Evaluation of sweating responses in patients with systemic connective tissue disorders using the quantitative sudomotor axon reflex test

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Abstract

In systemic connective tissue disorders (SCTDs), eccrine sweat glands are frequently attacked by immune cells, as evidenced by pathological observations. Sweating reflects vascular activity through the autonomic nervous system, while few studies have reported sweating ability in SCTDs or the relationship between sweating ability and Raynaud's phenomenon caused by sympathetic hyperreactivity. We performed the quantitative sudomotor axon reflex test on 85 patients diagnosed with systemic sclerosis, mixed connective tissue disease, systemic lupus erythematosus, Sjogren's syndrome, and dermatomyositis. Evaluations were performed once in summer and once in winter. We investigated the relationship of the axon reflex sweat volume and the reaction time to Raynaud's phenomenon, skin symptoms, and patient background. Most patients did not show a decrease in sweating compared to healthy participants, but patients with systemic sclerosis who were positive for anti-RNA polymerase III antibodies showed little or no sweating. One in three patients showed less sweating in summer than in winter, which is the opposite of the normal seasonal variation. Although no relationship was observed between the sweat volume and the total Raynaud's condition scores, patients with pain had more sweating than those without pain. These results suggested the possible utility of measuring sweating on autonomic peripheral circulatory disorders.

Introduction

Sweat glands play an essential role in seasonal heat acclimation and in maintaining systemic body homeostasis\(^1\). Therefore, to defend eccrine sweat glands from an autoimmune response, sweat glands are immune privileged\(^2\). Eccrine sweat glands in patients with systemic connective tissue disorders (SCTDs) exhibit histopathological abnormalities. In particular, lymphocyte infiltration and epithelial-mesenchymal transition were observed around the eccrine sweat glands of patients with systemic sclerosis (SSc), systemic lupus erythematosus (SLE), and Sjogren's syndrome (SS)\(^3–5\). Possibly related to the above findings, fatal heat stroke in a patient with diffuse scleroderma has been reported\(^6\). This led us to hypothesize that patients with SCTDs might have impaired sweating ability as well as an impaired capacity to respond to seasonal changes in temperature. Although there are several reports on sweating ability in patients with SS and atopic dermatitis\(^7,8\), no studies have been published focusing on seasonal changes in the sweating response and skin symptoms in patients diagnosed with SCTDs.

Eccrine sweat glands receive both cholinergic and adrenergic innervation and are controlled by the autonomic nervous system\(^9\). Aside from sweating, sympathetic nerve activity also regulates changes in body temperature according to the environment via vasoconstrictor nerves primarily responsive to noradrenaline, with the result that autonomic failure can affect both sweating ability and vasoactivity. The hyperactivation of the sympathetic nervous system in response to cold results in peripheral vasospasm and vasoconstriction in patients with SCTDs, known as Raynaud's phenomenon\(^10\). These responses cause pain, digital ulceration, and necrosis, resulting in a significantly reduced quality of life for those patients, especially in winter. Given the physiological functions of the sympathetic nervous...
system, autonomic abnormalities affect both sweating ability and vascular activity. Therefore, we speculate that there may be a relationship between sweating and Raynaud's phenomenon. In recent years, botulinum toxin, which is widely used as a treatment for hyperhidrosis\textsuperscript{11}, has been used to treat Raynaud's symptoms by blocking the sympathetic nervous system, and several systematic reviews and follow-up studies have validated its therapeutic effects\textsuperscript{12–14}. However, it is not clear whether the antiperspirant effect of botulinum toxin contributes to the improvements in Raynaud's symptoms or digital ulcers in patients with SCTDs.

The study aimed to evaluate sweating ability in patients with SCTDs by using the quantitative sudomotor axon reflex test (QSART) and to identify clinical profiles that include seasonal variations, disease-related differences, and associations with clinical factors such as Raynaud's phenomenon. This is the first study to provide a basis for understanding sweating ability in patients with SCTDs and contribute to developing treatment strategies for patients with autonomic peripheral circulatory disorders.

