Original Article
Clinicopathological features of pulmonary cryptococcosis with cryptococcal titan cells: a comparative analysis of 27 cases

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Received June 15, 2014; Accepted July 28, 2014; Epub July 15, 2014; Published August 1, 2014

Abstract: In addition to the typical size, Cryptococcus neoformans can enlarge its size to form titan cells during infection, and its diameter can reach up to 100 μm. Clinical reports about cryptococcal titan cells are rare. Most studies focus on aspects of animal models of infection with titan cells. Herein, we report the clinical and imaging characteristics and histopathologic features of 3 patients with titan cells and 27 patients with pathogens of typical size, and describe the morphological characteristics of titan cells in details. Histologically, 3 patients with titan cells show necrosis, fibrosis and macrophage accumulation. The titan cells appear in necrotic tissue and between macrophages, and have thick wall with unstained halo around them and diameters range from 20 to 80 μm with characteristic of narrow-necked single budding. There are also organisms with typical size. All 27 patients with normal pathogens show epithelioid granulomatous lesions. There is no significantly difference in clinical and imaging feature between the two groups. Cryptococcus neoformans exhibits a striking morphological change for the formation of titan cells during pulmonary infection, which will result in misdiagnosis and under diagnosis. The histopathological changes may be new manifestation, which need to be further confirmed by the study with animal models of infection and the observation of more clinical cases. Careful observation of the tissue sections is necessary.

Keywords: Cryptococcus neoformans, titan cells, cryptococcosis, pulmonary infection, histopathology

Introduction

Fungus Cryptococcus neoformans (CN) is an opportunistic pathogen, and also capable of infecting immunocompromised and immunocompetent individuals. Cryptococcal infection can lead to pneumonia, meningitis and skin lesions for human beings. Pulmonary cryptococcosis (PC) results from inhalation of fungal cells with subsequent lung infection and pneumonia. Because the symptoms and radiographic findings in PC are variable and nonspecific, PC is often misdiagnosed as ordinary pneumonia, lung cancer and diffuse lung disease. In recent years, the incidence of PC is rising due to the abuse of broad-spectrum antibiotics, the wide use of cytotoxic agents and immunosuppressant and the high prevalence of the immune deficiency diseases such as AIDS.

Recent studies have revealed a dramatic morphological change of CN for the formation of pathogenic giant cells - titan cells, which size reach up to 100 μm in diameter [1-3]. Currently, the studies about cryptococcal titan cells are mainly confined to aspects of animal models of infection [2-4], and the research of pathogenesis is still in the early stage, the clinical reports are rare and with no clinical and pathologic description in details. Herein, we report 3 PC cases with titan cells, and analyze retrospectively the clinical, radiological and pathological data of 27 PC cases with pathogens of typical size (ordinary PC patients) in order to further
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understand the disease and its clinicopathological features.

Patients and methods

All cases (n = 30) were hospitalized patients (n = 21) and patients for consultation (n = 9) in Affiliated Drum Tower Hospital of Nanjing University Medical School from 2004 to 2013. These cases include 3 PC cases with titan cells and 27 ordinary PC cases. We collected clinical information and imaging data of all patients.

All specimens were submitted for standard histological processing. Tissue sections were stained by hematoxylin and eosin (HE). Special staining methods, including Gomori methenamine silver/Grocott’s silver (GMS) and periodic acid-Schiff (PAS), were performed to evaluate these findings further.

Results

Pertinent clinical, image and histopathological features of 3 cases with cryptococcal titan cells are summarized in Table 1.

Clinical information

Three PC cases with titan cells aged 43, 56 and 72 years respectively, including 2 males and 1 female. Both chief complaints were cough and expectoration, which lasted for 1-3 months. Underlying diseases in 2 patients were autoimmune liver disease, Sjogren’s syndrome and idiopathic thrombocytopenia. Both were medicated with immunosuppressant drugs for years. Another case without underlying disease firstly complained of hemoptysis and fever with the highest temperature of 39.6°C.

