Concise report

The power Doppler ultrasonography score from 24 synovial sites or 6 simplified synovial sites, including the metacarpophalangeal joints, reflects the clinical disease activity and level of serum biomarkers in patients with rheumatoid arthritis

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Abstract

Objective. We evaluated the significance of the power Doppler ultrasonography (PDUS) score by comparing it with serum biomarkers and clinical disease activity.

Methods. We measured the PDUS scores of 24 synovial sites in 12 joints in 22 RA patients. For convenience, the PDUS scores of six synovial sites in six joints were also examined. Each joint was scored for a power Doppler (PD) signal on a scale from 0 to 3. The PDUS scores are the sums of the PD signal scores for the 24 synovial sites or the 6 synovial sites. On the same day, serum variables as well as clinical disease activity were evaluated.

Results. The PDUS scores from the 24 joint sites were significantly positively correlated with DAS of 28 joints (DAS-28), simplified disease activity index (SDAI), clinical disease activity index (CDAI) and serum biomarkers including MMP-3, VEGF and tissue inhibitor of metalloproteinases-1 (TIMP-1). Accordingly, the PDUS scores from the six synovial sites greatly correlated with those from the 24 joint sites. Clinical disease activities as well as serum variables were also clearly correlated with the PDUS scores from the six synovial sites.

Conclusion. The standard as well as the simplified PDUS scores well reflected clinical disease activity and serum variables, including angiogenic factors. Our data reaffirm the utility of ultrasonography for monitoring disease activity in patients with RA.

Key words: Ultrasonography, Power Doppler, Rheumatoid arthritis, Vascular endothelial growth factor.

Introduction

The greater resolution of superficial musculoskeletal structures offered with the use of high-frequency transducers, along with the high sensitivity of current colour Doppler and power Doppler (PD) US, have led to increasing use of US in rheumatic diseases [1]. Naredo et al. [2] reported 12-joint simplified PD ultrasonographic assessment as the original US scoring system. Recently, Kurosaka et al. [3] examined a relatively large number of patients and found a correlation of PD signal intensity with...
DAS of 28 joints (DAS-28) and serum angiogenic factors, although they did not include the power Doppler ultrasonography (PDUS) score of the finger joints of the hand, which are frequently affected by RA.

We focused on the association of the PDUS score including the MCP joints with serum biomarkers as well as clinical disease activity. We found that the PDUS score of 24 synovial sites at 12 joints reflects the clinical disease activity and serum biomarkers. Second, for convenience, we reduced the number of joints to six synovial sites at six joints and also found that the PDUS score of six synovial sites at six joints is clearly correlated with the clinical disease activity and serum biomarkers.

Materials and methods

RA patient and healthy control samples
Twenty-two RA patients were selected to be enrolled in the present study from the Unit of Translational Medicine, Department of Immunology and Rheumatology, Graduate School of Biomedical Sciences, Nagasaki University. All of the patients fulfilled the 1987 criteria of the ACR for RA [4]. The patients underwent clinical, laboratory and PDUS evaluation on the same day. We also collected serum samples from eight healthy controls without musculoskeletal disorder. Subjects’ written consent was obtained according to the Declaration of Helsinki, and the design of the work was approved by the Institutional Review Board of Nagasaki University.

Clinical and laboratory assessment
Clinical evaluation was performed by Japan College of Rheumatology-certified rheumatologists (A.K. and K.E.), who were blinded to the PDUS findings. Disease activity was evaluated by DAS-28, simplified disease activity index (SDAI) and clinical disease activity index (CDAI). In using DAS-28, 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 [5].

The following laboratory variables were assessed: RF (Dade Behring, Marburg, Germany; cut-off value, 14 IU/ml), anti-CCP antibodies (DIASTAT Anti-CCP, Axis-Shield, Dundee, UK; cut-off value, 4.5 IU/ml), CRP (Eiken Chemical Co. Ltd, Tokyo, Japan), ESR, VEGF (Quantikine, R&D Systems, Abingdon, UK), MMP-3 (Daichi Pure Chemicals, Fukuoka, Japan), MMP-9 (Biotrak ELISA System, GE Healthcare, USA) and tissue inhibitor of metalloproteinases-1 (TIMP-1; Biotrak ELISA System, GE Healthcare, USA). Clinical disease activity as well as serum variables were evaluated on the same day as US examination.

US examination
Each patient underwent a US assessment by a Japan College of Rheumatology-certified rheumatologist (S.K.), who was blinded to the clinical and laboratory findings. Images from all the examinations were stored, and the US scoring reliability was examined by assessing 24 synovial sites in randomly selected patients at the end of the study. This assessment was carried out by Japan College of Rheumatology-certified rheumatologists (S.K., N.I., K.F. and T.O.) with consensus. A systematic multiplanar gray scale (GS) and PD examination of 12 joints was performed with the same scanner (TOSHIBA AplioXG; Toshiba Medical Systems Corporation, Tochigi, Japan) using a multifrequency linear transducer (12 MHz) according to the EULAR guidelines [6]. The US score included the following 24 synovial sites at 12 joints: bilateral elbows (anterior and posterior recess), wrists (dorsal and carpal recess), second and third MCP joints (dorsal and palmar recess), knees (suprapatellar and lateral parapatellar recess) and ankles (anterior tibiotar recess, medial tendon sheaths and lateral tendon sheaths). Signs of OA were not detected by US and X-ray in the examined joints.