**Results**

The characteristics of patients and healthy participants are summarized in Table 1. Among 19 patients with SS, 12 patients had a comorbidity: eight and five patients had SSc and SLE, respectively, one of whom had both SSc and SLE. Among 86 patients, seven and 11 patients were unable to perform the QSART in the summer and winter, respectively. Consequently, the number of seasonal paired data was 67. Systemic corticosteroids, prostacyclin derivatives, serotonin receptor antagonists, and cholinergic agents were used in 25, 22, 14, and 3 patients, respectively. No parasympathomimetic agents were used in the study participants.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SSc (n = 48)</th>
<th>MCTD (n = 7)</th>
<th>SLE (n = 17)</th>
<th>SS (n = 19)</th>
<th>DM (n = 7)</th>
<th>Control (n = 11)</th>
</tr>
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<tr>
<td>Sex, n</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>7</td>
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<td>Age, years</td>
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<td>60.4 ± 9.3</td>
<td>63.7 ± 9.3</td>
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<td>15</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
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<tr>
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<td>11</td>
<td>16</td>
<td>0</td>
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</tr>
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<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mi2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
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<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Characteristics of study participants. Values are means ± standard deviation. SSc, systemic sclerosis; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; DM, dermatomyositis; ACA, anti-centromere antibody; Topo1, anti-topoisomerase 1 antibody; RNAP, anti-RNA polymerase III antibody; U1RNP, anti-U1RNP antibody; DNA, anti-DNA antibody; Sm, anti-Sm antibody; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; MDA5, anti-MDA5 antibody; Tif1, anti-Tif-1γ antibody; Mi2, anti-Mi2 antibody.

Axon reflex sweat volume
Comparison by disease types
We investigated axon reflex sweat volumes (ARSVs) in patients with SSc, mixed connective tissue disease (MCTD), SLE, SS, and dermatomyositis (DM) and healthy controls (see Supplementary Fig. S1a online). All disease groups showed as much or more sweating than healthy participants in both summer and winter. We analysed the mean differences in ARSVs between participants with each disease type and healthy participants (see Supplementary Table S1 online). We did not observe a significant difference in axonal reflex sweat volume in any disease group compared to healthy controls.

**Seasonal comparisons**

We investigated ARSVs as participant-wise ratios of the volume in summer to that in winter (Fig. 1a). For all patients, the geometric mean of the seasonal ratios (95% Confidence Interval: 95% CI) was 1.49 (1.20–1.87), indicating that ARSVs were larger in summer than in winter. This trend was also observed in the healthy control participants, with a geometric mean of 1.90 (0.99–3.66). However, some patients showed higher sweat volume in winter than in summer. There were 23 patients, corresponding to 34% in the seasonal paired data (Fig. 1b).

Patients who exhibited more perspiration in winter than in summer were found among all disease groups except for patients with DM (see Supplementary Fig. S1b online). In a comparison with the healthy participants, we observed a slight association of this phenomenon with having a diagnosis of SSc (OR [95% CI], 6.14 [0.40–94.72]). However, we did not observe an association between increased axial reflex sweat volume in winter compared with summer and smoking history, illness duration, finger temperature, nailfold capillary changes, skin sclerosis severity, skin symptoms (including digit ulcers, chilblains, subcutaneous calcifications, and telangiectasia), or disease complications (see Supplementary Table S2 and Figure S2 online).

**Relationship with skin sclerosis**

We investigated the relationship between the ARSV and the degree of skin sclerosis, defined by the modified Rodnan total skin thickness score (MRSS), in patients with SSc (Fig. 2). MRSS scores were dichotomized as low scores (≤ 10) and high scores (> 10), which represents weak disease and moderate to severe disease, respectively. The groups with MRSSs of ≤ 10 and > 10 included patients with scores of 0 to 10 and 13 to 26 in summer and 0 to 9 and 13 to 25 in winter. We observed lower ARSVs in the group with an MRSS > 10 than in the group with an MRSS ≤ 10. The geometric mean difference [95% CI] was 3.53 [1.51–8.26] in summer and 7.25 [3.65–14.40] in winter.

