Differences in clinical characteristics of invasive tracheobronchial aspergillosis according to the presence of invasive pulmonary aspergillosis

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Abstract

Introduction: The association of invasive tracheobronchial aspergillosis (ITBA) with invasive pulmonary aspergillosis (IPA) is not well-established. We aimed to compare clinical characteristics between patients who exhibited ITBA with IPA and those who exhibited isolated ITBA (iITBA) and to evaluate the usefulness of serum or bronchial galactomannan (GM) tests in diagnosing ITBA.

Methods: This retrospective single-center case-control study was conducted over a period of four years. Fifteen patients were enrolled after the confirmation of the presence of ITBA using bronchoscopy-guided biopsy (iITBA = 7 vs. ITBA+IPA = 8). Clinical characteristics of patients and results of serum or bronchial GM test were compared between the two groups. Mortality was assessed through 6-month follow-up data.

Results: The ITBA+IPA group showed a higher prevalence of hematologic malignancy (75% vs 14%; \( P = 0.029 \)), greater number of patients with multiple bronchial ulcers (75% vs. 14%; \( P = 0.029 \)), lower platelet counts (63,000/μL vs. 229,000/μL; \( P < 0.001 \)), and significantly higher mortality (63% vs. 0%; \( P = 0.026 \)) than the iITBA group. In the ITBA+IPA group, 57% of patients tested positive for serum GM assay, whereas in the iITBA group, patients tested negative (\( P = 0.070 \)). The bronchial GM level was high in both the iITBA and ITBA+IPA groups, but there was no significant difference between groups.

Conclusions: Patients with ITBA+IPA had a greater number of hematologic malignancies with lower platelet counts and worse prognoses than did those with iITBA. Bronchoscopic findings and bronchial GM test results were more useful than serum GM test results for diagnosing ITBA.
Introduction

Airborne *Aspergillus* spores are dispersed in the atmosphere and are small enough (2–3 μm) to be easily inhaled into the peripheral airways.\(^1\)\(^,\)\(^2\) During construction and indoor renovation, there is an increase in indoor and outdoor fungal contaminations.\(^3\)\(^,\)\(^4\) Inhaled spores result in various *Aspergillus*-related lung diseases based on different host-dependent anatomical and immunological factors, including saprophytic infections, allergic pulmonary diseases, invasive diseases, and toxic reactions.\(^5\) Invasive aspergillosis usually develops in immunocompromised hosts, such as those with hematologic malignancy with neutropenia.\(^6\)

Invasive tracheobronchial aspergillosis (ITBA) is a rare clinical form of invasive aspergillosis, in which the infection is primarily limited to the tracheobronchial tree.\(^7\) Fungal infection has been known to be common in patients with immune suppression, but ITBA has also been reported recently in relatively immunocompetent patients with chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), and influenza and those admitted to the intensive care unit (ICU) with non-neutropenia.\(^8\)\(^-\)\(^12\) As a result, interest in ITBA is increasing, but its pathophysiology and association with invasive pulmonary aspergillosis (IPA), another common form of invasive aspergillosis, has not been established well. ITBA co-exists with IPA; however, in some cases, it exists without IPA. In a previous study, such cases have been defined as those of isolated ITBA (iITBA).\(^13\)

Although the serum galactomannan (GM) assay is useful for diagnosing various *Aspergillus*-related diseases, it has limited benefits over the bronchial lavage GM assay for
patients with ITBA. Furthermore, chest imaging is not a useful tool for detecting iITBA without lung parenchymal abnormalities.

Based on previous findings, we aimed to compare the clinical characteristics between patients who exhibited ITBA with IPA and those who exhibited iITBA as well as to evaluate the usefulness of the serum and bronchial GM assays.

