographic and clinical data of the study population.

	All (*n* = 30)	Diagnosed PsA after baseline assessment (*n* = 10)	Still suspected PsA (*n* = 6)	Diagnosed PsA (*n* = 14)
Female (*n*)	22	8	4	10
Age in years	57.03 ± 11.01	50.4 ± 4.9	55.3 ± 13.3	62.5 ± 10.2
Duration of psoriasis	25 ± 17.4	20.7 ± 13.8	29 ± 17.9	27 ± 19.1
Duration of joint symptoms	12.3 ± 8.9	8.5 ± 6.3	16.4 ± 12.3	13.75 ± 7.7
BMI (kg/m^2)	30.3 ± 6.2	32.9 ± 6.4	29.1 ± 5.4	29.0 ± 5.7
PASI	2.3 ± 2.47	3.6 ± 2.9	2.5 ± 2.37	1.4 ± 1.62
NAPSI right	3.9 ± 5.5	6.7 ± 7.2	0.7 ± 1.5	3.42 ± 4.5
NAPSI left	4.9 ± 6.5	8.2 ± 7.1	1.8 ± 4.1	4.2 ± 6.1
TJC (0/28)	5.9 ± 5.7	6.9 ± 6.7	5 ± 6.4	5.6 ± 4.3
SJC (0/28)	1.8 ± 2.7	2.9 ± 3.8	1.5 ± 2.1	1.2 ± 1.6
DAS28	4.3 ± 1.2	4.8 ± 1.2	3.5 ± 1.2	4.2 ± 1.0
Erosion by MSUS	8 (27%)	0 (0%)	2 (33%)	6 (43%)
Systemic therapy	25 (83.3%)	8 (80%)	4 (67%)	13 (93%)
Current MTX medication	11 (36.7%)	5 (50%)	0 (0%)	6 (42.9%)
MTX in medical history	24 (80%)	10 (100%)	3 (50%)	11 (78.6%)
Biologicals	13 (43.3%)	4 (40%)	2 (33.3%)	7 (50%)

Data are reported by mean ± SD or n (%). PsA, psoriatic arthritis; BMI, body mass index; PASI, Psoriasis Area and Severity Index; NAPSI, Nail Psoriasis Severity Index; TJC, tender joint count; SJC, swollen joint count; DAS28, Disease Activity Score 28; MTX, methotrexate; MSUS, musculoskeletal ultrasound.

TABLE 2 | Prevalence of joints with pathological findings.

		Group I *n* = 130		Group II *n* = 78		Group III *n* = 182	
CE	BL	33 (25.4%)	$p = 0.35$	4 (5.1%)	$p = 0.15$	28 (10.6%)	$p = 0.76$
	FU	44 (33.8%)		15 (19.2%)		53 (14.1%)	
GSUS	BL	15 (11.5%)	$p = 0.005$*	8 (10.3%)	$p = 0.027$*	44 (16.7%)	$p = 0.001$*
	FU	95 (73%)		34 (43.6%)		141 (37.5%)	
PDUS	BL	6 (4.6%)	$p = 0.006$*	3 (3.8%)	$p = 0.31$	8 (3.0%)	$p = 0.004$*
	FU	23 (17.7%)		5 (6.4%)		23 (6.2%)	
FOI any phase	BL	60 (46%)	$p = 0.046$*	41 (52.6%)	p = n.a.	184 (69.7%)	p = n.a.
	FU	115 (88.5%)		36 (46.2%)		159 (42.3%)	
PVM	BL	24 (40%)	$p = 0.1$	14 (34.2%)	$p = 0.51$	58 (31.5%)	$p = 0.72$
	FU	47 (40.9%)		12 (33.3%)		51 (32.1%)	
p1	BL	7 (11.7%)	$p = 1.0$	10 (24.4%)	$p = 0.15$	22 (12.0%)	$p = 0.06$
	FU	4 (3.5%)		5 (13.9%)		7 (4.4%)	
p2	BL	28 (46.7%)	$p = 0.037$*	16 (39%)	$p = 0.74$	89 (48.4%)	$p = 0.82$
	FU	55 (47.8%)		17 (47%)		89 (56.0%)	
p3	BL	1 (1.7%)	$p = 0.26$	1 (2.4%)	$p = 0.56$	15 (8.2%)	$p = 0.55$
	FU	8 (7.0%)		2 (5.6%)		12 (7.6%)	

*Percentages in FOI PVM, p1, p2, p3 refer to all joints affected in FOI. The percentages in CE, GSUS, and PDUS refer to all joints considered in this group, *$p \leq 0.05$. n = Number of wrist and joints examined in this group; group I, diagnosed PsA after baseline assessment; group II, still suspected PsA; group III, diagnosed PsA; BL, baseline; FU, follow-up; CE, clinical examination; GSUS, ultrasound in grayscale mode; PDUS, ultrasound in power Doppler mode; FOI, fluorescence optical imaging; p1–p3, FOI phases 1–3. n.a. = not available.*

Group II – Suspected Psoriatic Arthritis

At FU, FOI showed a comparable number of affected joints in the group of suspected PsA (52.6% in BL, 46.2% in FU, see **Table 2**).

The distribution of the changes seen over the 3 phases was similar. However, in 2014, only half as many joints were detected in p1 as in 2011 (24.4% vs. 13.9%; p = n.s.).

At both study points, only a minimal number of increased signal intensities were found in p3 (2.4% in BL, 5.6% in FU, see **Table 2**).

At FU, no typical joint involvement pattern could be identified. A similar distribution of the affected finger joints with MCP (30%), PIP (31%), and DIP (23.3%) was found, with the wrists being slightly less affected (13.3%, **Table 3**).

Also in this group, the FU examination showed a significantly increased prevalence of affected joints by GSUS ($p = 0.027$, see **Table 2**).

Group III–Diagnosed Psoriatic Arthritis

Ultrasound in grayscale mode in the FU examination detected a significantly increased the prevalence of affected joints ($p = 0.001$), whereas the prevalence of affected joints in FOI was lower (BL: 69.7% vs. FU: 42.3%; p = n.s.).

TABLE 3 | Pattern of joint involvement according to all examination methods (CE, GSUS, and FOI).

		Group I *n* = 130	Group II *n* = 78	Group III *n* = 182
Wrist	BL	27 (23.5%)	14 (25%)	41 (15.5%)
	FU	17 (6.1%)	12 (13.3%)	30 (7.9%)
MCP	BL	17 (14.8%)	7 (12.5%)	46 (17.4%)
	FU	44 (15.9%)	27 (30.0%)	80 (21.3%)
PIP	BL	57 (49.6%)	26 (46.4%)	103 (39.0%)
	FU	114 (41.2%)	28 (31%)	140 (37.2%)
DIP	BL	17 (14.8%)	8 (14.28%)	74 (28%)
	FU	104 (37.5%)	21 (23.3%)	126 (33.5%)

N = Number of wrists and finger joints examined in this group; group I, diagnosed PsA after baseline assessment; group II, still suspected PsA; group III, diagnosed PsA; BL, baseline; FU, follow-up; MCP, metacarpophalangeal joint; PIP, proximal interphalangeal joint; DIP, distal interphalangeal joint.

This cohort showed the highest prevalence of signal enhancement in FOI phase 3, compared to the other groups (8.2% in BL, 7.6% in FU, see **Table 2**).

The involvement of PIP and DIP joints was mainly detected in p2 showing increased signal intensities in 80.0 and 94.3% of DIP joints in 2011 (BL) and 2014 (FU), respectively. Accordingly, the rate of affected PIP joints in p2 was 70.6% in 2011 and 84.6% in 2014 (data not shown).

Both the BL and the FU examination showed a typical pattern of joint involvement with accentuated affection of PIP (BL: 39%, FU: 37.2%) and DIP joints (BL: 28%, FU: 33.5%) (**Table 3**).

Association of Detected Newly Affected Joints by Musculoskeletal Ultrasound (GSUS/PDUS) and Fluorescence Optical Imaging in Patients in Group I Compared to Group II

Musculoskeletal ultrasound and FOI (PVM, p2) were associated with the detection of newly affected joints at FU (FOI: AUC 0.78; GSUS: AUC 0.77) more likely in group I compared to group II. More in detail, the GSUS examination method showed acceptable AUC for the PIP (PIPIII 0.72; PIPV 0.77), respectively. FOI in PVM demonstrated a similar AUC for DIPIV (0.78) and PIPII (0.72). Also, for FOI in p2, acceptable AUC in the DIPs could be determined (0.78–0.79) (see **Table 4**).

