Mycopathologia
DOI 10.1007/s11046-017-0212-y

Pulmonary Aspergillus Overlap Syndromes

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Received: 4 June 2017 / Accepted: 15 October 2017
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Abstract
Background Broadly, there are three main categories in pulmonary aspergillosis: chronic forms of aspergillosis; allergic bronchopulmonary aspergillosis; and invasive aspergillosis (IPA). IPA has been further subdivided into angioinvasive and airway-invasive aspergillosis. Aspergillus overlap syndromes is defined as the occurrence of more than one form aspergillus disease in a single individual.
Objectives To help clinicians correctly deal with AOS.
Methods Retrospectively study the clinical findings of nine patients presenting with AOS.
Results Four cases were diagnosed as angioinvasive aspergillosis complicated with ABPA, three cases as IPA overlap aspergilloma, and two cases as ABPA with AWIA. All the patients presented with cough and expectoration. In three patients with IPA overlap aspergilloma, two had hemoptysis, two had wheezing and fever. All of patients with IPA overlap ABPA had wheezing, dyspnea, and fever, three had sputum plugs, two had hemoptysis, and five patients had mucopurulent discharge and rhonchi in auscultation. Their total IgE ranged from 129 to 2124 IU/ml (676.5 ± 557.33 IU/ml). Fungal culture in sputum showed A. Fumigatus in three patients. All the six patients with IPA overlap ABPA applied steroid therapy and antifungal therapy. Three of them received two or more antifungal drugs successively, and three received combinational therapy. All the patients improved except one diagnosed ABPA overlap IPA.
Conclusions Clinical manifestation of AOS is not typical. Poor first-line therapeutic effects and complicated diagnosis criteria require clinicians to be aware of AOS when facing patients with aspergillosis.

Keywords Aspergillus overlap syndromes · Aspergilloma · Allergic bronchopulmonary aspergillosis · Invasive pulmonary aspergillosis

Introduction

Aspergillus is a ubiquitous mold that can affect the lung in numerous ways. Broadly, there are three main categories in pulmonary aspergillosis: chronic (and saprophytic) forms of aspergillosis, allergic bronchopulmonary aspergillosis (ABPA), and invasive pulmonary aspergillosis (IPA) [1]. IPA has been further subdivided in recent years to angioinvasive and airway-invasive aspergillosis (AWIA) [2]. Chronic pulmonary aspergilloma includes simple aspergilloma, chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis

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Published online: 24 October 2017

(CFPA), aspergillus nodule, and subacute invasive aspergillosis (SAIA) [3]. ABPA is the most frequently recognized manifestation of allergic aspergillosis, caused by the immunological reactions mounted against A. fumigatus [4]. Invasive aspergillosis, the most serious category, is usually developed in immunocompromised hosts and can be disseminated and cause death within weeks. However, sometimes there is considerable overlap between the main categories of aspergillus-related lung disease (ARLD). More than one form of ARLD often coexists, that is defined as aspergillus overlap syndromes (AOS). In this study, we describe the clinical findings and outcome of nine cases with concurrence of more than one form of ARLD, review current progress of aspergillosis to provide the basis for diagnosis and treatment of AOS, and propose some hypothesis to be further discussed.

Materials and Methods

Patients

The data were collected and retrospectively analyzed from nine cases diagnosed with coexistence of more than one category of pulmonary aspergillosis between March 2006 and November 2012 in Zhongshan Hospital, Fudan University. The study was approved by the Local Ethics Committee (approval number: B2016-047) and obtained a written informed consent from all patients.

Diagnostic Criteria

ABPA was diagnosed according to the criteria proposed by the ISHAM ABPA working group [4], including predisposing conditions, A. fumigatus-specific IgE/IgG or serum total IgE, radiological manifestation, and peripheral blood eosinophil count. The diagnosis of pulmonary aspergilloma is based on clinical and radiological features, combined with serological or microbiologic evidence of aspergillus spp. [3]. IPA was diagnosed according to the guidelines proposed by IDSA (2016 update) [1], and revised EORTC/MSG (European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group) definition [5] was applied to make proven, probable, or possible diagnosis. AWIA was diagnosed by pathological feature in which aspergillus organisms were deeply invaded into the basement membrane of the bronchial tree [2].

