Influence of recent exercise and skin temperature on ultrasound Doppler measurements in patients with rheumatoid arthritis—an intervention study

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Objective. Use of ultrasound Doppler (USD) in diagnosing and treatment monitoring of patients with RA has increased considerably. Hyperaemia is an integral part of the inflammatory response, and the amount of USD activity in an inflamed synovium may therefore be used to quantify the inflammatory activity. It is unclear, however, whether the hyperaemia alone reflects the disease activity or may be influenced by other factors.

Methods. Twenty-nine patients with RA underwent USD examination of the wrist before and immediately after three interventions. The interventions were carried out on three separate days. The interventions were (i) isometric exercise of the muscles of the hand and forearm, (ii) heating and (iii) cooling of the hand. The amount of Doppler in the wrist joint was quantified by measuring the percentage of colour in the synovium—the colour fraction (CF). The CF values estimated before and after each intervention were compared to see if any intervention affected the amount of Doppler in the synovium.

Results. The CF decreased significantly after cooling of the hand (P = 0.018 and < 0.0001). Despite being highly significant, the numerical decrease in CF was only modest, 0.78–1.33 percentage points. The other interventions did not affect the CF significantly, with P-values of 0.65 and 0.59 in the heating intervention and 0.49 in the exercise intervention.

Conclusions. Cooling of the hand should, if possible, be avoided before a USD examination of the wrist in patients with RA, because the amount of Doppler activity might be affected by low skin temperatures.

Key words: Ultrasonography, Rheumatoid arthritis, Synovium, Inflammation, Wrist.

Introduction

RA is characterized by inflammation of the synovium [1]. The inflammatory process is reflected in increased blood flow in the synovium [2]. Thus, the amount of blood flow in the synovium can be used as a parameter of disease activity.

Ultrasound Doppler (USD) measures blood flow in tissues, and thereby USD might be used as a measure of disease activity in RA. This assumption is supported by the findings of correlation between the amount of USD signal and histopathological signs of inflammation [2]. Furthermore, USD measurements have shown concurrent validity with validated predictors of disease activity in RA [3].

Because tissue temperature and recent physical activity affect the blood flow, it is reasonable to expect that these factors could affect the USD measurements. However, information is sparse on the possible influence of factors other than the disease activity on USD measurements. Data on the influence of skin cooling indicate a decrease of the USD signal after skin cooling [4–6].

The accuracy of USD as a measure of disease activity in RA depends on clarification of various physiological conditions, which might affect the measurements [7]. Thus, it is necessary to rule out that the changes in USD activity merely reflect the changes in physiological conditions.

The aim of this study was to investigate whether the USD measurements in a group of patients with RA were affected by recent physical activity and various skin temperatures.

Materials and methods

Participants

Patients from a rheumatological outpatient clinic fulfilling the ACR criteria for RA [8] participated in the study. Inclusion criteria were USD activity in a wrist joint and absence of a known heart disease or any malignancy. In all patients, a disease activity score in 28 joints (DAS28) was obtained. The local ethics committee in Frederiksberg, Copenhagen, approved the study, and all patients gave informed consent.

US examination

Examinations were performed with a Logiq 9 (GE Medical, Milwaukee, WI, USA) using a 14 MHz centre frequency linear array transducer. The same preset was used for all US examinations. The preset had the Doppler adjusted for maximum sensitivity for low flow: pulse repetition frequency of 0.4 kHz, lowest wall filter on 45 Hz and 7.5 MHz Doppler frequency, and Doppler gain just below the noise level.

The patients had their most affected wrist examined. An investigator (K.E.) trained in musculoskeletal US performed all the examinations. The joint was scanned from the dorsal side in the radial, central and ulnar positions and from the volar side in the central position. All scans were performed in the longitudinal plane.

US images were selected according to a protocol with excellent reliability [9]. With this protocol, specific anatomic landmarks had to be present in the images. After identifying the anatomic...
landmarks in the grey scale image, the Doppler was activated, and while keeping the landmarks in the image, the transducer was adjusted until the scan plane with the maximum Doppler activity was identified. The transducer was held in this position for a couple of heart cycles, whereupon the image was frozen. With the cine-loop function, the frames with maximum and minimum Doppler activity (Fig. 1), corresponding to systole and diastole, were stored in Digital Imaging and Communications in Medicine (DICOM) format.