**Methods**

In the present study, we used the clinical data warehouse appliance (uICE, Ulsan University Hospital Information of Clinical Ecosystem) in connection with the electronic medical records at the Ulsan University Hospital (UUH). We enrolled consecutive patients who were diagnosed with histologically confirmed ITBA based on the examination of one or more endobronchial lesions via bronchoscopic biopsy in the department of internal medicine of UUH from January 2008 to December 2011. Between 2008 and 2011, construction work for new buildings was carried out at UUH, and the study period was set based on the fact that aspergillosis infections had increased in our hospital during that period. We submitted the proposal to UUH institutional review board (IRB), for which exemption has been awarded (application ID: UUH-IRB-2021-02-015).

The presence of IPA was determined using the European Organization for the Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria. Patients with host risk factors, suggestive computed tomography (CT) findings, and mycological proof were diagnosed with (probable) IPA. ITBA was defined according to the EORTC definition, which was based on the presence of tracheobronchial lesions observed during bronchoscopy and the
presence of pathologic findings such as endobronchial tissue invasion and damage by
*Aspergillus* hyphae. Unlike IPA, ITBA cannot be diagnosed using non-invasive tests such as CT, making bronchoscopy accompanied by biopsies necessary and making diagnosis without clinical suspicion difficult. According to bronchoscopic and pathologic findings, ITBA can be divided into three types: ulcerative, pseudomembranous, and obstructive. According to another classification, iITBA can be divided into four types: superficial infiltration type (type I); full-layer involvement type (type II); occlusion type (type III); and mixed type (type IV). In this study, patients with ITBA and IPA were assigned to the ITBA+IPA group, whereas those with ITBA but without IPA were assigned to the iITBA group. Bronchoscopy-guided biopsy with bronchial washing was performed by experienced pulmonologists after identifying the presence of tracheobronchial lesions. Mucosal lesions in patients with ITBA were classified into four different types based on the bronchoscopic appearance of intraluminal lesions.

Data on clinical characteristics of the patients (i.e., age, sex, and underlying disease), laboratory findings (including culture results and GM assay), and radiologic findings were retrospectively collected from their medical records. In addition, information regarding specific respiratory symptoms or signs was collected. Six-month mortality rate was assessed using follow-up data.

The GM assay was performed using the Platelia *Aspergillus* antigen ELISA (Bio-Rad Laboratories, Marnes-la-Coquette, France) and photometric detection (STRATEC Biomedical...
AG Gemini Combo, Birkenfeld-Grafenhausen, Germany) at 450 nm. Indices (optical density [OD] of the sample/OD of the cutoff control) of ≥0.4 were considered positive.19

The clinical characteristics of the patients and results of the serum and bronchial GM assays were compared between the two groups (iITBA vs. ITBA+IPA). Proportions were compared using the chi-squared test or the Fisher’s exact test. Considering that the number of samples is small, the Mann–Whitney U test was used for continuous variables. A P value of < 0.05 was considered statistically significant. The data were analyzed using the SPSS software, version 24 (SPSS, Inc., Chicago, IL, USA).

Results

Fifteen patients who met our criteria were identified using uICE based on the presence of Aspergillus infection, which was histologically confirmed via bronchoscopic biopsy between January 2008 and December 2011. Among them, eight were diagnosed, based on chest CT reports, with IPA (iITBA = 7 vs. ITBA+IPA = 8). ITBA classification according to the endobronchial lesion type was as follows: type I, 11 patients; type II, 1 patient; type III, 0 patients; and type IV, 3 patients.

There were no statistically significant differences in baseline characteristics such as age, sex, and symptoms between the two groups. The ITBA+IPA group showed a higher prevalence of hematologic malignancy (75% [n = 6] vs. 14% [n = 1]; P = 0.029) than did the iITBA group. Other underlying diseases included solid tumors, systemic lupus erythematosus with systemic corticosteroid, chronic liver disease, DM, and chronic lung disease.
With regard to laboratory findings, white blood cell count and C-reactive protein level were not significantly different between the groups, but platelet counts were lower in the ITBA+IPA group than in the iITBA group (63,000/μL vs. 229,000/μL; \(P = 0.000\)). Moreover, the number of patients with multiple bronchial ulcers was higher in the ITBA+IPA group than in the iITBA group (75% \([n = 6]\) vs. 14% \([n = 1]\); \(P = 0.029\)).

