Infection and Drug Resistance

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ORIGINAL RESEARCH

Value Evaluation of Quantitative *Aspergillus fumigatus*-Specific IgG Antibody Test in the Diagnosis of Non-neutropenic Invasive Pulmonary Aspergillosis

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Background: The role of *Aspergillus*-specific IgG antibody test in the diagnosis of non-neutropenic invasive pulmonary aspergillosis (IPA) is still uncertain, and related studies are also limited.

Purpose: This study aims to evaluate the quantitative test value of *Aspergillus fumigatus*-specific IgG antibody in non-neutropenic IPA, which could provide additional evidence for related clinical diagnosis.

Methods: This prospective study collected clinical data of suspected IPA patients from January, 2020 to December, 2022, and patients were divided into two groups, IPA and non-IPA. The study analyzed clinical characteristics and diagnostic value of *Aspergillus*-specific IgG antibody test, using the receiver operating characteristic (ROC) curve to evaluate diagnostic efficacy.

Results: The study enrolled 59 IPA cases and 68 non-IPA cases, the average admission age of IPA group was 63.2±9.6 (33–79), and the gender ratio (male:female) of IPA group was 42:17. The proportion of patients with history of smoking and COPD were higher in IPA group (59.3% vs 39.7%, P=0.027; 33.9% vs 14.7%, P =0.011, respectively). The level of *Aspergillus fumigatus*-specific IgG antibody in IPA group was significantly higher than non-IPA group (202.1±167.0 vs 62.6±58.0, P<0.001). The area under the ROC curve was 0.799 (95%CI: 0.718, 0.865 P<0.001), and the cut-off with best diagnostic efficacy was 91 AU/mL.

Conclusion: Immunological test plays an important role in the diagnosis of pulmonary aspergillosis, and *Aspergillus*-specific IgG antibody test has the good diagnostic value in non-neutropenic IPA.

Keywords: *Aspergillus*, invasive pulmonary aspergillosis, IgG, non-neutropenic, diagnosis

Introduction

Aspergillus is one of the pathogenic fungi which may lead to life-threatening invasive infections. Approximately 50 species of *Aspergillus* are pathogenic to humans, most commonly *Aspergillus fumigatus*, which accounts for more than 90% of *Aspergillus* infections.[1,2] Therefore, it is essential to deal with different issues of *A. fumigatus* on clinical work. Aspergillosis is defined as different kinds of diseases caused by *Aspergillus*, and pulmonary aspergillosis (PA) is the main clinical manifestation.[3] The spectrum of PA is wide, including IPA, various kinds of chronic pulmonary aspergillosis (CPA) and allergic bronchopulmonary aspergillosis (ABPA), and IPA is the most severe pattern, which is more common in severely immunosuppressed patients.[4] People's innate immune system plays an important role in immune response against *Aspergillus*, specifically, IL-1β and tumor necrosis factor (TNF) engage immune and nonimmune cells, balance of Th1 and Th2 cytokines modulates the intensity of the response, and anti-inflammatory cytokines suppress inflammation during recovery.[5]

Received: 20 January 2024
Accepted: 16 May 2024
Published: 23 May 2024

In recent years, the morbidity and mortality of PA have gradually increased, and the increase of IPA cases is one of the main reasons for this phenomenon, especially those non-neutropenic cases. Global data in 2017 indicated that there were approximately 250,000 new IPA cases each year. Previous studies have shown that the incidence of IPA was 15%, and the mortality rate could be as high as 30~80% in patients with immune deficiency.[6,7]

Therefore, early diagnosis and timely treatment are essential to reduce mortality and improve prognosis of IPA. At present, IPA diagnosis should consider clinical features, radiology, histopathology, immunology – mainly refers to specific antigen tests, including galactomannan (GM) test, 1, 3-β-D-glucan (BDG) test (G test) – and microbiology all together, and exclude alternative diagnosis.[6] Among the above diagnostic methods, immunological tests have advantages of convenient operation, high sensitivity and specificity, short-time duration and less trauma.[6,8] Previous studies have proved that GM test in serum and bronchial alveolar lavage fluid (BALF) have higher sensitivity and specificity for diagnosis of IPA than CPA.[9] G test has high sensitivity but limited specificity in a wide range of invasive fungal infections like IPA, and its sensitivity is low in CPA.[10,11]

