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# Clinical utility of basophil activation test in diagnosis and predicting severity of mugwort pollen-related peach allergy

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ABSTRACT

*Background:* Cross-reactivity between pollen and plant foods results in low specificity of food-IgE and skin prick testing, which may cause over-diagnosis. A test that can accurately diagnose pollen-related food allergy and identify patients at risk of developing severe reactions is needed. This study evaluates basophil CD63 expression as a biomarker for diagnosis and predicting severity of mugwort pollen-related peach allergy.
*Methods:* Based on their allergic reactions to peach, an oral allergy symptom group (OAS, n = 15), a systemic reaction group (SR, n = 23), a peach-sensitized but tolerant group (PST, n = 21) and a non-peach-sensitized nonallergic group (NSE, n = 10) were identified among mugwort pollen allergic patients. Measurements of specific IgE to peach and its components, and basophil activation test (BAT) were performed.
*Results:* Upon stimulation with peach extract, BAT in peach-allergic patients (OAS and SR groups) showed a significant dose-dependent upregulation of CD63 compared with PST patients, but showed no difference between SR and OAS groups. BAT to Pru p 3 could discriminate not only between sensitization and clinical allergy, but also between OAS and systemic reactions. BAT to Pru p 3 revealed 92% sensitivity, 95% specificity, 92% positive predictive value, and 92% negative predictive value. Receiver operating characteristic curves showed that BAT to Pru p 3 had the largest area under the curve.
*Conclusions:* In the diagnosis of mugwort pollen-related peach allergy, BAT to Pru p 3 is superior to testing for IgE specific for peach and its components. Additionally, basophil activation can predict clinical severity.

## Background

Peach allergy is common in China, especially among patients with mugwort pollen allergy. It usually presents with severe allergic reactions. Jiang et al. reported 907 patients with anaphylaxis, in which 33% of reactions were caused by fruits/vegetables, with peach being the most common trigger. Among those peach-induced anaphylaxis cases, 71% were allergic to mugwort.[1] Mugwort pollen is the most important contributor to allergic rhinoconjunctivitis and asthma in late summer and autumn in China, especially in the northern region. In addition, patients with mugwort pollen allergy may develop allergic reactions to fruits and vegetables, most commonly peach. Recently, Art v 3, a lipid transfer protein (LTP) from mugwort, was identified as the sensitizer in a Chinese population with peach allergy, in which Pru p 3 (peach LTP) was the major allergen.[2,3] In sharp contrast to LTP-associated peach allergy in the Mediterranean area—in which sensitization to LTPs seems independent of any pollen hypersensitivity[4]—LTP-associated peach allergy in China mainly originates from primary sensitization to mugwort pollen.[5,6] In addition to Pru p 3, other peach components, namely, Pru p 1 (pathogenesis-related protein 10, PR-10), Pru p 2 (thaumatin-like protein, TLP) and Pru p 4 (profilin), have been identified.[7–9]

The diagnosis of food allergy is mainly based on a history of food-induced allergic reaction, skin prick testing (SPT) and the measurement of food-specific IgE (sIgE).[10,11] However, SPT and sIgE have the problems of misdiagnosis and risk stratification, as they are not accurate enough in predicting which kind of reaction the patient may experience in the future.

*Abbreviations:* BAT, basophil activation test; LTP, lipid transfer protein; sIgE, specific IgE; DBPCFC, double-blind placebo-controlled food challenge; SPT, skin prick testing; OAS, oral allergy syndrome; SR, systemic reaction; ROC, receiver operating characteristic; AUC, area under the curve; SABA, short-acting $\beta_2$-agonist.

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Although double-blind placebo-controlled food challenge (DBPCFC) is the gold standard in the diagnosis of a food allergy, it is not included in standard patient management, because it is time-consuming and poses a risk of causing anaphylaxis.[12,13] Thus, diagnostic tests that could usefully discriminate between sensitized and symptomatic subjects, and identify patients at a higher risk of developing severe systemic symptoms, would improve the clinician's ability to provide wise dietary counsel to patients.

