Concise report

Greyscale and power Doppler ultrasonographic evaluation of normal synovial joints: correlation with pro- and anti-inflammatory cytokines and angiogenic factors

Joanne Kitchen1,* and David Kane1

Abstract

Objective. US is a promising tool in evaluating RA synovitis, but abnormal US findings have been reported in small subsets of normal joints in healthy subjects. This study aimed to systematically assess greyscale US (GSUS) and power Doppler US (PDUS) findings in 40 peripheral joints—the 28-joint DAS (DAS28) set, ankles and MTP joints—in healthy subjects. A composite score of abnormal US findings in 40 joints was compared with serum levels of pro-inflammatory cytokines.

Methods. US of 60 standard views in 40 joints was performed in 30 healthy subjects (total 3600 images). GSUS and PDUS were scored semi-quantitatively (0–3). Serum samples were obtained at the time of US and analysed for IL-1α, IL-1β, IL-2, IL-6, IL-8, VEGF, TNF-α and IFN-γ using biochip array technology.

Results. GSUS abnormalities were more frequent than PDUS abnormalities [mean total GSUS score = 20.07 (range 6–45; maximum potential score = 180), mean total PDUS score = 4.8 (range 0–13)]. GSUS score increased with increasing age (Spearman’s ρ = 0.383, P = 0.037). A PDUS signal >1 was observed only in the wrist (8%) and MTP1 (3%). GSUS scores did not correlate with any pro-inflammatory cytokine level. The total PDUS score correlated significantly with serum VEGF (r = 0.395, P = 0.046).

Conclusion. PDUS signals >1 are rarely seen in normal synovial joints. GSUS synovitis, but not PDUS, may reflect age-related joint changes. PDUS correlated with VEGF, providing further evidence of a central role for VEGF in synovial neo-angiogenesis.

Key words: ultrasound, musculoskeletal ultrasound, power Doppler, GSUS, greyscale, cytokines, healthy joints, vascular endothelial growth factor.

Introduction

Rheumatologists increasingly use US for the detection and quantification of synovitis in RA [1]. Current high-resolution US technology provides a spatial resolution of ≤0.1 mm and modern power Doppler US (PDUS) tools are sensitive enough to detect normal blood flow in joints [2]. It is important to differentiate between physiological and pathological findings when interpreting US images. A small number of studies have attempted to define musculoskeletal US features of normal joints. Schmidt et al. [3] quantitatively measured capsule thickness in 102 healthy subjects, but this study was limited to greyscale US (GSUS) measurements and did not include all 28-joints of the DAS (DAS28) used in evaluating RA. GSUS studies of healthy MCP joints have generally demonstrated normal [grade 0 (G0)] synovium [4], but synovial hypertrophy [5], effusions and increasingly abnormal scores in women with age [6] have been observed. GSUS features of normal PIP joints include effusions (21% of 368 joints) [7] and synovial hypertrophy (30–40% of 120 joints) [6]. Other studies report GSUS abnormalities in the elbows (5%) [8], knees (up to 77%) [3], ankles (33%) and MTP joints...
joints (34%) [9]. PDUS studies of asymptomatic healthy joints report abnormalities in the MCP joints (0–18%) [5, 10–12], PIP joints (<1%) [7] and wrist joints [12]. No study has systematically evaluated all peripheral synovial joints in healthy subjects using an established semi-quantitative score for GSUS and PDUS.

This study systematically evaluated the DAS28 joint set, ankles and MTP joints using standardized European League Against Rheumatism (EULAR) US protocols and a previously validated scoring system in order to establish the GSUS and PDUS features of peripheral joints in asymptomatic healthy subjects. A composite GSUS and PDUS synovitis score of 40 joints was compared with serum pro-inflammatory cytokine levels to assess whether these US abnormalities correlate with markers of systemic inflammation.

**Methods**

**Subjects**

Thirty subjects (15 male, 15 female) participated after completing a screening questionnaire to ensure they had no history of arthritis, previous joint trauma requiring medical investigation or treatment, previous joint surgery or current joint symptoms of pain, swelling or early morning stiffness. Volunteers with a history of any inflammatory condition were excluded. Only two subjects with any significant medical history were included—one on anti-hypertensive medication (well controlled) and the other on thyroxine for treated hypothyroidism (and no history of arthralgias or myalgias). Occupational data were obtained on all subjects. All subjects gave written informed consent following approval by the Adelaide and Meath Hospital ethics committee.