Immunological tests also involve antibody tests, including *Aspergillus*-specific immunoglobulin M (IgM), IgG, IgE, IgA antibody tests. Nevertheless, related studies with *Aspergillus*-specific antibody tests' diagnostic value in IPA are still limited. Herbrecht et al found that elevated levels of *Aspergillus*-specific IgG antibodies were detected in up to 60% of IPA patients.[12] The previous study has found that *Aspergillus*-specific IgG antibody test has the certain diagnostic value for CPA and IPA, while the specificity is higher in IPA than CPA.[13]

Thus, there is no uniform antibody test widely used for the diagnosis of IPA, and further exploration is necessary. This study aims to evaluate the quantitative diagnostic value of the *A. fumigatus*-specific IgG antibody test in non-neutropenic IPA, with a view to provide much more reference for related clinical work.

Materials and Methods

Materials and Data Collection

This was a prospective, single-center and controlled study, 59 IPA cases and 68 non-IPA cases admitted to the First Affiliated Hospital, Zhejiang University School of Medicine from January, 2020 to December, 2022 were finally enrolled in this study (Figure 1). The enrollment, diagnosis and all testing results were evaluated by experienced respiratory physicians in our hospital.

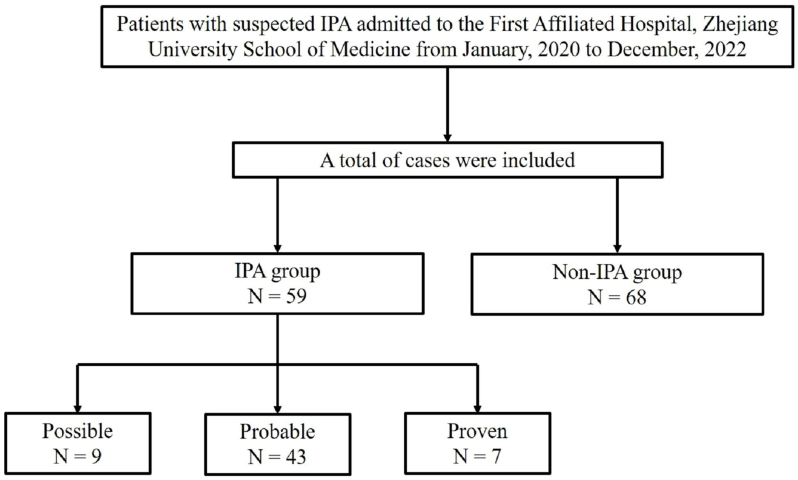

Figure 1 The flow chart of the study. The criteria of possible IPA: (1) Patients' age ≥18; (2) immune insufficiency (such as congenital immunodeficiency, long-term glucocorticoid treatment (glucocorticoid treatment time ≥3 weeks in the past 60 days),[13] long-term immunosuppressive therapy after solid organ transplantation, radiotherapy and chemotherapy for malignant tumors, etc) or others with emerging risk factors of IPA, such as end-stage COPD, liver cirrhosis, etc; (3) The time for appearance of suspected clinical symptoms or abnormal imaging manifestations of IPA was ≤1 month; (4) abnormal infiltrative manifestations in pulmonary CT images. On the basis of the possible IPA, proven IPA criteria should also meet: histopathological evidence or positive culture result from sterile environment (excluding BALF), probable IPA criteria should meet: mycologic evidence such as GM test, positive culture result (qualified specimen from sputum, BALF, bronchial brush), *Aspergillus* PCR, etc.

Suspected IPA patients were recruited in the study, the inclusion criteria were: (1) patients' age ≥18; (2) immune insufficiency (such as congenital immunodeficiency, long-term glucocorticoid treatment (glucocorticoid treatment time ≥3 weeks in the past 60 days),[14] long-term immunosuppressive therapy after solid organ transplantation, radiotherapy and chemotherapy for malignant tumors, etc) or others with emerging risk factors of IPA, such as end-stage COPD, liver cirrhosis, etc; (3) The time for appearance of suspected clinical symptoms or abnormal imaging manifestations of IPA was ≤1 month; (4) abnormal infiltrative manifestations in pulmonary computed tomography (CT) images. The inclusion criteria are exactly possible IPA's criteria.