The basophil activation test (BAT) is an in vitro test that could reflect the IgE-mediated pathophysiology of food allergy. The expression of CD63 is highly specific for IgE-mediated basophil activation.[14] In resting basophils, CD63 is barely detectable on the surface membrane. Upon challenge with specific allergens, CD63 becomes highly expressed at the surface of activated basophil cells. CD63 expression correlated with histamine release during anaphylaxis.[15] Thus, detection of CD63 on the surface of basophil cells could be a useful biomarker for predicting clinical allergy and severity.

Several studies have suggested that BAT is a useful tool for the diagnosis of food allergies, with sensitivity ranging from 77 to 98% and specificity 75–100%.[16–19] To date, there are no data available regarding BAT in the diagnosis of mugwort pollen-related food allergy.

The aim of this study was to investigate the BAT in discriminating between sensitized and symptomatic peach-allergic subjects and predicting clinical severity in mugwort pollen-related peach allergy. Furthermore, we assessed the utility of BAT in response to peach extract and to Pru p 3, respectively.

## Methods

### *Study population*

Subjects allergic to mugwort pollen were prospectively recruited from January to May 2016 at the Department of Allergy, Peking Union Medical College Hospital. Based on their clinical reactions after ingesting peach, mugwort pollen-related peach-allergic (MPRPA), peach-sensitized but tolerant (PST) and non-peach-sensitized nonallergic (NSE) individuals were consecutively enrolled. All the individuals experienced mugwort pollen allergy, confirmed by a positive skin test and IgE for mugwort pollen as well as a clinical history of autumnal pollinosis. Mugwort pollen-related peach-allergic patients (MPRPA) reported immediate adverse reactions occurring within 1 h after the ingestion of peach and a positive skin prick test and/or specific IgE to peach. According to clinical symptoms after ingesting peach, MPRPA patients were subcategorized into 2 groups as follows: group 1, symptoms localized to the oral mucosa (oral allergy syndrome, OAS); group 2, systemic reactions (SR) including general urticaria, angioedema, laryngeal angioedema, respiratory difficulty, gastrointestinal disorders or circulatory symptoms of anaphylaxis. Peach-sensitized but tolerant (PST) patients were identified who had positive IgE to peach, but were able to eat peach without developing allergic symptoms. This group therefore was distinct from the two MPRPA subgroups in which peach ingestion elicited clinical reactions.

All patients underwent clinical evaluation, sIgE to peach and its components, and basophil activation test. Written informed consent was obtained and the Ethics Committee of Peking Union Medical College Hospital approved the study.

### *Determination of allergen-specific IgE*

Quantifications of sIgE to mugwort pollen, peach and its allergenic components (Pru p 1, Pru p 3 and Pru p 4) were performed with ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE antibodies ≥0.35 kUA/L were considered positive.

### *Basophil activation test*

The BAT was performed with the Flow 2 CAST kit (Alpco Diagnostics, Windham, New Hampshire) as previously described.[16] Peach extract was prepared using a previously described protocol.[20] Heparinized whole blood was stimulated for 15 min at 37 °C with increasing concentrations (1 ng/mL–10 μg/mL) of peach extract and its major allergen Pru p 3 (12.5–100 ng/mL, Alpco), respectively. Polyclonal goat antihuman IgE (1 μg/mL, Alpco), monoclonal antibody recognizing the high-affinity IgE binding receptor (FcεRI) and N-formyl-methionyl-leucyl-phenylalanine were used as positive controls. Before erythrocyte lysis, cells were stained with CD63-FITC and CCR3-PE. Basophils were gated as $SSC^{low}/CCR3^{+}$, and among these, the $CD63^{+}$ cells were termed *activated basophils*. At least 300 basophils were analyzed using FloMax software (version 2.82, QA GmbH, Munster, Germany), and basophil activation was expressed as the % CD63 positive basophils (% $CD63^{+}$).

### *Statistical analysis*

Data analysis was performed using the statistical package SPSS/PC+ (SPSS, Chicago, IL, USA). Categorical variables were analyzed by Pearson's chi-squared test, and continuous variables were compared between groups by the Mann-Whitney *U* test. Analysis of receiver operating characteristic (ROC) curves was performed for sIgE, component testing and BAT to calculate the area under curve (AUC) to obtain the most accurate measurement. Differences were considered statistically significant at $P < .05$.

