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## Original article

# Usefulness of antigen-specific IgE probability curves derived from the 3gAllergy assay in diagnosing egg, cow's milk, and wheat allergies

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## ABSTRACT

**Background:** Specific IgE (sIgE) antibody detection using the Siemens IMMULITE® 3gAllergy™ (3gAllergy) assay have not been sufficiently examined for the diagnosis of food allergy. The aim of this study was to evaluate the utility of measuring sIgE levels using the 3gAllergy assay to diagnose allergic reactions to egg, milk, and wheat.

**Methods:** This retrospective study was conducted on patients with diagnosed or suspected allergies to egg, milk and wheat. Patients were divided into two groups according to their clinical reactivity to these allergens based on oral food challenge outcomes and/or convincing histories of immediate reaction to causative food(s). The sIgE levels were measured using 3gAllergy and ImmunoCAP. Predicted probability curves were estimated using logistic regression analysis.

**Results:** We analyzed 1561 patients, ages 0–19 y (egg = 436, milk = 499, wheat = 626). The sIgE levels determined using 3gAllergy correlated with those of ImmunoCAP, classifying 355 patients as symptomatic: egg = 149, milk = 123, wheat = 83. 3gAllergy sIgE levels were significantly higher in symptomatic than in asymptomatic patients ( $P < 0.0001$ ). Predictive probability for positive food allergy was significantly increased and correlated with increased sIgE levels. The cut-offs for allergic reaction with 95% predictive probability as determined by the 3gAllergy probability curves were different from those of ImmunoCAP.

**Conclusions:** Measurements of sIgE against egg, milk, and wheat as determined by 3gAllergy may be used as a tool to facilitate the diagnosis of food allergy in subjects with suspected food allergies. However, these probability curves should not be applied interchangeably between different assays.

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## Introduction

Egg, milk, and wheat are common causative foods in food allergy,<sup>1</sup> accounting for the top three causes of food allergies in Japan.<sup>2</sup> The levels of allergen-specific IgEs (sIgEs) produced in response to food are useful predictors of clinical reactions caused by food allergens.<sup>3–9</sup> Previously, we found that egg white (EW)-, milk-, and wheat-sIgE levels were associated with positive symptoms in children suspected of having a food allergy, and obtained decision points (cutoff values) for each of these food allergens using

the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden).<sup>10,11</sup> These sIgE decision points can be used to help clinicians decide when oral food challenges (OFCs) are appropriate.<sup>12–14</sup>

Currently, several assays for measuring sIgE antibodies are used by commercial laboratories. The Siemens IMMULITE® 3gAllergy™ (3gAllergy) assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) is one such assay; however, limited publications are available regarding its clinical usefulness for the diagnosis of food allergy in comparison to those for ImmunoCAP. The 3gAllergy readout is reported as IU<sub>A</sub>/mL, whereas that of ImmunoCAP is reported as kU<sub>A</sub>/L. However, even though the results reported using the same sIgE level measures, the sIgE levels are not considered diagnostically interchangeable because some studies have shown discrepancies among results obtained with different assays.<sup>15,16</sup>

Therefore, to determine whether the 3gAllergy assay represents a valid measure of sIgE levels in food allergy, this study aimed to

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evaluate the utility of measuring sIgE levels using the 3gAllergy assay to diagnose allergic reactions to egg, milk, and wheat.

## Methods

### Study design

This retrospective study was conducted on patients with suspected allergies to egg, milk, and wheat who visited the Department of Pediatrics of Sagami National Hospital from December 2009 to May 2012 and were tested within six months of the first visit for sIgE to EW, milk, and wheat (Fig. 1).

Inclusion criteria consisted of patients who had been confirmed as symptomatic or asymptomatic after ingesting egg, milk, or wheat. Patients were defined as symptomatic if they displayed a positive reaction to OFC in the hospital, or by convincing history of immediate allergic reaction (urticaria, pruritus, wheals, eyelid or lip swelling, oral cavity irritation, coughing, wheezing, dyspnea, abdominal pain, vomiting, and diarrhea) owing to intake of the causative food(s) within 1 year before and after blood sampling. Patients were defined as asymptomatic if they could ingest half of a cooked egg (3.1 g egg protein), 100 mL milk (3.4 g milk protein), or 100 g boiled udon noodles (Japanese wheat noodles composed of 2.6 g wheat protein) without exhibiting an immediate allergic reaction within 6 months after blood sampling.