Differences Between Baseline and Follow-Up in the Mean Number of Affected Joints Detected by the Different Methods

The mean number of joints detected as affected ($\geq$1) differed significantly between the three groups for FOI in p2 at BL (p = 0.013) and FU (p = 0.013). The *post hoc* test by the Sidak method resulted in a significant difference between group I and group III at baseline (p = 0.028) and the groups I and II at the FU (p = 0.010). Regarding GSUS examination method, we also found a significant difference in the number of affected joints between the three groups at the time of FU (p = 0.003). The *post hoc* test showed a significant difference between the groups I and II (p = 0.002) and groups II and III (p = 0.013).

Agreement Rates of Fluorescence Optical Imaging With GSUS and Clinical Examination

Both in 2011 and 2014, agreement of CE (swollen joints) and FOI was good to very good in groups II and III (see **Table 5**). In group III, agreement rates in 2011 ranged from 52.8 to 88.8%, with highest accordance found in p1 and p3. Also in 2014, the agreement of CE and FOI was highest in p1 (92.9%) and p3 (88.5%). In group II, agreement rates in 2011 ranged from 80.8 to 100% and from 75.6 to 92.3% in 2014, respectively. In this group, highest agreement rates were found in the MCP and DIP joints depending on the individual phases of FOI with highest agreement found in p3 where mostly negative results were present. Agreement rates in group I extended from 71.2 to 95% at BL and from 52.9 to 89.7% at FU with highest rates for p1 and p3.

In all 3 groups, the agreement of FOI and GSUS was better at BL than at FU, which depends on the individual phases of FOI.

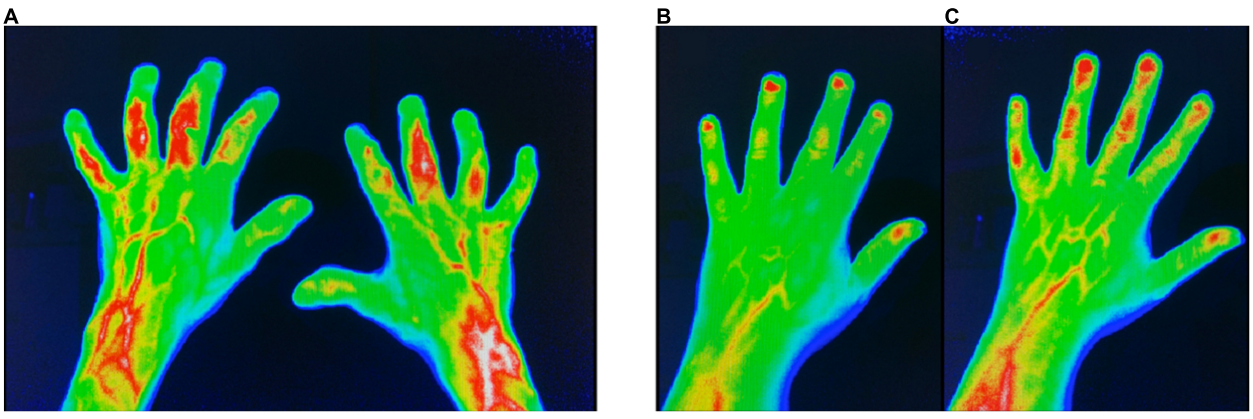

FIGURE 1 | FOI in group I (change of diagnosis from suspected to confirmed PsA). **(A)** FOI in p3 with moderate signal intensities in the left PIP3 + 4 and right PIP3 + 4 joints at FU. **(B)** FOI in p2 with low signal intensities in PIP 3–5 at baseline. **(C)** FOI in p2 with moderate signal intensities in PIP 3–5 and DIP 2–4 at FU. FOI, fluorescence optical imaging; p1–p3, FOI phases 1–3; PIP, proximal interphalangeal joint; DIP, distal interphalangeal joint.

TABLE 4 | Association of GSUS, PDUS, and FOI with newly suspected joints in FU with regard to the change of diagnosis (group I).

	MCP				PIP				DIP			
	II	III	IV	V	II	III	IV	V	II	III	IV	V
GSUS	0.52	0.72	0.70	0.70	0.68	0.72	0.67	0.77	0.60	0.62	0.65	0.62
PDUS	0.43	0.50	0.50	0.45	0.65	0.55	0.55	0.55	0.72	0.60	0.55	0.60
FOI PVM	0.50	0.50	0.50	0.56	0.72	0.64	0.64	0.58	0.67	0.69	0.78	0.58
FOI p1	0.50	0.50	0.50	0.50	0.56	0.61	0.56	0.50	0.56	0.50	0.50	0.50
FOI p2	0.50	0.50	0.33	0.47	0.58	0.72	0.58	0.67	0.78	0.79	0.69	0.75
FOI p3	0.50	0.50	0.50	0.50	0.56	0.61	0.61	0.56	0.50	0.56	0.50	0.42

AUC, area under the curve; GSUS, ultrasound in grayscale mode; PDUS, ultrasound in power Doppler mode; FOI, fluorescence optical imaging; PVM, PrimaVista Mode; p1–p3, FOI phases 1–3; MCP, metacarpophalangeal joint; PIP, proximal interphalangeal joint; DIP, distal interphalangeal joint.

TABLE 5 | Agreement rates (%, prevalence-adjusted bias-adjusted kappa) of FOI and GSUS (GSUS as standard of reference) vs. CE; FOI and GSUS (CE as standard of reference) for BL and FU examination.

		Group I				Group II				Group III			
		BL	kappa	FU	kappa	BL	kappa	FU	kappa	BL	kappa	FU	kappa
GSUS + FOI	PVM	79.5	0.59	49.6	−0.05	74.4	0.48	61.5	0.10	62.6	0.26	38.5	−0.22
	p1	83.8	0.68	27.3	−0.53	76.7	0.53	55.1	0.03	71.4	0.43	25.3	−0.48
	p2	76.1	0.52	49.6	0.01	74.4	0.48	55.1	−0.03	56.2	0.15	49.4	0.02
	p3	87.2	0.74	28.2	−0.48	91.0	0.81	58.9	0.00	69.8	0.40	23.6	−0.50
CE + FOI	PVM	75.9	0.52	56.4	0.13	82.7	0.65	82.0	0.64	66.5	0.33	72.5	0.45
	p1	91.3	0.83	88.9	0.78	80.8	0.62	91.0	0.82	85.2	0.70	92.9	0.85
	p2	71.2	0.42	52.9	0.06	80.8	0.62	75.6	0.51	52.8	0.05	53.3	0.08
	p3	95.2	0.90	89.7	0.79	100	1	92.3	0.85	88.8	0.74	88.5	0.77
CE + GSUS		85.6	0.71	30	−0.24	92.3	0.88	53.8	0.01	76.4	0.52	26.4	−0.42

Group I, diagnosed PsA after baseline assessment; group II, still suspected PsA; group III, diagnosed PsA; BL, baseline; FU, follow-up; CE, clinical examination; FOI, fluorescence optical imaging; PVM, FOI PrimaVista Mode; p1–p3, FOI phases 1–3; GSUS, ultrasound in grayscale mode.

In group III, agreement rates of GSUS and FOI ranged from 56.2 to 71.4% in 2011 and from 23.6 to 49.4% in 2014, respectively. Corresponding agreement rates in group II ranged from 74.4 to 91% in 2011, with the highest agreements found in p3, and from 55.1 to 61.5% in FU. Also in group I, GSUS and FOI showed lower agreement rates ranging from 27.3 to 49.6% in 2014 with p2 and PVM exhibiting the highest agreement rates. The best agreement rate was found for PIPV (p2 and PVM) with 88.9%.

Safety

No side effects to the FOI examination or to indocyanine green (ICG) were detected during the study.

DISCUSSION

As far as we know, this is the first study presenting FU data on FOI results in patients with PsA or rather early PsA. Since PsA – as chronic, progressive disease in the majority of patients–results in radiological damage in up to 47% of patients at a median interval of 2 years (26), there is a great need for an objective and sensitive screening tool. Thus, the aim of this study was to explore the value of FOI to distinguish between acute and chronic disease stages for screening purpose.

We found that newly detected joints by MSUS and FOI (PVM, p2) in FU were positively associated with the change of diagnosis from suspected to confirmed PsA. These results match the findings of significantly increased number of joints with pathological findings in group I in FU, with the DIP joints being particularly affected.

The number of joints detected as affected in FOI p2 at the two study points differed significantly between the three groups (p = 0.013 at BL and FU) and between groups I and II at FU (p = 0.010). This indicates that FOI is able to distinguish between patients with clear and suspected PsA. Phase 2 seems to be most sensitive for this purpose, which underlines the importance of this phase for subclinical inflammation, as described previously (12).

