Assessment of microvascular changes in Raynaud’s phenomenon and connective tissue disease using colour Doppler ultrasound

M. Keberle, H.-P. Tony1, R. Jahns1, M. Hau, R. Haerten2 and M. Jenett

Institut für Röntgendiagnostik, University of Würzburg, Würzburg, 1 Medizinische Poliklinik, University of Würzburg, Würzburg and 2 Siemens AG, Medical Engineering Group, Erlangen, Germany

Abstract

Objective. We used colour Doppler ultrasound (CDU) to differentiate primary from secondary Raynaud’s phenomenon (pRP and sRP, respectively) and to assess digital vascular damage in patients with connective tissue disease (CTD).

Methods. Vascularity in the nailbeds of 15 healthy controls and 35 patients with CTD (systemic sclerosis or systemic lupus erythematosus) was quantified using a multi-D array transducer before and after cold and warm challenge, respectively. The results were compared with the clinically evaluated initial skin lesions. Vascularity was compared similarly between 10 pRP and 22 sRP patients.

Results. Vascularity at ambient temperature differed between healthy subjects and sRP patients as well as between healthy subjects and CTD patients without initial skin lesions. Patients with pRP had normal vascularity at ambient temperature but differed from healthy controls in response to a dynamic temperature challenge. CDU confirmed the clinical evaluation in 89.4% of the patients with RP and in 78.0% of the skin lesions.

Conclusion. The novel CDU technique presented here makes it possible to discriminate between pRP and sRP and to quantify vascular changes in CTD patients.

Key words: Connective tissue disease, Raynaud’s phenomenon, Ultrasound, Doppler studies.

Raynaud’s phenomenon (RP) is the most typical early manifestation in more than 90% of patients with systemic sclerosis (SSc) and in about a third of all patients with systemic lupus erythematosus (SLE) [1, 2]. To date, the diagnosis of RP is based mainly on clinical criteria [2]. However, in clinical routine it is often difficult to differentiate between RP and cold-intolerance or between primary (pRP) and secondary RP (sRP) in patients with underlying connective tissue disorders (CTD), such as SSc and SLE. In addition, more than 95% of patients with SSc and about 50% of those with SLE suffer from disease-related digital skin lesions [1, 2]. In SSc, intimal hyperplasia is caused mainly by the overproduction of collagen and, therefore, often results in structural narrowing of the smaller digital arteries [2]. In SLE, the pathomechanisms leading to structural damage are still not fully understood. One mechanism might be direct interaction of antiphospholipid antibodies with membranes of the vascular endothelial cells, resulting in non-inflammatory thickening of small vessels by intimal proliferation [1]. Both mechanisms can lead to alterations in the microvascular lumina.

Various methods, including nailfold microscopy, laser Doppler flow monitoring, thermography and plethysmography, have been used to evaluate distal digital vascularity and to assess the microvascular damage that has accumulated [3]. However, due to lack of availability, feasibility and reproducibility, none of the above methods has been generally accepted in clinical routine [3]. In our search for a convenient method to evaluate peripheral vascularity, we used colour Doppler ultrasound (CDU) together with novel ultrasound array technology in order to visualize the smallest vessels in the nailbed, which have an extremely low blood flow. We checked whether this method would be useful in differentiating pRP from sRP, as a possible clinical application. In addition, we evaluated the method in the assessment of both the presence and the severity of digital vascular damage in CTD by comparing the digital skin lesions found on initial clinical evaluation with the vascular changes determined by CDU. Our results hold promise as the basis for a means of identifying those patients with or without RP who have underlying CTD...
Assessment of vascularity by colour Doppler ultrasound

at an early stage in the course of the disease. A standardized examination, including functional tests, was established and the results were categorized for clinical use.

Patients and methods

Forty-five patients were included in our study after informed consent had been obtained from them (Table 1). Twenty-two of the patients with CTD (11 with SSc, 11 with SLE) had sRP. The CTD patients were classified according to the criteria provided by the American College of Rheumatology [4, 5]. All patients with RP presented with clinically typical symptoms, such as episodic digital ischaemia associated with cold exposure and digital blanching, cyanosis and rubor after rewarming. pRP patients were required to have a 2-yr history of RP without any signs of CTD, trauma or neurological diseases or the use of RP-associated drugs. They were also required to have repeated negative tests for antinuclear antibodies, anti-SSA, anti-RNP, antitopoiso merase type 1 and anti-centromere antibodies. In addition, 15 healthy subjects who were matched for gender and age served as controls (Table 1). None of the patients or healthy controls, with the exception of one patient with borderline hypertension and one patient with dietary controlled diabetes mellitus (type IIb), had evidence of concomitant vascular disease. Only non-smokers and individuals who were not being treated with vasoactive drugs were admitted to the study.