**Image analysis**

The USD activity was quantified with the colour fraction (CF). The CF is the number of colour pixels divided by the total number of pixels in a region of interest (ROI) [10]. The ROI was defined by specific anatomic landmarks surrounding the synovial tissue in the wrist. This method has shown excellent test–retest reliability [9] (Fig. 1). The CF was calculated in the eight images from each examination (maximum and minimum Doppler activity in each position). Subsequently, the average CF value was calculated.

The image evaluation was carried out by another person than the one performing the US examinations. The CF calculation was made in Image-Pro Analyser version 6.3 (MediaCybernetics, Bethesda, MD, USA).

**Interventions**

All patients performed all interventions randomly on three separate days. Interventions were made at the wrist with maximum Doppler activity. Scanning was done immediately before (pre-scan) and after (post-scan) the intervention. Before pre-scans, the patient rested for 15 min in room temperature. Before temperature interventions, baseline skin temperature was obtained at the dorsal part of the wrist (Memolog 600j Novo Diagnostic Systems, Hadsoud, DK). Patients were not allowed to smoke 1.5 h before the interventions.

The interventions were:

(i) hand exercise: five times maximum grip strength on a Digital Hand Dynamometer (North Coast Medical).

(ii) Heating: heating to 37°C between two warm packings with a temperature of 72°C.

(iii) Cooling: cooling to 22°C between two bags of crushed ice. If the temperature of 22°C was not achieved in 20 min, the actual temperature was noted, the ice was removed and the post-scan was performed.

(iv) Cooling: cooling for 10 min in a bucket with crushed ice. If the patient could not manage for 10 min, the intervention was stopped, the time was noted and the post-scan was performed.

**Statistics**

A linear mixed model was applied using the SAS system. To rule out any location-specific effects, the initial analysis focused on the fixed-effects analysis of location (four levels: dorsal radial, dorsal central, dorsal ulnar and volar central) and time (two levels for exercise: Scan 1 and 2A; three levels for both heating and cooling: Scan 1, 2A and 2B), analysing whether there was a time × location interaction: The estimates were adjusted for the baseline (Scan 1) values.

If no time × location interactions were present and no significant main effect of location, CF measurements for all locations were pooled, and a subsequent analysis was performed to focus on the main effects of time (three levels: Scan 1, 2A and 2B). *P*-value of <0.05 was considered as statistical significance. The day-to-day variation in baseline CF measurements was calculated as the S.D. of the three baseline scans for each subject and averaged across the group.

**Results**

**Demographic and clinical data**

Twenty-nine patients with RA, 3 men and 26 women, had participated in the study. The mean ± S.D. of age was 64 ± 11 years; mean disease duration was 12 ± 12 years; and mean DAS28 was 3.4 ± 1.33. Fifteen patients were treated with an anti-TNF-α 11 in addition with MTX, 11 were treated with MTX, SSZ and/or prednisolone. Three patients were on no medication.

**Hand exercise**

Because of the pain during exercise, three patients did not wish to participate in the grip strength intervention.
Heating
The mean baseline skin temperature before heating was 32.63°C (range 28.26–34.99°C). Fifteen of the patients did not reach a skin temperature of 32°C within 20 min; mean temperature for those patients was 24.79°C. Nine patients did not manage to keep the hand in the crushed ice for 10 min; mean skin temperature for these patients did not differ from the other 20 patients (24.71 ± 2.31°C and 24.19 ± 1.83°C, respectively) (Table 1).

Cooling
The mean baseline skin temperature before cooling was 32.63°C (range 28.26–34.99°C). Fifteen of the patients did not reach a skin temperature of 32°C and did not undergo the intervention. After paraffin bath, the mean temperature was 34.4°C. Three patients did not manage to keep the hand in paraffin for 15 min.

US data
A total of 84 pre-scan US examinations were performed in 29 participants with a mean CF of 3.62 ± 3.65 (range 0.1–16)%.

Hand exercise
There was no significant time × location interaction (P = 0.486), and no main effect of location (P = 0.928). There was no significant change in the mean CF pooled across locations following exercise (main effect of time: P = 0.49). After the exercise intervention, the mean CF increased to 0.22% (P = 0.49; Table 1).

Heating
There was no significant time × location interaction (P = 0.981), and no main effect of location (P = 0.266). There was no significant change in the mean CF pooled across locations (main effect of time: P = 0.62). In the heating intervention, the mean CF decreased to 0.21% from baseline (P = 0.65) when heated up to 37°C, whereas, it increased to 0.24% (P = 0.59) from baseline after heating in 50°C warm paraffin (Table 1).

