

Collective Outbreak of Severe Acute Histoplasmosis in Immunocompetent Chinese: The Clinical characteristics and Continuous Monitoring of Serum Cytokines/Chemokines

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Research Article

Keywords: histoplasmosis, pulmonary, infection, fungi, outbreak, cytokines

Posted Date: March 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-275931/v1>

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Abstract

Background: Acute histoplasmosis is a rare fungal disease and is likely to be misdiagnosed in clinic. In this paper we attempted to find some easy potential specific diagnostic and disease severity predictors of acute histoplasmosis, on the basis of so far the biggest collective outbreak of severe acute histoplasmosis by inhaled fungi in Chinese.

Methods: 10 immunocompetent Chinese were infected when they were working in Guyana, South America. Their clinic characteristics, as well as their blood samples were collected for analysis, and then Person rank correlation was employed to check the associations between the indicators and their clinic conditions.

Results: At admission, their CRP, PCT, LDH and CysC were increased, CD4/CD8 was decreased (<1), and β 2-MG was increased in 9 of them. For cytokines and chemokines, IL-4 was always $<1.3\text{pg/ml}$, and the IL-12, INF- α , TNF- α were always less than reference values. The results of Person rank correlation analysis showed that β 2-MG, CysC, G-test, N/L, LDH were in positive associations to CRP, PCT, which were significantly correlated to the disease severity as well. Then IL-6, IL-8, IL-10 were in positive associations to CRP, PCT, β 2-MG, CysC, G-test, N/L, and LDH, too.

Conclusions: β 2-MG, CysC, N/L, LDH, IL-6, IL-8, IL-10 may be reasonably potential diagnostic and disease severity predictors of acute histoplasmosis, but more examinations are still needed for further verification. More relevant indicators should also be taken into account for improving the accuracy of diagnosis and differential diagnosis.

1. Background

Histoplasma capsulatum (*H. capsulatum*) the causative agent to histoplasmosis, is found in association with birds, bats or their droppings. Exposure to *H. capsulatum* typically occurs by inhalation of fungal spores, following disruption of soil or other contaminated material for histoplasma's habitats^[1]. *H. capsulatum* is a thermally dimorphic fungus, the average incubation period is 1 ~ 3 weeks, and clinical manifestations can range from asymptomatic infection to severe, life-threatening disease depending on host status, inoculum size, and other factors^[1]. Acute pulmonary histoplasmosis is the most common symptomatic manifestation and is often self-limited, especially among immunocompetent persons^[2].

Histoplasmosis is a rare tropical endemic and fungal infectious disease, which is endemic in the Mississippi and Ohio river basins, Latin America, Africa and parts of Asia^[3]. According to the time of onset, it can be divided into acute (< 1 month), subacute (1 ~ 3 months) and chronic (> 3 months). In China, it is often reported sporadic or chronic. Because the disease is relatively rare in China, it is difficult to be distinguished from tuberculosis, pulmonary metastasis tumour and other pulmonary fungal infections in clinic.

As *histoplasma capsulatum* culture growth is often shown at more than 1 week, histoplasma antigen is a useful tool and rapid detection for diagnosis of acute histoplasmosis^[4, 5], however, it's likely unavailable in non-epidemic countries like China. Several studies have reported elevated level in alkaline phosphatase, Westergren sedimentation rate, lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin expression, and fungal (1–3)- β -Dglucan in histoplasmosis patients, but none of these markers is specific^[6, 7]. So the acute histoplasmosis is likely to be missed and even misdiagnosed in clinic.

In this study, in order to explore some easily available and specific markers of histoplasmosis in non-epidemic country, we focus on an acute histoplasmosis outbreak of Chinese, in which 10 Chinese were infected. So far, this outbreak is still the biggest among immunocompetent Chinese. We collected the symptoms, signs, laboratory examination and imaging data of 10 patients with acute histoplasmosis, analyzed their clinical characteristics, compared their cytokine and chemokine levels at different time points, and finally performed a Pearson rank correlation analysis to the laboratory examination indicators. Results show that some of the markers expressed certain positive or negative rank correlations to the patient condition, which suggests those markers might be potential indicators of histoplasmosis for clinical diagnosis and prognosis. We hope these results of this paper could provide researchers better insights to the understanding of this disease.