Patient subgroups

In order to distinguish the two subtypes of RP, healthy controls were compared either with patients who had pRP or with those CTD patients who presented sRP. Another aspect of our study was to assess early quantitative changes in the peripheral vasculature of CTD patients irrespective of RP (not a direct comparison of SSc with SLE patients). Therefore, all CTD patients were divided into two subgroups according to the presence of initial disease-related digital skin lesions, such as oedematous scleroderma and/or skin thickening. Clinical examination revealed 15 CTD patients with initial digital skin lesions (13 with SSc, two with SLE), whereas 20 of the CTD patients had no clinically detectable alteration of the digital skin (five with SSc, 15 with SLE). Patients with manifest ulcerations and/or gangrene were not included in the study.

Ultrasound examination

All CDU examinations were performed by the same person (MJ), who was not aware of the patients’ diagnoses. In control subjects and patients, both the second and third right finger were examined. In patients with disease-related digital skin damage, at least one of the two fingers examined had initial lesions. The examinations were performed according to the following protocol. After 20 min of acclimatization at ambient temperature (19–21°C, baseline vascularity) the hand to be studied was positioned stably on a soft pillow. Dorsoflexor scans of the nailbed (sagittal and transverse; Fig. 1A and B, respectively) and of the fingertip (transverse; Fig. 1C) were obtained in a standardized manner and stored digitally on a picture archiving and communication system. To avoid misinterpretation of colour signals caused by motion artefacts, in some cases pulsed Doppler was used for clarification. In addition, the distal radial and ulnar arteries and the digital arteries were analysed in all patients in order to exclude a haemodynamically relevant stenosis of these vessels. Patients with such stenoses were not included.

Dynamic challenge

To induce hyperaemia, control subjects and patients placed their right hand into warm water (40°C) for 5 min before CDU was repeated. After at least 15 min of re-equilibration to baseline conditions at ambient temperature, the subjects placed their right hand into cold water (10°C) for 5 min to induce vasoconstriction before CDU was repeated. In all cases the dynamic challenges were well tolerated.

Technical aspects of the ultrasound examination

The studies were performed with a Sonoline Elegra Advanced ultrasound machine equipped with a newly developed multi-D linear array transducer (MDA) (VFX 13–5; Siemens Medical Systems, Ultrasound Group, Issaquah, Washington, USA). The VFX 13–5 covers a high frequency range (up to 13 MHz) and is designed for the high-resolution imaging of superficial structures. This broadband transducer is capable of transmitting very short ultrasound pulses and has the advantage of high axial resolution. MDA transducers comprise multiple rows of transducer elements. Conventional linear arrays have a single row of elements and generate non-homogeneous elevation beam profiles, i.e. profiles that are perpendicular to the scanning plane. The elevation beam profile is typically wider in the near depth range as well as in the far depth range, and is focused only in the mid range. Using multi-D technology, the elevation beam profile is depth-controlled. For echoes from near the surface, only the inner row of elements is activated, whereas for echoes from deeper structures multiple rows of elements are employed. This results in a reduced slice thickness, particularly in the near range. Consequently, lateral resolution in the elevation direction is increased, partial volume artefacts are reduced, and contrast resolution in the image is improved. The VFX 13–5 transducer is therefore

---

**Table 1. Study population**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Male/female ratio</th>
<th>Mean age (yr) ± SD</th>
<th>Mean disease duration (yr) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>15</td>
<td>1:4</td>
<td>33 ± 10</td>
<td>—</td>
</tr>
<tr>
<td>Patients</td>
<td>45</td>
<td>1:5.4</td>
<td>38 ± 17</td>
<td>5.6 ± 2.6</td>
</tr>
<tr>
<td>pRP</td>
<td>10</td>
<td>1:4</td>
<td>36 ± 13</td>
<td>3.6 ± 1.2</td>
</tr>
<tr>
<td>SSc</td>
<td>18</td>
<td>1:5</td>
<td>42 ± 20</td>
<td>5.8 ± 2.7</td>
</tr>
<tr>
<td>SLE</td>
<td>17</td>
<td>1:7.5</td>
<td>37 ± 15</td>
<td>5.4 ± 2.4</td>
</tr>
</tbody>
</table>

---
particularly suited to tissue and colour flow imaging of structures close to the skin surface. Multi-D technology is widely available and the costs are similar to those associated with the use of other high-frequency transducers.

