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Clinical manifestations and outcome analysis of invasive pulmonary aspergillosis infection: a retrospective study in 43 nonneutropenic patients

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Abstract

Objective: To investigate clinical characteristics of early-onset invasive pulmonary aspergillosis (IPA) in nonneutropenic patients.

Methods: Retrospective analysis was used to investigate clinical manifestations and auxiliary examination characteristics in 43 patients with IPA and 51 patients with community-acquired pneumonia in the early stage.

Results: Risk factors of IPA were dust and mold exposure, bronchiectasis, old pulmonary tuberculosis, and nasosinusitis. The incidence rate of complex clinical manifestations was 60.47% during the first week of IPA. The incidence rate of white blood cell (WBC) count $>20.0 \times 10^9$/L was 51.16%. Lung CT findings indicated incidence rates of single or multiple nodules with a halo sign and central airway expansion with ground glass opacity were 27.9% and 37.21%, respectively. Mortality rates of patients with IPA given empirical and targeted antifungal treatments were 12.0% and 42.9%, respectively.

Conclusions: Bronchiectasis, old pulmonary tuberculosis, nasosinusitis, and dust and mold exposure may increase the risk of IPA. Single or multiple nodules with a halo sign and central airway expansion with ground glass opacity may be early-stage lung CT findings in patients with IPA. A WBC count $>20.0 \times 10^9$/L may aid in early diagnosis, and empirical antifungal therapy may reduce mortality in patients with IPA.

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Introduction

Invasive pulmonary aspergillosis (IPA) is an infectious pulmonary disease with a definite pathogeny, caused mainly by inhalation of *Aspergillus* conidia via the airway. Although the etiopathogenesis of IPA is clear and drug treatment is available, the prognosis remains poor. According to recent studies, mortality caused by direct IPA and IPA coinfection with primary diseases ranges from 54% to 90%.[1] One of the most important reasons for the high mortality rate is delayed initial treatment of IPA owing to difficulty in its clinical diagnosis during the early disease stages.[2] In this study, we retrospectively analyzed the clinical data of patients with confirmed IPA in the respiratory medicine department of our hospital. We investigated the risk factors for IPA infection, summarized the clinical features of early-stage IPA, and analyzed the impact of early empirical or targeted therapy on disease outcome. This study aimed to review and summarize the common clinical features of patients with IPA, especially nonneutropenic patients, in the early stage of disease, to provide medical evidence regarding criteria for the early diagnosis of IPA.

Methods

Characteristics of the study population

The diagnostic criteria for IPA were based on the 2013 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) diagnostic criteria for IPA and diagnostic standards of the Infectious Diseases Society of America.[3,4] Among the patients with IPA, 12 were diagnosed with IPA using laboratory diagnostic evidence from direct microscopic observation of lung biopsy specimens. In addition, 31 patients with IPA were confirmed via the following concurrent clinical diagnostic criteria: (1) lung computed tomography (CT) scans showing one of the three characteristics of clear-bordered lesions with or without a halo sign, an air crescent sign, or an intrapulmonary cavity; (2) molds found in sputum, bronchoalveolar lavage fluid, or bronchial brushing specimens; and (3) serum *Aspergillus* galactomannan (GM) antigen testing yielding positive results. The diagnostic criteria for CAP were based on the 2013 Guidelines for the Diagnosis and Management of Community-Acquired Pneumonia of the Respiratory Society of the Chinese Medical Association. Treatment assessment was based on the 2008 EORTC/MSG evaluation criteria for invasive fungal disease targeted treatment.[5] The medical records of all patients were collected from the day of onset to day 72 after hospital discharge. Clinical outcomes were classified as effective (complete remission or partial remission), ineffective (stable disease or deterioration), or death (owing to various reasons directly or indirectly related to IPA).

Clinical data collection

For all patients, we collected information regarding previous history of underlying diseases and clinical manifestations from the day of onset to day 72 after hospital discharge, along with information about auxiliary examinations and treatment. The CAP cohort analysis method was used to analyze the correlation between underlying diseases and IPA and changes in clinical manifestations. Patients were divided into an empirical antifungal therapy group and a targeted antifungal therapy group on the basis of their early condition. We analyzed differences in the course and effect of treatment between the two groups.