Data Collection

Routine blood tests and total IgE measurement were routinely performed in clinical laboratory. Pyogenic and fungal cultures were performed for detection of sputum. All of nine cases were underwent thoracic HRCT scanning at the time of diagnosis and during follow-up. The bronchopulmonary segments were obtained at the appropriate lobar and segmental bronchus, according to their relationships with major and minor fissures [6, 7].

Bronchiectasis in patients with ABPA was classified as 'central' when the lesson was confined to the medial half of lung or midway between hilum and chest wall [8, 9]. A high concentration of mucus was considered if mucus plugs were visually denser than the normal skeletal muscle [10]. Aspergilloma was defined by the presence of eccentric soft tissue opacity within bronchiectatic cavity. Radiological manifestation of IPA shows scattered, patchy shadows and halo sign.

Seven of these nine patients underwent bronchoscopy and six took histopathological examination. Another patient was undergoing surgical operation due to suspicion of tumor, and the aspergillosis was finally diagnosed after postoperative pathology.

Results

Five male cases and four female cases with the age between 27 and 70 years old were included in our case series. Four cases were diagnosed with angioinvasive aspergillosis complicated with ABPA. Three cases were diagnosed with IPA complicated with aspergilloma. Two cases were diagnosed with ABPA complicated with AWIA. According to the histopathological examination, six cases were diagnosed 'proven' aspergillosis. Other three cases were diagnosed 'probable' aspergillosis due to positive microorganism examination results.

Among three patients diagnosed as IPA complicated with aspergilloma, one case was previously

infected with classical tuberculosis. Among six cases diagnosed as IPA complicated with ABPA, one case had a history of asthma for 12 years and two cases had chronic obstructive pulmonary diseases. According to their medical history, we found one patient had close contact with wood chips and glue for more than 10 years as a carpenter. Two patients took inhaled corticosteroid (ICS) for more than 2 years. To further establish Aspergillus fumigatus etiology, we tested serum immunoglobulin and the percentage of T lymphocytes and natural killer cells (NK cells) to detect the patients' immune function in six patients. But only one patient who had poor prognosis had lower serum immunoglobulin level and defect in cellular immunity. Her serum immunoglobulin G (IgG) was 3.51 g/L, IgA was 0.54 g/L and $CD3^+$ T lymphocytes accounted for 40.0%, $CD4^+$ Th lymphocytes 11.0%, $CD8^+$ Ts lymphocytes 23.0%, and NK cells 8.0%. No patients had neutropenia (the neutrophil count ranged from 2×10^9/L to 11.62×10^9/L) or abnormal results of autoantibodies.

Cough and expectoration were the most frequent clinical symptoms. In three cases with IPA overlapped with aspergilloma, two cases had haemoptysis, two cases had wheezing and fever, but no one had sputum plugs or dyspnoea. In six cases with IPA (including angioinvasive aspergillosis and AWIA) overlapped with ABPA, most of them suffered from wheezing, dyspnea, and fever; three cases had sputum plugs and two cases had hemoptysis.

Among three cases of aspergilloma complicated with IPA, the eosinophil count was ranged from 0.15×10^9/L to 0.5×10^9/L ($0.32 \pm 0.18\times10^9$/L). Only one case was detected positive for *A. Fumigatus* infection after sputum culture. All included cases were positive for *Aspergillus* spp. IgE/IgG measurement. Radiological examination showed a fungal ball and air crescent sign in their radiological examination besides scattered, patchy shadows, multiple nodules, and halo sign which suggesting invasive aspergillosis.

Six cases diagnosed with ABPA and complication of IPA had eosinophil count between 0 and 0.42×10^9/L ($0.16 \pm 0.18 \times 10^9$/L) in the routine blood tests. The total IgE levels were ranged from 129 to 2124 IU/ml (676.5 ± 557.33 IU/ml) (normal count < 200 IU/ml). Four of ABPA cases had high eosinophil count. Fungal culture in sputum showed positive for *A. Fumigatus* in three cases. In their radiological examination, toothpaste, finger-in-glove opacities were observed in three cases, and tramlines and signet-ring sign suggesting the presence of bronchiectasis were also observed. Figure 1a shows multiple diffuse patchy and high-density shadows in lung with air bronchogram in one of the patients who had received the enhanced CT scanning (Fig. 1a).

