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# Comparison of Clinical Test Results in Pollen-food Allergy Syndrome: Multicenter Case Series Study

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## Abstract

***Background***

The crude antigen-specific immunoglobulin E (IgE) test does not necessarily have a high sensitivity and specificity for the diagnosis of pollen-food allergy syndrome (PFAS). The skin prick-to-prick test (PPT) has a high sensitivity; however, it can induce allergic symptoms, similar to the oral food challenge (OFC). A new *in vitro* test for the diagnosis of PFAS is required. This study aimed to compare the results of the basophil activation test (BAT) to the results of the other tests in PFAS.

***Methods***

To compare the sensitivities of the crude antigen-specific IgE test, PPT, and BAT-CD203c for diagnosing PFAS, we performed these tests on patients with PFAS. To examine the correlation between threshold doses and basophil CD203c expression levels, we also performed OFC on the patients and determined their threshold doses according to the results. We compared basophil CD203c expression levels in patients with oral symptoms and those with systemic symptoms in PFAS.

***Results***

Ten patients with PFAS induced by eating peach, watermelon, or tomato were analyzed. BAT-CD203c, crude antigen-specific IgE test, and PPT showed sensitivities of 93.3%, 86.7%, and 60%, respectively. There was a significant negative correlation between threshold doses and basophil CD203c expression levels. Basophil CD203c expression levels for the patient with systemic symptoms induced by eating peach were markedly higher than those for patients with oral symptoms.

***Conclusions***

BAT-CD203c has a high sensitivity in the diagnosis of PFAS and may be useful for predicting the threshold doses. BAT-CD203c may distinguish systemic and oral symptoms in peach allergy.

Key Words: Basophil degranulation test; Food allergy; Peach; Tomato; Watermelon

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## Introduction

Pollen-food allergy syndrome (PFAS) is a food allergy based on cross-reactivity between pollens and food antigens. Its typical symptoms are isolated in the oropharynx, and systemic reactions are unusual[1]. The prevalence rates of PFAS have been reported to be 13%-58% in adults and 4.2% in children[2]. There is a concern that the number of children with PFAS will increase in the future as pollinosis develops at a younger age[3]. The symptoms of PFAS are mostly subjective, and a detailed medical history is important for the diagnosis of PFAS[4,5]. However, it is sometimes difficult to diagnose PFAS in children who have difficulty explaining their symptoms and medical history. Therefore, a superior test to assist the diagnosis of PFAS is needed.

Some objective tests for diagnosing PFAS have been verified, such as the allergen-specific immunoglobulin E (IgE) test, skin prick test, and oral food challenge (OFC)[1,6]. The crude antigen-specific IgE test has been reported to have a sensitivity of approximately 20%-70% and a specificity of approximately 60%-90%[7]. Therefore, it does not necessarily have high sensitivity and specificity for the diagnosis of PFAS. In addition, the crude antigen-specific IgE test result may be negative, even if systemic symptoms appear in PFAS. For example, the sensitivity of the peach-specific IgE test for severe peach allergy has been reported to be approximately 70%[8]. Therefore, the crude antigen-specific IgE test is insufficient to predict the risk of systemic symptoms. The allergen component-specific IgE test has been reported to be useful for predicting the risk of systemic symptoms[9,10]. However, it has not been sufficiently covered by medical insurance in Japan. The skin prick test, especially the prick-to-prick test (PPT), which is performed using fruit and vegetable pulps, has been reported to have higher sensitivity in the diagnosis of PFAS than the allergen-specific IgE test[7]. However, it is difficult to predict the risk of systemic symptoms using the skin prick test and crude antigen-specific IgE test[8], and the skin prick test has a high risk of inducing allergic symptoms in patients who have had systemic symptoms after ingestion of the causative food. If it is difficult to diagnose PFAS even after taking a detailed medical history and performing an allergen-specific IgE test and skin prick test, OFC is finally performed. A double-blind placebo-controlled food challenge (DBPCFC) is recommended because the symptoms of PFAS are mostly subjective[11]. Patients with systemic symptoms can be diagnosed with PFAS even with an open food challenge. Because OFC has a high risk of inducing allergic symptoms, a new *in vitro* test for the diagnosis of PFAS is needed.

