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

Miwa Ashida MD1 | Shimpei Morimoto PhD2,3,4 | Mariko Yozaki MT1 | Daisuke Ehara MD1 | Yuta Koike MD, PhD1,5 | Hiroyuki Murota MD, PhD1,5

1Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
2Department of Immunology and Rheumatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
3Innovation Platform & Office for Precision Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
4Clinical Research Center, Nagasaki University Hospital, Nagasaki, Japan
5Leading medical research core unit, life-sciences innovation, Nagasaki university graduate school of biomedical sciences, Nagasaki, Japan

Correspondence
Hiroyuki Murota, Department of Dermatology, Nagasaki University Graduate School of Biomedical Sciences, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.
Email: h-murota@nagasaki-u.ac.jp

Funding information
Mitsubishi Tanabe Pharma Corporation, Grant/Award Number: MTPS20190606019

Abstract
Background: In systemic connective tissue disorders, eccrine sweat glands are frequently attacked by immune cells, as evidenced by pathological observations.

Aims: Sweating affects vascular activity through the autonomic nervous system, while few studies have reported sweating ability in systemic connective tissue disorders or the relationship between sweating ability and Raynaud’s phenomenon caused by sympathetic hyperreactivity.

Materials & methods: 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 between the axon reflex sweat volume or the reaction time and Raynaud’s phenomenon assessed by a Raynaud’s condition score, skin symptoms such as nailfold capillary changes, skin sclerosis severity, digital ulcers, chilblains, subcutaneous calcifications, and telangiectasia, and patient background.

Results: 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.

Conclusion: This is the first exploratory observational study of sweating ability in patients with systemic connective tissue disorders, revealing several clinical factors associated with acetylcholine-induced sweating.
1 | INTRODUCTION

Sweat glands play an essential role in seasonal heat acclimation and in maintaining systemic body homeostasis. Therefore, to defend eccrine sweat glands from an autoimmune response, sweat glands are immune privileged. 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). Possibly related to the above findings, fatal heat stroke in a patient with diffuse scleroderma has been reported. 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, 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. 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. 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, 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. 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.

2 | MATERIALS

This study was conducted according to the study protocol, which is available at the Japan registry of clinical trials.

2.1 | 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, Mixed connective tissue disease (MCTD), SLE, SS, and Dermatomyositis (DM). Patients with SSc, MCTD, SLE, and SS who met the diagnostic criteria for those diseases were included in this study. The study included 11 healthy individuals as controls. The normal use of either oral or topical medications was not restricted for any patient.

2.2 | 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. 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 axon reflex sweat volume (ARSV), and the time until the start of sweating was recorded as the sweat latency. There are no defined

KEYWORDS
acetylcholine, systemic connective tissue disorders, Raynaud phenomenon, seasonal variation, sweating
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.

### 2.3 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 2 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 Raynaud’s condition score (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), color (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 to 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 modified Rodnan total skin thickness scores (MRSS).

### 2.4 Statistical analysis

The differences between subgroups of participants, defined by corresponding clinical factors, are reported as the mean difference and Wald’s 95% Confidence Interval (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/mrmts/QSARTConnTisDis).

#### Table 1: Characteristics of study participants

| Characteristic | Ssc (n = 48) | MCTD (n = 7) | SLE (n = 17) | SS (n = 19) | DM (n = 7) | Control (n = 11) |
|----------------|-------------|-------------|-------------|------------|-----------|----------------|
| Sex, n         |             |             |             |            |           |                |
| Male           | 7           | 2           | 2           | 3          | 3         | 4              |
| Female         | 41          | 5           | 15          | 16         | 4         | 7              |
| Age, years     | 63.5 ± 11.8 | 61.1 ± 5.9  | 54.1 ± 1.1  | 60.4 ± 9.3 | 63.7 ± 9.3 | 47.9 ± 6.2     |
| Duration of illness, years | 15.8 ± 9.8 | 20.0 ± 9.8  | 16.8 ± 6.7  | 13.0 ± 22.1 | 13.3 ± 6.3 |
| Autoantibody, n|             |             |             |            |           |                |
| ACA            | 25          | 0           | 1           | 3          | 0         |                |
| Topo1          | 13          | 0           | 0           | 1          | 0         |                |
| RNAP           | 7           | 0           | 1           | 4          | 0         |                |
| U1RNP          | 4           | 7           | 10          | 6          | 0         |                |
| DNA            | 2           | 1           | 15          | 6          | 0         |                |
| Sm             | 1           | 0           | 3           | 0          | 0         |                |
| SS-A           | 9           | 3           | 11          | 16         | 0         |                |
| SS-B           | 2           | 0           | 1           | 7          | 0         |                |
| MDA5           | 0           | 0           | 0           | 0          | 2         |                |
| Tif1           | 0           | 0           | 0           | 0          | 2         |                |
| Mi2            | 0           | 0           | 0           | 0          | 1         |                |
| Unknown        | 3           | 0           | 0           | 0          | 3         |                |

Note: Values are means ± standard deviation.