GM tests and G tests of serum or bronchoalveolar lavage fluid (BALF) were performed in some of the patients. But only one patient got positive results in both tests. Six patients were accepted bronchofiberscopy. Under bronchofiberscopy, we observed that part of trachea cavity was covered with pale yellow film and left lower bronchial lumen was blocked by light yellow protrusion, which was taken out for later histopathological examination (Fig. 2). The histopathological examination of five patients showed hypha along with positive periodic acid-Schiff and Gomori methenamine silver stain. Another patient accepted surgical resection, showing aspergilloma.

All the patients were received antibiotics before admitted into our hospital. two cases of IPA overlapped with aspergilloma patients had received surgical resection before they applied antifungal therapy. As to antifungal therapy, one patient had applied liposomal amphotericin B for about 1 month and aspergillosis relapsed after 1-month drug discontinuance. He then took itraconazole. The other two cases were continuously received treatment with caspofungin and voriconazole.

All of six patients with IPA overlapped with ABPA were received steroid therapy, and one case of them suffered from osteoporosis. They were simultaneously received antifungal therapy. Three of them were received two or more antifungal drugs successively, and three cases were received combinational antifungal therapy. The patient of Fig. 1 had improved clinical symptoms and laboratory index after about 3 weeks of voriconazole treatment. But inflammation was not absorbed as shown on chest CT (Fig. 1b). Then oral prednisone 30 mg/days was added. Two weeks later, his chest CT showed the absorption of inflammation (Fig. 1c). After 2 months of voriconazole treatment, the patient suffered abnormal liver function and received liposomal amphotericin B for alternative antifungal therapy in conjunction with reduced doses of prednisone. In addition, almost all of the cases were applied antibiotics and symptomatic therapy. All of the cases improved in sputum fungal culture, clinical and radiological examination during