The exclusion criteria were: (1) neutropenic patients (defined as neutrophils $\leq 1.8\times10^9$/L);[15] (2) received anti-*Aspergillus* treatment ≥3 days in the past 3 months; (3) accompanied by hematological malignant tumors; (4) patients who were positive for human immunodeficiency virus (HIV); (5) patients with past history of *Aspergillus* infection or colonization.

The patients with suspected IPA were divided into two groups: IPA and non-IPA, and the diagnostic criteria of IPA was according to the modified version by The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) in 2019.[14] Possible IPA should meet inclusion criteria, on the basis of the former, proven IPA needed histopathological evidence or positive culture result from sterile environment (excluding BALF), while probable IPA required mycologic evidence such as GM test, positive culture result (qualified specimen from sputum, BALF, bronchial brush), *Aspergillus* PCR, etc.

The patients' inpatient medical records were collected. The collected data included current medical history, past history, personal history, other laboratory test results, and so on (Table 1 and Table 2).

Table 1 Clinical Baseline Information of IPA and Non-IPA Patients

Items	IPA n (%)	Non-IPA n (%)	*P*-value
Cases	59	68	
Admission age	63.2±9.6	61.1±12.4	0.286
Age range	33–79	32–85	–
Gender (male/female)	42/17	46/22	0.666
Smoking history	35 (59.3)	27 (39.7)	0.027
Allergic history	10 (16.9)	6 (8.8)	0.169
Operation history	26 (44.1)	30 (44.1)	0.995
Other positive pathogens	39 (66.1)	46 (67.6)	0.854
Concrete positive pathogens			
Pseudomonas aeruginosa	5 (8.5)	2 (2.9)	0.331
Candida. Spp	17 (28.8)	13 (19.1)	0.199
Cryptococcus neoformans	0	4 (5.9)	0.166
Staphylococcus aureus	0	2 (2.9)	0.499
Acinetobacter baumannii	1 (1.7)	2 (2.9)	1.000
Stenotrophomonas maltophilia	3 (5.1)	3 (4.4)	1.000
Klebsiella pneumoniae	5 (8.5)	4 (5.9)	0.825
Influenza virus	6 (10.2)	2 (2.9)	0.192
EB virus	3 (5.1)	7 (10.3)	0.449
CMV	2 (3.4)	4 (5.9)	0.810
Others[a]	20 (33.9)	25 (36.8)	0.736
History of pulmonary diseases			
COPD	20 (33.9)	10 (14.7)	0.011
Bronchiectasis	3 (5.1)	3 (4.4)	1.000
Pulmonary tuberculosis	9 (15.3)	6 (8.8)	0.263
Lung cancer	9 (15.3)	11 (16.2)	0.887
Asthma	5 (8.5)	1 (1.5)	0.151
Others[b]	11 (18.6)	23 (33.8)	0.054

(*Continued*)

Table 1 (Continued).

Items	IPA n (%)	Non-IPA n (%)	*P*-value
Other comorbidities			
Hypertension	22 (37.3)	22 (32.4)	0.560
Diabetes mellitus	10 (16.9)	16 (23.5)	0.359
Heart disease	7 (11.9)	10 (14.7)	0.639
Autoimmune disease	7 (11.9)	13 (19.1)	0.263
Liver cirrhosis	0	4 (5.9)	0.166
Other malignant tumor	4 (6.8)	10 (14.7)	0.155
Solid organ transplantation	1 (1.7)	6 (8.8)	0.172
Others[c]	25 (42.4)	40 (58.8)	0.064
History related with immune function			
Immunosuppressive agents	3 (5.1)	10 (14.7)	0.074
Long-term hormone	15 (25.4)	17 (25.0)	0.956
Radiation/Chemotherapy for malignant tumors	9 (15.3)	13 (19.1)	0.566
Clinical symptoms			
Cough	50 (84.7)	52 (76.5)	0.242
Expectoration	41 (69.5)	44 (64.7)	0.568
Fever	36 (61.0)	36 (52.9)	0.360
Hemoptysis	7 (11.9)	4 (5.9)	0.232
Shortness of breath	37 (62.7)	24 (35.3)	0.002
Oppression in chest	31 (52.5)	25 (36.8)	0.074
Chest pain	5 (8.5)	5 (7.4)	1.000

Notes: [a]Hepatitis B virus, *Escherichia coli, Mycobacterium tuberculosis*, etc. [b]Interstitial lung disease, lung abscess, focal organizing pneumonia, etc. [c]Nervous system diseases, metabolic diseases, mental diseases, etc. **Abbreviations**: CMV, cytomegalovirus; EB, Epstein-Barr; COPD, chronic obstructive pulmonary disease.

