

Original Article

Ultrasonographic examination of rheumatoid arthritis patients who are free of physical synovitis: power Doppler subclinical synovitis is associated with bone erosion

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Abstract

Objective. To investigate the characteristics of power Doppler (PD) subclinical synovitis in patients with rheumatoid arthritis (RA) who achieve clinical remission free from physical synovitis.

Methods. Twenty-nine RA patients were consecutively enrolled. All of the patients had achieved clinical remission [simplified disease activity index (SDAI) ≤ 3.3] for at least 6 months at the musculoskeletal ultrasonography (MSKUS) examination. Additionally, none of the patients exhibited tender joints at 68 sites or swollen joints at 66 sites. MSKUS of bilateral wrist and finger joints, including the 1st – 5th MCP joints, 1st IP joint and 2nd -5th PIP joints, was performed, and the findings obtained by gray scale (GS) and PD were graded on a semi- quantitative scale from 0 to 3.

Results. Median disease duration upon the introduction of disease-modifying antirheumatic drugs (DMARDs) was 3 months, and that at MSKUS examination was 21 months. The percentages of patients with PD synovitis in at least one joint were PD grade ≥ 1 ; 58.6 %, PD grade ≥ 2 ; 31.0 %, PD grade 3; 6.9 %. The use of biological agents was low in patients with a PD synovitis grade ≥ 2 ($p < 0.05$). The presence of US bone erosion was high by patient ($p < 0.05$) and by joint ($p < 0.0001$) with PD synovitis as compared to those without PD synovitis. However, no correlations were found between PD synovitis measures and serum biomarkers including angiogenesis factors.

Conclusion. PD subclinical synovitis correlates with several clinical characteristics, whereas conventional serum biomarkers are not useful to indicate the presence of subclinical PD synovitis.

Abbreviations

ACPA: anti-cyclic citrullinated peptide antibody

ACR: American College of Rheumatology

CDAI: clinical disease activity index

CRP: C-reactive protein

DAS: disease activity score

DAS28: 28-joint count disease activity score

DMARDs: disease-modifying antirheumatic drugs

ESR: erythrocyte sedimentation rate

EULAR: European League Against Rheumatism

GS: gray scale

IP: interphalangeal

JCR: Japan College of Rheumatology

MCP: metacarpophalangeal

MMP-3: matrix metalloproteinase-3

MRI: magnetic resonance imaging

MSKUS: musculoskeletal ultrasonography

PD: power Doppler

PtGA: patient global assessment

PIP: proximal interphalangeal

RA: rheumatoid arthritis

RF: rheumatoid factor

SDAI: simplified disease activity index

SJC: swollen joint counts

sRANKL: soluble receptor activator of NF κ B ligand

TJC: tender joint counts

VEGF: vascular endothelial growth factor

Introduction

Recently, the outcome for patients with rheumatoid arthritis (RA) has improved due to improvements in therapy, and “clinical remission” is a realistic therapeutic goal [1]. There are several definitions of “clinical remission” based on various composite scores, such as the Disease Activity Score (DAS), 28-joint count DAS (DAS28), Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI) and a Boolean definition as recently proposed by American College of Rheumatology (ACR) or European League Against Rheumatism (EULAR) [2]. The latter three criteria based on SDAI, CDAI and the Boolean definition are more stringent than those based on DAS28 [2]. The RA patients achieving remission according to the SDAI, CDAI or Boolean definition have a greater chance of structural remission than the patients achieving DAS28 remission; however, some reports have pointed out the presence of radiographic progression despite the achievement of these types of clinical remission [3-5], which reflects the inadequate sensitivity of the conventional approaches to detecting synovitis.