The basophil activation test (BAT) is a less invasive test than PPT and OFC. In addition, it is a more useful method for identifying rare allergens compared to the allergen-specific IgE test, in which identifiable allergens are limited[12]. Basophils are activated by allergen-bound IgE antibodies, release inflammatory mediators such as histamine, and produce Th2-type cytokines [interleukin (IL)-4, IL-13] to induce allergic symptoms[13]. The basophil surface markers, CD63 and CD203c, indicate basophil activation, and BAT detects the levels of these markers using a flow cytometer[14]. The BAT has been reported to have high sensitivity and specificity for the diagnosis of peanut, milk, egg, and wheat allergy[15-17]. Regarding the usefulness of BAT in diagnosing PFAS, there are some reports demonstrating the sensitivity and specificity of BAT[18-21]. However, the allergens examined in these reports are limited. Regarding the usefulness of BAT in predicting the severity of PFAS, some studies have examined the difference in BAT between systemic and oral symptoms in PFAS[20,21]. However, the conclusions remain unclear. Furthermore, there are no reports examining the correlation between threshold doses and basophil CD203c expression levels in PFAS.

This study aimed to compare the result of BAT to the results of the other tests for PFAS.

## Methods

***Study design and study population***

This was a multicenter case series study. Between October 2019 and March 2020, we enrolled patients who visited the Department of Pediatrics, Osaka City University Hospital or Fujitani Clinic in Osaka Prefecture and were diagnosed with PFAS. All patients were diagnosed with PFAS because they had a history of oral symptoms due to ingestion of raw fruits or vegetables and tested positive for specific IgE against the pollen that had been reported to be cross-reactive with the causative food. They underwent the crude antigen-specific IgE test, PPT, and BAT against the causative foods, and we examined the sensitivity of each test for the diagnosis of PFAS. OFC was performed on the patients who consented to the test, and their threshold doses were determined based on the results of OFC. We examined the correlation between threshold doses and basophil CD203c expression levels. Patients with systemic symptoms also underwent the crude antigen-specific IgE test, PPT, and BAT against the causative foods, and we compared the results with those of patients who had only oral symptoms. Furthermore, to compare basophil CD203c expression levels in patients with PFAS and healthy subjects, we also performed BAT-CD203c in healthy subjects who did not have food allergies.

***Specific immunoglobulin E test***

Allergen-specific IgE was measured using ImmunoCAP™ (Thermo Fisher Diagnostics, Uppsala, Sweden). We defined allergen-specific IgE antibody titers of $<0.1$ $U_A$/mL as 0 $U_A$/mL and of $\geqq 100$ $U_A$/mL as 100 $U_A$/mL. We also defined allergen-specific IgE antibody titer of $\geqq 0.35$ $U_A$/mL (CAP class 1) as positive.

***Prick-to-prick test***

Patients were instructed to stop taking antihistamines 3 days before undergoing PPT. Bifurcated Needle® (Allergy Laboratories, Ohio, Inc., USA) was used as the pricking needle. After piercing the pulp of raw fruits or vegetables with a pricking needle, it was pressed against the skin of the patient's forearm[22]. Allergen Scratch Extract Positive control (Torii) histamine dihydrochloride® (Torii Pharmaceutical Co., Ltd., Japan) and saline were used as positive and negative controls, respectively. Swelling more than twice the size of the positive control was defined as 4 (+), equivalent to that of 3 (+), half of that as 2 (+), less than half of that and larger than the size of the negative control as 1 (+), and equivalent or smaller than that of negative control as 0 (−).We defined 2 (+) or more as a positive response.

***Extraction of fruit and vegetable allergens***

In brief, the pulp of raw fruits or vegetables was minced and pureed in a food processor, and extraction buffer was added to it. The particles were separated by centrifugation for 10 min at 2000 rpm. The supernatant was collected for use as an extract and dialyzed against phosphate-buffered saline (PBS) overnight. The extracts were freeze-dried and stored at −80℃ until use. The frozen extracts were reconstituted in PBS, and the protein concentration in each extract was measured with a spectrophotometer (NanoDrop™ 2000c, Thermo Fisher Scientific, Wilmington, DE, USA) and diluted to 10 and 100 μg/mL with PBS at the time of use.

