INTRODUCTION

Invasive fungal infection as an opportunistic infection in compromised hosts who have experienced organ transplantation, chemotherapy, or long-term administration of immunosuppressants has remained a significant concern for a long time (1). In particular, invasive fungal infection caused by Aspergillus spp. is a life-threatening illness in severely immunosuppressive as well as slightly impaired immunological conditions. The former scenario is represented by acute invasive pulmonary aspergillosis (IPA) and typically occurs in neutropenic patients with hematologic malignancy who frequently have a severe prognosis (2). Pathologically, IPA is characterized by fungal invasion through lung parenchyma and develops usually via nodular lesions that appear as a nodule with a halo sign on a chest computed tomography (CT) scan (3). The latter scenario is known as chronic pulmonary aspergillosis (CPA) and usually needs long-term antifungal treatment; it occasionally results in fatal respiratory failure and hemoptysis (4). The pathophysiology of CPA has not been as fully clarified as that of IPA. This chronic form of pulmonary aspergillosis, with the exception of simple aspergilloma, has been reported as chronic necrotizing pulmonary aspergillosis (CNPA) and semi-invasive pulmonary aspergillosis among other terms (5,6). In 2003, Denning et al. proposed a new clinical entity of CPA including chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), and CNPA (7). Although these accepted classifications were mainly constructed on the basis of radiographic findings, immunological conditions, and duration of disease, very few histopathological implications have been delineated (8). To elucidate the mechanism of the progression of CPA, we conducted a histopathological analysis and evaluated correlations with clinical features.

MATERIALS AND METHODS

This study included 30 cases clinically diagnosed with CPA consisting of 16 autopsy cases and 14 surgical specimens obtained from the Japanese Red Cross Medical Center and Toho University Omori Medical Center during 2000–2011. Cases with simple aspergilloma were excluded from this study. The clinical features of the cases were reviewed and included preexisting conditions, major symptoms, disease duration, and chest CT images. Sequential CT images were obtained from 27 cases. The lungs were fixed with formalin in both autopsy and surgical resection cases. Paraffin sections were prepared and stained with hematoxylin and eosin, Grocott-Gomori’s methenamine silver nitrate, elastic fiber stain, or Periodic acid-Schiff reagent for light microscopy examination. Detailed examinations focused on the structure of the cavity wall, fungal invasion, epithelial lining, erosion, necrosis, neutrophil in-
RESULTS

Clinical characteristics of the 30 subjects (28 men) are summarized in Table 1. The mean age of all subjects was 70.3 (range 29–86) years. All patients had preexisting diseases, and 15 had multiple underlying conditions. Sequeleae of tuberculosis was the most common underlying condition (17/30, 56.7%), followed by diabetes mellitus (8/30, 26.7%), interstitial lung disease (5/30, 16.7%), bronchial asthma (3/30, 10.0%), nontuberculous mycobacterial infection (3/30, 10.0%), pulmonary cyst (2/30, 6.7%), bronchiectasis (2/30, 6.7%), chronic heart failure (2/30, 6.7%), chronic obstructive pulmonary disease (1/30, 3.3%), dilated cardiac myopathy (1/30, 3.3%), rheumatoid arthritis (1/30, 3.3%), cerebral infarction (1/30, 3.3%), and hepatocellular carcinoma (1/30, 3.3%). Additionally, 5 patients had received steroid administration for interstitial lung disease, and 4 had prior thoracic surgery. The most common symptoms were cough, hemoptysis, and hemoptyisis (15 cases), followed by dyspnea (13 cases), fever (4 cases), sputum (3 cases), and chest pain (1 case).