Table 2 Laboratory Testing Results of IPA and Non-IPA Patients

Items	IPA	Non-IPA	*P*-value
Cases	59	68	
Aspergillus-specific IgG antibody (AU/mL)	202.1±167.0	62.6±58.0	<0.001
Negative/middle/positive	17/9/33	53/8/7	–
WBC ($\times10^9$/L)	10.9±6.3	9.2±5.0	0.089
Neutrophils ($\times10^9$/L)	9.2±6.3	7.5±4.7	0.078
Eosinophils ($\times10^9$/L)	0.1±0.2	0.1±0.1	0.505
Monocytes ($\times10^9$/L)	0.6±0.4	0.6±0.6	0.833
Lymphocytes ($\times10^9$/L)	1.0±0.7	1.0±0.6	0.823
RBC ($\times10^9$/L)	3.8±0.6	3.8±0.7	0.682
Hb (g/L)	112.4±19.7	114.9±21.3	0.495
PLT ($\times10^9$/L)	279.2±111.6	238.1±128.2	0.059
ALT (U/L)	44.2±54.2	42.2±84.8	0.876
AST (U/L)	42.2±53.5	31.8±30.0	0.580
Albumin (g/L)	32.1±6.6	34.8±5.9	0.016
Creatine (μmol/L)	66.1±33.8	89.0±88.1	0.307
LDH (U/L)	361.8±249.3	286.2±126.0	0.140
CRP (mg/L)	80.2±88.0	64.7±75.2	0.292
ESR (mm/h)	58.7±26.7	45.7±31.1	0.021
PCT (ng/mL)	0.5±1.0	3.6±10.1	0.071

Abbreviations: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobulin; PLT, platelets; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; PCT, procalcitonin.

Aspergillus fumigatus-specific IgG Antibody Test

The antibody used in this study was *A. fumigatus*-specific IgG antibody, which reflected patient's immune response to spores, and it was detected by means of enzyme-linked immunosorbent assay (ELISA) kit (Dynamiker, Tianjin, China). Optical density (OD) values were passed through the microplate reader (Bio-Rad iMark, Hercules, California, USA).

The recommended positive cut-off value of *A. fumigatus*-specific IgG antibody kit was 120 AU/mL, the antibody level (<80 AU/mL) was considered a negative result, and 80–120 AU/mL was the middle result.

Other Laboratory Tests

Peripheral blood was used as the test specimen for other relevant laboratory tests, and these tests were all examined by the department of laboratory medicine in hospital. The normality of each index was determined in accordance with the hospital's own instrument inspection standards.

Statistical Analysis

All data in this study were statistically analyzed using SPSS 20.0 software (IBM Corporation, Armonk, NY, USA), GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) and MedCalc 18.2 (MedCalc Software Ltd, Ostend, Belgium) Software were also used to complete the figures. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD). The Kolmogorov–Smirnov test was used to determine whether the normal distribution condition was met. If $P>0.05$, then the normal distribution was met, and the *t*-test was used for comparison between groups. If the normal distribution was not met, nonparametric rank sum test was used for comparison between groups. The count data was expressed in terms of frequency and percentage, and chi-squared test or Fisher's exact test was used for comparison between groups.

Sensitivity, specificity, Youden's index, positive predictive value (PPV) and negative predictive value (NPV) were acquired by calculation. ROC curve was used to evaluate diagnostic efficacy.