For each patient, day 72 of follow-up after hospital discharge was used as the observation endpoint. For patients who died during this study, the time of all-cause death was regarded as the endpoint. The mortality rate was calculated according to the number of deaths caused by reasons directly or indirectly related to IPA or CAP. This study was approved by the institutional review board of the medical ethics committee of Harbin Medical University. Informed consent was waived by the institutional review board because this was a retrospective study. All patient information was anonymized

Statistical analysis

We used SAS 9 software to perform the statistical analysis (SAS Institute Inc., Cary, NC, USA). Data from independent analyses are shown as mean ± standard deviation (SD). Statistical analysis of measurement data between the two groups was performed using a Student *t*-test (two-tailed). Chi-squared and Fisher's exact tests were used to analyze frequency and percentage. Univariate and multivariate logistic regression analysis was used for risk factor analysis and reported as odds ratios (ORs) and 95% confidence intervals (CIs) with standard error. Univariate logistic regression models were created and calculated for logarithmized signal ratios above the mean using each characteristic as a variable. Using a step-down approach, multivariate models were established by including variables according to Akaike's information criterion. Dependent variables were explained using independent variables with the Cox & Snell and Nagelkerke R-squared statistic and predicted using a classification table. A p value < 0.05 was considered statistically significant. Analysis was not carried out if the number of positive cases was fewer than two.

Results

Demographic and clinical characteristics

In this study, we included the clinical data of 43 patients diagnosed with IPA in the respiratory medicine department of the First Affiliated Hospital of Harbin Medical University from 1 December 2015 to 31 December 2017. This cohort comprised 27 men and 16 women aged 33 to 82 years, with average age 47 ± 9 years. For comparison analysis, we also collected clinical data of 51 patients with community-acquired pneumonia (CAP) who were hospitalized during the same period, comprising 31 men and 20 women aged 21 to 77 years, with average age 40 ± 11 years.

The average time to reach a definitive diagnosis in the 43 patients with IPA was 8.02 days. Two patients were lost to follow-up during the 72 days of follow-up. Thirty-nine patients were treated with anti-*Aspergillus* therapy, with an average duration of 14.05 days and a total cure rate of 35.9%. Twelve patients died owing to reasons directly or indirectly related to IPA; the mortality rate was 29.3%. Twenty-five patients were treated with empirical antifungal therapy lasting for 13.08 days, with a

Table 1. Comparison of the course and effect of empirical and targeted antifungal treatment in 39 patients with invasive pulmonary aspergillosis.

Variable	Empirical therapy group (n = 25)	Targeted therapy group (n = 14)	P value
Treatment course (days)	13.08 ± 4.43	13.81 ± 6.74	0.6761
Cure rate (%)	40.0	28.6	0.1916
Mortality rate (%)	12.0	42.9	0.0286

total cure rate of 40% (10/25) and a mortality rate of 12% (3/25). Another 14 patients were treated with targeted antifungal therapy for 13.81 days, with a total cure rate of 28.6% (4/14) and a mortality rate of 42.9% (6/14) ($p = 0.1916$ and 0.0286, respectively). No statistically significant differences were observed between the treatment courses and cure rates, but the mortality rate of the two treatment methods was significantly different, as shown in Table 1. Of the 51 enrolled patients with CAP, 5 were lost to follow-up during the 72 days. The average time to definitive CAP diagnosis was 2.53 days. The total cure rate and mortality rate was 78.4% (40/51) and 5.9% (3/51), respectively.

