Proliferative Synovitis, an Ultrasound Pattern Associated with ACPA positive Patients, Erosive Disease and Enhanced Need to Change Therapy in Rheumatoid Arthritis

Julio Ramírez (julramga@gmail.com)
Hospital Clinic de Barcelona Servei de Reumatologia https://orcid.org/0000-0002-7047-8056

Ana Belen Azuaga-Piñango
Hospital Clinic de Barcelona Servei de Reumatologia

Beatriz Frade-Sosa
Hospital Clinic de Barcelona Servei de Reumatologia

Roberto Gumucio-Sanguino
Hospital Clinic de Barcelona Servei de Reumatologia

Katherine Cajiao-Sanchez
Hospital Clinic de Barcelona Servei de Reumatologia

Andrea Cuervo
Hospital Clinic de Barcelona Servei de Reumatologia

Raquel Celis
Hospital Clinic de Barcelona Servei de Reumatologia

Jose Antonio Narvaez
Hospital Bellvitge Servei de Radiologia

Virginia Ruiz-Esquide
Hospital Clinic de Barcelona Servei de Reumatologia

Raul Castellanos-Moreira
Hospital Clinic de Barcelona Servei de Reumatologia

Andres Ponce
Hospital Clinic de Barcelona Servei de Reumatologia

Jose Gómez-Puerta
Hospital Clinic de Barcelona Servei de Reumatologia

Jose Luis Pablos
Hospital 12 de Octubre, Servicio de Reumatologia

Raimon Sanmarti
Hospital Clinic de Barcelona Servei de Reumatologia

Juan D Cañete
Hospital Clinic de Barcelona Servei de Reumatologia

Research article

Keywords: Rheumatoid Arthritis, Ultrasound, Magnetic Resonance Imaging, Biomarkers, Synovium

Posted Date: June 23rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37243/v1

Abstract

Objectives: To analyze ultrasound (US) differences between rheumatoid arthritis (RA) patients according to their autoantibody status and characterize the clinical, immunological and radiological features associated with the US pattern of seropositive patients.

Methods: We collected clinical and immunological data along with bilateral hand US images of RA patients. Serum biomarkers, MRI of dominant hand and immunostaining of synovial biopsies were performed.

Results: Two hundred and five RA patients were collected (84.8% seropositive). No significant differences in disease activity/therapy were found according to autoantibodies status. An extreme proliferative US pattern, encompassing synovial hypertrophy grade II-III with Power Doppler signal that we called US Proliferative Synovitis (US PS) was present in 55.5% of seropositive and 16.1% of seronegative patients, (p=0.0001). In the multivariate analysis, erosions [OR 4.90 CI 95% (2.17-11.07). p=0.0001] and ACPA [OR 3.5 CI 95% (1.39-10.7), p=0.09] but not RF status [OR 0.74 CI 95% (0.31-1.71), p=0.483] were independently associated with the presence of US PS.

Ninety-four per cent of joints with US PS scored 2-3 in RAMRIS synovitis sub-index. At synovial level, US PS was significantly associated with higher density of vessels (p=0.042). Moreover, significantly higher serum levels of angiogenic and inflammatory cytokines were found in patients with US PS.

After a mean of 46 months of follow-up, US PS was independently associated with change of therapy (OR 2.63, 95% CI 1.20-5.77, p=0.016).

Conclusions: ACPA+ RA was associated with US PS. This US pattern significantly detected erosive disease and an enhanced need to change therapy in the long-term.

Introduction

Ultrasound (US) is a non-invasive imaging technique playing an important role for the assessment of patients with rheumatoid arthritis (RA). The use of US is relevant along the different stages of the disease, more sensitive and reliable than physical examination and has a potential role to guide therapeutic interventions [1].

US findings in patients with chronic inflammatory arthritis include synovial proliferation or synovial hypertrophy (SH), neovessels or Power Doppler (PD) signal, tenosynovitis or soft tissue oedema. However, these imaging findings are not always present and may differ depending on the specific diagnosis. Different US patterns have been described for early RA and psoriatic arthritis (PsA) [2]. Whereas synovial involvement was the hallmark of RA patients, soft tissue changes were more frequently found in early PsA.

Seropositive (sero+) RA is a very well-defined disease. Its genetic, synovial pathology and immunological profile are well known. A worse clinical prognosis and more radiological progression seem to characterize sero + RA patients [3–4]. However, up to 30% of RA patients are negative for rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). These patients are more heterogeneous and not so well defined as sero + RA [5].

Furthermore, we and others have previously reported by arthroscopy a different synovial vascular pattern for sero + RA, characterized by straight vessels, and seronegative (sero-) RA, defined by tortuous vessels, similar to PsA pattern [6–7]. Interestingly, those different synovial vascular patterns were associated with different radiographic damage, with straight and mixed patterns having significantly more erosive disease. However, another immunohistological study in our center could not find differences in synovial vessel density according to ACPA status [8].

Few imaging studies have analyzed differences in RA according to autoantibody status [9–10]. In a recent study using Magnetic Resonance imaging (MRI), bone marrow oedema was associated with the combination of RF and ACPA. However, no association between autoantibodies serum levels and osteitis score was found [9].

We hypothesize that, similarly to as it is visualized by arthroscopy, sero- RA patients could have a different US pattern as compared to sero + RA, characterized by less synovial proliferation. The aim of this study was to analyze differences in the US synovial pattern between patients with sero + and sero- RA. Specifically, we aimed to characterize the clinical, immunological and radiological features of the US pattern associated with sero + RA.