**Relationship with Raynaud’s phenomenon**

We analysed the association of the ARSV with the severity of Raynaud’s activity, defined by the Raynaud’s condition score (RCS). The total scores were dichotomized as low scores (0–7) and high scores (8–16) (Fig. 3a). Values for the subcomponents of attack, pain, colour, and duration were dichotomized into those without symptoms (0) and those with symptoms (≥ 1) (Fig. 3b). We did not observe a clear relationship between the dichotomized total RCS and the sweat volume. However, there was a clear relationship between the pain score and the sweat volume. In summer, the geometric mean of the ratio
[95% CI] was 1.61 [0.97–2.66]; in winter, the geometric mean of the ratio [95% CI] was 2.29 [1.54–3.39] (see Supplementary Table S3 online). No apparent relationship was observed between the score for each of the other subcomponents and the sweat volume.

**Sweat latency**

**Comparison by disease types or specific autoantibodies**

We investigated the sweat latencies for each disease (Fig. 4a). In the summer, all patients with MCTD, SLE, and DM began to sweat within 160 s. In contrast, three of the patients with SSc (7%) and two of the patients with SS (11%) did not begin to sweat, even after 300 s (the time limit for this measurement). In the winter, patients with SSc and SS showed the same trends as seen in the summer, and two of the patients with MCTD (29%) also showed this trend.

Since the sweat latency of SSc patients was prolonged compared with that of patients with other diseases, we compared the sweat latencies between groups of patients with each specific antibody for SSc (Fig. 4b). In the summer, 21 of the anti-centromere antibody (ACA)-positive patients (91%), all anti-topoisomerase 1 antibody (Topo1)-positive patients (100%), and 19 of the anti-U1RNP antibody (U1RNP)-positive patients (90%) began to sweat within 100 s. However, only three of the anti-RNA polymerase III antibody (RNAP)-positive patients (50%) responded within 100 s, and the remaining three patients (50%) did not begin to sweat within the 300-s time limit. In the winter, RNAP-positive patients showed the same trend as seen in the summer.

**Analysis regarding an attenuated response to acetylcholine**

In Fig. 4, patients who did not sweat during the observation period were considered to have an attenuated response to acetylcholine. Therefore, we further analysed the details of these patients (Table 2). There were nine patients (11%) with a latency of > 300 s for whom sweating was barely measurable, including six patients with SSc (67%) but none with SLE or DM. Of these six patients with SSc, four were RNAP positive (44%), one was Topo1 positive (11%), and the other had an unknown antibody. None of the patients were ACA positive. We observed a slight association between the attenuated response to acetylcholine and cold fingers below 32°C in summer (the OR [95% CI] was 15.3 [3.17–73.31]) (see Supplementary Table S4 and Fig. S3 online).
### Table 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Latency ≥ 300 s (n = 9)</th>
<th>Latency &lt; 300 s (n = 76)</th>
<th>OR</th>
<th>(95% CI)</th>
<th>p value (H0: Estimate = 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axon reflex sweat volume, mg/5 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>0.57 ± 0.50</td>
<td>1.49 ± 1.27†</td>
<td>-</td>
<td>(-3.82 to 0.45)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Winter</td>
<td>0.09 ± 0.05</td>
<td>1.10 ± 0.74</td>
<td>-</td>
<td>(-1.19 to 0.81)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sex, n</td>
<td>Male</td>
<td>1</td>
<td>12</td>
<td>0.67</td>
<td>(0.08 to 5.83)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8</td>
<td>64</td>
<td>0.67</td>
<td>(0.08 to 5.83)</td>
</tr>
<tr>
<td>Disease, n</td>
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<td>42</td>
<td>1.14</td>
<td>(0.26 to 4.99)</td>
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<tr>
<td></td>
<td>MCTD</td>
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<td>5</td>
<td>4.06</td>
<td>(0.66 to 24.91)</td>
</tr>
<tr>
<td></td>
<td>SLE</td>
<td>0</td>
<td>17</td>
<td>0.19</td>
<td>(0.01 to 3.23)</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>3</td>
<td>16</td>
<td>1.88</td>
<td>(0.42 to 8.33)</td>
</tr>
<tr>
<td></td>
<td>DM</td>
<td>0</td>
<td>7</td>
<td>0.49</td>
<td>(0.03 to 9.25)</td>
</tr>
<tr>
<td>Antibody, n</td>
<td>ACA</td>
<td>0</td>
<td>25</td>
<td>0.11</td>
<td>(0.01 to 1.99)</td>
</tr>
<tr>
<td></td>
<td>Topo1</td>
<td>1</td>
<td>12</td>
<td>0.67</td>
<td>(0.08 to 5.83)</td>
</tr>
<tr>
<td></td>
<td>RNAP</td>
<td>4</td>
<td>3</td>
<td>30.42</td>
<td>(5.28 to 175.00)</td>
</tr>
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<td></td>
<td>U1RNP</td>
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<td>18</td>
<td>0.92</td>
<td>(0.18 to 4.83)</td>
</tr>
<tr>
<td></td>
<td>DNA</td>
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<td>12</td>
<td>0.20</td>
<td>(0.02 to 1.71)</td>
</tr>
<tr>
<td></td>
<td>Sm</td>
<td>2</td>
<td>20</td>
<td>1.11</td>
<td>(0.05 to 23.11)</td>
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<tr>
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<td>SS-A</td>
<td>3</td>
<td>26</td>
<td>0.96</td>
<td>(0.22 to 4.16)</td>
</tr>
<tr>
<td></td>
<td>SS-B</td>
<td>2</td>
<td>5</td>
<td>4.06</td>
<td>(0.66 to 24.91)</td>
</tr>
</tbody>
</table>