Recent studies assumed that FOI p3 shows increased capillary permeability in which the dye ICG (indocyanine green) is more persistent than normal, which represents chronic changes that only develop in the course of disease (10, 27). This is consistent with our finding that patients with diagnosed PsA showed the highest prevalence of signal enhancement in FOI p3. Correspondingly, we found erosions in 43% of this group in the GSUS. Unlike group II with unchanged suspected PsA where almost no changes could be found in FOI p3, signal enhancement in p3 was three times more frequent than at the time of the baseline study in group I. Interestingly, the signal enhancements found at FU in this phase were mainly slight changes (grade 1). In case that only higher-grade changes (grade $\geq$2) had been

considered as an evaluation criterion, this information would have been lost, since only one joint in this cohort showed a higher-grade change in FOI p3 in the FU. This could be possibly taken into account for diagnostic and consequently further therapeutic decisions.

Comparable studies (10, 12) already described that especially the flooding in and the washing out of ICG may depend on an increased and dysregulated microcirculation, which leads to the assumption that phase 1 visualizes active inflammation. In contrast to Erdmann-Keding et al. (15), the group with unchanged suspected PsA did not show higher-grade changes in FOI p1. However, this may be explained by a falsification of the results due to a systematic therapy existing at the time of FU in these patients. Glimm et al. detected statistically significant reduction in FOI sum score (FOIAS – fluorescence optical imaging activity score) when they investigated FOI as a tool for therapy monitoring in patients with early and active rheumatoid arthritis (RA) under DMARD therapy in a 1-year FU period (10). Therefore, it is possible that our study may have found fewer changes in FOI phase 1, since 67% of the patients with unchanged suspected PsA already received systematic therapy at the time of FU (whereas only 29% received csDMARDs (conventional synthetical) and 23% bDMARDs (biologic) at baseline).

We found good to very good association of FOI and CE (swollen joints) with FOI p1 and p3, which shows the highest accordance for the groups II and III. This result is consistent with the findings of Werner et al., which showed highest agreement between FOI p1 and swollen and tender joints indicating that p1 displays joints with high clinical activity (9). The good agreement of FOI p3 and CE in group III can be explained by already existing chronic joint changes, which are reflected in the FOI in p3 as chronic capillary leakage. In group II, there were almost no changes in FOI p3 in clinically unaffected joints. This is in line with the findings of a systematic literature review (SLR) by Zabotti et al. presenting that the risk of PsA development in PsO patients with arthralgia was about two times greater than in subjects without arthralgia (28).

Disagreement of FOI p2 and CE results from a higher rate of positive findings in FOI (see also **Supplementary Data** and **Supplementary Table 1**). This may underline the importance of this phase for subclinical inflammation, which cannot yet be detected by clinical investigation. It is also consistent with the findings of Werner et al. who found positive findings in FOI in 45% of clinically asymptomatic joints (9). The visualization of changes in microcirculation and vascularization by FOI may enable the detection of a very early PsA disease state in a pre–subclinical phase – in transition to a clinical stage (29). This is underlined by the hypothesis that non-specific musculoskeletal symptoms in patients with psoriasis may actually represent a preclinical phase of PsA (30, 31). In addition, Faustini et al. reported that the risk for developing PsA was as high as 60% if patients had subclinical synovitis and symptoms related to arthralgia highlighting the importance to establish an early PsA diagnosis (32).

Unlike ultrasound, FOI examination can be performed by medical assistants and does not require the presence of a medical physician. Although the analysis must be performed by a physician, it can be undertaken at a different time and thus offers flexibility. Even if the injection of ICG is necessary for the procedure of FOI, it has been shown that ICG is well tolerated and side effects (i.e., anaphylactic reaction) occur rarely (1:42,000) (33). FOI can therefore be regarded as a safe examination method and is also associated with a low expenditure of time. All these advantages of the FOI method allow an easy integration of the examination into the clinical routine.

Study limitations include the relatively low number of patients in this FU study. To further investigate FOI's ability to detect early PsA, larger-scale studies with several FU examinations are necessary. This study characterizes a pilot study to (further) explore the role of FOI as a screening tool for PsA development. Furthermore, patients of all three groups were under systemic therapy at the time of the FU, which implies that our findings might not reflect the "natural state" of disease. However, of the patients from group II who received systemic therapy at the time of FU, one-third received it exclusively for psoriatic skin lesions and another one-third for symptomatic joint pain. A further limitation of the study might have arisen from a possible interference of the results by osteoarthritis (OA), which might have led to false-positive results due to the inflammatory effect of (early) OA.

This work supports the findings of the baseline study and therefore provides further evidence that FOI is able to distinguish between acute and chronic disease stages. Hence, FOI can be considered as an useful screening tool for the early diagnosis of PsA. Since a delay in diagnosis impacts on long-term joint damage and functional disability (4, 5), its application in daily routine can help to diagnose early PsA in time to prevent progressive joint damage. An integration of this method as screening for prompt recognition of patients demanding a further referral can contribute to achieve this goal.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Medical Ethical Committee of the Charité – Universitätsmedizin, Berlin, Germany. All patients provided informed consent to participate in the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JB, A-MG, JK, and SO made substantial contributions to the conception and design of the work, the acquisition, analysis, and interpretation of data for the work. JB, A-MG, GK, ME-K, G-RB, PH, JK, and SO were drafting the work and revising it critically

for important intellectual content. All authors provide approval for publication of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.845545/full#supplementary-material

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Women in Rheumatology in the Arab League of Associations for Rheumatology Countries: A Rising Workforce

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Background: An increase in women physicians in the medical workforce is witnessed in recent decades, paralleled by an increase in leadership positions and, to a lesser extent, in academic advancement.

Objectives: This study aims to evaluate the women rheumatologists (WR) workforce and to identify the challenges faced by WR in the Arab League of Associations for Rheumatology (ArLAR) countries.

Methods: We collected data from members of special interest groups from 16 ArLAR countries about the number of rheumatologists in the national societies and boards – including WR –, and the estimated percentage of WR involved in academia. Also, a sample of WR was identified based on their established leadership positions and invited to a structured interview addressing their career path and the gender-related challenges.

Results: The proportion of WR varied widely across the ArLAR countries, with a mean of 56%. Moreover, WR constituted 47% of the society's board members and roughly 49% of WR were involved in academia. However, only 37% of the current society presidents were females. Structured interviews indicated that WR place a high value on patient care and teaching, followed by research and publications. The primary reported gender-related challenge was balancing work with family demands. Moreover, some WR reported gender-related discrimination incurred by colleagues, patients, and administrations.

Conclusion: WR constituted more than half of the current rheumatology workforce in the ArLAR countries, with a lower – but steadily growing – proportion of WR in leadership positions. As they embrace their growing role in the workforce, WR must benefit from all the provided tools, from learning from the experience of current women leaders in the field to using the latest technology such as social media platforms to empower them to reach gender equity.

Keywords: rheumatology, workforce, leadership, female, women empowerment, social media

INTRODUCTION

Gender disparities in medicine have always been a subject of debate, as women have faced several challenges in achieving gender equity, especially in reaching and maintaining leadership positions and academic advancement (1). However, an increase in women physicians in the medical workforce is witnessed in recent decades (2–4), and this rise in the workforce is mirrored by an increasing proportion of women becoming program directors and division heads in different branches of medicine (5). However, in academia, women are still less likely to be promoted to the rank of associate or full professor and have fewer publications with first and senior authorship (6). Moreover, women are under-represented as first and senior authors for publications reporting industry-sponsored studies and randomized controlled trials (7), whereas gender parity is more balanced in the first authorship for investigator-led research publications. One of the reasons behind this discrepancy could be that women engage in clinician-educator tracks rather than research tracks (1), and that they take a less important part in industry-collaborative research than men.

In the Arab countries, the number of female physicians has also been steadily increasing with time, which is paralleled by an increase in leadership positions (8, 9) in different medical specialties. However, according to our knowledge, there are no specific studies about the female rheumatology workforce in the Arab countries of the Middle East and North Africa (MENA) region. The Arab League of Associations for Rheumatology (ArLAR) includes 16 countries with 384 million inhabitants. These countries extend over 13 million km^2, 2 continents (Asia and Africa), and 4 time zones (UTC+0 to UTC+4) (10, 11) and share the same language and some cultural similarities. Notably, these countries are connected through an organized network that facilitates the conduction of collaborative research.

This study aims to evaluate the women rheumatologists (WR) workforce and to identify the challenges faced by WR in the ArLAR countries.

MATERIALS AND METHODS

Women Rheumatologists Workforce

For the purpose of this study, the workforce was defined as the WR practicing in ArLAR countries. Rheumatology fellows, nurses, and assistants were not included in the current analysis. Data were collected in 16 Arab countries that are part of the ArLAR in November and December 2021.