## Results

### *Study population*

In total, 69 mugwort pollen allergic subjects were enrolled. The median age was 26 years (range 11–58 years) and 48% were male. Among the study population, 38 mugwort pollen allergic patients were clinically allergic to peach (MPRPA), 31 subjects were able to eat peach (21 PST and 10 NSE). Based on their peach-induced symptoms, the 38 MPRPA patients were categorized into 15 OAS and 23 SR patients. Of patients with systemic reactions, 3 patients (13%) experienced expiratory dyspnea, 2 patients (9%) developed shock, and 11 patients (48%) were treated in the emergency department. Demographic characteristics of the study population are presented in Tables 1 and 2.

Regarding mugwort related respiratory symptoms of peach-allergic patients as a whole (OAS and SR), 95% had rhinitis and 53% asthma. The frequency of asthma among SR patients was higher than that of OAS patients, but there was no significant difference (33% OAS *vs* 65% SR, $P = .052$, Table 2). Considering the severity of asthma, the major frequency of asthma patients receiving short-acting $\beta_2$-agonists (SABAs) during mugwort pollen season was found in SR patients (7% OAS *vs* 35% SR, $P = .033$, Table 2).

**Table 1**
Demographic and molecule sensitization profiles of participants.

| Characteristic | MPRPA (n = 38) | Peach tolerant (n = 31) | | *P* value[a] |
|---|---|---|---|---|
| | | PST (n = 21) | NSE (n = 10) | |
| Age (y) | 22 (11–52) | 29 (13–58) | 27 (18–42) | .37 |
| Males | 20 (52.6) | 9 (42.9) | 4 (40) | .06 |
| tIgE | 232.5 (19–1810) | 241.5 (39–1462) | 72 (38–152) | .85 |
| sIgE | | | | |
| Peach | 11.2 (0.57–100) | 3.46 (0.38–38.5) | 0.01 (0–0.08) | **< .001** |
| Pru p 1 | 0.01 (0–25.1) | 0.01 (0–5.2) | 0.01 (0–0.02) | .65 |
| Pru p 3 | 8.64 (0.03–78.2) | 0.85 (0–32.7) | 0.01 (0–0.03) | **< .001** |
| Pru p 4 | 0.01 (0–32.7) | 0.01 (0–15.6) | 0.01 (0–0.02) | .81 |

Values are expressed as median (range) or numbers (percentages).
*MPRPA*, mugwort pollen-related peach-allergic patients; *PST*, peach-sensitized but tolerant patients; *NSE*, non-peach-sensitized nonallergic patients.
[a] *P* value refers to the comparison between MPRPA and PST patients.

**Table 2**
Demographic and clinical features of the study population according to severity groups.

| | Peach allergy (n = 38) | | *P* value |
|---|---|---|---|
| | OAS (n = 15) | SR (n = 23) | |
| Age (y) | 19 (11–47) | 28 (6–51) | .25 |
| Males | 9 (60) | 11 (47.8) | .52 |
| tIgE | 257 (21–1720) | 326 (30–1421) | .89 |
| sIgE | | | |
| Peach | 9.7 (0.68–100) | 11.8 (1.2–50.3) | .27 |
| Pru p 1 | 0.01 (0–25.8) | 0.01 (0–16.9) | .43 |
| Pru p 3 | 6.8 (0.03–45.6) | 11.3 (0.04–78.2) | **.042** |
| Pru p 4 | 0 (0–45.2) | 0.02 (0–18.4) | .91 |
| Mugwort pollen allergy | | | |
| Ocular symptoms | 12 (80) | 18 (78.3) | .90 |
| Nasal symptoms | 15 (100) | 21 (91.3) | ND |
| Asthma | 5 (33.3) | 15 (65.2) | .052 |
| Asthma treatment | | | |
| SABAs | 1 (6.7) | 8 (34.8) | **.033** |
| Management in ED | 0 | 2 (8.7) | ND |

Values are expressed as medians (range) or numbers (percentages).
*OAS*, oral allergy syndrome; *SR*, systemic reaction; *SABAs*, short-acting $\beta_2$-agonists; *ED*, emergency department; *ND*, not different.