**Musculoskeletal US examination**

Standard GSUS and PDUS of the DAS28 joint set, ankles and MTP joints were performed in preselected imaging planes according to the EULAR guidelines [13]. A MyLab70 XVG system (Esaote, Genoa, Italy) with a 6–18 MHz broadband linear array transducer was used for all imaging. Each joint was dynamically scanned using standardized presets and representative GSUS (B mode) and PDUS images were obtained. The scans were performed in a temperature-controlled room after the subject had been resting for at least 30 min. No subjects were taking NSAIDs. Both dorsal and palmar views of the MCP and PIP joints were performed, resulting in 60 GSUS and 60 PDUS images from 40 joints for each subject. Images were scored using a previously validated 0–3 semi-quantitative scale for both B mode and PDUS [14]. Pathology was defined according to the OMERACT criteria [15]. The maximum grade of either effusion or synovial hypertrophy visualized was ascribed to each image. This resulted in a theoretical maximum score of 180 for both GSUS and PDUS (assuming a score of 3 in every joint view). Doppler presets were standardized with a Doppler frequency of 14.3 MHz, gain of 54% and a pulse repetition frequency of 750 Hz. Scanning was performed with the subject relaxed, using a thick layer of US gel and applying minimal pressure to the joints.

**Proteomic analysis**

Serum samples were obtained at the time of US examination and stored at –80°C. Samples were analysed for 26 of 30 control subjects for IL-1α, IL-1β, IL-2, IL-6, IL-8, VEGF, TNF-α and IFN-γ. The proteomic analysis was carried out using the Evidence Investigator (Randox, Antrim, Northern Ireland, UK), which uses biochip array technology to perform simultaneous detection of multiple analytes from a single sample.

**Statistical analysis**

Data were analysed using SPSS version 18 software (IBM, Armonk, NY, USA). Differences between groups were analysed using the Mann–Whitney U test. Correlations were assessed by Spearman’s correlation coefficient.

**Results**

**Demographics**

Thirty healthy subjects [mean age 38.9 years (±11.7; range 22–63)] underwent GSUS and PDUS of 40 joints, resulting in 3600 images for analysis. No subject was involved in heavy manual labour during the study and all were asymptomatic. Results are summarized in Table 1 and Fig. 1.

**GSUS results**

The mean total GSUS score was 20.07 (range 6–45, maximum potential score 180). Using the standard semi-quantitative 4-point scale, both 0 and 1 GSUS values are considered normal, allowing for minor degrees of physiological fluid.

We found that 95.7% of dorsal and 98.7% of palmar MCP joints were normal (G0/1). Changes noted on dorsal MCP joints were synovial hypertrophy, predominantly over MCP2 and MCP3 [more frequent on the dominant hand (48% vs 32%)]. Palmar aspect changes commonly included synovial hypertrophy or effusion at MCP2 (55%) and effusions at MCP4 (38%). PIP joints were assessed from dorsal and palmar views, with 97.3% G0/1. We found that 68.4% of MTP1 and 93.7% of MTP2–5 had G0/1 change. G2 changes were seen in MTP2 (n = 6), MTP3 (n = 5) and MTP4 (n = 2). Two MTP2s had G3 GSUS. Fifty-one per cent of wrists had small effusions and one wrist had G2 changes. Five per cent of posterior elbows had G1 effusions, but the remainder were normal. No fluid or synovial hypertrophy was seen in the shoulders. SF (G0/1) was seen in the knee at both the suprapatellar (100%) and lateral (98.3%) recesses, in keeping with published data [3]. One subject had a unilateral G2 knee effusion. Fifty-five ankles were G0, five had small effusions distally.
Correlations of GSUS scores with age, sex and clinical status

Although subjects were age matched, GSUS scores were significantly higher in males than in females ($P = 0.014$). Age and GSUS score correlated significantly (Spearman’s $r = 0.383$, $P = 0.037$). Ten subjects had G2 scores in joints other than MTP1 or the thumb IP joint. Six subjects had G2 MCP joints. Of these, two had asymptomatic OA (Heberden’s nodes but no pain), one had a family history of RA and another had a distant history of resolved joint stiffness.

PDUS results

The mean total PDUS score was 4.8 (range 0–13). PDUS signal in normal joints was rarely observed and tended to be single vessels (G1). There was no significant difference in PDUS scores between male and female subjects ($P = 0.753$) and no significant correlation between