The data regarding the number of rheumatologists registered in the national societies and the number of rheumatologists in the national society boards – including the number of WR specifically – and the estimated percentage of WR involved in academia were requested by e-mail from society members working in special interest groups within the ArLAR. In addition, the gender of the past and current president of the national society was recorded. Finally, the size of the population by country was retrieved from the 2020 World Bank source (12). The data were presented descriptively using numbers and percentages.

Current Challenges for Women in Rheumatology

Women rheumatologists from 17 Arab countries (16 ArLAR countries and Bahrain) were identified based on their current or past known leadership positions (past or current society presidents, society board members, and WR with high academic positions such as dean of medicine). The selection of the rheumatologists was based on a convenience sampling technique, closely related to their leadership positions, associated to a quota sampling technique, where at least one woman rheumatologist was invited from each Arab country. Each WR was interviewed by one of the authors using a live interview on the Zoom platform. In case of the impossibility of doing a live interview, responding by e-mail was proposed. The interview was based on a structured questionnaire including 12 questions compiled from previous interviews conducted by the authors (**Supplementary Data**). The questionnaire comprised two parts: the first one corresponding to the rheumatology career in general and the second part to the specific challenges faced due to the female gender. The questions were formulated in English, and the interview was conducted in mixed language (English and Arabic). Data were collected over 2 months (November and December 2021).

All the answers were transcribed in a Microsoft Excel document and classified by question. There was no specific software used in the qualitative analysis. Thereafter, a qualitative analysis of the responses to each question was done separately. The answers were analyzed by the authors and grouped into homogeneous themes or domains. The number of times a theme recurred was also recorded. Data was published anonymously.

RESULTS

Women Rheumatologists Workforce

Globally, the Arab countries have 3,454 registered rheumatologists for a total of 382 million inhabitants, indicating a proportion of 0.90 rheumatologists per 100,000 inhabitants (or one rheumatologist for 110,596 inhabitants). However, this proportion varied widely among countries and ranges from 0.23 in Libya to 1.8 rheumatologists per 100,000 inhabitants in Tunisia (**Table 1**).

The proportion of women within the national rheumatology societies was 56.46% (1,922/3,404), ranging from 7.14% in Palestine to 70.91% in Tunisia, with a median of 42.35%.

Within the national society board committees, women constituted 47.1% of all board members (65/138), with a wide range going from 0% in Palestine to 100% in Sudan and a median value of 42.86%.

The percentage of WR involved in academia was estimated to be 49.33%, ranging from 15% in Jordan to 100% in Palestine (based on only one woman rheumatologist).

As for the leadership position within the national society, the past president was a female in 26.7% of the societies (4 presidents

TABLE 1 | Rheumatology workforce in the ArLAR countries.

Country	Country population (in millions)	Number of rheumatologists in the national society	Number of WR in the society (% from all rheumatologists)	Number of rheumatologists in the society board	Number of WR in the society board (% from all board)	Percentage of WR active in academia	Sex of the past society president	Sex of the current society president
Algeria[#]	43.8	614	260 (42.4%)	23	14 (60.9%)	50%	Male	Female
Egypt	102.3	1,388	944 (68.0%)	14	3 (21.43%)	89%	Male	Male
Iraq	40.2	263	88 (33.5%)	7	1 (14.3%)	30%	Male	Male
Jordan	10.2	34	8 (23.5%)	5	1 (20.0%)	15%	Male	Male
Kuwait	4.3	35	18 (51.4%)	4	3 (75.0%)	50%	Female	Female
Lebanon	6.8	51	18 (35.3%)	7	3 (42.9%)	39%	Male	Male
Libya	6.9	16	11 (68.8%)	5	3 (60.0%)	40%	Male	Female
Morocco	36.9	370	239 (64.6%)	14	12 (85.7%)	62%	Male	Male
Oman	5.1	15	6 (40.0%)	7	2 (28.6%)	50%	Female	Male
Palestine	4.8	14	1 (7.1%)	4	0 (0%)	100%	Male	Male
Qatar	2.9	30	10 (33.3%)	10	3 (30.0%)	50%	NA*	Female
Saudi Arabia	34.8	246	96 (39.0%)	9	2 (22.2%)	NA	Male	Female
Sudan	43.8	44	27 (61.4%)	4	4 (100%)	33%	Female	Male
Syria	17.5	64	40 (62.5%)	7	3 (42.7%)	17%	Female	Female
Tunisia	11.8	220	156 (70.9%)	5	4 (80.0%)	66%	Male	Male
UAE*	9.9	50 (approximate)	NA	13	7 (53.8%)	NA	Male	Male
Total	382	3,454	1,922 (56.5%)	138	65 (47.1%)	49.4%	4 female/15 26.7%	6 female/16 37.5%

NA, not available; WR, women rheumatologists.
[#]Data pooled from three societies.
**Data pooled from two societies.*

in 15 countries), whereas the current president is a female in 37.5% of the societies (6 presidents in 16 countries) (**Table 1**).

Current Challenges for Women in Rheumatology

The authors invited 19 WR to participate in the qualitative structured interview; 15 rheumatologists from 14 Arab countries accepted (78.95%), 1 did not respond, and 3 declined for technical problems or lack of time (**Figure 1**). The interview was performed live over Zoom with 11 WR and over email with 4 WR. The live interviews' duration ranged ted from 17 to 21 min, with an average duration of 19 min. The participants' age ranged from 45 to 70 years. Two of the interviewees were previous dean of medicine, five were current Societies' presidents, five were head of departments, and were three Societies' president-elect. All of the interviewees were involved in academic activities, in addition to their clinical work, and they were from the following countries: Algeria, Bahrain, Egypt, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Qatar, Saudi Arabia, Sudan, Syria, and Tunisia.

Regarding their career in general, the first reason for choosing rheumatology was the intellectual interest in the specialty *per se*, considering it as challenging, analytical, and multisystemic. Some WR chose rheumatology because of an unmet need for this specialty in their respective countries, and a few chose it because of the convenient lifestyle (**Table 2**). In 13 WR, the influence of a mentor who had an impact on their career choice was reported. Most of the time, the mentor was a male, and his origin was balanced between local and international.

Very diverse elements shaped the WR's careers, topped by the dedication to patients as a first component. Also, many WR reported the importance of their parent or partner's support, the role of international training and networking, and the added value of training in research and epidemiology.

Among the most cited skills that helped the WR be a role model and inspiring doctor were professionalism and commitment. In addition, communication was considered an important skill, as well as being a good team player, having good work ethics, and being empathic. Moreover, continuous update of knowledge was considered an essential personal skill.

When asked about their main achievement so far, most WR cited their teaching role, transmitting knowledge to young rheumatologists and being a role model for them.

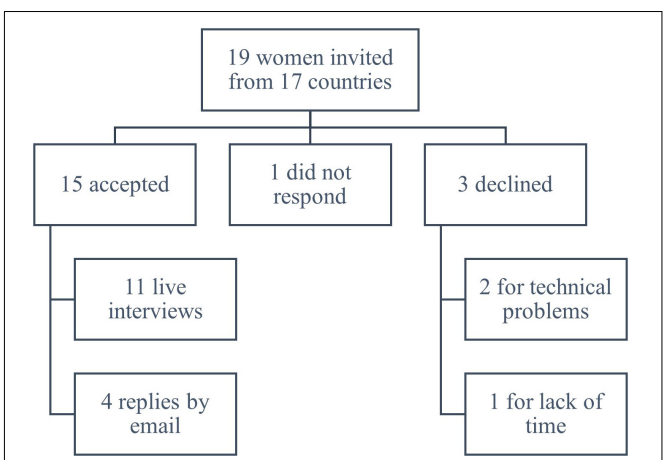

FIGURE 1 | Flowchart of interview participants from the 14 ArLAR countries.

TABLE 2 | Summary of the interviews with women rheumatologists from the ArLAR countries regarding the rheumatologist's career journey.

