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Original Article

Clinical utility of the basophil activation test in the diagnosis of sweat allergy

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AD, atopic dermatitis; AIGA, acquired

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ASwST, autologous sweat skin test;

BAT, basophil activation test;

CholU, cholinergic urticaria; HRT, histamine

release test; HU, histamine release units;

QR, Semi-purified sweat antigen

ABSTRACT

Background: Many patients with atopic dermatitis and cholinergic urticaria display an immediate-type allergy to autologous sweat. Although the histamine release test (HRT) using semi-purified sweat antigen (QR) was available for the detection of immediate sweat allergy, the existence of HRT low responders could not be disregarded. Furthermore, it has not been established whether the results of the HRT are consistent with the autologous sweat skin test (ASwST). We aimed to compare the HRT and basophil activation test (BAT) for the diagnosis of immediate sweat allergy.

Methods: The HRT and BAT were performed on 47 subjects (35 ASwST positive, 12 negative) whose symptoms had worsened on sweating. For the BAT, blood was incubated with QR or crude sweat and CD203c upregulation was assessed. A commercial HRT was performed and histamine release induced by QR was quantified.

Results: When excluding non-responders for anti-IgE antibody, the BAT using QR and the HRT had a sensitivity of 100% and 44% and specificity of 75% and 100%, respectively. The BAT and HRT had a positive predictive value of 91.3% and 100% and negative predictive value of 100% and 30%, respectively. The BAT detected 0% non-responders, whereas the HRT identified 22.5%. When using crude sweat for the BAT, the false-positives observed when using QR were not detected.

Conclusions: The BAT using QR displayed a higher sensitivity and negative predictive value and a lower number of non-responders compared with the HRT. Furthermore, the BAT using crude sweat can also be an alternative tool for the ASwST.

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Introduction

The concept of hypersensitivity to human sweat was first described in 1953.¹ Subsequently, the issue of sweat allergy was not spotlighted again until 1989, when Adachi *et al.* used the RAST and detected IgE antibody to sweat in atopic dermatitis (AD) patients.² This immediate-type allergic reaction to sweat was also confirmed in patients with cholinergic urticaria (CholU).³ In 2002, Hide *et al.* observed that basophils of patients with AD released histamine when mixed with sweat, and this response was inhibited by the removal of IgE on the basophils.⁴ In addition, Tanaka *et al.*

demonstrated a high specificity and sensitivity of the basophil-histamine release test to the semi-purified sweat antigen in AD patients compared with healthy controls and patients with psoriasis vulgaris.⁵ Furthermore, Hiragun *et al.* identified that MGL_1304, produced by *Malassezia globosa*, a fungus present on the human skin, is a major allergen in human sweat.⁶

Recently, the clinical significance of the immediate-type allergic reaction to sweat has become more important, because CholU patients can be classified as with or without sweat allergy.^{7,8} It should be noted that CholU patients with sweat allergy occasionally present with severe anaphylactic shock.^{9,10} In previous studies, we and Adachi used the autologous sweat skin test (ASwST) as a gold-standard tool to detect sweat allergy.^{3,7} However, it is difficult and time-consuming to obtain a sufficient amount of autologous sweat from each patient for the ASwST. Therefore, we sought to establish a simple blood test for the diagnosis of sweat allergy.

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In Japan, although a histamine release test (HRT) using QR, which is a purified human sweat antigen containing MGL_1304,¹¹ was a commercially available and useful clinical tool for detecting immediate-type sweat allergy, this commercial test has been discontinued since April 2018. Additionally, the number of HRT low-responders cannot be disregarded and there is no report to our knowledge on whether the results of the HRT are consistent with the ASwST. Recent reports have indicated the clinical efficacy of the basophil activation test (BAT) in allergy diagnosis.^{12,13} Here, we report a clinical trial of adopting the BAT using QR or crude sweat to detect sweat allergy.

Methods

Study population

In total, 47 patients whose symptoms worsened because of sweating were enrolled at the Dermatological Institute of Kobe University Hospital. Of these, 6 subjects had AD, 32 ChIU, and 9 had both. To compare the clinical utility between the HRT and BAT for sweat allergy detection, 47 patients with ($n = 35$) or without ($n = 12$) sweat allergy as confirmed by a positive or negative ASwST result, respectively, were enrolled in the present study from June 2015 to August 2018. The institutional ethics committee of Kobe University Hospital approved this study (Approval No. 1617, 180186) and all subjects provided written informed consent. The characteristics of the patients are described in Table 1. The female ratio, AD patient rate, total RAST, and specific IgE to *Malassezia*, *Candida*, and *Pityrosporum*, were significantly higher in the ASwST-positive group than the negative group.

Intracutaneous test with autologous sweat

An intracutaneous test was performed as previously described.^{7,14} In brief, samples of autologous diluted sweat of 1/100 and 1/1000 (0.02 mL) and 0.9% sterile saline (0.02 mL) were separately injected intradermally into the forearm of each subject. The wheal diameters were measured after 15 min. We defined patients as having strong hypersensitivity to sweat when the wheal diameter induced by 1/100 or 1/1000-diluted sweat was ≥ 6 mm. In this study, we used ASwST as a golden standard tool detected sweat allergy. In this paper, we defined sweat as liquid collected from human skin surface which may contain a small amount of both exogenous and endogenous substances.

Purification of the sweat antigen

Semi-purified sweat antigen (QR) was obtained from human sweat of healthy volunteers as described in previous studies.^{6,15}

Briefly, sweat collected from healthy volunteers was mixed with 20 mM Tris to adjust to pH 8.0 and loaded onto an anion-exchange column (monoQ HR 10/10, Amersham Biosciences, Piscataway, NJ, USA), which was previously equilibrated with 20 mM Tris-HCl, pH 8.0, and eluted by a linear gradient of NaCl up to 1 M using an FPLC systemTM (AKTA explorer 10S and SMART system, Amersham Biosciences). The eluted fractions with histamine-releasing activity were collected, diluted with 0.1% trifluoroacetic acid, and loaded onto a reversed-phase chromatography column (SOURCE 15RPC ST 4.6/100, Amersham Biosciences). The column-bound material was separated by elution using a linear acetonitrile gradient.

