Raynaud’s phenomenon: a mirror of autoimmune disease

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CHAPTER 2

Digital ischemia during cooling is independently related to nailfold capillaroscopic pattern in patients with Raynaud’s phenomenon

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ABSTRACT

Objectives: The aim of the study is to assess the association between plethysmographically measured vasospasms during stepwise cooling and recovery, as an index for digital ischemia, and nailfold capillaroscopic pattern (NCP) severity in patients with primary or secondary Raynaud’s phenomenon (RP) including systemic sclerosis (SSc).

Methods: In 381 consecutive patients with suspected RP without a history of digital ulcers, NCP (assessed by widefield videocapillaroscopy), cooling and recovery fingertip photo-electric plethysmography, and clinical characteristics were analyzed. NCP were graded as follows: normal, non-specific, early, and active. Mean ischemia time was defined as the mean time of perfusion loss during cooling and recovery of five fingers.

Results: In the patients with loss of perfusion during cooling and recovery the NCP was normal in 152, non-specific in 96, early in 61, and active in 39 patients. Mean ischemia time was positively associated with NCP severity, p<0.05 for each two or three grade increase and independent of underlying SSc. The difference was most pronounced during recovery.

Conclusions: We demonstrate that the degree of vasospasm and ischemia provoked by stepwise cooling and recovery are positively associated with NCP in patients with Raynaud’s phenomenon of different etiology and without a history of digital ulcers.
INTRODUCTION

Raynaud’s phenomenon (RP) is a common disorder characterized by cold induced vasospasms of the digital arteries. In a small proportion of patients it occurs secondary to an underlying disease, such as systemic sclerosis (SSc). Patients with secondary RP (SRP) are prone to develop digital ischemia and digital ulcers. In patients with advanced SSc fingertip perfusion at room temperature clearly correlates with microangiopathy severity, as assessed by nailfold capillaroscopic pattern (NCP). This may indicate severe vasospasms or structural narrowing of the digital arteries. Whether this association is restricted to advanced SSc or may already be present less severe cases has not been previously investigated.

We aim to describe the degree of vasospasm and ischemia provoked by stepwise cooling and recovery of 5 digits of consecutive patients with suspected RP who underwent cooling and recovery fingertip photo-electric plethysmography (PPG) at our centre and hypothesize that the severity of NCP is positively associated with cold induced ischemia.

PATIENTS AND METHODS

The study was approved by the local ethics committee (Medisch Ethische Toetsingscommissie Groningen, the Netherlands). Since the study does not fall under the Dutch law of medical research in humans (WMO), the local ethics committee has provided exemption from written informed consent. Consecutive patients with suspected RP (n=381), based on discoloration and/or discomfort of the hands provoked by cold, without a history of digital ulcers, in whom objective confirmation was deemed indicated by the treating physician, were included. Patients were assessed between November 2008 and August 2013 and were analysed according to standardised procedures as described below. In all patients NCP and PPG were performed. Also clinical characteristics were retrospectively collected. These consisted of laboratory and in case of (suspected) SRP functional studies, including pulmonary function tests and esophageal scintigraphy initially, and if deemed necessary by the treating physician followed by high resolution computed tomography (HRCT) and cardiac ultrasound.
**Nailfold capillaroscopic pattern (NCP)**

