Original Article
Clinical in vestigation of misdiagnosis of invasive pulmonary aspergillosis in 26 immunocompetent patients

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Abstract: The clinical features of invasive pulmonary aspergillosis (IPA) in immunocompromised patients is well studied in the past decades. While the manifestations of IPA in immunocompetent patients remain unclear.The purpose of this study was to determine the clinical and radiological manifestations of invasive pulmonary aspergillosis (IPA) in patients without immunosuppression, as well as the reasons for the misdiagnosis of IPA. We retrieved and retrospectively reviewed the records of 102 patients from whom surgical lung specimens of chronic inflammatory granulomas were harvested. 26 patients were eventually diagnosed with pulmonary aspergillosis on Grocott methenamine silver staining. We investigated these patients in detail. We found that the rate of misdiagnosis before the lung surgery was as high as 73%. The most common symptom was hemoptysis, and the main feature in radiology was nodule or mass lesion. Air crescent sign or Halo sign were not common in our study. The atypical radiological manifestations and non-specific clinical findings make the diagnosis of IPA difficult and lead to a high misdiagnosis rate.

Keywords: Invasive pulmonary aspergillosis, immunocompetent, radiological features, clinical characteristics

Introduction

Invasive pulmonary aspergillosis (IPA) is an opportunistic infection that occurs in severely immunocompromised patients, especially, patients who have undergone hematopoietic stem cell transplantation (HSCT) or have hematological malignancy [1, 2]. According to the guidelines in China [3]. The Standardizing the diagnosis and treatment of invasive pulmonary fungal diseases and those of 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) [4], the diagnosis of fungal infection is classified as proven, probable, and possible invasive fungal disease in immunocompromised patients. The final diagnosis of IPA depends on the combination of various elements, including host factors, radiological signs, clinical symptoms, mycological results, and histopathological findings. The above guidelines emphasize the importance of host factors in the diagnosis of fungal infection. However, many individuals without any risk factors are infected with Aspergillus [5-8], and it is difficult to establish a diagnosis of aspergillosis in patients without host factors.

In the current study, we assessed the clinical and radiological findings in patients who were eventually diagnosed with aspergillosis in order to determine the reason for the initial misdiagnosis, and to provide clinicians with more information about the diagnosis of aspergillosis.

Material and methods

Subjects

Surgical lung specimens of chronic granulomatous inflammation harvested between August

2005 and July 2013 were identified from the archives of Anhui Provincial Hospital (Hefei, China). The search yielded 102 surgical lung specimens of chronic inflammatory granulomas. These paraffin-embedded tissues were sliced and stained again in order to determine the underlying etiology. The staining methods used were as follows: hematoxylin-eosin (HE) staining to show the tissue structure, Grocott methenamine silver (GMS) staining to confirm Aspergillus infection, and acid-fast staining to recognize tuberculosis infection.

The medical records of the patients from whom the specimens were harvested were retrospectively reviewed for age, sex, presenting symptoms, treatment, and radiological manifestations. The laboratory data abstracted included pulmonary-function tests and white blood cell count. In total, we identified 26 patients who had been diagnosed with aspergillosis on histopathological examination with GMS staining, according to the guidelines used in China and the EORTC/MSG guidelines [3, 4]. This study was approved by the ethics committee of Anhui Provincial Hospital.

Standard and radiological definitions

All the patients in our study were diagnosed with proven pulmonary aspergillosis according to the EORTC/MSG guidelines [4]. Currently accepted classical host factors for aspergillosis [3, 4] include the following: (1) a history of neutropenia (neutrophil count < 500 cells/mm^3) for more than 10 days before the onset of fungal disease, (2) allogeneic stem cell transplantation, (3) corticosteroid use for > 3 weeks with a mean minimum dose of 0.3 mg/kg/day prednisone or its equivalent (except for, allergic bronchopulmonary aspergillosis treatment), (4) use of drugs that suppress T-cells, during the past 90 days, for example, cyclosporine, tumor necrosis factor-α blockers, specific monoclonal antibodies, and nucleoside analogues, and (5) inherited severe immunodeficiency disease, such as severe combined immunodeficiency and chronic granulomatous disease. Chronic obstructive pulmonary disease (COPD) was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease standard [9].