HRT

The HRT was performed using the HRT Shionogi kit (Shionogi & Co., Ltd., Osaka, Japan),^{7,16} and assays were performed within 72 h after blood sampling from 40 patients (4 subjects had AD, 27 ChIU, and 9 had both). Venous peripheral blood samples of 20 μ L from patients with worsening symptoms because of sweating and anti-basophil antibodies conjugated to magnetic beads were added to the wells of a 96-well plate and incubated for 10 min at room temperature on a plate mixer. Antibody-binding basophils in each well were then trapped with a chandelier-shaped magnet and transferred to another microplate, where the basophils were stimulated at 37 °C for 1 h with QR, anti-IgE antibody (clone; HE-35A),¹⁷ or vehicle. The reaction was terminated by adding 200 μ g/mL digitonin. The final QR concentrations used were 1.3, 4, 10, 20, and 40 HU (histamine release units). Antigen-induced basophil histamine release content, total histamine content, and spontaneous histamine release content into the medium were determined using an enzyme-linked immunosorbent assay with a characteristic detection profile. The anti-IgE antibody- and QR-induced histamine release rates (%) were calculated as: (antigen-induced histamine release – spontaneous histamine release)/(total histamine content – spontaneous histamine release) $\times$ 100. A cut-off value was set at 20% and an anti-IgE-antibody induced histamine release rate of $<20\%$ was regarded as a non-responder. The results were expressed as an index value (class 0: $<20\%$ at any concentration, class 1: $\geq 20\%$ at a concentration of 40 HU, class 2: $\geq 20\%$ at a concentration of 20 HU, class 3: $\geq 20\%$ at a concentration of 10 HU, class 4: $\geq 20\%$ at a concentration of 4 HU). Class 2 or higher was considered to be positive according to the manufacturer's instructions.¹⁸

BAT

Whole blood (up to 2 mL) was obtained from 29 patients (1 subject had AD, 21 ChIU, and 7 had both) using ethylenediaminetetraacetic acid-containing tubes and assays were

Table 1
Characteristics of the study population.

Demographic features	ASwST positive ($n = 35$)	ASwST negative ($n = 12$)	<i>P</i> value
Age (y)	25 (17–48)	43 (17–63)	0.05
Female sex. No. (%)	22 (62.8%)	2 (16.6%)	0.007 [#]
Atopic disease. No. (%)	15 (42.8%)	0 (0%)	0.004 [#]
Cholinergic urticaria. No. (%)	31 (88.5%)	10 (83.3%)	0.63 [#]
Total IgE (IU/mL)	911 (106–43138)	78.1 (3–664)	<0.001
sIgE to <i>Malassezia</i> (UA/mL)	2.705 (0.1–89.5)	0.1 (0.1–3.43)	0.002
sIgE to <i>Candida</i> (UA/mL)	0.48 (0.1–56.8)	0.1	0.01
sIgE to <i>Pityrosporum</i> (UA/mL)	0.68 (0.1–88.6)	0.1 (0.1–9.74)	0.008

Values are shown as numbers (percentages) or medians (range). Statistics were performed using Fisher's exact test (#) or Mann–Whitney *U* test. ASwST, autologous sweat skin test; sIgE, specific IgE.

performed within 24 h after blood sampling. The Allergen Kit (Beckman Coulter, Fullerton, CA, USA) was used to quantify basophil CD203c expression according to the manufacturer's instructions.¹⁹ In brief, we used QR, crude sweat, or anti-IgE antibody (clone; E124-2-8D) (4 µg/mL) as positive controls and PBS as a negative control for basophil stimulation. R-phycoerythrin-cyanine 7 (PE-Cy7)-conjugated anti CD3, fluorescein isothiocyanate (FITC)-conjugated anti-CRTH2, and R-phycoerythrin (PE)-conjugated anti-CD203c antibodies were used to detect basophils and their activation. The final QR concentrations used were 1.3, 4, 10, 20, and 40 HU. Sweat was collected from one AD patient and this was used as the crude sweat because it had standard activity when comparing the sweat from the other patients. The sweat was diluted in PBS to final concentrations at 0.001, 0.01, 0.1, and 0.3 µg/mL protein. Then, red blood cells were lysed, and the remaining leukocytes were analyzed by FACSverse (BD Biosciences, San Jose, CA, USA). Flow cytometric results were analyzed by FlowJo software (FlowJo, LLC, Ashland, OR, USA). Basophils were identified by their characteristic forward and side scatter, by the expression of basophil activation, and by the absence of CD3 (Supplementary Fig. 1). The double-gated cells were defined on the CRTH2/CD203c plot as basophils. The results of antibody stimulation were expressed as the CD203c^{high} basophils (%). The CD203c^{high} basophils (%) of the negative control was gated at ≤5%.²⁰ Participants who responded to anti-IgE stimulation with <10% CD203c^{high} basophils were designated non-responders.²¹ Positive criteria were calculated to exclude nonspecific reactions similar to the HRT. QR- or crude sweat-induced basophil activity was calculated as: (antigen-induced CD203c^{high} basophils (%) – spontaneous CD203c^{high} basophils (%))/(CD203c^{high} basophils against IgE antibody stimulation (%) – spontaneous CD203c^{high} basophils (%)) × 100. ROC curves was

used to determine the cut-off for positive BAT results and the value was set at 10.

Statistical analysis

Statistical analysis was performed using Fishers' exact test for categorical variables and Mann–Whitney *U* test or unpaired *t* test for continuous variables. ROC analyses were used to compare diagnostic accuracy between the BAT and HRT. All statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Two-sided *P*-values of <0.05 were considered statistically significant.

Results

Comparison of basophil activity and histamine release using QR

Both the HRT, which was previously available for commercial use in Japan, but has been discontinued since April 2018, and the BAT, which might be an alternative to the HRT, were performed using the same QR.