NCP was assessed by widefield videocapillaroscopy as described previously. In short, we used an Olympus BHMJ FW-32362 (Tokyo, Japan), with a Grundig FA-85 Z/W video camera (Fürth/Bay, Gemarny) and an Osram XBO 75W xenon lamp (Berlin, Germany). Immersion oil was applied to the skin to increase transparency. The 5x objective was used for the images with a total of 180x enlargement. NCP was examined after at least 15 minutes adaptation at a room temperature of 23°C. The middle and ring finger of both hands were studied. NCP was performed by one of five vascular technicians, supervised by a medical specialist and in case of uncertainty a consensus based conclusion was made. The pattern was classified based on the patterns defined by Cutolo et al. The pattern was deemed “normal” if the mean number of dilated capillaries per finger was ≤3 and no giant capillaries were observed (n=172). The non-specific pattern was defined as a mean number of >3 dilated capillaries in the absence of giant capillaries (n=105). An “early” pattern was defined as at least one giant capillary per finger, but without loss of capillaries or hemorrhages (n=64). Giant capillaries were visually judged by the observer as typical scleroderma like homogeneously enlarged loops. An “active” pattern was defined as ≥1 giant capillaries in total combined with loss of capillaries (less than a mean of 20 capillaries per 3mm nailfold per finger) and/or hemorrhages (n=40). None of the patients had a ”late” pattern (none or few giant capillaries, neovascularisation). Since the setup used in this study could not measure capillary dimensions, the definition of giant capillaries, because of the typical appearance, was less prone to mistake than judging dilation only.

**Cooling and recovery fingertip photo-electric plethysmography (PPG)**

After microscopy, cooling and recovery fingertip PPG was performed as described previously. Photoplethysmography is an optical technique that typically uses infrared light for the transcutaneous registration of beat-to-beat blood volume changes in the microvascular bed of the skin. It provides information on the peripheral circulation at the site where the PPG cuff is placed, the fingertips. Obstructions anywhere in the vasculature upstream of the cuff potentially affect the signal. This is why it is presumed that also provides information about the macrovasculature and not only the microvasculature. In cooling plethysmography assessments, a prerequisite is that the plethysmogram in all fingers (or toes) is normal after prolonged (up to 30 minutes) warming-up of the hands (or feet) at 33°C. If a normal plethysmogram is not present...
in these conditions, a cooling is not performed. This is to ensure that a fixed vascular obstruction is not present. Data acquisition was performed using a Biopac MP-100 system (Biopac Systems Inc., Goleta (CA), USA) with five PPG100C amplifiers and PPG200C sensors, an ECG100C amplifier with ECG-cables, an SKT100C amplifier and TSD202C temperature sensor, and AcqKnowledge 3.8.2 software. All signals (ECG, temperature and five fingertip PPG’s) were sampled at 200 Hz and stored in a file for off-line processing with dedicated software to determine pulsations in each finger.

One hand was submerged in water to the level of the radiocarpal joint. The water temperature was lowered in steps of 3°C from 33°C until 6°C, or until the point at which the cold was not tolerated anymore. During each step there was a stabilization period of four minutes for each temperature, the whole cooling procedure taking 45 minutes if finished until 6°C. At the end of stabilization 15 seconds of signals were analyzed to calculate perfusion of all individual fingers. After all cooling steps, the hand was taken out of the water, smoothly dried with a towel and then rested on a dry towel to track perfusion recovery in open air for 10 minutes. The perfusion was recorded during the last 15 seconds of every minute during recovery. The cooling and recovery was positive for RP when two or more fingers lost perfusion during the same step of cooling, or one or more fingers lost perfusion and stayed abnormal during two or more subsequent steps, or when the perfusion did not restore within the ten minutes of recovery period. In the 15 s recordings, PPG pulses are analyzed from R-peak (detected in the ECG) up to 600 ms. Mean and SD of the amplitude of the pulses is determined and a signal-to-noise ratio (S/N) is calculated as mean amplitude divided by the SD of the amplitude. Perfusion is defined as S/N>15. Automatic detection of perfusion is visually checked by a vascular technician. This is necessary because in the range 10<S/N<15, the signal can be related (perfusion) or unrelated (no perfusion) to the heart beat.

If the patient did not tolerate the pain any longer, abnormal perfusion was assumed one step of 3°C after the lowest measured temperature. We calculated the number of fingers with normal perfusion for each time point (figure 2A). For all fingers together, we calculated the area under this function of time (AUC) using the trapezoid approximation of the area. The AUC was calculated during the period of cooling and during the period of recovery separately. Additionally, the “time point of loss of perfusion” of the separate fingers was assessed, as well as the “time point of recovery” of perfusion during
rewarming. The time in minutes between loss of perfusion and recovery of one finger is referred to as the “ischemia time”: the total time of abnormal perfusion (ischemia) in that finger. If recurrence of perfusion did not occur within the recovery of ten minutes, ten minutes were counted for the time point of recovery. The “mean ischemia time” was calculated as the mean time of the ischemia time of all five fingers.

