Allergy Asthma Immunol Res. 2021 Jan;13(1):141-153
https://doi.org/10.4168/aair.2021.13.1.141
pISSN 2092-7355·eISSN 2092-7363

Allergy, Asthma & Immunology Research AAIR

Original Article

Agreement Between Predictive, Allergen-Specific IgE Values Assessed by ImmunoCAP and IMMULITE 2000 3gAllergy™ Assay Systems for Milk and Wheat Allergies

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Received: Feb 29, 2020
Revised: May 29, 2020
Accepted: Jun 16, 2020

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ABSTRACT

Purpose: ImmunoCAP® (ImmunoCAP) and IMMULITE® 2000 3gAllergy™ (3gAllergy) systems are major quantitative allergen-specific immunoglobulin E (sIgE) assay methods. Due to the heterogeneous nature of allergenic extracts and differences in the assay format, quantitation of allergen-sIgEs is not expected to correlate well between different methods. However, we have recently reported good agreement between the methods in the diagnosis of egg allergy. This study aimed to determine and correlate the predictive values of sIgE by the two systems in the diagnosis of milk and wheat allergies.
Methods: Children who had undergone oral food challenge (OFC) for the diagnosis of milk and wheat allergies were enrolled. The OFCs were performed to diagnose either true allergy in the 1-year-old group (A) or tolerance in the 2- to 6-year-old group (B). Milk, casein and β-lactoglobulin, and wheat and ω-5 gliadin sIgE values were measured using the 2 systems. The predictive accuracy of each sIgE for the OFC outcome was assessed using receiver operating characteristic (ROC) curves. The probability of a positive OFC outcome was estimated by logistic regression analysis.
Results: A total of 395 patients were recruited from 7 primary care clinics and 19 hospitals in Japan. Milk and wheat OFCs were performed for 87 and 102 group A patients, and 124 and 82 group B patients, respectively. ROC analysis yielded similar areas under the curve for the 2 assays (0.7–0.9). The log-transformed sIgE data showed a strong linear correlation with the estimated probabilities ($R > 0.9$).
Conclusions: The 2 systems may be interchangeable for diagnosis of milk and wheat allergies in young children.

Keywords: Milk hypersensitivity; wheat hypersensitivity; immunologic tests; food allergy; iImmune tolerance immunoglobulin E; allergens

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Disclosure

Takao Fujisawa, Mizuho Nagao and Setsuko Ito received lecture fees from Thermo Fisher Scientific and Siemens Healthcare Diagnostics. Yasunori Sato received lecture fees from Siemens Healthcare Diagnostics. Yasmeen Al Hawi and Kanae Furuya declare that they have no conflicts of interest.

INTRODUCTION

Three major commercial assay systems have been used worldwide to quantify serum allergen-specific immunoglobulin E (sIgE) levels: Phadia ImmunoCAP® (ImmunoCAP), Hycor Turbo-MP™ (Hycor) and Siemens Immulite® 2000 3gAllergy™ (3gAllergy). They utilize the same basicmethod for sIgE measurement that was developed about 50 years ago,[1] but employ different solid phases, supply sources for allergenic extracts and anti-human IgE antibody detection labels that emit signals through either fluorescence (ImmunoCAP), colorimetry (Hycor) or chemiluminescence (3gAllergy) reactions.[2] For the harmonization of the laboratory procedures, inter-laboratory, intra-method and inter-dilution agreement of the 3 assays were tested and found to be excellent, with coefficients of variation (CVs) below 15%, but the inter-method CVs were unacceptably high.[3] Sato *et al.*[4] evaluated the predicted probability of egg, milk and wheat-sIgE by ImmunoCAP and 3gAllergy in a large retrospective cohort, but they found that the 2 assays gave different probability curves. Thus, it is believed that sIgE levels determined by the different assays should not be considered interchangeable.[4-6]

However, in regular clinical practice with access to different assay systems, a clinician may receive discordant reports from laboratories that employ different assay systems, and interpretation of the "different" data[7] can be very difficult. It would be ideal that the sIgE assays could be harmonized to give compatible results. Recently, we reported a strong correlation of the egg-sIgE level and predicted probabilities between the ImmunoCAP and 3gAllergy assay systems.[8] That study carefully tested assay performance and agreement based on standardized oral food challenge (OFC) outcomes. Pearson's correlation coefficients were 0.97 for egg white sIgE and 0.95 for ovomucoid sIgE in the logarithmically transformed data. The predicted probability of a positive OFC in individual patients also correlated strongly between the 2 systems with R = 0.98 for egg white and 0.93 for ovomucoid.[8] The compatibility can be extended to other allergen specificities by employing a similar methodology.

The present study aimed to identify and correlate the predictive values of sIgE with the 2 different systems, ImmunoCAP and 3gAllergy, which are often used in diagnosis of milk and wheat allergies. With a multi-center study design, the patient populations tested in this study were those most likely to be encountered at general pediatric clinics.

MATERIALS AND METHODS

This was a prospective and observational study. Suspected or confirmed food allergy patients aged from 1 to 6 years who had undergone milk or wheat OFC were recruited from August 2012 to August 2015 at multiple sites, including 7 primary care clinics and 19 hospitals across Japan. All patients were on an elimination diet for either milk or wheat due to a suspected or diagnosed allergy prior to the study. OFCs were performed as a requisite diagnostic procedure as part of food allergy management.

The enrolled patients were divided into 2 groups according to age. Group A included patients at the age of 1 year who were suspected to have milk or wheat allergy because of positive sIgE to milk or wheat, which had been tested for infantile eczema/atopic dermatitis (AD). Group A also included those for whom milk or wheat had been eliminated for more than 6 months due to previous allergen-induced symptoms. Group B included patients aged 2–6 years who had been

https://doi.org/10.4168/aair.2021.13.1.141

diagnosed with food allergy based on a documented history of apparent milk/wheat-induced symptoms with corresponding sensitization or a positive OFC. These patients had been on a total elimination diet for more than 12 months prior to inclusion in the study. The OFCs' aims were to confirm true allergy in group A and natural outgrow or tolerance in group B.