We used standard frequencies of 12.0 MHz for B-mode and 9.0 MHz for colour-mode scanning with the focus at 5 mm. The ultrasound system was optimized to detect the lowest signal possible by selecting a low pulse-repetition frequency (551 Hz), a low wall filter, and a high priority (14). Colour gain was adjusted at the beginning of each examination by selecting the highest value at which the colour image was still unaffected by artefacts.

Quantification of colour signals and anatomical definition of the region of interest

To determine vascularity on the three scans obtained from each of the fingers examined, we defined a region of interest (ROI), which was located between the fingernail and the bony surface of the distal phalanx between the nailfold proximally and the end of the fingertip distally (sagittal scan of the nailbed), which did not include the lateral digital arteries in the transverse plane (transverse scan of the nailbed just distal to the nailfold), and which was surrounded by the skin surface (transverse scan of the fingertip just distal to the end of the phalanx) (Fig. 1A–C). Vascularity was obtained by computer-aided ROI analysis using specialized software (Quanticon, version 3.08; EchoTech, Hallbergmoos, Germany). After positioning the ROIs on the three scans, the absolute number of colour signals detected was assumed to represent the vascularity within the region analysed (colour signals per ROI). Because the vasculature of the nailbed was situated mainly in the centre of the ROI, we did not express our measurements as relative values, to avoid falsification by different finger sizes.

Statistics

Separate analyses were carried out for the results obtained for the second and third fingers (Wilcoxon test), and for the results obtained for female and male control individuals (U-test). Each baseline examination was performed twice to determine intra-observer variability. Moreover, within a 3-month period all persons included in the study were examined for a second time by another person (MK) to determine inter-observer variability. For each of the groups (healthy controls vs patients with pRP or patients with sRP, and controls vs CTD patients with or without initial digital skin lesions), the mean vascularity was determined (± s.d.) at baseline, after cold challenge and after warm challenge (descriptive statistics). Data for control subjects obtained before and after a cold or a warm challenge were analysed by simple analysis of variance (ANOVA). In the same manner, two-factor ANOVA was used to analyse the data obtained for controls and CTD patients with or without initial digital skin lesions, and also the data obtained for controls and patients with pRP and sRP.
Assessment of vascularity by colour Doppler ultrasound

(before and after the challenges). Because of the clinically important aim of differentiating pRP from sRP in patients who do not present with typical symptoms of CTD, only data for patients without obvious digital skin lesions were analysed; therefore, five patients with concomitant digital skin lesions out of 22 patients with sRP were excluded from the statistical analysis.

To compare the ultrasound results with the clinical diagnoses (healthy controls, pRP and sRP patients; controls, CTD patients with and without initial digital skin lesions), we performed discriminant analysis using the predictors before and after both the cold and the warm challenge.

Results

Control subjects

At ambient temperature, all but one of the healthy controls had excellent peripheral vascularity. As expected, vascularity decreased after the cold challenge, whereas the warm challenge led to a further increase in peripheral vascularity (Figs 2 and 3). The mean baseline vascularity of the control subjects was 22 031 ± 10 682 colour signals per ROI; after the cold challenge the mean vascularity decreased to 11 055 ± 6096 colour signals per ROI (P < 0.001) (Fig. 2). In contrast, the warm challenge led to an increase in vascularity to 29 982 ± 10 518 colour signals per ROI (P < 0.001) (Fig. 3). In this preliminary comparison, we found no significant differences in mean vascularity between female and male control individuals, either at baseline or after a dynamic challenge.

Raynaud’s phenomenon

At ambient temperature, healthy controls and patients with pRP showed similar vascularities, which were clearly distinguishable from the lower vascularities determined in patients suffering from sRP associated with CTD (Fig. 2). The cold challenge induced decreases in all three groups (healthy controls and patients with pRP and sRP); however, this decrease in vascularity was more pronounced in pRP and sRP patients.

In patients with pRP, the mean baseline vascularity was 25 467 ± 3063 colour signals per ROI; after the cold challenge the mean vascularity decreased to 23 02 ± 2175 colour signals per ROI (P < 0.001) (Fig. 4), and after the warm challenge it increased to 30 090 ± 8448 colour signals per ROI (P < 0.01). Compared with healthy control subjects starting from a similar baseline vascularity, the decrease in vascularity induced by the cold challenge was significantly different (P < 0.001) (Fig. 2).

In patients with sRP, the mean baseline vascularity was 79 98 ± 6934 colour signals per ROI. After the cold challenge the mean vascularity decreased to 63 9 ± 489 colour signals per ROI (P < 0.001) (Fig. 5), and after the warm challenge it increased to 19 411 ± 7993 colour signals per ROI (P < 0.001). In comparison with healthy controls, even at ambient temperatures sRP patients...
had a lower vascularity \( (P < 0.001) \); this difference was even more pronounced after the cold challenge \( (P < 0.001) \) (Fig. 2).