Methods

Patients

Observational study. Consecutive patients from the Rheumatology Department (Hospital Clinic, Barcelona, Spain) meeting American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2010 criteria for RA [11] were enrolled. Clinical, immunological, demographic and US images of patients were collected. Seropositivity was defined as the presence of RF (>50 UI/ml) and/or ACPA (>50 UI/ml) at least twice at any time of the disease course. Probable RA or overlap syndromes were excluded. Before undergoing US, MRI, serological test or synovial biopsies, informed consent was signed. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Ultrasound Assessment

All sonographic assessments were performed using high-sensitivity US equipment (Acuson Antares®, Siemens AG, Erlangen, Germany) with a linear probe of frequency range from 8 to 14 MHz. Intraarticular infiltrations were not allowed one month previous to the US assessment. US findings were defined

according to published OMERACT definitions [12]. An experienced sonographer evaluated 6 joints of each hand (including MCP joints and wrists) for SH and intra-articular PD signals according to EULAR guidelines [13]. All evaluations were scanned on the dorsal aspect using longitudinal midline and transversal planes. PD calibrations and SH/PD assessment have been previously described [14].

We evaluated the morphology of synovial tissue. Specifically, we looked to identify an extreme proliferative pattern defined as the marked thickness of synovial membrane with PD signal inside the edges of the joint (Figure 1. A). Synovial thickening should bulge over the line linking tops of the periarticular bones and with/without extension to one of the bone diaphysis, similarly to the definition of synovial proliferation grade II or III adopted by Szkudlarek et al [15]. We adopted the term “US proliferative synovitis” (US PS) to define these features. On the contrary, the flat growth of synovial filling the angle between the periarticular bones with PD signal inside the joint, was defined as “flat synovitis”, a concept equivalent to SH grade I (Figure 1.B). Both US patterns included the presence of PD signal.

Four rheumatologists, blinded to clinical data and autoantibody status of patients, scored all the images. Interobserver reliability was evaluated before patients’ inclusion by scoring SH/PD in 50-recorded images of joints from 20 RA patients. Interobserver correlations were all good to excellent (range 0.608-0.831).

Histological and immunohistochemical assessments

All the US-guided synovial biopsies were performed in a case day surgery according to the technique previously described by Kelly et al [16]. Biopsies were taken from selected patients who signed the informed consent. 6-8 synovial biopsies were taken per procedure.

Paraffin embedded slides were prepared from synovial tissues and stained with an automated immunostainer (TechMate 500 Plus; Dako, Cambridge, UK). In short, we immunostained with anti-CD3, CD20, CD117, CD15, CD68, hsp-47 and CD31 antibodies as previously described [17].

Digital image analysis

The stained slides were scored by digital image analysis by an independent observer. Each stained slide was scored by dividing it in different regions. Within each region, the number of stained cells per area and the percentage of stained cells were measured in at least 20 high-power fields using the AnalySIS®Imaging processing program (Olympus®) as previously described [18].

MRI Assessment

Selected patients who signed informed consent were scanned on a 1.5 Tesla system (Siemens Aera, Siemens Medical, Erlangen, Germany) using a dedicated wrist coil. MRI protocol was previously reported [19].

Images were reviewed on a standard Dicom (Digital Imaging and Communication in Medicine) compliance workstation. Images were scored by two independent radiologists blinded to image time

point and patient identity using the Rheumatoid Arthritis Magnetic Resonance Imaging Scoring (RAMRIS) system [20].

Intraobserver reliability kappa values were 0.82, 0.83 and 0.90 for the RAMRIS semiquantitative scoring of synovitis, bone marrow oedema, and erosions, respectively; interobserver kappa values were 0.69, 0.74 and 0.84 [18].

Quantification of biomarkers of inflammation/angiogenesis.

Cytokines and angiogenic mediators were analyzed using Quantibody® Human Custom Array (RayBiotech, Norcross, GA, USA)1 [8]. Detection limits for cytokines are displayed on the manufacturer's website **[21].** After sample dilution, the effect of RF on the final results was estimated to be around 1% [22].

Calprotectin serum levels were determined using an ELISA Test Kit [CALPROLAB Calprotectin ELISA (ALP) CALPRO AS, Norway] in accordance with the manufacturer’s protocol [23].

Clinical Follow-up

At baseline and every fourth months patients underwent complete clinical and biological assessment. Treatment change was defined as the change in any conventional synthetic Disease-modifying drugs (csDMARDs) or biological synthetic Disease-modifying drugs (bDMARDS) (but not glucocorticoids) across the observational period.

Statistical analysis

Epidemiological, clinical and US variables were compared between patients with or without RF or ACPA and with US PS or flat synovitis. Numerical variables were described as mean and standard deviation (SD) and categorical variables as frequencies and percentages. T- student test was used to compare the distribution of numerical variables between groups. Chi-quared test was used to compare categorical variables. To ascertain independent associations between variables we used multivariate analysis. For all tests, p values ≤ 0.05 were considered significant. All analyses were performed using the SPSS 18.00 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of the cohort

Two hundred and five RA patients were collected. Demographic and clinical characteristics are shown in Table 1. All patients had predominant hand involvement at US assessment. Mean age was 57.1 (SD 14.1) years and the mean time of disease evolution was 113.3 (SD 105.7) months. Mean Disease Activity Score 28-joint count C reactive protein (DAS28-CRP) was 2.55 (SD 1.03). Overall, 173 out of 205 (84.8%) patients were sero + for either RF or ACPA autoantibodies. One hundred and eight patients (53.7%) had

radiographic erosions in hand/feet. At baseline, 75.6% were taking csDMARDs, 34.3% bDMARDS, and 49.3% low doses of glucocorticoids (≤ 5 mg of prednisone or equivalent).