Patients were excluded if they met any of the following criteria: 1) Apparent symptoms after ingestion of either milk or wheat within 3 months before the OFC; 2) Performance of a milk or wheat OFC within 3 months before the current OFC; 3) Uncontrolled AD or asthma; and 4) Other chronic diseases.

Values of ≥ 0.1 kU_A/L by ImmunoCAP and ≥ 0.1 IU_A/mL by 3gAllergy for milk sIgE and wheat sIgE were used as standards for sensitization.

Before the OFC, the patients were invited to join the study in order to test the validity and performance of the 2 sIgE assays (ImmunoCAP and 3gAllergy). Prior to enrollment, written informed consent from the parents or guardians, along with informed assent from children aged > 3 years were obtained. The study protocol was reviewed and approved (approval number 24-12) by the Institutional Review Board of Mie National Hospital (principal investigator site).

OFC

OFCs were performed in a single-blind manner. For the milk OFC, group A and B patients consumed a total volume of 50 mL or 200 mL of skimmed milk (BeanStalksnow Co., Ltd., Tokyo, Japan; protein content = 3.3 g/100 mL), respectively. For the wheat OFC, group A and B patients consumed wheat in the form of cooked udon noodles (Sugakiya Co., Ltd., Toyoake, Japan; protein content = 2.6 g/100 g) in respective total amounts of 40 g and 80 g, which are the regular serving amounts for children at those ages.

The challenge food was divided into 6 graded doses (2/100, 4/100, 8/100, 16/100, 32/100, and 38/100), and each increased dose was administered at 15- to 30-minute intervals.

An OFC was judged positive if there was any objective clinical reaction, such as urticaria, angioedema, rhino-conjunctivitis, cough, wheezing, vomiting and diarrhea, and/or a decrease in blood pressure. Intense abdominal pain (self-rated as 1 or 2 using a 5-grade pain intensity face scale) was considered positive even if it was the only sign observed. An OFC was judged negative if no symptoms were observed for 2 hours after ingestion of the total amount of prepared allergen at challenge. Full emergency equipment and medications were ready during the course of all procedures. Antihistamines were discontinued 72 hours before the OFC.

sIgE measurement

We measured the levels of sIgE to milk, casein and β-lactoglobulin (BLG) for milk allergy and to wheat and ω-5 gliadin (OM5G) for wheat allergy by using the ImmunoCAP (Thermo Fisher Scientific, Phadia AB; Uppsala, Sweden) and 3gAllergy systems (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The ImmunoCAP system employs a fluorescent enzyme immunoassay (FEIA) utilizing cellulose-based solid-phase antigens.[9] The 3gAllergy system employs a chemiluminescent enzyme immunoassay (CLEIA) utilizing liquid antigens.[10] The ImmunoCAP readouts are reported in kilo-units of antibody per liter (kU_A/L), with a range of 0.1 to 100 kU_A/L, while 3gAllergy readouts were reported in international units of antibody per milliliter (IU_A/mL), with a range of 0.1 to 500 IU_A/mL. Sample collection and sIgE measurement

https://doi.org/10.4168/aair.2021.13.1.141

were performed on the same day as the OFC or within 4 weeks before the OFC. Serum specimens were stored at −20°C and sent to the SRL laboratory (Hachioji, Tokyo, Japan), where the assays were performed according to the respective manufacturer's instructions.

Evaluation of AD

AD was diagnosed based on the criteria of the Japanese Dermatological Association (Tsu, Japan).[11] The severity of AD was evaluated based on the simplified criteria of the Japanese Guideline for Atopic Dermatitis: mild if only mild rash was observed, irrespective of the body site; moderate if rash with severe inflammation was observed over less than 10% of the body surface area; severe if rash with severe inflammation was observed over 10% to 30% of the body surface area; and most severe if rash with severe inflammation was observed over more than 30% of the body surface area.[12]

Evaluation of other co-morbid allergic diseases

We employed the diagnoses of co-morbid diseases made by the physicians who cared for the subjects. We asked caregivers of the subjects, "Has your doctor ever diagnosed your child as having asthma/allergic rhinitis?"

Statistical analysis

Categorical data are expressed in frequencies and proportions, while continuous data are expressed in means and standard deviations (SD). Receiver operator characteristic (ROC) analysis was used to determine the area under the curve (AUC). The highest value of the Youden index was used to determine the optimal cutoff point. Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and positive and negative likelihood ratios (LR+ and LR−) were calculated. We used logistic regression analysis to predict the relationships between OFC outcomes and sIgE levels measured by both ImmunoCAP and 3gAllergy. The relationships were graphically represented by probability curves. Pearson's correlation coefficient was used to measure the degree of linear correlation of the log-transformed serum sIgE values and predicted probabilities measured by ImmunoCAP and 3gAllergy. We compared OFC-positive and OFC-negative groups using the Mann-Whitney *U* test; $P < 0.05$ was considered statistically significant. All statistical analyses were planned *a priori* and performed using the SAS software program, version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Demographics of the patients

As shown in **Tables 1** and **2**, male patients constituted about 60% of the study population in both the milk and wheat groups. The median age of patients who underwent milk OFC was 16 and 48 months in groups A and B, respectively, while the mean ages in the wheat OFC groups were 17 and 48 months, respectively. A history of milk-elicited symptoms was found in 50.6% of group A patients and 63.7% of group B patients. In the wheat OFC group, a history of wheat-elicited symptoms was found in 44.1% of group A patients and 63.4% of group B patients. Co-morbid AD was found in 78.9% and 78.0% of the patients in the milk and wheat OFC groups, respectively. The AD symptoms were mild and controlled in most cases. Physician-diagnosed asthma was found in 4.7% and 9.1% of group A patients, and 31.5% and 37.5% of group B patients, with milk and wheat OFCs, respectively (**Tables 1** and **2**).

https://doi.org/10.4168/aair.2021.13.1.141

Table 1. Demographic and clinical characteristics of the study population with milk OFC