Characteristics of the participants and their sweating response to acetylcholine. Values are means ± standard deviation. † The number of participants was 69 because 7 patients could not be examined.

OR, odds ratio; CI, confidence interval; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; DM, dermatomyositis; ACA, anti-centromere antibody; Topo1, anti-topoisomerase 1 antibody; RNAP, anti-RNA polymerase III antibody; U1RNP, anti-U1RNP antibody; DNA, anti-DNA antibody; Sm, anti-Sm antibody; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; MDA5, anti-MDA5 antibody; Tif1, anti-Tif-1γ antibody; Mi2, anti-Mi2 antibody. The p values were calculated via Welch's method or Fisher's exact method.
### Characteristics of the participants and their sweating response to acetylcholine. Values are means ± standard deviation. † The number of participants was 69 because 7 patients could not be examined. OR, odds ratio; CI, confidence interval; SSc, systemic sclerosis; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; DM, dermatomyositis; ACA, anticytromere antibody; Topo1, anti-topoisomerase 1 antibody; RNAP, anti-RNA polymerase III antibody; U1RNP, anti-U1RNP antibody; DNA, anti-DNA antibody; Sm, anti-Sm antibody; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; MDA5, anti-MDA5 antibody; Tif1, anti-Tif-1γ antibody; Mi2, anti-Mi2 antibody. The p values were calculated via Welch's method or Fisher’s exact method.

<table>
<thead>
<tr>
<th>Latency ≥ 300 s (n = 9)</th>
<th>Latency &lt; 300 s (n = 76)</th>
<th>OR (95% CI)</th>
<th>p value (H0: Estimate = 0)</th>
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<tbody>
<tr>
<td>MDA5</td>
<td>0</td>
<td>2</td>
<td>1.57 (0.07 to 35.21)</td>
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<tr>
<td>Tif1</td>
<td>0</td>
<td>2</td>
<td>1.57 (0.07 to 35.21)</td>
</tr>
<tr>
<td>Mi2</td>
<td>0</td>
<td>1</td>
<td>2.65 (0.10 to 69.82)</td>
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<tr>
<td>Unknown</td>
<td>1</td>
<td>5</td>
<td>1.78 (0.18 to 17.16)</td>
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### Discussion

We employed the QSART to assess sweating ability. A low ARSV and/or prolonged sweat latency in the QSART can be used to diagnose abnormalities of the postganglionic sympathetic fibres or eccrine sweat glands associated with poor acetylcholine-induced sudomotor responses\(^\text{16,17}\). The sweating responses of individuals living in Japan are more pronounced in summer than in winter\(^\text{18}\). The changes in sweating activity measured by the QSART confirmed the involvement of the peripheral nervous system in altering sudomotor activity during seasonal acclimation\(^\text{19}\).