Table 1
Demographic and clinical characteristics of RA patients according to US pattern.

	Total Patients	Ultrasound Pattern		p value
	n = 205	Proliferative (n = 101)	Flat (n = 104)	
Female, n (%)	162 (79.4)	79 (78.2)	83 (80.6)	0.576
Age, mean (SD) years	57.1 (± 14,1)	56.3 (± 12.0)	58.0 (± 15.9)	0.400
Current Smoker, n (%)	47 (26.9)	22 (25.6)	25 (28.1)	0.736
Disease duration, mean (SD) months	113.3 (± 105.7)	127.7 (± 111.1)	99.3 (± 99.3)	0.057
Erosive disease, n (%)	108 (53.7)	73 (72.3)	35 (35.0)	**0.0001**
ACPA, n (%)	153 (75.4)	89 (88.1)	64 (62.1)	**0.0001**
RF, n (%)	99 (68.3)	78 (77.2)	63 (61.2)	**0.010**
DAS 28–CRP, mean (SD)	2.55 (± 1.03)	2.66 (± 1.04)	2.44 (± 1.02)	0.170
GC, n (%)	99 (49.3)	45 (45.5)	54 (52.9)	0.324
csDMARDs, n (%)	152 (75.6)	81 (81.8)	71 (69.6)	**0.050**
bDMARD, n (%)	69 (34.3)	35 (35.4)	34 (33.3)	0.769
Total US score (SH + PD)	14.9 (± 11.5)	18.8 (± 11.8)	11.1 (± 9.9)	**0.0001**

ACPA: anti-citrullinated protein antibodies, bDMARD: biological disease-modifying antirheumatic drugs, CRP: C-reactive protein, csDMARDs: conventional synthetic disease-modifying antirheumatic drugs, DAS28-CRP: Disease Activity Score 28-joint count C reactive protein, GC: glucocorticoids, PD: power Doppler, RF rheumatoid factor, SD: Standard Deviation; SJC: swollen joints count, SH: Synovial hypertrophy, TJC: tender joint count, US: Ultrasound.

Clinical differences between seropositive and seronegative RA patients

Overall, no significant differences were found between sero + and sero- patients in terms of disease activity (SJC, tender joint count [TJC], CRP, DAS28) or therapy (glucocorticoids, csDMARDs, bDMARD). Disease duration was longer (p = 0.005) and the proportion of patients taking glucocorticoids was higher in ACPA + group (p = 0.009). Erosive disease was significantly more frequent in RF+ (60.0%, p = 0.009) and ACPA+ (58.6%, p = 0.021) patients.

Ultrasound differences between seropositive and seronegative RA patients

No significant differences in US scores (SH, PD and global scores) were found according to RF and/or ACPA status. US PS was present at least in one joint in 55.5% of sero + patients (55.3% in RF + and 58.2% in ACPA + patients) and 16.1% of sero- patients (p = 0.0001). Globally, 101 (49.2%) out of 205 patients had US PS (Fig. 1. A). 95% were FR /ACPA+. Only five patients with sero- RA had this expansive US pattern (p = 0001). In the univariate analysis, significantly more patients with US PS had erosive disease (72.3% vs 35.0%, p = 0.0001) and more of them were taking csDMARD (81.8% vs 69.6%, p = 0.050) although significant differences in disease activity were not found (Table 1).

One hundred and four patients (50.8%) did not have US PS, but they showed a flat synovial pattern, a concept equivalent to SH grade I.

US proliferative synovitis is independently associated with ACPA status and erosive disease

After adjusting for clinical (disease duration, current smoking, erosive disease and DAS28-CRP) serological (RF and ACPA) and therapy variables (csDMARDs, bDMARDs and glucocorticoids), only erosive disease [OR 4.90, CI 95% (2.17–11.07), p = 0.0001] and ACPA [OR 3.5, CI 95% (1.39–10.7), p = 0.09] but not RF status [OR 0.74, CI 95% (0.31–1.71), p = 0.483] were independently associated with the presence of US PS.

US proliferative synovitis is associated with higher density of synovial vessels and synovial cell infiltrates

To better characterize US PS, we performed synovial biopsies in a subgroup of 23 patients with either proliferative (13 patients) or flat (10 patients) synovitis. Synovium of patients with US PS had significantly higher density of vessels (p = 0.042) and higher, but non-significant, trend in density of B, T, mast cells and macrophages (Fig. 2).

Likewise, serological biomarkers showed a trend towards higher serum concentration of angiogenic (Activin A, bFGF, IL18, IL20, PIGF, SDF-1 and VEGF-D) and pro-inflammatory (IL23) cytokines in patients with US PS (Fig. 3).

MRI assessment

To verify the concordance between MRI and US on the concept of proliferative and flat synovitis, 42 patients underwent MRI of the dominant hand, 17 of them (40.4%) with US PS. At patient level, no significant differences regarding RAMRIS and its individual components (erosion, synovitis and bone oedema) were found according to the presence of US PS.