**Clinical and laboratory characteristics**

Patients were classified primary RP (PRP, n=111) if NCP was normal and autoantibodies were negative (antinuclear antibodies with a titre <1:80, performed by indirect immunofluorescence (n=318), and extractable nuclear antigens <10U/ml, performed by fluorescent-enzyme immuno-assay (n=263)). If autoantibodies were not tested they were presumed normal. Remaining patients were labelled SRP consisting of no classifiable underlying diseases (n=107), classifiable SSc patients divided into early SSc based on the LeRoy criteria for early SSc\(^{11}\) (n=95) or definite SSc (all limited cutaneous) based on the 2013 ACR/EULAR classification criteria\(^{12}\) (n=10), and classifiable other underlying diseases (n=25) i.e. mixed connective tissue disease (n=8), systemic lupus erythematosus (n=9), and Sjögren’s disease (n=8). Please refer to figure 1 for a schematic representation. Patients with non-specific NCP were classified as SRP with no classifiable underlying disease. This is because these changes may be seen in patients with several other diseases associated with Raynaud’s phenomenon.\(^{13}\) Also this is in line with the study of Bernero et al., this demonstrated that patients with “not-specific capillary alterations” progressed to a scleroderma pattern in 18% of the cases during 5 year follow-up.\(^{14}\) Potential systemic involvement was adjudicated in most patients with SRP. Possible lung involvement was defined as diffusion capacity of the lung for carbon monoxide (uncorrected for alveolar volume) (DLCO, n=113) or forced vital capacity (FVC, n=117) <80% assessed with a pulmonary function test. If necessary, when DLCO or FVC was abnormal or judged by the physician, HRCT (n=26) and/or cardiac ultrasound (n=86) were performed as additional tests. Definite pulmonary involvement was defined as an interstitial lung disease (ILD) pattern on HRCT or documented pulmonary hypertension by mean pulmonary artery pressure ≥25mmHg at rest on right heart catheterisation. Oesophageal involvement was assessed by oesophageal scintigraphy with Tc-99M colloid (n=77). Skin involvement was defined as puffy fingers, calcinosis, telangiectasia, distal- or proximal sclerodactyly as described by the treating physician.
Figure 1. Flow chart of classification of patients with suspected Raynaud’s phenomenon. Patients with other classifiable underlying disease like mixed connective tissue disease, SLE or Sjögren’s disease, have enough manifestations of the disease to be classified as such, judged by treating physician. LeRoy criteria for early SSc: objectively diagnosed RP with SSc type NCP or SSc specific autoantibodies. Patients with definite SSc have 9 points or more in the classification system of the 2013 ACR/EULAR criteria. Patients without classifiable underlying disease do not fulfil any of the criteria above but do have a non-specific NCP or positive autoantibodies.

Statistics
Statistical analysis was carried out using IBM SPSS Statistics version 22. The normal distribution was tested with a Q-Q plot. ANOVA was used for trend between NCP categories. The independent t-test and the Mann Whitney U test were used if applicable to compare unpaired groups of variables. Binary logistic regression was used for binary dependent variables, ordinal regression was used for ordinal dependent variables, using a stepwise forward approach including only variables in the analysis with a crude p<0.100. Data are described as mean ± standard deviation (SD) if there is a normal distribution or as median with the inter quartile range (IQR). P-values<0.05 were considered statistically significant.
RESULTS

Patient characteristics
Patients characteristics are outlined in table 1, listed by diagnosis (n=381). These still include 33 patients without digital ischemia on the cooling and recovery PPG (9%). These patients visited our centre for arthralgia (n=6), suspected systemic disease (n=6), suspected RP (n=8), or another reason though with complaints resembling RP (n=13). This group was excluded in the following statistical analyses because no ischemia was present during the cooling and recovery PPG. For puffy finger (p=0.025), sclerodactyly (p=0.001), telangiectasia (p=0.029), SSc specific autoantibodies (p=0.018) and autoantibodies (p=0.004) a positive association with the NCP was found for trend. After ten minutes recovery 150 patients still had no reperfusion, included 54 (36%) with normal, 43 (45%) with non-specific, 29 (48%) with early and 24 (62%) with active NCP.