***Basophil activation test (BAT-CD203c)***

To quantify basophil CD203c expression, BAT was performed using a commercial kit (Allergenicity Kit, Beckman Coulter, Fullerton, CA, USA) in a manner similar to that in a previous study[17]. In brief, ethylenediaminetetraacetic acid-containing whole blood was incubated with each antigen extract at protein concentrations of 10 and 100 μg/mL for 10 min at 37℃ after the addition of activation solution

and the mixture of phycoerythrin-cyanine 7-labeled anti-CD3, fluorescein isothiocyanate-labeled anti-CRTH2, and phycoerythrin-labeled anti-CD203c for cell activation and staining of cell surface antigens. Anti-IgE antibody at 10 μg/mL was used as a positive control, and PBS was used as a negative control. The samples were analyzed using a flow cytometer (Gallios™, Beckman Coulter). Basophils were identified based on their forward- and side-scatter properties, the absence of CD3 expression, and the presence of CRTH2 expression. Upregulation of CD203c on basophils was determined using a threshold defined by the fluorescence of unstimulated cells (negative control). The ratio of CD203c expression level induced by a food allergen to that induced by PBS was expressed as the CD203c stimulation index (SI), and CD203c SI ≧2 was defined as positive[16]. Patients with CD203c expression levels induced by an anti-human IgE antibody <10% were defined as low responders and excluded from the analysis.

***Oral food challenge***

OFC was performed openly. The patients ingested 20g of raw fruits or vegetables at 5-min intervals and up to 100g. OFC was discontinued if subjective or objective symptoms were observed, and total intake was defined as the threshold dose.

***Statistical analyses***

Sensitivity was calculated as the positivity rate for each test in the patients with PFAS. The correlation between threshold doses and BAT-CD203c SI was analyzed using Spearman's rank correlation coefficient. The difference in BAT-CD203c SI between patients with PFAS and healthy subjects was analyzed using the Mann-Whitney U test. Statistical significance was set at $p<0.05$. Statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

***Ethics statement***

This study was approved by the Ethical Committee of Osaka City University Graduate School of Medicine (#4411). Informed consent was obtained from the patients or their guardians.

## Results

***Characteristics of patients***

Ten patients were enrolled and underwent clinical testing (Table 1). Patients aged 8 to 19 (median, 12) years were included, and six (60%) were male. Food allergens included peach (70%), watermelon (60%), and tomato (20%). Half of the patients had atopic dermatitis and bronchial asthma, and all patients had allergic rhinitis. The median total IgE levels were 1200 (range: 360-2700) IU/mL, and the median pollen-specific IgE levels were higher in the order Japanese cedar (83 $U_A$/mL), alder (64.7 $U_A$/mL), and orchard grass (2.13 $U_A$/mL). No low responders were found in BAT.

***Sensitivity of tests***

The sensitivities of the crude antigen-specific IgE antibody test, PPT, and BAT-CD203c against food allergens in the diagnosis of PFAS were examined (Table 2). The sensitivities of the crude antigen-specific IgE antibody test were 85.7%, 83.3%, and 100% for peach, watermelon, and tomato, respectively. Similarly, the sensitivities of PPT, BAT-CD203c in the extract at 10 μg/mL, and BAT-CD203c in the extract at 100 μg/mL were 71.4%, 50%, and 50%; 14.3%, 33.3%, and 100%; and 85.7%, 100%, and 100% for peach, watermelon, and tomato, respectively. For total food allergens, BAT-CD203c in the extract at 100 μg/mL showed the highest sensitivity (93.3%), followed by the crude antigen-specific IgE antibody test (86.7%), PPT (60%), and BAT-CD203c in the extract at 10 μg/mL