In the ITBA+IPA group, 57% of the patients tested positive for serum GM, whereas all patients with iITBA were negative. The serum GM level was higher in the ITBA+IPA group than in the iITBA group [median (range), 0.906 (0.270–4.334) vs. 0.396 (0.080–0.321); \(P = 0.002\)]. The bronchial GM level was high in both the iITBA and ITBA+IPA groups and did not show a statistically significant difference [median (range), 2.640 (0.780–5.670) vs. 3.70 (0.296–5.294); \(P = 0.937\)]. A direct comparison of the serum and bronchial GM levels showed that the levels were significantly higher in the bronchial samples than in the serum samples of all patients [median (range), 3.70 (0.296–5.670) vs. 0.321 (0.080–4.334); \(P = 0.001\)] (Figure 1).

The six-month mortality rate after the time of diagnosis was significantly higher in the ITBA+IPA group than in the iITBA group (63% \([n = 5]\) vs. 0% \([n = 0]\); \(P = 0.026\)).

### Table 1. Baseline characteristics of patients with isolated invasive tracheobronchial aspergillosis and invasive tracheobronchial aspergillosis with invasive pulmonary aspergillosis

<table>
<thead>
<tr>
<th></th>
<th>Isolated ITBA (n = 7)</th>
<th>ITBA with IPA (n = 8)</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>62 (42–73)</td>
<td>58 (46–72)</td>
<td>.536</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>4 (57%)</td>
<td>6 (75%)</td>
<td>.608</td>
</tr>
<tr>
<td>Underlying diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematologic malignancy</td>
<td>1 (14%)</td>
<td>6 (75%)</td>
<td>.029</td>
</tr>
<tr>
<td>Non-hematologic disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solid tumor</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Disease / Finding</td>
<td>iTBA</td>
<td>ITBA + IPA</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chronic liver disease</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chronic lung diseases</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough/sputum</td>
<td>4/5</td>
<td>3/2</td>
<td>NA</td>
</tr>
<tr>
<td>Fever</td>
<td>4 (57%)</td>
<td>4 (50%)</td>
<td></td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2 (29%)</td>
<td>3 (38%)</td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory finding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC, median (range)</td>
<td>8,620 (280–19,100)</td>
<td>1,935 (60–17,970)</td>
<td>.281</td>
</tr>
<tr>
<td>ANC &lt; 500, N</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>229 k (155–487 k)</td>
<td>63 k (22–118 k)</td>
<td>.000</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>3.54 (0.09–22.34)</td>
<td>17.19 (2.51–25.75)</td>
<td>.281</td>
</tr>
<tr>
<td><strong>Bronchoscopic finding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of multiple ulcers</td>
<td>1/7 (14%)</td>
<td>6/8 (75%)</td>
<td>.029</td>
</tr>
<tr>
<td>Type I/II/III/IV</td>
<td>5/1/0/1</td>
<td>6/0/0/2</td>
<td>NA</td>
</tr>
<tr>
<td>Bronchial GM positivity</td>
<td>6/6 (100%)</td>
<td>5/6 (83%)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum GM positivity</td>
<td>0/6 (0%)</td>
<td>4/7 (57%)</td>
<td>.070</td>
</tr>
<tr>
<td>Fungal culture</td>
<td>A. fumigatus (4)</td>
<td>A. fumigatus (3)</td>
<td>A. terreus (1)</td>
</tr>
<tr>
<td>Mortality due to aspergillosis</td>
<td>0/7 (0%)</td>
<td>5/8 (63%)</td>
<td>.026</td>
</tr>
</tbody>
</table>

ITBA, Invasive tracheobronchial aspergillosis; IPA, invasive pulmonary aspergillosis; SLE, systemic lupus erythematosus; DM, diabetes mellitus; WBC, white blood cell; CRP, C-reactive protein; GM, galactomannan. *Used the chi-square test or the Fisher exact test for categorical variables and the Mann-Whitney U test for continuous variables.
Figure 1. Bronchial and serum galactomannan levels in patients with iITBA and ITBA+IPA. Plots indicate individual data points and median ± interquartile ranges of the mean galactomannan levels (horizontal line and vertical bar). P value for Mann–Whitney U test (P = 0.001).