---

**TABLE 1** The number of joints with each score in healthy subjects

<table>
<thead>
<tr>
<th>Joint</th>
<th>$n$</th>
<th>GS 0 (%)</th>
<th>GS 1 (%)</th>
<th>GS 2 (%)</th>
<th>GS 3 (%)</th>
<th>PD 0 (%)</th>
<th>PD 1 (%)</th>
<th>PD 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP</td>
<td>600</td>
<td>403 (67.2)</td>
<td>178 (29.7)</td>
<td>19 (3.2)</td>
<td>–</td>
<td>557 (92.8)</td>
<td>43 (7)</td>
<td>–</td>
</tr>
<tr>
<td>PIP</td>
<td>600</td>
<td>467 (77.8)</td>
<td>117 (19.5)</td>
<td>16 (2.7)</td>
<td>–</td>
<td>576 (96)</td>
<td>24 (4)</td>
<td>–</td>
</tr>
<tr>
<td>Wrist</td>
<td>60</td>
<td>28 (47)</td>
<td>31 (51)</td>
<td>1 (2)</td>
<td>–</td>
<td>35 (58.3)</td>
<td>20 (33.3)</td>
<td>5 (8.3)</td>
</tr>
<tr>
<td>Elbow</td>
<td>60</td>
<td>57 (95)</td>
<td>3 (5)</td>
<td>–</td>
<td>–</td>
<td>58 (96.7)</td>
<td>2 (3.3)</td>
<td>–</td>
</tr>
<tr>
<td>Shoulder</td>
<td>60</td>
<td>60 (100)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>60 (100)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Knee</td>
<td>120</td>
<td>91 (75.8)</td>
<td>28 (23.3)</td>
<td>1 (1.7)</td>
<td>–</td>
<td>119 (99.2)</td>
<td>1 (0.8)</td>
<td>–</td>
</tr>
<tr>
<td>Ankle</td>
<td>60</td>
<td>55 (91.7)</td>
<td>5 (8.3)</td>
<td>–</td>
<td>–</td>
<td>60 (100)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MTP1</td>
<td>60</td>
<td>7 (11.7)</td>
<td>34 (56.7)</td>
<td>16 (26.7)</td>
<td>3 (5)</td>
<td>43 (71.7)</td>
<td>9 (15)</td>
<td>8 (13.3)</td>
</tr>
<tr>
<td>MTP2</td>
<td>240</td>
<td>149 (62)</td>
<td>76 (31.7)</td>
<td>13 (5.4)</td>
<td>2 (0.8)</td>
<td>231 (96.3)</td>
<td>9 (3.7)</td>
<td>–</td>
</tr>
</tbody>
</table>

Data are given as $n$ (%). $n$: number of joint views scanned in that region (MCP and PIP had both dorsal and palmar views, the knee included both a suprapatellar and a lateral recess view); GS: greyscale; PD: power Doppler.

**Fig. 1** Homunculus illustrating the percentage of normal joints that showed GSUS abnormalities and power Doppler signal.

GSUS: greyscale US; G1: grade 1; G2: grade 2; G3: grade 3; MCPJ: metacarpophalangeal joint; PIPJ: proximal interphalangeal joint; MTP: metatarsophalangeal joint.
age and PDUS score (Spearman’s $r=0.298$, two-tailed $P=0.11$).

We found 93.3% of MCP joints had a G0 PDUS score. Ninety-six per cent of PIP joints had G0 with 4% G1. Twelve of 24 G1 PIP scores were in the thumb. Despite the high frequency of GSUS changes in MTP1 joints, 71.7% had G0 PDUS. MTP2–5 demonstrated no PDUS score in 98.3% of joints, a G1 score in 3.7% and none had pathological scores (G2–G3).

The majority of wrist scans were G0 (58.3%). Thirty-three had G1 and 8.3% had G2 PDUS scores. A G1 PDUS score was detected in 2 of 60 (3.3%) elbow scans. No PDUS signal was demonstrated in the shoulders. Most knee scans, at both the suprapatellar and lateral recesses, showed no PDUS signal (99.2%). The knee joint with G1 score at the lateral recess had G2 GSUS changes present. No PDUS signal was detected in any of 60 ankles.

Results of the Randox high-sensitivity cytokine array

Median cytokine results (in pg/ml) were IL-2, 1.96 [inter-quartile range (IQR) 1.63–4.27]; IL-4, 1.94 (0.97–2.83); IL-6, 0.96 (0.53–2.82); IL-8, 13.82 (8.73–172.2); IL-10, 0.59 (0.3–1.57); IL-1α, 0.2 (0.11–0.43); IL-1β, 1.41 (0.01–3.4); VEGF, 146.62 (49.66–283.78); TNF-α, 11.8 (8.02–18.98) and IFN-γ, 1.74 (1.03–3.57).

Serum cytokine levels were correlated with total 40-joint GSUS and PDUS scores. There was a significant correlation between VEGF and PDUS score in normal subjects (Spearman’s $r=0.395$, $P=0.046$). There was a significant negative correlation between the TNF-α and PDUS score ($r=−0.405$, $P=0.04$). There was no correlation with any other cytokines and GSUS or PDUS score.