Question	Summary of responses (number of response recurrence)
How did you become interested in rheumatology?	Intellectual interest in the specialty: challenging, multisystemic, analytical (8) Unmet need in the country (4) Convenient lifestyle (3) Personal or familial musculoskeletal experience (2) Specific patients' cases (2) Research opportunities, choice by default
Did you have any mentors?	International mentor (5), local mentor (4), both local and international mentors (4) No mentor (2)
What are the top 3 elements that shaped your career?	Dedication for patients (5) Parent/partner support (4) International training and networking (4) Discipline and commitment (3), continuous medical education (3) Training in research (3) Ambition (3) and love for the challenge (3) Love of transmitting knowledge/teaching (3) Personal experience as a patient (1), ethics and religious beliefs (1), having a good mentor (1)
What 5 skills helped you to be a role model and inspiring doctor?	Professional and committed (7) Good communicator and good listener (6) Good team player (5) Have good work ethics and respect for patients (5), empathic (5) Continuously updating knowledge (5) Enthusiastic and motivated for work (4), passion for teaching and sharing knowledge (4) Patient (3), positive and confident (3), humble, friendly, and kind (3) Strategic thinker (2) Ambitious (1), strong (1), leader (1)
What has been your main achievement so far?	Teaching of young rheumatologists and being a role model for students and colleagues (9) Having success within the national rheumatology society (8) Being a member of an international organization/network (7) Promoting research and publications (6) Establishing a rheumatology unit/managerial position (6) Assuming an academic leadership position (4) Raising a family (3) Promoting patient education (2)
What is your current professional focus?	Improving research (10), improving rheumatology training (5) Assuming an academic leadership position (4) Promoting and being an advocate for rheumatology (3) Leading national and regional organization (ArLAR) (3) Managing a rheumatology unit (3), promoting patient education (3) Improving personal skills (such as musculoskeletal ultrasound) (1) Narrowing the gender gap in rheumatology (1)
What is next for you? Is there a dream job as a rheumatologist?	Four persons state that they have reached their highest potential Boost research and publications (5) Promote rheumatology in the country (3) and the region (2) Develop a rheumatology excellence center (2), establish a private career (2) Leading the national society (1), teach the new generation (1) Establish a women-based task force to support women research and training (1)

Also, many WR considered their active part in national and international societies as a significant achievement. Moreover, promoting research and publications was considered the main achievement in many.

The current professional focus of most WR was improving research, followed by improving rheumatology training and assuming an academic position.

As for the challenges that the interviewees faced because of being women (**Table 3**), it is interesting to note that four women reported no gender-related obstacles. For the rest of WR, the primary obstacle was balancing work with family demands, leading to a need to work more to prove oneself and to loss of career opportunities in some cases. The WR also faced reluctance from male colleagues to have a woman leading the team and from male patients to gain their confidence and be at ease during a clinical exam. However, some women reported that female patients were more likely to consult a female physician, which has been an advantage since most rheumatic diseases are more prevalent in females. Also, WR reported under-appreciation and delay in career advancement from the administration because of their gender.

Nevertheless, WR managed to reach a good work-life balance using time management, early planning, delegating, and having support from the family, although they reported that many compromises had to be made, and priorities had to be set. They provided some motivational advice for the new generation: "Have faith," "Set your goals, be persistent, and you will fulfill your dreams." Most of them are satisfied with the current status of WR in the ArLAR countries and are very optimistic about their future. However, since a higher proportion of rheumatologists are

TABLE 3 | Summary of the interviews with women rheumatologists from the ArLAR countries regarding the challenges faced as a woman and how to overcome them.

Question	Summary of responses
Did you face any obstacles because you are a woman? If yes, name the top 3.	Four out of 15 women said that there were no obstacles during their careers. Balancing work with family demands (6) Reluctance from male colleagues to have a woman leading the group (3) Getting the confidence of male patients and examining them (2) Under-appreciation, and delay in career advancement from administration (2) Criticism over being a woman active on social media
How did you manage your work-life balance?	Time management and early planning (11) Delegating to teams at work and to the family at home (5) Support from husband (5) from family (5) Need to do compromise and set priorities (5) Have a dedicated time for family as a priority (4) Have personal time (4), for example, sports activities, reading, exercising, traveling, and hobbies Compartmentalization of personal and professional life
Do you have any tips for the new rheumatologists about that?	Be confident (2), set goals (2) Be strong, persistent, motivated Accept failure, update knowledge
How do you see the future of women rheumatologists in our region?	Very qualified current workforce (5) and promising future for WR (9) Collaboration among countries to make a higher impact No concern about women but concern about rheumatology as a threatened discipline (2) Although a majority, 30% of WR do not work because of family or because they cannot afford to have a private clinic Universities do not believe in women taking leadership positions

women today, and since WR tend not to work or work only as part-time (30% as per one's estimation), and due to many other non-gender-related challenges, they are concerned about the future of rheumatology as a discipline. Therefore, they propose that women and men rheumatologists join forces to advocate strongly for their specialty.

DISCUSSION

In the ArLAR countries, the proportion of WR (56% in the current study) was higher than the ones reported in other countries: 52% in Canada in 2021 (13), 50% in Australia and New Zealand in 2019 (14), 49% in Latin America in 2020 [49.2% (15)], 47% in the United Kingdom in 2018 (16), and 41% in the United States in 2015 (2). Nevertheless, according to the 2015 American College of Rheumatology (ACR) workforce survey, it was anticipated that women will make up to 57% of the United States rheumatology workforce by 2030 (2). This higher proportion of women in the ArLAR countries could be related to the unmet need for rheumatology specialists in these countries or the convenient lifestyle of rheumatologists.

As females are reported to treat 30% fewer patients than their male counterparts according to the 2015 ACR workforce survey or to stay at home in up to 30% in some Arab countries, the need for rheumatologists, in general, is expected to rise in the future if the female to male ratio increases (2). Thus, the concern of some of our interviewees about the future of rheumatology as a discipline is well-founded.

In general, the global number of rheumatologists per inhabitant (0.9/100,000) was similar to that in Latin America [1 per 106,838 inhabitants in a cross-sectional study from 19 countries in 2020 (15)] and most European countries [ranging from 0.5 to 0.93/100,000 (17)]. However, it was lower than the number of rheumatologists in the United States [1.9 per 100,000 inhabitants as per the 2015 American College of Rheumatology (ACR) Rheumatology Workforce Study Report (2)] and in France [3.8/100,000 (18)].

Despite the increasing women to men ratio in rheumatology, WR from the ArLAR region reported obstacles in academic advancement and leadership positions. At the international level, gender bias is obvious in the authorship of scientific articles. Overall, men have a higher publication rate than women across different scientific disciplines (3, 19). Moreover, women authors receive fewer citations (20, 21) and are also under-represented in first and senior authorship positions in articles published in medical journals, even in disciplines such as family medicine which are enriched for women practitioners (3, 22, 23). Nevertheless, according to this study, 49% of WR were involved in academic positions in the ArLAR countries, compared to 41% in the United States, according to a cross-sectional on 6,125 rheumatologists (6).

According to the interviews, it was perceived, first, that WR in the ArLAR countries tended to give a higher value to patient care and teaching and being close mentors to their students. This preference for being closer to the patients and to the students was also reported by others, as women seem to engage in clinician-educator tracks (1). Subsequently, it implied seeking fewer managerial and leadership positions. Second, priority given for family responsibilities in general, and motherhood duties in particular, was mentioned several times by the interviewees. In fact, 3 out of the 15 women mentioned that that their main achievement so far was raising a family. Also, having an optimal work-life balance was the primary obstacle faced by WR in the ArLAR countries, as it was mentioned by 6 out of the 15 interviewed women. This was not specific to the region but is reported by others, as demonstrated in the recent ACR 2021 session with WR leaders from all over the world (24).

As a response to these challenges, WR cited time management and early planning as a first solution. To help reach an optimal work-life balance, WR in the current study and the ACR session cited the role of family support and delegation as a second solution. In addition, good mentors were mentioned in most cases as having a positive impact on the career path of the WR, as were the hard work and pursuing higher levels of education, especially abroad when coming from developing countries. Finally, it was clear that there was a need to compromise and to set priorities, often citing family as a priority and sometimes advising to have personal time as well. As per most of the interviewees, the role of WR will continue to grow in the future. One of the means of growth may be their engagement on social media (SoMe). In a survey among 233 rheumatologists from 47 countries (25), 83% of respondents were active users on at least one SoMe platform, with an average weekly use of 6 hours and a majority of use related to work. Nevertheless, 72% of respondents were aged 30–39 years, and the results may not be extrapolated to older generations. Lack of knowledge about how to use SoMe was the most common reason for not using it, as found in studies conducted in Arab countries, where lack of technical knowledge of SoMe and how to use it positively and safely within privacy settings were the main barriers to using social networks (26). This highlights the need to understand better the value of SoMe and the opportunity to educate potential users on how to use it positively to facilitate learning and inter-professional relationships (27).

The current study has some limitations. The count of WR did not consider whether they worked part or full-time. Also, the level of academic positions was not available. Moreover, the selection criteria of the women for the interviews may be subjective as may be the estimation of what a "successful" WR is. Nevertheless, choosing women with leadership positions (dean of medicine, president of society, and head of department) could be used a surrogate for success definition. Also, since participants are already successful figures, the study may have missed the less successful WR who might have faced more difficult challenges not reported here.

