Evaluation of a commercial quantitative Aspergillus fumigatus-specific IgM assay for the diagnosis of invasive pulmonary aspergillosis

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Abstract
Invasive pulmonary aspergillosis (IPA) is a common fungal infection with high mortality rates in immunocompromised patients. Early diagnosis of IPA is still challenging because of its nonspecific clinical symptoms and radiological presentations.

To evaluate the clinical value of a commercial Aspergillus fumigatus-specific IgM antibody assay in diagnosis of IPA, a multicenter prospective study was performed in 12 hospitals in Zhejiang Province, China, from January 1 to December 31, 2016. A total of 59 patients were enrolled in this study, including 30 IPA and 29 non-IPA patients. The sensitivities of IgM assay were 30.0%, 26.7%, 23.3%, and 20.0%, and the specificities were 79.3%, 86.2%, 86.2%, and 96.6% at the cutoff values of 50, 60, 70 and 80 AU/mL, respectively. The area under the curve of the IgM assay revealed by the receiver-operating characteristic analysis was 0.511 in the IPA cases. This study is the first to evaluate the clinical performance of a commercial A. fumigatus-specific IgM antibody assay that uses envelopes galactomannan extracted from A. fumigatus as the sole antigen in diagnosis of IPA in China.

In conclusion, the A. fumigatus-specific IgM antibody assay has limited value and should not be a prior recommendation for IPA diagnosis.

Abbreviations: A. fumigatus = Aspergillus fumigatus, AU = arbitrary units, AUC = area under the curve, BALF = bronchoalveolar lavage fluid, BDG = (1→3)-ß-D-glucan, CI = confidence interval, COPD = chronic obstructive pulmonary disease, CT = computed tomography, ELISA = enzyme-linked immunosorbent assay, EORTC/MSG = The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group, GM = galactomannan, IPA = invasive pulmonary aspergillosis, LFD = lateral-flow device, NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value, ROC = receiver-operating characteristic curve.

Keywords: invasive pulmonary aspergillosis, IPA, serum Aspergillus fumigatus-specific IgM antibody assay

1. Introduction
Aspergillus spp. are ubiquitous in the environment. Aspergillus fumigatus (A. fumigatus) is the most common pathogen among...
quantitative approach with high diagnostic value for IPA is urgently needed.

Numerous and different antigens may be produced by *Aspergillus* during the growth cycle, and their corresponding antibodies would be produced after interacting with the immune system. The IgM antibody is noticeably produced in the early stages of infection. Recently, a new commercial *A. fumigatus*-specific IgM antibody assay that uses GM extracted from *A. fumigatus* as the sole antigen became available in China. We wondered whether this commercial *Aspergillus*-specific IgM antibody assay could play a role in diagnosis of IPA. The purpose of this study was to evaluate the clinical value of the IgM antibody assay in IPA diagnosis.

2. Patients and methods

This prospective study was performed in 12 hospitals in Zhejiang Province, Eastern China from January to December, 2016. The core institute for the study was the First Affiliated Hospital, School of Medicine, Zhejiang University, a 2000-bed referral hospital in Hangzhou. The institutional review board of Clinical Research of the First Affiliated Hospital, School of Medicine, Zhejiang University approved the study protocol (Number: 20158443), and all methods were performed in accordance with the approved guidelines and regulations. Written informed consent was obtained from all of the patients. Laboratory technicians who were responsible for testing the samples could not identify individual participants or access clinical data, whereas other authors had access to information that could identify individual participants during or after data collection.

Adult inpatients with severe immunocompromised conditions, clinical symptoms, or radiological findings <1 month, and an infiltration of chest CT were included. Patients who had received treatment for *Aspergillus* for >3 days in the last 3 months, patients who had malignant hematological diseases, or patients refusing enrollment were excluded from the study. Flow of participants was showed in Figure 1. CT data were examined and agreed by 2 experienced radiologists. IPA diagnosis was determined according to revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group.

All of the enrolled patients received examinations including at least 1 sputum culture/smear and 1 serum GM test. Alternative examinations included bronchoscopy (BALF culture/smear/GM, biopsied tissue culture/pathology) and percutaneous CT-guided lung biopsy (biopsied tissue culture/pathology). Enrolled patients were classified as IPA (proven and probable) and non-IPA patients according to revised definitions of invasive fungal disease from the EORTC/MSG Consensus Group. Clinical data including disease history, clinical manifestations, diagnostic results, and treatment were also collected for every patient and reported to the core unit for further confirmation. The enrollment and definitive diagnosis were determined by 2 experienced respiratory physicians in the core unit.

2.1. Serological analysis

The serum *A. fumigatus*-specific IgM antibody level was detected on all of the samples by Dynamiker *A. fumigatus* IgM assay (Dynamiker, Tianjin, China). Plate enzyme-linked immunosor-
In the present study, we evaluated the clinical performance of a commercial *Aspergillus* IgM antibody assay in diagnosis of IPA. Our results suggest that serum *A. fumigatus*-specific IgM antibody assay can offer little assistance to current diagnostic methods in IPA.

Compared with the non-IPA patients, the IPA patients had a higher frequency of COPD history. The possible reason may be the increased incidence of IPA in patients with COPD in recent years. Tacccone et al. studied the epidemiology of invasive aspergillosis in critically ill patients and showed that the most common comorbidity condition was COPD. The IPA and non-IPA patients had no significant difference in respiratory symptoms, and the reason could be that IPA patients presented nonspecific clinical symptoms. Our study enrolled suspected IPA patients as the control rather than the healthy patients, which resulted in the similar host factors between IPA and non-IPA patients.