Discussion

This is the first study to evaluate GSUS and PDUS in 40 asymptomatic synovial joints of healthy subjects, comprising the most relevant joints in the assessment of patients with RA, using established semi-quantitative scores. GSUS scores $\geq 1$ were more frequent than PDUS scores in all normal joints. In MCP and PIP joints, GSUS scores were consistent with previously published literature [5, 6, 18] and most MCP joints in healthy subjects were normal (G0–1). Most PIP joints were normal, including 22% with small G1 physiological effusions, comparable to 21% prevalence in a previous study [7]. MTP1 was the only joint to demonstrate G3 synovial hypertrophy/effusion, probably due to high rates of asymptomatic osteoarthritic changes. Our study frequently detected abnormalities in the MTP joints (48% $\geq$ G1, 11% G2), which has been described elsewhere [3, 9]. A synovitis score of $\geq$ G2 appears to indicate abnormality in the MCP and PIP joints, but synovitis is present at a low frequency in normal MTP joints. The majority of wrist and elbow scans were normal, but 5% of elbows showed small (G1) effusions, consistent with the literature. Fluid or synovial hypertrophy was not seen in the posterior glenohumeral scans of normal shoulders, a site that has a reported high specificity for synovitis. Physiological fluid in healthy knee joints has been described [3], and this was noted in 32% of knees. Overall a G2 score was an infrequent finding in normal wrists and knees and was not observed in the normal elbow, shoulder and ankle, suggesting that it is a reliable indicator of pathology in these joints.

This is the first study to report a positive correlation between the GSUS synovitis score and increasing age in men and women. This is unsurprising, as changes due to OA can often be seen in asymptomatic joints, the prevalence of which increases with age. Higher incidence of abnormal GSUS findings with increasing age is probably due to synovial and capsular thickening associated with an early stage of OA and has been described in normal subjects [17]. However, PDUS scores did not increase with age, suggesting that PDUS signals from joints may be more specific for assessment of inflammation, particularly in older subjects. One limitation of our study is that subjects were volunteers of employment age and hence had a lower mean age (38.9 years) than patients with new-onset inflammatory arthritis (48.8 years in our cohort).

A PDUS signal in normal joints was rare. Initial studies [11] reported G0 in control MCP joints. Our findings (G1 in 6.7% of MCP joints) are in keeping with recent studies where PDUS signal was detected in 5.2–18% of normal MCP joints, using machines with higher Doppler sensitivity [5, 12, 18]. PDUS signal in normal PIP joints is rare (<1% joints in one study [18], 2.2% in another). Our study reflects the current literature, with no PDUS in 96% of PIP joints. Fifty per cent of G1 Doppler signals were seen in the palmar aspect of the thumb IP joint and likely represent a normal nutrient artery. This could not be reliably determined to be intra- or extracapsular. Despite the high frequency of GSUS changes in MTP1 joints, 71.7% showed no PDUS flow and PDUS was not a feature of other normal MTP joints.

Doppler signal was commonly detected in wrist joints (41.7%), a finding reported elsewhere [18]. No PDUS signal was demonstrated in the shoulder, consistent with previous studies, or in the ankles or knees, except in one knee where a G1 signal was seen in conjunction with GSUS effusion and synovial hypertrophy in a middle-aged female with a distant history of joint injury. Thus we may conclude that PDUS signal is rare and of low grade in small joints. PDUS is not observed in the normal elbow, shoulder, knee or ankle: this is likely to reflect the difficulty of detecting PDUS in deep joints (even in inflammatory disease), but also because the subjects were healthy. Doppler scores of 1 or 2 may be seen in normal wrists, and caution is advised in overinterpreting PDUS in the wrist joints.

This study is the first to compare levels of serum cytokines implicated in the pathogenesis of synovial inflammation with a 40-joint US score. GSUS scores did not correlate with any of the serum cytokines measured. PDUS correlated with VEGF and there was a weak negative correlation between PDUS score and TNF-α level. Serum TNF-α level has not been shown to be a good marker of inflammation in inflammatory arthritis [19, 20]. VEGF has been demonstrated to have a central role in the
an angiogenic process in rheumatoid synovium. By using PDUS to develop a total semi-quantitative score for vascularity in 40 joints, this study provides further evidence that serum VEGF levels are a potential biomarker of total synovial vascularity. We intend to confirm this correlation between total PDUS score and serum VEGF in different patient groups before and after therapy. There was no relationship with the remainder of the cytokines.

**Rheumatology key messages**

- Low-grade greyscale US changes occur in healthy subjects and increase with age and in males.
- A power Doppler US signal >1 is not observed in normal joints and reliably measures synovial inflammation except in wrists.
- Total power Doppler US in 40 joints correlated with serum VEGF concentration, providing evidence of a role in synovial neo-angiogenesis.

**Funding:** The biochip analysis was supported by a rheumatology fellowship awarded through the Royal College of Physicians in Ireland, which was supported by Merck Sharpe and Dohme (Ireland), and through a grant from the Meath Foundation, Dublin.

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

**References**