In this regard, musculoskeletal ultrasonography (MSKUS) is an ideal modality to detect subclinical joint inflammation that may lead to further radiographic progression since MSKUS is more sensitive than physical examination for detecting joint injury in patients with RA [6-8]. Varying kinds of joint injury including articular synovitis, tenosynovitis and bone erosion can be recorded by gray scale (GS) and power Doppler (PD) techniques [9, 10]. We have recently reported that the presence of PD synovitis of grade ≥ 2 is a very RA-specific MSKUS finding among patients with early arthritis [11]. As stated in previous reports, including those by us as well as other investigators, PD synovitis has been suggested to reflect the pathologic alterations of rheumatoid synovial inflammation in patients with RA better than GS synovitis [8, 12-14]. The qualitative importance of subclinical synovitis, as first described by Brown et al., is that the joints with PD signals may develop continued structural deterioration irrespective of the achievement of good clinical status in established RA patients with a median disease duration of 7 years [8].

Recently, more detailed information regarding PD synovitis in RA patients with good clinical status has been accumulated [8, 14-16]. Saleem et al. have revealed that PD synovitis remains in long-standing established RA patients achieving clinical remission evaluated by SDAI or Boolean definition [15]. They have also shown that those patients in whom PD synovitis remains will develop clinical flare-ups during treatment with conventional disease-modifying antirheumatic drugs (DMARDs) [16]. Residual PD synovitis is also predictive of clinical flare-ups in early-stage RA patients of mean disease duration of 3.8 months treated by conventional DMARDs [14]. These data strongly suggest that the RA patients in clinical remission with residual PD synovitis do not achieve “true” remission and are at risk for subsequent structural deterioration and flair. However, the subjects in the above studies exhibited slightly tender or swollen joints upon physical examination even if they had achieved clinical remission [8, 14-19]. Therefore, it appears to be desirable to select RA patients who have achieved sustained clinical remission without any tender or swollen joints in order to examine the characteristics of “real” subclinical PD synovitis in individuals who are almost completely free from synovitis by physical examination. We have serially selected these kinds of patients and tried to characterize the residual subclinical PD synovitis in association with biomarkers.

Materials and Methods

Patients

Twenty-nine RA patients who fulfilled the 2010 RA classification criteria [20] were consecutively recruited in the present study. All of the patients achieved clinical remission ($SDAI \leq 3.3$) for at least 6 months upon MSKUS examination. Furthermore, all of the patients exhibited no tender joints among 68 sites and no swollen joints among 66 sites upon MSKUS examination. They were recruited from the Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University from July 2011 through February 2012. Patients gave their informed consent to be subjected to the protocol that was approved by the Institutional Review Board of Nagasaki University. Serum samples were collected and stored at -20°C upon MSKUS examination until the assay.

Clinical and laboratory assessment

Clinical evaluation was performed by Japan College of Rheumatology (JCR)-certified rheumatologists (H.N. and A.K.) who were blinded to the MSKUS findings. Agreement for the presence of tender joints and swollen joints by physical examination between the 2 rheumatologists were very high: Kappa coefficient of distribution of tender joints was 0.92 and that of swollen joints was also 0.92 in both wrist and finger joints of other active 5 RA patients. Disease activity was evaluated by the DAS28- erythrocyte sedimentation rate (ESR), SDAI, CDAI and Boolean remission. In using DAS28-ESR, we followed the criteria set by the European League against Rheumatism (EULAR), and in using CDAI and SDAI we followed the method recommended by Smolen and colleagues [21]. Boolean remission is defined by all of the following parameters being ≤ 1 [tender joint count (TJC) ≤ 1 , swollen joint count (SJC) ≤ 1 , patient global assessment (PtGA) ≤ 1 cm, and C-reactive protein (CRP) ≤ 1 mg/dL] [22].

The following laboratory variables were assessed: rheumatoid factor (RF) (Dade Behring, Marburg, Germany; cutoff value, 14 IU/ml), anti-cyclic citrullinated peptide antibodies (ACPA) (DIASAT Anti-CCP, Axis-Shield, Dundee, UK; cutoff value, 4.5 U/ml), CRP (Eiken Chemical Co. Ltd., Tokyo, Japan), ESR, matrix metalloproteinase-3 (MMP-3) (Daiichi Pure Chemicals, Fukuoka, Japan), vascular endothelial growth factor (VEGF) (Quantikine, R&D Systems, Abingdon, UK), angiopoietin-2 (Quantikine, R&D Systems, Abingdon, UK), and soluble receptor activator of NF κ B ligand (sRANKL) (BIOMEDICA, Wien, Austria). Clinical disease activity as well as serum variables were evaluated on the day of the MSKUS examination.