Results

A total of 129 adult patients (≥18 years old) with suspected IPA from January, 2020 to December, 2022 were enrolled in this study (Figure 1, Table 1). There were 59 IPA cases (including 9 possible, 43 probable and 7 proven) and 68 non-IPA cases, while the specific diseases in the latter group included tuberculosis, bacterial infection, pneumocystis jiroveci pneumonia, focal organizing pneumonia, metastatic cancer and so on. In 43 probable IPA patients, 38 patients had positive culture results and five other patients had positive GM test results, seven proven IPA patients were all diagnosed with histopathological evidence. Three patients were diagnosed with *Aspergillus flavus* and one with *Aspergillus niger* in those with clear non-*A. fumigatus* species. The average admission age of IPA group was 63.2±9.6 (33–79) years old, and the gender ratio was 42:17 (male:female). Compared with the non-IPA group, the proportion of patients with history of smoking and COPD in IPA group was higher (59.3 vs 39.7%, P=0.027; 33.9 vs 14.7%, P=0.011, respectively), meanwhile, patients in the IPA group were more likely to have shortness of breath (62.7 vs 35.3%, P=0.002).

In the results of other related laboratory tests (Table 2), the level of albumin in IPA group was significantly lower than non-IPA group (32.1±6.6 vs 34.8±5.9, $P=0.016$). In addition, although there was significant difference in the level of erythrocyte sedimentation rate (ESR) between two groups ($P<0.05$), the average level of ESR was at inflammatory level. Therefore, the significance here was of little value.

The levels of *A. fumigatus*-specific IgG antibody in the IPA and non-IPA groups were shown in Figure 2. The level of specific IgG antibody in the IPA group was significantly higher than the non-IPA group (202.1±167.0 vs 62.6±58.0, $P<0.001$, Figure 2A). In this study, the positive rates in IPA and non-IPA groups were 55.9–71.2%, 10.3–22.1%, respectively. There was no significant difference in the level of specific IgG antibody among possible, probable and proven IPA groups ($P=0.205$, Figure 2B).

The ROC curve of specific IgG antibody test in the diagnosis of IPA was shown in Figure 3, the sensitivity, specificity, Youden's index, PPV, and NPV values under different cut-off values were listed in Table 3. In the ROC curve, the area under the ROC curve (AUROC) was 0.799 (95%CI: 0.718– 0.865 $P<0.001$), which indicated that the *A. fumigatus*-

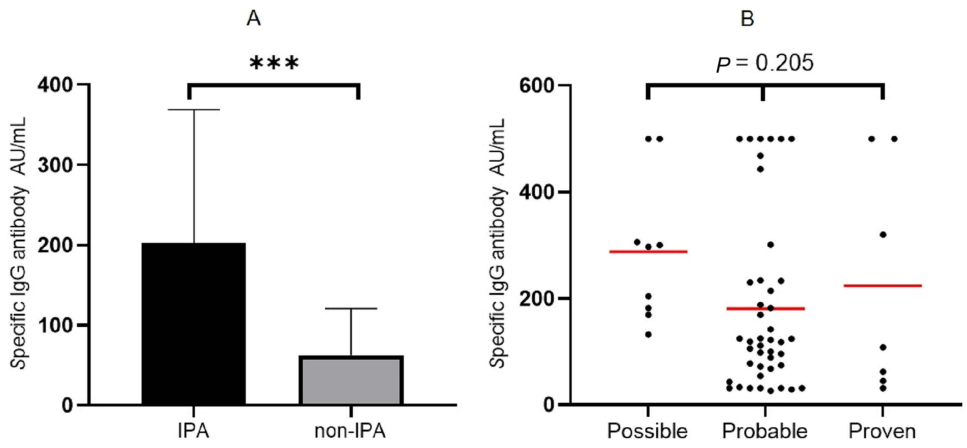

Figure 2 (**A**) The level of *Aspergillus fumigatus*-specific IgG antibody in IPA and non-IPA patients. (**B**) The level of *A. fumigatus*-specific IgG antibody in possible, probable and proven IPA patients. ***p < 0.001.