For the *A. fumigatus*-specific-IgM assay, the sensitivities were 20.0% to 30.0%, and the specificities were 79.3% to 96.6% at diagnostic cutoffs of 50 to 80 AU/mL. The ROC analysis showed the *A. fumigatus* specific-IgM assay had limited clinical value in diagnosis of IPA, which was in consistent with results from other existing commercial IgM kits. IgM antibody production may not occur during the course of IPA, as most of IPA patients were in immunocompromised conditions. Detection of *Aspergillus*-specific IgM antibodies is not accepted as the widely used IPA diagnostic criteria. Kappe et al. studied IgM antibody levels in IPA patients, and only 2 of 26 patients showed positive IgM.

### Table 1

| Characteristics                  | IPA (n = 30) | Non-IPA (n = 29) | P       |
|----------------------------------|-------------|-----------------|---------|
| Sex (M/F)                        | 22/8        | 19/10           | 0.514   |
| Mean (age ± SD, y)               | 64.1 ± 17.9 | 56.1 ± 11.8     | 0.049   |
| Previous pulmonary diseases      |             |                 |         |
| COPD (yes/no)                    | 9/21        | 0/29            | 0.004*  |
| Tuberculosis (yes/no)            | 0/30        | 1/28            | 0.492   |
| Bronchietasis (yes/no)           | 0/30        | 1/28            | 0.492   |
| Other (yes/no)                   | 7/23        | 3/26            | 0.326   |
| Respiratory symptoms             |             |                 |         |
| Productive cough (yes/no)        | 23/6        | 17/12           | 0.089   |
| Hemoptysis (yes/no)              | 1/28        | 3/26            | 0.604   |
| Fever (yes/no)                   | 16/13       | 18/11           | 0.594   |
| Host factors                     |             |                 |         |
| T cell immunosuppressants (yes/no)| 5/25        | 9/20            | 0.195   |
| Prolonged use of corticosteroids (yes/no)| 6/24       | 4/25            | 0.773   |
| Treatment for malignant diseases (yes/no)| 15/15    | 10/19           | 0.228   |
| Recent history of neutropenia (yes/no)| 4/26       | 5/24            | 0.956   |

COPD = chronic obstructive pulmonary disease, IPA = invasive pulmonary aspergillosis, SD = standard deviation.

*P < .05.

### Table 2

| Examinations                                      | IPA  | Non-IPA | P       |
|---------------------------------------------------|------|---------|---------|
| Pathological examination                          | 1    | 1       |         |
| Host factors, chest CT and positive               | 11   | 1       |         |
| Sputum culture and serum GM                       | 4    | 1       |         |
| Serum                                             | 3    | 1       |         |
| BALF GM                                           | 4    | 1       |         |
| Sputum culture and BALF GM                        | 2    | 1       |         |
| BALF culture and GM                               | 1    |         |         |

BALF = bronchoalveolar lavage fluid, CT = computed tomography, GM = galactomannan, IPA = invasive pulmonary aspergillosis, No. = number.

### Table 3

| Cut-off, AU/mL | Sensitivity | Specificity | PPV     | NPV     | Youden index |
|----------------|-------------|-------------|---------|---------|--------------|
| 50             | 30.0 (15.4–49.6) | 79.3 (59.7–91.3) | 60.0 (32.9–82.5) | 52.3 (36.9–76.3) | 0.003         |
| 60             | 26.7 (13.0–46.2) | 86.2 (67.4–95.5) | 66.7 (35.4–87.7) | 53.2 (38.2–67.6) | 0.129         |
| 70             | 23.3 (10.6–42.7) | 86.2 (67.4–95.5) | 63.6 (31.6–87.6) | 52.1 (37.4–66.5) | 0.095         |
| 80             | 20.0 (8.4–39.1)  | 96.6 (80.4–99.8) | 85.7 (42.0–99.2) | 53.8 (39.9–76.5) | 0.166         |

IPA = invasive pulmonary aspergillosis, NPV = negative predictive value, PPV = positive predictive value.
antibodies, which agreed with the poor sensitivities shown in our current study. The sensitivities of the A. fumigatus–specific IgM assay on all of the above cut-offs was not competitive compared with other widely used diagnostic methods. The sensitivity of GM assay ranges from 36.4% to 97.0% in BALF sample and 11.6% to 90.9% in serum samples. The sensitivity of PCR-based assays ranges from 66.7% to 95.5%[5,20,21] and 77% to 81.8% for Aspergillus-specific LFD tests.[4,19] In regard to the specificity of the A. fumigatus-specific IgM assay, the overall result is acceptable at 80 AU/mL (96.6%) when comparing it with the GM assay (at least 85%)[11,18,20] PCR assays (92.5%–98.7%)[5,20,21] and Aspergillus-specific LFD tests (92%–98.0%).[18,21]

To our best of knowledge, this is the first study to evaluate the clinical performance of a commercial A. fumigatus IgM antibody kit that uses GM extracted from A. fumigatus as sole antigen in the diagnosis of IPA. In conclusion, this serum A. fumigatus-specific IgM antibody assay has a limited value and should not be recommended for IPA diagnosis.

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