Cooling
There was no significant time × location interaction (P = 0.105) and no main effect of location (P = 0.165). There was a significant main effect of time in the CF pooled across locations (P = 0.006). After cooling to a mean skin temperature of 23.44°C, the decrease in CF from baseline was 0.78% (P = 0.018), and after adding the ice the mean decrease in CF from baseline was 1.33% (P < 0.0001) (Table 1).

Day-to-day variation
The mean s.d. for baseline CF values in the study population was 1.45% with a 95% empirical data interval from 0.17 to 5.92%.

Table 1. Changes in CF after interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Baseline temperature</th>
<th>Change in CF from baseline*, %</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Hand exercise, n = 26</td>
<td></td>
<td>0.22</td>
<td>0.49</td>
<td>-0.87, 0.43</td>
</tr>
<tr>
<td>(ii) Heating to 37°C, n = 29</td>
<td>32.6°C</td>
<td>0.21</td>
<td>0.65</td>
<td>-0.71, 1.12</td>
</tr>
<tr>
<td>(iii) Paraffin bath</td>
<td></td>
<td>0.24</td>
<td>0.59</td>
<td>-1.15, 0.67</td>
</tr>
<tr>
<td>(mean 34.4°C), n = 29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii) Cooling to -22°C</td>
<td>32.6°C</td>
<td>-0.78</td>
<td>0.02*</td>
<td>0.14, 1.42</td>
</tr>
<tr>
<td>(mean 23.4°C), n = 29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii) Ice cooling</td>
<td></td>
<td>1.33</td>
<td>0.001*</td>
<td>0.68, 1.98</td>
</tr>
<tr>
<td>(mean 24.5°C), n = 29</td>
<td></td>
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</table>

*Percentage point. *Statistically significant. CF: colour fraction.

Discussion
Our results strongly indicate that only cooling of the skin affects the amount of USD activity in the synovium in RA patients. This is in accordance with previous results in the materials published earlier [4, 5]. In contrast, heating and hand exercise did not affect the CF in any systematic way. The decrease in CF after cooling was statistically significant; however, the numerical decrease was only modest, and was well within the obtained variation of 1.45% in the CF in the baseline examinations.

As mentioned earlier, our results are in accordance with the other two studies investigating the effect of skin cooling on the USD measurements, which likewise found a decrease of the Doppler signal in the synovium in RA patients [4, 5]. Furthermore, we performed a pilot study on 14 RA patients in which we also found a significant reduction of CF after cooling [6]. Thus, despite the modest decrease of the CF after cooling, it seems reasonable but not essential to recommend that patients with RA do not have cold hands when USD examination was performed. It seems of no consequence that patients have warm hands or have performed recent physical activity.

The interaction between DAS28 and USD in this study is interesting to note. Thus, all participants by definition had USD activity, and despite this, some patients (nine) had a DAS28 < 2.6 indicating disease remission. This is in concordance with other studies showing USD activity in patients in clinical remission [11, 12]. It has been shown that patients with subclinical Doppler activity have increased the risk of developing erosions indicating a future important role of USD in the assessment of disease activity in patients with RA [11]. The expected increasing use of USD in the assessment of disease activity in RA patients emphasizes the importance of validation of Doppler measurements, which has also been stressed by the OMERACT US group [13].

It could be argued that the relatively small study cohort is a limitation of our results. However, our results were consistent in a cohort covering a broad spectrum of both disease activity, assessed by DAS28, and disease duration. The variation in our study population indicates that the results may be extrapolated to RA patients in general. A strength of the study is that the three interventions were executed in all 29 patients (26 in the hand exercise intervention), reducing individual variations.

To our knowledge, this is the first study investigating the effect of various physiological stimulations on the USD measurements in RA. Our results show that the USD measurements in the wrist joint are only slightly affected by the cooling of the skin of RA patients. No other physical stimulation can affect the USD. The results indicate that the accuracy of USD measurements in RA is good, which underlines its usefulness in diagnosis, disease progression assessment and monitoring of treatment. Studies on other physiological or pharmaceutical stimuli with possible effect on regional perfusion are required to fully clarify the accuracy of USD measurements in RA.

Rheumatology key messages

- Although modest, local skin cooling affects USD measurements in RA.
- This study has increased the validity of USD in detecting increase in hyperaemia.

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