

Comparison between ImmunoCAP and multiple antigen simultaneous tests for measuring *Aspergillus*-specific Immunoglobulin E levels in *Aspergillus*-sensitized patients

Kazunobu Kuwabara, MD, PhD¹, Tatsuyoshi Yokoi, MD¹, Takazumi Yoshida, MD¹, Mamoru Shiga, MD, PhD¹, Masahiro Hirose, MD, PhD¹, Rieko Kondo, MD, PhD¹, Kayoko Matsunaga, MD, PhD², Masashi Nakamura, PhD^{2,3}, Takahiko Horiguchi, MD, PhD¹

¹Department of Internal Medicine Fujita Health University Banbuntane Houtokukai Hospital, Nagoya, Aichi, Japan, ²Department of Integrative Medical Science for Allergic Disease, Fujita Health University School of Medicine, Toyoake, Aichi, Japan, ³General Research and Development Institute, Hoyu Co., Ltd.

Abstract

Objectives: *Aspergillus* sensitization is important for patients with asthma. In Japan, the methods applied to measure allergen-specific immunoglobulin E (IgE) levels in blood are the single antigen test, ImmunoCAP (IC), and multiple antigen simultaneous tests, View Allergy® (VA) or MAST IV® (MA). Here, we report the concordance rates (CR) for *Aspergillus*-specific IgE levels between IC and VA or MA.

Methods: *Aspergillus*-specific IgE levels in serum samples from 34 male and 23 female patients with bronchial asthma were measured by ImmunoCAP, View Allergy® (both Thermo Fisher Scientific, Uppsala, Sweden) and MAST IV® (Hitachi Chemical Diagnostics, Inc. Mountain View, CA, USA). Results of Class 1 or greater were regarded as positive, and the CRs between the methods were assessed.

Results: Of the 57 patients, 24 were found to be positive for *Aspergillus*-specific IgE by IC, and 5 had allergic bronchopulmonary aspergillosis (ABPA). Significant intraclass correlations were observed between IC and VA ($r=0.964$, $p<0.001$) and between IC and MA ($r=0.620$, $p<0.001$). Between IC and VA, the CR, positive concordance ratio, and negative concordance ratio was 98.2%, 100%, and 96.9%, respectively; between IC and MA, these values were 77.2%, 45.8%, and 100%, respectively. All five patients with ABPA were found to be positive for *Aspergillus*-specific IgE by VA, whereas only three of these patients (60%) were found to be positive by MA.

Conclusions: In patients with asthma, measurements obtained by IC were more concordant with those obtained by VA compared with those obtained by MA.

Keywords: Asthma, ImmunoCAP, *Aspergillus*

Introduction

In Japan, the mortality from bronchial asthma (BA) was 4.5%–5.0% per 100,000 individuals between 1975 and 1994. Mortality from BA began to decrease in 1997 and reached 1.2 per 100,000 population (1,510 deaths) in 2015.¹ However, some patients still suffer from poorly controlled asthma. According to a report from the Netherlands, 3.6% of adult patients with asthma are classified as having severe refractory asthma despite adequate adherence to treatment with inhaled corticosteroids and long-acting bronchodilators with correct inhalation technique.² In this circumstance, the assessment and treatment of patients with refractory asthma is an extremely important issue for the therapeutic management of BA.

In addition to well-known risk factors for refractory asthma, such as obesity and gastroesophageal reflux, fungal sensitization can also induce this condition, as was recently proposed in the concept of severe asthma with fungal sensitization (SAFS).³

Sensitization to fungi is thought to lead to the development of refractory BA, and sensitization to *Aspergillus* is particularly common. Because several studies have demonstrated that sensitization of immunoglobulin E (IgE) to *Aspergillus fumigatus* is a risk factor for reduction and acute deterioration of pulmonary function,^{4,5} caution should be taken to detect the development of allergic bronchopulmonary aspergillosis (ABPA) during treatment of refractory asthma. In Japan, because several epidemiological studies have shown that approximately 10% of patients with asthma are sensitized to *A. fumigatus*,^{6,7} early and correct recognition of sensitization to *Aspergillus* is also important for management of patients with asthma.