Identification of potential risk factors for IPA

In the IPA group, 16 patients were exposed to decaying grass; dust; moldy clothes, books, or food; or polluted water. The underlying diseases in these patients included 12 cases of bronchiectasis, 11 old pulmonary tuberculosis, 11 nasosinusitis, 9 diabetes, 7 chronic obstructive pulmonary disease (COPD), 3 bronchial asthma, 2 lung cancer, and 1 case of pulmonary fibrosis. Some patients had more than two diseases, and only five patients had no underlying diseases. In the CAP group, underlying diseases in patients included 9 cases of COPD, 8 cardiovascular or cerebrovascular disease, 5 diabetes, 4 old pulmonary tuberculosis, 4 bronchiectasis, 4 nasosinusitis, and 3 cases of lung cancer. Similarly, some patients had more than two diseases. Additionally, 19 patients had no underlying diseases. The results of univariate and multiple logistic regression analyses (43 patients with IPA and 51 with CAP), after removing confounding factors (e.g., age, sex), suggested that bronchiectasis (OR: 0.111, 95% CI: 0.024–0.513, $p < 0.01$), old pulmonary tuberculosis (OR: 0.105 95% CI: 0.020–0.540, $p < 0.01$), nasosinusitis (OR: 0.072, 95% CI: 0.014–0.373, $p < 0.01$), and contact with dust (OR: 0.015, 95% CI: 0.003–0.089 $p < 0.01$) were related to the risk of IPA, as shown in Tables 2 and 3.

Analysis of clinical manifestations of IPA onset

Clinical manifestations in the 43 patients with IPA during the first week after onset are shown in Tables 4 and 5. Of patients in the IPA group, 60.47% had typical clinical manifestations such as fever, wheezing, hemoptysis, and rhonchi; this group exhibited significant differences among other symptoms and physical signs ($p < 0.01$). This finding suggested that these symptoms and signs might be pathognomonic manifestations of IPA in the early stages. In the first week after onset, 51.16% (22/43) of patients had a WBC count $>20.0 \times 10^9$/L in at least one routine blood examination ($p < 0.01$) and 51.16% (22/43) had a GM value between 0.5 and 1.5 ($p < 0.01$). Lung CT scans showed that incidence rates of single or multiple nodules with halo signs in the lungs and central airway expansion with ground glass opacity

Table 2. Univariate logistic regression analysis for risk of invasive pulmonary aspergillosis.

Variable	Estimated value	SE	Chi-squared value	P	OR	95% CI
Bronchiectasis	−1.5148	0.6220	5.9311	0.0149	0.220	(0.065, 0.744)
Secondary diabetes	−0.8900	0.6019	2.1868	0.1392	0.411	(0.126, 1.336)
Bronchial asthma	1.2040	0.6952	2.9991	0.0833	3.333	(0.853, 13.021)
Old tuberculosis	−1.3745	0.6276	4.7963	0.0285	0.253	(0.074, 0.866)
Lung cancer	0.2689	0.9375	0.0823	0.7743	1.309	(0.208, 8.219)
COPD	0.1460	0.5538	0.0694	0.7921	1.157	(0.391, 3.426)
Sinusitis	−1.3960	0.6272	4.9534	0.0260	0.248	(0.072, 0.847)
Contact with dust and mold	−2.3866	0.6730	12.5764	0.0004	0.092	(0.025, 0.344)

SE, standard error; COPD, chronic obstructive pulmonary disease.

Table 3. Results of multivariable logistic regression analysis of the correlation between underlying diseases and disease risk in 43 patients with invasive pulmonary aspergillosis.

Variable	Estimated value	SE	Chi-squared value	P	OR	95% CI
Bronchiectasis	−2.1999	0.7820	7.9146	0.0049	0.111	(0.024, 0.513)
Old tuberculosis	−2.2549	0.8362	7.2726	0.0070	0.105	(0.020, 0.540)
Sinusitis	−2.6244	0.8355	9.8667	0.0017	0.072	(0.014, 0.373)
Exposure to dust	−4.1711	0.8930	21.8192	<0.0001	0.015	(0.003, 0.089)

SE, standard error; OR, odds ratio; CI, confidence interval.

Table 4. Comparison of clinical manifestations and leukocyte count in the first week of invasive pulmonary aspergillosis infection.