Twenty-two patients had received antifungal therapy that involved micafungin (12 patients), itraconazole (10 patients), amphotericin B or liposomal amphotericin B (10 patients), and voriconazole (9 patients). The average duration of the disease until surgery for surgical subjects was 16.6 months (range 2 months to 5 years), whereas that of the autopsy group was 13.8 months.
(range 1.5 months to 7 years), for which the recorded causes of death included respiratory failure in 14 patients and hemoptysis in 2 patients. Seven of the 16 autopsy cases involved exacerbation of respiratory failure, with 3 requiring mechanical ventilation and 1 requiring respiratory support using noninvasive positive pressure ventilation. The cultures were positive for *Aspergillus fumigatus* in 7 cases, *Aspergillus niger* in 2 cases, and *Aspergillus* spp. in 1 case. Bronchoalveolar lavage testing was not required for any case.

Radiologic findings of the subjects are summarized in Table 2. The right upper lobe was the most commonly affected site, followed by the left upper lobe, right lower lobe, and bilateral upper lobes, each with a prevalence of 60%, 27%, 7%, and 7%, respectively. The major CT findings consisted of a cavity (100%), fungus ball (70%), consolidation (67%), and ground glass opacity (GGO) (40%). Outlines of the sequential changes in CT findings from 27 subjects based on serial images revealed extension of consolidation in 19 cases (70%), extension of consolidation with GGO in 7 cases (26%), enlargement of the cavity in 7 cases (26%), enlargement of the fungus ball in 1 case (4%), and disappearance of the fungus ball in 1 case (4%). No sequential changes were found in 4 of the 27 cases (15%). Although all extended consolidations involved the marginal area of the cavity, 10 of the 19 cases showed extension along the bronchi, 3 exhibited development in the subpleural area, and 6 showed newly developed consolidation and/or GGO across the opposite lung. Representative findings from the index cases are shown in Figs. 1–3.

Pathological features of the patients are summarized in Table 3. Sections of lung tissue from 29 of the 30 subjects showed cavities containing fungus balls, which were usually fragile and consisted of a brownish yellow

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### Table 2. Radiologic features

<table>
<thead>
<tr>
<th>Distribution of XP finding</th>
<th>(n = 30)</th>
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<tbody>
<tr>
<td>RUL</td>
<td>18 (60%)</td>
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<tr>
<td>LUL</td>
<td>8 (27%)</td>
</tr>
<tr>
<td>RLL</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>BUL</td>
<td>2 (7%)</td>
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<tr>
<td>Initial CT findings</td>
<td>(n = 30)</td>
</tr>
<tr>
<td>cavity</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>fungus ball</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>consolidation</td>
<td>20 (67%)</td>
</tr>
<tr>
<td>GGO</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Serial CT findings</td>
<td>(n = 27)</td>
</tr>
<tr>
<td>extension of consolidation</td>
<td>19 (70%)</td>
</tr>
<tr>
<td>extension of consolidation with GGO</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>enlargement of cavity</td>
<td>7 (26%)</td>
</tr>
<tr>
<td>enlargement of fungus ball</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>disappearance of fungus ball</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>no change</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Distribution of extended opacities</td>
<td>(n = 19)</td>
</tr>
<tr>
<td>peri cavity extension</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>peri bronchial extension</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>extension to opposite lung</td>
<td>6 (32%)</td>
</tr>
<tr>
<td>subpleural extension</td>
<td>3 (16%)</td>
</tr>
</tbody>
</table>

RUL, right upper lobe; LUL, left upper lobe; RLL, right lower lobe; BUL, bilateral upper lobes; GGO, ground glass opacity.

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Fig. 1. Sequential chest x-ray and CT findings of case 13. Initially thickening of pleura and slight infiltration was seen in both upper lungs (A,D). Then cavity including fungus ball appeared in the left upper lung. And Infiltration extended to left lower lung (B). Serial chest CT showed cavity including fungus ball like and consolidation and GGO adjacent cavity and lower left lung (E,F). Finally, extensive infiltration in the right lung appeared before death (C).
mass that broke easily into fragments. Histopathological examination revealed hyphae that were tightly conglom- erated without any space inside the fungus ball. Occasionally, the surface of the fungus ball was coated with eosinophilic materials representing the Splendore-Hoeppli phenomenon. Although hyphae frequently contacted the eroded surface of the cavity, we could not detect the invasion of hyphae in all subjects. The lumina of the cavities and connecting bronchi were filled with massive inflammatory exudate including neutrophils and necrotic tissues. In contrast, there was inflammatory exudate filling the alveoli, including the area without fungal organisms, consisting of granulation tissue and/ or collagen fibers and elastin (Fig. 4), indicating OP. Various degrees of filling with exudation into the alveolar space involving OP were observed in most cases (Fig. 5). OP was observed in the extended area across the opposite lung in 6 cases. Oxalate crystal deposition in pulmonary parenchyma was observed in 2 cases.