Characteristics	Group A (n = 87)	Group B (n = 124)	Total (n = 211)
Sex (male)	59/87 (67.8)	79/124 (63.7)	138/211 (65.4)
Age (month)	16 (12–23)	48 (24–84)	32 (12–84)
OFC performed at			
Hospital	49/87 (56.3)	90/124 (72.6)	139/211 (65.9)
Clinic	38/87 (43.7)	34/124 (27.4)	72/211 (34.1)
Clinical evidence of milk allergy			
Sensitization to milk*	87/87 (100.0)	124/124 (100.0)	211/211 (100.0)
History of milk-induced symptoms†	44/87 (50.6)	79/124 (63.7)	123/211 (58.3)
Positive oral foods challenge†	2/87 (2.3)	22/124 (17.7)	24/211 (10.9)
History of eczema in infancy	71/85 (83.5)	106/123 (86.2)	177/208 (85.1)
Diagnosis of atopic dermatitis	67/82 (81.7)	94/122 (77.0)	161/204 (78.9)
Severity of atopic dermatitis			
Mild	61/82 (74.4)	72/122 (59.0)	133/204 (65.2)
Moderate	6/82 (7.3)	21/122 (17.2)	27/204 (13.2)
Severe‡	0/82 (0.0)	1/122 (0.8)	1/204 (0.5)
History of recurrent wheezing	12/85 (14.1)	55/124 (44.4)	67/209 (32.1)
Diagnosis of asthma	4/85 (4.7)	39/124 (31.5)	43/209 (20.6)

Values are presented as number (%) or median (range).
sIgE, specific immunoglobulin E; OFC, oral food challenge.
*Milk-sIgE ≥ 0.1 (ImmunoCAP or 3gAllergy); †The present OFCs were performed more than 6 months after the induction of symptoms and previous OFC; ‡There were no 'most severe' subjects.

Table 2. Demographic and clinical characteristics of the study population with wheat OFC

Characteristics	Group A (n = 102)	Group B (n = 82)	Total (n = 184)
Sex (male)	65/102 (63.7)	59/82 (72.0)	124/184 (67.4)
Age (mon)	17 (12–23)	48 (24–81)	22 (12–81)
The place that was examined			
Hospital	76/102 (74.5)	73/82 (89.0)	149/184 (81.0)
Clinic	26/102 (25.5)	9/82 (11.0)	35/184 (19.0)
Clinical evidence of milk allergy			
Sensitization to wheat*	102/102 (100.0)	82/82 (100.0)	184/184 (100.0)
History of wheat-induced symptoms†	45/102 (44.1)	52/82 (63.4)	97/184 (52.7)
Positive oral foods challenge†	3/102 (2.9)	16/82 (19.5)	19/184 (10.3)
History of eczema in infancy	90/101 (89.1)	59/81 (72.8)	149/182 (81.9)
Diagnosis of atopic dermatitis	84/99 (84.8)	54/78 (69.2)	138/177 (78.0)
Severity of atopic dermatitis			
Mild	79/84 (94.0)	46/54 (85.2)	125/138 (90.6)
Moderate	5/84 (6.0)	7/54 (13.0)	12/138 (8.7)
Severe‡	0/84 (0.0)	1/54 (1.9)	1/138 (0.7)
History of recurrent wheezing	15/99 (15.2)	36/80 (45.0)	67/179 (28.5)
Diagnosis of asthma	9/99 (9.1)	30/80 (37.5)	39/179 (21.8)

Values are presented as number (%) or median (range).
sIgE, specific immunoglobulin E; OFC, oral food challenge.
*Wheat sIgE ≥ 0.1 (ImmunoCAP or 3gAllergy); †The present OFCs were performed more than 6 months after the induction of symptoms and previous OFC; ‡There were no 'most severe' subjects.

OFC outcomes

Milk OFC

A total of 244 patients underwent milk OFC. Thirty-three of the patients were excluded from the study due to refusal to ingest the designated volume of milk in the OFC or incomplete records. The remaining 211 patients were included in the final study population, of whom 152 patients had a positive reaction (Failed) and 59 a negative reaction (Passed). Among the patients who failed the milk OFC, 57 were from group A and 95 from group B (**Supplementary Fig. S1A**). Most of those who failed had cutaneous and respiratory manifestations (**Supplementary Table S1**). Adrenaline was injections to10.5% (n = 6) of group A patients and 11.6% (n = 11) of group B patients. One male patient had cardiovascular manifestations with hypotension, but he was given immediate treatment and recovered fully.

https://doi.org/10.4168/aair.2021.13.1.141

Wheat OFC

A total of 206 patients were scheduled for the wheat OFC, but 22 patients were excluded for the above reasons, and a few were not sensitized. Of the remaining 184 patients, 131 had a positive reaction (Failed) and 53 had a negative reaction (Passed). The Failed patients consisted of 62 patients from group A and 69 patients from group B (**Supplementary Fig. S1B**). Most of the patients who failed had cutaneous and/or respiratory manifestations (**Supplementary Table S1**). Adrenaline was injected to 11.3% (n = 7) of Group A patients and 18.8% (n = 13) of group B patients. Group A and B patients had 2 cases of cardiovascular events: tachycardia in 1 patient and hypotension in 1 patient. Also, 1 group B patient experienced neurological manifestations, consisting of temporary loss of consciousness. All patients with adverse reactions were given immediate treatment and recovered fully, without any sequelae.

Diagnostic performance of sIgE assays by ImmunoCAP and 3gAllergy

ROC analysis was performed to determine the diagnostic accuracy of the 2 sIgE tests for OFC outcomes.

Milk

The milk-sIgE, casein-sIgE and BLG-sIgE values detected by ImmunoCAP and 3gAllergy in patients who failed the milk OFC were significantly higher than in the patients who passed (**Supplementary Table S2**). As shown in **Table 3**, ImmunoCAP and 3gAllergy showed similar AUCs in both groups, with a slightly higher value for 3gAllergy. In comparison of groups A and B, the AUCs and LR+ were higher in group B, suggesting better performance in diagnosing milk allergy in older patients.