Group	IPA (n=43)	CAP (n=51)	Chi-squared value	P value
Symptoms and signs			25.7546	< 0.0001
Fever + wheezing and/or rhonchi	8	14		
Fever + cough and/or rhonchi + hemoptysis	5	18		
Fever + wheezing and/or rhonchi + hemoptysis	26	6		
Other	4	13		
Leukocyte count in routine blood testing			13.892	0.0031
WBC $< 4.0 \times 10^9$/L	5	11		
WBC 4.0–10.0 $\times 10^9$/L	5	13		
WBC 10.1–20.0 $\times 10^9$/L	11	19		
WBC $> 20.0 \times 10^9$/L	22	8		
GM test			51.0013	< 0.0001
GM < 0.5	6	9		
GM 0.5–1.5	22	3		
GM > 1.5	10	0		
No GM detected	5	39		

IPA, invasive pulmonary aspergillosis; CAP, community-acquired pneumonia; WBC, white blood cells; GM, galactomannan.

Table 5. Results of comparison analysis of lung CT scans in the first week of invasive pulmonary aspergillosis infection.

Group	IPA (n = 43)	CAP (n = 51)	Chi-squared value	P value
Lung CT			35.5490	<0.0001
Single or multiple nodules with halo sign	12	6		
Central airway expansion with ground glass opacity	16	4		
Partial consolidation with halo sign	7	5		
Cavities with an air crescent sign	3	0		
Other	5	36		

IPA, invasive pulmonary aspergillosis; CAP, community-acquired pneumonia; CT, computed tomography.

Table 6. Results of comparison analysis of lung CT scans for three groups of patients with IPA, taken at different times after onset of invasive pulmonary aspergillosis.

Group	0–7 days after onset (n = 43)	7–14 days after onset (n = 32)	>14 days after onset (n = 35)	Chi-squared value	P value
Single nodule or multiple nodules with a halo sign	12	7	3	37.294	<0.0001
Central airway expansion with ground glass opacity	16	5	2		
Partial consolidation with halo sign	7	4	2		
Cavities with an air crescent sign	3	13	13		
Other	5	3	15		

were 27.9% (12/43) and 37.21% (16/43), respectively, which were different from those of other imaging signs ($p < 0.01$).

Analysis of lung CT scans in patients with IPA at different times after onset

We performed a statistical analysis of lung CT scans from the 43 patients with IPA; scans were obtained at different times after onset. We regarded each lesion as a single analysis object because various lesions were seen in the CT images of patients with IPA. The CT scan results showed a process of dynamic change. We divided the CT scans into three groups: those taken 0 to 7 days after onset, 7 to 14 days after onset, and more than 14 days after onset. Statistically significant differences were found among the three groups ($p < 0.0001$), as shown in Table 6. We adjusted the numerical value of α to avoid type I errors. After adjustment to $\alpha = 0.0167$ (0.05/3), statistically significant differences were found between the groups 0 to 7 days after onset and 7 to 14 days after onset ($p = 0.0091$). However, no statistically significant differences were found between the groups 7 to 14 days after onset and more than 14 days after onset ($p = 0.0173$).

Discussion

We analyzed the pathogeny and underlying diseases of patients with IPA in this retrospective study. The risk of IPA onset was

increased in patients with a clinical history, indicating the possibility of inhaling *Aspergillus* conidia and including elements such as contact with decaying grass; dust; moldy clothes, books, or food; or polluted water. In addition, we examined the lung CT scans of patients 1 week after IPA onset and found areas of bronchiectasis near the center of the hilus pulmonis. The appearance of areas of bronchiectasis—nonthickened bronchial walls and surrounding ground glass opacity—was different from the appearance prior to disease onset. Therefore, we hypothesized that this development could have resulted from inhaling *Aspergillus* conidia into the bronchial lumen; however, the specific mechanism requires further research. Analysis of the correlation between the presence of underlying diseases and IPA risk showed that bronchiectasis, nasosinusitis, and old tuberculosis were positively correlated with IPA onset. These underlying diseases are chronic structural lung diseases.[6] Therefore, we hypothesized that long-term chronic lung inflammation permits *Aspergillus* to readily colonize the lungs and damage the respiratory immune defense mechanism, resulting in infection by the colonizing pathogen.[7] CT scans of patients with IPA who had chronic structural lung diseases mainly showed single or multiple nodules with a halo sign in the first week after onset. These imaging findings were consistent with pathological changes of partial tissue necrosis, surrounding inflammatory cell infiltration, and blood capillary exudate resulting from infection by colonizing bacterial pathogens.[8]