Figure 1 shows representative positive and negative FACS results of the BAT using QR of ASwST-positive (also positive for BAT) and ASwST-negative (also negative for BAT) subjects. Then, to validate the correlation between the ASwST and BAT using QR, we categorized the subjects into positive or negative ASwST results, then we analyzed the relationship between the ASwST, BAT, and HRT. Basophil CD203c reactivity (BAT) to QR was significantly higher in the ASwST-positive group than the -negative group at all dilution concentrations. It should be noted that the higher the antigen concentration, the more significant the difference we observed

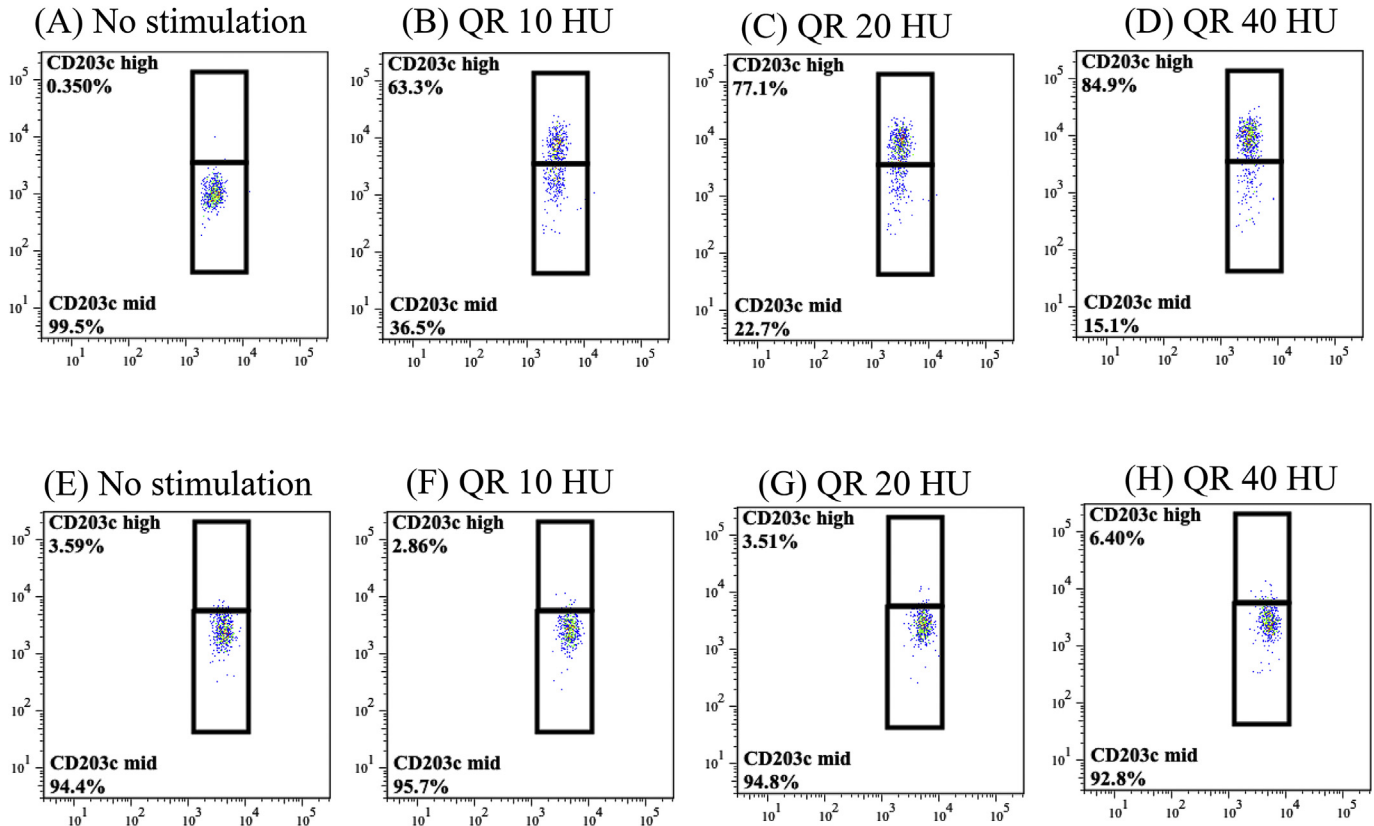


Fig. 1. Representative results of the basophil activation test using QR. (A–D) Autologous sweat skin test (ASwST)-positive subject, (E–H) ASwST-negative subject. Final QR concentration is as follows: (A, E) without stimulation, (B, F) QR 10 histamine release units (HU), (C, G) QR 20 HU, (D, H) QR 40 HU.

(Fig. 2). One and two false-positive subjects (ASwST negative, BAT positive) were observed at 4 HU and higher concentrations, respectively.

Given that the BAT using QR displayed a good relationship with the ASwST, we also investigated whether the HRT related well with the ASwST. In the HRT, we could not detect any significant difference between the two groups because of the considerable number of false-negatives (ASwST positive, HRT negative) (Fig. 3). Indeed, 14 (56%) of the ASwST-positive group were false-negatives in the HRT. Of the two patients who were false-positive in the BAT assay, one each was negative or a non-responder in the HRT assay.

Next, to compare diagnostic accuracy, we evaluated and summarized the parameters of the BAT using 20 HU QR and the HRT (Table 2). The BAT and HRT had a sensitivity of 100% and 44% and specificity of 75% and 100%, respectively, when non-responders, whose basophils did not respond to anti-IgE antibody, were excluded. The BAT and HRT displayed a positive predictive value of 91.3% and 100% and negative predictive value of 100% and 30%, respectively, when non-responders, whose basophils did not respond to anti-IgE antibody, were excluded. In addition, the BAT detected 0% non-responders, whereas HRT resulted in 22.5%. In addition, the ROC analyses revealed that the BAT was more consistent with the ASwST than the HRT as determined by the area under curve; BAT = 0.90; HRT = 0.72 (Fig. 4A, B).