Ischemia and reperfusion time and NCP
The mean number of fingers with normal perfusion for each time point in each NCP group is illustrated in figure 2A. The percentage of patients with early perfusion loss, and with later (or lack of) recovery increased with the severity of NCP, indicating a more severe vasospastic reaction. Mean ischemia time was positively associated with NCP severity (please refer to figure 2). For the cooling and recovery periods alone, the AUC also decreased with NCP severity (please refer to figure 2A and, for detailed data, to the supplementary table S1). When analysing NCP severity as dependent variable the mean ischemia time (p<0.001), age (p<0.001), sclerodactyly (p=0.001), and puffy fingers (p=0.024) were independent predictors, where gender, antinuclear antibodies titre ≥1:80, SSc specific autoantibodies and telangiectasia were not significant.

Ischemia and reperfusion time and patient diagnosis
Mean ischemia time was significantly greater in all different patient subgroups with SRP (20.0±8.9 min) compared with PRP (17.7±8.8 min; p=0.026). The AUC during the period of recovery alone was significantly lower (p=0.011), while a trend was observed in AUC during cooling (p=0.052). In the patients classified as definite SSc, mean ischemia time (31.1±4.5 min) was significantly greater than in the other SRP patient subgroups without definite SSc (19.5±8.7 min; p<0.001). The AUC during the periods of cooling and recovery alone was significantly lower in the patients with definite SSc (p<0.001 and
Table 1. Characteristics of patients (n=381) listed by diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Primary RP</th>
<th>No classifiable underlying disease</th>
<th>Early SSc (LeRoy 2001)</th>
<th>Definite SSc (ACR/EULAR 2013)</th>
<th>Other classifiable disease</th>
<th>No ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>111</td>
<td>107</td>
<td>95</td>
<td>10</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>Age in years</td>
<td>44 ±18</td>
<td>38 ±18</td>
<td>39 ±15</td>
<td>60 ±15</td>
<td>38 ±15</td>
<td>36 ±15</td>
</tr>
<tr>
<td>Female gender</td>
<td>65 (59)</td>
<td>67 (63)</td>
<td>70 (74)</td>
<td>8 (80)</td>
<td>24 (96)</td>
<td>26 (79)</td>
</tr>
<tr>
<td>Weight (n=309) in kg</td>
<td>73 ±16</td>
<td>73 ±19</td>
<td>66 ±14</td>
<td>67 ±13</td>
<td>71 ±20</td>
<td>74 ±16</td>
</tr>
<tr>
<td>Normal</td>
<td>111 (100)</td>
<td>26 (24)</td>
<td>2 (2)</td>
<td>0 (0)</td>
<td>13 (52)</td>
<td>20 (61)</td>
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<tr>
<td>Non-specific</td>
<td>0 (0)</td>
<td>81 (76)</td>
<td>2 (2)</td>
<td>1 (10)</td>
<td>12 (48)</td>
<td>9 (27)</td>
</tr>
<tr>
<td>Early</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>57 (60)</td>
<td>4 (40)</td>
<td>0 (0)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Active</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>34 (36)</td>
<td>5 (50)</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Skin thickening</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (4)</td>
<td>10 (100)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- Puffy fingers</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>4 (4)</td>
<td>1 (10)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>- Sclerodactyl</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (90)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>Fingertip lesions</td>
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<td>0 (0)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Telangiectasia</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>5 (50)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ILD pattern on HRCT</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (20)</td>
<td>2 (8)</td>
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<tr>
<td>Pulmonary hypertension</td>
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<td>0 (0)</td>
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<tr>
<td>SSc specific autoantibodies</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>5 (5)</td>
<td>6 (60)</td>
<td>0 (0)</td>
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<tr>
<td>Positive autoantibodies</td>
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<td>42 (39)</td>
<td>38 (40)</td>
<td>9 (90)</td>
<td>22 (88)</td>
<td>6 (18)</td>
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<td>Esophagael involvement</td>
<td>2 (2)</td>
<td>4 (4)</td>
<td>6 (6)</td>
<td>7 (70)</td>
<td>5 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DLCO&lt;80% (n=118)</td>
<td>7 (6)</td>
<td>9 (8)</td>
<td>23 (24)</td>
<td>7 (70)</td>
<td>9 (36)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>FVC&lt;80% (n=122)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (20)</td>
<td>3 (12)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>FVC&lt;80% (n=122)</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (20)</td>
<td>3 (12)</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