CONCLUSION

The proportion of WR varied widely across the ArLAR countries, with a mean of 56%, higher than the one reported from the rest of the world. Moreover, WR constituted 47% of the society's board

members. However, only 37% of the current society presidents were females, with an increasing trend in leadership positions over time. Roughly 49% of WR were involved in academia, but the level of involvement was not reported. Structured interviews indicate that WR place a high value on patient care and teaching, followed by research and publications. The primary reported gender-related challenge was balancing work with family demands. Moreover, some WR reported gender-related discrimination incurred by colleagues, patients, and administrations. Nevertheless, they provided valuable advice on overcoming these challenges and kept an optimistic view of their role in the future. As they embrace their growing role in the workforce, WR must benefit from all the tools provided to them, from learning from the experience of current women leaders in the field to using available technology such as social media platforms to empower them to reach gender equity.

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

NZ, IH, and LE designed the study, collected the data, analyzed the results, drafted the manuscript, and revised it for intellectual content. All authors approved the version to be published and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.880285/full#supplementary-material

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Pregnancy in women with Hereditary Angioedema due to C1-inhibitor deficiency: Results from the ITACA cohort study on outcome of mothers and children with *in utero* exposure to plasma-derived C1-inhibitor

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Background: In women with Hereditary Angioedema (HAE) due to C1-inhibitor (C1INH) deficiency (C1INH-HAE), pregnancy counseling and treatment can be challenging. Despite the evidence of the immediate favorable outcome and safety of plasma-derived (pd)C1INH concentrate, there are no data regarding any difference among women who underwent or not pdC1INH during pregnancy or on children with *in utero* exposure to pdC1INH. The present interview study aimed at analyzing outcome of C1INH-HAE mothers and children according to pdC1INH-exposure during pregnancies.

Methods: C1INH-HAE women who experienced at least 1 pregnancy were included from seven centers of the Italian Network for Hereditary and Acquired Angioedema (ITACA). The interview study retrospectively analyzed pregnancies who underwent (group 1) or not (group 2) pdC1INH. The overall goals of the study included immediate and long-term outcomes, in terms of outcomes in the time interval between pregnancy and survey.

Results: A total of 168 pregnancies from 87 included women were analyzed. At term delivery (>37 gestation-week, GW) has been registered in 73.8% of cases, while spontaneous abortion (SA) occurred in 14.2% of cases with a mean GW 7 ± 2. The group 1 including pdC1INH-treated pregnancies comprised a third of the cohort (51/168, time interval 1.5 ± 10.4 yrs), while the group 2 represented 69.6% (117/168, time interval 32.8 ± 14 yrs). The same prevalence of SA occurred when comparing group 1 (11.7%) with group 2 (15.4%) with a similar

GW at SA. The group 1 was older at the pregnancy time and younger at the interview than the group 2 ($P < 0.01$ for both); moreover, the group 1 showed a higher prevalence of cesarean delivery ($P < 0.0001$). The overall prevalence of obstetrical syndromes was similar between two groups: however, gestational diabetes was described only in pdC1INH-untreated pregnancies. *In utero* pdC1INH-exposed children ($n = 45$) did not show differences compared with unexposed ones ($n = 99$) in neonatal short-term outcomes.

Conclusion: Through appropriate management and counseling, most of C1INH-HAE women undergo successful pregnancy and delivery. For pregnant C1INH-HAE women being treated with pdC1INH, our findings are reassuring and might lead to an improvement of both the knowledge about treatments and the experience of HAE itself.

KEYWORDS

C1-inhibitor, hereditary angioedema, education, counseling, pregnancy

Introduction

Hereditary angioedema (HAE) resulting from the defect of C1 inhibitor (C1INH) is an autosomal dominant disease characterized by recurrent attacks of cutaneous and submucosal swelling in any site which are generally self-limiting within 72 h (1, 2). The defect of C1INH comprises either a deficiency (type I C1INH-HAE) or a dysfunction (type II C1INH-HAE) of the protein, allowing for a dysregulated plasma kallikrein activity within the kallikrein–kinin pathway, and thus for the overproduction of bradykinin with, in turn, the consequent activation of the bradykinin B2 receptors (1, 2). The resulting increased vascular permeability induces angioedema attacks that recognize several triggers including stress, infections, and estrogens (3, 4). In this context, women with C1INH-HAE experience a greater incidence of attacks and a stronger clinical severity with poorer quality of life than male patients (5). Women can be asymptomatic until puberty, while the exposure to increased concentrations of estrogens–both endogenous (puberty, menstrual cycle, and pregnancy) and/or exogenous (hormonal medications)—can trigger recurrent angioedema attacks (6). Although no epidemiologic studies showed a higher prevalence of reproductive failure in HAE women, previous evidence described few cases of C1INH-HAE women with abnormalities in both complement components and ovarian function, thus suggesting an intriguing interplay between kallikrein–kinin pathway and fertility (7). Moreover, as documented by data from the literature, a dysregulated complement system (CS) acts as a key factor in the pathogenesis of several obstetrical complications including early pregnancy loss, pre-eclampsia, and pre-term birth (8, 9). Therefore, in women with C1INH-HAE, pregnancy can be challenging because of the complex network among dysregulated plasma kallikrein activity, estrogens, and fertility. Nevertheless, reproductive outcome in C1INH-HAE women shows variable course, with the plasma-derived (pd)C1INH being the only specific treatment of angioedema attacks during pregnancy for both on demand and prophylaxis management (4, 5). Despite the evidence on the immediate favorable outcome as well on the safety of pdC1INH concentrate during pregnancy/labor (10–12), no data have been registered concerning differences among pregnancies who underwent or not pdC1INH or children with/without *in utero* exposure to pdC1INH.

Hence, the main aim of the present interview study was to explore outcomes of C1INH-HAE mothers and their children according to the exposure to pdC1INH during pregnancies in both therapeutic regimens: on demand versus long-term prophylaxis.

Patients and methods

We performed a retrospective cross-sectional study via an interview regarding pregnancy data of C1INH-HAE women who had experienced at least 1 pregnancy. The study was performed during a 12-month period (2021). Seven centers of the Italian Network for Hereditary and Acquired Angioedema (ITACA) participated in the study (13). Inclusion criteria were: (a) female patients with a defined diagnosis of C1INH-HAE (1, 2), and 2) history of at least one pregnancy. Exclusion criteria consisted of pre-pubertal age of female patients, female infertility, no wish for offspring, no consent to study. The interview study thus analyzed pregnancies that underwent (group 1) or not (group 2) pdC1INH.

The overall goal of the study was to compare the immediate pregnancy outcomes in accordance with the *in-utero* exposure

to pdC1INH. The primary outcomes were: delivery (at-term or pre-term), occurrence of obstetrical syndromes for mothers, birth weight and Apgar for children. In addition, secondary outcomes included the prevalence of C1INH-HAE diagnosis from both mothers and living children, as well as other concomitant diseases at the last follow up. A semi-structured survey was developed, and the main items were selected to obtain data on pregnancy outcomes and pdC1INH treatment. Both quantitative and qualitative data were collected by using the semi-structured interview that was applied by experienced researchers at each ITACA center involved in the study. Data about C1INH-HAE comprised: age at onset of symptoms and diagnosis, triggers, frequency of angioedema attacks, C1INH-HAE diagnosis before pregnancy, C1INH-HAE treatment during pregnancies. Specific questions on pregnancies included: time of the occurrence of pregnancies and mother's age at pregnancy, type of pregnancy (spontaneous, assisted reproductive techniques, e.g. *in vitro* fertilization), occurrence of spontaneous abortion [SA, defined as a spontaneous pregnancy loss before 20 gestation-week (GW)], at term delivery (> 37 GW), pre-term delivery (≤ 37 GW) (14, 15), obstetrical syndromes [including hypertensive disorders of pregnancy (HDP), eclampsia, gestational diabetes], placental abnormalities (16–18). HAE severity was defined in accordance with frequency of angioedema attacks: high disease activity was defined as ≥1 attack/4 weeks. Both long-term prophylaxis (LTP) and short-term prophylaxis (STP), as well as on demand treatment of attacks during pregnancy were registered.

We included the following newborn outcomes: birth weight (normal ≥2.5 kg, low <2.5 kg, high ≥4.5 kg), Apgar score (normal range 7–10), breastfeeding (yes/no), C1INH-HAE diagnosis, congenital abnormalities, concomitant diseases (autoimmune systemic diseases and allergy) at the time of the survey (17, 19). The Local Ethics Committees approved the study and every patient provided informed consent at each ITACA center involved in the study.