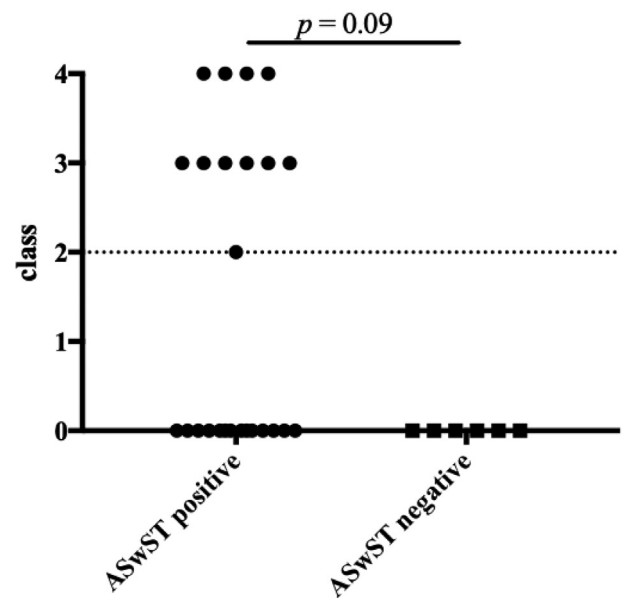


Fig. 3. Comparison of the histamine release test between the autologous sweat skin test (ASwST)-positive and -negative groups. The dotted line represents the cutoff. Statistical analyses were performed using the Mann–Whitney *U* test.

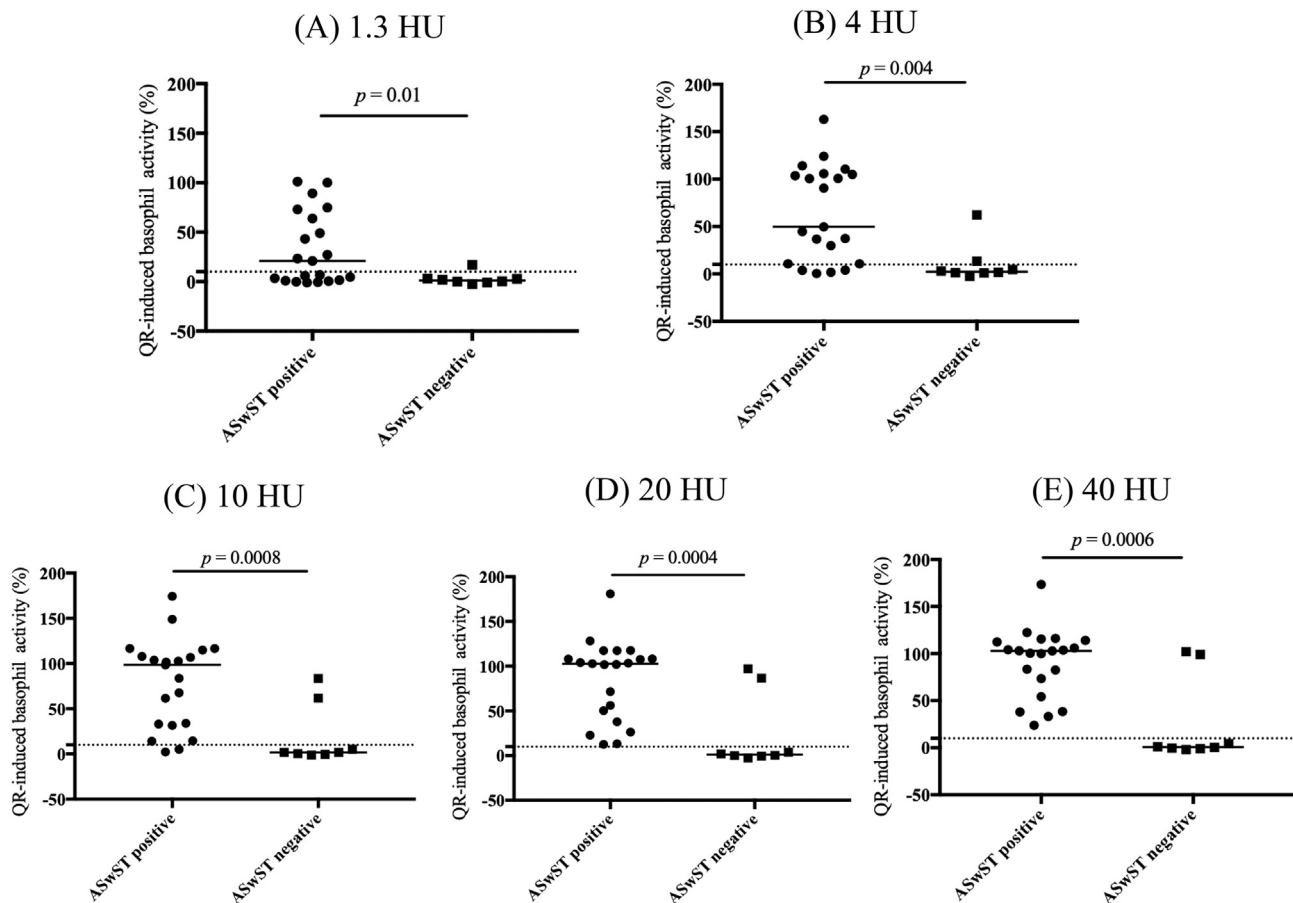


Fig. 2. Comparison of the QR-induced basophil activation test between the autologous sweat skin test (ASwST)-positive and -negative groups. The solid line presents the median and the dotted line the cutoff. Final QR concentration is as follows: (A) 1.3 histamine release units (HU), (B) 4 HU, (C) 10 HU, (D) 20 HU, (E) 40 HU. Statistical analysis was performed using the Mann–Whitney *U* test.

Table 2

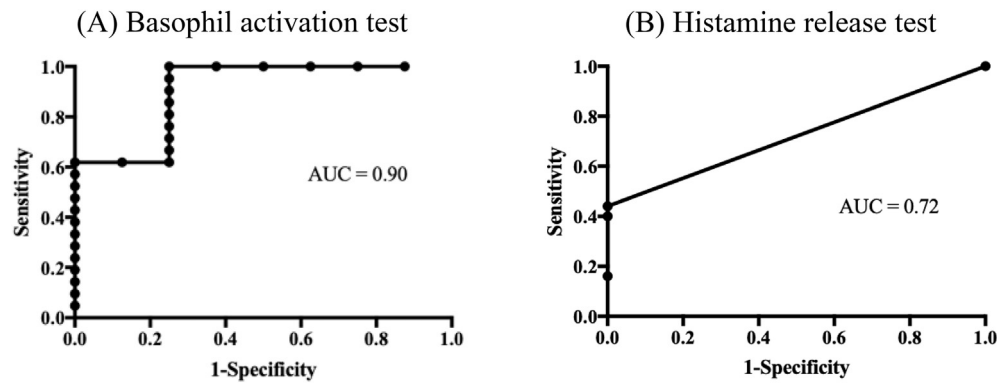
Performance of different tests in the diagnosis of sweat allergy.