On the basis of the clinical evaluation, CDU yielded a correct classification in 89.4% of the patients analysed (including healthy controls and patients with pRP or sRP) (Table 2).

**Connective tissue disorders with and without initial digital skin lesions**

At ambient temperature, all patients who suffered from CTD with initial disease-related digital skin lesions had an extremely low vascularity. Interestingly, the subgroup of CTD patients who had no obvious digital skin lesions also had reduced vascularity that was intermediate between those of healthy subjects and CTD patients with digital skin lesions (Fig. 3). After the warm challenge, vascularity increased in healthy controls but also in both patient subgroups; this increase was clearly more pronounced in healthy controls than in CTD patients.

Especially CTD patients with digital skin lesions had only a limited elevation of vascularity after the warm challenge. The mean baseline vascularity of CTD patients without digital skin lesions was \( 6260 \pm 7531 \) colour signals per ROI (Fig. 5); after the warm challenge it increased to \( 18510 \pm 8640 \) colour signals per ROI \( (P < 0.001) \), and after the cold challenge it decreased to \( 1517 \pm 2290 \) colour signals per ROI \( (P < 0.001) \). When comparing

---

**Table 2. Percentages of correctly classified (bold numbers) and missed diagnoses in the different study groups, as determined by CDU**

<table>
<thead>
<tr>
<th></th>
<th>Controls (CDU)</th>
<th>Primary RP (CDU)</th>
<th>Secondary RP (CDU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls ( (n = 15) )</td>
<td>93.3</td>
<td>0</td>
<td>6.7</td>
</tr>
<tr>
<td>Primary RP ( (n = 10) )</td>
<td>0</td>
<td>80.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Secondary RP ( (n = 17) )</td>
<td>0</td>
<td>9.1</td>
<td>90.9</td>
</tr>
</tbody>
</table>
this patient subgroup with the healthy controls, the vascularity at baseline and after the dynamic challenges both differed significantly ($P < 0.001$) (Fig. 3).

In CTD patients presenting with initial digital skin lesions, the mean baseline vascularity was $711 \pm 1311$ colour signals per ROI. After the warm challenge it increased to $10343 \pm 3623$ colour signals per ROI ($P < 0.001$) (Fig. 6), and after the cold challenge it decreased significantly to $446 \pm 401$ colour signals per ROI ($P < 0.01$). Upon comparison of patients with or without digital skin lesions, the vascularity at baseline and after the dynamic challenges both differed significantly ($P < 0.001$) (Fig. 3).

On the basis of the clinical evaluation, CDU yielded a correct classification in 78.0% of the cases analysed (including healthy controls and patients with or without initial digital skin lesions) (Table 3).

Table 3. Percentages of correctly classified (bold numbers) or missed diagnoses within the different study groups (controls, patients with connective tissue disease without and with initial digital skin lesions), as determined by CDU

<table>
<thead>
<tr>
<th></th>
<th>Controls (CDU)</th>
<th>No skin lesions (CDU)</th>
<th>With skin lesions (CDU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls ($n = 15$)</td>
<td>80.0</td>
<td>20.0</td>
<td>0</td>
</tr>
<tr>
<td>No skin lesions ($n = 20$)</td>
<td>10.0</td>
<td>60.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Skin lesions ($n = 15$)</td>
<td>0</td>
<td>0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Further analyses

Intra-observer variability was negligible ($r = 0.97$; baseline examinations only). Equally, inter-observer variability was low and yielded excellent coefficients of $r = 0.93$ (baseline), $r = 0.72$ (cold challenge) and $r = 0.82$ (warm challenge). Vascularities of the second and the third right fingers of the same hand did not differ significantly either between controls and patients or between baseline or dynamic conditions.