2. Patients And Methods

Patients:

The 10 patients were sent as miners from Chongqing to Guyana, South America, where they worked and then found infected. They returned to China on April 10th 2019 and were then treated in Chongqing Public Health Medical Treatment Center.

Image examination and Laboratory Analysis:

When patients were admitted, they received CT scanings of their heads, chests and abdomens. All patients had their blood and sputum samples cultured, but only 5 of them got their bone marrow smear examined, and only 1 of them had his bronchoalveolar lavage fluid (BALF) cultured and bronchoscopic frozen lung biopsy performed. The examination of blood routine, liver and kidney function, electrolyte, CRP, procalcitonin (PCT), and fungal D-glucan G-test (G-test) were tested regularly for the 10 patients.

Measurement of serum cytokines and chemokines:

Ethylenediamine tetracetic acid (EDTA) blood samples were collected as described above, preserved in ice packs, and transported immediately to a biosafety level 2 laboratory in the First Affiliated Hospital of Chongqing Medical University. Serum was separated by centrifugation (2000 g for 10 min) at 4°C and stored in 3 mL aliquots at -80°C. Inflammatory cytokines and chemokines IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, TNF- α , IFN- γ were measured simultaneously by multiplex bead-based flow cytometry using human inflammatory cytokine and chemokine cytometric bead array reagents, respectively (4-color FACSCalibur flow cytometer; BD Biosciences). Serum concentrations of IL-27, IL-33, and HMGB1 were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems).

Relevance

Pearson rank correlation analysis was performed between any two of the laboratory indicators, including CRP, PCT, G-test, CD4/CD8, white blood cell count (WBC), the ratio of neutrophils to lymphocytes (N/L), platelet (PLT), β 2-microglobulin (β 2-MG), Cystatin C (CysC), LDH, which were measured using the blood samples taken from the 10 patients during the first month in hospitalization. Then the Pearson rank correlation analysis was performed again between cytokines, chemokines and the indicators above. Subjectively, Pearson rank correlation coefficient is categorized into three groups: weak association (-0.3 ~ 0.3), moderate association (-0.7~-0.3, 0.3 ~ 0.7), and strong association (-1~-0.7, 0.7 ~ 1).

Diagnosis:

Fungi growth was found in the BALF and blood culture of No.1 patient, then they were cultured for 37 days on Sabouraud medium in two culture dishes under 25°C and 37°C, respectively. Fungi grew into white cotton-like colonies under 25°C, but into smooth and cheese-like colonies under 37°C. Within colonies, blastospores of 3 ~ 5 μ m diameter made the cotton blue staining and hexamine silver staining positive (Fig. 1). Consistently, the frozen section of lung specimen suggested multifocal histiocytosis with granulomatous lesion and local tissue necrosis. According to the revised definition of invasive fungal disease^[9], thus the No.1 patient was diagnosed as acute histoplasmosis. According to the identical inducement, common epidemiological characteristics, similar clinical and imaging manifestations, and the same positive urine histoplasmosis antigen test of all the 10 patients, as a result, they were all diagnosed as acute histoplasmosis.

Treatment:

The patients were treated according to the 2007 edition of the American Society of infectious diseases diagnosis and treatment guideline for the histoplasmosis^[8]. At first, all the patients were treated with liposomal amphotericin B, then with itraconazole. After that, however, due to their intolerable side effects to liver and kidney, voriconazole was used instead for maintenance to all patients.