After analysing the specific joints with US PS, all but one scored 2 (58.8%) or 3 (35%) in the MRI synovitis sub-index. On the contrary, joints with US flat synovitis predominantly scored 1 in MRI synovitis sub-index, 10 patients scored 2 and none of the joints with US flat synovitis scored 3. No remarkable findings were found on MRI erosion or MRI bone oedema sub-indexes between joints with either US PS or flat synovitis.

Clinical Follow-up

After a mean time of follow-up of 46 months (SD 38.8), 65 out of 205 patients (31.6%) had changed the baseline therapy and 83 (40.3%) had at least one flare.

After adjusting for the presence of RF, ACPA, DAS28-CRP, disease duration, time of follow-up and therapy, US PS was independently associated with the change of baseline therapy (OR 2.60, 95% CI 1.10–5.77, p = 0.018).

Interestingly, serum levels of calprotectin at the end of the follow-up were numerically higher in patients with US PS (2.5 mg/dl versus 1.9 mg/dl), although significant differences were not reached (p = 0.093).

Discussion

This study is focused on US differences according to autoantibody status in RA patients. After performing over 400 hands US in RA patients, we observed that ACPA+ (but not RF) had a significantly more proliferative pattern at synovial level that we called US PS, equivalent to SH grade II-III, whereas sero- patients had predominantly a flatter US pattern, equivalent to SH grade I. The presence of US PS also identified a subgroup of RA patients with erosive disease, more synovial vessels, higher serum levels of both angiogenic and inflammatory biomarkers and an enhanced need for changing the baseline therapy in the long-term.

Inflammation and angiogenesis of the synovial membrane are the hallmarks of arthritis. Initially, synovitis was thought to be non-specific, with no differences among different subtypes of arthritis. However, immunohistochemistry studies have shown that vessels morphology and cell infiltration may differ between RA and spondyloarthritis (SpA) patients. Whereas straight vessels and lining hyperplasia have been related to RA, tortuous vascular pattern and infiltrates with predominant innate immune cells such as neutrophils and mast cells have been related to PsA and SpA [24–25]. Comparing PsA and sero-RA, histological analysis of synovial tissue composition has revealed similarities in cell distribution. Synovial tissue of PsA patients has been found to be enriched in CD117 + cells in the sublining area. Conversely, synovial tissue of sero- RA patients has been found to be enriched in CD138 + cells [26]. Therefore, the study of synovial tissue could be relevant to provide clues for a definitive diagnosis in undifferentiated arthritis [27].

Few studies have focused on imaging differences in RA according to autoantibody status. A recent study using US found that PD perfusion patterns were different in sero + and sero- RA. The difference appeared to be ACPA, but not RF dependent, suggesting that the pathophysiological process is different in ACPA + and ACPA- RA [10].

Gatehold et al found that erosion load differed significantly between sero + and sero- RA. Joint space narrowing scores were greater in sero + RA. The qualitative comparison showed that sero- RA patients displayed periarticular ossifications, carpal shortening, and sparing of the carpo-metacarpal joints, whereas sero + RA patients had more carpo-metacarpal damage and less shortening [28].

In this study, we observed that US PS was independently associated with ACPA status and not influenced by disease duration, therapy or disease activity. The concept of US PS makes reference to the upper outgrowth of synovial with a convex upper edge and corresponds to SH grade II and III (always with PD). In most cases, US PS adopted a characteristic morphology, with capsular distension and convex shape on the upper top. Conversely, sero- RA patients generally showed a flatter outgrowth of synovial tissue similar to what can be seen in PsA, a concept closer to SH grade I with PD.

We identified an independent association between US PS with erosive disease, even after analyzing only sero + patients (data not shown). In addition, more of them had to change the baseline therapy due to lack of response in the long-term. Therefore, US PS might be considered as a bad prognosis finding to considerate when making therapeutic decisions, although this issue should be definitely addressed in prospective studies.

After verifying the independent association of US PS with both ACPA status and erosive disease, we sought to better characterize this US pattern using a double approach with MRI and immunochemistry. First, we verified that US PS at joint level corresponded almost unanimously to grade 2–3 MRI synovitis whereas US flat synovitis corresponded predominantly to grade 1 MRI synovitis. Second, we observed a strong trend to higher density of B, T, mast cells and macrophages, and a significantly higher density of vessels in synovial biopsies from US PS, reflecting substantial divergences between these two patterns of synovitis beyond the US appearance.

Few studies have analyzed synovial tissue differences in ACPA+/- patients. Gómez-Puerta et al did not find significant differences in synovial cell infiltrate or lymphoid neogenesis according to ACPA status [8]. Conversely, Orr C et al demonstrated that synovial B cell infiltrates and lymphoid aggregates were significantly higher in ACPA + patients [29]. We observed that patients with US PS also had a different synovial pattern and showed a worse response to the therapy. However, neither ACPA nor RF status were relevant for synovial infiltrates or determining clinical outcomes (data not shown). This data suggests that the presence of US PS, better than autoantibodies status, reflects a differential aspect at synovial level and a worse prognosis in the long-term.

Moreover, higher serum levels of angiogenic and inflammatory biomarkers were found in patients with US PS, confirming differences not only at local but also at peripheral level. We had previously seen higher levels of serum biomarkers in RA versus PsA patients in clinical remission [30]. Our RA patients with US flat synovitis, most of them RF and ACPA-, showed lower levels of serum biomarkers, resembling PsA patients [30]. Therefore, sero- RA could represent a different entity, standing between sero + RA and PsA, as it has also been suggested in recent studies analyzing serum metabolomes and lipidomes [31].