Diagnostic test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Non-responder (%)
BAT (n = 29)	100% (21/21)	75% (6/8)	91.3% (21/23)	100% (6/6)	0% (0/29)
HRT (n = 40)	44% (11/25)	100% (6/6)	100% (11/11)	30% (6/20)	22.5% (9/40)

BAT results: when stimulated with QR 20 HU.

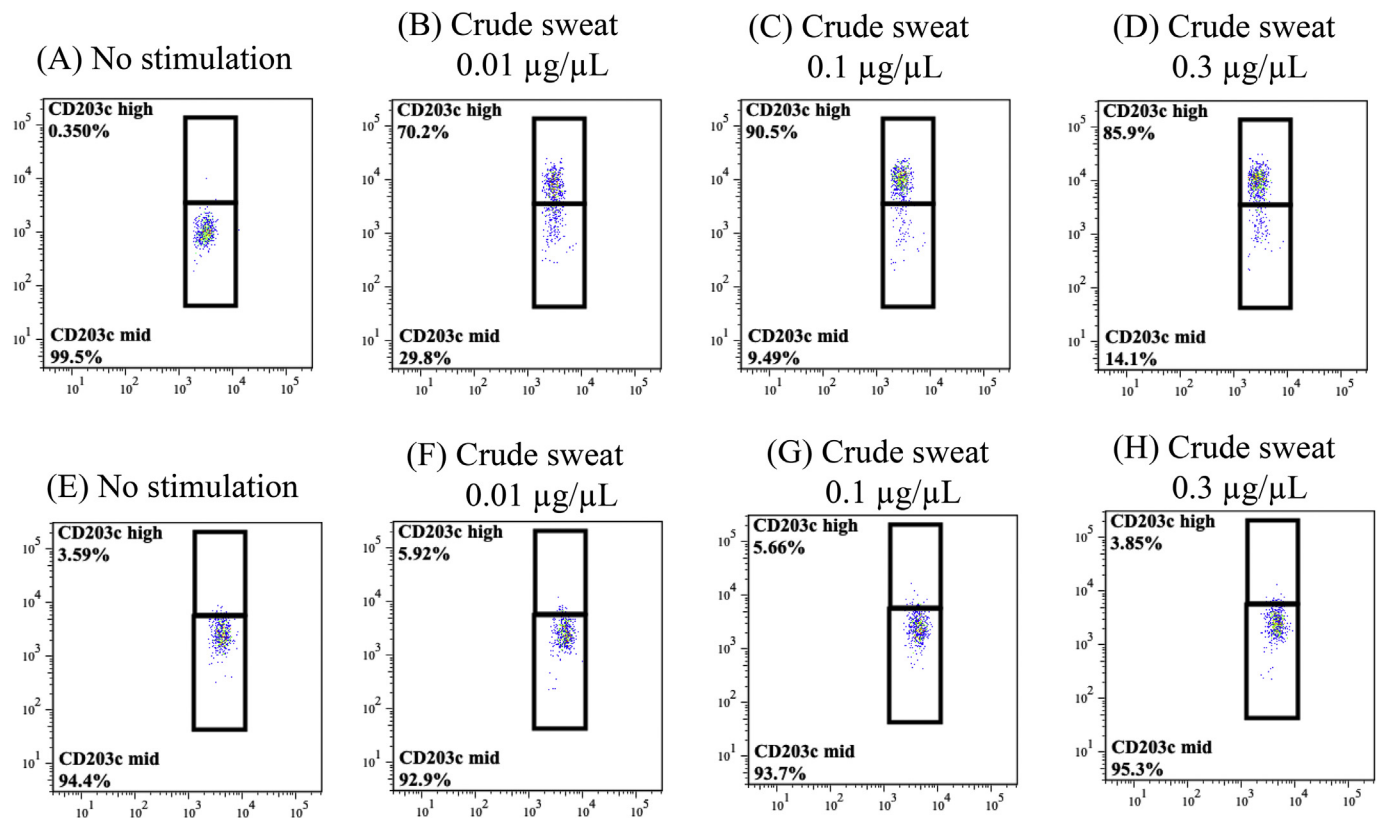
HRT-positive criteria: $\geq 20\%$ at a concentration of 20 HU.

BAT, basophil activation test; HRT, histamine release test; HU, histamine release units.

**Fig. 4.** Receiver operating characteristics analyses. (A) Basophil activation test using 20 histamine release units QR, (B) Histamine release test. AUC, area under curve.*Comparison of basophil activity using QR and crude sweat*

Although we extracted QR as the fractions displaying the strongest histamine-releasing activity within crude sweat,⁶ we considered the possibility that other fractions present in crude

sweat also contributed to the positive results in the ASwST. Therefore, to validate this hypothesis, we also performed the BAT using crude sweat. Figure 5 shows representative results of the positive and negative FACS results of the BAT using crude sweat. Basophil CD203c reactivity (BAT) to crude sweat was significantly

**Fig. 5.** Representative results of the basophil activation test using crude sweat. (A–D) Autologous sweat skin test (ASwST)-positive subject, (E–H) ASwST-negative subject. Final crude sweat concentration is as follows: (A, E) without stimulation, (B, F) crude sweat 0.01 $\mu\text{g}/\mu\text{L}$, (C, G) crude sweat 0.1 $\mu\text{g}/\mu\text{L}$, (D, H) crude sweat 0.3 $\mu\text{g}/\mu\text{L}$.

higher in the ASwST-positive group than negative group at concentrations other than 0.001 $\mu\text{g}/\text{mL}$ protein (Fig. 6). Furthermore, we observed no false-positive subjects when using crude sweat. In addition, the BAT using crude sweat at a dilution concentration of ≥ 0.1 $\mu\text{g}/\text{mL}$ showed a greater significant difference ($P < 0.0001$) (Fig. 6C, D) than the BAT using QR (Fig. 2).