Prognosis:

9 of the 10 patients recovered after therapy and were followed up continuously. While the No.1 patient was in deep coma when he was admitted, and was assessed vegetative by neurologists. Two month later his symptoms of acute pulmonary histoplasmosis remitted, but he was still vegetative. He was then transferred to another local hospital for rehabilitation treatment and missed follow-up.

3. Results

General data:

The ages of the 10 patients range from 30 to 56 years (median age 45 years). One of the 10 used to have tuberculosis and gastroesophageal reflux (both already cured). All patients had experience of neither HIV infection, nor long-term use of glucocorticoid and immunosuppressant. When they got infected in South America, they were working in an abandoned mine tunnel without respiratory protection, where lived a large number of bats with much bat excrement on the ground. The exposure time of them ranges from 0.2 hours to 96.0 hours. Their jobs include soil cleaning (4 patients), flame cutting (2), dockers (2) and site supervisors (2). During the incubation period (about 9 ~ 13 days), they got fevers of unknown origin successively after their first entrance into the tunnel. One of them underwent septic shock and acute respiratory distress syndrome (ARDS) after 4 days' fever, whose spontaneous heartbeat recovered after 15 minutes' cardiopulmonary resuscitation when the respiratory and cardiac arrest occurred (Table 1).

Table 1
General information, clinical and imaging findings of 10 patients with acute histoplasmosis.

	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	NO. 9	NO. 10
General information										
age(year)	42	50	50	48	40	56	43	47	32	30
incubation period(day)	11	9	11	13	12	12	11	10	11	12
work	soil cleaning	soil cleaning	soil cleaning	soil cleaning	dockers	site supervisors	flame cutting	site supervisors	dockers	flame cutting
exposure time(hour)	48	36	54	96	0.2	0.3	4	0.3	0.2	2
symptoms and signs										
fever	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
chills	coma	no	yes	no	no	no	no	yes	no	no
insomnia	coma	yes	no	yes	yes	yes	no	yes	yes	yes
dizzy	coma	no	yes	yes	yes	yes	no	yes	yes	yes
headache	coma	no	yes	no	yes	no	yes	yes	yes	yes
cough	coma	yes	yes	yes	yes	yes	yes	yes	yes	yes
sputum	coma	yes	yes	no	no	yes	yes	yes	no	no
dyspnea	coma	yes	yes	yes	yes	yes	no	no	no	no
thoracodynia	coma	no	no	no	yes	no	no	no	no	no
anorexia	coma	yes	no	no	yes	no	yes	yes	yes	yes
nausea	coma	no	no	no	no	no	no	yes	no	yes
vomit	coma	no	no	no	no	no	no	yes	no	yes
diarrhea	no	no	no	no	no	no	no	yes	no	no
rash	yes	no	no	yes	no	no	no	no	yes	no
imaging findings										
hepatomegaly	no	no	no	no	yes	no	no	no	no	no
splenomegaly	no	yes	yes	yes	yes	yes	no	yes	yes	yes
lung moist rales	yes	yes	no	yes	no	no	no	no	no	no
node	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
solid	yes	yes	no	no	no	no	no	no	no	no
ground-glass opacity	yes	yes	no	yes	yes	yes	yes	yes	yes	yes
mediastinal lymphadenectasis	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
pleural effusion	yes	no	no	no	yes	yes	no	no	no	yes
pericardial effusion	yes	no	no	yes	no	yes	no	no	no	no
meningeal thickening	yes	no	no	no	no	no	no	no	no	no

Symptoms and signs:

The symptoms of patients in hospital were mostly recorded in 4 aspects, which include systemic poisoning, nervous system, respiratory system and digestive system (Table 1). Body temperature of the 10 patients lied in 38.0 ~ 39.4 °C. Three of them had persistent fever, while the others had intermittent fever. Fever, dizziness, headache, insomnia, cough were the most common. The main signs include: skin rash, splenomegaly, moist rale of lung.

Imaging manifestations:

1) Chest CT scanning showed (Fig. 2): i) multiple nodular shadows; ii) consolidation shadows; iii) ground glass opacity shadows; iv) mediastinum and/or hilar lymphadenopathy; v) pleural effusions; vi) pericardial effusions.