MSKUS assessment

Each patient underwent a MSKUS assessment on the same day as the clinical evaluation by a JCR-certified rheumatologist (S.K.) who was blinded to the clinical findings (S.K. is also an instructor of MSKUS certified by JCR with 7 years experience of MSKUS). Images from all the examinations were stored, and the US scoring reliability was examined in randomly selected patients at the end of the study. A systematic multiplanar GS and PD examination of 22 joints was performed with the same scanner (TOSHIBA AplioXG) using a multifrequency linear transducer (12 MHz). The ultrasound score included the following 22 joints: bilateral wrists (intra-carpal, radio-carpal and ulno-carpal recesses) and finger joints including the 1st -5th

metacarpophalangeal (MCP) joints, the 1st interphalangeal (IP) joint and the 2nd-5th proximal interphalangeal (PIP) joints (dorsal recess). All joint regions were sonographically examined in a standardized manner according to the European League Against Rheumatism (EULAR) [9] and JCR guidelines. These are the same sites where magnetic resonance imaging (MRI) has been performed in patients with early arthritis, as we previously described [23, 24].

Each joint was scored for GS and PD on a semi-quantitative scale from 0 to 3 [10] (synovial hypertrophy in GS: grade 0 = absence, no synovial thickening; grade 1 = mild, minimal synovial thickening filling the angle between the periarticular bones without bulging over the line linking the tops of the bones; grade 2 = moderate, synovial thickening bulging over the line linking the tops of the periarticular bones but without extension to at least one bone diaphysis; grade 3 = marked, synovial thickening bulging over the line linking the tops of the periarticular bones and with extension to at least one of the bone diaphyses; PD signals: grade 0 = absence, no synovial flow; grade 1 = mild, single-vessel signals; grade 2 = moderate, confluent signals in less than half of the synovial area; grade 3 = marked, signals in more than half of the synovial area), and the presence or absence of tenosynovitis was noted. These scores corresponded to the maximum score for GS and PD obtained from any of the synovial sites evaluated at each joint. Tenosynovitis is defined by the presence of abnormal hypoechoic or anechoic material with or without fluid inside the tendon sheath with positive PD signals in two perpendicular planes [25]. Erosion is defined by a cortical break seen in two perpendicular planes [10].

Statistical analyses

Within-group comparisons were made using Mann-Whitney's U test and the χ^2 test (Fisher's exact probability test when appropriate). Correlations were assessed with Spearman's correlation coefficient. The overall significance level for statistical analysis was 5% (two-sided). *P* values less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics in 29 RA patients achieved definitive clinical remission

The median (range) values of age, disease duration at introducing DMARDs, disease duration at MSKUS examination and remission duration at MSKUS examination were 57 (30-80) years-old, 21 (11-300) months, and 10 (6-26) months, respectively. The prevalence rates of ACPA and RF were 86.2 % and 89.7 %, respectively. Twenty-seven patients (93.1%) were treated with conventional DMARDs (23 patients treated by methotrexate and 4 patients by salazosulfapyridine) and 12 patients (41.4%) were treated with biological agents (5 patients treated by infliximab, 5 patients by etanercept, 1 patient by adalimumab and 1 patient by tocilizumab). DAS28-ESR, CDAI and SDAI were very low; the median (range) of DAS28-ESR was 1.64 (0.53-2.30), that of CDAI was 0.60 (0-2.00), and that of SDAI was 0.66 (0.01-2.03). All patients achieved Boolean remission. In addition, none of the patients exhibited tender or swollen joints.

