CHAPTER 5

Nailfold capillaroscopy abnormalities are associated with BMI in subjects with Raynaud’s phenomenon

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Submitted
ABSTRACT

Objectives: To investigate the occurrence of nailfold capillary microscopy (NCM) abnormalities in RP patients in different BMI categories.

Method: NCMs were retrospectively assessed in 351 RP patients, without an underlying systemic disease. Patients were divided by BMI (kg/m²) category (BMI<18.5, BMI 18.5-25, BMI ≥25). NCM was quantitatively assessed. A standardized cooling experiment was performed in 152 patients.

Results: Lowest count of capillaries (BMI<18.5: 20.9 (± 3.2), BMI 18.5-25: 22.2 (± 2.7), BMI ≥25: 23.2 (± 2.0), p<0.001), highest counts of dilated capillaries (BMI<18.5: 5.1 (± 4.0), BMI 18.5-25: 4.2 (±3.7), BMI ≥25: 2.6 (±3.1), p=0.001), and giant capillaries (BMI<18.5: 1.0 (± 2.2), BMI 18.5-25: 0.5 (±1.2), BMI ≥25: 0.2 (±1.1), p=0.001) were found in underweight RP patients. Patients with a BMI ≥25 kg/m² showed a shorter mean recovery time as compared to the other groups (BMI< 18.5: 6.01 min (± 3.4), BMI 18.5-25: 5.81 min (± 3.6), BMI ≥25: 4.23 min (±3.3), p=0.029).

Conclusion: These findings indicate that microvascular abnormalities are associated with BMI and occur independently of a connective tissue disease. These preliminary results might suggest a protective role of (perivascular) fat in Raynaud’s phenomenon and warrants future studies.
INTRODUCTION

Raynaud’s phenomenon (RP) is a frequently occurring disorder, characterized by an episodic discoloration of the extremities in response to cold exposure or emotional stress. RP can be either primary (idiopathic), which often occurs in young females, or secondary to many diseases and conditions.\textsuperscript{1-3} Although the exact pathogenesis is incompletely understood, body mass index (BMI) is thought to play an important role in the pathogenesis of RP.

A recently conducted large cohort study in a Danish population (n=75008) showed that the risk of RP increases by each decrease of a BMI unit.\textsuperscript{4} Microvascular changes, assessed with nailfold capillary microscopy (NCM), were also found to co-occur with certain eating disorders (e.g., anorexia nervosa), in the absence of an underlying auto-immune disease.\textsuperscript{5} Mueller et al. have demonstrated that the presence of an abnormal NCM is associated with poor survival in RP patients without previously known connective tissue disease (CTD).\textsuperscript{6} The presence of microvascular changes are also associated with the severity of RP, as documented with cooling plethysmography, indicating that mechanisms (other than underlying CTDs) may play an important role.\textsuperscript{7}

The current study aims to investigate the occurrence of NCM abnormalities in RP patients with a low BMI, normal BMI, and high BMI. In addition, we will also investigate the difference in digital perfusion during a standardized cooling and recovery experiment in relation to BMI.

MATERIALS AND METHODS

Participants
NCM results and clinical characteristics were retrospectively assessed in patients with RP who presented between November 2008 to August 2013. Laboratory tests (e.g., serological tests) and, in the case of suspected secondary RP, functional test (e.g., pulmonary function tests, oesophageal scintigraphy, high-resolution CT and cardiac ultrasound) were also obtained. The diagnosis of RP was confirmed by the treating physician, and patients were included when no clinical (i.e., negative ANA and no signs
of organ involvement) evidence of an underlying connective tissue disease was found after at least 4 years follow-up. This study was approved by the local medical ethics committee (METc 2015/175, Groningen, The Netherlands). Given that the study does not fall under the Dutch law of medical research in humans, the local ethics committee has provided exemption from written informed consent.

**Clinical and laboratory assessments**

Patient characteristics (including sex, age, body weight and height) were collected. Patients were divided by BMI category: underweight (BMI<18.5 kg/m²), healthy weight (BMI 18.5-25 kg/m²), and overweight (BMI ≥25 kg/m²). NCM of the middle and ring finger (of both hands) was assessed in all patients as described previously. All images of nailfolds were taken at 180x magnification. The number of capillaries (per 3mm) was counted for each finger, and the mean number of capillaries per finger per subject was derived. As the set-up used in this study could not measure capillary dimension, dilated and giant capillaries were only judged visually by an experienced observer. Patterns were deemed abnormal if the mean capillary count was <18, or mean number of dilated capillaries per finger was >3, or any giant capillaries were observed.