2) Cranial CT scanning (Fig. 3a) showed that, in 1 patient both the thickness and density of meninges of bilateral frontotemporal, parietal and occipital lobes were significantly increased, while no obvious abnormality was found in other 9.

3) Abdominal CT scanning (Fig. 3b) showed splenomegaly in 8 patients, hepatomegaly in 1 patient, while no obvious effusion and lymph node swelling in the abdominal cavity in all patients. Details were shown in Table 1.

The results of laboratory examination:

When the 10 patients just return, blood gas analysis indicated type I respiratory failures in 3 of them, and the serum G-test increased in 9 of them. Their CRP, PCT, LDH and CysC were found increased, their ratio of CD4/CD8 was found decreased lower than "1", and their β 2-MG was found increased in 9 of them. As for their WBC, it's found lower than normal reference range in 1 patient, however, during treatment, the WBC of 3 patients occasionally fluctuated upward beyond. After treatment, the symptoms remitted, the CRP, PCT, LDH, β 2-MG, and CD4/CD8 all gradually got back into their normal ranges, CysCs were fluctuated for the renal failures by drugs.

Serum cytokine and chemokine concentrations.

It's shown that, IL-4 was always < 1.3pg/ml for all of them, and the IL-12, INF-a, TNF-a were always less than reference values as well except for those of No.1 patient at the initial stage, while the other serum cytokines and chemokines varied widely with time and patients. Specially for the No.1 patient, whose condition was the most severe, his cytokines and chemokines were significantly higher than those of others, except for IL-4, IL-12, INF-a, TNF-a.

Pearson Rank Correlation of the indicators

Pearson rank correlation analysis suggested that: β 2-MG, CysC, G-test, N/L were in positive moderate associations to CRP, PCT, and LDH was in positive weak associations to CRP, while was in positive moderate association to PCT. For cytokines and chemokines, IL-6, IL-8, IL-10, IL-27 were nearly in positive moderate associations to CRP, PCT. Details were shown in Table 2 and Table 3.

Table 2
Pearson rank correlation between any two of the laboratory indicators, including CRP, PCT, G-test, CD4/CD8, WBC, N/L, PLT, β 2-MG, CysC, LDH

	CRP		PCT		G-test		CD4/CD8		WBC		N/L		PLT		β 2-MG
	mean	median	mean	median	mean	median	mean	median	mean	median	mean	median	mean	median	mean
CRP			0.49	0.53	0.46	0.47	0.27	0.13	0.11	0.02	0.5	0.54	0.38	0.53	0.4
PCT	0.49	0.53			0.57	0.52	0.08	0.08	0.08	0.04	0.48	0.5	0.2	0.17	0.6
G-test	0.46	0.47	0.57	0.52			0.41	0.41	0.02	0.12	0.43	0.42	0.27	0.19	0.1
CD4/CD8	0.27	0.13	0.08	0.08	0.41	0.41			0.18	0.13	0.02	0.08	0.15	0.15	0.1
WBC	0.11	0.02	0.08	0.04	0.02	0.12	0.18	0.13			0.37	0.47	0.32	0.43	0.1
N/L	0.5	0.54	0.48	0.5	0.43	0.42	0.02	0.08	0.37	0.47			0.28	0.36	0.4
PLT	0.38	0.53	0.2	0.17	0.27	0.19	0.15	0.15	0.32	0.43	0.28	0.36			0.2
β 2-MG	0.49	0.56	0.61	0.58	0.16	0.19	0.18	0.18	0.15	0.13	0.42	0.43	0.22	0.27	
Cysc	0.47	0.52	0.46	0.38	0.32	0.21	0.17	0.17	0.24	0.18	0.53	0.58	0.24	0.22	0.7
LDH	0.28	0.28	0.53	0.5	0.46	0.45	0.55	0.55	0.04	0.04	0.09	0.02	0.06	0.14	0.2