Twenty-seven ordinary PC patients include 19 males and 8 females with a mean age of 53 years (24 to 80 years). Excepting 11 cases without underlying disease, other patients had suffered from malignant tumor, Sjogren’s syndrome, chronic bronchitis, tuberculosis, diabetes, hypertension, cerebral infarction and smoking for decades. Three patients had a history of exposure to pigeons or parrots, and 2 patients had traveled abroad. Fifteen cases had abnormal chest radiograph during routine physical examination, including 7 patients being treated for malignancy. The other patients (n = 12) complained of cough, sputum, fever, chest pain and tightness, which lasted for 4 days to 8 months. All patients had no headaches, vomiting and other symptoms of meningeal irritation. Admission diagnosis found pneumonia, diffuse lung disease, tuberculosis, lung cancer, metastatic cancer and occupying lesions, and one patient was diagnosed with acute lymphoblastic leukemia with pulmonary fungal infection.

All patients showed negative results for HIV on admission examination. Fungal culture showed a negative result for sputum.

Radiographic examination

Chest radiographic examination for cases with titan cells exhibited nodules, consolidation, or ground-glass attenuation and patchy shadows. All lesions were involved in bilateral lung, and subpleural lesions were visible. One of the cases was accompanied with cavitary lesions. Enhanced CT scan showed a negative result.

The chest X-ray/initial CT scan findings of 20/27 ordinary PC patients were collected. Nine patients showed pulmonary nodules/masses, and most of nodules had poorly defined margins. Seven patients were observed to have patchy airspace consolidation. Four patients exhibited a mixed pattern with nodules and patchy reticular opacity. Other associated findings included cavitation (n = 2) and pleural effusion (n = 1). The majority of patients had lesions in the peripheral lung zone. Thirteen patients had the lesions involving unilateral lung, 7 patients had the lesions with bilateral lung involvement. Single lobe involvement was found in 13 patients, of whom 7 patients involved the lower lobe. CT scan showed mild enhancement in only one case.

Macroscopic and histopathologic features

All samples of PC cases with titan cells were CT-guided percutaneous transthoracic needle biopsy (PTNB) pulmonary specimens, with case 1 followed by lobectomy. Two cases had similar histological features, and showed lots of necrosis, fibrosis and monocyte-macrophage aggregation by HE staining. There were large amounts of pathogens surrounded by a transparent unstained area within necrosis, even at lower magnification. Pathogens appeared as round or oval fungal organisms with variable size, shell-shaped, blue-gray or red dye thick-wall at high
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PTNB, percutaneous transthoracic needle biopsy.
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magnification (Figure 1A). Many macrophages accumulated with vacuoles of variable size surrounding the necrosis, and fibrosis with minor lymphocyte infiltration also could be seen (Figures 1B and 2A). PAS and GMS staining of the section outlined the fungal cells with red dyed and brown-black dyed walls within necrosis (Figures 1C and 2B) and between macrophages (Figure 2C), and showed single typical narrow-necked budding, expressing as a normal size cell attached to a titan cell (Figures 1D and 2B). The organisms were strikingly different in size. The titan cells size could reach up to 80 µm, and the small cells were 2 to 20 µm in diameter (Figures 1 and 2). The specimens from case 1 with followed pulmonary segmentectomy showed necrotic foci of varying sizes by microscope, which were surrounded by fibers and collagen with large amounts of macrophage reactions, while focal lymphocytic infiltration was found in the edge of the fibers.

Multinuclear giant cells, neutrophils and eosinophils were not observed. In the necrotic zone and the surrounding alveolar space, many round or oval fungal pathogens of varying sizes can be observed at lower magnification, which manifested as larger size, thick and red-stained wall, shell-like shape and unstained transparent zone around them and were more apparent by PAS staining. The specimens from case 3 showed epithelioid granuloma with organizing pneumonia and small focal necrosis, and a lot of macrophages infiltrated under fibrotic background around the necrotic foci (Figure 3A), and a handful of pathogenic giant cells shown by special staining were observed (Figure 3B). However, the pathogens in multinuclear giant cells of epithelioid granuloma had typical size of common fungi.