Both HRT and BAT were carried out in 22 out of 47 patients (Supplementary Fig. 2). Of these, 16 (72.7%) patients showed positive reaction to the ASwST (A) and remaining 6 (27.2%) negative reaction (B). Of ASwST positive patients, 6 (37.5%) patients showed positive reactions to all of BAT using QR and crude sweat and HRT. Eight (50%) patients showed positive reactions to BAT using QR and crude, whereas HRT detected negative or non-responder reaction. Remaining 2 (12.5%) patients was positive in only BAT using QR (crude-BAT negative, HRT negative or non-responder). On the other hands, of ASwST negative patients, 3 (50%) patients showed negative to all tests. The remaining three showed negative reactions to only BAT using QR and crude sweat (HRT non-responder), only BAT using crude sweat and HRT (QR-BAT positive), and only BAT using crude sweat (QR-BAT positive, HRT non-responder), respectively.

Additionally, of the 41 patients with ChouU, 6 patients with acquired idiopathic generalized anhidrosis (AIGA) were included. We described the responsiveness of ASwST, BAT and HRT in patients

with AIGA (Supplementary Fig. 3). Of these, 5 (83.3%) patients showed negative reaction to the ASwST and remaining 1 positive reaction (16.6%). Although the results of BAT using crude sweat were consistent with the ASwST, one positive was detected when using QR in ASwST negative group. In addition, HRT correctly detected negative in two (40%) patients.

Discussion

In this study, we first compared the HRT and BAT using the same sweat antigen, QR, to diagnose sweat allergy. We demonstrated that the BAT displayed higher sensitivity and negative predictive value compared with the HRT. Additionally, the HRT yielded less power of detection because of a considerable number of false-negatives. The only drawback that has to be considered in the BAT is the detection of false-positives at a high QR concentration. Furthermore, we investigated whether, in addition to semi-purified QR, crude sweat could also detect sensitization in other fractions.²² When using crude sweat, we detected no false-positives in contrast to when using QR. Although the BAT using crude sweat is suitable as a substitute for the ASwST, the antigenicity may change depending on the origin of the sweat. Hence, the limitation of this study is that the sweat collected from only one AD patient was used as crude sweat. It is advisable that a standard crude sweat antigen

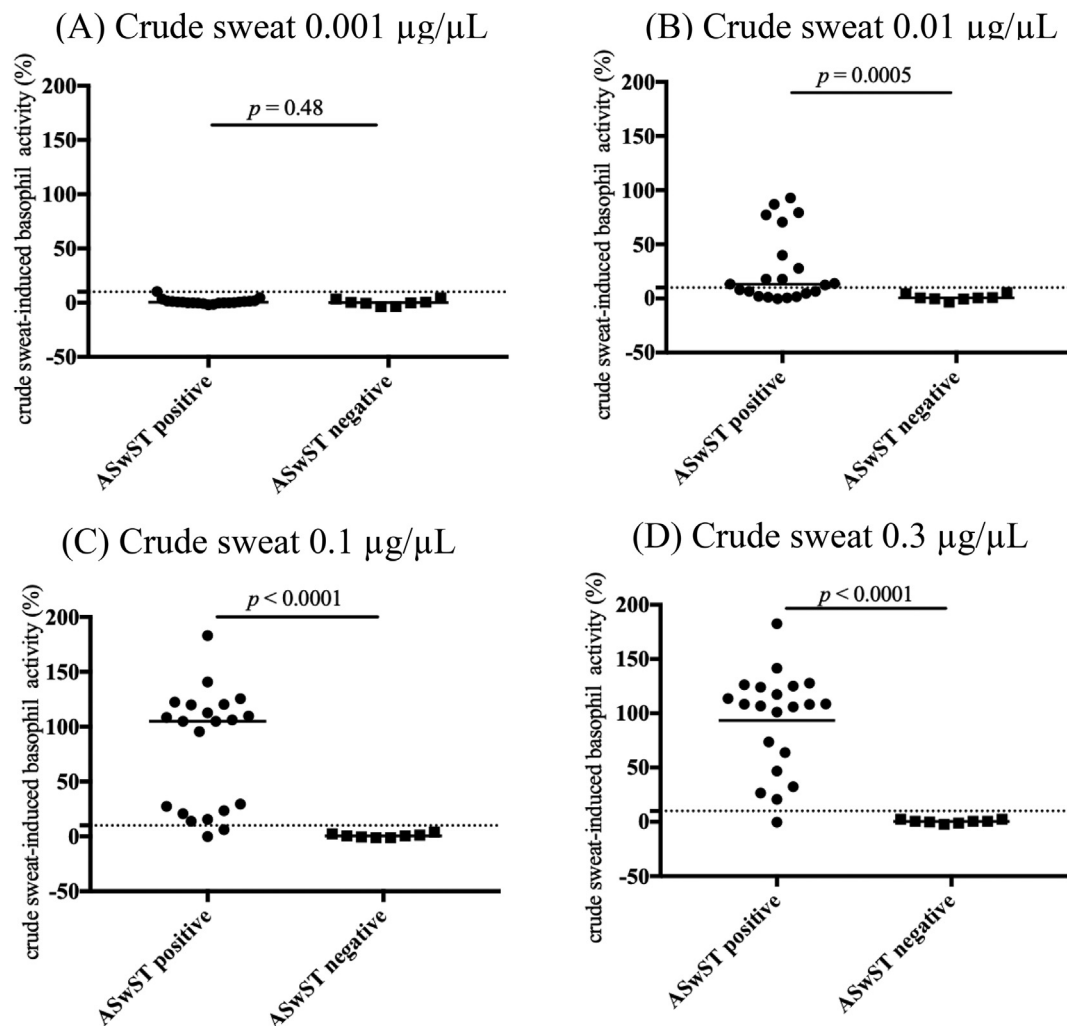


Fig. 6. Comparison of crude sweat-induced basophil activation test between the autologous sweat skin test (ASwST)-positive and -negative groups. The solid line presents (A–C) the median or (D) the mean and the dotted line the cutoff. Final crude sweat antigen concentration is as follows: (A) 0.001 $\mu\text{g}/\text{mL}$, (B) 0.01 $\mu\text{g}/\text{mL}$, (C) 0.1 $\mu\text{g}/\text{mL}$, (D) 0.3 $\mu\text{g}/\text{mL}$. Statistical analysis was performed as follows: (A–C) Mann–Whitney *U* test, (D) unpaired *t* test.

that does not differ among testing facilities, such as QR, should be developed in the future.