For the exclusion criteria, patients were excluded if they had never consumed the causative food(s) because of only positive sIgE levels, if they only ingest the causative food(s) less than the above-mentioned doses within 6 months after blood sampling, if they kept avoiding the causative food(s) with no immediate allergic reaction within 1 year before and after blood sampling, or if data were missing.

### Oral food challenge

OFC was performed by either a single-blinded test or an open test, according to the Japanese Pediatric Guideline for Food Allergy 2012.<sup>17</sup> Egg was given as a pumpkin-flavored cupcake using either an egg yolk or half of a whole egg mixed with 40 g pumpkin and 5 g

sugar, and cooked in a 1000 watt microwave for 90 s. Milk was administered either as 48 g yogurt or prepared as a pumpkin-flavored cupcake (25 mL milk mixed with 40 g pumpkin and 5 g sugar, cooked in a 1000 watt microwave for 90 s). Wheat was consumed as 15–100 g boiled udon noodles cooked in 100 °C water for 1 min (Tablemark, Tokyo, Japan).<sup>18</sup> Each challenge food was given in gradually increasing amounts in three meals, with 30 min between each administration. The challenge was terminated either when the entire challenge dose had been consumed, or when indications of a hypersensitivity reaction occurred. If an allergic reaction was induced, patients were treated according to severity using medications, fluid resuscitation, histamine H1 receptor antagonists, steroids, inhalation of  $\beta$  2 stimulants, or intramuscular injection of adrenaline.

### Measurement of serum-specific IgE

Blood samples were drawn during the course of regular clinic visits and were stored at  $-84$  °C in the hospital refrigerator. Allergen-sIgE levels were measured using the 3gAllergy and ImmunoCAP assays. Both assays used sera from blood sampled at the same time. ImmunoCAP assay was measured on the same day of blood sampling. The 3gAllergy assay was performed using frozen sera. The 3gAllergy assay uses biotinylated allergen extracts in liquid format to capture sIgE antibodies. These sIgE antibodies form antibody-antigen complexes on streptavidin coated beads via avidin-biotin binding. The captured sIgE antibodies are then detected by an enzyme-labeled anti-IgE antibody.<sup>19</sup> The 3gAllergy readout is reported as IU<sub>A</sub>/mL according to the CLSI, Clinical Laboratory Standards Institute, guideline.<sup>20</sup> The range of 3gAllergy readouts was 0.10–500 IU<sub>A</sub>/mL. In this study, to simplify calculations, 0.05 IU<sub>A</sub>/mL was substituted for all values <0.1 IU<sub>A</sub>/mL and 501 IU<sub>A</sub>/mL was used for all values >500 IU<sub>A</sub>/mL.

In the ImmunoCAP assay, allergen extracts are absorbed into a cellulose sponge and detected following the addition of an enzyme-labeled antibody. The assay range available in Japan at the time of the study was 0.35–100 kU<sub>A</sub>/L; however for the purposes of this study, 0.15 kU<sub>A</sub>/L was used in calculations for all values <0.35 kU<sub>A</sub>/L, and 101 kU<sub>A</sub>/L was used in place of all values >100 kU<sub>A</sub>/L.