Wheat

The wheat-sIgE and OM5G-sIgE values detected by ImmunoCAP and 3gAllergy in patients who failed the wheat OFCs were significantly higher than in the passed patients (**Supplementary Table S3**). The AUCs with ImmunoCAP and 3gAllergy for the wheat OFC outcomes were similar (**Table 4**). ImmunoCAP showed slightly higher AUCs than 3gAllergy

Table 3. Diagnostic performance of sIgE assays in predicting milk OFC outcomes

Patient group	sIgE assay	AUC	95% CI	Optimal cut-off point*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR−
Group A	Milk									
	ImmunoCAP	0.798	0.695–0.902	2.36	80.7	73.3	85.2	66.7	5.45	0.26
	3gAllergy	0.840	0.748–0.931	1.96	87.7	70.0	84.7	75.0	5.75	0.18
	Casein									
	ImmunoCAP	0.785	0.682–0.888	3.58	66.7	83.3	88.4	56.8	5.73	0.40
	3gAllergy	0.813	0.716–0.911	4.74	73.7	80.0	87.5	61.5	5.89	0.33
	BLG									
	ImmunoCAP	0.678	0.565–0.792	1.23	40.4	93.3	92.0	45.2	5.04	0.64
	3gAllergy	0.730	0.622–0.838	1.12	64.9	80.0	86.0	54.5	4.65	0.44
Group B	Milk									
	ImmunoCAP	0.873	0.792–0.953	5.33	84.2	82.8	94.1	61.5	14.3	0.19
	3gAllergy	0.923	0.868–0.979	5.51	92.6	82.8	94.6	77.4	17.2	0.09
	Casein									
	ImmunoCAP	0.881	0.812–0.949	5.25	77.9	86.2	94.9	54.3	15.1	0.26
	3gAllergy	0.901	0.840–0.961	11.00	80.0	86.2	95.0	56.8	16.0	0.23
	BLG									
	ImmunoCAP	0.690	0.587–0.792	1.62	47.4	89.7	93.8	34.2	7.58	0.59
	3gAllergy	0.765	0.668–0.863	1.43	77.9	65.5	88.1	47.5	6.54	0.34

sIgE, specific immunoglobulin E; OFC, oral food challenge; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; BLG, β-lactoglobulin.
*Units are kU_A/L for ImmunoCAP and IU_A/mL for 3gAllergy.

https://doi.org/10.4168/aair.2021.13.1.141

Table 4. Diagnostic performance of sIgE assays in predicting wheat OFC outcomes

Patient group	sIgE assay	AUC	95% CI	Optimal cut-off point*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR−
Group A	Wheat									
	ImmunoCAP	0.861	0.790–0.931	7.030	74.2	85.0	88.4	68.0	4.946	0.304
	3gAllergy	0.813	0.731–0.894	4.704	62.9	85.0	86.7	59.6	4.194	0.436
	OM5G									
	ImmunoCAP	0.846	0.770–0.923	0.550	72.6	85.0	88.2	66.7	4.839	0.323
	3gAllergy	0.806	0.720–0.892	0.354	83.9	67.5	80.0	73.0	2.581	0.239
Group B	Wheat									
	ImmunoCAP	0.757	0.590–0.924	4.190	87.0	61.5	92.3	47.1	2.261	0.212
	3gAllergy	0.732	0.566–0.898	3.480	84.1	61.5	92.1	42.1	2.186	0.259
	OM5G									
	ImmunoCAP	0.739	0.619–0.860	0.410	63.8	84.6	95.7	30.6	4.145	0.428
	3gAllergy	0.799	0.694–0.904	0.117	76.8	76.9	94.6	38.5	3.329	0.301

sIgE, specific immunoglobulin E; OFC, oral food challenge; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio; OM5G, ω-5 gliadin.
*Units are kUA/L for ImmunoCAP and IUA/mL for 3gAllergy.

for wheat-sIgE, while 3gAllergy showed higher AUCs for OM5G-sIgE than ImmunoCAP. In contrast to the milk OFCs, AUCs were higher in group A than in group B, suggesting that sIgE's predictive ability is higher when the test is conducted at a younger age.

Predictive probability for a positive OFC

The relationships between the OFC outcomes and sIgE levels by ImmunoCAP and 3gAllergy were estimated by logistic regression analysis and shown as probability curves (**Supplementary Figs. S2** and **S3**). Overall, all the curves with the 2 tests for milk OFC and wheat OFC were considerably similar in shape, indicating comparable diagnostic performance with the assays. As is well known, the risk for a positive OFC was clearly dependent on the sIgE level. To determine the utility in clinical decision-making, the milk-, casein-, BLG-, wheat- and OM5G-sIgE levels were calculated for each assay method at 90%, 80%, 50%, and 10% predicted probability, respectively, for a positive OFC (**Supplementary Tables S4** and **S5**).

Correlations of ImmunoCAP and 3gAllergy

We used Pearson's coefficient to determine the correlations between the sIgE values and predicted probabilities measured with ImmunoCAP and 3gAllergy. Highly positive correlations were found with R>0.9 between the log-transformed values for milk-sIgE (**Fig. 1A**), casein-sIgE (**Fig. 1B**), BLG-sIgE (**Fig. 1C**), wheat-sIgE (**Fig. 2A**) and OM5G-sIgE (**Fig. 2B**) with ImmunoCAP and 3gAllergy. Based on these results, equations for transformation and a conversion table were created (**Supplementary Tables S6** and **S7** in the Additional files). Highly positive correlations were also found between the predicted probabilities based on the values for milk-sIgE (**Fig. 1D**), casein-sIgE (**Fig. 1E**), BLG-sIgE (**Fig. 1F**), wheat-sIgE (**Fig. 2C**) and OM5G-sIgE (**Fig. 2D**) with ImmunoCAP and 3gAllergy.