In the present study, we assessed both sweating ability and its association with the clinical severity of Raynaud’s symptoms in patients with SCTDs. We found that none of the disease groups showed an apparent decrease in sweat volume compared to healthy participants (Supplementary Table S1 online). However, it was a novel finding that approximately one in three patients (34%) showed less sweating in summer than in winter (Fig. 1). This phenomenon was more common in patients with SSc than in healthy participants.

Because of the unique seasonal changes in sweating ability shown in the present study, we anticipated that patients with SCTDs have a dysregulated sweating ability due to abnormal peripheral nerve responses. While problems associated with heat adaptation are major factors for heat stroke\(^\text{20}\), there are no specific data available on the risk of heat stroke in patients with SCTDs. Li et al\(^\text{21}\) noted that left uncontrolled, recent trends in global warming will lead to an increased risk of heat stroke in 1.2 billion people by the year 2100. According to this assumption, we should pay attention to the relationship between global warming and seasonal perspiration in patients with SCTDs.
We focused on RNAP-positive SSc patients because they may have characteristic sweating abnormalities that are not present in other patient groups. Patients with RNAP-positive SSc had prolonged sweat latencies (Fig. 4), with 44% of them showing a poor response to acetylcholine (Table 2). Furthermore, RNAP-positive patients showed both less sweating and smaller seasonal differences than ACA-positive or Topo1-positive patients (see Supplementary Fig. S4a, Fig. S4b online). Patients with a high degree of skin stiffness showed less sweating than patients with a low degree of skin stiffness or no skin stiffness (Fig. 2); 57% of patients with an MRSS > 10 were RNAP positive.

Autoantibodies reactive with RNA polymerase (RNAP) III are confirmed to be strongly associated with diffuse or extensive cutaneous involvement and renal crisis\textsuperscript{22,23}. Severe and rapidly progressive cutaneous fibrosis may attenuate the response to acetylcholine by disrupting and reducing the number of eccrine sweat glands and nerve fibres. In some patients with SS, eccrine sweat gland dysfunction is associated with autoimmune mechanisms mediated by CD8 T cells\textsuperscript{24} or M3 receptor-specific autoantibodies\textsuperscript{25}. As we did not perform pathological assessment of eccrine glands, we cannot exclude the possibility that RNAP is directly associated with eccrine gland dysfunction. Further research on sweat gland impairment and the autonomic nervous system in RNAP-positive SSc patients may lead to a better understanding of peripheral circulation in patients with SCTDs.

Our results also indicated that patients with a higher degree of the pain in the RCS evaluation had a higher sweat volume (Fig. 3 and Supplementary Table S3). Regarding this phenomenon, we anticipated that the neuronal transmitters that convey pain signals might be involved in sweating ability. It has been reported that patients with Raynaud’s symptoms exhibit abnormal responses to pain-associated neurotransmitters, including substance P, glutamate, and calcitonin gene-related peptides\textsuperscript{26}, which may contribute to Raynaud-related pain. On the other hand, substance P and calcitonin gene-related peptide are expressed in normal sweat gland secretory cells or around the sweat glands\textsuperscript{27,28} and contribute to gland secretion in response to harmful stimuli. Taken together, these findings suggest that the response to neurotransmitters might link the pain in Raynaud’s phenomenon and increased sweating in winter.

Increased winter sweating with severe pain in Raynaud’s phenomenon might explain the phenomenon of increased winter sweating in some SCTD patients shown in Fig. 1. Tabata et al. studied sweating in SSc patients by using capillaroscopy and reported that 7 out of 21 patients developed increased sweating, although they did not perform a seasonal analysis\textsuperscript{29}. The mechanism by which the overactivity of the sympathetic nerves that causes Raynaud’s phenomenon affects sweating remains to be explored in additional studies involving a larger patient cohort, autonomic function tests, and pathological examination.