The definitions of chest imaging findings used in our study followed the guidelines of the Fleischner Society [10] and were as follows: (1)

A soft-tissue opacity that completely covered the background of the lung was defined as a "nodule" if its diameter was < 3 cm and as a "mass" if its diameter was ≥ 3 cm. (2) A "ground-glass opacity" was defined as a hazy area of increased opacity in the lung, with preservation of bronchial and vascular margins. (3) The "halo" sign was defined as the computed tomography (CT) finding of a ground-glass opacity surrounding a nodule or mass. (4) An air crescent was a collection of air in a crescent shape that separated the wall of a cavity from an inner mass. (5) Consolidation appeared as a homogeneous increase in pulmonary parenchymal attenuation that obscured the margins of vessels and airway walls. (6) A cavity was defined as a gas-filled space, seen as a lucency or low-attenuation area, within an area of pulmonary consolidation, a mass, or a nodule. (7) The "tree-in-bud" pattern represented centrilobular branching structures that resembled a budding tree. (8) Bronchial dilatation was assessed with respect to the accompanying pulmonary artery ("signet ring" sign), and was associated with a lack of tapering of the bronchi and the presence of bronchi within 1 cm of the pleural surface.

The available radiographic data were reviewed by an expert radiologist and a chest expert. The histological findings were retrospectively reexamined by an expert pathologist and an expert microbiologist. We defined immune-competent as patients without any of the host factors listed above.

Statistical analysis

The data were summarized and analyzed. Continuous variables were summarized as either means and standard deviations or medians with interquartile ranges. For categorical variables, the percentages of patients in each category were calculated. All analyses were performed using SPSS for Windows (version 16.0).

Results

Demographic characteristics and underlying diseases

In all, 26 patients were diagnosed with proven pulmonary aspergillosis, according to the GMS staining findings. There were slightly more female patients (n = 15) than male patients (n

Table 1. Demographic characteristics and underlying diseases

Characteristic	Value
Mean age, y (range)	48 (27-77)
Female sex, No. (%)	15/26 (57.7)
Underlying diseases, No. (%)	14/26 (53.8)
Tuberculosis, No. (%)	4/26 (15.4)
Diabetes mellitus, No. (%)	3/26 (11.5)
Bronchiectasis, No. (%)	3/26 (11.5)
Pulmonary sequestration, No. (%)	2/26 (7.7)
Cancer, No. (%)	1/26 (3.8)
Chronic liver disease, No. (%)	1/26 (3.8)

Table 2. Clinical manifestations and diagnosis

Characteristic	Number (%)
Clinical manifestations	
Hemoptysis	16/26 (61.5)
Cough	13/26 (50.0)
Expectoration	7/26 (26.9)
Dry cough	6/26 (23.1)
Bloody phlegm	3/26 (11.5)
Chest stuffiness	3/26 (11.5)
Thoracodynia	4/26 (15.4)
Melosalgia	1/26 (3.8)
Fever	2/26 (7.7)
No symptoms	1/26 (3.8)
Neutropenia	0
Diagnosis	
Misdiagnosis	19/26 (73.1)
Hemoptysis of unknown origin	1/19 (5.3)
Lung cancer	10/19 (52.6)
Tuberculosis	4/19 (21.1)
Inflammatory pseudotumor	3/19 (15.8)
Bronchiectasis	1/19 (5.3)

= 11), with a female-to-male ratio of approximately 1:0.73. The mean age was 48 years (range, 27-77 years). No patient had classic host factors.

Of the 26 patients, 14 (53.8%) had underlying diseases, including tuberculosis (4 patients), bronchiectasis (3 patients), diabetes mellitus (3 patients), pulmonary sequestration (2 patients), breast cancer (1 patient), and chronic liver disease (1 patient; **Table 1**).

Clinical manifestations, laboratory tests and diagnosis

The clinical manifestations, laboratory tests, and diagnosis are summarized in **Table 2**. Hemoptysis was the most common complaint (16 patients, 61.5%), followed by cough (13 patients, 50.0%; dry cough, 6 patients, 23.1%). Thoracodynia and bloody phlegm were present in 4 patients (15.4%) and 3 patients (11.5%), respectively. Only 3 patients (11.5%) had chest stuffiness. On admission, none of the patients had fever.