DISCUSSION

This study aimed to determine and correlate the predictive values of sIgE with 2 commonly available assay systems, ImmunoCAP and 3gAllergy, in the diagnosis of milk and wheat allergies within the most common patient populations at pediatric clinics. The results showed a strong correlation in log-transformed values and predicted probabilities between the 2 methods. This signifies that the results of the 2 assay methods are comparable and may be interchangeable, unlike what other studies have found.

https://doi.org/10.4168/aair.2021.13.1.141

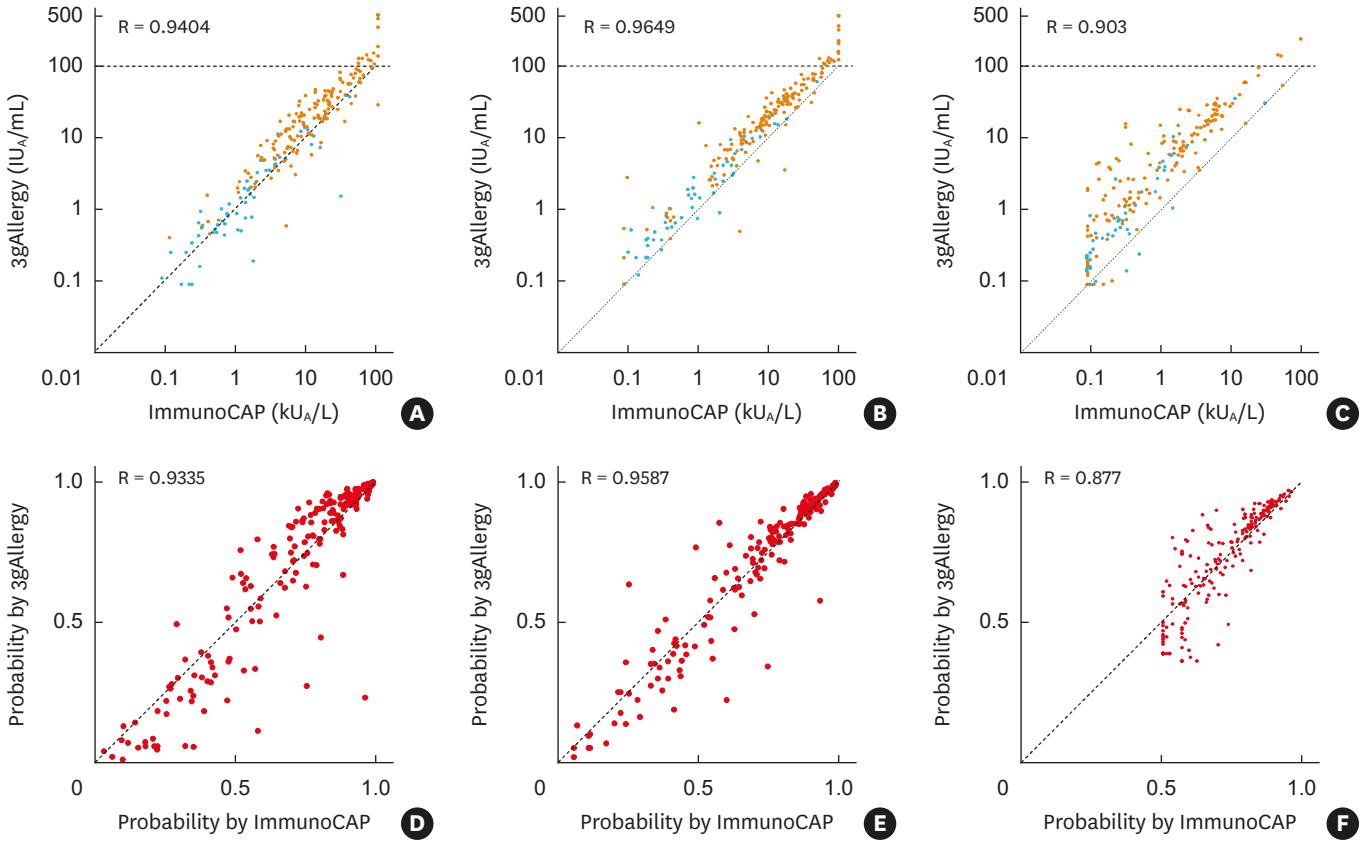

Fig. 1. Correlation between the log-transformed values for milk sIgE (A), casein sIgE (B), and BLG sIgE (C) by ImmunoCAP and 3gAllergy. Pearson's Rs were 0.9404 (95% CI, 0.9225–0.9542; $P < 0.0001$) (A), 0.9649 (95% CI, 0.9542–0.9731; $P < 0.0001$) and 0.9030 (95% CI, 0.8746–0.9252; $P < 0.0001$) (C). Red dots indicate patients with a positive milk OFC (Failed), and blue dots indicate patients with a negative (Passed) milk OFC. Correlation between probabilities predicted by ImmunoCAP and 3gAllergy (D, E, F). Estimated probabilities by ImmunoCAP and 3gAllergy for a positive milk OFC at given milk-sIgE (D), casein-sIgE (E) and BLG-sIgE (F) levels. Pearson's Rs were 0.9335 (95% CI, 0.9136–0.9489; $P < 0.0001$) (D), 0.9587 (95% CI, 0.9462–0.9684; $P < 0.0001$) (E) and 0.8770 (95% CI, 0.8416–0.9048; $P < 0.0001$) (F). Dotted diagonal lines connect from the origin of the axis at 0.01 to the point of 100 (A, B, and C) and from the origin of the axis at 0 to the point of 1 (D, E, and F).
sIgE, specific immunoglobulin E; BLG, β-lactoglobulin; OFC, oral food challenge; CI, confidence interval.