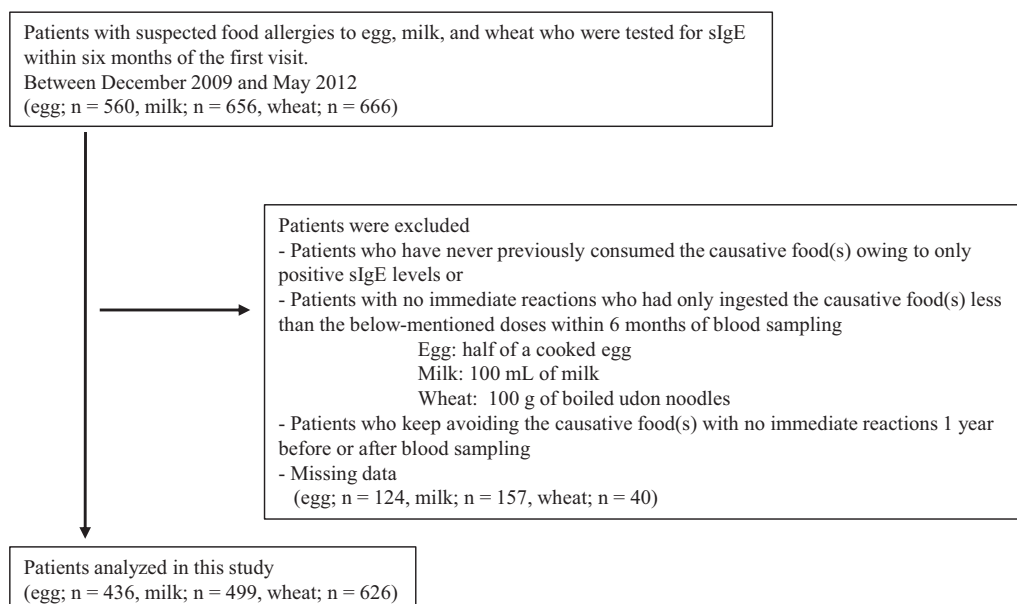


Fig. 1. Schematic of this study.

### Statistical analysis

All data were analyzed according to the intention-to-treat principle. For the baseline characteristics, summary statistics were constructed employing frequencies and proportions for categorical data, and means and standard deviations (SD) for continuous variables. Spearman's correlation coefficient was estimated between 3gAllergy and ImmunoCAP. We compared patient characteristics using the Fisher's exact test for categorical outcomes and t-tests or the Wilcoxon rank sum test for continuous variables, as appropriate. A logistic regression model was used to evaluate the sIgE titers necessary to predict the probability of inducing symptoms. The most optimal cut-off value was determined as the one with highest Youden's Index. All statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA) and the R statistical program, version 3.10 (<https://cran.r-project.org>).

### Ethical considerations

This study was approved by the Institutional Review Board at Sagami National Hospital (Kanagawa, Japan, No.2013/24). Children and their parents were provided with an oral and written explanation of the objectives of the study, and written informed consent to participate was obtained for each child.

### Results

#### Patient characteristics

Of the 1561 patients enrolled in this study, 436 were tested for egg, 499 for milk, and 626 for wheat (Table 1). The mean ages at blood sampling ( $\pm$ SD) were  $2.4 \pm 3.0$  for egg,  $1.8 \pm 2.8$  for milk, and  $1.1 \pm 2.0$  for wheat; the majority of patients were very young children less than two years of age. OFC was performed for 108 patients for egg, 71 for milk, and 32 for wheat. A total of 149/287, 123/376, and 83/543 patients were symptomatic/asymptomatic for egg, milk, or wheat, respectively.

#### Comparison of 3gAllergy and ImmunoCAP

Strong correlations were observed between the sIgE levels measured using the 3gAllergy and ImmunoCAP systems for all allergens tested. The Spearman's coefficient ( $r_s$ ) was 0.95 for EW, 0.94 for milk, and 0.95 for wheat (Fig. 2A–C). However, some patients with negative sIgE levels as determined by ImmunoCAP showed positive sIgE levels when 3gAllergy was used. For milk and wheat, some patients with negative sIgE levels as determined by 3gAllergy showed positive sIgE levels when ImmunoCAP was used. In addition, the mean ratios between the 3gAllergy and ImmunoCAP results ( $\pm$ SEM) were  $3.6 \pm 0.1$  for EW sIgE;  $1.1 \pm 0.1$  for CM sIgE; and  $0.6 \pm 0.1$  for wheat sIgE. When the levels determined by the 3gAllergy assay were converted into ImmunoCAP units, they

**Table 1A**

Patient characteristics at the time of blood sampling: egg.