No patient had a history of corticosteroid therapy. Antibiotic treatment was prescribed to 5 patients before admission, 2 of whom had been on long-term antibiotic treatment since they developed bronchiectasis. Three patients underwent bronchoscopy. In one of these patients, bronchoscopy of the left, upper lobe, anterior segment bronchus (LB3) revealed granulomatous obstructions. Subsequent histological examination revealed chronic inflammation. Laboratory tests showed that none of the patients had neutropenia, even though some patients had a low white blood cell count.

Of the 26 patients, only 7 (26.9%) were diagnosed with aspergillosis infection on admission. Thus, the rate of misdiagnosis was 73.1%. The most common misdiagnoses were lung cancer, tuberculosis, and inflammatory pseudotumor. A 47 year-old patient, who had had melosalgia since > 1 month, was diagnosed with lumbar disc herniation. In this patient, multiple nodules were found in both lungs on a radiographic examination performed prior to a scheduled arthroscopic microdiscectomy. The melosalgia was relieved after lung surgery.

Imaging examinations

All 26 patients underwent chest radiography on admission. The radiological characteristics were diverse. There was a nodule or a mass in 10 patients (most lesions measured < 3 cm in diameter). All nodules (**Figure 1A**, **1B**) were of uneven density and contained a central, necrotic area of low density. Four