Table 3

Pearson rank correlation between cytokines, chemokines and the following indicators, including CRP, PCT, G-test, CD4/CD8, WBC, N/L

	CRP		PCT		G-test		CD4/CD8		WBC		N/L		PLT		β 2-M
	mean	median	mean	median	mean	median	mean	median	mean	median	mean	median	mean	median	mean
1L-1 β	0.04	0.02	0.36	0.32	0.25	0.24	0.02	0.02	0.04	0.05	0.16	0.05	-0.02	-0.19	0.29
IL-2	0.3	0.24	0.17	0.2	0.15	0.12	-0.68	-0.68	0	0.05	0.05	0.07	-0.46	-0.59	0.23
IL-5	0.25	0.22	-0.01	-0.15	0.04	0.08	-0.45	-0.45	0.27	0.25	-0.07	-0.14	0.04	0.02	0.17
IL-6	0.53	0.59	0.54	0.53	0.38	0.31	-0.61	-0.61	0.08	-0.08	0.39	0.48	-0.43	-0.5	0.38
IL-8	0.5	0.62	0.22	0.37	0.21	0.22	-0.43	-0.43	-0.12	0.15	0.13	0.2	-0.24	-0.37	0.18
IL-10	0.37	0.24	0.6	0.68	0.44	0.64	-0.55	-0.55	-0.19	-0.21	0.23	0.19	-0.32	-0.43	0.42
IL-17	0.25	0.25	0.2	0.24	0.01	0.04	-0.41	-0.41	0.02	-0.18	0.13	0.15	-0.3	-0.5	0.27
IL-27	0.6	0.68	0.47	0.48	0.51	0.38	-0.52	-0.52	-0.35	-0.22	0.3	0.33	-0.5	-0.59	0.16
IL-33	0.21	0.31	0.24	0.47	0.15	0.3	-0.3	-0.3	-0.04	-0.13	0.2	0.32	-0.21	-0.41	0.28
IFN- γ	0.37	0.37	0.19	0.2	0.21	0.21	-0.64	-0.64	0.04	0.14	0.18	0.22	-0.36	-0.42	0.34
HMGB1	0.1	0.21	0.3	0.31	0.15	0.22	0.01	0.01	0.09	0.1	0.07	0.03	-0.2	-0.22	0.1

4. Discussion

Acute histoplasmosis is a rare fungal disease. Staffolani S, et al^[10] reviewed the literatures on acute histoplasmosis in immunocompetent travelers. They found most of the reported travelers were from America and Europe to Africa and South America. The only exception they found was traveling from Taiwan to Indonesia. By then, no immunocompetent travelers from Chinese mainland was found reported by them. In recent years, an increasing number of sporadic and chronic histoplasmosis cases started to occur in Chinese mainland, with 90%~95% of the reported patients were asymptomatic in immunocompetent persons. This outbreak reported in our paper is so far the biggest collective exposure in Chinese, with the most 10 acute histoplasmosis patients.

The infection of acute histoplasmosis is usually mild and self-limiting in immunocompetent people, however, apparently in rare cases, the disease can progress to a severe form with high morbidity^[11-14]. The most frequent symptoms were fever, cough, headache and chest pains. Constitutional symptoms (myalgia, sweats, weight loss, anorexia etc.) were also commonly reported. All the 10 patients we reported had accordant clinical symptoms with the literatures. But the conditions of the severer one of the 10 were significantly much worse than those reported in literatures^[2,15-25]. This is possibly because China is a non-epidemic country of histoplasmosis, so most Chinese had no immunity to acute histoplasmosis. The 10 patients, with neither any immunity nor any protective measures, entered the abandoned tunnel and inhaled a large amount of pathogenic fungi in a short time, thus doubtlessly, their conditions are likely to be much worse.