The IA, tenosynovial and intrabursal PD signals were graded on a semi-quantitative scale from 0 to 3 (Grade 0 = absence, no synovial flow; Grade 1 = mild, ≤3 isolated singles; Grade 2 = moderate, >3 isolated singles or confluent signal in less than half of the synovial area; Grade 3 = marked, signals in more than half of the synovial area). These scores corresponded to the maximum score for PD signals obtained from any of the synovial sites evaluated at each joint, as documented by Naredo et al. [2]. The sum of the PD signal scores obtained from each joint was used as the PDUS score, as reported by Naredo et al. [2]. The 12-joint (12j)-PDUS score was the sum of the scores of the above 24 synovial sites at 12 joints. In an attempt to expand the convenience of ultrasonography in clinical practice, we have chosen six synovial sites from six joints including the bilateral wrists (dorsal recess) and second and third MCP joints (dorsal recess). The six-joint (6j)-PDUS score was the sum of the six synovial sites.

Statistical analyses
Within-group comparisons were made using the Mann-Whitney U-test. Correlations were assessed with Spearman’s correlation coefficient test. The overall significance level for statistical analysis was 5% (two-sided). P < 0.05 was considered statistically significant.

Results

Patient characteristics
The demographic and clinical characteristics of 22 RA patients (5 males and 17 females) are as follows. The mean (s.d.) (range) age of the patients was 64 (9) (48–81) years. The mean (s.d.) (range) of disease duration was 2.3 (2.5) (0.25–10) years, which corresponded to relatively early-stage disease. RF and anti-CCP antibodies were positive in 15 (68.2%) and 18 (81.8%) patients, respectively. They received either synthetic DMARDs (n = 14), a combination of synthetic DMARDs plus TNF inhibitor (n = 1) or TNF inhibitor monotherapy (n = 1). Six patients were not treated with DMARDs. The mean tender joint counts (TJCcs), swollen joint counts (SJCs), ESR, CRP, DAS-28, SDAI and CDAI were 9.2, 8.0, 58.8, 2.38, 5.69, 30.6 and 28.3, respectively, which indicate that...
patients with relatively high disease activity were included in the present study.

Twelve 6j-PDUS scores and serum biomarkers

The median (range) of PDUS scores was 13.5 (1–39). Serum VEGF, MMP-3, MMP-9 and TIMP-1 were significantly higher in RA patients than in healthy controls—the mean levels of serum biomarkers; RA patients vs healthy controls (P-value, Mann–Whitney U-test)—VEGF: 695 vs 308 pg/ml (P < 0.0001), MMP-3: 185 vs 30 U/l (P < 0.001), MMP-9: 1962 vs 55 pg/ml (P < 0.0001) and TIMP-1: 214 vs 160 pg/ml (P < 0.05).

The correlations of 12j-PDUS scores with disease activity and serum biomarkers

The correlation of DAS-28 with SDAI or CDAI was extremely high, indicating that the physical examination was well performed (Table 1). The 12j-PDUS scores from 24 synovial sites were significantly positively correlated with TJC, SJC, ESR, CRP, DAS-28, SDAI, CDAI, serum VEGF, MMP-3 and TIMP-1, whereas they were not correlated with serum MMP-9 (Table 2). In particular, DAS-28 (r = 0.72, P < 0.001) and serum VEGF (r = 0.82, P < 0.01) strongly correlated with PDUS scores.

The correlations between clinical disease activity and serum biomarkers are shown in Table 1. All serum biomarkers correlated with inflammatory markers such as CRP and ESR. With regard to angiogenic factors, VEGF correlated well with the variables other than MMP-9 or TIMP-1 (Table 1).

Six 6j-PDUS scores can be an alternative for 12j-PDUS scores

The 6j-PDUS scores were strongly correlated with 12j-PDUS scores (r = 0.92, P < 0.0001). Accordingly, 6j-PDUS scores were significantly correlated with TJC (r = 0.50, P < 0.05), SJC (r = 0.44, P < 0.05), ESR (r = 0.57, P < 0.05), DAS-28 (r = 0.67, P < 0.01), SDAI (r = 0.55, P < 0.05), CDAI (r = 0.54, P < 0.05) and serum VEGF (r = 0.62, P < 0.01), whereas they were not correlated with serum MMP-3, MMP-9 and TIMP-1. Although these associations were slightly weaker than those with 12j-PDUS scores, the tendencies of 6j-PDUS and 12j-PDUS scores were very similar to each other.