A study by Wood *et al.*[5] compared 3 sIgE assay systems (ImmunoCAP, Immulite and the Turbo radioallergosorbent test [RAST]) using 60 samples for peanut and 20 for soy. They found differences among the systems and concluded that the Immulite assay system overestimated and TurboRAST underestimated IgE results compared to ImmunoCAP. However, they tested for linear correlations using only raw values for sIgE. As is well known, logarithmically transformed, but not raw, data follow a Gaussian distribution, so their results may not be valid. In addition, sIgE data were not generated by using a standardized OFC. A study by Wang *et al.*[6] compared the same 3 systems for the measurement of sIgE to egg, milk, peanut, cat, birch and *Dermatophagoides farina* in 50 serum samples. They also noted some discrepancy among the assays, but they also performed only linear regression analysis. For food allergens, they evaluated the data utilizing 50% and 95% positive-predictive decision points for clinical reactivity that had been determined by other groups using the ImmunoCAP assay.[13,14] They did not determine the decision points for the other assays, and it is obvious that diagnostic accuracy cannot be achieved by using "foreign" decision points. In the present study, we carefully identified predictive values for not only ImmunoCAP but also 3gAllergy based on standardized OFC, and there was a strong correlation between the predictive probabilities with the 2 assay systems. In addition, the use of ImmunoCAP as a standard or reference for the

https://doi.org/10.4168/aair.2021.13.1.141

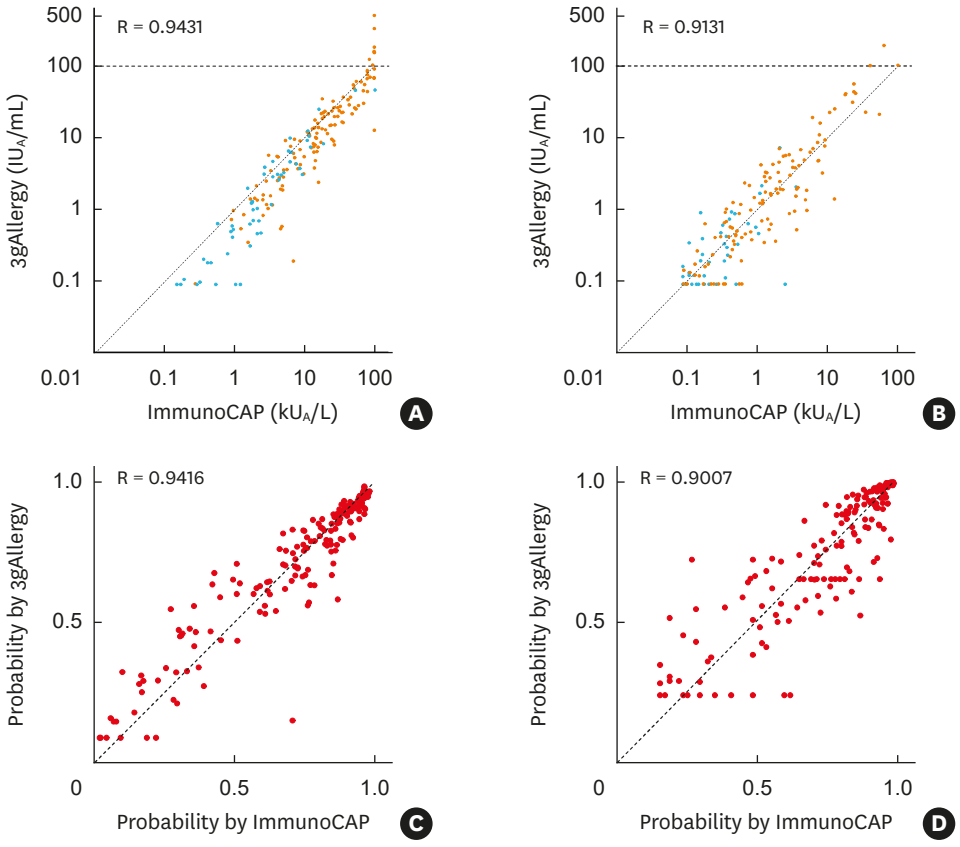

Fig. 2. Correlation between the log-transformed values for wheat-sIgE (A) and OM5G-sIgE (B) by ImmunoCAP and 3gAllergy. Pearson's Rs were 0.9431 (95% CI, 0.9245–0.9571; $P < 0.0001$) (A) and 0.9131 (95% CI, 0.8854–0.9344; $P < 0.0001$) (B). Red dots indicate patients with a positive (Failed) wheat OFC, and blue dots indicate patients with a negative (Passed) wheat OFC.
Correlation between the probabilities predicted by ImmunoCAP and 3gAllergy (C, D). Estimated probabilities by ImmunoCAP and 3gAllergy for a positive wheat OFC at given wheat-sIgE (C) and OM5G-sIgE (D) levels. Pearson's Rs were 0.9416 (95% CI, 0.9226–0.9560; $P < 0.0001$) (C) and 0.9007 (95% CI, 0.8693–0.9249; $P < 0.0001$) (D). Dotted diagonal lines connect from 0.01 (origin) to 100 on the axis (A, B), and from 0 (origin) to 1 on the axis (C, D).
sIgE, specific immunoglobulin E; OM5G, ω-5 gliadin; CI, confidence interval; OFC, oral food challenge.

other assay systems was not appropriate because the basic concept of these 3 assay systems is to measure the relative binding of sIgE to the allergen, not exact molecular amounts.

Interestingly, the AUC results in the milk group were better in older patients with both assay methods, while they were worse in the wheat group. In other words, the diagnostic performance of both assay methods is better when testing is conducted at an older age for milk allergy and at a younger age for wheat allergy. Our results for milk allergen are in line with those reported by Komata *et al.*,[15] who demonstrated better performance of the ImmunoCAP assay for egg and milk sIgE in patients older than 2 years. In the wheat assay, however, we assume that younger patients tend to react more strongly to the water-insoluble components of the wheat allergens (gliadins and glutenins), leading to better performance. A study by Battais *et al.*[16] showed that younger wheat-allergy patients (2–3 years old) had more sIgE antibodies to gliadin fractions detected by the RAST system than older patients (5 years and older). OM5G-sIgE presents a good marker for the diagnosis of immediate-type wheat allergy in children[17,18] and wheat-dependent exercise-induced anaphylaxis in adults.[19] Our results correspond well with those earlier findings.

https://doi.org/10.4168/aair.2021.13.1.141

A strength of our study was that we recruited a population of patients at various ages from multiple centers across Japan. They are very likely to be examined at pediatric clinics, where a physician must aware whether or not a toddler has 'true' milk or wheat allergy, and also whether or not a preschool-aged child has 'outgrown' an allergy. The predictive values established in this study fit those clinical needs.