Data shown as mean ± standard deviation or as number (% of diagnosis group)

n=the number of patients in whom the variable was documented or tested
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Figure 2 A. Mean number of digits with normal perfusion during the photo-electric plethysmography during cooling and recovery. Area under the curve (AUC) during cooling and during recovery decreased with severity of the nailfold capillaroscopic pattern (NCP) (p=0.001 and p=0.005 for trend). AUC during cooling was significantly different between normal and early (p=0.001), normal and active (p=0.011), non-specific and early (p=0.034). AUC during recovery was significantly different between normal and non-specific (p=0.008), normal and early (p=0.013), normal and active (p=0.001). B. Box plot for ischemia time of separate NCP subgroups. Mean ischemia time is positively associated with NCP severity (p<0.001 for trend).
p=0.002 respectively) compared with other SRP patient subgroups. For detailed data of mean ischemia time and AUC of different patient diagnosis subgroups please refer to the supplementary table S1.

**Separate analysis of middle and ring finger**

When analysing the middle and ring finger from the hand that was cooled, mean ischemia time was positively associated with NCP severity of that separate finger as well as with the other fingers on the ipsi- and contralateral hand (p<0.005 for trends; please refer to supplementary table S2). Furthermore, the NCP of the different fingers (middle and ring finger of the cooled hand, but also the middle and ring finger of the non-cooled hand) were strongly interrelated (correlation coefficient for all above 0.6). In the index finger, the most severe vasospasms were observed (supplementary figure S1).

Mean ischemia time was positively associated with NCP severity of that separate finger. Since the NCP of the individual fingers were strongly interrelated, this association also remained present when correlating the mean ischemia time of one finger with NCP of another finger.

**DISCUSSION**

The current study demonstrates that the degree of vasospasm and ischemia provoked by stepwise cooling and recovery appears to be positively associated with the NCP observed with widefield videocapillaroscopy in unselected patients with RP of different etiology and without a history of digital ulcers. To the best of our knowledge, this study is the first to assess the relation between microvascular abnormalities and vasospasm of digital arteries and arterioles in patients with a wide spectrum of RP ranging from PRP with a normal NCP, to SSc with extensive NCP abnormalities. Although our study included only a small number of classifiable SSc patients (n=10) because of the unselected approach, it was apparent that the degree of vasospasm was most severe in patients with definite SSc. However, the association of NCP with the degree of vasospasm remained significant after correction for the presence of organ involvement, stressing the importance of microvascular damage even before the diagnosis of SSc is made.
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Our findings extend those of previous studies in patients with severe SSc. It has been reported that the severity of NCP is associated with the severity of digital ischemia\textsuperscript{15} and that certain characteristics of NCP predict the development of digital ulcers.\textsuperscript{16} Also fingertip blood perfusion was shown to be negatively related to NCP.\textsuperscript{2} In that specific study, measurements were performed at room temperature, and no cooling was performed, which may indicate some degree of structural, but not temperature dependent, vasospasm abnormalities. Previous MRI studies of hands of patients with SSc have shown that the superficial palmar arch and more distally the common proper palmar digital arteries and proper palmar digital arteries, that supply the superficial palmer arch, are affected by structural narrowing or even occlusion.\textsuperscript{17-19} More proximally, occlusion of the ulnar artery is also frequently observed.\textsuperscript{18,20} This reinforces the idea that not only microvascular but also macrovascular abnormalities exist and determine the severity of digital ischemia in SSc.