The specimens in 27 ordinary PC patients were selected from PTNB in 15 cases, pulmonary
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lobectomy/segmentectomy in 8 cases, thoracoscopic resection of lung lesions in 3 cases and transbronchial lung biopsy in one case. Gross examination in 8 patients undergoing pulmonary lobectomy/segmentectomy showed as follows: intact pleura on the surface of the lung, and single or multiple lung nodules with the maximum diameter ranging from millet size to 4.5 cm, and gray, grayish yellow or taupe and solid section, most of which were found at subpleural region of the lung. Histological sections findings in 27 PC patients showed epithelioid granulomatous lesions, among them, 21 patients were found to be accompanied by non-necrotizing epithelioid granuloma, 6 cases by necrosis including 3 cases with abscess (Figure 4A),

Figure 2. Case 2. A. Monocyte-macrophage accumulation accompanied with fibrosis and focal necrosis (HE×100). B. PAS staining exhibited the pathogenic giant cells and small cells with viable size, and red arrow denoted the typical budding pattern (×400). C. PAS staining showed the titan cells between macrophages (×400).

Figure 3. Case 3. A. The specimen of PTNB showed epithelioid granulomatous inflammation with small focal necrosis (HE×100). B. Two titan cells were observed by GMS staining, and more pathogenic small cells were seen (×400).
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11 cases by organizing pneumonia. Granulomatous lesions were composed by epithelioid cells, fibrocytes, fibroblasts, multinuclear giant cells, lymphocytes and a few plasmocytes. On HE section, some round or oval pathogens of varying numbers may be seen indistinctly in multinuclear giant cells, epithelioid cells, tissue space and necrotic tissues. Orginisms were shown as the vesicle shape or pale pink appearance, and around which translucent halo was found. PAS and GMS staining showed that the pathogens were 4-7 µm small cells in diameter and have typical narrow-necked budding (Figure 4B). Moreover, the size of the pathogens in one case was varying with the diameter of 2-20 µm (Figure 4C).

Follow-up

Follow-up visit was conducted by calling and consulting the archival data of the patients. The follow-up visit lasted from 6 to 80 months.

In 3 PC patients with titan cells, case 2 was lost to follow-up because he discharged from hospital voluntarily, and the other two patients exhibited an improved and stable condition after treatment. Among 27 ordinary PC patients, 8 cases undergoing lobectomy/segmentectomy and 3 cases undergoing thoracoscopic resection of lung lesions are in good condition currently after orally taking antifungal agents. Except for four patients who were lost to follow-up, 4 patients with malignancy continued to progress, and the remaining cases exhibited improved and stable condition after anti-fungal treatment.

Discussion

As shown in the majority of journal literatures and reference books in pathology, the average diameter of the CN cells was 4-7 µm with varying sizes (range: 2-20 µm), and single narrow-necked budding was often found. Most of pathogens were surrounded by one layer of polysaccharide capsule with varying thickness, and the cell wall can be well displayed by PAS and GMS staining [5]. Excepting occasional reports of CN pseudohyphal form [6-8], it was shown that CN had three morphological and phenotypic variations: changes in capsule
structure, changes in capsule size, and changes in the total size of the cell which can be achieved by the formation of cryptococcal titan cells or microforms [9]. Only a few reports showed that cryptococcal titan cells were found in the clinical samples [10, 11]. Titan cells were found firstly in the lung, brain and cerebrospinal fluid of patients with cryptococcosis with the diameter of 40-60 μm and even more then 60 μm. However, in the culture condition in vitro, these pathogens would revert to the small cells with common forms.

Morphologically, the most significant features of cryptococcal titan cells are that cell volume increases strikingly, their diameter can reach up to 100 μm and daughter cells with normal size are reproduced by budding [2, 3]. The capsules of titan cells are more dense than those of typical cells, and the thickness of the cell wall can be up to 2-3 μm while typical cell wall thickness is 50-100 nm. Titan cells are mononuclear polyploidy, while the typical cells are haploid. Titan cells are not easy to be swallowed by macrophages for the larger volume [12].