Residual subclinical synovitis on MSKUS in 29 RA patients achieved definitive clinical remission

The numbers (percentages) of patients with subclinical synovitis present in at least one joint were GS grade ≥ 1 ; 21 (72.4%), GS grade ≥ 2 ; 10 (34.5%), GS grade 3; 3 (10.3%), PD grade ≥ 1 ; 17 (58.6%), PD grade ≥ 2 ; 9 (31.0%), and PD grade 3; 2 (6.9%). The median (range) GS and PD scores were 2 (0 - 15) and 1 (0 - 12), respectively.

Comparison of clinical characteristics between patients with subclinical PD synovitis and patients without PD synovitis

The clinical characteristics were compared between patients with subclinical PD synovitis and patients without PD synovitis, and between patients with PD grade ≥ 2 and patients with PD grade 1 or 0 (Table 1). Age, gender, disease duration upon the introduction of DMARDs, disease duration at MSKUS examination, remission duration, the prevalence of ACPA and RF, the usage of conventional DMARDs, and disease activity were not different among the groups. In addition, parameters evaluating clinical disease activity at MSKUS examination were comparably very low in each group (middle part of Table 1). However, in the patients with PD grade ≥ 2 as compared with patients without, the usage of biological agents was significantly low ($p = 0.032$). The same tendency was also observed between patients with and patients without PD synovitis ($p = 0.12$). In addition, regarding to PtGA, PtGA was 0 in 10 patients whereas that was more than 0 in the remaining 19 patients. The percentage of patients having PD synovitis (70% in patients with PtGA 0 whereas 53 % in patients with PtGA more than 0) and total PD score (mean score 2 in patients with PtGA 0 whereas 1 in patients with PtGA more than 0) were not different between the above 10 patients and 19 patients.

Comparison of MSKUS findings between patients with subclinical PD synovitis and patients without PD synovitis

The MSKUS findings were also compared between patients with subclinical PD synovitis and patients without PD synovitis, and between patients with PD grade ≥ 2 and patients with PD grade 1 or 0 (Table 1). As suspected, total PD score was significantly high in the patients with PD synovitis ($p < 0.0001$) and the

patients with PD grade ≥ 2 ($p = 0.0001$). Additionally, total GS score was significantly higher in the patients with PD synovitis ($p < 0.0001$) and the patients with PD grade ≥ 2 ($p = 0.001$). Furthermore, the percentage of patients with MSKUS bone erosion was significantly higher in the patients with PD synovitis ($p = 0.032$) and the patients with PD grade ≥ 2 ($p = 0.0007$). We have confirmed the association of PD synovitis and US bone erosion by analyzing their coexistence in a total of 638 joints from 29 patients. As shown in Table 2, there was a marked association between PD synovitis and MSKUS bone erosion ($p < 0.0001$).

Comparison of serum biomarkers between patients with subclinical PD synovitis and patients without PD synovitis

Table 3 summarizes the data. Serum concentrations of MMP-3, VEGF, angiopoietin-2 and sRANKL in the present 29 patients were comparable with those in normal subjects. In addition, there were no differences in these biomarkers regardless of the presence of PD synovitis or PD grade ≥ 2 . Furthermore, no correlation was observed between total PD score and any of the biomarkers (Figure 1). Since some characteristics distributed in the patients with PD synovitis or PD grade ≥ 2 were determined, we have tried to confirm contributions of these characteristics by logistic regression analysis [SAS system®, version 9.2 (SAS Institute Inc., Cary, NC, USA)]. However, we were not able to obtain definitive results, probably due to the small sample size (data not shown).

Discussion

Subclinical synovitis is defined as joint inflammation determined not by physical examination but by MSKUS or MRI [6-8, 14-17, 26]. The importance of subclinical synovitis, especially as determined by PDUS, has been strengthened by several reports showing that its presence is predictive of further radiographic progression [14, 27] or clinical flare-ups [14, 27]. To more strictly assess the role of subclinical PD synovitis in patients with RA, none of the patients in the present study exhibited tender or swollen joints upon physical examination, indicating that our data represent the real nature of subclinical PD synovitis as well as patients who have achieved definitive clinical remission with subclinical PD synovitis. To our knowledge, this is the first examination of patients with early-stage RA involved in “true” subclinical PD synovitis.