Abbreviations: ACA, anti-centromere antibody; DM, dermatomyositis; DNA, anti-DNA antibody; MCTD, mixed connective tissue disease; MDA5, anti-MDA5 antibody; Mi2, anti-Mi2 antibody; RNAP, anti-RNA polymerase III antibody; SLE, systemic lupus erythematosus; Sm, anti-Sm antibody; SS, Sjogren’s syndrome; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; SSc, systemic sclerosis; Tif1, anti-Tif-1γ antibody; Topo1, anti-topoisomerase 1 antibody; U1RNP, anti-U1RNP antibody.
3 | RESULTS

The characteristics of patients and healthy participants are summarized in Table 1. Among 19 patients with SS, 12 patients had a co-morbidity: 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.

3.1 | Axon reflex sweat volume

3.1.1 | Comparison by disease types

We investigated ARSVs in patients with SSc, MCTD, SLE, SS, and DM and healthy controls (see Figure S1a). All disease groups showed as much or more sweating than healthy participants in both summer and winter. We analyzed the mean differences in ARSVs between participants with each disease type and healthy participants (see Table S1). We did not observe a significant difference in axonal reflex sweat volume in any disease group compared to healthy controls.

3.1.2 | Seasonal comparisons

We investigated ARSVs as participant-wise ratios of the volume in summer to that in winter (Figure 1a). For all patients, the geometric mean of the seasonal ratios [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 (Figure 1b).

Patients who exhibited more perspiration in winter than in summer were found among all disease groups except for patients with DM (see Figure S1b). 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 Table S2 and Figure S2).

3.1.3 | Relationship with skin sclerosis

We investigated the relationship between the ARSV and the degree of skin sclerosis, defined by the MRSS, in patients with SSc (Figure 2). The scores were dichotomized as low scores (≤10) and high scores (>10). The groups with MRSSs of ≤10 and >10 included patients with scores of 0–10 and 13–26 in summer and 0–9 and 13–25 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.

3.1.4 | Relationship with Raynaud’s phenomenon

We analyzed the association of the ARSV with the severity of Raynaud’s activity, defined by the RCS. The total scores were dichotomized as low scores (0–7) and high scores 8–16 (Figure 3a). Values for
the subcomponents of attack, pain, color, and duration were dichotomized into those without symptoms (0) and those with symptoms (≥1) (Figure 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 Table S3). No apparent relationship was observed between the score for each of the other subcomponents and the sweat volume.

3.2 | Sweat latency

3.2.1 | Comparison by disease types or specific autoantibodies

We investigated the sweat latencies for each disease (Figure 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 (Figure 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.

3.2.2 Analysis regarding an attenuated response to acetylcholine

In Figure 4, patients who did not sweat during the observation period were considered to have an attenuated response to acetylcholine. Therefore, we further analyzed 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 Table S4 and Figure S3).

4 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 fibers or eccrine sweat glands associated with poor acetylcholine-induced sudomotor responses. Twenty, thirty The sweating responses of individuals living in Japan are more pronounced in summer than in winter. Thirty-one The changes in sweating activity measured by the QSART confirmed the involvement of the peripheral nervous system in altering sudomotor activity during seasonal acclimation.

In this 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 (Table S1). However, it was a novel finding that approximately one in three patients (34%) showed less sweating in summer than in winter (Figure 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 this 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, there are no specific data available on the risk of heat stroke in patients with SCTDs. Li et al. 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 (Figure 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 Figure S4a,b). Patients with a high degree of skin stiffness showed less sweating than patients with a low degree of skin stiffness or no skin stiffness (Figure 2); 57% of patients with an MRSS >10 were RNAP positive.