This study has some limitations. First, four observers scored US images, which could enter too much variability, although intercorrelation ratio was good to excellent. Second, only wrist and MCP US images were evaluated. Considering that hand involvement is the hallmark of RA, this protocol should be enough to capture the disease activity. We do not know if these results could be reproduced in big joints, although the presence of synovial fluid (barely seen in small joints) would hamper a proper evaluation of synovial proliferation.

Conclusions

The presence of US PS, encompassing SH grade II and III with PD, was independently associated with ACPA + RA. This US pattern identified a subgroup of RA patients with erosive disease, higher vessels density at synovial level and a greater need for changing the therapy in the long-term.

Abbreviations

ACPA, Anti-Citrullinated Peptide/Protein Antibodies; ACR, American College of Rheumatology; bDMARDs, biological Disease-Modifying Antirheumatic Drugs; bFGF, basic Fibroblast Growth Factor; csDMARDs, conventional synthetic Disease-Modifying Antirheumatic Drugs; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; DMARDs, disease-modifying antirheumatic drugs; ELISA, Enzyme-Linked ImmunoSorbent Assay; EULAR, European League Against Rheumatism; IL, interleukin; mdc, minimum detectable change; MCP, Metacarpophalangeal; MHz, MegaHertz; MRI, Magnetic Resonance Imaging; OR, Odds Ratio; PD, Power Doppler; PlGF, Placental Growth Factor; PsA, Psoriatic Arthritis; RA, Rheumatoid Arthritis; RAMRIS, Rheumatoid Arthritis Magnetic Resonance Imaging Score; RF, Rheumatoid Factor; SD, Standard Deviation; SDF-1, Stromal Cell-Derived Factor 1; Sero-, Seronegative; Sero+, Seropositive; SH, Synovial Hypertrophy; SJC, Swollen Joint Count; SpA, Spondyloarthritis; TJC, Tender Joint Count; US, Ultrasound; USPS, Ultrasound Proliferative Synovitis; VEGF-D, Vascular Endothelial Growth Factor-D.

Declarations

Ethical Approval Form

Comité Ético de Investigación Científica del Hospital Clínic de Barcelona, Spain (2011/6490).

Consent for publication

Signed consent for publication was obtained from all patients.

Availability of data and consent to participate

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding Information:

This study was supported by grants PI11/1890 (JDC), RIER RD16/0012/0010 (JDC) and RIER RD16/0012/001 (JLP), from the Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain (co-financed by FEDER, European Union “Una manera de hacer Europa”).

Authors’ contributions

JR had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis. JR was responsible for the study design. JR, ABAP, BFS, RGS, CSK, AC, RC, AP, JAN, VRE, RCM and JGP performed data acquisition, analysis, interpretation, and final approval of the manuscript. Manuscript preparation was by JR, JDC, JLP and RS. All authors read and approved the final manuscript.

References

1. - do Prado AD, Staub HL, Bisi MC, et al. Ultrasound and its clinical use in rheumatoid arthritis: where do we stand? Adv Rheumatol. 2018;58:19.
2. Zabotti A, Salvin S, Quartuccio L, et al. Differentiation between early rheumatoid and early psoriatic arthritis by the ultrasonographic study of the synovio-entheseal complex of the small joints of the hands. Clin Exp Rheumatol. 2016;34:459–65.
3. Machold KP, Stamm TA, Nell VP, et al. Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. Rheumatology. 2007;15:342–9.
4. - Van den Broek M, Dirven L, Klarenbeek NB, et al. The association of treatment response and joint damage with ACPA-status in recent onset RA: a subanalysis of the 8-year follow-up of the best study. Ann Rheum Dis. 2012;71:245–8.
5. - McGonagle D, Watad A, Savic S. Mechanistic immunological based classification of rheumatoid arthritis. Autoimmun Rev. 2018;17:1115–23.
6. Salvador G, Sanmarti R, Gil-Torregrosa B, et al. Synovial vascular patterns and angiogenic factors expression in synovial tissue and serum of patients with rheumatoid arthritis. Rheumatology. 2006;45:966–71.
7. Cañete JD, Rodriguez JR, Salvador G, et al. Diagnostic usefulness of synovial vascular morphology in chronic arthritis. A systematic survey of 100 cases. Semin Arthritis Rheum. 2003;32:378–87.
8. Gómez-Puerta JA, Celis R, Hernández MV, et al. Differences in synovial fluid cytokine levels but not in synovial tissue cell infiltrate between anti-citrullinated peptide/protein antibody-positive and -negative rheumatoid arthritis patients. Arthritis Res Ther. 2013;15:R182.