patients had lobulation and spiculation, which were difficult to distinguish from tumors (**Figures 1C**, **1D**, **2A** and **2B**). Five patients were found to have patches on CT. Cavity formation was reported in 6 patients, all of whom had thin-walled cavities. The air crescent sign was seen in 2 patients (**Figure 3C**, **3D**), while the halo sign was found in 4 patients (**Figure 1A**, **1B**). Consolidation (**Figure 2C**, **2D**) with air bronchograms was reported in 2 patients. In addition, 4 patients showed bronchiectasis.

Multiple features were observed in the same individual, such as nodules and patches. None
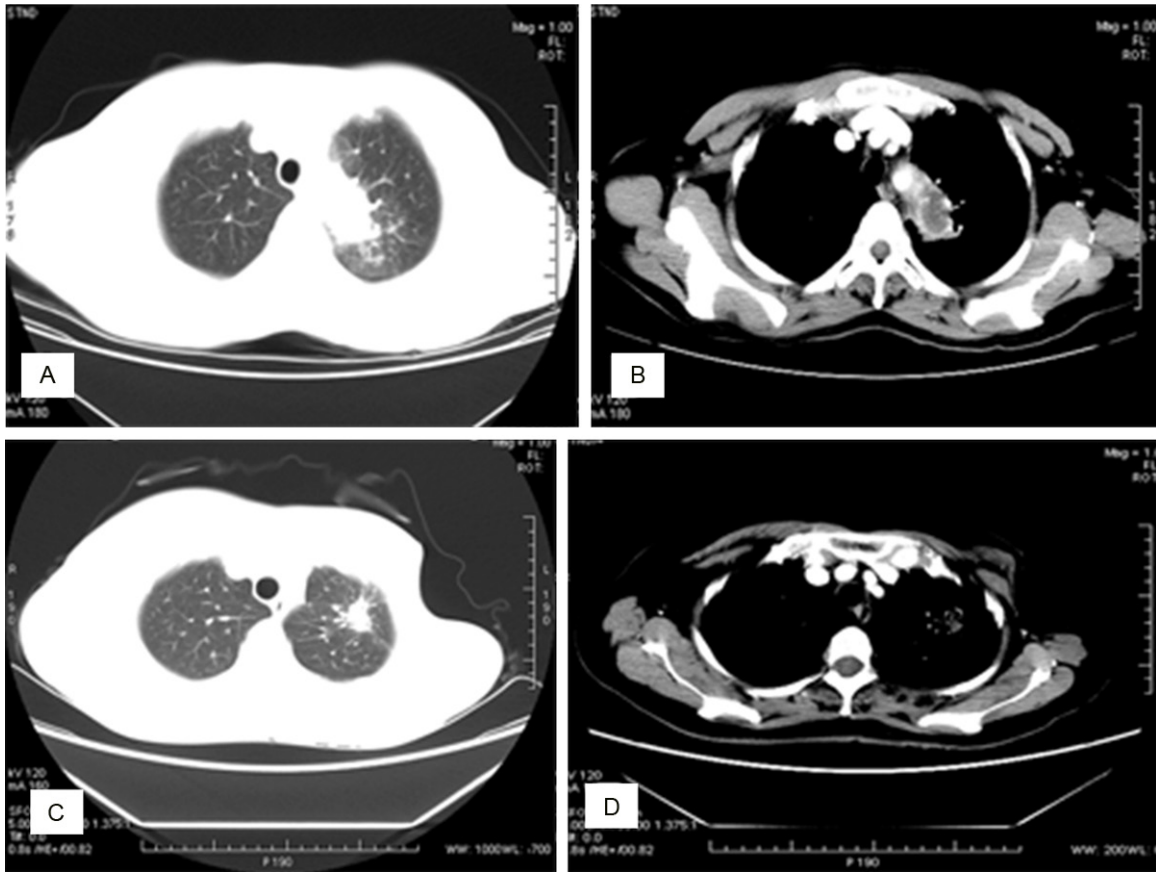
Figure 1. (A, B) A 43-year-old woman with hemoptysis with no obvious cause. Chest CT shows a nodule with lobulation, and the halo sign. Intravenous contrast-enhanced CT reveals that the nodule is heterogeneously enhanced and contains an area of low density (necrosis), measuring 2*2.5 cm. The postoperative pathological examination revealed an Aspergillus infection. (C, D) A 56-year old woman was diagnosed with breast cancer and underwent surgery 11 years ago. Thereafter, she had underwent several cycles of chemotherapy. Three years ago, she developed intermittent hemoptysis. CT shows a lesion with high density and an irregular boundary and spiculation in the left upper lung, surrounded by patchy. In the mediastinal window, the lesion is partly subtracted.