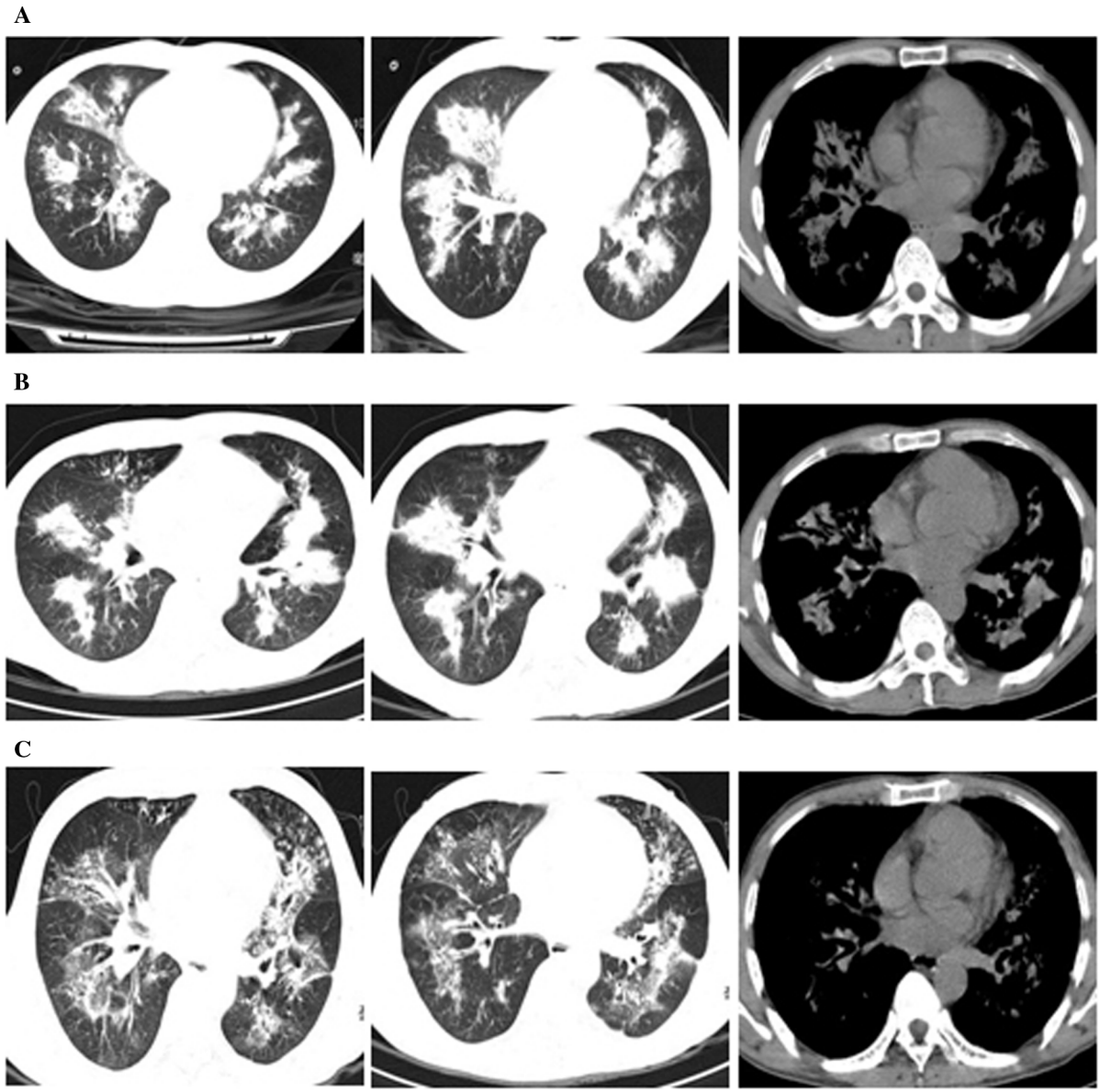

Fig. 1 Chest CT appearance of patient. **a** Pictures before antifungal treatment. **b** Pictures after 3 weeks treatment with voriconazole without oral prednisone. **c** Pictures after 2 weeks treatment with oral prednisone

follow-up, except for one case of ABPA complicated with IPA, who applied voriconazole and steroid after poor therapeutic effects of itraconazole.

Discussion

AOS is defined as the occurrence of more than one form of aspergillus disease in a single individual. This can be simultaneous or sequential (patient with one aspergillus disease developing another) [11]. The mechanism for the development of AOS remains unknown. We assumed that is contributed by multiple factors, such as the presence of severe underlying lung diseases, corticosteroid therapy, aspergillus fungal load, genetic factors, or just coincidence [12]. The contribution of neutrophils to anti-Aspergillus immunity was also mentioned [13], but in our case series, no

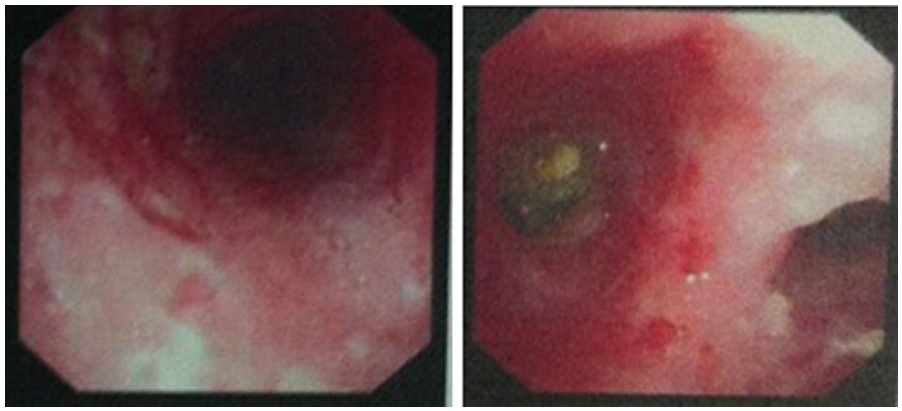

Fig. 2 Pictures under bronchofiberscopy. Part of the trachea cavity was covered with pale yellow film, and left lower bronchial lumen is blocked by light yellow protrusion

patients had neutropenia. The immunologic function in all the patients was also sound except for the patient who died finally. As shown above, the most of our patients were treated with antibiotics prior to definitive diagnosis. It was proposed that the use of multiple broad-spectrum antibiotics altered microbiological flora. Whether the occurrence and deterioration of IPA are related to the application of broad-spectrum antibiotics remains unknown. One patient in our study had structural lung disease and received steroid therapy, which might contribute to the progression of IPA [11].