9. Boeters DM, Nieuwenhuis WP, Verheul MK, et al. MRI-detected osteitis is not associated with the presence or level of ACPA alone, but with the combined presence of ACPA and RF. Arthritis Res The. 2016;18:179.
10. - Gadeholt O, Feuchtenberger M, Wech T, et al. Power-Doppler perfusion phenotype in RA patients is dependent to anti-citrullinated peptide antibody status, not on rheumatoid factor. Rheum Int. 2019;39:1019–25.
11. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62:2569–81.
12. - Wakefield RJ, Balint PV, Szkudlarek M, Filippucci E, Backhaus M, D'Agostino MA, Sanchez EN, Iagnocco A, Schmidt WA, Bruyn GA, et al. Musculoskeletal ultrasound including definitions for ultrasonographic pathology. J Rheumatol. 2005;32:2485–7.
13. - Backhaus M, Burmester GR, Gerber T, et al. Guidelines for musculoskeletal ultrasound in rheumatology. Ann Rheum Dis. 2001;60:641–9.
14. -Ramírez J, Ruíz-Esquide V, Pomés I, et al. Patients with rheumatoid arthritis in clinical remission and ultrasound-defined active synovitis exhibit higher disease activity and increased serum levels of angiogenic biomarkers. Arthritis Res Ther. 2014;16:R5.
15. - Szkudlarek M, Court-Payen M, Jacobsen S, et al. Interobserver agreement in ultrasonography of the finger and toe joints in rheumatoid arthritis. Arthritis Rheum. 2003;48:955–62.
16. Kelly S, Humby F, Filer A, et al. Ultrasound-guided synovial biopsy: a safe, well-tolerated and reliable technique for obtaining high-quality synovial tissue from both large and small joints in early arthritis patients. Ann Rheum Dis. 2013;74:611–7.
17. - Ramírez J, Celis R, Usategui A, et al. Immunopathologic characterization of ultrasound-defined synovitis in rheumatoid arthritis patients in clinical remission. Arthritis Res Ther. 2016;18:74.
18. Haringman JJ, Vinkenoog M, Gerlag DM, et al. Reliability of computerized image analysis for the evaluation of serial synovial biopsies in randomized controlled trials in rheumatoid arthritis. Arthritis Res Ther. 2005;7:R862-7.
19. - Ramírez J, Narváez JA, Ruiz-Esquide V, et al. Clinical and sonographic biomarkers of structural damage progression in RA patients in clinical remission: A prospective study with 12 months follow-up. Semin Arthritis Rheum. 2017;47:303–9.
20. - Ostergaard M, Peterfy C, Conaghan P, et al. OMERACT rheumatoid arthritis magnetic resonance imaging studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. J Rheumatol. 2003;30:1385–6.
21. **RayBiotech**. http://www.raybiotech.com.
22. - Churchman SM, Geiler J, Parmar R, et al. Multiplexing immunoassays for cytokine detection in the serum of patients with rheumatoid arthritis: lack of sensitivity and interference by rheumatoid factor. Clin Exp Rheumatol. 2012;30:534–42.

23. - Inciarte-Mundo J, Victoria Hernández M, Ruiz-Esquide V, et al. Serum Calprotectin Versus Acute-Phase Reactants in the Discrimination of Inflammatory Disease Activity in Rheumatoid Arthritis Patients Receiving Tumor Necrosis Factor Inhibitors. Arthritis Care Res (Hoboken). 2016;68:899–906.
24. Reece R, Cañete JD, Parson WJ, et al. Distinct vascular patterns of early synovitis in psoriatic, reactive and psoriatic arthritis. Arthritis Rheum. 1999;42:1481–4.
25. - Noordenbos T, Yeremenko N, Gofita I, et al. Interleukin-17-positive mast cells contribute to sinovial inflammation in spondyloarthritis. Arthritis Rheum. 2012;64:99–109.
26. - Alivernini S, Bruno D, Tolusso B, et al. Differential synovial tissue biomarkers among psoriatic arthritis and rheumatoid factor/anti-citrulline antibody-negative rheumatoid arthritis. Arthritis Res Ther. 2019;21:116.
27. - Alivernini S, Tolusso B, Petricca L, et al. Synovial predictors of differentiation to definite arthritis in patients with seronegative undifferentiated peripheral inflammatory arthritis: microRNA signature, histological, and ultrasound features. Front Medicine. 2018;5:186.
28. - Gadeholt O, Hausotter K, Eberle H, et al. Differing X-ray patterns in seronegative and seropositive rheumatoid arthritis. Clin Rheumatol. 2019;38:2403–10.
29. - Orr C, Najm A, Biniecka M, et al. Synovial immunophenotype and Anti-Citrullinated Peptide Antibodies in Rheumatoid Arthritis Patients. Arthritis Rheum. 2017;69:2114–23.
30. - Ramírez J, Inciarte-Mundo J, Cuervo A, et al. Differing local and systemic inflammatory burden in polyarticular psoriatic arthritis and rheumatoid arthritis patients on anti-TNF treatment in clinical remission. Clin Exp Rheumatol. 2017;35:74–9.
31. - Souto-Carneiro M, Tóth L, Behnisch R, et al. Differences in the serum metabolome and lipidome identify potential biomarkers for seronegative rheumatoid arthritis versus psoriatic arthritis. Ann Rheum Dis. 2020;79:499–506.