In conclusion, most patients did not show decreased sweating compared to healthy participants, but RNAP-positive patients with SSc had impaired sweating. One in three patients with an SCTD showed more sweating activity in winter than in summer, which is the opposite of the regular change. Although sweat volume was not associated with the total RCS, the pain of Raynaud’s phenomenon increased the volume of sweating.
A limitation of this study was the small sample size for each disease. Our study did not consider the effects of regularly used drugs, including external agents such as moisturizers, the obscurity of patients’ answers about Raynaud’s symptoms, the practice of sports, the living environment, and patients’ physical constitutions. In SSc patients, the reduced permeability of acetylcholine due to the hardness of the skin should be considered. Further study in combination with other autonomic nervous system assessments and more detailed patient backgrounds can provide a better understanding of the signs and biomarkers associated with peripheral nerve disorders and contribute to the development of treatment strategies for patients with autonomic peripheral circulatory disorders.

**Materials And Methods**

This study was conducted according to the study protocol\(^{30}\), which is available at the Japan registry of clinical trials.

**Participants**

The study population comprised 85 Japanese patients in the dermatology department of Nagasaki University Hospital with established diagnoses of SCTDs associated with Raynaud’s phenomenon, including those diagnosed with SSc, MCTD, SLE, SS, and DM. Patients with SSc, MCTD, SLE, and SS who met the diagnostic criteria for those diseases were included in this study\(^{31-34}\). The study included 11 healthy individuals as controls. The normal use of either oral or topical medications was not restricted for any patient.

**QSART**

To assess the effect of seasonal changes on the sweating response, the QSART was conducted during the summer (June 2019–September 2019, mean, minimum, and maximum temperatures of 25.6°C, 17.5°C, and 37.3°C, respectively) and during the winter (December 2019–March 2020, mean, minimum, and maximum temperatures of 10.6°C, 0.8°C, and 21.2°C, respectively).

The QSART was developed by Low et al\(^{17}\) and involves the flow of dry air into a capsule followed by the measurement of the sweat volume. This is performed by quantifying the moisture levels in the outflowing air with a high-sensitivity hygrometer. In this study, the participants rested in a thermostatic chamber (room temperature, 23–26°C; room humidity, 40–60%) for at least 30 min prior to the physiological examination. The QSART was performed using a SKN-2000 (Skinos Co., Ltd., Nagano, Japan), and acetylcholine was delivered to the dermis of each participant’s forearm by iontophoresis for 5 min with a current of 5 mA. The amount of sweat measured for 5 min was recorded as the ARSV, and the time until the start of sweating was recorded as the sweat latency. There are no defined standard reference values for the QSART. Therefore, in this study, the QSART values of healthy volunteers were used as the reference values. The results for sweat latency (the interval of time required for sweating) were evaluated by graphic representation with a Kaplan–Meier method.
Assessment of the clinical severity of Raynaud’s phenomenon and other skin symptoms

On the same day, the patients were interviewed regarding their Raynaud’s symptoms during the previous two weeks. Information regarding their skin symptoms (nailfold capillary changes, skin sclerosis severity, digital ulcers, chilblains, subcutaneous calcifications, or telangiectasia) was collected, and the surface temperatures of their fingers were recorded using thermography. Information on autoantibodies and patient complications was referenced from each patient’s medical record.

The severity of Raynaud’s symptoms was assessed using a revised version of the RCS with values for the frequency of attacks (0, none; 1, once per 2 weeks; 2, once per week; 3, once per 2 days; 4, every day), pain (0, none; 1, fairly rare; 2, rare; 3, sometimes; 4, always), colour (0, none; 1, red; 2, purple; 3, sometimes white; 4, always white), and the duration of Raynaud’s phenomenon (0, none; 1, < 15 min; 2, 15–30 min; 3, 30–60 min; 4, > 60 min); scores ranged from 0–16 points. Nailfold capillary observations via dermoscopy were classified into one of four phases: normal, early, active, or late. The severity of skin sclerosis in scleroderma patients was assessed using the MRSS.