In our patient group 196 of the 348 patients (56%) with ischemia during cooling had at least a mean of three or more widened capillaries per nailfold in the NCP. Although our hospital is a tertiary referral centre, it seems unlikely that all of these patients suffer from a underlying connective tissue disease. Bernero et. al. demonstrated that 18% of the patients with non-specific capillary alternations progressed to a scleroderma pattern in the next five years [14]. Since we did not perform a formal follow-up of the PRP patients, it cannot be ruled out that a small percentage may have developed features of SSc later on. In PRP patients, subtle changes have been found in the NCP in comparison to a healthy control group by Bukhari et. al.\textsuperscript{21} In that study, loop dimensions were measured using 600x magnification. They observed a gradual increase in arterial, venous, and total loop as well as capillary width, but not in capillary density. This may suggest that the abnormal NCP is not strictly a result of an underlying disease but may also be influenced by repeated episodes of digital ischemia.

Our observation of a differential involvement of separate fingers, with the index finger showing the most severe vasospasms, with especially prolonged recovery, is in line with previous reports of DU in SSc patients. Observational studies have shown that digit 2 and 3 are most frequently affected by DU.\textsuperscript{1,22} Unfortunately, we only assessed NCP in digit 3 and 4 as it is custom at our hospital, and could therefore not assess whether NCP was
most advanced in digit 2. However, as all fingers NCP findings were highly correlated with NCP in all other fingers, we do not expect this relation to be present.

Additionally, it was previously reported that the thumb is frequently spared in RP using thermography technique. This could not be confirmed with our PPG data. This might indicate that thumb sparing may be a result of a difference in dermal perfusion and not due to arteriolar perfusion as measured with PPG.

Digital ischemia was objectively documented by cooling and recovery PPG, this procedure is more precise than the more common procedure where PPG measurements are done before and after a moment of cooling at one fixed low temperature. This allowed us to precisely assess the degree of ischemia by calculating the “ischemia time” and the AUC of the cooling procedure. As digital ischemia was more severe in patients classified as SSc, it appears to be an expression of severity of the underlying disease. A limitation of this technique is sometimes poor patient acceptability. Several of the patients did not complete the cooling because of pain. We registered this as an abnormal PPG one step after stopping the measurement, although objective documentation of ischemia development lacks. A limitation of our retrospective study is that no standardized scoring protocol for physical and additional examinations was used, for example Rodnan skin score. No selection bias existed because all patients were included that had microscopy to assess the NCP and also had a cooling and recovery PPG. The microscopy was judged by five different observers and our wide field capillaroscopy did not allow measurements of capillary dimensions. However, capillaries were scored according to the same protocol. Although proximal vaso-occlusive disease was not excluded using duplex or magnetic resonance imaging, all patients (except one) had normal perfusion after warming up at 33°C at the start of the subsequent cooling PPG.

In conclusion our results demonstrate indirect evidence for the association between degree of digital ischemia during cooling and microvascular abnormalities in patients with PRP and SRP, already in those patients with early stages of connective tissue disease. This may implicate a role of digital artery vasospasms and recurrent ischemia early in the development of nailfold microangiopathy. To investigate whether a causal relation exists between the severity of RP and occurrence of nailfold abnormalities, a prospective study with sequential NCP measurement is warranted.
REFERENCES


SUPPLEMENTAL DATA

**Table S1.** Mean ischemic time of five fingers and the area under the curve of mean number of digits with normal perfusion during cooling and recovery