Although sensitization to *Aspergillus* can be determined by blood or skin tests, the blood test is more frequently conducted to measure *Aspergillus*-specific IgE levels because of its simplicity and minimal invasiveness. ImmunoCAP (IC) is commonly performed to measure allergen-specific IgE levels owing to the large quantity of accumulated data on this assay.⁸ However, because the IC is a single-antigen test, a multiple antigen simultaneous test is often applied to measure allergen-specific IgE levels in daily clinical practice. At present, allergen-specific IgE multiple antigen simultaneous tests are performed in Japan using either MAST IV® (MA) or VIEW Allergy® (VA). The initial version of the MAST kit allowed the measurement of only 16 allergen types, but the MAST IV®, which includes *Aspergillus*

Received 11 November, 2017, Accepted 13 February, 2018.

Corresponding author: Kazunobu Kuwabara, MD, PhD, Department of Internal Medicine Fujita Health University Banbuntane Houtokukai Hospital, 3-6-10 Otobashi, Nakagawa-ku, Nagoya, Aichi 454-8509 Japan
E-mail: kuwabara.1980@gmail.com

Table 1 Comparison of inspection methods

	ImmunoCAP	View Allergy®	MAST IV®
Manufacturer	Thermo Fisher Diagnostics, Ltd.	Thermo Fisher Diagnostics, Ltd.	Hitachi Chemical Co., Ltd.
Measurement principle	FEIA	FEIA	CLEIA
Results	U _A /mL	Class (0–6)	Class (0–6)

FEIA: Fluorescence Enzyme Immunoassay

CLEIA: Chemiluminescent Enzyme Immunoassay

as a test item, was recently released. Additionally, VA, a new multiple allergen-specific IgE assay, was released in 2016. VA is sold by the same manufacturer and includes the same antigen as IC.⁹

MA and IC are different in terms of the applied measurement principle, so the results were assessed after class conversion. The measurement principles of these three test methods are shown in Table 1. In the present study, because previous studies reported that measurements obtained by MA sometimes do not agree with those obtained by IC, we assessed the concordance rates of *Aspergillus*-specific IgE levels between the IC and the newly available multiple antigen simultaneous test methods, i.e., VA and MA.

Methods

We used serum samples obtained from 57 patients with BA who visited Fujita Health University Second Teaching Hospital between February and September 2017 and provided consent to participate in this study. The diagnosis of BA was made by pulmonologists according to the 2015 Japanese Asthma Prevention and Management Guidelines,¹⁰ and the treatment level required for each patient was classified according to the 2017 Global Initiative for Asthma (GINA) guidelines.¹¹ ABPA was defined as a condition that was diagnosed by pulmonologists according to the Patterson criteria¹² and that presented with chest imaging findings of central bronchodilatation and infiltration. This study was conducted after review and approval by the Medical Research Ethics Committee of Fujita Health University (Approval No. HM16-371).

The *Aspergillus*-specific IgE levels in the serum samples were measured separately by IC, VA, and MA. Measurement of *Aspergillus*-specific IgE antibody levels by IC and VA was outsourced to Thermo Fisher Diagnostic K.K., and measurement by MA was outsourced to testing centers (BML, Inc. and SRL, Inc.). Results of Class 1 or greater, as determined by IC, MA, and VA, were regarded as positive, and concordance rates of IC with MA and VA were assessed. The class conversion table for each test method is shown in Table 2.

The statistical analysis software used was StatMate version 3.19 (ATMS, Co., Ltd., Tokyo, Japan). Two independent groups were compared by the Mann–Whitney U test. The degree of association between two variables was determined using Spearman's rank correlation coefficients. Differences across three or more independent groups were assessed by the Kruskal–Wallis test. Values of $p < 0.05$ were considered to indicate a statistically significant difference.

Results

This study included 34 men (mean ± standard deviation for age: 57.5 ± 20.77 years) and 23 women (49.8 ± 15.07 years). Of the 57 patients, 8 (14.0%), 6 (10.5%), 24 (42.1%), 11 (19.2%), and 8

Table 2 Patient background

	Asthma Patient
Age (years), mean ± SD	54.5 ± 19.0
Gender (male:female), n (%)	34 (60%):23 (40%)
GINA treatment step, n (1:2:3:4:5)	8:6:24:11:8
Duration of asthma (years), median (IQR)	12 (3–30)
Total IgE (IU/mL), median (IQR)*	520 (172–1538)

BA: bronchial asthma, IQR: interquartile range, SD: standard deviation

* The same serum as used in this study.