of the patients had mediastinal lymphadenopathy. Eight patients underwent intravenous contrast-enhanced CT, of whom 4 showed no significant enhancement. The remaining 4 patients had mild-to-obvious enhancement of the lesion. Of the 26 patients, 2 (7.7%) had bilateral lung lesions; half of the patients (n = 13) had lesions in the right lung. We also observed one case of aspergilloma, which typically appears as an ovoid or round opacity located within a lung cavity (**Figure 3A**, **3B**). The radiological features are listed in **Table 3**.

Pathology

Histologically, IPA is characterized by necrosis of the lung parenchyma followed by acute or chronic inflammatory cell infiltration and especially, the presence of hyphae invading lung tissue.

Occasionally, vascular invasion or dissemination to other organs can also be observed. Morphologically, Aspergillus typically grow as septate hyphae with characteristic branching at 45° angles, which can easily be observed on HE staining, but is best observed with GMS staining (**Figure 4**).

Discussion

An early diagnosis of IPA based on histopathological or mycological evidence is difficult to establish [11, 12], especially, in the absence of host factors. The demonstration of invasive hyphae on histological examination or a positive culture from a normally sterile environment is diagnostic of proven invasive fungal disease. The diagnosis of IPA requires a combination of host factors, and clinical and mycological crite-
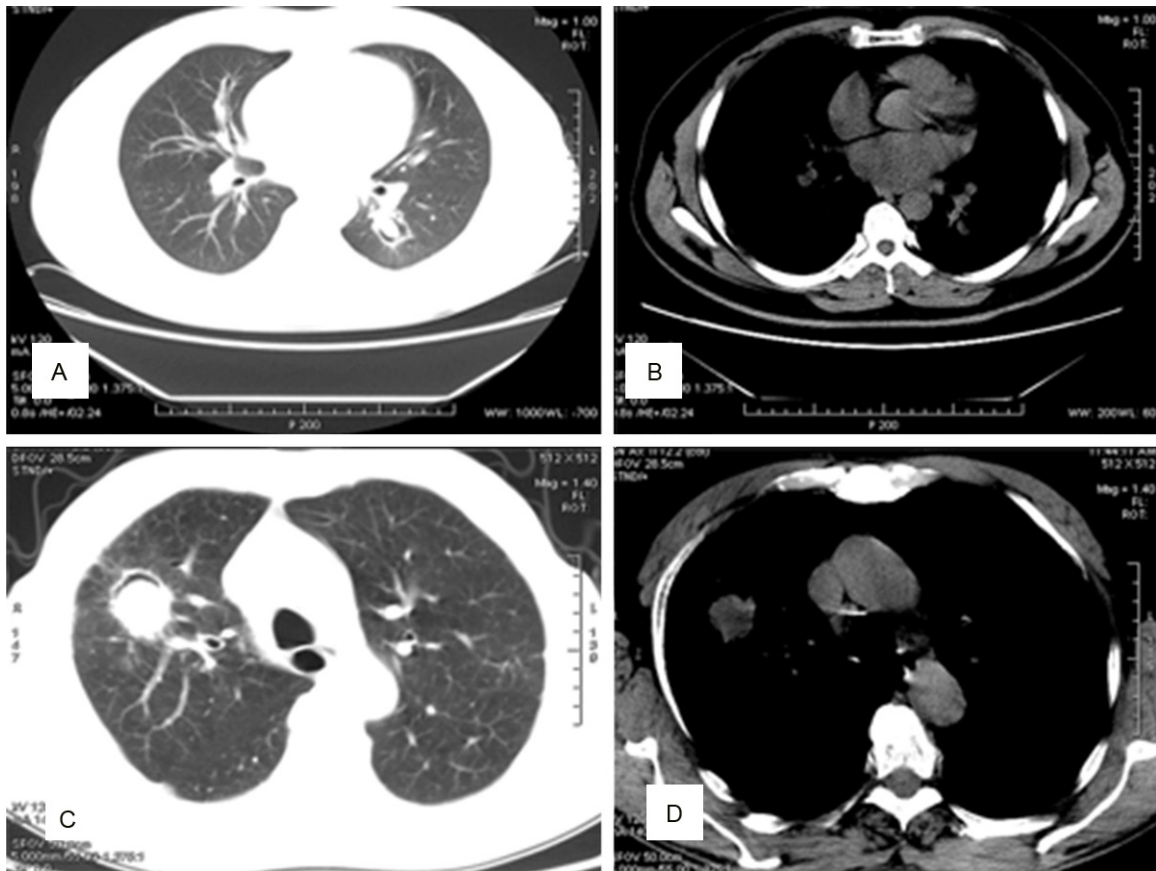
Figure 2. (A, B) A 35-year old woman with a more than 2-year history of hemoptysis. A mass is observed in the right lung. The lesion has a clear boundary and lobulation, and measures 3*2.5 cm. (C, D) In the right lung, consolidation is observed with an ill-defined border and uneven density, within which cavity formation can also be observed.

ria. It is very difficult to obtain evidence to confirm pulmonary aspergillosis in the absence of host factors. Thus, the incidence of aspergillosis and the clinical and radiological manifestations of IPA in individuals without classic host factors remain unclear.

Our investigation indicates that hemoptysis is an important symptom; this symptom has also been reported in immunocompromised patients [13, 14]. In addition, cough and thoracodynia were common symptoms. Some patients had no symptoms at all. According to our investigation, the clinical findings in IPA patients without host factors are non-specific and contribute little to the diagnosis. None of our patients had classic host factors, but 14 of 26 patients (53.8%) had underlying diseases. Several studies [15-19] have indicated that patients with underlying diseases, such as cystic fibrosis and COPD, are at risk for pulmonary aspergillosis.