Autoantibodies reactive with RNAP are confirmed to be strongly associated with diffuse or extensive cutaneous involvement and renal crisis. Severe and rapidly progressive cutaneous fibrosis may attenuate the response to acetylcholine by disrupting and reducing the number of eccrine sweat glands and nerve fibers. In some patients with SS, eccrine sweat gland dysfunction is associated with autoimmune mechanisms mediated by CD8 T cells or M3 receptor-specific autoantibodies. 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.

| TABLE 2 | Characteristics of the participants and their sweating response to acetylcholine |
|----------|-------------------------------------------------------------------------------------------------|
|          | Latency ≥ 300 s (n = 9) | Latency < 300 s (n = 76) | OR | (95% CI) | p Value (H0: Estimate = 0) |
| Axon reflex sweat volume, mg/5 min |
| Summer | 0.57 ± 0.50 | 1.49 ± 1.27<sup>a</sup> | - | (-3.82 to 0.45) | <0.001 |
| Winter | 0.09 ± 0.05 | 1.10 ± 0.74 | - | (-1.19 to 0.81) | <0.001 |
| Sex, n |
| Male | 1 | 12 | 0.67 | (0.08 to 5.83) | 1.00 |
| Female | 8 | 64 | 0.67 | (0.08 to 5.83) | 1.00 |
| Disease, n |
| SSc | 6 | 42 | 1.14 | (0.26 to 4.99) | 1.00 |
| MCTD | 2 | 5 | 4.06 | (0.66 to 24.91) | 0.159 |
| SLE | 0 | 17 | 0.19 | (0.01 to 3.23) | 0.194 |
| SS | 3 | 16 | 1.88 | (0.42 to 8.33) | 0.412 |
| DM | 0 | 7 | 0.49 | (0.03 to 9.25) | 1.00 |
| Antibody, n |
| ACA | 0 | 25 | 0.11 | (0.01 to 1.99) | 0.053 |
| Topo1 | 1 | 12 | 0.67 | (0.08 to 5.83) | 1.00 |
| RNAP | 4 | 3 | 30.42 | (5.28 to 175.00) | <0.001 |
| U1RNP | 2 | 18 | 0.92 | (0.18 to 4.83) | 1.00 |
| DNA | 0 | 12 | 0.20 | (0.02 to 1.71) | 0.150 |
| Sm | 2 | 20 | 1.11 | (0.05 to 23.11) | 1.00 |
| SS-A | 3 | 26 | 0.96 | (0.22 to 4.16) | 1.00 |
| SS-B | 2 | 5 | 4.06 | (0.66 to 24.91) | 0.159 |
| MDA5 | 0 | 2 | 1.57 | (0.07 to 35.21) | 1.00 |
| Tif1 | 0 | 2 | 1.57 | (0.07 to 35.21) | 1.00 |
| Mi2 | 1 | 1 | 2.65 | (0.10 to 69.82) | 1.00 |
| Unknown | 1 | 5 | 1.78 | (0.18 to 17.16) | 0.500 |

Note: Values are means ± standard deviation. The p values were calculated via Welch’s method or Fisher’s exact method.
Abbreviations: ACA, anti-centromere antibody; CI, confidence interval; DM, dermatomyositis; DNA, anti-DNA antibody; MCTD, mixed connective tissue disease; MDA5, anti-MDA5 antibody; Mi2, anti-Mi2 antibody; OR, odds ratio; RNAP, anti-RNA polymerase III antibody; SLE, systemic lupus erythematous; Sm, anti-Sm antibody; SS, Sjogren’s syndrome; SS-A, anti-SS-A antibody; SS-B, anti-SS-B antibody; SSc, systemic sclerosis; Tif1, anti-Tif-1γ antibody; Topo1, anti-topoisomerase 1 antibody; U1RNP, anti-U1RNP antibody.

<sup>a</sup>The number of participants was 69 because 7 patients could not be examined.
Our results also indicated that patients with a higher degree of the pain subcomponent in the RCS had a higher sweat volume (Figure 3 and Table S3). Regarding this phenomenon, we anticipated that the neuronal transmitters that convey pain signals might be involved in sweating activity. 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,39 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 glands40,41 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 Figure 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.42 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 take into account 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.

ACKNOWLEDGMENT
Mitsubishi Tanabe Pharma Corporation provided financial support to conduct the research and publish the article.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.
15. Ashida M, Koga T, Morimoto S, Yozaki M, Ebara D, Koike Y, et al. Evaluation of sweating responses in patients with collagen disease using the quantitative sudomotor axon reflex test (QSART): a study protocol for an investigator-initiated, prospective, observational clinical study. BMJ Open. 2021;11(10):e050690.

16. Asano Y, Jinnin M, Kawaguchi Y, Kuwana M, Goto D, Sato S, et al. Diagnostic criteria, severity classification and guidelines of systemic sclerosis. J Dermatol. 2018;45(6):633–91.

17. Tanaka Y, Kuwana M, Fuji T, Kameda H, Muro Y, Fuji K, et al. 2019 Diagnostic criteria for mixed connective tissue disease (MCTD): From the Japan research committee of the ministry of health, labor, and welfare for systemic autoimmune diseases. Mod Rheumatol. 2021;31(1):29–33.

18. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, et al. 2019 European League Against Rheumatism/ American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol (Hoboken, NJ). 2019;71(9):1400–12.

19. Sumida T, Azuma N, Moriyama M, Takahashi H, Asashima H, Honda F, et al. Clinical practice guideline for Sjögren’s syndrome 2017. Mod Rheumatol. 2018;28(3):383–408.

20. Low PA, Caskey PE, Tuck RR, Fealey RD, Dyck PJ. Quantitative sudomotor axon reflex test in normal and neuropathic subjects. Ann Neurol. 1983;14(5):573–81.

21. Merkel PA, Herlyn M, Martin RW, Anderson JJ, Mayes MD, Bell P, et al. Measuring disease activity and functional status in patients with scleroderma and Raynaud’s phenomenon. Ann Rheum Dis. 2002;61(9):2410–20.

22. Hasegawa M. Dermoscopy findings of nail fold capillaries in connective tissue diseases. J Dermatol. 2011;38(1):66–70.

23. Rodnan GP, Lipinski E, Lukswick J. Skin thickness and collagen content in progressive systemic sclerosis and localized scleroderma. Arthritis Rheum. 1979;22(2):130–40.

24. Bretz F. Multiple comparisons using R. Boca Raton: CRC Press; 2011.

25. Welch BL. The generalisation of student's problems when several different population variances are involved. Biometrika. 1947;34(1–2):28–35.

26. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc. 1955;50(272):1096–121.

27. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models. Blom J Biometrische Zeitschrift. 2008;50(3):346–63.

28. Todorov V, Filzmoser P. An object-oriented framework for Robust multivariate Analysis. J Stat Softw. 2009;32(3):1–47.

29. Kassambara A, Kosinski M, Biecek P. survminer: Drawing Survival Curves using ‘ggplot2’. 2020. Available from: http://www.sthda.com/english/rpkgs/survminer/.

30. Murota H. Old and new approaches for assessing sweating. Curr Probl Dermatol. 2016;51:22–9.

31. Nakamura Y, Okamura K. Seasonal variation of sweating responses under identical heat stress. Appl Hum Sci. 1998;17(5):167–72.

32. Shin YO, Lee JB, Kim JH. Seasonal acclimation in sudomotor function evaluated by QSART in healthy humans. Korean J Physiol Pharmacol. 2016;20(5):499–505.

33. Epstein Y, Yanovich R. Heatstroke. N Engl J Med. 2019;380(25):2449–59.

34. Li D, Yuan J, Kopp RE. Escalating global exposure to compound heat-humidity extremes with warming. Environ Res Lett. 2020;15(6):064003.

35. Harvey GR, Butts S, Rands AL, Patel Y, McHugh NJ. Clinical and serological associations with anti-RNA polymerase antibodies in systemic sclerosis. Clin Exp Immunol. 1999;117(2):395–402.

36. Okano Y, Steen VD, Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. Ann Intern Med. 1993;119(10):1005–13.

37. Katayama Y. Dry skin manifestations in Sjögren syndrome and atopic dermatitis related to aberrant sudomotor function in inflammatory allergic skin diseases. Allergol Int. 2018;67(4):448–54.

38. Naito Y, Matsumoto I, Wakamatsu E, Goto D, Sugiyama T, Matsumura R, et al. Muscarinic acetylcholine receptor autoantibodies in patients with Sjögren’s syndrome. Ann Rheum Dis. 2005;64(3):510–1.

39. Bunker CB, Foreman JC, Dowd PM. Digital cutaneous vascular responses to histamine and neuropeptides in Raynaud’s phenomenon. J Invest Dermatol. 1991;96(3):314–7.

40. Tainio H, Vaalasti A, Rechardt L. The distribution of substance P-, CGRP-, galanin- and ANP-like immunoreactive nerves in human sweat glands. Histochim J. 1987;19(6–7):375–80.

41. Zancanaro C, Merigo F, Crescimanno C, Orlandini S, Osculati A. Immunohistochemical evidence suggests intrinsic regulatory activity of human eccrine sweat glands. J Anat. 1999;194(Pt 3):433–44.

42. Tabata K, Jinnin M, Furukawa K, Tani S, Okuhira H, Mikita N, et al. Finger sweating levels evaluated by video capillaroscopy system are increased in patients with systemic sclerosis compared to prec clinical stage patients. Drug Discov Ther. 2021;14(6):325–9.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ashida M, Morimoto S, Yozaki M, Ebara D, Koike Y, Murota H. Evaluation of sweating responses in patients with systemic connective tissue disorders using the quantitative sudomotor axon reflex test. J Cutan Immunol. Allergy. 2022;5:208–216. https://doi.org/10.1002/cia2.12269