The chest CT manifestations of acute histoplasmosis were various. The most common signs were nodular infiltrates^[26]. In the study, chest CT images of 4 patients (case No.1~4) showed diffuse miliary nodules in both lungs of them, as they were soil cleaners who underwent longer exposure and inhaled more pathogenic fungi. The pulmonary nodules of the 4 were plenty and tiny, with diameters mostly less than 5 mm. Conversely, CT images of the other 6 patients doing other jobs showed scattered nodular shadows in both lungs, because they were exposed shorter and inhaled fewer fungi. Their pulmonary nodules were few and big in the subpleural and bipulmonary lobes, diameters of which ranged from 2~15mm. Thus apparently the manifestations of pulmonary nodules were closely related to the inhaled fungi amount and exposure time.

The abdominal CT manifestations of acute histoplasmosis include splenomegaly sometimes with focal splenic lesions, and lymph node enlargement^[27]. Although diffuse hypodense lesions were found less common in spleen, they were taken as specific signs of histoplasmosis infection^[28]. Of the 10 patients we reported, splenomegalies were found in 8 as per their abdominal CT images, analogous to those reported in literatures. The splenomegaly might be attributed to the spread of *H. capsulatum* infection along the reticuloendothelial system.

It's reported 5%~20% of disseminated histoplasmosis can affect the central nervous system, especially in immunosuppressive patients^[29]. Once there was even a rare case of brain abscess with histoplasmosis in an adult with normal immune function^[30]. As for the case No.1 patient we reported, when he was admitted, he was in deep coma with his cranial CT image showing apparent meningitis inside, which indicates the histoplasmosis affects not only the brain parenchyma but also the meninges when it spreads to nervous system.

As *H. capsulatum* culture growth is often shown at more than 1 week, histoplasma antigen is a useful tool and rapid detection for diagnosis of acute histoplasmosis^[6], however, it's likely unavailable in non-epidemic countries like China. As when the 10 patients we reported at admission, their CRP, PCT, LDH, β 2-MG, Cysc, and G-test were increased, their CD4/CD8 was decreased, their WBC and N/L kept relatively constant, and their cytokines and chemokines were increased except for IL-4, IL-12, INF- α , TNF- α , and their CT images were presented as nodular infiltrates in lungs. In this case, comprehensive multi-analysis by incorporating more indicators might help the diagnosis of histoplasmosis. Thus if any other patient was exposed to histoplasma recently and showed similar conditions on the foregoing manifestation, it might be reasonable to be considered as histoplasmosis.

β 2-MG is concerned with the presentation and stabilization of major histocompatibility complex (MHC) I antigen on the cell surface, which is important in adjusting antigen presentation and immunity^[31]. It has been reported that β 2-MG is an independent predictor of pulmonary infection in hemodialysis patients^[32], which was found increased in 9 of the 10 patients we reported, consistently. With the progress of treatment and the remission of conditions, β 2-MG got back into normal range, and during this remission β 2-MG showed positive moderate rank correlation to the CRP and PCT, which were significantly correlated to the disease severity as well. Therefore, it sounds reasonable to take β 2-MG as another potential predictor of acute histoplasmosis. Besides, when the lungs were damaged, LDH was found increased in serum in former study^[33], and in all patients we reported as well, consistently. Likewise, LDH also showed positive rank correlation to CRP and PCT, thus LDH in serum may also be taken as a potential predictor. Similar conclusions could be drawn to the N/L, but not to WBC, which suggested that N/L may be more sensitive than WBC in acute histoplasmosis infection. As for the CysC, it showed positive rank correlation to not only CRP, PCT but also G-test, N/L, β 2-MG and LDH, thus similarly it's also a potential predictor. Of the 10 patients we reported, although their urea nitrogen and creatinine were in the normal range, their CysCs were increased to a certain extent. With the administration of liposome amphotericin B, some patients experienced kidney damage but their CysCs did not increase significantly, indicating that histoplasmosis infection might have potential damage to kidney and CysC might be insensitive to drugs. However, considering about limitation from the number of cases we reported, more case studies and verifications are still required for the applicability of those potential predictors.