Egg	Total (n = 436)	Symptomatic (n = 149)	Asymptomatic (n = 287)	P-value
Age (years)	$2.4 \pm 3.0$	$2.0 \pm 2.8$	$2.6 \pm 3.1$	0.0073
Male sex, no. (%)	267 (61)	87 (58)	180 (63)	0.38
Atopic eczema, no. (%)	249 (57)	88 (59)	161 (56)	0.55
Asthma, no. (%)	66 (15)	25 (17)	41 (14)	0.49
Egg white sIgE (ImmunoCAP, kU <sub>A</sub> /L)	$11.4 \pm 21.5$	$24.82 \pm 28.8$	$4.46 \pm 11.4$	<.0001
Egg white sIgE (3gAllergy, IU <sub>A</sub> /mL)	$43.1 \pm 86.3$	$95.84 \pm 119$	$15.74 \pm 41.2$	<.0001

**Table 1B**

Patient characteristics at the time of blood sampling: milk.

Milk	Total (n = 499)	Symptomatic (n = 123)	Asymptomatic (n = 376)	P-value
Age (years)	$1.8 \pm 2.8$	$2.6 \pm 3.4$	$1.6 \pm 2.6$	0.017
Male sex, no. (%)	301 (60)	86 (69)	215 (57)	0.014
Atopic eczema, no. (%)	333 (67)	87 (71)	246 (65)	0.28
Asthma, no. (%)	57 (11)	30 (20)	27 (7)	<.0001
Milk sIgE (ImmunoCAP, kU <sub>A</sub> /L)	$7.2 \pm 18.9$	$25.2 \pm 31.5$	$1.33 \pm 3.6$	<.0001
Milk sIgE (3gAllergy, IU <sub>A</sub> /mL)	$13.9 \pm 45.2$	$52.3 \pm 79.3$	$1.39 \pm 4.6$	<.0001

**Table 1C**

Patient characteristics at the time of blood sampling: wheat.

Wheat	Total (n = 626)	Symptomatic (n = 83)	Asymptomatic (n = 543)	P-value
Age (years)	$1.1 \pm 2.0$	$1.4 \pm 2.3$	$1.1 \pm 1.9$	0.68
Male sex, no. (%)	370 (59)	60 (72)	310 (57)	0.0087
Atopic eczema, no. (%)	426 (68)	57 (69)	369 (68)	0.89
Asthma, no. (%)	42 (7)	8 (10)	34 (6)	0.25
Wheat sIgE (ImmunoCAP, kU <sub>A</sub> /L)	$5.60 \pm 17.4$	$28.66 \pm 32.5$	$2.07 \pm 9.8$	<.0001
Wheat sIgE (3gAllergy, IU <sub>A</sub> /mL)	$4.76 \pm 28.0$	$29.51 \pm 69.4$	$1.19 \pm 10.4$	<.0001

increased approximately 4-fold for EW and 2-fold for milk, but decreased by approximately half for wheat in comparison with ImmunoCAP (data not shown).