Nevertheless, the study has several limitations. First, single-blind OFCs without any placebo were performed instead of double-blind placebo-controlled food challenges (DBPCFCs), the gold standard for diagnosis of food allergy. That approach may have caused biased results, but we judged an OFC to be positive only when objective clinical reactions were confirmed. DBPCFCs are very time-consuming and expensive, and they are not practical in real-world clinical settings. Secondly, we did not consider the possible influence of other co-morbid allergies. However, we did a separate statistical analysis not shown here by multivariate logistic regression analysis, which found no association of food allergies with AD or asthma, since our patients' conditions were very well controlled according to the inclusion criteria. We should mention that severe AD patients are not suitable for OFC and that predictive values are needed only for controlled patients. Thirdly, the 2 methods are not "directly" interchangeable in consideration of the differences in the allergen sources, coating methods and antigen-antibody reaction status. Thus, monitoring of sIgE levels should be performed using a single assay method. In real-world clinical settings; however, a physician sometimes receives data generated by 2 different methods, and a conversion table (**Supplementary Table S6**) would be useful.

In conclusion, the ImmunoCAP and 3gAllergy systems showed similar assay performances. Based on strong correlations between the log-transformed values and predicted probabilities, the 2 systems may be interchangeable for the diagnosis of milk and wheat allergies in young children. Since new assay methods have been developed, further studies to test compatibility across the methods needs to be performed.[20]

ACKNOWLEDGMENTS

The authors would like to thank the many IPAD-3g investigators who participated in the study: Drs. Keigo Kainuma, Junya Hirayama and Yu Kuwabara (Mie National Hospital); Drs. Takahiro Ito, Mari Morimoto and Atsushi Yamashita (Allergy Center and Institute for Clinical Research; Mie National Hospital); Dr. Jun Atsuta (Atsuta Pediatric Clinic); Drs. Tatsuki Fukuie and Ryuhei Yasuoka (Department of Pediatrics; Hamamatsu University School of Medicine); Dr. Chiho Tatsumoto (Aozora Pediatric Clinic); Drs. Toshio Katsunuma and Masako Watanabe (Department of Pediatrics; Jikei University Daisan Hospital); Drs. Masanori Ikeda, Kazuko Sugai and Kazuhiro Sekimoto (Department of Pediatrics; National Hospital Organization Fukuyama Medical Center); Drs. Satoshi Sato, Miki Sato and Tae Hijikata (Department of Pediatrics, Tokyo Medical University Hospital); Drs. Yutaka Suehiro, Yukiko Hiraguchi, Yuko Ebishima, Saeko Shimodera and Shouko Yoshino (Department of Pediatrics; Clinical Center of Immunology and Allergy, Osaka Saiseikai Nakatsu Hospital); Drs. Naoki Shimojo, Fumiya Yamaide, Takeshi Yamamoto, Koki Chiba and Kanako Kudo (Department of Pediatrics; Chiba University Graduate School of Medicine); Dr. Reiko Tokuda (Tokuda Family Clinic); Dr. Tomoko Ohtani (Department of Pediatrics; Tokyo Women's Medical University Medical Center East); Drs. Kazunobu Ouchi, Tokio Wakabayashi and Sahoko Ono (Department of Pediatrics; Kawasaki Medical School Hospital); Drs. Kenichi Tokuyama and Eiji Morita (Department of Pediatrics; Saitama Medical University Hospital);

Dr. Hiroyuki Kojima (Higashikoiwa Wanpaku Clinic); Dr. Yasuhiro Shimauchi (Department of Pediatrics; Mitoyo General Hospital); Dr. Hiroshi Nakano (Department of Pediatrics; Minamitama Hospital); Dr. Hiroko Murasugi (Tenshodo Clinic); Drs. Hidetsugu Mizuuchi and Atsue Ubuka (Department of Pediatrics; National Hospital Organization Minami Okayama Medical Center); Dr. Kazumi Hiraba (Mokubo Pediatric Clinic); Dr. Hiroyasu Okahata (Department of Pediatrics; JA Hiroshima General Hospital); Drs. Shigemi Yoshihara and Hironobu Fukuda (Department of Pediatrics; Dokkyo Medical University); Drs. Hirokazu Arakawa, Takumi Takizawa, Hisako Yagi, Harumi Koyama and Tomohiro Utsunomiya (Department of Pediatrics; Gunma University Graduate School of Medicine); Dr. Norimasa Fukuda (Grimm Pediatrics and Allergy Clinic); Drs. Toshimi Nakamura and Yoko Yamashita (Department of Pediatrics; Kanazawa Medical University); Drs. Tetsuro Kitamura and Michimasa Fujiwara (Department of Pediatrics; Nippon Kohkan Fukuyama Hospital); and Dr. Ayumi Kinoshita (Department of Pediatrics; National Hospital Organization Shikoku Medical Center for Children and Adults).

This study was supported by a grant from Japan Allergy Foundation.

SUPPLEMENTARY MATERIALS

Supplementary Table S1
Symptoms provoked in OFC-positive (Failed) subjects

Click here to view

Supplementary Table S2
Comparison of milk-, casein-, and BLG-sIgE values between the milk OFC-positive (Failed) and -negative (Passed) patients in milk OFC

Click here to view

Supplementary Table S3
Comparison of wheat-sIgE and OM5G-sIgE values between the wheat OFC-positive (Failed) and -negative (Passed) patients

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Supplementary Table S4
Milk-, casein-, and BLG-sIgE values with estimated 90% and 10% predicted probabilities of a positive (Failed) milk OFC

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Supplementary Table S5
Wheat-sIgE and OM5G-sIgE values with estimated 90% and 10% predicted probabilities of a positive (Failed) wheat OFC

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Supplementary Table S6

Conversion table for ImmunoCAP to 3gAllergy (milk)

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Supplementary Table S7

Conversion table for ImmunoCAP to 3gAllergy (wheat)

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Supplementary Fig. S1

OFC flow diagram showing a cascade of 'Passed' (negative OFC) and 'Failed' (positive OFC) outcomes.