*Level of peach sIgE differentiates between allergic and tolerant patients but does not correlate with severity of allergic reactions*

The median sIgE level to peach from clinically allergic patients was 11.2 kUA/L (range 0.57–100 kUA/L), whereas the median sIgE level from peach sensitized but tolerant (PST) patients was 3.46 kUA/L (range 0.38–38.5 kUA/L). The sIgE levels differed significantly in allergic and tolerant patients ($P < .001$, Table 1). Among the 38 MPRPA patients, the sIgE levels of SR and OAS patients were similar ($P = .27$, Table 2), indicating that the type of clinical allergic response could not be predicted based on sIgE quantification.

Total IgE (tIgE) levels were not different between allergic and tolerant patients, and there was no significant correlation between tIgE and the severity of allergic reactions (Tables 1 and 2).

*Pru p 3 sIgE differs between allergic and tolerant patients and correlates with severity of allergic reactions*

The median sIgE level to Pru p 3 from MPRPA patients was 8.64 kUA/L (range 0.03–78.2 kUA/L), which differed significantly from sIgE levels of the PST patients (0.85 kUA/L, range 0–32.7 kUA/L; $P < .001$, Table 1). SR patients had higher values for Pru p 3 than OAS patients ($P = .042$, Table 2).

The sIgE levels to Pru p 1 and Pru p 4 were not different between allergic and tolerant patients, and there was no significant correlation between these two sIgEs and the severity of allergic reactions (Tables 1 and 2).

*BAT to Pru p 3 discriminates between allergic and tolerant patients and correlates with severity of allergic reactions*

It is important to determine the optimal concentration that provokes the maximum cellular activation for each allergen.[17] Five concentrations of peach extract were used to challenge the basophils in vitro. In MPRPA patients, basophils showed increased expression of CD63 with increasing concentrations of peach extract from 1 ng/mL up to 100 ng/mL followed by a plateau. Compared to the basophils of MPRPA patients, the basophils from PST and NSE patients did not significantly respond to peach (Fig. 1). In our study the optimal concentration of peach extract was 100 ng/mL, while the optimal concentration of Pru p 3 was 25 ng/mL. CD63 expression from the MPRPA basophils stimulated by peach or Pru p 3 was significantly higher than that from PST and NSE subjects ($P < .001$, Fig. 1). Spearman's correlation coefficients for sIgE and the percentage of $CD63^+$ basophils for peach and Pru p 3 were 0.36 and 0.49, respectively.

No significant differences were detected between the OAS and SR groups by comparing CD63 expression using several peach extract concentrations ($P = .28–.42$, Fig. 2). However, after stimulation with 25 ng/mL Pru p 3, the SR group showed a higher proportion of $CD63^+$ basophils than OAS patients ($P < .001$, Fig. 2).

*BAT is superior to sIgE and component testing in discriminating between allergic and tolerant patients and predicting severity of allergic reactions*

Receiver operating characteristic curves were used to compare the performance of sIgE, component testing and BAT in the diagnosis of mugwort pollen-related peach allergy (MPRPA) (Fig. 3). The BAT after stimulation with Pru p 3 (AUC 0.96, 95% confidence interval 0.916–1.000, $P < .001$) had the largest AUC compared with BAT at 100 ng/mL peach (AUC 0.90, 95% confidence interval 0.804–0.997, $P < .001$), sIgE to peach (AUC 0.73, 95% confidence interval 0.592–0.873, *P*

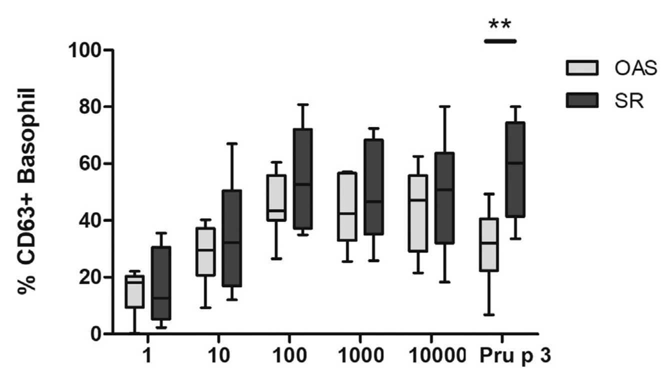


**Fig. 2.** BAT at different doses of peach extract (1 ng/mL–10 μg/mL) and Pru p 3 (25 ng/mL) in OAS (n = 15) versus SR (n = 23) groups. **P < .001. OAS, oral allergy syndrome; SR, systemic reaction.