A standardized cooling and recovery experiment was performed in 152 patients (n=22 for BMI<18.5 kg/m²; n=87 for 18.5-25 kg/m²; n= 43 for ≥25 kg/m²) as described previously. In short, the left hand of the subject, with photo-electric plethysmography (PPG) sensors attached to all five fingers, was submerged in water to the level of the radiocarpal joint. The cooling procedure started with water at 33°C and every 4 minutes the water was cooled by 3°C. After the cooling steps, the hand was taken out of the water, and recovery of perfusion was tracked for 10 min. Perfusion was recorded during the last 15 seconds of every 4 minutes during cooling, and during the last 15 seconds of every minute during recovery. Mean ischemic time was defined as the mean time of perfusion loss during cooling and recovery, whereas mean recovery time was defined as the mean time of perfusion loss during recovery.

**Statistical analysis**

Statistics were performed with The Statistical Product and Service Solutions (SPSS; version 22, Released 2013, IBM Corp., Armonk, NY). Non-parametric tests (X², Mann-Whitney U or Kruskal Wallis) were used in case of non-normally distributed data
to determine group differences. Pairwise comparisons were performed, adjusted by the Bonferroni correction for multiple testing. Correlations between variables were determined by either Pearson or Spearman correlation coefficients. A p-value below 0.05 was considered significant.

RESULTS

A total of 351 patients with RP were enrolled in the present study. None of the patients showed signs of an underlying connective tissue disease. The median age was 40.6 (IQR 24.6-52.9) with a male/female ratio of 111/240. Forty-five patients were underweight (BMI <18.5 kg/m²), 219 had a healthy weight (BMI 18.5-25 kg/m²), and 87 patients were overweight (BMI ≥25 kg/m²) (Table 1).

Table 1. Patients characteristics displayed per body mass index (BMI) (kg/m²) category

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>N</th>
<th>Age (median, IQR)</th>
<th>Female gender, n(%)</th>
<th>Smoking (previous and current), n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;18.5 kg/m²</td>
<td>45</td>
<td>22.5 (17.5-44.9)</td>
<td>34 (75.6%)</td>
<td>13 (28.9%)</td>
</tr>
<tr>
<td>BMI 18.5-25 kg/m²</td>
<td>219</td>
<td>38.1 (24.0-52.2)</td>
<td>160 (71.7%)</td>
<td>79 (35.4%)</td>
</tr>
<tr>
<td>BMI ≥25 kg/m²</td>
<td>87</td>
<td>49.7 (39.6-58.9)</td>
<td>49 (55.7%)</td>
<td>17 (19.3%)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>&lt;0.001</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Relations BMI and NCM

The observed NCM abnormalities are displayed per group in Figure 1. The mean ±SD number of capillaries for all patients was 22.3 ±2.7 per finger and patients with a BMI<18.5 kg/m² showed significantly lower counted capillaries (20.9 ±3.2) compared to the other groups (BMI 18.5-25 kg/m²: 22.2 ±2.7, BMI ≥25 kg/m²:23.2 ±2.0, p<0.001). Underweight RP patients also had the highest mean count of dilated capillaries (BMI<18.5 kg/m²: 5.1 ±4.0, BMI 18.5-25 kg/m²: 4.2 ±3.7, BMI ≥25 kg/m²: 2.6 ±3.1, p=0.001), and giant capillaries (BMI<18.5 kg/m²: 1.0 ±2.2, BMI 18.5-25 kg/m²: 0.5 ±1.2, BMI ≥25 kg/m²: 0.2 ±1.1, p=0.001). Post-hoc analysis of the pairwise comparisons is show in figure 2.
Relation BMI and digital ischemia

The mean number of digits with normal perfusion for each BMI group, assessed with PPG during cooling and recovery experiment, are depicted in Figure 2. The mean ischemic time did not significantly differ between groups (17.82 ±9.59 min for BMI < 18.5 kg/m²; 17.93 ±8.74 min for BMI 18.5-25 kg/m²; 18.64 ±8.64 min for BMI ≥25 kg/m², p=0.93). However, there was a significant difference between groups in mean recovery time (6.0 ±3.4 min for BMI < 18.5 kg/m²; 5.8 ±3.6 min for BMI 18.5-25 kg/m²; 4.2 ±3.3 min for BMI ≥25 kg/m², p=0.03). Post-hoc analysis only revealed a significant difference in mean recovery time between BMI ≥25 kg/m² and BMI 18-25 kg/m² (p=0.04).