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Supplementary Fig. S2

Predicted probability derived from logistic regression analysis of milk OFC outcomes. Estimated probability curves for positive (Failed) OFC at a given milk sIgE level by ImmunoCAP (A) and 3gAllergy (B), casein sIgE level by ImmunoCAP (C) and 3gAllergy (D) and BLG sIgE level by ImmunoCAP (E) and 3gAllergy (F) are depicted. Shaded areas indicate the range of 95% CI. Blue and red shaded areas indicate groups A and B, respectively.

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Supplementary Fig. S3

Predicted probability derived from logistic regression analysis of wheat OFC outcomes. Estimated probability curves for positive (Failed) OFC at a given wheat sIgE level by ImmunoCAP (A) and 3gAllergy (B), OM5G sIgE level by ImmunoCAP (C) and 3gAllergy (D) are depicted. Shaded areas indicate range of 95% CI. Blue and red shaded areas indicate groups A and B, respectively.

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REFERENCES

1. Wide L, Bennich H, Johansson SG. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 1967;2:1105-7.
PUBMED | CROSSREF
2. Hamilton RG, Williams PB; Specific IgE Testing Task Force of the American Academy of Allergy, Asthma & Immunology; American College of Allergy, Asthma and Immunology. Human IgE antibody serology: a primer for the practicing North American allergist/immunologist. J Allergy Clin Immunol 2010;126:33-8.
PUBMED | CROSSREF
3. Hamilton RG. Proficiency survey-based evaluation of clinical total and allergen-specific IgE assay performance. Arch Pathol Lab Med 2010;134:975-82.
PUBMED | CROSSREF
4. Sato S, Ogura K, Takahashi K, Sato Y, Yanagida N, Ebisawa M. Usefulness of antigen-specific IgE probability curves derived from the 3gAllergy assay in diagnosing egg, cow's milk, and wheat allergies. Allergol Int 2017;66:296-301.
PUBMED | CROSSREF

5. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. Ann Allergy Asthma Immunol 2007;99:34-41.
PUBMED | CROSSREF
6. Wang J, Godbold JH, Sampson HA. Correlation of serum allergy (IgE) tests performed by different assay systems. J Allergy Clin Immunol 2008;121:1219-24.
PUBMED | CROSSREF
7. Bulat Lokas S, Plavec D, Rikić Pišković J, Živković J, Nogalo B, Turkalj M. Allergen-specific IgE measurement: intermethod comparison of two assay systems in diagnosing clinical allergy. J Clin Lab Anal 2017;31:e22047.
PUBMED | CROSSREF
8. Furuya K, Nagao M, Sato Y, Ito S, Fujisawa T; IPAD3g investigators. Predictive values of egg-specific IgE by two commonly used assay systems for the diagnosis of egg allergy in young children: a prospective multicenter study. Allergy 2016;71:1435-43.
PUBMED | CROSSREF
9. Johansson SG. ImmunoCAP Specific IgE test: an objective tool for research and routine allergy diagnosis. Expert Rev Mol Diagn 2004;4:273-9.
PUBMED | CROSSREF
10. Li TM, Chuang T, Tse S, Hovanec-Burns D, El Shami AS. Development and validation of a third generation allergen-specific IgE assay on the continuous random access IMMULITE 2000 analyzer. Ann Clin Lab Sci 2004;34:67-74.
PUBMED
11. Saeki H, Nakahara T, Tanaka A, Kabashima K, Sugaya M, Murota H, et al. Clinical practice guidelines for the management of atopic dermatitis 2016. J Dermatol 2016;43:1117-45.
PUBMED | CROSSREF
12. Katoh N, Ohya Y, Ikeda M, Ebihara T, Katayama I, Saeki H, et al. Clinical practice guidelines for the management of atopic dermatitis 2018. J Dermatol 2019;46:1053-101.
PUBMED | CROSSREF
13. Perry TT, Matsui EC, Kay Conover-Walker M, Wood RA. The relationship of allergen-specific IgE levels and oral food challenge outcome. J Allergy Clin Immunol 2004;114:144-9.
PUBMED | CROSSREF
14. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. J Allergy Clin Immunol 2004;114:387-91.
PUBMED | CROSSREF
15. Komata T, Söderström L, Borres MP, Tachimoto H, Ebisawa M. The predictive relationship of food-specific serum IgE concentrations to challenge outcomes for egg and milk varies by patient age. J Allergy Clin Immunol 2007;119:1272-4.
PUBMED | CROSSREF
16. Battais F, Mothes T, Moneret-Vautrin DA, Pineau F, Kanny G, Popineau Y, et al. Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat. Allergy 2005;60:815-21.
PUBMED | CROSSREF
17. Ito K, Futamura M, Borres MP, Takaoka Y, Dahlstrom J, Sakamoto T, et al. IgE antibodies to omega-5 gliadin associate with immediate symptoms on oral wheat challenge in Japanese children. Allergy 2008;63:1536-42.
PUBMED | CROSSREF
18. Ebisawa M, Shibata R, Sato S, Borres MP, Ito K. Clinical utility of IgE antibodies to ω-5 gliadin in the diagnosis of wheat allergy: a pediatric multicenter challenge study. Int Arch Allergy Immunol 2012;158:71-6.
PUBMED | CROSSREF
19. Morita E, Matsuo H, Mihara S, Morimoto K, Savage AW, Tatham AS. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. J Dermatol Sci 2003;33:99-104.
PUBMED | CROSSREF
20. Kim SR, Park KH, Lee JH, Kim BJ, Hwang JH, Lim KJ, et al. Validation of PROTIA™ Allergy-Q 64 Atopy® as a specific IgE measurement assay for 10 major allergen components. Allergy Asthma Immunol Res 2019;11:422-32.
PUBMED | CROSSREF