#### Predicted probability curves derived from sIgE using the 3gAllergy assay

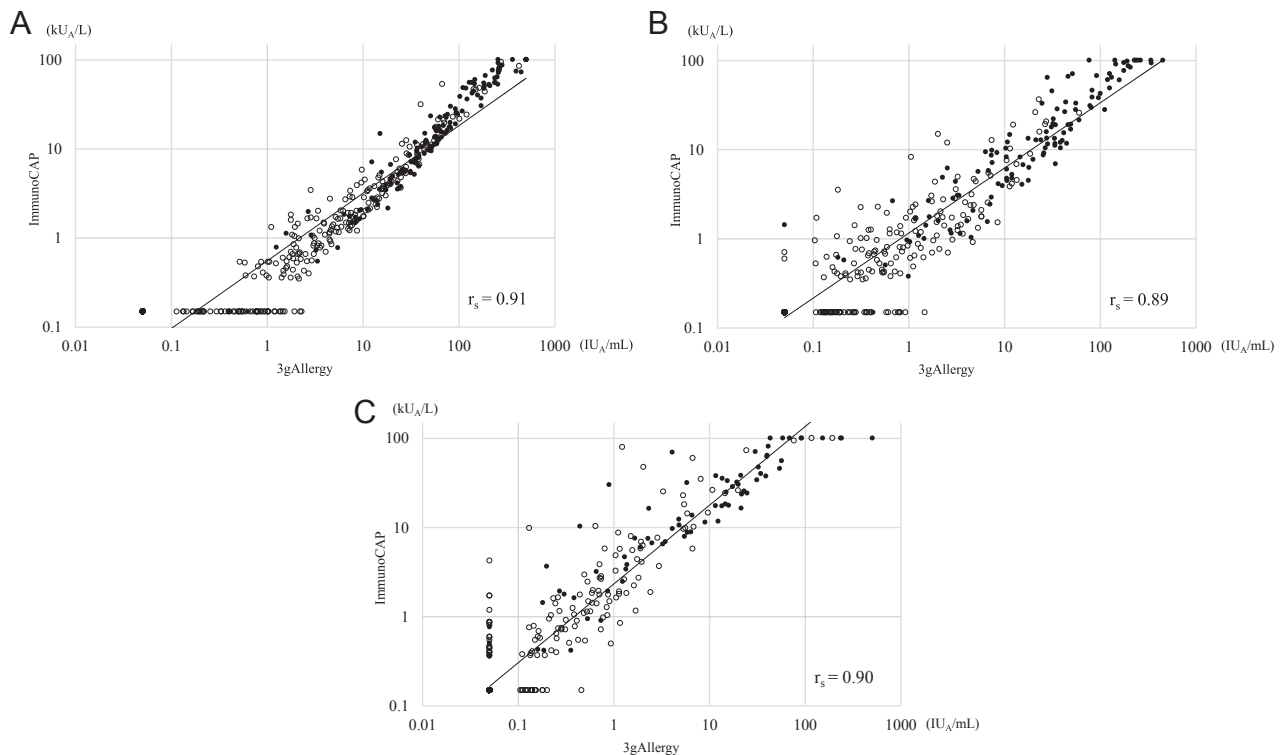
The sIgE levels obtained using the 3gAllergy and ImmunoCAP assays were significantly higher for symptomatic than for asymptomatic patients (Table 1). Based on receiver operating characteristic analysis, both assays showed equivalent accuracy for predicting food allergy (Supplementary Table 1). The relationship between the sIgE level and the occurrence of an allergic reaction to food was investigated using a logistic regression model. A significant relationship was observed between the outcome and the concentration of sIgE levels to EW, milk, and wheat for both assay methods (Fig. 3A–C). However, the probability determined by the sIgE levels using the 3gAllergy assay was different from that of ImmunoCAP for each food.

In addition, we calculated the sIgE levels for predictive decision points for food allergy. For the 3gAllergy assays, 95% predicted probability for positive food allergy was obtained at 100.0 IU<sub>A</sub>/mL for milk and at 115.0 IU<sub>A</sub>/mL for wheat, but could not be calculated for EW. The 95% predicted probability using ImmunoCAP could be obtained only for milk, at a value of 42.7 kU<sub>A</sub>/L. The sIgE levels required to obtain the predicted probability also differed between the 3gAllergy and ImmunoCAP assays (Supplementary Table 2).

### Discussion

This study evaluated the utility of measuring sIgE antibody levels for food allergy diagnosis using 3gAllergy probability curves. By doing so, we have demonstrated that 3gAllergy is clinically useful for diagnosing food allergies, as indicated by correlation of the levels of sIgE to EW, milk, and wheat to the probability of allergen-induced symptoms. Probability curves constructed using the same samples on the ImmunoCAP system provided similar results.

The observed levels of sIgE antibody to EW, milk, and wheat showed significant correlation between 3gAllergy and ImmunoCAP



**Fig. 2.** Comparison of 3gAllergy and ImmunoCAP. Correlation of sIgE between 3gAllergy and ImmunoCAP assays for each group of food allergic patients. (A) Egg white ( $n = 436$ ); (B) Milk ( $n = 497$ ); (C) Wheat ( $n = 626$ ). Symptomatic patients are shown with filled circles, and asymptomatic patients are shown with open circles.