NS, not significant; ITBA, invasive tracheobronchial aspergillosis; iITBA, isolated ITBA; IPA, invasive pulmonary aspergillosis

Discussion

In this study, the ITBA+IPA group showed a higher rate of hematologic malignancies with lower platelet counts, higher prevalence of multiple bronchial ulcers, and worse prognosis than did the iITBA group. The results of bronchial GM assay were positive in both the iITBA and ITBA+IPA groups, but those of the serum GM assay were positive only in the ITBA+IPA group. Furthermore, the serum GM level in the ITBA+IPA group was higher than that in the iITBA group.

The high proportion of patients with hematologic malignancies in the ITBA+IPA group seems to be related to an uncontrolled hematologic disease and chemotherapy, which can cause long lasting immune suppression without recovery. Although the temporal relationship between ITBA and IPA is not clear, it can be hypothesized that these forms may represent different stages of the same disease process that and the rate of progression to IPA is high if there is a severe immune suppression in patients with ITBA.7,20 Recent studies showed that the mortality rate was higher in patients with ITBA+IPA (90%) than in those with IPA (32%) or ITBA alone (iITBA, 22%).11,12 Similar results were observed in our study, and this suggests that the prognosis becomes worse when ITBA co-exists with IPA; therefore, the early diagnosis and treatment of iITBA before the development of IPA are crucial. In general,
multiple or extensive endobronchial lesions are thought to indicate a more advanced and severe stage of the disease than is a single lesion. Higher number of bronchial ulcers found in the ITBA+IPA group might progress to ITBA+IPA as opposed to being remained as an ITBA, in which *Aspergillus* infection limited to superficial mucosa without further invasion to the bronchial tissue and vessel.

The diagnosis of ITBA without IPA is difficult and delayed because of its non-specific clinical presentation and radiological findings, in addition to unavailability or poor interpretation of serological biomarkers. By definition, the typical chest CT findings associated with IPA, such as nodules with halo signs or cavities, are not observed in patients with iTBA. It may be possible to diagnose fungal diseases in patients with immunodeficiency because they suspected to contract opportunistic infections, but it is more difficult in immunocompetent individuals because clinicians tend to hardly suspect it.\(^{11,21}\) Respiratory *Aspergillus* infection is a well-known complication in severely and chronically immunocompromised hosts. However, mildly immunocompromised hosts, such as those with COPD, advanced liver cirrhosis, or diabetes, has been reported to be at a risk of contracting ITBA or IPA.\(^8\text{-}^{10}\) In addition, ITBA has recently been reported in patients with systemic viral infection such as influenza and severe fever thrombocytopenia syndrome.\(^{11,21}\) Therefore, attention should be paid to whether ITBA has occurred; if suspected, bronchoscopy should be considered early for diagnosis and treatment.