Statistical analysis

The differences between subgroups of participants, defined by corresponding clinical factors, are reported as the mean difference and Wald’s 95% CI or its adjusted version of simultaneous inference keeping the duality with the p value computation. The p values reported with the mean differences were calculated via Welch’s method or Dunnett’s method. The dependency between two binomials is reported using the odds ratio (OR) and its 95% CI. The p value for the independence between two binomials was calculated via Fisher’s exact method. Statistical analyses were performed using either GraphPad Prism (ver. 5, GraphPad Software, San Diego, LA, USA) or R (ver. 3.6.0, The R Foundation, Vienna, Austria). The following R libraries were used in relevant analyses: multcomp ver. 1.4–13, robustbase ver. 0.93–6, and survminer ver. 0.4.8. The R source code for the analyses is available from a GitHub repository (https://github.com/mrmtshmp/QSARTConnTisDis).

Declarations

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Author contributions

HM and MA have made substantial contributions to the study conception and design. MA, MY, YK and DE were involved in the acquisition of data. SM and MA contributed to the analysis and interpretation of the data. HM, MA, YK, and SM assisted in drafting the manuscript or revising.
All authors have given final approval of the version to be published and agreed to be accountable for all aspects of the work.

**Competing interests**

The authors have no conflicts of interest directly relevant to the content of this article.

**Ethics declaration**

This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Clinical Research Review Board of Nagasaki University (Reference number: CRB19-001). All participants provided informed consent prior to participating in the study.

**Data availability statement**

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**References**


**Figures**
Figure 1

The axonal reflex sweat volume (ARSV) and its seasonal differences. (a) Plots of changes in the ARSV measured by the quantitative sudomotor axon reflex test in summer and winter. The graph on the left and the right shows data for patients with systemic connective tissue disorders (SCTDs) (67 paired sets) and healthy controls (11 paired sets), respectively. Most patients with SCTDs sweated as much or more than healthy controls, but some patients sweated more in winter than in summer. (b) The number of patients with SCTDs and healthy controls in the groups with more sweating in winter than in summer (summer < winter) and summer than in winter (summer > winter).
Figure 2

Plots of the axonal reflex sweat volume (ARSV) and the degree of skin sclerosis. (a) and (b) represent the distributions of ARSVs by the modified Rodnan total skin thickness scores (MRSS) in patients with systemic sclerosis in summer and winter, respectively. The horizontal axis shows the dichotomized score. The MRSS was dichotomized as \( \leq 10 \) and >10. The group with an MRSS >10 showed lower ARSVs than the group with an MRSS \( \leq 10 \) in both summer and winter. The boxes indicate the interquartile ranges. The middle lines within the boxes represent the medians. The whiskers above/below the boxes are the ranges of the observed values from the 75% quartiles/25% quartiles to the maximums/minimums within 1.5 times the interquartile ranges.
Figure 3

Plots of the axonal reflex sweat volume (ARSV) according to the Raynaud's condition score (RCS). (a) and (b) represent the distributions of ARSVs by the total RCS and the score for the pain subcomponent, respectively. The horizontal axis shows the dichotomized score. The total RCS was dichotomized as 0–7 and 8–16. The scores for pain were dichotomized as 0 and ≥1. The top and bottom panels represent summer data (n = 78) and winter data (n = 73), respectively. A relationship was not found between the dichotomized total RCS and the sweat volume. However, a clear relationship was found between the pain score and the sweat volume. The p values were calculated via Fisher's exact method. The boxes and whiskers are drawn by the same rule in Figure 2.
Figure 4

Sweat latencies according to disease types or scleroderma-specific autoantibodies. Sweat latency represents the time from acetylcholine involvement to the beginning of sweating. (a) and (b) represent Kaplan–Meier curves for the sweat latency by the disease type and scleroderma-specific autoantibody, respectively. The vertical axis shows the patient cumulative probability of sweating (%), and the horizontal axis shows the time since the administration of acetylcholine (s). The top and bottom panels represent the summer data and winter data, respectively. SSc, systemic sclerosis; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SS, Sjogren’s syndrome; DM, dermatomyositis; ACA, anti-centromere antibody; Topo1, anti-topoisomerase 1 antibody; RNAP, anti-RNA polymerase III antibody; U1RNP, anti-U1RNP antibody. In both summer and winter, the sweat latency tended to be prolonged in the patients with SSc, especially in RNAP-positive patients.

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