<table>
<thead>
<tr>
<th>Nailfold capillaroscopic pattern</th>
<th>Mean ischemic time of five fingers (min)</th>
<th>Area under the curve during cooling (min)</th>
<th>Area under the curve during recovery (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=152)</td>
<td>17.4&lt;sup&gt;a,b&lt;/sup&gt; ±8.9</td>
<td>104&lt;sup&gt;a,b&lt;/sup&gt; (81-130)</td>
<td>34&lt;sup&gt;a,b,c&lt;/sup&gt; (15-45)</td>
</tr>
<tr>
<td>Non-specific (n=96)</td>
<td>19.2&lt;sup&gt;a&lt;/sup&gt; ±8.0</td>
<td>98&lt;sup&gt;b&lt;/sup&gt; (72-124)</td>
<td>27&lt;sup&gt;d&lt;/sup&gt; (9-39)</td>
</tr>
<tr>
<td>Early (n=61)</td>
<td>21.7&lt;sup&gt;d&lt;/sup&gt; ±8.7</td>
<td>82&lt;sup&gt;d&lt;/sup&gt; (62-110)</td>
<td>28&lt;sup&gt;d&lt;/sup&gt; (6-38)</td>
</tr>
<tr>
<td>Active (n=39)</td>
<td>22.8&lt;sup&gt;c,d&lt;/sup&gt; ±10.0</td>
<td>90&lt;sup&gt;d&lt;/sup&gt; (46-114)</td>
<td>20&lt;sup&gt;d&lt;/sup&gt; (0-37)</td>
</tr>
</tbody>
</table>

**Diagnosis**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mean ischemic time (min)</th>
<th>Area under the curve during cooling (min)</th>
<th>Area under the curve during recovery (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary RP (n=111)</td>
<td>17.7 ±8.8</td>
<td>102 (82-130)</td>
<td>33 (15-45)</td>
</tr>
<tr>
<td>No classifiable underlying disease (n=107)</td>
<td>18.0 ±8.5</td>
<td>102 (74-130)</td>
<td>32 (10-42)</td>
</tr>
<tr>
<td>Early SSc (n=95)</td>
<td>21.3 ±8.9</td>
<td>90 (62-38)</td>
<td>27 (6-38)</td>
</tr>
<tr>
<td>Definite SSc (n=10)</td>
<td>31.1 ±4.5</td>
<td>63 (38-70)</td>
<td>6 (0-9)</td>
</tr>
<tr>
<td>Other classifiable disease (n=25)</td>
<td>18.8 ±8.0</td>
<td>106 (78-122)</td>
<td>27 (19-38)</td>
</tr>
</tbody>
</table>

Data shown as mean (± standard deviation) or median (IQR)  
Significantly different (p<0.05) from NCP 'active, 'early, 'non-specific and 'normal  
RP: Raynaud’s phenomenon, SSc: systemic sclerosis

**Table S2.** Mean ischemic time per nailfold capillaroscopic group per finger

<table>
<thead>
<tr>
<th>Nailfold capillaroscopic pattern</th>
<th>Middle finger</th>
<th>Ring finger</th>
</tr>
</thead>
<tbody>
<tr>
<td>number</td>
<td>Mean ischemic time (min)</td>
<td>number</td>
</tr>
<tr>
<td>Normal</td>
<td>124 16.8 ±10.2</td>
<td>114 13.6 ±11.1</td>
</tr>
<tr>
<td>Non-specific</td>
<td>114 18.2 ±9.8</td>
<td>122 15.9 ±11.0</td>
</tr>
<tr>
<td>Early</td>
<td>17 22.5 ±11.9</td>
<td>17 19.3 ±11.4</td>
</tr>
<tr>
<td>Active</td>
<td>30 22.5 ±9.2</td>
<td>28 20.3 ±10.8</td>
</tr>
</tbody>
</table>

Data shown as number or as mean (± standard deviation)
Supplemental Figure S1. Perfusion loss and recovery period for every nailfold capillaroscopic pattern. Percentage of patients with A. perfusion loss during cooling period and B. restoration of perfusion during recovery period for every finger (digit 1 to 5) and for the whole hand (loss of perfusion is then defined as more than one finger with abnormal perfusion as measured by the PPG).
Digital ischemia and nailfold capillaroscopy