AOS can be briefly classified into aspergilloma overlap ABPA, ABPA overlap IPA, and aspergilloma overlap IPA. Among ABPA patients, aspergilloma formation may be due to the fact that bronchiectasis in ABPA and aspergillus-induced immune responses create a suitable environment for aspergillus colonization. Long-term glucocorticoid treatment could accelerate the formation of aspergilloma. A genetic polymorphism will lead to excessive aspergillus colonization, so that ABPA patients develop aspergilloma successively [14–16]. It was also previously reported that the activation of immune responses by aspergillus ball at lesion sites might account for the development of ABPA with aspergilloma in some cases [11, 15]. Furthermore, ABPA overlap IPA was also reported previously by other researchers, in which nine cases were discovered from 1980 to 2012 and one case was newly discovered [11]. The results suggested that the use of steroid might lead to immune dysregulation and facilitate invasion of aspergillus from dilated airway into lung parenchyma. Among six patients diagnosed ABPA overlap IPA, two had a history of ICS application. As to aspergilloma overlap IPA, we have already admitted a patient who had took oral prednisone due to scleroderma for 36 years and had poor control of hyperglycemia developed aspergilloma complicated with IPA. To our knowledge, aspergilloma remains relatively stable in patients and rarely causes IPA. The long-term oral steroid therapy and poor control of diabetes may cause invasion of aspergillus. There were only a few of cases reported, and some of which occurred after removal of aspergilloma, as demonstrated in two cases of our study. The mechanism needs to be further studied.

There is no typical clinical symptom of AOS. Laboratory tests showed the elevated inflammatory index. ABPA patients complicated with IPA had higher levels of total IgE. Currently, 1000 IU/ml was applied as a cutoff value [4]. In our six cases of ABPA overlap with IPA, only one case showed level higher than 1000 IU/L. Whether or not the reason of inconspicuously increased IgE level is due to the IPA overlap in the six cases still needs to be further verified in more cases of ABPA complicated with IPA. On the other hand, the results of sputum culture depend on status of patient immune system. At least one aspergillus isolate can be detected from sputum of patients with normal immune status. But for patients with impaired immune function, that may suggest IPA [12, 17]. Since clinical symptoms and laboratory measurements are not specific, AOS was often diagnosed based on radiological manifestation, fiber bronchoscopy, and histopathological examination.

All the patients have radiological examination in diagnosis and follow-up. Aspergilloma is a round mass within cavity that is made of thicken wall and filled with air, called air crescent sign [18]. The typical HRCT images of ABPA are fleeting opacities or finger-in-glove opacities [4]. IPA is manifested in HRCT as multiple patchy of nodular opacities, tree-in-bud sign resembling tuberculosis, and halo sign [1, 2, 19]. AOS should be considered when two manifestations mentioned above were identified in one patient's radiological image. In our study, all of HRCT images among our nine patients showed patchy nodular opacities, indicating pulmonary inflammation. Aspergilloma overlap IPA patient's typical image showed a high-density mass. ABPA overlap IPA patient's typical image showed double-track sign that represents bronchiectasis and high-attenuation mucus plug.

There are no treatment guidelines and few references for AOS patients. Voriconazole is the first-line antifungal agent in the treatment of IPA, while caspofungin can be applied in the primary treatment of IPA in high-risk neutropenic patient and salvage therapy of aspergillosis [1, 20]. Steroid therapy is often applied to ABPA patients in conjunction with antifungal agents [4]. Aspergilloma patients are usually followed up in outpatient service or take an operation if necessary. Since AOS patients have a worse prognosis and more severe symptoms [14, 21], more research and expert discussion are needed to guide clinical AOS treatment.