In our investigation, 4 main radiological findings were identified: nodules and masses (10 patients), cavity (6 patients), patches (5 patients), and bronchiectasis (4 patients). Our results are similar to those of a survey conducted by Qin et al. [20] in patients with liver transplantation; they found that the main radiological manifestations were nodules and patchy consolidation. Franquet et al. [21] have reported that mass-like lesions are the predominant finding in immunocompromised individuals. A large epidemiological survey of fungal infections in China determined that the main radiological manifestations of pulmonary aspergillosis were cavity, consolidation, and pleural effusion [22]. Masses and nodules are occasionally difficult to distinguish from malignancies, especially, when the lesion exhibits lobulation and spiculation or a diffuse and bilateral distribution in the lungs. In our study, 10 patients were misdiagnosed with lung cancer. A recent report has indicated that nodules in pulmonary asper-
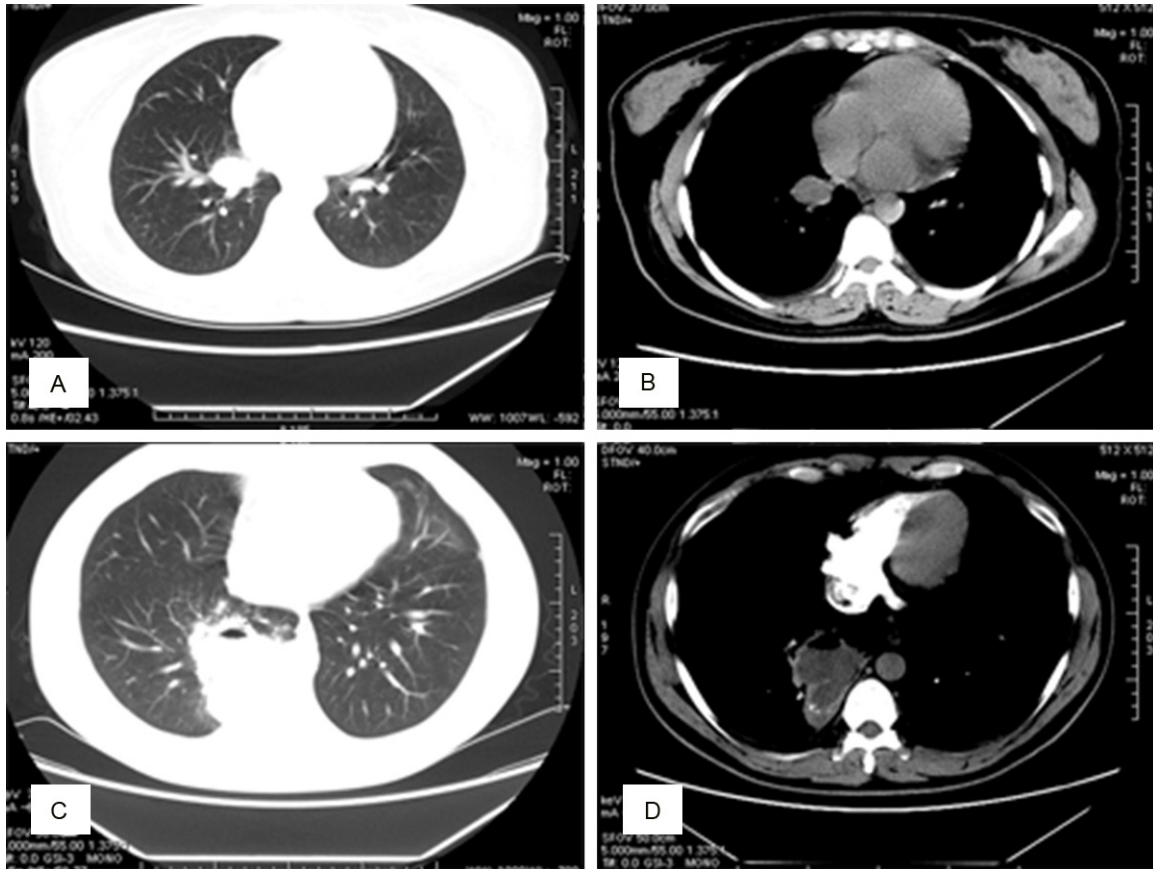
Figure 3. (A, B) A 37-year-old man with bloody sputum since > 3 months. Chest CT reveals an ovoid opacity located within a lung cavity. (C, D) A 59-year old man with cough and bloody sputum. Chest CT reveals a round, high-density, soft-tissue lesion (diameter, 4.2 cm) in the right, upper lung. A curved air shadow, i.e., the air crescent sign, is seen within the lesion. In the mediastinal window, subtraction is not obvious.

Table 3. Imaging examinations

Characteristic	Number (%)
Nodule or mass lesion	10/26 (38.5)
Nodules	7/26 (26.9)
Masses	3/26 (11.5)
Lobulation and spiculation	4/10 (40.0)
Halo sign	4/26 (15.4)
Cavity	6/26 (23.1)
Consolidation	3/26 (11.5)
Air bronchogram	2/26 (7.7)
Air crescent sign	2/26 (7.7)
Patch	5/26 (19.2)
Bronchiectasis	4/26 (15.4)

gillosis may mimic cancer, with a high 18F-fluorodeoxyglucose (FDG) uptake on positron-emission tomography (PET-CT) examinations [23].

The air crescent sign commonly occurs in IPA, especially, in neutropenic patients, during recovery from the disease [24]. One study reported that the air crescent sign was seen in 48% of patients with IPA [25]. In our study, however, only 2 (7.7%) patients exhibited the air crescent sign. The halo sign is also a classic finding in pulmonary aspergillosis. Reginale et al. [26] evaluated the significance of the halo sign in IPA, and found that most (61%) of their patients (patients with allogeneic HSCT, hematological malignancy, or recent neutropenia) had the halo sign, but consolidation, nodules, cavities, and the air crescent sign were less common. In our investigation, 4 (15.4%) patients had the halo sign.