Figures

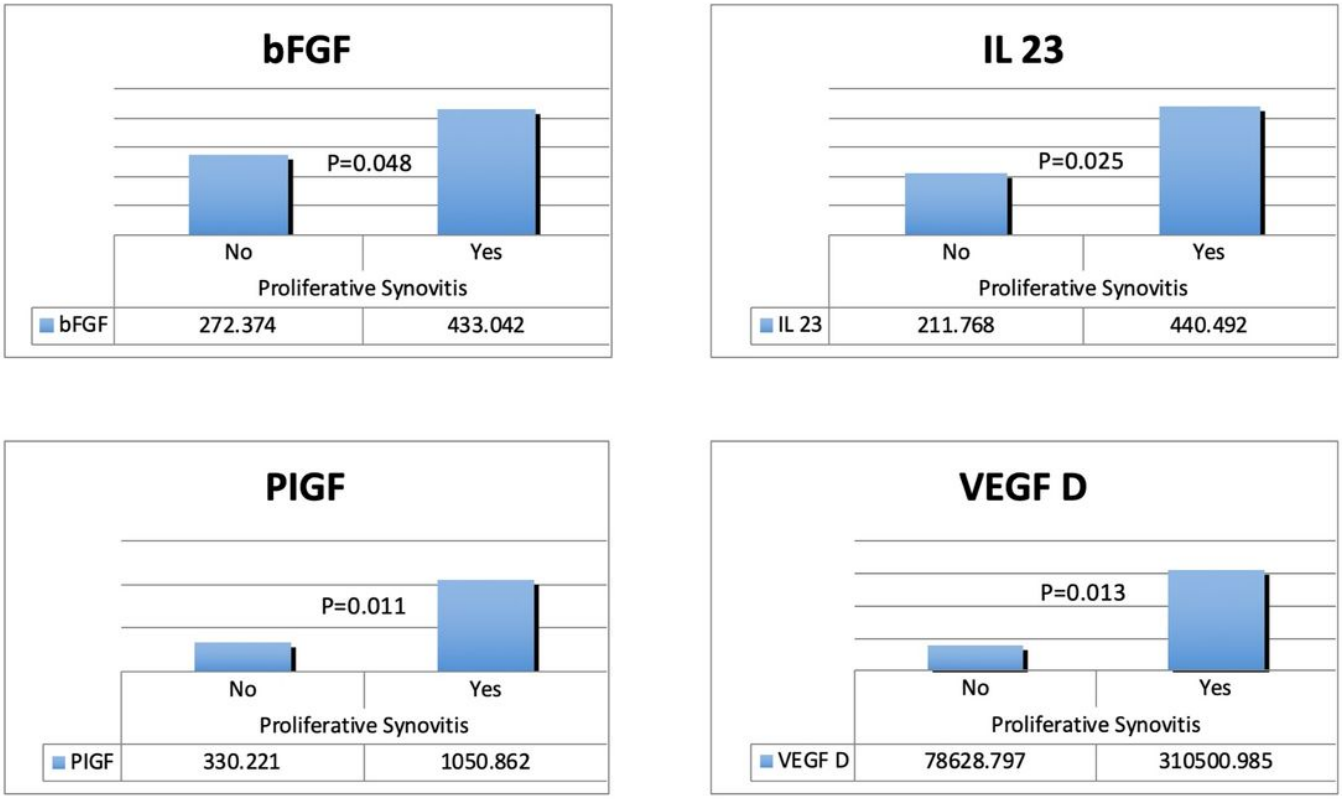

Figure 1

Serum levels of angiogenic and inflammatory biomarkers in patients with RA. bFGF: basic Fibroblast Growth Factor; IL: Interleukin; PIGF: Placental Growth Factor; VEGF D: Vascular Endothelial Growth Factor D. Serum levels of cytokines are shown in pg/ml.