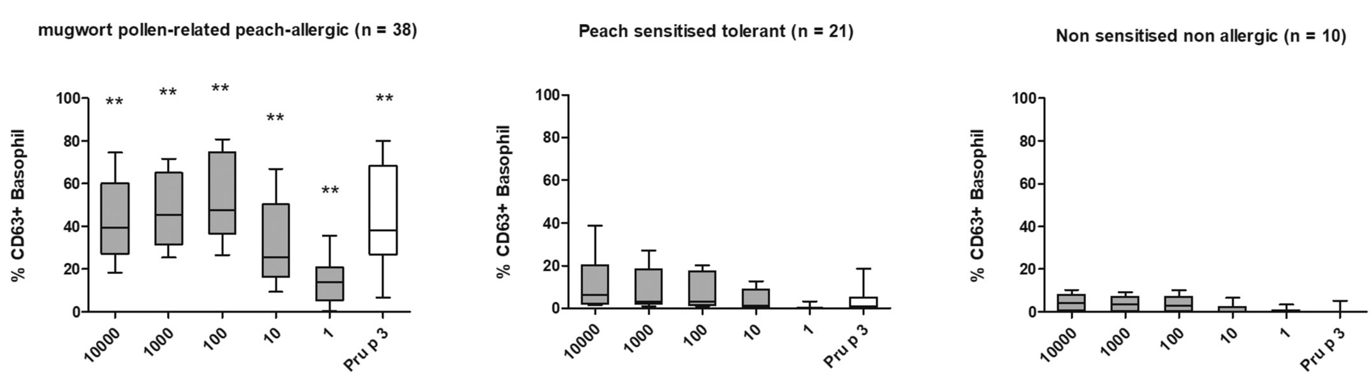


**Fig. 1.** BAT upon stimulation with peach extract (1 ng/mL–10 μg/mL) and Pru p 3 (25 ng/mL) in mugwort pollen-related peach-allergic (MPRPA, n = 38), peach-sensitized but tolerant (PST, n = 21) and non-peach-sensitized nonallergic group (NSE, n = 10) patients. **P < .001.

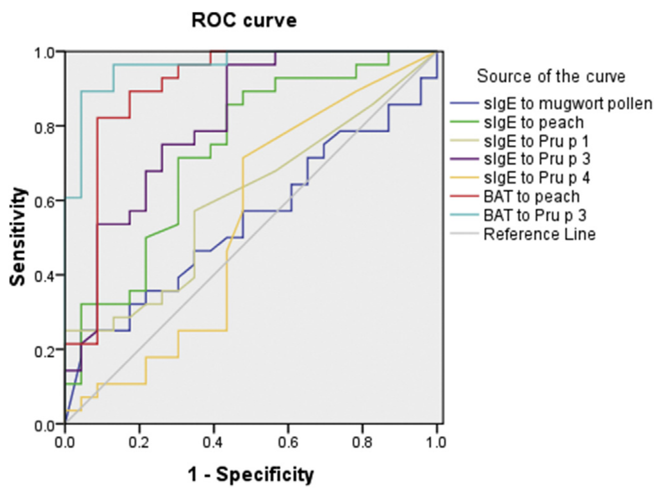


**Fig. 3.** ROC curve analysis for specific IgE, component testing and BAT in predicting peach allergy. BAT stimulated with 25 ng/mL Pru p 3 had the largest area under ROC curve (AUC 0.96, 95% CI 0.916–1.000, P < .001) compared with BAT at 100 ng/mL peach (AUC 0.90, 95% CI 0.804–0.997, P < .001), specific IgE to peach (AUC 0.73, 95% CI 0.592–0.873, P = .005), and specific IgE to Pru p 3 (AUC 0.81, 95% CI 0.690–0.932, P < .001). AUC, the area under curve; CI, confidence interval.

= .005), and sIgE to Pru p 3 (AUC 0.81, 95% confidence interval 0.690–0.932, $P < .001$, Fig. 3). BAT to Pru p 3 revealed the best diagnostic performance, with 92.3% sensitivity, 94.6% specificity, 92.3% positive predictive value (PPV), and 91.7% negative predictive value (NPV) (Table 3).