A few reports have explored the characteristics of PD synovitis in Caucasian early-stage RA patients who have achieved clinical remission [14, 28]. Although clinical disease activity was lower in the present study in comparison with these reports, since some of the patients in previous reports exhibited tender or swollen joints, the percentage of patients with PD synovitis was higher in the present study than in the previous reports [14, 28]. The positivity rates of ACPA (29% in ref. 14) and RF (39% in ref. 14 and 41% in ref. 28) in the present cases were much higher than in the previous cases, which may influence the results. Alternatively, Japanese RA patients might be more susceptible to joint inflammation as compared with Caucasian RA patients. However, these points need to be clarified in further studies.

In addition, we have revealed the characteristics of early-stage RA patients with subclinical PD synovitis. With regard to therapies, the absence of PD synovitis was likely to be associated with biological agents. There is increasing evidence that biological agents are superior to conventional DMARDs in terms of radiographic progression [1, 29, 30]. Since the existence of PD signals is a risk factor for further radiographic progression in patients with RA [8, 27], the suppression of PD signals by biological agents may explain the preferential protective effect toward joint damage as compared with conventional DMARDs. Furthermore, the percentage of patients with US bone erosion was higher in those with subclinical PD synovitis than in those without PD synovitis. Additionally, the frequency of the joints with US bone erosion was much higher in the joints with PD signals as compared with the joints without PD signals. These data indicate that the coexistence of PD signals with US bone erosion is a characteristic feature of US images in patients with early-stage RA even after they have achieved definitive clinical remission. It would be reasonable for patients with PD synovitis to show a high GS score in the present study, since the severity of the PD score usually correlates with that of the GS score in patients with RA. Considering that the presence of PD signals predicts further radiographic progression [8, 27], subclinical PD synovitis is thought to be pathologically still active, and is thus supposed to coexist with US bone erosion, possibly leading to further joint damage. However, longitudinal observation is necessary to confirm the above speculation.

PD signals with GS thickening of synovial tissues in RA patients reflect synovial cell hyperplasia with neovascularization [31, 32]. Therefore, high serum concentrations of MMP-3, RANKL, and angiogenesis factors along with acute phase reactants are commonly observed in patients with RA [33-35]. In this regard, we and other investigators have revealed increments of serum VEGF, angiopoietin-1 and angiopoietin-2 in RA patients that are correlated with clinical disease activity [36, 37]. Scire et al. previously reported a correlation

of PD measures with CRP in patients with early-stage RA, although this correlation was very low as compared with active disease [14]. In comparison to the results by Scire et al., CRP, ESR, MMP-3, VEGF, angiopoietin-2 and RANKL remained in the normal ranges regardless of the presence or absence of subclinical PD synovitis in the present study. Furthermore, there were no correlations between PD sores and CRP, ESR, MMP-3, VEGF, angiopoietin-2 or RANKL in the present study. Since all of the present cases achieved more stringent remission as compared with the cases in the report by Scire et al. [14], no correlation could be determined. At this point, the presence of US bone erosion or a higher GS score is believed to be a very relevant finding for predicting the presence of subclinical PD synovitis in patients who have achieved definitive clinical remission. Alternatively, the use of conventional biomarkers may not be enough to identify the remnant disease activity, and more global analysis may be warranted to seek the biomarkers that differentiate the presence or absence of imaging remission.

There are some limitations in the present study. We were not able to validate the multivariate analysis, probably due to the small sample size. Also, this is not a randomized control trial but an observational study; therefore, the choice of DMARDs depended on each physician's preference. These differences might affect the distribution of subclinical PD synovitis. Larger-scale randomized controlled trials are needed to confirm our present findings. However, as stated by the Targeted Ultrasound Initiative Group [38], the suppression of residual PD synovitis is suggested to be a target to achieve in imaging remission. Thus, our present data may emphasize the importance of subclinical PD synovitis and suggest that it may be an ideal surrogate marker in the attempt to achieve complete remission in patients with RA, especially in the earlier stages of the disease though longitudinal studies containing plain radiographic progression are necessary to clarify the meaning of present study.