For ABPA patients, steroid therapy is an important risk factor for developing IPA [11, 22]. A large number of ABPA patients used ICS for treatment, while fewer patients developed IPA, so there were insufficiently evidences showing that steroid therapy was responsible for secondary IPA. It suggests that the doses of steroid can be reduced with combinational use of antifungal agents [14]. But it was also presumed that combinational use of itraconazole and methylprednisolone could lead to an immunosuppressive state of patient immune system and develop IPA [11]. All of our six ABPA overlap IPA patients were received steroid therapy, and five of them were recovered. An obvious absorption in lung shadow was observed in one of our patients after oral prednisone was added. So, we think it would be beneficial to treat the ABPA overlap IPA patients with both steroid and antifungal agents. But as to the patients with poor antifungal therapeutic effects, who have a long term or high dose of steroid therapy, whether it is necessary to reduce steroid therapy needs to be further discussed.

Most of nine patients were received different antifungal agents successively, and three patients were received combinational therapy. Although application of itraconazole, caspofungin, or liposomal amphotericin B in primary treatment can play a therapeutic effect on some AOS patients, for AOS, especially one category overlapped with IPA, of which the risk is greater and the mortality rate is higher, we should apply voriconazole in primary therapy as far as possible. In addition, the course of the treatment of IPA is at least 6–12 weeks [1], so most of the patients should take oral voriconazole or itraconazole as sequential treatment. We have taken a lesson from the patient in whom aspergillosis relapsed due to withdrawal of liposomal amphotericin B after 1-month application. With regard to combinational therapy, three of our patients took voriconazole combined with caspofungin, voriconazole combined with liposomal amphotericin B, and itraconazole combined with liposomal amphotericin B, respectively. However, combinational antifungal therapy is controversial for the therapy of AOS. Most of the combinations are amphotericin B or azole antifungal drugs combined with echinocandins that have less adverse effects and different drug target [23]. Combination antifungal agents in primary therapy and salvage therapy have different outcomes [24–26]. More research should be implemented to further address this issue in AOS patients.

Voriconazole and itraconazole have severe adverse effects [27], so hepatic and renal function tests should be routinely performed during follow-up. Liver damage occurred in one of our patients treated with voriconazole. Currently, it was suggested that therapeutic drug monitoring (TDM) should be performed in patients receiving trizole-based therapy for IA [1]. In patients who have a relapse, we suggest that TDM should be done to monitor plasma drug concentration.

One of our patients underwent drug sensitivity test and showed drug resistance to caspofungin, so alternative antifungal drugs were administered. Other four patients were also received second-line of antifungal agents after first-line antifungal agents were not effective. Similarly, drug resistance was also reported previously [28]. We assumed that drug resistance

might occur more frequently in patients with overlap phenotypes, rather than only single phenotypes. What's more, in patients who have a relapse after substandard treatment, drug sensitivity test should be performed to detect drug resistance. Although antifungal susceptibility testing (AFST) is not recommended by Infectious Diseases Society of America (IDSA) as clinical routine treatment [1], it should be taken into consideration in AOS patients.

Conclusions

There are few of clinical research for AOS in the recent years. But poor first-line therapeutic effects and complicated diagnosis criteria require clinicians to be aware of AOS when facing patients with aspergillosis. Early diagnosis and timely treatment are essential to improve prognosis and reduce mortality of patients with AOS.

Acknowledgements The work was supported by Natural Science Foundation of Shanghai (13ZR1406600).

Funding The study was supported by Natural Science Foundation of Shanghai (13ZR1406600).