Statistical analysis

The data were entered anonymously into a database and a descriptive statistical analysis was performed. Continuous data were expressed as mean (SD) if the distribution was normal; categorical variables were expressed as counts and percentages. Continuous variables were compared by using either the parametric unpaired *T*-test or the non-parametric Mann–Whitney U test, as appropriate. Categorical variables were compared using the Chi-squared test or Fisher's exact test, as appropriate. The *p*-values <0.05 were considered significant. All statistical analyses were performed using the GraphPad Prism version 9 (GraphPad software).

Results

Characteristics of study cohort

Eighty-seven female patients out of 234 followed-up in the 7 ITACA centers participating in the study fulfilled the inclusion criteria. The remaining women ($n = 147$) were excluded mainly for no consent to interview (36.7%) or no wish for offspring (34%), whereas pre-pubertal age (17.7%) and primary infertility (11.6%) represented minor cases.

A total of 168 pregnancies related to 87 women were analyzed (Table 1). All included women were type I C1INH-HAE and, in almost all the pregnancies, we registered C1INH-HAE familial history. Nearly all pregnancies were spontaneous (98.2%), with first pregnancies in a half of cases (50.6%). At term deliveries were reported in 73.8% of cases, while SA occurred in 14.3% with a mean GW 7 ± 1.9. Pre-term deliveries (≤ 37 GW) occurred in 12% of pregnancies. Obstetrical complications were reported in a quarter of the whole cohort (Table 1) and were mainly represented by preterm delivery (PD) (58.8%) and placental abnormalities (26.4%), whereas complicated labor by HAE acute attacks occurred in 11.7% of the pregnancies. The occurrence of other therapies during pregnancy has been documented in rare cases (14/168) and was levothyroxine supplementation for concomitant thyroiditis in half the cases and low-dose aspirin as isolated intervention for preventing obstetrical syndromes in the remaining cases.

Pregnancy outcome according to pdC1INH treatment

Approximately one third of the pregnancies ($n = 51$) underwent treatment with pdC1INH (group 1), while 117 (69.6%) were not treated with pdC1INH (group 2). In group 1 pdC1INH was administered in accordance with the disease severity: in pregnancies with a high disease severity ($n = 7$, 13.7%) it was administered as LTP (1000 UI pdC1INH, every 4 days) plus on-demand, whereas in the remaining cases it was used exclusively on demand (86.3%, 1500 UI pdC1INH). The pdC1INH was used as STP in all cases of elective cesarean deliveries in group 1. In order to explore the potential effect of the total amount of pdC1INH used during pregnancy, we additionally analyzed the few pregnancies on LTP and no significant difference in terms of prevalence of obstetrical syndromes occurred between LTP-pregnancies and on demand C1INH-pregnancies. The time interval between pregnancy and survey in the group 1 was significantly lower than in the group 2 (1.5 ± 10.4 yrs vs. 32.8 ± 14 yrs, p 0.01).

The prevalence of SA was similar in group 1 and 2 and with no differences in GW at the SA (Table 1). There were neither any significant differences regarding at term pregnancies between the two groups (Table 1). However, the mothers' mean age at

TABLE 1 Data from pregnancies in the study sample.

	Pregnancies ($N = 168$)	pdC1INH, YES ($N = 51$)	pdC1INH, NO ($N = 117$)
Mothers' age at pregnancy, yrs (mean ± SD)	26.5 ± 7.9	29.8 ± 5.4**	26 ± 4
Mothers' age at the study, yrs (mean ± SD)	48.5 ± 16.9	41.3 ± 7.7**	55.2 ± 14.2
Mothers' C1INH-HAE diagnosis after pregnancy (*N*/%)	73/43.5	3/5.8****	70/59.8
First pregnancy (*N*/%)	85/50.6	24/47	61/52.1
Cesarean delivery (*N*/%)	43/29.9	25/49****	18/15.4
At term delivery, >37 GW (*N*/%)	124/73.8	37/72.5	87/74.4
GW at delivery (mean ± SD)	39.2 ± 1.7	38.9 ± 1.6	38 ± 1.3
SA (*N*/%)	24/14.3	6/11.7	18/15.4
GW at SA (mean ± SD)	7 ± 1.9	8 ± 1.7	9 ± 1.3
Obstetrical complications			
Preterm, ≤ 37 GW (*N*/%)	20/11.9	8/17.8	12/12.2
Gestational Diabetes (*N*/%)	5/3.5	–	5/5
HDP (N/%)	3/2	3/6.7	–
Placental abnormalities (*N*/%)	9/6.3	4/8.9	5/5
HAE acute attacks §(*N*/%)	4/2.7	1/2.3	3/3

C1INH, C1 inhibitor; HAE, hereditary angioedema; pdC1INH, plasma derived C1 inhibitor; GW, gestational week; SA, spontaneous abortion; HDP, hypertensive disorders of pregnancy; § at the delivery; **P < 0.01, ****P < 0.0001.

pregnancy was lower in group 2 than in group 1 ($P < 0.01$), while patients were younger at the time of the interview in group 1 ($P < 0.01$). Interestingly, group 1 showed a significantly lower prevalence of HAE diagnosis after pregnancy than group 2 ($P < 0.0001$), as well as a higher prevalence of cesarean delivery ($P < 0.0001$). The overall prevalence of obstetrical syndromes was similar in the two groups, whereas gestational diabetes was only described in pdC1INH-untreated pregnancies (Table 1).

Children outcomes according to the *in utero* pdC1INH exposure

Data from 67 living children from all pregnancies were recorded (Table 2). A normal birth weight and Apgar score were documented in both *in utero* pdC1INH exposed and unexposed newborns. Also, the prevalence of breastfeeding was similar in both groups of infants (Table 2). At the time of the survey the age of pdC1INH exposed subjects was significantly lower than the age of unexposed ones ($P < 0.01$). Accordingly, the C1INH-HAE diagnosis has not been carried out in a higher proportion in pdC1INH exposed subjects than in unexposed ones ($P < 0.001$) while a defined C1INH-HAE diagnosis was documented in a higher prevalence among unexposed subjects ($P < 0.01$).

TABLE 2 Data from newborns in accordance with pdC1INH treatment.

	pdC1INH, YES ($N = 45$)	pdC1INH, NO ($N = 99$)
Normal birth weight (*N*/%)	37/82.2	87/87.8
Breastfeeding (*N*/%)	28/62.2	79/79.8
C1INH-HAE affected (*N*/%)	14/31.2 **	59/59.6
C1INH-HAE not affected (*N*/%)	20/44.4	33/33.4
C1INH-HAE diagnosis not carried out (*N*/%)	11/24.4 ***	7/7
Congenital abnormalities (*N*/%)	0/0	2/2
Systemic autoimmune diseases (*N*/%)	0/0	2/2
Allergy (*N*/%)	2/4.4	10/10.1
Other (*N*/%)	1/2.2	7/7
Current age, yrs (mean ± SD)	11.5 ± 10.4 **	32.8 ± 14

C1INH, C1 inhibitor; HAE, hereditary angioedema; pdC1INH, plasma derived C1 inhibitor.
**P 0.01 and ***P 0.001 with the respect to pdC1INH NO group.

Discussion

Pregnancy outcomes in C1INH-HAE women

Pregnancy outcome in C1INH-HAE women is still a challenge considering the effects that hormones and pregnancy itself have on complement and kallikrein–kinin pathway activation, that may worsen disease activity (20, 21). Moreover, considering the role of immune-mediated pathways in women reproduction, the dysregulated kallikrein–kinin pathway in C1INH-HAE women might potentially predispose to a worse pregnancy outcome (8).

The disease inheritance as well as the impact of unpredictable recurrent acute angioedema attacks might influence the family planning (22). Therefore, nearly a quarter of the C1INH-HAE women followed-up at the involved ITACA centers was not eligible for the interview study because of no wish for offspring. However, they represented a relatively small group of the C1INH-HAE women, probably because the

presence of family history of HAE in almost all the registered pregnancies led to adequate self-awareness of the disease.

In accordance with data from the literature, reproductive failure in terms of infertility and SA was not prevalent in our sample of C1INH-HAE pregnancies (5, 23). We documented that nearly all pregnancies were spontaneous and three-quarters of them experienced at term delivery. The occurrence of PD and SA in our cohort recognized a prevalence similar to that of non-C1INH-HAE women of child-bearing-age and this result may be associated with adequate therapeutic management (5, 6, 24). According to the data collected, all pregnancies with obstetric complications experienced such comorbidities, including PDH and metabolic abnormalities, only during the weeks of gestation and represented a very small percentage (10%). A subanalysis of pregnant women who suffered from hypertension could provide interesting insights on the effects of comorbidities on pregnancy outcome: however, few cases have been documented in the present study. These issues could be adequately addressed by further investigations of a larger cohort with a prospective study design.