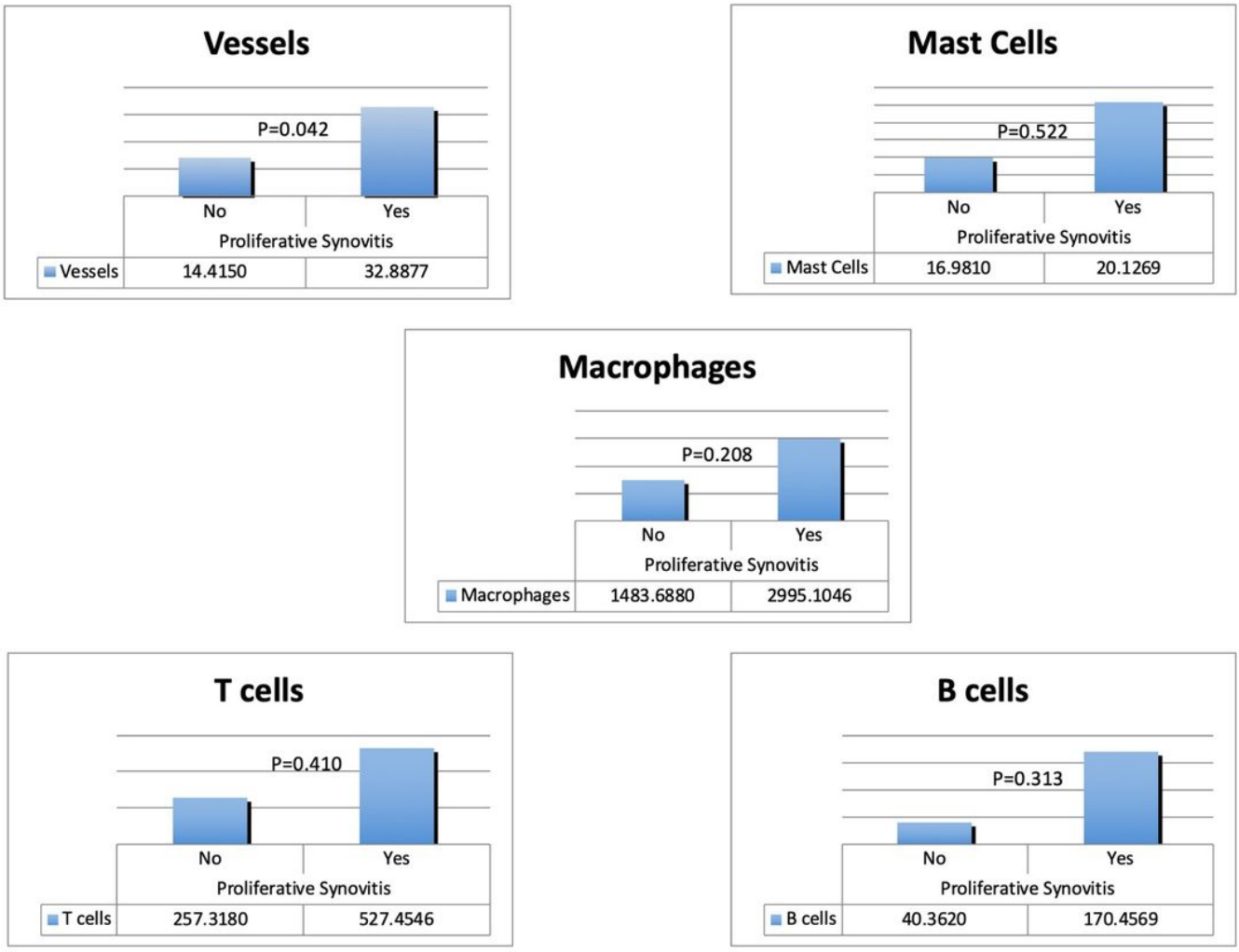

Figure 2

Synovial cells density in 23 patients with RA.

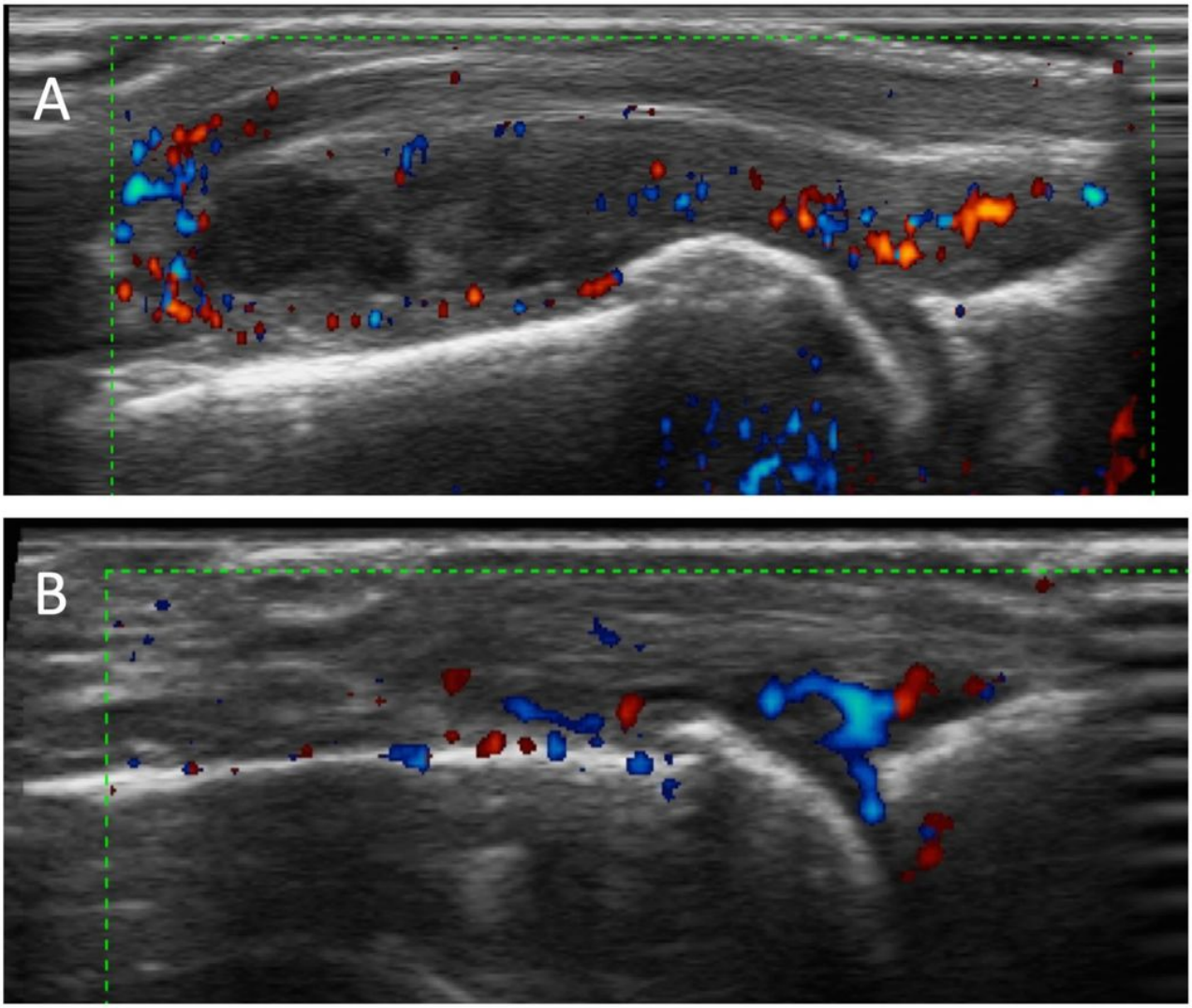

Figure 3

A: Ultrasound Proliferative Synovitis in MCP joint of a FR and ACPA positive RA patient. The synovial has an exophytic growth with globular shape and convexity in the upper limit of joint capsule. B: Ultrasound Flat Synovitis in MCP joint of a patient with FR and ACPA negative RA. Synovial Proliferation is restricted and the upper limit of the joint capsule has a flat shape.