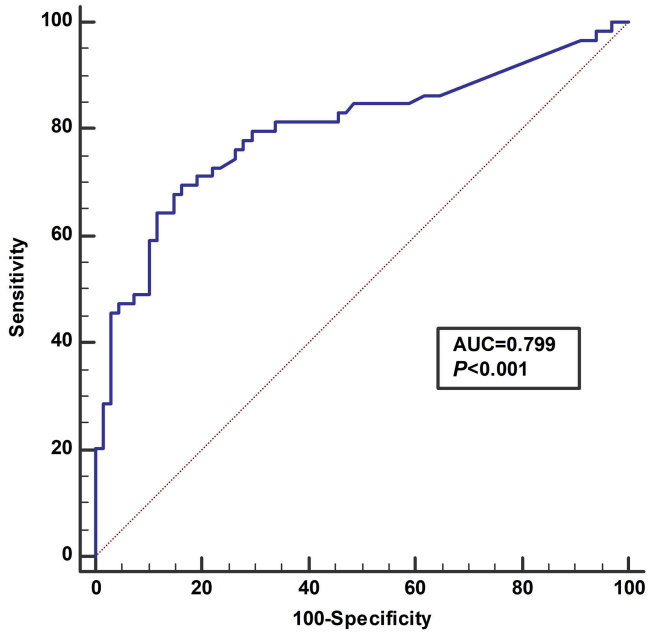

Figure 3 The ROC curve of *Aspergillus fumigatus*-specific IgG antibody test in the diagnosis of IPA.

specific IgG antibody had a good diagnostic performance for IPA. The cut-off with best diagnostic efficacy was 91 AU/mL, and in this instance, the sensitivity and specificity were 69.5% and 83.8%, respectively. The Youden's index was 0.533, and the PPV and NPV were 0.788 and 0.760 at this cut-off value.

Discussion

Although the *Aspergillus*-specific IgG antibody test is the cornerstone for the diagnosis of CPA, its role in IPA diagnosis is still uncertain and few data are available so far, especially non-neutropenic IPA.[16] Therefore, in this study, we prospectively compared clinical features between IPA and non-IPA patients, and evaluated the quantitative diagnostic value of the *A. fumigatus*-specific IgG antibody test in non-neutropenic IPA.

In terms of clinical characteristics, the study showed that patients with history of smoking and COPD were more likely to suffer from IPA. It is well-known that IPA mainly occurs in patients with specific risk factors, traditional factors include neutropenia, hematologic malignancies, solid organ transplantation, neoplasm and so on.[17] However, in recent

Table 3 Sensitivity and Specificity of *Aspergillus fumigatus*-Specific IgG Antibody Test at Different Cut-Offs in the Diagnosis of IPA

Cut-Offs (AU/mL)	Results				
	Sensitivity	Specificity	Youden's Index	PPV	NPV
72	0.746	0.735	0.481	0.710	0.769
82	0.712	0.794	0.506	0.750	0.761
91	0.695	0.838	0.533	0.788	0.760
100	0.644	0.853	0.497	0.792	0.734
111	0.593	0.882	0.475	0.814	0.714
122	0.542	0.897	0.439	0.821	0.693

Abbreviations: PPV, positive predictive value; NPV, negative predictive value.

years, the number of non-neutropenic patients in IPA is gradually increasing, including those with liver cirrhosis and end-stage COPD, etc.[18] The diagnosis of IPA in non-neutropenic patients is difficult because of nonspecific clinical features.[19] Patients in this study were all non-neutropenic, and people's innate immune system plays an important role in immune response against *Aspergillus*, which was the most crucial reason for sample selection in this study. Our study validated that patients with history of smoking and COPD were at high risk to suffer from IPA, which indicated that effective management with COPD patients was also good measure to prevent IPA.

Moreover, the level of *Aspergillus*-specific IgG antibody in the IPA group was significantly higher than non-IPA group, while there was no significant difference among possible, probable and proven IPA, which suggested that *Aspergillus*-specific IgG antibody could be potential diagnostic indicator for IPA. Previous study has found that serum *Aspergillus* IgG antibody detection may have certain clinical value in the diagnosis of IPA and CPA in non-neutropenic patients, but the sample size was small and the result in this study was in accordance with former research.[13]

It is well known that *Aspergillus*-specific IgG is considered the most reliable method for diagnosing CPA, when blood is used as a test sample, the sensitivity and specificity can reach up to 96% and 99%, respectively.[16,20] In addition previous studies have shown that the level of *Aspergillus*-specific IgG antibody may be elevated in 29–100% of IPA patients, and sensitivity is greater in non-neutropenic patients (48%).[21,22] More significantly, patients with known history of *Aspergillus* infection or colonization were excluded in this study. In our study, the positive rate in IPA group was not 100%, which may be related to different immune response against *Aspergillus* in the human body. But the positive rate in IPA group was relatively higher than non-IPA group, the sensitivity and specificity were 69.5% and 83.8% at the 91AU/mL cut-off value, which furtherly certified that *Aspergillus*-specific IgG antibody had good performance for diagnosing IPA, and provided statistical support by broadening sample size for this field.