The majority of patients with PC need to be confirmed by the biopsy or surgical intervention [13-16]. The histological characteristic of PC is changeable due to different immune states of the patients [5, 15, 17, 18]. In immunocompromised patients and immunocompetent individuals, the histopathology often display as the granulomatous lesions. There are few patients in whom organizing pneumonia is the main histological change [19]. In patients with early or severe immunosuppression, the histologic patterns emerged as minimal inflammation with plenty of extracellular organisms that efface the tissue architecture of lung [20]. The sections in all 27 ordinary PC patients in this paper showed epithelioid granulomas with/without necrosis and organizing pneumonia, and the pathogens was composed of 4-7 μm small cells in diameter in the majority of patients. All 3 patients with titan cells exhibited necrosis, fibrosis and mononuclear macrophage aggregation. Two patients were found to have large amounts of cryptococcal titan cells of varying sizes in the degenerated necrotic tissues and between macrophages. Their diameters were of 20-80 μm. Moreover, the narrow-necked single budding with small daughter cells and typical small cells with the diameter of 2-20 μm could also be observed. These pathogens were similar to the morphology of the cryptococcal titan cells described in literatures [2, 3, 21]. The specimens from case 1 who underwent the followed lobectomy also exhibited similar histological changes, and no epithelioid granulomas. The specimens from case 3 showed necrotic epithelioid granulomas with organizing pneumonia, but a few pathogenic giant cells were observed, while the majority of pathogens were small cells. The cryptococcal titan cells can be displayed obviously even by HE staining, which is related to obvious increase of the total cell size and significant thickening of cell wall and capsule. The distinct necrosis may be caused by the fact that the pathogenic giant cells cannot be swallowed by macrophages, but the accumulated macrophages can release large amounts of inflammatory mediators/cytokines to cause necrosis of the cells and tissues. It is notable that multinuclear giant cells could not be observed when large amounts of titan cells were appeared, comparing with epithelioid granulomatous lesions in ordinary PC patients. It is may be a new manifestation.

The diagnosis of PC due to cryptococcal titan cells for the 3 patients we described is based on several histological features. First, the sizes of the pathogens observed in the 3 patients are within the reported size range that the maximum diameter of titan cells can be up to 100 μm. Second, the cell wall and capsule of the titan cells is dense and thick and the CN of typical size can also be observed by HE and special staining. Third, definite single narrow-necked budding can be identified, and no other fungal forms such as endospores, hypha and pseudohypha. In addition, titan cells are found not only in the necrotic tissues, but also between the macrophages, moreover, all the 3 patients do not receive the treatment with antifungal agents when undergoing diagnostic biopsy. Therefore, it means that the morphological variation is not a degenerative phenomenon secondary to morphological adaptation and necrotic tissues, or long-term antifungal treatment. It is in accord with the morphology of cryptococcal titan cells described in literatures [3, 21], and the diagnosis of PC thus be determined.

The clinical and imaging manifestations of PC are diverse, and it often involves a variety of
lungs, especially malignancy [22]. The clinical presentation of PC are associated with the immune status of the patient, and may be totally asymptomatic and also the severe pneumonia with respiratory failure [23]. The most common symptoms include cough, expectoration, fever, chest pain, dyspnea, night sweat and hemoptysis [13, 24, 25]. The imaging manifestations are nonspecific, and include single or multiple pulmonary nodules/masses, segmental consolidation, diffuse interstitial infiltrates, cavitation and diffused mixed lesions [13, 24, 26]. The PC patients with titan cells complained mainly of cough and expectation in this report, and 15 ordinary PC patients did not exhibited obvious clinical symptoms, while the remaining 12 patients presented with nonspecific clinical manifestations. Imaging examination showed no significant difference between the PC patients with titan cells and ordinary PC patients, and lesions both involved the sub-pleural area. Nevertheless, the clinical and imaging manifestations of PC patients with titan cells need to be further explored.