**DISCUSSION**

This clinical review focused on the CT images and histological observation of 30 subjects with CPA to elucidate its pathophysiology. Clinical backgrounds, underlying conditions, and symptoms of our patients were similar to those recorded in the scientific literature and also showed a predominance of older men (7,9). None of the patients exhibited a severely immunocompromised condition, but they showed a mildly impaired immunological status. Sequelae of tuberculosis was found as the most common underlying condition, followed by diabetes mellitus, interstitial lung disease, bronchial asthma, and nontuberculous mycobacterium infection. Steroid administration and prior thoracic surgery were also recorded. Although pulmonary tuberculosis sequelae itself does not impair the systemic immune system, the pulmonary tuberculosis sequelae-induced anatomical reconstruction can lead to weakness and local dysfunction of airway clearance. In a previous study that used modeling to estimate the global burden of CPA as the sequelae of pulmonary tuberculosis, the prevalence of CPA varied widely, with a lower prevalence in developed countries compared with developing countries (10). However, as the morbidity of tuberculosis in Japan remains higher than that of other developed countries, the prevalence of CPA is still high. Analysis of chest radiographs showed that shadowed lesions were
Fig. 3. Sequential chest x-ray and CT findings of case 27. Initially, pleural thickening and cavity was seen in right upper lung (A,D). Then, extensive infiltration appeared in both lungs (B,E). Serial chest CT revealed massive consolidation in left lung and partial subpleural consolidation in right lung (F).

located predominantly in the right upper lobe. Some case report series and histopathological analyses of CPA also revealed a predominance of laterality (8,11–13). It is thought that this represents some relationship with the favored site of tuberculosis sequelae (14). The average duration of disease until autopsy was 13.8 months. Nevertheless, most of the patients who died had received antifungal therapy, and the present study revealed a severe prognosis. The main cause of death was respiratory failure. Chest CT images of the patients revealed cavities, but 30% of these cases did not include the fungus ball within this structure. Consolidation and GGO, usually seen around the cavity and extending sequentially along the bronchovascular bundle, were the second most common findings of CT. A unilateral consolidation and/or GGO in 6 cases finally involved the opposite lung. Most subjects experienced exacerbation of respiratory failure, and a histopathological examination of their lungs revealed OP. The present study demonstrated that the essential pathological feature of CPA consisted of a cavity including the fungus ball with erosion and OP. Fungal invasion into tissue was not observed. It is assumed that epithelial lining disorders such as disruption and destruction of the epithelium is caused by fungal growth of the fungus ball adjacent to the cavity wall and not by tissue invasion. Continuing inflammatory changes of ulceration then lead to dilation of the cavity by necrotic destruction of tissue and thickening of the cavity wall by massive exudation with fibrotic changes and OP. Additionally, OP extended widely around the cavity and in the peripheral area including the opposite lung. These ulcerations and OP are thought to be significant features of the pathophysiology of CPA. OP was also correlated with GGO and consolidation from CT findings in cases involving rapid deterioration. Our findings indicated that OP may sometimes lead to rapid expansion and a poor prognosis with respiratory failure.