The atypical radiological manifestations and non-specific clinical findings make the diagnosis of IPA difficult and lead to a high misdiagnosis rate. Patients with hemoptysis, cough, expectoration, or fever, who are found to have masses, nodules, cavities, patchy shadows, or consolidation with air bronchograms on radiological examination, especially, patients with
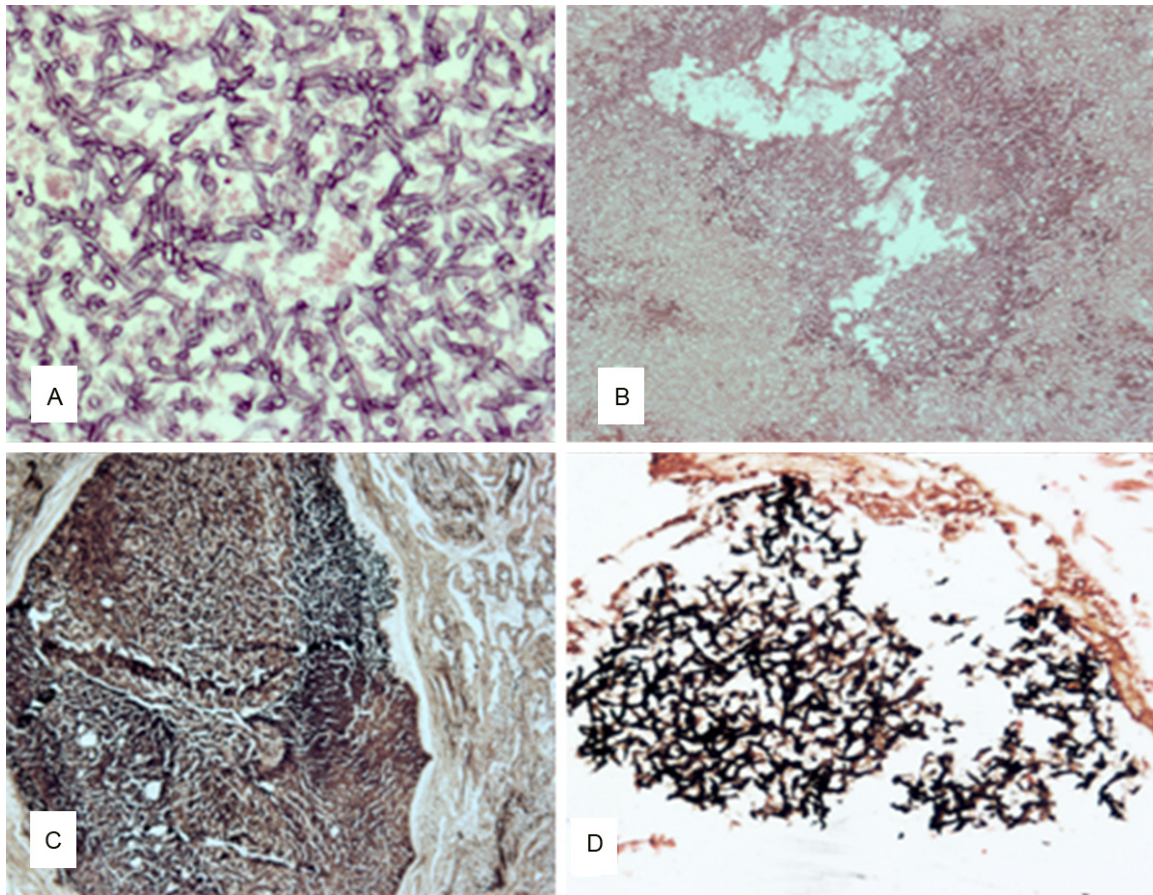
Figure 4. (A, B) Aspergillus hyphae on HE staining. There are numerous Aspergillus hyphae with 45° branching (A: original magnification, × 400; B: original magnification, × 200). (C) The lesion is surrounded by fibrous tissue (original magnification, × 100). (D) GMS staining shows fungal elements (original magnification, × 200).

underlying diseases, should undergo further examinations for pulmonary aspergillosis.

Disclosure of conflict of interest

None.

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