However, there were several "positive" cases in non-IPA group, which was likely to make an impact on sensitivity and specificity. It could be explained that non-IPA patients in this study mostly suffered from structural pulmonary diseases, and these patients may have *Aspergillus* sensitization in small airways. Besides, the best cut-off value was lower than the recommended value of kit in our study, which may be relevant to non-neutropenic IPA patients or sample size. Non-neutropenic IPA patients are increasing year by year, a lower cut-off value of this kit may should be considered to optimize diagnostic efficacy. Therefore, it is still necessary to increase the sample size in the future to confirm the reliability of this conclusion.

In the diagnosis of IPA, the level of GM in serum or BALF has been recommended as diagnostic marker among adults and children, especially those neutropenic patients, and the diagnostic sensitivity can reach 70%, while the sensitivity of GM test can be reduced to 20% in non-neutropenic patients.[6,17,23] It is worth mentioning that IPA patients in this study were all non-neutropenic, which extended the diagnostiv=c value of the specific IgG test in IPA. Yajie Lu discovered that the diagnostic value of *Aspergillus*-specific IgG antibody for IPA was superior to serum GM, and a little inferior to BALF GM in non-neutropenic IPA, which indicated that *Aspergillus*-specific IgG antibody test had the good diagnostic value in non-neutropenic IPA.[24] Dobias et al found that the specificity and PPV were highest by *A. fumigatus*-specific IgA and IgG antibody tests, and when a specific antibody test combined with GM test or G test, the sensitivity and NPV could be higher.[25] Although sensitivity

and specificity of *Aspergillus*-specific IgG antibody test were relatively high in our study, it was indispensable to explore diagnostic value of *Aspergillus*-specific IgG antibody test combined with other methods. Moreover, nowadays cytokine/chemokine are upcoming as a biomarker or immunotherapeutic strategies or personalized medicine. It has been observed that invasive aspergillosis involves Th1 and Th17 cell-type immunity via interferon-γ (IFN-γ), IL-1, IL-6, and IL-17, which may provide new perspective to carry out research on diagnosis and treatment of IPA.[26]

In addition, there are some new technologies in the diagnosis of IPA over the years. Lateral-flow device (LFD) is a qualitative test and can be used for point-of-care testing in IPA. Currently two products are on the market, studies have shown that when the above two point-of-care products are combined to diagnose non-neutropenic IPA, the sensitivity and specificity can reach 80%.[27,28] The samples used by LFD technology in the diagnosis of pulmonary aspergillosis are mainly blood and BALF, but recently some researchers have found that urine can also be a potential sample for the diagnosis of IPA.[29] Hence, studies focusing on diagnostic value of *Aspergillus*-specific IgG antibody test in different specimens and combination with new technology also could be important researching directions in the future.

To sum up, this study proved that *Aspergillus*-specific IgG antibody test had the good diagnostic value in non-neutropenic IPA. However, there were still some limitations in this study. First, the study only involved a single center and the sample size was limited, there may be heterogeneity in different centers. Second, the antibody kits used in this study were *A. fumigatus*-specific antibodies, which caused missed diagnosis for other types of *Aspergillus*. Furthermore, although patients with known history of *Aspergillus* infection or colonization were excluded in this study, it is not clear that whether the interference of colonization has been completely eliminated. Third, conclusions mentioned in the text only could be provided as references, which should be further explored with larger sample size in the future.

Conclusion

Immunological test plays an important role in the diagnosis of pulmonary aspergillosis, and *Aspergillus*-specific IgG antibody test has the good diagnostic value in non-neutropenic IPA.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

The Institutional Review Board of Clinical Research of the First Affiliated Hospital, School of Medicine, Zhejiang University approved the study protocol, and all methods were performed in accordance with the approved guidelines and regulations (IIT-2019-999). Written informed consent for experiment and publication was obtained from all patients. This study was carried out in accordance with the ethical standards of the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by research grants, including the Key R&D Plan of the Ministry of Science and Technology of China (2022YFC2504502), the Research and Development Program of Zhejiang Province (2023C03068), Medical and Health Technology Project of Zhejiang Province (WKJ-ZJ-2109). The funders had no involvement in the study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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