The low platelet counts in the ITBA+IPA group make it difficult to conduct invasive tests such as percutaneous needle aspiration biopsy (PCNA) for confirming suspected IPA lung lesions. If clinicians decide that direct identification of the fungus was necessary in spite of the risk of bleeding, bronchoscopy could be a safe option for obtaining samples. Our study showed that bronchial GM levels were significantly elevated in both groups. However, serum
GM was not detected in patients with iITBA; similar findings have been observed in previous studies.²², ²³ The low GM level in patients with ITBA is explained by the hypothesis that fungal invasion is limited to the surface layer of the airway mucosa and is not sufficient to facilitate the spread of *Aspergillus* antigen into circulating blood.²⁴ In invasive bronchial aspergillosis (classically observed in patients with COPD), GM is, therefore, not routinely detected in blood because neutrophils prevent translocation from the pulmonary compartment to the blood compartment.²⁵ This hypothesis was confirmed by a meta-analysis in which GM antigenemia presented low sensitivity in transplant recipients without neutropenia and more recently by van de Groep et al. using a population with severe influenza.²⁶ The diagnostic value of bronchoalveolar lavage (BAL) fluid GM for IPA in patients without neutropenia has been shown to be superior to that of serum GM.²⁷ BAL fluid GM also appears to be more sensitive than serum GM and lower respiratory tract *Aspergillus* isolation for the diagnosis of IPA in critically ill patients with COPD.²⁸ Based on this result, we suggest that the bronchial GM assay and related tests (including biopsy) can be more useful than the serum GM assay for diagnosing ITBA. We, therefore, recommend early detection of ITBA using bronchoscopy and that of iITBA using bronchial GM assay. In a recent study, the usefulness of bronchoscopy for diagnosing ITBA in patients with critical illness and no neutropenia was also evaluated.¹¹ For bronchoscopy, the sensitivity, specificity, positive and negative predictive values, and overall accuracy of changes suspected to be related to ITBA were 83.3%, 70.3%, 53.2%, 91.2%, and 74.1%, respectively; the diagnostic performance of bronchoscopy is better than that of BAL culture. It was also interesting that mycetoma and halo signs were observed only in the chest CT images of 4.5% and 10.4% of the study subjects, respectively. This indicates that suspected bronchoscopic finding has diagnostic value in iITBA.
Although research on ITBA has been rare, significant studies have recently been published. One of them is a multicenter, retrospective, observational study conducted by Remy Nyga et al. Unlike our study, this previous study first diagnosed IPA in patients with severe influenza and compared differences between two groups with or without ITBA. Ninety-day mortality rates and GM concentrations in BAL fluid were higher in the patients with IPA+ITBA than in those with IPA only, which is similar to the findings of our study. Together, results of the previous and current studies, indicated that ITBA+IPA is a more advanced disease and has worse prognosis than ITBA or IPA alone. However, considering our findings, higher mortality would be attributable to neutropenia and hematologic malignancies in the IPA+ITBA group.

Our study has some important limitations. Because ITBA is a rare disease, the study sample is small, which may have contributed to the low statistical power in the representation of the entire ITBA population. Nevertheless, the environmental management of our hospitals was strengthened during construction and the air circulation was changed with HEPA filters in hematology ward, and consequently, after 2012, the incidence of ITBA was further reduced, which increased the difficulty of expanding the study population. Additionally, a selection bias may have been introduced by the retrospective single-center design of the study. All bronchoscopic examinations were not conducted by a single examiner and interpretation of findings was not conducted after agreement of several clinicians; a group of sufficiently skilled pulmonologists performed these tasks. However, a single pulmonologist independently analyzed the type of ulcers present.

Although there are some limitations to this study, it presents interesting information. First,
ITBA is a relatively rare disease that has not been studied extensively. Second, most clearly defined ITBA than other studies, using a search strategy to find patients who biopsies have confirmed hyphae invasion. Third, we have tried to identify the characteristics and pathogenesis of ITBA. Fourth, we have suggested additional diagnostic methods such as bronchoscopy and bronchial GM.

Conclusions

Based on the findings of this study, we believe that attention is needed to diagnose and treat ITBA at an appropriate time in patients with immune suppression. Moreover, bronchoscopy and related tests (including bronchial GM assay, biopsy) can be more useful than the serum GM assay for diagnosing not only iITBA but also ITBA+IPA. In addition, in patients with hematologic malignancies showing low platelet counts, tests conducted with bronchoscopy can be a safe alternative to PCNA. Through this study, we were unable to provide sufficient explanation for the relationship between IPA and ITBA, and further studies are required.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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