Discussion

We have verified additional information regarding PDUS scores in patients with RA. First, our data included the small MCP joints. Since the second and third MCP joints are considered to be important areas for radiographic imaging of RA, as reported by Naredo et al. for US [2] and by OMERACT for MRI [7], our present data may reinforce the utility of PDUS. Although our present study includes relatively elderly patients, signs of OA were not detected in the examined joints, indicating that our results reflect rheumatoid inflammatory change.

Second, we have chosen other biomarkers. MMP-9 is important for the budding of endothelial cells, and TIMP-1 is an inhibitor of MMP-9; both are elevated in serum as well as in the synovial tissues of RA [8, 9]. As suspected, TIMP-1 was correlated with PDUS score and several other biomarkers, although its correlation was weaker than that of VEGF. MMP-9 tended to correlate with PDUS score; however, it did not reach statistical significance. Since the budding of endothelial cells is an early step in angiogenesis, MMP-9 may be important in the early phase of rheumatoid synovitis. The selection of very early-stage RA may be necessary to identify any association of MMP-9 with PDUS score.

Third, we have assessed SDAI and CDAI in the present study. The present study has revealed a clear correlation of PDUS score with SDAI and CDAI, although that of DAS-28 ESR was better. These data reinforce the validity of PDUS for the measurement of the disease activity of RA.

Fourth, for better clinical availability, we have reduced the number of sites examined by US to only six sites of the wrist and finger joints. These methods are simple and can save time that would be spent on scanning. Since the correlation of disease activity and PDUS was weaker than those with 24 synovial sites, further studies with larger numbers of patients should be necessary.

Recent investigations have found that the presence of the PDUS signal is a better predictor of further radiographic joint destruction than DAS-28 [10, 11].

**Table 1** Correlations between disease activity and serum biomarkers

<table>
<thead>
<tr>
<th></th>
<th>DAS-28</th>
<th>SDAI</th>
<th>CDAI</th>
<th>ESR</th>
<th>CRP</th>
<th>VEGF</th>
<th>MMP-3</th>
<th>MMP-9</th>
</tr>
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<tbody>
<tr>
<td>SDAI</td>
<td></td>
<td>0.93**</td>
<td>0.99**</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CDAI</td>
<td></td>
<td>0.93**</td>
<td></td>
<td>0.44*</td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td></td>
<td>0.64**</td>
<td>0.64**</td>
<td>0.59**</td>
<td>0.71**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td></td>
<td>0.64**</td>
<td>0.54*</td>
<td>0.51*</td>
<td>0.62**</td>
<td>0.70**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
<td>0.59**</td>
<td>0.54*</td>
<td>0.55*</td>
<td>0.57**</td>
<td>0.68**</td>
<td>0.60**</td>
<td></td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.61**</td>
<td>0.58**</td>
<td></td>
<td>0.43*</td>
<td>0.49*</td>
<td>0.29</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>MMP-9</td>
<td>0.27</td>
<td>0.23</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.39</td>
<td>0.42</td>
<td>0.37</td>
<td>0.52*</td>
<td>0.71**</td>
<td>0.58**</td>
<td>0.69**</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Correlations were assessed with Spearman’s correlation coefficient test. *P < 0.05, **P < 0.01, ***P < 0.0001.
Therefore, it is very important to search the variables that correlate with PDUS score. Among the biomarkers and clinical disease activity indices in the present study, however, DAS-28 was the strongest variable that correlates with PDUS scores. VEGF was best in the biomarkers although it was weaker than that of DAS-28. These data may suggest that comprehensive analysis is necessary to identify the best biomarkers to reflect the severity of the PDUS score.

In conclusion, PDUS, especially when focused on the area of the wrist and finger joints, is an excellent tool for the evaluation of disease activity in patients with RA. Our six-site evaluation method can be adequately tolerated in clinical practice.

Rheumatology key messages

- Standard as well as simplified PDUS scores reflected clinical disease activity and serum variables, including angiogenic factors.
- Our six-site evaluation method can be adequately tolerated in clinical practice.

Disclosure statement: The authors have declared no conflicts of interest.

References


TABLE 2 Correlations of PDUS score with disease activity and serum biomarkers

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender joints</td>
<td>0.52</td>
<td>0.017</td>
</tr>
<tr>
<td>Swollen joints</td>
<td>0.48</td>
<td>0.028</td>
</tr>
<tr>
<td>ESR</td>
<td>0.62</td>
<td>0.005</td>
</tr>
<tr>
<td>CRP</td>
<td>0.47</td>
<td>0.03</td>
</tr>
<tr>
<td>DAS-28</td>
<td>0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDAI</td>
<td>0.6</td>
<td>0.006</td>
</tr>
<tr>
<td>CDAI</td>
<td>0.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Serum VEGF levels</td>
<td>0.62</td>
<td>0.005</td>
</tr>
<tr>
<td>Serum MMP-3 levels</td>
<td>0.47</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum MMP-9 levels</td>
<td>0.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Serum TIMP-1 levels</td>
<td>0.54</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Correlations were assessed with Spearman’s correlation coefficient test.