Based on allergic reactions after ingesting peach, the 38 MPRPA patients were categorized into 15 OAS and 23 SR patients. Fig. 4 shows the ROC curve analysis for specific IgE to peach and its allergenic components and BAT in predicting systemic reactions to peach. BAT stimulated with Pru p 3 had the largest area (AUC 0.90, 95% confidence interval 0.762–1.000, $P = .001$) compared with BAT stimulated with 100 ng/mL peach (AUC 0.71, 95% confidence interval 0.511–0.900, $P = .08$), and specific IgE to Pru p 3 (AUC 0.73, 95% confidence interval 0.534–0.921, $P = .049$).

## Discussion

This study demonstrates that the use of BAT stimulated with Pru p 3 could discriminate between sensitized and allergic subjects, and predict the severity of allergic reactions to peach in mugwort-allergic patients. Mugwort is the most important allergenic pollen in late summer and autumn in China. Our previous study found that 72% of the subjects with mugwort pollinosis developed food allergy, and peach was the most common trigger, usually causing severe reactions.[21] Indeed, in this study population with clinical peach allergy, seven patients experienced dyspnea, five patients developed shock, and half of the patients were treated in the emergency department. Current management of food allergy relies on allergen avoidance and the prescription of epinephrine to patients with the risk of anaphylaxis. Knowing whether individual patients have a risk of developing severe reactions would improve the care for patients. Thus, diagnostic tests that could identify symptomatic peach allergic subjects and predict the potential risk of severe reactions are desirable.

It has been reported that component testing is useful for the diagnosis of food allergies and predicting the severity of allergic reactions, but little data is available in mugwort pollen-related food allergy.[22–24] Recent studies indicated that the BAT could discriminate between peanut allergic and tolerant children and predict severity of allergic reactions during oral food challenge.[25,26] However, the BAT is currently only used for clinical research, not as a routine clinical test. Before being relevant in clinical management, the diagnostic utility of BAT needs to be validated for specific food allergens and in different populations. In this study, the utility of sIgE, component testing and BAT were compared for diagnosing mugwort pollen-related peach allergy (MPRPA) and predicting the severity of allergic reactions. Our study showed that BAT stimulated by Pru p 3 is the best diagnostic test, and basophil activation given by % $CD63^+$ was the best predictor of allergy severity.

In our study, higher levels of sIgE to peach were noted in allergic subjects compared with tolerant subjects, but they were comparable in OAS and SR groups. Consistent with previous studies, sIgE positivity usually reflects a state of sensitization that is not well associated with severity of allergic reactions. But, the higher the levels of sIgE are, the more the risk of developing some form of clinical allergic reaction increases. However, in line with the study by Rossi et al.,[27] we observed that the levels of sIgE to Pru p 3 could not only discriminate between allergic and tolerant subjects, but also predict the severity of clinical symptoms. We found the detection of sIgE to Pru p 3 to be superior to sIgE to peach in diagnosing mugwort pollen-related peach allergy and predicting its severity.

To investigate the utility of BAT in mugwort pollen-related peach allergy, peach extract and Pru p 3, respectively, were used to challenge the basophils in vitro. In MPRPA patients, BAT showed a peach dose-dependent upregulation of CD63 followed by a plateau. The basophil activation to peach extract was higher in allergic patients than in tolerant subjects, but the BAT results were comparable in OAS and SR groups, limiting its utility in predicting severity. In contrast, the basophil activation to Pru p 3 correlated not only with clinical allergy but also with the severity of symptoms. Evaluating the diagnostic performance of each test by ROC-curve analysis, we observed that BAT stimulated with Pru p 3 had the largest AUC and the best diagnostic performance. The BAT at 100 ng/mL peach extract had higher sensitivity, but lower specificity. This is probably due to the cross-reaction between pollen and plant food allergen, thereby causing false positive results in some of mugwort-allergic patients. Perhaps for this reason, BAT to Pru p 3, the major peach allergen, is superior to BAT to peach extract in the diagnosis and assessment of severity in mugwort pollen-related peach allergy.

The present study focused on a comparison between symptomatic peach-allergic (MPRPA) and sensitized but tolerant (PST) subjects, addressing the possible effect of cross-reactivity on the performance of diagnostic tests. In line with previous studies of primary food allergy,[26,28] BAT could improve the diagnosis of mugwort pollen-related peach allergy over use of sIgE and component testing, and also predict clinical severity.