OP is defined histopathologically by the presence of intra-alveolar buds of granulation tissue, consisting of intermixed myofibroblasts and connective tissue (15). OP is considered a nonspecific response to many types of lung injury, including medications, toxins, radiation, connective tissue diseases, cancers, and several infections. Although bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and influenza virus are well-known causes of OP, few cases of OP related to pulmonary aspergillosis have been reported (12,13,16). OP induced by bacteria cannot be excluded. However, the
Aspergillus A. niger but also by A. fumigatus can invade bronchitis. In the present study, oxalate products have been reported (17,18). Although crystal depositions were seen mainly near the fungus ball and around the cavity wall where oxalic acid can cause parenchymal damage, crystal deposition was also observed with OP apart from the burden of hyphae (19–21). A. niger might be presumed to affect parenchyma by spreading oxalic acid or organisms that cause OP (22). It is then supposed that the organisms produced not only by A. niger but also by A. fumigatus can induce OP (23,24). The progression of OP may be associated with the transbronchial (via airways) spreading of some organisms produced from Aspergillus spp., and massive OP might occur. It was originally reported that a good response was obtained by treating OP with corticosteroids (15,16). However, there are no reports concerning the effectiveness of steroid treatment regarding the progression of CPA. In our study, steroid (30 mg/day prednisolone or 1 g methylprednisolone pulse therapy) was administered in 7 of 9 cases with exacerbation of CPA. Only 2 cases showed a slightly favorable response regarding respiratory symptoms but with no improvement in their radiological findings. Therefore, steroid treatment was not considered helpful for OP in CPA. The reason for the ineffectiveness of steroid treatment may be that OP in CPA differs from the simple serial exudative change as a post-infectious process from tissue damage. The detailed mechanism of OP formation is unknown. It is thought that elucidation of the cause of OP might lead to prognostic improvement of CPA.

CPA was originally reported by Gefter et al. and Binder et al. as CNPA and semi-invasive pulmonary aspergillosis (5,6). However, they described the fungal invasion required for the pathological diagnosis of CNPA, and both of their reports were regarded as clinical benchmarks that characterized chronic progression over several months and were accepted as intermediate features between simple aspergilloma and IPA. In 2003, Denning et al. proposed an entity of CPA including CCPA, CFPA, and CNPA. They also suggested that subacute invasive aspergillosis usually occurs in mild immunocompromised conditions (7). However, their study also described fungal invasion in CNPA, but...
detailed pathological features were not thoroughly delineated. Pathological features of fungal invasion of lung parenchyma have been considered a distinctive feature not only of IPA but also of CNPA (25). Hence, it is difficult to distinguish these conditions by pathological examination of biopsies. Furthermore, the present study has shown that fungal invasion of parenchyma was not seen in CPA. Therefore, all of our cases could be classified as CCPA. OP was therefore proved to be related to the severity of CPA rather than fungal invasion.

We analyzed the clinicopathological correlations of CPA. The essential pathophysiology of CPA can be understood as an active state of erosive cavities and/or bronchitis caused by contact with the fungus balls, which may play a significant role in producing the massive inflammatory exudate that spreads throughout the alveolar spaces and finally develops into OP without direct invasion of fungi into the lung tissue. The mechanism of tissue invasion, which is different from that of IPA, involves progression with destruction of parenchyma by ulceration and sometimes results in rapid deterioration with OP, which leads to a poor prognosis and potentially respiratory failure. New diagnostic and therapeutic strategies for CPA may result from an increased understanding of the pathophysiology of CPA. Furthermore, future investigations need to contribute to clinical practices that affect the prognostic implications of CPA (26,27).

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Conflict of interest Dr. Shibuya reports receiving research grants from Pfizer Inc., Janssen Pharmaceutical K.K., Dainippon Sumitomo Pharma Co., Astellas Pharma Inc., Taiho Pharmaceutical Co., and POLA-Pharma Inc. Other authors declare that they have no compet-
Fig. 5. (Color online) Histopathologic appearance of case 27. 68-year-old man who had severe respiratory failure. Macroscopic findings showed cavities surrounded dense grayish area. Pleural thickening could be seen (left, panoramic view of left whole lung). Histologic findings of the lesion (asterisk) showed organization in air space. Alveolar space adjacent and around the cavity was filled with inflammatory exudate and inflammatory granulation tissue which reflects to organizing pneumonia without the presence of fungal elements.

REFERENCES