**Table 1. Demographics and clinical test results of ten patients with PFAS**

| Subject | Age/Sex | AD | BA | AR | T-IgE | Japanese cedar sIgE | Alder sIgE | Orchard grass sIgE | Allergen | Crude antigen sIgE | PPT | BAT-CD203c SI (10 μg/mL) | BAT-CD203c SI (100 μg/mL) | Threshold doses |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8/F | + | − | + | 2700 | 90.7 | 100 | 11.5 | Peach | 84.4 | 2 | 1.02 | 39.36 | NA |
| | | | | | | | | | Watermelon | 5.8 | 2 | 4.22 | 66.79 | NA |
| 2 | 12/M | − | − | + | 2000 | 100 | 86.7 | 9.14 | Watermelon | 5.54 | 3 | 13.24 | 76.35 | 20g |
| | | | | | | | | | Tomato | 13.3 | 3 | 6.32 | 73.48 | NA |
| 3 | 9/F | + | + | + | 1400 | 57.5 | 17.8 | 0.48 | Peach | 15.0 | 2 | 3.13 | 39.84 | 40g |
| 4 | 14/M | + | + | + | 576 | 100 | 27.8 | 32.5 | Watermelon | 1.47 | 3 | 0.44 | 146.79 | NA |
| 5 | 19/F | + | + | + | 2339 | 29.0 | 0.89 | 2.16 | Watermelon | 0.82 | 0 | 1.62 | 157.86 | NA |
| 6 | 18/M | − | − | + | 835 | 75.3 | 53.3 | 2.09 | Peach | 10.4 | 2 | 0.56 | 21.35 | 100g |
| 7 | 12/F | − | − | + | 360 | 0.14 | 8.07 | 0.19 | Peach | 0.1 | 0 | 0.60 | 1.31 | 80g |
| | | | | | | | | | Watermelon | 0 | 0 | 1.21 | 81.0 | 40g |
| 8 | 11/M | − | − | + | 1207 | 100 | 100 | 24.7 | Peach | 50.5 | 4 | 1.40 | 15.55 | NA |
| | | | | | | | | | Watermelon | 1.78 | 0 | 1.47 | 17.65 | NA |
| 9 | 16/M | − | + | + | 873 | 43.7 | 76.1 | 2.0 | Peach | 27.7 | 0 | 1.21 | 16.63 | NA |
| | | | | | | | | | Tomato | 1.73 | 0 | 2.62 | 13.76 | NA |
| 10 | 12/M | + | + | + | 1192 | 100 | 92.9 | 1.47 | Peach | 59.1 | 2 | 1.33 | 18.03 | NA |

PFAS, pollen-food allergy syndrome; AD, atopic dermatitis; BA, bronchial asthma; AR, allergic rhinitis; T-IgE, total IgE; sIgE, specific IgE; PPT, prick-to-prick test; BAT, basophil activation test; SI, stimulation index; and NA, not available.

**Table 2. Results and sensitivities of tests in PFAS patients**

| Test | Median (range) | Sensitivity |
|---|---|---|
| Specific IgE ($U_A$/mL) | | |
| Peach | 27.7 (0.1-84.4) | 85.7% |
| Watermelon | 1.63 (0-5.8) | 83.3% |
| Tomato | 7.52 (1.73-13.3) | 100% |
| Total | 5.8 (0-84.4) | 86.7% |
| PPT (+) | | |
| Peach | 2 (0-4) | 71.4% |
| Watermelon | 1 (0-3) | 50% |
| Tomato | 1.5 (0-3) | 50% |
| Total | 2 (0-4) | 60% |
| BAT-CD203c SI (extract at 10 μg/mL) | | |
| Peach | 1.21 (0.56-3.13) | 14.3% |
| Watermelon | 1.54 (0.44-13.24) | 33.3% |
| Tomato | 4.47 (2.62-6.32) | 100% |
| Total | 1.40 (0.44-13.24) | 33.3% |
| BAT-CD203c SI (extract at 100 μg/mL) | | |
| Peach | 18.03 (1.31-39.84) | 85.7% |
| Watermelon | 78.67 (17.65-157.86) | 100% |
| Tomato | 43.62 (13.76-73.48) | 100% |
| Total | 39.36 (1.31-157.86) | 93.3% |

PPT, prick-to-prick test; BAT, basophil activation test; and SI, stimulation index.

(33.3%).

***Correlation between BAT-CD203c SI and threshold dose***

OFC was performed on four patients who consented to the test (peach: n=2, watermelon: n=1,

both peach and watermelon: n=1), and the correlation between threshold doses and BAT-CD203c SI was examined (Fig. 1). There was a significant negative correlation between threshold doses and BAT-CD203c SI in the extract at 10 μg/mL ($r_s$=−0.975, p=0.005). The correlation between threshold doses and BAT-CD203c SI in the extract at 100 μg/mL tended to be negative, although not significant ($r_s$=−0.718, p=0.172).