Discussion

Raynaud’s phenomenon

RP is an early symptom in the course of CTD and has considerable clinical effect. However, the clinically obvious diagnosis of RP is sometimes difficult to confirm by technical means and there are still no reliable diagnostic tests [2, 3]. There is a growing number of promising studies evaluating digital vascularity in RP patients by ultrasound [3, 6]. In a recent report, Naidu et al. [6] measured the decrease in digital artery diameter upon cold challenge by using high-frequency ultrasound with a predicted sensitivity of 96% and a specificity of 100%. However, the reproducibility of this technique was not evaluated [3, 6]. Reproducibility must be regarded as an important factor in the evaluation of peripheral digital vascular changes. Here, we show that our method is highly reproducible and yields a correct classification in about 90% of the individuals examined. The decrease in vascularity seen after a cold challenge allowed us to discriminate between healthy controls and most of the patients suffering from RP (Table 2). Nevertheless, it remains a major challenge to predict those patients who have RP with underlying CTD. Methods such as nailfold microscopy, laser Doppler flow monitoring, thermography and plethysmography have been used to detect and/or quantify disease-related digital microvascular damage [3]. Until now, none of these techniques has been generally accepted as the method of choice in this field, mainly because of their lack of availability, feasibility and reproducibility [3]. Nailfold microscopy is regarded as the most reliable method of detecting early microvascular changes in CTD patients. Even though nailfold microscopy is helpful in distinguishing pRP from sRP in systemic sclerosis [7] it hardly allows reliable quantification and is not well suited to follow-up. Thus, there is still a need for a sensitive and...
reproducible method for the quantititative evaluation of peripheral vascularity. In our study, the mean baseline vascularity of patients with pRP and healthy controls did not differ significantly (Fig 2). By contrast, the baseline vascularity of patients with sRP was significantly lower than that of healthy controls and/or patients with pRP. Thus, CDU holds promise as a method which can differentiate between pRP and sRP and provide information additional to that provided by nailfold microscopy on the quantitative microvascular changes in a reproducible manner. In future it will be interesting to compare CDU with nailfold microscopy and other methods. We see the role of the CDU technique described here as expanding the value of nailfold microscopy in diagnosis and in improving the follow-up of disease progression and the assessment of treatments.

Connective tissue disorders

Hyperplastic intimal changes in the peripheral digital arteries may result in substantially reduced vascularity in SSc and SLE, although the pathophysiological mechanism may be different [1, 8–10]. We obtained extremely low vascularity in CTD patients, a pathological finding that was even more pronounced in patients with initial digital skin lesions. It is important to note that CTD patients without any disease-related skin lesions also had a significantly lower vascularity than healthy controls. This suggests that structural changes in the vasculature occur at an early stage of the disease that might be easily quantified by ultrasound. Because most SSc patients had initial digital skin changes, we did not perform an additional subgroup analysis of SSc vs SLE patients in this preliminary study. In 22% of the patients, CDU analysis of nailfold vascularity was unable to predict the clinical classification of the study subgroups (controls, CTD patients with and without initial digital skin lesions) (Table 3). This may be expected, as the progression of (early) vascular damage to clinically evident digital skin lesions is a continuous process in CTD.

It has been reported that a warm stimulus can significantly increase peripheral vascularity in patients with SSc [3]. We also observed this phenomenon in all of our patients with CTD after a warm challenge. However, the inducible increase in vascularity was significantly lower in CTD patients than in healthy controls, suggesting a restricted vessel response after the dynamic challenge. This was probably a result of irreversible structural changes in the vasculature.

The assessment of disease status in SSc or SLE is important in prognosis and in guiding risk-adapted treatment. Several approaches, such as a modified health assessment questionnaire and an index of accumulated damage in SLE, have been presented [11, 12]. The method described here might be useful in quantifying the degree of digital vascular damage. It could be evaluated as a useful way of determining the progression of the underlying disease and could help in the assessment of responses to treatment over time in SSc and SLE.

Methodological aspects

Constant adjustment of the ultrasound parameters is essential. Pressure on digital vessels caused by the ultrasound transducer can be circumvented by ensuring that a thin layer of gel is maintained between the patient’s nail and the transducer. Results can be falsified by changes in ambient temperature, acclimatization time, the severity and duration of the dynamic challenges, and delay in image acquisition after the challenge. Even the patient’s sympathetic tone at the time of the test may remain poorly controllable in the assessment of digital vascularity. However, our standardized examination protocol resulted in a high level of reproducibility of the method, with low inter-observer variability. The ultrasound examination of two fingers [excluding the acclimatization time (20 min), the re-equilibration time (15 min) and the dynamic challenges (5 min each)] followed by the computer-aided determination of the vascularity takes about 15 min per subject and is easy to learn. High-resolution and CDU techniques are used increasingly. They are easy to perform and are no burden on the patient.

In conclusion, the novel CDU technique presented here appears to be able to detect and quantify early vascular damage in CTD patients, to unmask RP, and even to differentiate between pRP and sRP. In an ongoing study of patients suffering from CTD we are monitoring responses to treatment and the progression and/or regression of disease activity with the CDU method presented here. Further studies will be needed to assess the clinical value of CDU compared with the other diagnostic tools that are available.

References

7. Bukhari M, Herrick AL, Moore T, Manning J, Jayson...