Key messages

- PD subclinical synovitis still remains in RA patients achieving clinical remission free from physical synovitis.
- Conventional biomarkers may not be enough to identify the remnant disease activity.
- PD subclinical synovitis may be an ideal surrogate marker to accomplish complete remission of RA.

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none

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Figure Legends

Figure 1. Correlation between total PD score and serum biomarkers.

No correlation between total PD score and any of the biomarkers was observed. Correlations were assessed with Spearman's correlation coefficient.

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; MMP-3, matrix metalloproteinase-3; VEGF, vascular endothelial growth factor; Ang-2, angiotensin-2, sRANKL, soluble receptor activator of NF κ B ligand; PD, power Doppler

Figure 1.

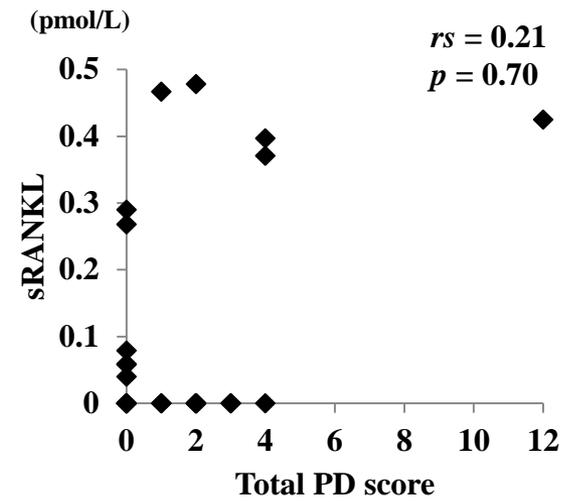
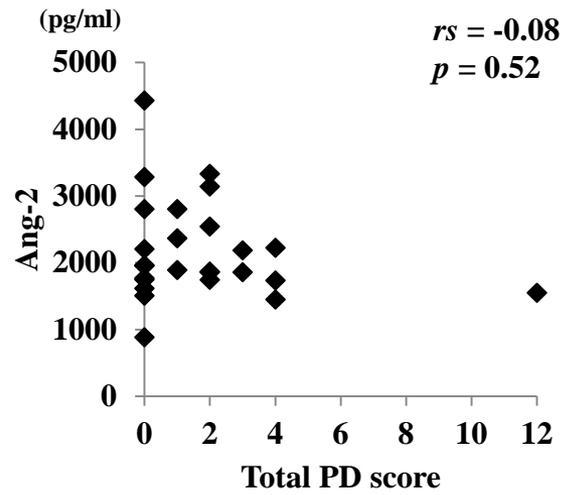
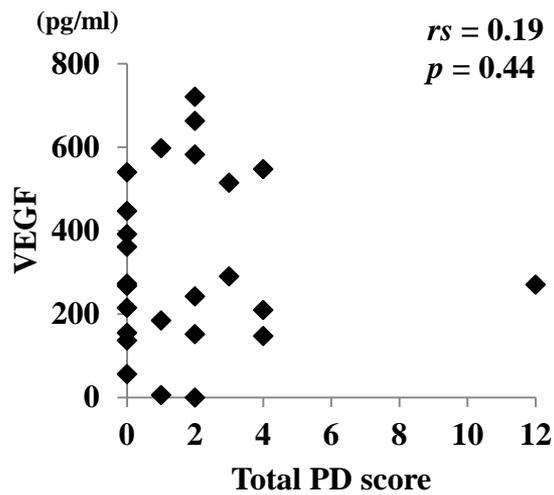
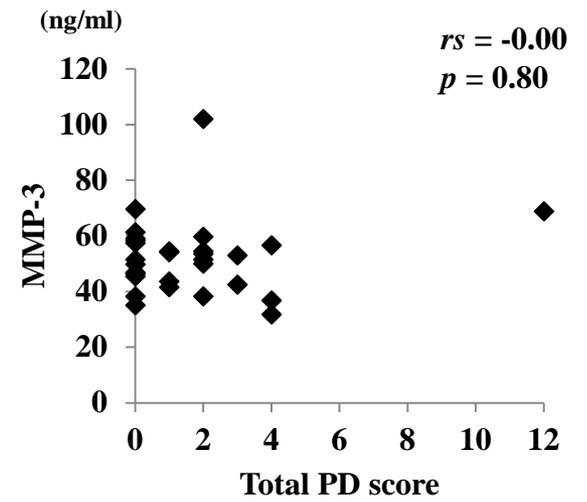
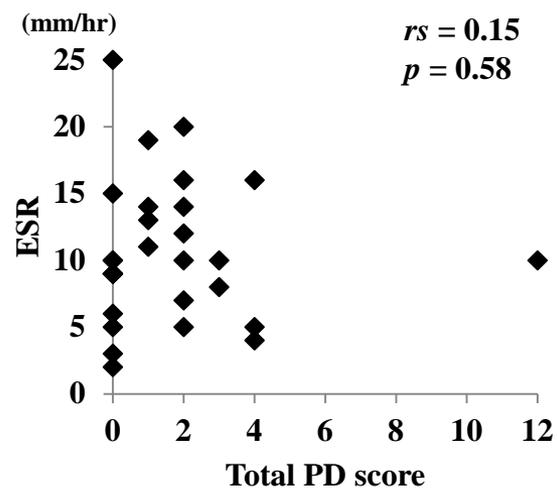
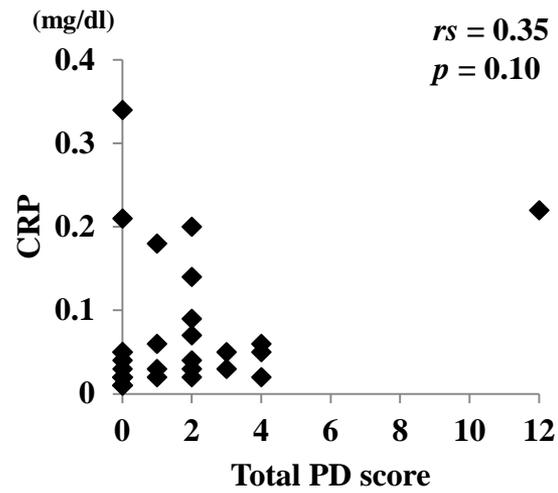