***Difference in laboratory results between systemic and oral symptoms in peach allergy***

The laboratory results of seven patients who had oral allergy symptoms (OAS) (OAS patients) after eating raw peach were compared with those of a patient who had systemic symptoms [systemic reaction (SR) patient] (Table 3). Total IgE was normal in the SR patient, but it was high in OAS patients. Peach-specific IgE was negative in the SR patient; in contrast, it was positive in six of the seven OAS patients. Specific IgEs against Pru p 1, 3, 4, and 7, which are peach allergen components, was measured in the SR and OAS patients. In the SR patient, Pru p 1-, 3-, and 4-specific IgE levels were negative, and the Pru p 7-specific IgE level increased to 0.67 $U_A$/mL (CAP class 1). Pru p 7 as peach gibberellin-regulated protein (GRP) has been identified[8], and GRP has been reported as food antigens that result in systemic symptoms[10]. Therefore, we diagnosed the SR patient as having peach GRP allergy. In contrast, among the OAS patients, Pru p 1-specific IgE was positive in six of the seven patients, Pru p 4-specific IgE was positive in two of the seven patients, and Pru p 3- and 7-specific IgE were negative in all patients. Alder-specific IgE was negative in the SR patient and positive in all OAS patients. PPT was positive in the SR patient and in five of the seven OAS patients. BAT-CD203c was performed on the SR patient, the seven OAS patients, and six healthy controls (Fig. 2). In healthy controls, BAT-CD203c SI in the extracts at both 10 and 100 μg/mL was negative. In the OAS patients, BAT-CD203c SI in the extract at 10 μg/mL was positive in one of the seven patients and in the extract at 100 μg/mL was positive in six of the seven patients. BAT-CD203c

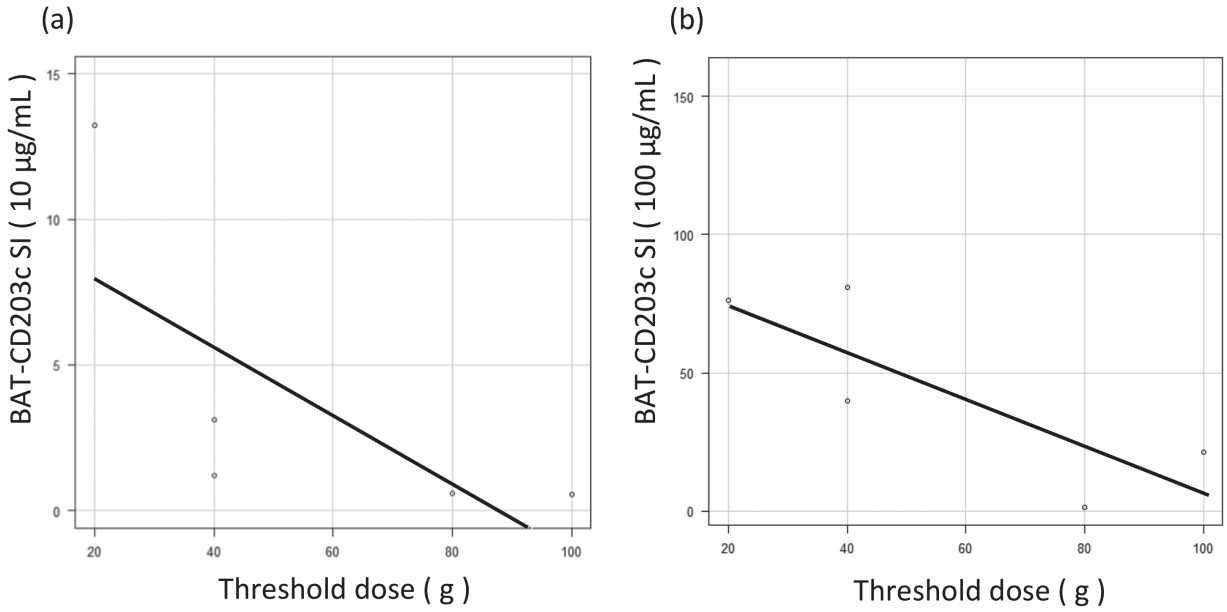


**Figure 1.** Correlation between BAT-CD203c SI and threshold dose in patients with PFAS. There was a significant negative correlation between threshold doses and BAT-CD203c SI in the extract at (a) 10 μg/mL (n=5, $r_s$=−0.975, p=0.005). The correlation between threshold doses and BAT-CD203c SI in the extract at (b) 100 μg/mL tended to be negative, although not significant (n=5, $r_s$=−0.718, p=0.172). BAT, basophil activation test; SI, stimulation index; and PFAS, pollen-food allergy syndrome.