Table 3 Class conversion table for ImmunoCAP, View Allergy®, and MAST IV®

Class	ImmunoCAP (U _A /mL)	View allergy® (Index)	MAST IV® (LC)
6	≥100.0	≥29.31	160–200
5	50.00–99.99	17.35–29.30	120–159
4	17.5–49.99	7.05–17.34	58.1–119
3	3.50–17.49	1.80–7.04	13.5–58.0
2	0.70–3.49	0.50–1.79	2.78–13.4
1	0.35–0.69	0.27–0.49	1.40–2.77
0	0–0.34	0–0.26	0–1.39

LC: lumicount

(14.0%) were assessed as needing Step 1, 2, 3, 4, and 5 treatment levels, respectively, based on the guidelines set by GINA. The median disease duration was 12 years (Q1–Q3: 3–30 years). The median total IgE level was 520 IU/mL (Q1–Q3: 172–1538 IU/mL) (Table 3). Of the 57 patients, 24 (42.1%) were found to be positive for *Aspergillus*-specific IgE by IC. This group included 5 patients with ABPA. The measurements obtained by IC were significantly correlated with those obtained by both VA ($r = 0.976$, $p < 0.001$) and MA ($r = 0.635$, $p < 0.001$). All 5 patients with ABPA ($n = 5/5$) were found to be positive for *Aspergillus*-specific IgE by VA, whereas 60% ($n = 3/5$) were found to be positive by MA (Figure 1). Between IC and VA, the concordance rate was 98.2%, the positive concordance ratio was 100%, and the negative concordance ratio was 96.9%. Between IC and MA, the concordance rate was 77.2%, the positive concordance ratio was 45.8%, and the negative concordance ratio was 100% (Figure 2).

Discussion

The antibody class IgE was first reported by Ishizaka et al. in 1967,¹³ and IC has been widely used for measuring allergen-specific IgE levels for more than 40 years. IC is regarded as the gold standard for the in vitro measurement of allergen-specific IgE levels.⁸ Thus, IC results are used as the benchmark against which the results of screening for allergen-specific IgE are compared.

This study found that, in patients with BA, *Aspergillus*-specific IgE levels measured by IC were more concordant with those

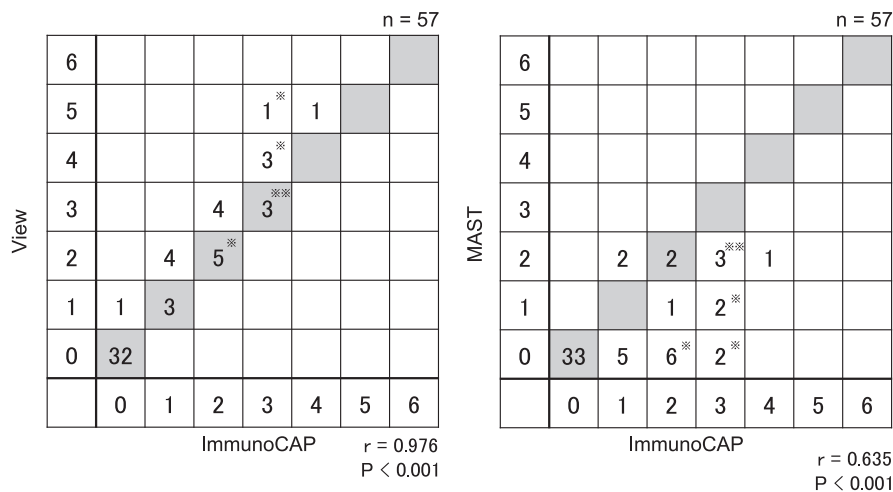


Figure 1 The degree of association between two variables was determined using Spearman’s rank correlation coefficients. Association of *Aspergillus*-specific immunoglobulin E classes determined by ImmunoCAP with those determined by VIEW Allergy and MAST IV. The numerical values in cells are the number of patients. ImmunoCAP showed significant correlations with both test methods. Of the 5 patients with allergic bronchopulmonary aspergillosis included in this study, 5 were found to be positive by VIEW Allergy, and 3 were found to be positive by MAST IV.
*, Including 1 ABPA patient, **, Including 2 ABPA patients