Table 1. Comparison of clinical characteristics and MSKUS findings between patients with PD-positive synovitis and patients without PD-positive synovitis.

	PD negative (n=12)	PD positive (n=17)	<i>P</i>	PD grade 0/1 (n=20)	PD grade 2/3 (n=9)	<i>p</i>
Age; years ^a	50.5 (30-72)	57.0 (35-80)	0.36	59.5 (30-80)	56 (35-74)	0.49
Gender; Female / Male	9 / 3	13 / 4	0.70	15 / 5	7 / 2	0.63
Disease duration at introduction of DMARDs; months ^a	3 (1-36)	3 (1-36)	0.95	3 (1-36)	2 (2-6)	0.85
Disease duration at MSKUS examination; months ^a	20 (12-67)	21 (11-300)	0.64	20 (11-67)	21 (11-300)	0.46
Duration of remission; months ^a	11 (6-24)	10 (6-26)	0.95	9.5 (6-26)	12 (6-21)	0.48
Positivity of ACPA; n (%)	10 (83.3)	15 (88.2)	0.56	16 (80.0)	9 (100)	0.20
Positivity of RF; n (%)	10 (83.3)	16 (94.1)	0.75	18 (90.0)	8 (88.9)	0.78
Conventional DMARDs therapy; n (%)	10 (83.3)	17 (100)	0.16	18 (90.0)	9 (100)	0.47
Biological therapy; n (%)	7 (58.3)	5 (29.4)	0.12	11 (55.0)	1 (11.1)	0.03
Concomitant steroid; n (%)	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Tender joint counts; n/68	0	0	-	0	0	-
Swollen joint counts; n/66	0	0	-	0	0	-
PtGA; mm ^a	3 (0-10)	3 (0-10)	0.38	3 (0-10)	0 (0-10)	0.13
EGA; mm ^a	3 (0-8)	3 (0-10)	0.54	3 (0-10)	1 (0-10)	0.27
CRP; mg/dl ^a	0.03 (0.01-0.34)	0.05 (0.02-0.22)	0.09	0.03 (0.01-0.34)	0.05 (0.02-0.22)	0.07