SI in the extract at 100 μg/mL was significantly higher in the OAS patients than in healthy controls. In the SR patient, BAT-CD203c SI in the extract at both 10 and 100 μg/mL was positive, and BAT-CD203c SI in the extract at 10 μg/mL was markedly higher than that in the OAS patients and healthy controls.

**Table 3. Demographics and sensitization of patients with peach allergy**

| | Peach allergy | |
|---|---|---|
| | SR (n=1) | OAS (n=7) |
| Age (y) | 24 | 12 (8-18) |
| Female | 1 | 3 (43) |
| Total IgE (IU/mL) | 35 | 1192 (360-2700) |
| Specific IgE ($U_A$/mL) | | |
| Peach | 0 | 27.7 (0.1-84.4) |
| Pru p 1 | 0 | 44.8 (0-100) |
| Pru p 3 | 0 | 0 (0-0.34) |
| Pru p 4 | 0 | 0 (0-46) |
| Pru p 7 | 0.67 | 0 (0-0.29) |
| Japanese cedar | 6.38 | 75.3 (0.14-100) |
| Alder | 0 | 76.1 (8.07-100) |
| PPT (+) | 2 | 2 (0-4) |

Values in OAS are expressed as median (range) or numbers (percentages). SR, systemic reaction; and OAS, oral allergy syndrome.

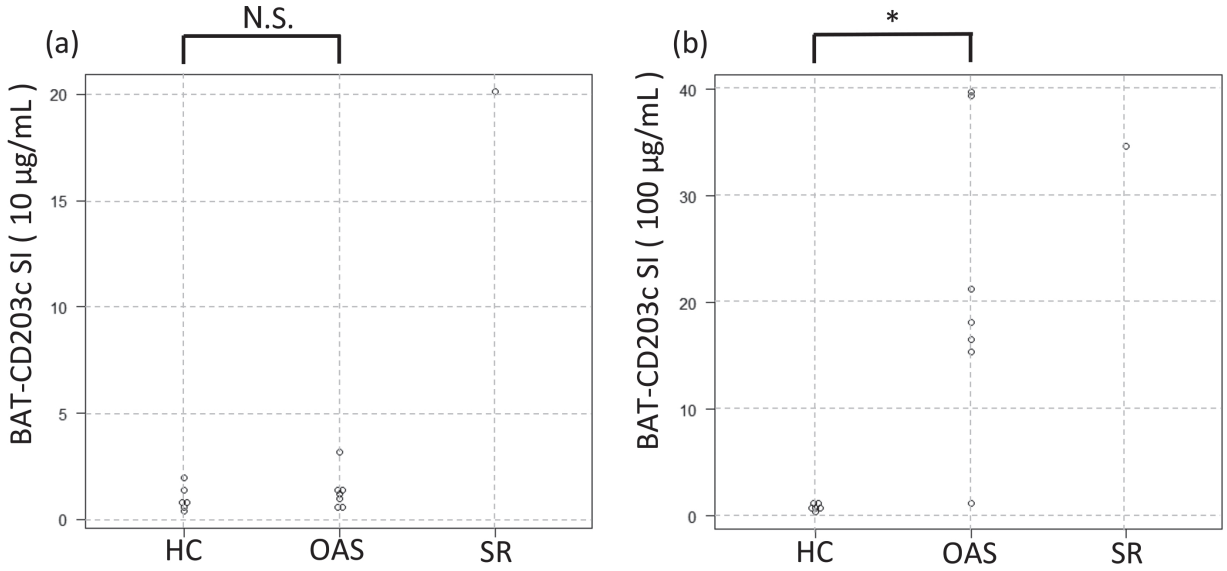


**Figure 2.** Comparison of BAT-CD203c SI in peach allergy and healthy control. BAT-CD203c was performed on the SR patient, the seven OAS patients, and six healthy controls. BAT-CD203c SI in the extract at (b) 100 μg/mL was significantly higher in the OAS patients than in healthy controls. In the SR patient, BAT-CD203c SI in the extract at both (a) 10 and (b) 100 μg/mL was positive, and BAT-CD203c SI in the extract at (a) 10 μg/mL was markedly higher than that in the OAS patients and healthy controls. *$p<0.05$. BAT, basophil activation test; SI, stimulation index; SR, systemic reaction; OAS, oral allergy syndrome; HC, healthy control; and N.S., not significant.