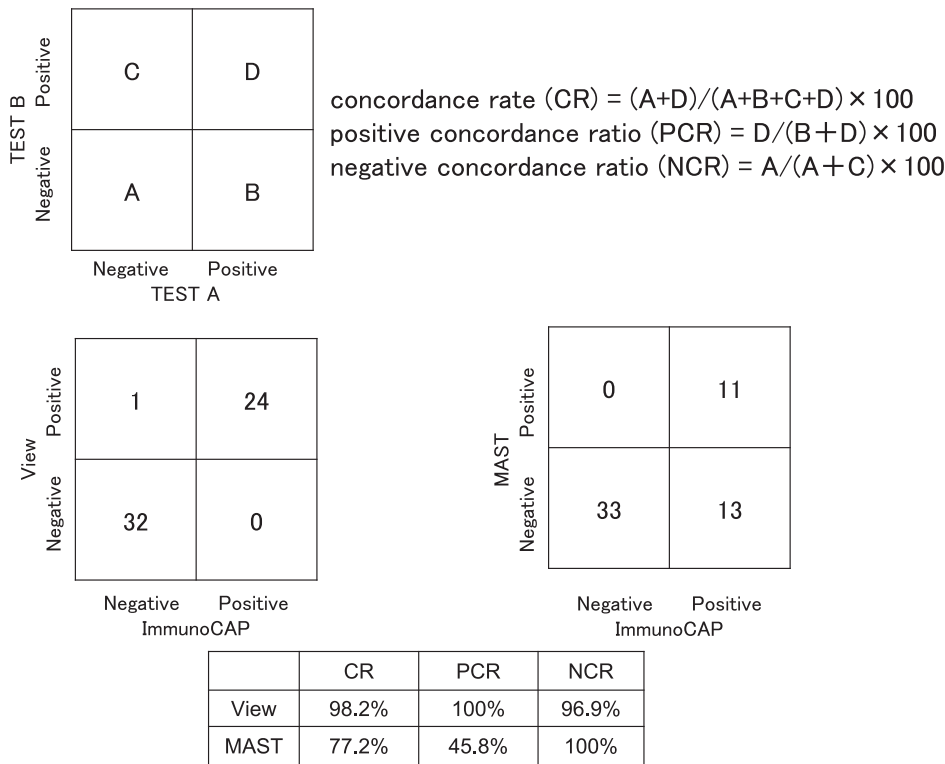


Figure 2 Concordance rates, positive concordance ratios, and negative concordance ratios of *Aspergillus*-specific immunoglobulin E levels between ImmunoCAP and VIEW Allergy and between ImmunoCAP and MAST IV. The numerical values in cells are the number of patients.
CR; concordance rate, PCR; positive concordance ratio, NCR; negative concordance ratio

measured by VA than with those measured by MA. The higher concordance rate between IC and VA is presumably attributable to the fact that both test methods were developed by the same manufacturer and use the same antigen.⁹ Unfortunately, we could not obtain information on the *Aspergillus* strain used for MA. Because it has been reported that fungal antigen components are likely to differ among culture methods, deviations in the test results were assumed to be attributable to differences in the

antigen components of the test kits and the inactivation of antigens during test kit production.¹⁴
Of the five patients with ABPA, 60% (3/5) were identified as positive for *Aspergillus*-specific IgE by MA, whereas VA identified all ABPA patients (5/5) as positive. A recent report indicated that many patients with ABPA are sensitized to Asp f 1 and Asp f 2, based on component analysis.¹⁵ The differing sensitivity of the MA and VA tests for patients with ABPA may be attributable to

differences in the content of these components.

For optimal management of patients with asthma, it is important to determine whether they are sensitized to inhalant antigens, such as fungi, mites, and pollen. The allergen-specific IgE multiple antigen simultaneous test is widely applied in clinical practice because it allows the examination of sensitizations to a wide range of antigens, including not only inhalant antigens but also food antigens and latex. Recently, it has become increasingly important to clarify *Aspergillus* sensitization in asthma patients, and the screening method used in the initial phase of asthma treatment requires a high level of sensitivity. Based on reports demonstrating an increase in *Aspergillus*-specific IgE during medical treatment and occasional ABPA development during asthma treatment,^{16,17} *Aspergillus*-specific IgE should be monitored over time in the treatment of asthma, and it is therefore important to confirm *Aspergillus* sensitization in ABPA diagnosis.