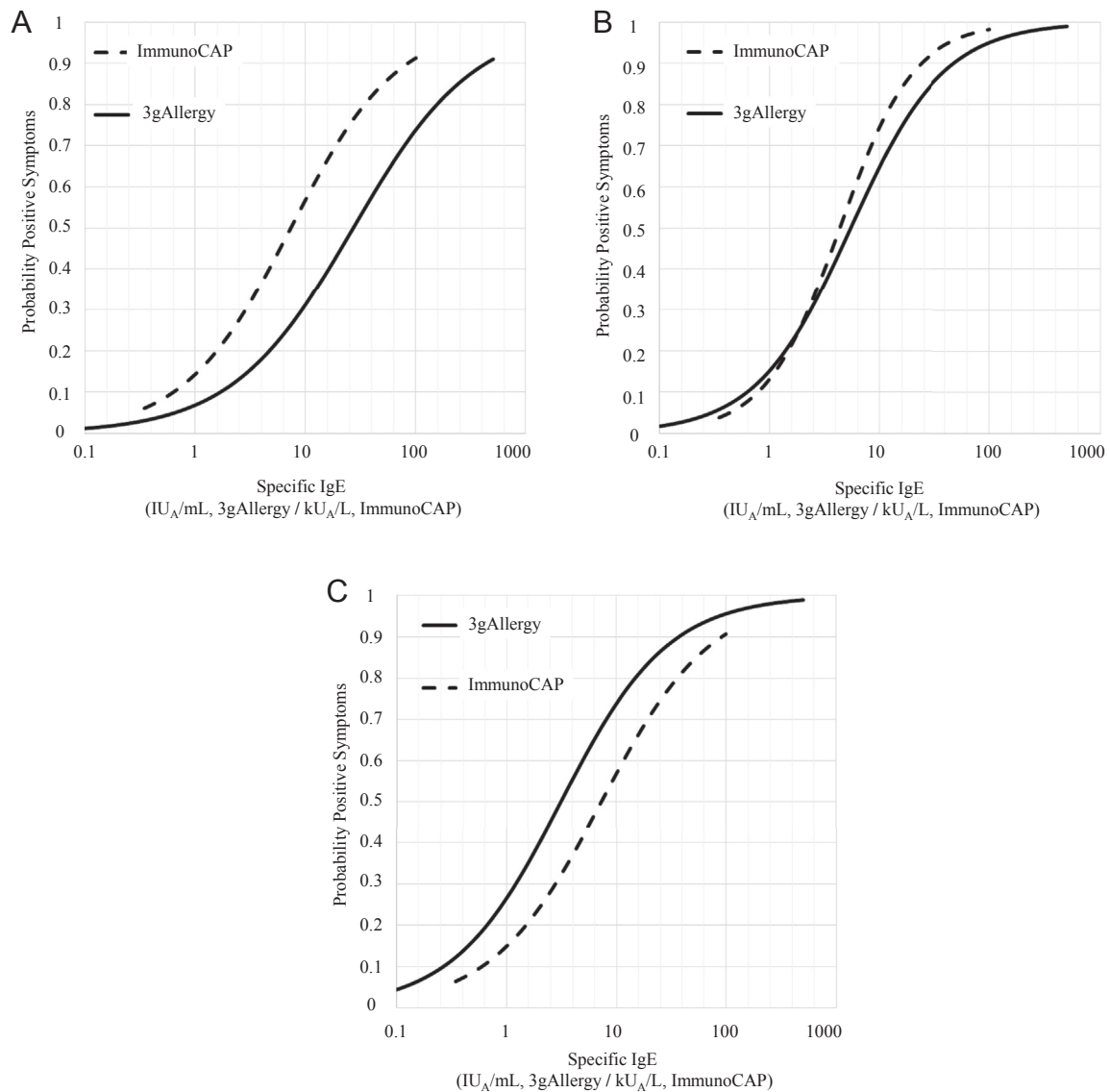
assays. This is consistent with an earlier study by Hamilton *et al.*,<sup>21</sup> which also determined that egg and milk-sIgE levels were correlated between 3gAllergy and ImmunoCAP; this was found to be true for peanuts as well. Recently, a study of egg allergy in early childhood demonstrated a strong correlation for detection of sIgE antibodies to egg and ovomucoid between these two methods.<sup>22</sup> Both methods were thus reported to be potentially useful as a supportive diagnostic tool in young children who require a confirmed diagnosis of egg allergy. In our study, in addition to EW, the milk and wheat sIgE levels measured by 3gAllergy were associated with the probability that symptoms would be induced with OFC, indicating that sIgE antibody titers can be predictive of the occurrence of immediate-type reactions. These results are similar to those obtained in studies using ImmunoCAP.<sup>3,4,10,11</sup> Therefore, we have clearly shown that the sIgE values determined using 3gAllergy can be helpful for diagnosing allergies to egg, milk, and wheat.