Previous reports stated that the HRT detects 10–20% non-responders,^{21,23} and in the present study we detected 22.5%. By contrast, whereas the percentage of non-responders for the BAT has been reported as approximately 5–10%,²⁴ we detected no non-responders in our analysis. Our study is consistent with these previous ones, in that the BAT detected less non-responders than the HRT.

The BAT has been validated as a reliable tool for the diagnosis of IgE-mediated allergies.¹² There are only a limited number of studies comparing the HRT and BAT. Larsen *et al.* recently reported such a comparative study in the diagnosis of peanut allergy and concluded that the BAT displayed a significantly higher CDsens, defined as the inverted value for the threshold allergen concentration multiplied by 100,²⁵ compared with the HRT.²¹

Although the BAT displayed superior specificity, negative predictive value, and non-responder detection rate in the present study, we need to consider the detection of false-positives at relatively high QR concentrations. Analysis using a combination of the HRT and BAT may improve diagnostic accuracy.

Recently, the clinical significance of immediate-type allergic reaction to sweat has become more important, because ChIU patients can be classified as with or without sweat allergy.⁸ It should be noted that ChIU patients with sweat allergy might present with severe anaphylactic shock.^{9,10} Furthermore, in the treatment of ChIU patients with sweat hypersensitivity and resistance to conventional therapy, the effectiveness of rapid desensitization with autologous sweat was demonstrated.¹⁴ By contrast, in AD patients, Ilves *et al.* reported that the wheal reaction to autologous sweat was significantly associated with the clinical severity, total serum IgE, and specific IgE level against *Malassezia* spp.²⁶ Furthermore, patients with a high HRT classification displayed discomfort to sweating.²⁷ By contrast, no association between the ASwST and AD disease severity was reported.⁴ In daily clinical practice, many AD patients complain of the worsening of their disease by sweating. It has been reported that the leakage of sweat followed by mast-cell activation contribute to AD pathogenesis.²⁸ Therefore, while the clinical directive may need to be changed for individual patients who complain of the worsening of their disease by sweating, the BAT may provide a promising bench-to-bedside interactive tool for AD and ChIU patients. In the ASwST, autologous sweat must be collected and the examination procedure is somewhat complicated, whereas the patients require only a blood test in the BAT and HRT. In particular, the BAT may be a promising detection tool for immediate-type sweat allergy, because of its higher sensitivity and negative predictive value and lower number of non-responders.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.09.003>.

Conflict of interest

The authors have no conflict of interest to declare.

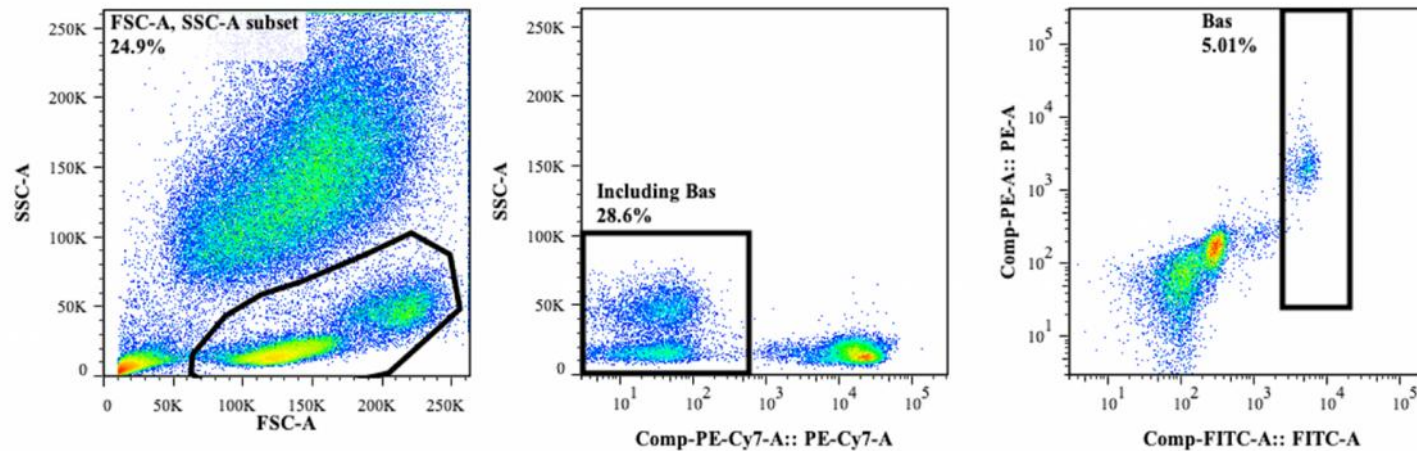
Authors' contributions

YO, KW, AF, MH and CN wrote the manuscript. YO, KW, AF, SI, MH and KO contributed to data collection. YO, KW, and AF performed the statistical analysis and

interpretation of the results. KI and MH supplied material and designed the study. All authors read and approved the final manuscript.