Authors' Contribution SC designed the study, LY collected and analyzed the data, LY and JZ wrote the paper, and JZ and SC contributed to the review of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval For this type of study, formal consent is not required.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Patterson TF, Thompson GR, Denning DW, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the infectious diseases society of America. Clin Infect Dis. 2016;63(4):e1–60.
2. Chabi ML, Goracci A, Roche N, et al. Pulmonary aspergillosis. Diagn Interv Imaging. 2015;96(5):435–42.
3. Denning DW, Cadranel J, Beigelman-Aubry C, et al. Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. Eur Respir J. 2015;47(1):45–68.
4. Agarwal R, Sehgal IS, Dhooria S, et al. Developments in the diagnosis and treatment of allergic bronchopulmonary aspergillosis. Expert Rev Respir Med. 2016;10(12):1317–34.
5. De Pauw B, Walsh TJ, Donnelly JP, et al. 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. Clin Infect Dis. 2008;46(12):1813–21.
6. Osborne D, Vock P, Godwin JD, et al. CT identification of bronchopulmonary segments: 50 normal subjects. AJR Am J Roentgenol. 1984;142(1):47–52.
7. Frija J, Schmit P, Katz M, et al. Computed tomography of the pulmonary fissures: normal anatomy. J Comput Assist Tomogr. 1982;6(6):1069–74.
8. Reiff DB, Wells AU, Carr DH, et al. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol. 1995;165(2):261–7.
9. Hansell DM, Strickland B. High-resolution computed tomography in pulmonary cystic fibrosis. Br J Radiol. 1989;62(733):1–5.
10. Agarwal R, Aggarwal AN, Gupta D. High-attenuation mucus in allergic bronchopulmonary aspergillosis: another cause of diffuse high-attenuation pulmonary abnormality. AJR Am J Roentgenol. 2006;186(3):904.
11. Maturu VN, Agarwal R. Acute invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis: case report and systematic review. Mycopathologia. 2015;180(3–4):209–15.
12. Kousha M, Tadi R, Soubani AO. Pulmonary aspergillosis: a clinical review. Eur Respir Rev. 2011;20(121):156–74.
13. Askew DS, Kontoyiannis DP, Clemons KV. Advances against aspergillosis: biology, host response, diagnosis and treatment. Mycopathologia. 2014;178(5–6):321–4.
14. Shah A, Panjabi C. Allergic bronchopulmonary aspergillosis: a perplexing clinical entity. Allergy Asthma Immunol Res. 2016;8(4):282.
15. Agarwal R, Aggarwal AN, Garg M, et al. Allergic bronchopulmonary aspergillosis with aspergilloma: an immunologically severe disease with poor outcome. Mycopathologia. 2012;174(3):193–201.
16. Montani D, Zendah I, Achouh L, et al. Association of pulmonary aspergilloma and allergic bronchopulmonary aspergillosis. Eur Respir Rev. 2010;19:349.
17. Zmeili OS, Soubani AO. Pulmonary aspergillosis: a clinical update. QJM. 2007;100(6):317–34.
18. Buckingham S, Hansell D. Aspergillus in the lung: diverse and coincident forms. Eur Radiol. 2003;13(8):1786–800.
19. Brad H, Tbompson WSJR. Varied radiologic appearances of pulmonary aspergillosis. RadioGraphics. 1995;15:1273–84.
20. Garnacho-Montero J, Olaechea P, Alvarez-Lerma F, et al. Epidemiology, diagnosis and treatment of fungal respiratory infections in the critically ill patient. Rev Esp Quimioter. 2013;26(2):173–88.
21. Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and

proposal of new diagnostic and classification criteria. Clin Exp Allergy. 2013;43(8):850–73.
22. Ganassini A, Cazzadori A. Invasive pulmonary aspergillosis complicating allergic bronchopulmonary aspergillosis. Respir Med. 1995;89:143.
23. Panackal AA. Combination antifungal therapy for invasive aspergillosis revisited. Med Mycol Open Access. 2016;2(2):16.
24. Marr KA, Schlamm HT, Herbrecht R, et al. Combination antifungal therapy for invasive aspergillosis: a randomized trial. Ann Intern Med. 2015;162(2):81–9.
25. Raad II, Zakhem AE, Helou GE, et al. Clinical experience of the use of voriconazole, caspofungin or the combination in primary and salvage therapy of invasive aspergillosis in haematological malignancies. Int J Antimicrob Agents. 2015;45(3):283–8.
26. Panackal AA, Parisini E, Proschan M. Salvage combination antifungal therapy for acute invasive aspergillosis may improve outcomes: a systematic review and meta-analysis. Int J Infect Dis. 2014;28:80–94.
27. Frange P, Bougnoux ME, Lanternier F, et al. An update on pediatric invasive aspergillosis. Médecine et Maladies Infectieuses. 2015;45(6):189–98.
28. Mu X, Yu J, Nie L, et al. Mixed types of pulmonary aspergillosis. Zhonghua Jie He He Hu Xi Za Zhi. 2014;37(9):677–81.