ESR; mm/hr ^a	9 (2-25)	11 (4-20)	0.13	10.5 (2-25)	8 (4-14)	0.10
DAS28-ESR ^a	1.60 (0.53-2.30)	1.74 (0.97-2.17)	0.17	1.73 (0.53-2.30)	1.60 (0.97-1.90)	0.09
CDAI ^a	0.65 (0-1.80)	0.60 (0-2.00)	0.48	0.60 (0-1.80)	0.10 (0-2.00)	0.25
SDAI ^a	0.77 (0.01-2.01)	0.62 (0.02-2.03)	0.81	0.75 (0.01-2.01)	0.24 (0.02-2.03)	0.45
Boolean remission; n(%)	12 (100)	17 (100)	-	20 (100)	9 (100)	-
MSKUS findings						
Total GS score ^a	0 (0-2)	4 (1-15)	<0.0001	1 (0-11)	4 (2-15)	0.001
Total PD score ^a	0	2 (1-12)	<0.0001	0 (0-4)	3 (2-12)	0.0001
Tenosynovitis; n (%)	2 (16.7)	4 (23.5)	0.51	3 (15.0)	3 (33.3)	0.53
Bone erosion; n (%)	1 (8.3)	8 (47.1)	0.03	2 (10.0)	7 (77.8)	<0.001

^aMedian (range)

Within-group comparisons were assessed with Mann-Whitney's U test and χ^2 test

MSKUS, musculoskeletal ultrasonography; RF, rheumatoid factor, ACPA, anti-cyclic citrullinated peptide antibody; PGA, patient global assessment; EGA, evaluator global assessment; DAS28, disease activity score 28; CDAI, clinical disease activity index; SDAI, simplified disease activity index; GS, gray scale; PD, power Doppler

Table 2. Comparison between PD positive synovitis and MSKUS bone erosion

	PD negative	PD positive	
Bone erosion negative	600	21	P<0.0001
Bone erosion positive	7	10	

Within-group comparisons were assessed with χ^2 test.

PD, power Doppler

Table 3. Comparison of serum biomarkers between patients with PD-positive synovitis and patients without PD-positive synovitis.

	PD negative (n=12)	PD positive (n=17)	<i>p</i>	PD grade 0/1 (n=20)	PD grade 2/3 (n=9)	<i>P</i>
MMP-3 (ng/ml)	50.7 (35.1-69.6)	53.0 (31.8-102)	0.79	53.8 (35.1-69.6)	50 (31.8-102)	0.35
VEGF (pg/ml)	268 (56.9-540)	271 (0-721)	0.45	270 (0-721)	257 (147-664)	0.91
Angiopietin-2 (pg/ml)	1954 (885-4429)	1895 (1449-3335)	0.80	2085 (885-4429)	1803 (1449-2543)	0.08
ampli-sRANKL (pmol/L)	0.4 (0-0.290)	0 (0-0.478)	0.90	0 (0-0.478)	0 (0-0.425)	0.96

Serum levels (median (range)) of healthy controls (n=10); VEGF 291(32-602) pg/ml, angiopietin-2 1827(1230-2587) pg/ml, ampli-sRANKL 0.009 (0-0.308) pmol/L

Within-group comparisons were assessed with Mann-Whitney's U test.

PD, power Doppler; MSKUS, musculoskeletal ultrasonography; MMP-3, matrix metalloproteinase-3; VEGF, vascular endothelial growth factor; sRANKL, soluble receptor activator of NF κ B ligand