This is the first study that investigates BAT in mugwort pollen-related food allergy. Limitations of the study include the small sample size and lack of an independent cohort. In addition, the diagnosis of peach allergy was based on clinical history and a skin prick test and/or specific IgE,

**Table 3**
Optimal cutoffs for peach-sIgE, Pru p 3-sIgE and BAT to peach and Pru p 3 with the largest area under the ROC curve in predicting peach allergy.

| | Cutoff | AUC | Sensitivity | Specificity | PPV | NPV | LR+ | LR- |
|---|---|---|---|---|---|---|---|---|
| Peach-sIgE | 4.06 | 0.73 | 76.9% | 66.7% | 71.4% | 72.7% | 2.3 | 0.3 |
| Pru p 3- sIgE | 0.64 | 0.81 | 92.3% | 66.7% | 76.5% | 87.5% | 3 | 0.1 |
| % $CD63^+$ (peach) | 19.4 | 0.90 | 100% | 86.3% | 81.3% | 100% | 7.3 | – |
| % $CD63^+$ (Pru p 3) | 13.0 | 0.96 | 92.3% | 94.6% | 92.3% | 91.7% | 17.1 | 0.08 |

ROC, receiver operating characteristic; AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio; % $CD63^+$, percentage of CD63-positive basophils.

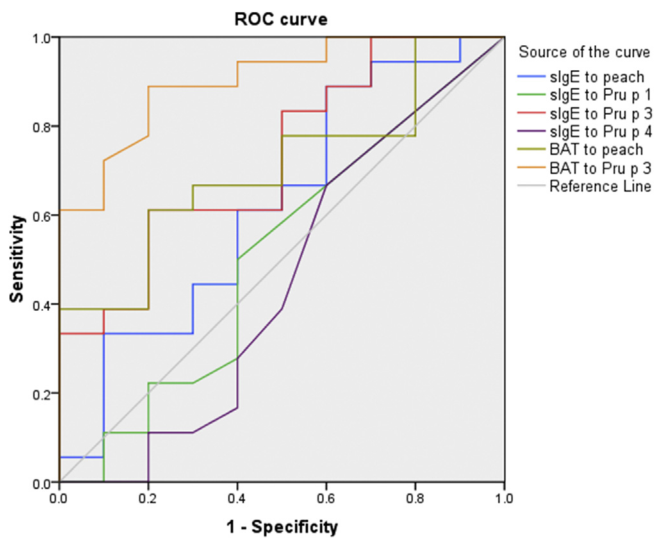


**Fig. 4.** ROC curve analysis for specific IgE to peach and its allergenic components and BAT in predicting systemic reactions to peach. BAT stimulated with 25 ng/mL Pru p 3 had the largest area under ROC curve (AUC 0.90, 95% CI 0.762–1.000, P = .001) compared with BAT at 100 ng/mL peach (AUC 0.71, 95% CI 0.511–0.900, P = .08), and specific IgE to Pru p 3 (AUC 0.73, 95% CI 0.534–0.921, P = .049). AUC, the area under curve; CI, confidence interval.

rather than a double-blind placebo-controlled food challenge. It is difficult to obtain ethical approval in China for a food challenge in patients at risk of anaphylaxis.

## Conclusions

In conclusion, BAT to Pru p 3 proved to be superior to other diagnostic tests in discriminating between sensitized and allergic subjects, and predicting the severity of allergic reactions to peach in mugwort-allergic patients. These data provide new evidence that supports efforts to introduce BAT into routine allergy diagnosis.

## Declarations

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### *Competing interests*

The authors declare that they have no competing interests.

### *Authors contributions*

Shan Deng performed the tests, drafted the main body of the manuscript and prepared the figures and tables. Jia Yin designed the study and revised the article. Both authors read and approved the final manuscript.

### *Ethics approval and consent to participate*

The Ethics Committee of Peking Union Medical College Hospital approved the study. All the patients have signed a written informed consent form.

### *Consent for publication*

All the participants have signed the informed consent for publication of their clinical and laboratory data without name.

### *Availability of data and materials*

Raw data analyzed during the current study are available on reasonable request.

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