Our group has previously reported that the sIgE level corresponding to a 95% predicted probability for positive symptoms according to ImmunoCAP was 25.5 kU<sub>A</sub>/L for EW and 50.9 kU<sub>A</sub>/L for milk,<sup>10</sup> and other studies have reported lower cutoffs for the 95% predicted probability.<sup>3,4,23</sup> Although the decision points obtained from current study were not identical to the previously reported results, it is possible that the probability of eliciting an allergic response might be affected by age, application criteria, severity, distribution of sIgE antibodies, or the positivity rate of the patient group.<sup>24,25</sup> In this study, most subjects were young children; therefore, the decision points were not suitable for long-term follow-up. Notably, the probability curves generated by 3gAllergy for EW-, milk-, and wheat-sIgE differed from those for ImmunoCAP. Consequently, the cutoff predicting the probability of a positive reaction was different for each method, as was the 95% predicted probability used to define the clinical decision point for each method. Similar to our study, Furuya *et al.* reported that the levels required to obtain 90% predicted probability using the 3gAllergy

assay were higher than those required using ImmunoCAP for EW and ovomucoid.<sup>22</sup>

In addition, our study showed that the sIgE antibody levels were higher as measured by the 3gAllergy assay than by ImmunoCAP for EW and milk, but were lower for wheat. Similarly, Wang *et al.* reported in their study of 50 patients that the sIgE levels according to 3gAllergy were higher than those determined by ImmunoCAP by 4.85-fold for EW, 2.33-fold for milk, and 1.86-fold for peanuts.<sup>16</sup> Wood *et al.* also reported similar results for peanuts.<sup>15</sup> Conversely, we found that the sIgE antibody levels generated to wheat were lower for as determined by 3gAllergy than for ImmunoCAP, corroborating the result of Nagao *et al.*<sup>26</sup> Several reports have pointed out that such differences might be caused by the differences in the assay format and allergen extract sources between 3gAllergy and ImmunoCAP.<sup>16,22,27,28</sup> Furthermore, Nagao *et al.* suggested that this might be attributable to the differences in the composition of the wheat allergen used in the assay because the amount of gliadin (present in the alcohol soluble fraction) might be lower in 3gAllergy.<sup>26</sup> We also found that some patients with negative sIgE levels as determined by ImmunoCAP showed positive sIgE levels when 3gAllergy was used. Previous studies suggested that 3gAllergy uses biotinylated allergen extracts in a liquid format to capture sIgE antibodies, which had higher sensitivity than other assays.<sup>22,29</sup> Together, these results indicate that clinicians need to know the method used to correctly interpret the reported sIgE measurements.

We note three limitations of this study. First, the serum samples were collected from patients visiting a single facility, and these were analyzed retrospectively. Second, currently, the assay range of ImmunoCAP available in Japan is 0.1–100 kU<sub>A</sub>/L or greater. There is a possibility that the results of this study were influenced by the limited assay range. Third, we did not try to determine the reason underlying the observed differences in the sIgE levels between the two methods. Further studies should be conducted prospectively in multiple facilities to confirm the general applicability of our



**Fig. 3.** Comparison of fitted predicted probability curves for positive symptoms between 3gAllergy and ImmunoCAP assays. (A) Egg white ( $n = 436$ ); (B) Milk ( $n = 497$ ); (C) Wheat ( $n = 626$ ).

findings. It would also be valuable to try to determine the source of allergen heterogeneity between the different manufacturer methods.

In conclusion, the sIgE levels determined by 3gAllergy correlate well with those determined using ImmunoCAP. Measurement of sIgE levels using 3gAllergy was successfully utilized for diagnosing patients with food allergies to egg, milk, and wheat. However, our results demonstrated that these probability curves should not be applied interchangeably between the different assays. It is expected that further evaluation will likely demonstrate diagnostic utility of the 3gAllergy assay for other foods as well.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2016.06.012>.

## Conflict of interest

The authors have no conflict of interest to declare.

## Authors' contributions

SS and ME designed the study and wrote the manuscript. KO, KT and NY contributed to the data collection. YS performed the statistical analysis and interpreted the results. All authors read and approved the final manuscript.

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