Figure 1. Percentage of patients with abnormalities on nailfold capillary microscopy, depicted for each BMI category. P-value represents the post-hoc analysis, adjusted by the Bonferroni correction for multiple testing. Abbreviations: NCM= nailfold capillary microscopy; BMI= body mass index.
DISCUSSION

To the best of our knowledge, this is the first study to investigate potential mechanisms of Raynaud’s phenomenon in relation to BMI. The main finding of the current study was that underweight RP patients more frequently showed NCM abnormalities. In addition, patients with a higher BMI showed a significantly shorter mean recovery time as compared to the other groups. Our findings indicate that the occurrence of microvascular abnormalities in underweight RP patients can occur independently of the presence of a connective tissue disease, and have to be attributed to other mechanisms.

Despite the extensive research on this subject, the exact pathogenesis of RP still remains incompletely understood. The current understanding of this rather complex phenomena is that the imbalance between vasoconstriction and vasodilatation is purely functional and that structural vascular changes do not occur in PRP patients. In our current cohort, patients in the BMI category <18.5 kg/m² not only had a lower capillary count, but more frequently showed dilated capillaries and giant capillaries. In addition, BMI
was found to be negatively associated with mean capillary count and the occurrence of dilated and giant capillaries. Bukhari et al. have previously compared NCM of PRP patients, SSc patients, and healthy controls and found capillary dimensions to be increased and capillary density to be decreased, even in PRP patients.\textsuperscript{13} Therefore, we postulate that vascular abnormalities can indeed occur in underweight RP patients without an underlying connective tissue disease, and, thereby, low body weight may be considered as an additional risk factor of RP.\textsuperscript{1,14} Further supporting our postulation, Klein-Weigel et al. have reported that dilated efferent capillary loops and venoles were more frequently present in symptomatic anorexia nervosa patients compared with non-symptomatic patients.\textsuperscript{5} Another study reported that anorexia nervosa is indeed associated with peripheral vascular abnormalities (e.g., dysregulation of peripheral vasoconstriction/vasodilation, acrocyanosis, arterial vasospasm).\textsuperscript{15} Palova et al. have demonstrated that not only flow-mediated vasodilatation is decreased in patients with anorexia, but also that this abnormality improves following renourishing.\textsuperscript{16} Therefore, a compensatory heat conserving, selective peripheral vasoconstriction is a plausible and perhaps reversible cause of RP in these underweight patients. In a recently conducted study from our center, it was found that the degree of vasospasm and ischemia, was positively associated with the NCM.\textsuperscript{7} Therefore, our observed NCM abnormalities may also be explained as a compensation for the reoccurring vasospasms, and perhaps the occurrence of prolonged attacks, due to the higher sensitivity to cold temperatures.

Interestingly, underweight RP patients in our study were substantially younger compared to the other two groups. Although not knowing the exact cause of the low BMI in our cohort, we do know that eating disorders affect between 1-10% of adolescent and college-age women.\textsuperscript{17} Therefore, we have to take into account the possibility that presence of underlying eating disorders may also have influenced our results.

Despite the fact that the association between RP and BMI has been previously reported,\textsuperscript{18} the underlying mechanisms are less frequently discussed. Our findings indicate that patients with a higher BMI have a significantly shorter mean recovery time compared to underweight patients and patients with a healthy BMI. This finding might suggest a protective role of fatty tissue against vasospastic events. In support, it was previously reported that flow-mediated dilation improved after long-term oestrogen replacement therapy in postmenopausal women with secondary RP.\textsuperscript{19} As oestrogen levels may
be increased in obese patients, due to peripheral aromatization of testosterone to oestrogen\textsuperscript{20}, this finding sheds light on the possible protective factor of an increased BMI in RP.

A limitation of this retrospective study is that we only assessed NCM in 4 fingers and not in 8, which was common practice in our centre at the time of data collection. Despite this limitation, we were able to demonstrate clear differences between BMI groups and we believe that the inclusion of additional fingers would not have changed the results. In addition, other factors that could have potentially influenced BMI (i.e., decreased muscle mass) were not investigated in the current study. We have assessed NCM only once per patient, therefore, possibly missing NCM changes over time, which could indicate progression. However, as these patients did not have signs of an underlying systemic disease (e.g., organ involvement and serological evidence) at baseline and no signs of an underlying disease developed over a follow-up period of at least 4 years, progression to a systemic disease remains very unlikely.

In conclusion, our findings indicate that microvascular abnormalities are inversely associated with BMI and occur independently of a connective tissue disease. The role of BMI in RP severity is further underlined by the shorter mean recovery time after cold challenge in patients with a high BMI. This study indicates that the role of (perivascular) fat, as well as other potential body weight related mechanisms in subjects with RP, deserve further study.

**ACKNOWLEDGEMENTS**

The authors would like to express their gratitude towards A.M. van Roon, S. C. van de Zande and A.I. van Gessel for their important technical contribution during the course of this research.
Chapter 5

REFERENCES