The use of pdC1INH during pregnancy

One-third of all pregnancies in our population underwent pdC1INH for both treatment of acute HAE attacks and for prophylaxis, regardless of a specific gestational trimester. It might be surprising that two-thirds of all pregnancies had not received pdC1INH; however, more than half of them were classified as affected by C1INH-HAE after pregnancy and were therefore managed differently (5). Nevertheless, group 2 C1INH-HAE diagnosed pregnancies did not use pdC1INH due to low disease severity, suggesting that although pregnancy has been considered as a triggering factor for HAE attacks, it may have a good effect on HAE disease activity (5). As previously described, it is rare for clinical manifestations of C1INH-HAE to present for the first time during pregnancy; however, C1INH levels decrease during pregnancy in relation to increased plasma volume, and transient low levels of C1INH have been described in pregnant women without C1INH-HAE, making its diagnosis difficult during pregnancy (5). In our cohort, few group 1 pregnancies received pdC1INH without a definite diagnosis of C1INH-HAE because they had experienced angioedema attacks for the first time during pregnancy. However, the presence of a family history of HAE represented a high index of suspicion in all these cases, which allowed appropriate treatment with pdC1INH of the angioedema attacks. After pregnancy, all of these women received a certified diagnosis of C1INH-HAE. As documented, our results support the use of pdC1INH to achieve a favorable outcome in women with C1INH-HAE who experience worsening disease severity during gestation (23, 25–28).

Additionally, international guidelines support the use of pdC1INH as a long-term prophylaxis also in pregnant C1INH-HAE women with histories of miscarriage and/or high-risk pregnancies with a suggested dosage being the same as in nonpregnant patients despite the pregnancy weight gain (5). Consistent with our results, there were no differences in SA or PD rates between pdC1INH-treated and untreated pregnancies and no differences in delivery week in at term pregnancies. The reasons for PD have not been further defined as they were not related to acute HAE attacks or documented cardiovascular and/or metabolic disorders. Metabolic disorders presented a different prevalence between pdC1INH-treated and untreated pregnancies, showing a higher frequency in the latter group probably due to the use of steroids to treat angioedema attacks in group 2 women without a definite diagnosis of C1INH-HAE during pregnancy.

Furthermore, there was a significant difference between the groups with respect to the mean age of the women at the time of the study, since those who had received pdC1INH in pregnancy were younger than those who had not received pdC1INH in pregnancy. This difference suggests that women with C1INH-HAE who had a more recent pregnancy were more willing to treat angioedema attacks or had more medications available than women who had had a pregnancy in the past. In addition, our findings indirectly highlighted a gradual reduction in diagnostic delay over time in C1INH-HAE patients, as women in group 1 were younger and showed a higher rate of C1INH-HAE diagnosis before pregnancy compared to women in group 2.

Delivery in pregnant C1INH-HAE women

Even though cute attacks of HAE complicated few deliveries, management of HAE during labor requires special consideration because it may be exacerbated (6, 25). As expected, since all pregnancies with cesarean delivery and pdC1INH-STP were treated with pdC1INH (at least as STP during delivery), elective cesarean delivery with pdC1INH-STP was more common for pdC1INH-treated than untreated pregnancies. Cesarean delivery is not recommended in women with C1INH-HAE, so its higher prevalence in pregnancies treated with pdC1INH and therefore in younger women with HAE could reflect an increase in the indication of cesarean sections over time (22, 29–31).

As has been published, in women with C1INH-HAE, vaginal deliveries are preferred over cesarean sections, and in this setting, epidural anesthesia is preferred over general anesthesia to reduce the risk of acute attacks of angioedema (6). Nevertheless, labor and delivery only rarely induce an attack, which could occur either during labor or within 48 h of delivery (32). In our view, elective cesarean section, although not absolutely advisable, could allow for adequate and targeted management of delivery in selected women with C1INH-HAE,

mainly those with high severity of the disease and/or on LTP, to avoid emergent deliveries that could be complicated by difficult-to-treat angioedema attacks. Furthermore, pdC1INH should always be available in the delivery room, also in vaginal deliveries (5, 6).

Children outcomes and intrauterine pdC1INH exposure

Live birth data documented normal birth weight and Apgar score in neonates exposed and unexposed to pdC1INH *in utero*. These data directly support the immediate safety of pdC1INH during pregnancy as reported by evidence from the literature (23, 26). At the time of the survey study, the occurrence of a definite C1INH-HAE diagnosis had occurred at a lower rate among pdC1INH-exposed than unexposed neonates, resulting in a lower prevalence of C1INH-HAE in the first group of babies (32–34). The reason was related to the younger age of the C1INH-HAE women who underwent pdC1INH during pregnancy and, therefore, the younger age of their children. In C1INH-HAE, prenatal diagnosis in established pregnancy is only rarely requested and can only be performed if the disease-causing mutation of the affected parent is known (5). Nevertheless, C1INH-HAE diagnosis in neonates and infants can be performed by using biochemical tests that may be inconclusive in very young children (< 12 months). Genetic testing is thus a safer and more direct tool to determine whether a child has inherited the disease and in newborns it can be performed on umbilical cord or peripheral blood (5). In addition, biochemical and genetic testing of asymptomatic children with affected parent should be performed because presymptomatic C1INH-HAE children are at risk of unexpected attacks and the early diagnosis can help to ensure the adequate treatment (28). However, live birth data among neonates described that the occurrence of childhood deficiencies was uncommon and independent of intrauterine exposure to pdC1INH.

C1INH-HAE could produce autoimmunity due to the consumption of early components of the classical complement pathway, as in patients with genetic C1 or C2 deficiencies (35). It can be hypothesized that pdC1INH replacement therapy may have a modulatory impact on autoimmune diseases in C1INH-HAE (occurrence and/or severity) by increasing C1-INH, C4, and/or C2 as suggested in some studies (36). Similarly, the role of other complement components such as C3a and C5a as potential effectors in type 1 hypersensitivity reactions, as well as crosstalk between mast cells and complement suggest that complement activation may also synergize with classical IgE responses, possibly affecting allergic disorders (37, 38). Consequently, we analyzed the prevalence of autoimmune and allergic diseases in the two groups of children at the last follow-up (interview-time), and there were no differences. However, the different ages and the restricted population represent relevant limitations for these results that should certainly be confirmed by future clinical investigations. Further analysis on the occurrence of allergic and/or autoimmune diseases in a larger HAE sample and/or in the general population should be addressed in prospective studies that stratify children according to the co-diagnosis of C1INH- HAE.

Limitations and strengths

The main limitation of our results is represented by the retrospective design of the study that included women whose pregnancy had occurred more than 30 years earlier. The long-time interval in some cases could have given rise to a forgetting bias and consequently a lack of data and objective information on the use of pdC1INH, concomitant treatments and/or obstetric complications. For instance, the total amount of pdC1INH used during pregnancies was not available from our collected data due to bias related to the retrospective design. However, the exact amount of pdC1INH administered in each pregnancy would certainly have improved the quality of the results: therefore, further investigations with a prospective design should focus on the specific accumulated amount of received pdC1INH and its possible correlation with the outcomes.

Nevertheless, the retrospective design allowed us to focus on multiple data at the same time and long-term patient history, which could provide information on the course and burden of the disease, outcomes and therapeutic management over the years. Anyway, the main strength of the present study is represented by its multicenter design, which made it possible to obtain a representative and relevant sample of patients with such a rare disease.

Conclusions

Reproductive planning is a persistent concern for women with inherited and rare diseases (29–31). As known, mechanisms of reproductive failure involve immune-mediated pathways including dyregulated complement and kallikrein–kinin pathway, mainly locally, at the site of implant, also in women without C1INH-HAE (8, 39, 40). Accordingly, the ultimate goal for C1INH-HAE management, particularly during pregnancy, is to achieve disease remission and no attacks and thus to use appropriate treatments making the complete control of HAE a realistic possibility for patients (41–43). For pregnant C1INH-HAE women being treated with pdC1INH, our findings are reassuring and might lead to an improvement of both the knowledge about treatments and the experience of HAE itself.

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 study involving human participants was reviewed and approved by each local committee at the involved ITACA Centers. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

PT, GS, RS, and MCa contributed to the conceptualization and scope. PT, RS, and MCa read and approved the final version. All authors collected and analyzed data, contributed to the article, and approved the submitted version.

Conflict of interest

Author MCa received speaker/consultancy fees from BioCryst, CSL Behring, Pharming, and Takeda. Authors PT and MCa received speaker/consultancy fees from CSL Behring. Author RS received grants from CSL Behring and Takeda.

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

Publisher's note

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