## Discussion

In this study, we found that BAT-CD203c using crude antigen extracts showed high sensitivity in the diagnosis of PFAS. Moreover, the higher the BAT-CD203c SI, the lower the threshold dose. We suggest that BAT-CD203c using peach extracts may distinguish between systemic and oral symptoms in peach allergy.

It has been reported that BAT has a high sensitivity and specificity in the diagnosis of peanut, milk, egg, and wheat allergies[15-17] and has a high diagnostic accuracy for PFAS[18-21]. In this study, we showed that BAT has a high sensitivity for the diagnosis of PFAS, a result consistent with those of previous studies. There have been no reports examining the correlation between threshold doses and BAT-CD203c. In this study, we reported for the first time that there was a significant negative correlation between threshold doses on the basis of the results of OFC and BAT-CD203c SI for PFAS. Therefore, BAT may be useful for predicting the threshold doses of food allergens for PFAS. There have been some reports examining the difference in BAT between systemic and oral symptoms for PFAS[20,21]. However, there has been no consensus on this topic. In fruit and vegetable allergies, lipid transfer protein (LTP) and GRP have been reported as food antigens that result in systemic symptoms and sometimes anaphylaxis[9,10]. Pru p 3 as peach LTP and Pru p 7 as peach GRP have been identified[8,23]. Fruit peels contain more LTP, whereas fruit pulp contains more GRP. It has been suspected that the causative antigen of severe peach allergy in Japan is mainly Pru p 7 because most Japanese people eat peeled peaches[10]. In recent years, it has been reported that cypmaclein, a cypress GRP, is cross-reactive with Pru p 7[24]. In this study, the patient who had systemic symptoms after eating raw peaches was diagnosed as having GRP allergy because of an increase in the Pru p 7-specific IgE level. In this patient, BAT-CD203c in the peach extract at 10 μg/mL was markedly higher than that in the patients who had only oral symptoms and healthy controls. This suggests that BAT-CD203c may be useful in predicting the risk of systemic symptoms in PFAS.

Currently, there is no unified standard for the concentration of antigen extracts used in BAT. In this study, BAT was performed in extracts at two concentrations (10 and 100 μg/mL), and the results at each concentration were analyzed. As a result, there were some differences in the results between the two concentrations. First, the sensitivity of BAT in diagnosing PFAS was high at 100 μg/mL, whereas it was low at 10 μg/mL, except for tomatoes. Second, BAT-CD203c SI in peach OAS was significantly higher only at 100 μg/mL than in healthy controls. Third, there was a significant negative correlation between threshold doses and BAT-CD203c SI in the extract at 10 μg/mL. Fourth, BAT-CD203c SI in the peach extract at 10 μg/mL was markedly higher in the SR patient than in the OAS patients and healthy controls; however, the SI at 100 μg/mL did not differ between the SR patient and the OAS patients. Therefore, we suggest that BAT in extracts at a higher concentration (100 μg/mL) and a lower concentration (10 μg/mL) may be useful for diagnosing PFAS and for predicting the severity of PFAS, respectively. BAT should be performed simultaneously at multiple antigen concentrations for one causative food. In contrary, PPT, which was reported to have high sensitivity in the diagnosis of PFAS, was less sensitive than BAT and the crude antigen-specific IgE test in this study. This result is possibly attributed to the fact that some patients did not comply with discontinuation of antihistamines before PPT and that the doctor who performed PPT was not the same person at the two medical institutions.

This study has several limitations. First, in this study, the sensitivity of each test could be obtained, but the specificity could not be obtained, which was insufficient to evaluate the diagnostic

accuracy. However, by comparing the sensitivities of the tests in this study, it was found that BAT is a useful test for the exclusion diagnosis of PFAS. Second, the number of patients in this study was small, and only one patient had systemic symptoms due to the ingestion of a raw peach. Therefore, it was insufficient to compare BAT-CD203c SI in SR patients with that in OAS patients. Long-term prospective studies are required in the future. Third, in this study, open OFCs were performed instead of DBPCFC. This may have affected the results because PFAS mainly causes subjective symptoms such as itching and tingling in the oropharyngeal area. The medical institutions where DBPCFC can be performed are limited; therefore, large multicenter studies will be needed in the future.

In conclusion, BAT-CD203c has high sensitivity for the diagnosis of PFAS and may be useful for predicting the severity of PFAS. We suggest that BAT is a reliable tool for predicting PFAS.

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