When a simultaneous multi-parameter screening of allergen-specific IgE is used to determine the sensitization status of a BA patient, it is important to consider that the results may not be consistent among tests. This difference is clinically relevant because the gold standard Immuno CAP, rather than the initial screening method, is often used for IgE measurement during asthma management. Our results indicate that VA, which achieved consistent results in comparison with historical data and exhibited a high sensitivity even in ABPA patients, is superior as a screening method for assessing *Aspergillus* sensitization in asthma patients.

Conflict of Interest

No potential COI to disclose.

Acknowledgements

We would like to express our sincere appreciation to Thermo Fisher Diagnostics in this study.

References

1. Ichinose M, Sugiura H, Nagase H, Yamaguchi M, Inoue H, Sagara H, Tamaoki J, Tohda Y, Munakata M, Yamauchi K, Ohta K. Japanese guidelines for adult asthma 2017. *Allergol Int* 2017; 66: 163–89.
2. Hekking P, Wener R, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015; 135: 896–902.
3. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006; 27: 615–26.
4. Fairs A, Agbetile J, Hargadon B, Bourne M, Monteiro WR, Brightling CE, Bradding P, Green RH, Mutalithas K, Desai D, Pavord ID, Wardlaw AJ, Pashley CH. IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am J Respir Crit Care Med* 2010; 182: 1362–8.
5. Goh K, Yli ACA, Lapperre TS, Chan AK, Chew FT, Chotirmall SH, Koh MS. Sensitization to *Aspergillus* species is associated with frequent exacerbations in severe asthma. *J Asthma Allergy* 2017; 10: 131–40.
6. Adachi M, Inoue H, Tamura G, Sano Y, Ohta K, Nakagawa T, Ito K, Baba K, Hirata K, Tohda Y, Nakajima S, Takahashi K, Asai S, Miyamoto T. Sensitizing allergens of patients with bronchial asthma in Japan. *Allergology & Immunology* 2006; 13: 548–54 (in Japanese).
7. Ohira D, Shiga M, Kuwabara K, Ban N, Hata H, Nasu T, Hirose M, Kondo R, Horiguchi T. Importance of insect inhalant allergens (including cockroach allergen) in adult bronchial asthma. *Occupational and Environmental Allergy* 2013; 20: 59–68 (in Japanese).
8. Akdis C, Agache I. Global atlas of allergy. *European Academy of Allergy and Clinical Immunology* 2014: 166.
9. Harita M. Fundamental study on multiple allergen specific IgE assay: "View allergy 39". *Japanese Journal of Medicine and Pharmaceutical Science* 2016; 73: 721–6 (in Japanese).
10. Japanese Society of Allergology. *Asthma Prevention and Management Guidelines (JGL)*. Tokyo: Kyowa Kikaku; 2015 (in Japanese).
11. Global Initiative for Asthma. *GINA Report. Global Strategy for Asthma Management and Prevention* 2017; 2017: 43. <<http://ginasthma.org/gina-reports>>. (Accessed November 2, 2017).
12. Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis and the evaluation of the patient with asthma. *J Allergy Clin Immunol* 1988; 81: 646–50.
13. Ishizaka K, Ishizaka T, Hornbrook MM. Physico-chemical properties of human reaginic antibody: IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 1966; 97: 75–85.
14. Esch RE. Manufacturing and standardizing fungal allergen products. *J Allergy Clin Immunol* 2004; 113: 210–5.
15. Tanimoto H, Fukutomi Y, Yasueda H, Takeuchi Y, Saito A, Watai K, Sekiya K, Tsuburai T, Asano K, Taniguchi M, Akiyama K. Molecular-based allergy diagnosis of allergic bronchopulmonary aspergillosis in *Aspergillus fumigatus*-sensitized Japanese patients. *Clin Exp Allergy* 2015; 45: 1790–800.
16. Agarwal R. Role of inhaled corticosteroids in the management of serological allergic bronchopulmonary aspergillosis (ABPA). *Intern Med* 2011; 50: 855–60.
17. Greenberger PA, Miller TP, Roberts M, Smith LL. Allergic bronchopulmonary aspergillosis in patients with and without bronchiectasis. *Ann Allergy* 1993; 70: 333–8.

Copyright©2018 Kazunobu Kuwabara, MD, PhD et al. 

This is an Open access article distributed under the Terms of Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.