Cryptococcal titan cells detected in the clinical specimens may result in a misdiagnosis. The patients infected by other pathogens with giant forms in fungus have rarely been reported. The pathogens mainly include Candida albicans, Blastomyces dermatitidis, genus Emmonsia, Paracoccidioides brasiliensis, Coccidioides posadasii and Rhinosporidium seeberi [23, 27-32]. Giant blastoconidia can be seen in very few cases with Candida albicans infection with their diameters of up to 30 µm, and such infections are common in immunocompromised patients receiving the treatment with cytotoxic drug. The diameter of the pathogens in necrotic tissues and abscess can reach up to 20-40 µm in case of Blastomyces dermatitidis infection, and HE sections showed that the yeast forms had thick, doubly refractile cell walls and prominent nuclear content; meanwhile, the organism was black by GMS staining and exhibited broad-based budding. Genus Emmonsia can be giant cells and cause Adiaspiromycosis in human. The adiaspore size ranged from 25 to 400 µm for Emmonsia crescents. With the HE staining, the thick walls of the adiaspores appear to be composed of two layers: a narrow eosinophilic outer layer and a chitinous thicker inner hyaline layer. The adiaspores are usually empty. Emmonsia parva infection in human is rare, and requires 40°C to produce adiaspores, which are 10 to 25 µm in diameter. Paracoccidioides brasiliensis are round or oval yeast cells with the diameter of from 3 to 30 µm and occasionally as great as 60 µm. It is often identified that single and multiple narrow-necked blastoconidia attached to the parent cell in a “teardrop” budding by GMS staining. Coccidioides immitis and Coccidioides posadasii can expand to form giant spherules with the diameter of 30-150 µm. The spherule can evolve a large amount of endospores. The mature spherules would rupture and release endospores into the infected tissues. The spherules and endospores may appear simultaneously. The diameter of endospores is generally 2-5 µm. GMS and PAS staining can display endospores and immature spherules. Rhinosporidium seeberi may result in Rhinosporidiosis. The spongilla are round or oval with clear boundary of wall, and their diameters can reach up to 200-350 µm, so that they can be seen with the unaided eye in tissue sections. The mature endospores are 8 to 10 µm in diameter, and released into the surrounding tissues following the rupture of the spongilla.

After CN spores or desiccated yeast cells are inhaled and lodged into the lung from the environment, the majority of organisms may be swallowed and eliminated by the mononuclear phagocyte of host. However, formation of titan cells may make the pathogens escape and adapt the host immune response, so that they can survive for long time in the host to cause pulmonary infection [1]. However, the mechanism for the formation titan cells is unclear. Many studies indicated that it is a phenomenon of morphological adaptation induced by the interactions between the pathogens and host cells [1-3]. Thermal effect may be one of factors causing gigantism of the yeast cells [10]. The yeast cells isolated from human brain abscess and then cultured at 25°C in vitro were the small cells, while the diameter of such cells can be about 25 µm at 35°C. DNA replication without concomitant cell fission may be a potential mechanism for cell size increase and may cause that the cells contain mononuclear with polyploidy [2, 3]. Moreover, it may be related to cAMP/protein kinase A (PKA) signaling pathway [2], some g-protein coupled receptors (such as Gpr5, Ste3a), and transcription factor Rim101 may be also involved in the process [33]. In
addition, the formation of titan cells may be a common response to phagocytic cells through sensing of phospholipids in the membrane of the pathogens [34].

The intracellular cryptococcal cells were easier to disseminate to CNS compared to extracellular pathogens [35]. The daughter cells produced by titan cells have a stronger virulence and resistance to oxidative/nitrosative stress [2, 3]. Hence, titan cell production inhibits phagocytosis to promote CN survival and enhance dissemination of CNS [36]. Garcia-Rodas considered that the formation of titan cells was related to a reduction in permeability consistent with an increase in capsule density, which may lead to decrease of the penetrability of antifungal agents [4]. In 3 PC patients with titan cells, case 1 exhibited a poor response to oral antifungal agents after diagnostic procedure of PTNB, and then had stable condition after receiving lobectomy combined with antifungal agents, which indicated the resistance of titan cells to antifungal agents. Case 3 and most of ordinary PC patients who only received antifungal therapy obtain a better efficacy. This result may provide a clinical evidence for the resistance of cryptococcal titan cells to antifungal agents. Therefore, during cryptococcal infection, titan cells in large numbers may have dual effects: resulting in resistance to antifungal agents on one hand, and preventing the dissemination of the pathogens into the CNS on the other hand.

Our cases highlight that cryptococcal titan cells should be kept in mind during differential diagnosis when we observe organisms of giant cells in tissue sections. Titan cells might cause misdiagnosis and underdiagnosis. Because of the rare clinical cases of the cryptococcal infection with titan cells, the morphological changes and its effects on the clinical and pathological processes need further investigation in the animal models of infection and observation of more clinical cases.

Acknowledgements

This work was supported by Medical Science and Technology Development Program Fund of Nanjing City (YKK10071).

Disclosure of conflict of interest

None.
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