Aspergillus enters the lungs mainly via bronchial inhalation and further invades the bronchi, lung tissues, and blood vessels, leading to inflammation, tissue damage, and necrosis.[9,10] Animal models demonstrate a specific inflammatory reaction after bronchial instillation of *Aspergillus* conidia, the manifestations of which change over time in the lung and include bronchitis, pulmonary alveolar inflammatory exudate, blood vessel injury, and hemorrhagic infarction of lung tissue.[11] As reported by Kyo et al.,[12] the number and growth rate of bacteria and the extent of tissue damage in patients with IPA changes with the infection duration. We analyzed the clinical manifestations of our 43 patients with IPA 1 week after onset and found that 37.2% of them presented with fever, wheezing and lung rhonchi, and hemoptysis during the first week of IPA infection. Considering the pathological process of *Aspergillus* infection, we found that the temporal kinetics of these clinical manifestations and pathological damage processes were identical. Thus, we concluded that complex clinical manifestations such as fever, wheezing, and pulmonary rhonchi and hemoptysis occurring in the early stages of disease could be early clinical features of IPA. In addition, we analyzed the characteristics of routine blood samples taken in the early stage of disease and found that, unlike patients with CAP, the incidence rate of WBC count $>20.0 \times 10^9$/L was 65.1% in patients with IPA during the first week after onset. However, this change differed from the change in WBC count of routine blood samples from patients with agranulocytosis.[13,14] The reason for this discrepancy requires further research, and proving whether these results are specific to patients with IPA will require additional data.

In our study, 38 patients underwent serum GM testing. GM values were mostly between 0.5 and 1.5 (58.8%). A study by Nouer et al.[15] suggested that GM values between 0.5 and 1.5 could increase the sensitivity of *Aspergillus* detection because high values can affect the positive rate and sensitivity of the test. We believe that, apart from the small sample size, this result might stem from a low blood β-GM content owing to a small amount of

Aspergillus in the nidus of infection during early-stage disease. Changes in the GM value could, therefore, be associated with the duration of illness.

In this study, we primarily analyzed the changing characteristics of lung CT scans from patients with IPA and demonstrated a dynamically changing process. At 0 to 7 days after onset, lung CT scans showed new areas of bronchiectasis around the lung hilus, combined with ground glass opacity and single or multiple nodular shadows with a halo sign. After treatment, parts of these new areas of bronchiectasis gradually shrank, and areas of ground glass opacity gradually disappeared. In some patients, consolidation shadows and pleural effusion appeared around the areas of bronchiectasis. The nodular shadows with halo signs gradually shrank or disappeared 8 to 14 days after onset, and some formed cavities with air crescent signs. Considering these dynamically changing characteristics, we hypothesized that new areas of bronchiectasis with ground glass opacity and nodular shadows with halo signs visible on lung CT scan could be early features of *Aspergillus* infection.[16]

The EORTC/MSG (2013) has proposed clinical evidence to confirm IPA diagnosis and has presented the basis of targeted therapy and empirical therapy.[3] In this study, we analyzed the impact of empirical antifungal therapy and targeted therapy on disease outcome and showed that both treatments had the same impact on the cure rate but a different impact on the mortality rate ($p < 0.05$). Thus, empirical antifungal therapy could reduce the mortality rate of IPA.

A limitation of this study was that the small sample size reduced the robustness of the conclusions. In addition, owing to incompleteness of the dynamic observation data, some aspects could not be analyzed in depth. However, the results of this research clearly suggested that nonneutropenic patients who contact moldy objects or dust or who have chronic structural lung diseases carry a risk of IPA. In addition, these results suggested that in early stages of IPA, specific clinical and lung CT scan manifestations may be observed. Thus, the present results could provide medical evidence for the early diagnosis of IPA in nonneutropenic patients.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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