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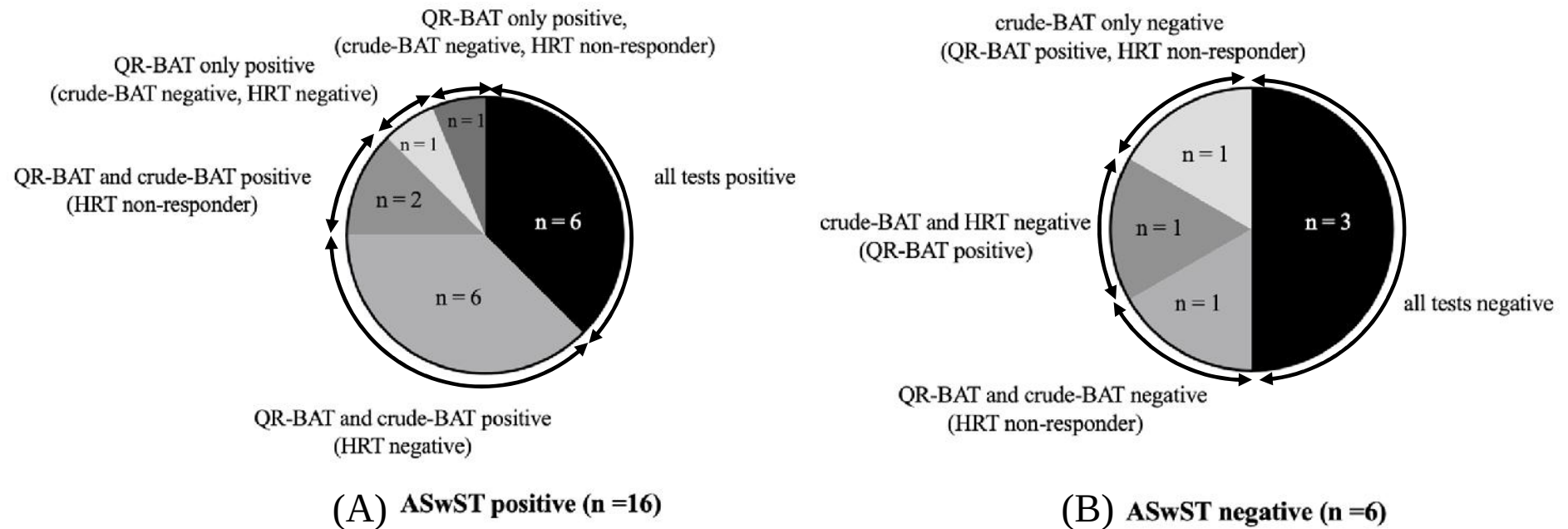


Supplementary Fig. 1.

Representative images of flow cytometry for detecting basophils.

On the forward scatter (FSC)/side scatter (SSC) plot (left), the basophil scatter gate and leukocyte gate are defined. On the phycoerythrin-cyanine 7 (PE-Cy7)/SSC plot (middle), the CD3-negative group is defined. On the fluorescein isothiocyanate (FITC)/CD203c plot (right), the CRTH- and CD203c-positive group is defined as basophils.

A: area, Comp: compensation

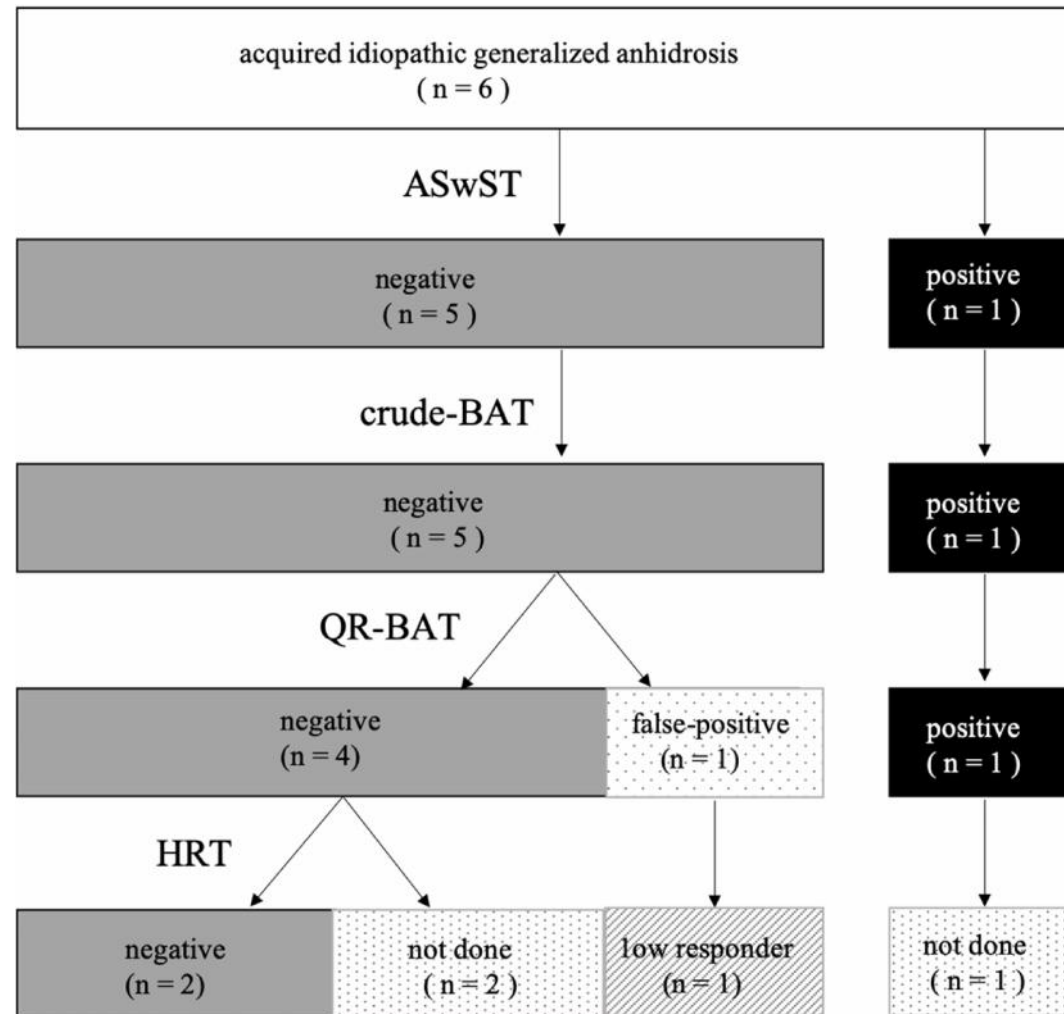


Supplementary Fig. 2

The relationship basophil activation test using QR and using crude sweat and histamine release test in the same patient.

(A) Autologous sweat skin test (ASwST)-positive patients

(B) ASwST-negative patients



Supplementary Fig. 3

The responsiveness of autologous sweat skin test, basophil activation test using QR and crude sweat, and histamine release test in patients with acquired idiopathic generalized anhidrosis.