Cytokines, particularly chemokines, are key recruitment mediators of leukocytes and other inflammatory cells leading to pulmonary damage, and have been implicated in the pathogenesis of ARDS/diffuse alveolar damage^[34]. At present, the monitoring of cytokines and chemokines in sepsis is commonly applied besides ARDS^[35]. In addition, in Severe Acute Respiratory Syndrome (SARS)^[36] and 2019 novel coronavirus infection disease (COVID-19)^[37], the monitoring of cytokines and chemokines were usually employed. For the COVID-19 patients, the serum IL-6, IL-8, and TNF- α were significantly increased, and were taken as independent predictors for survivals^[37]. But so far there were few studies about the monitoring and correlation of cytokines and chemokines in histoplasmosis patients. Hirayama T, et al^[38], found that the serum IL-2 receptor (sIL-2R) were increased and well correlated to the disease status in acute histoplasmosis patients, although sIL-2R is actually nonspecific to inflammation. In this study, we tested the IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-27, IL-33, TNF- α , IFN- γ , and HMGB1 of the 10 patients, totally 12 cytokines and chemokines, regularly for 8 months. This is perhaps the first study based on a continuous monitoring of a series of cytokines and chemokines in histoplasmosis. We found that all the patients' serum IL-4 were lower than the reference range. Their IL-12, INF- α , TNF- α were always lower than the reference range except for those of No.1 patient at early stage. Other cytokines and chemokines increased at beginning but gradually decreased with the disease remission. The IL-6, IL-8, and IL-10 increased relatively consistently and they all had positive rank correlation to CRP, PCT, G-test, N/L, β 2-MG, CysC, and LDH. Thus the IL-6, IL-8, IL-10 were reasonably sensitive to the severity of acute histoplasmosis. However, due to the limitations from the number of cases, the examination intervals, inconsistency of some of the control conditions (e.g. severity, immunity, therapy of individuals, etc.), more case studies and verifications are still required.

While pulmonary due to histoplasma in immunosuppressed patients is more common in Epidemic area, systemic representation of this fungal infection in immunocompetent patients is rare and self-limiting in China. To our knowledge, this is the first collective acute histoplasmosis infection in immunocompetent patients, and with the largest number of patients in Chinese. Physicians should think of acute histoplasmosis when people had fevers, cough in histoplasma endemic regions. More examinations and imaging tests should be employed, and more relevant indicators should be taken into account for improving the accuracy of diagnosis and differential diagnosis.

Abbreviations

H. capsulatum, Histoplasma capsulatum; CT, computer tomography; CRP, C-reactive protein; LDH, lactate dehydrogenase; BALF, bronchoalveolar lavage fluid; HE, Hematoxylin-Eosin; PAS, Periodic Acid-Schiff; PCT, procalcitonin; G-test, fungal D-glucan G-test; EDTA, Ethylenediamine tetraacetic acid; WBC, white blood cell count; N/L, the ratio of neutrophils to lymphocytes; PLT, platelet; β 2-MG, β 2-microglobulin; CysC, Cystatin C; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; ARDS, acute respiratory distress syndrome; MHC, major histocompatibility complex; SARS, Severe Acute Respiratory Syndrome (SARS); COVID-19, 2019 novel coronavirus infection disease.

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. The study was submitted to and approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University (Number: 2020-008). Written informed consent for participation was obtained from all participants before the start of the study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was not funded.

Authors' contributions

Yin-yin Peng: wrote the paper; Shu-liang Guo: designed this study; Xiao-feng yan, Lv-lang Zhang, Jing Wang, Guo-dan Yuan, Gang Qing, Lu-lu Xu, Ju Cao: collected the data, test the indicators, and analysis them. Shu-liang Guo: checked the papers.

Acknowledgement:

Authors thank the editors of this journal for their nice help during the manuscript preparation.

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Figures

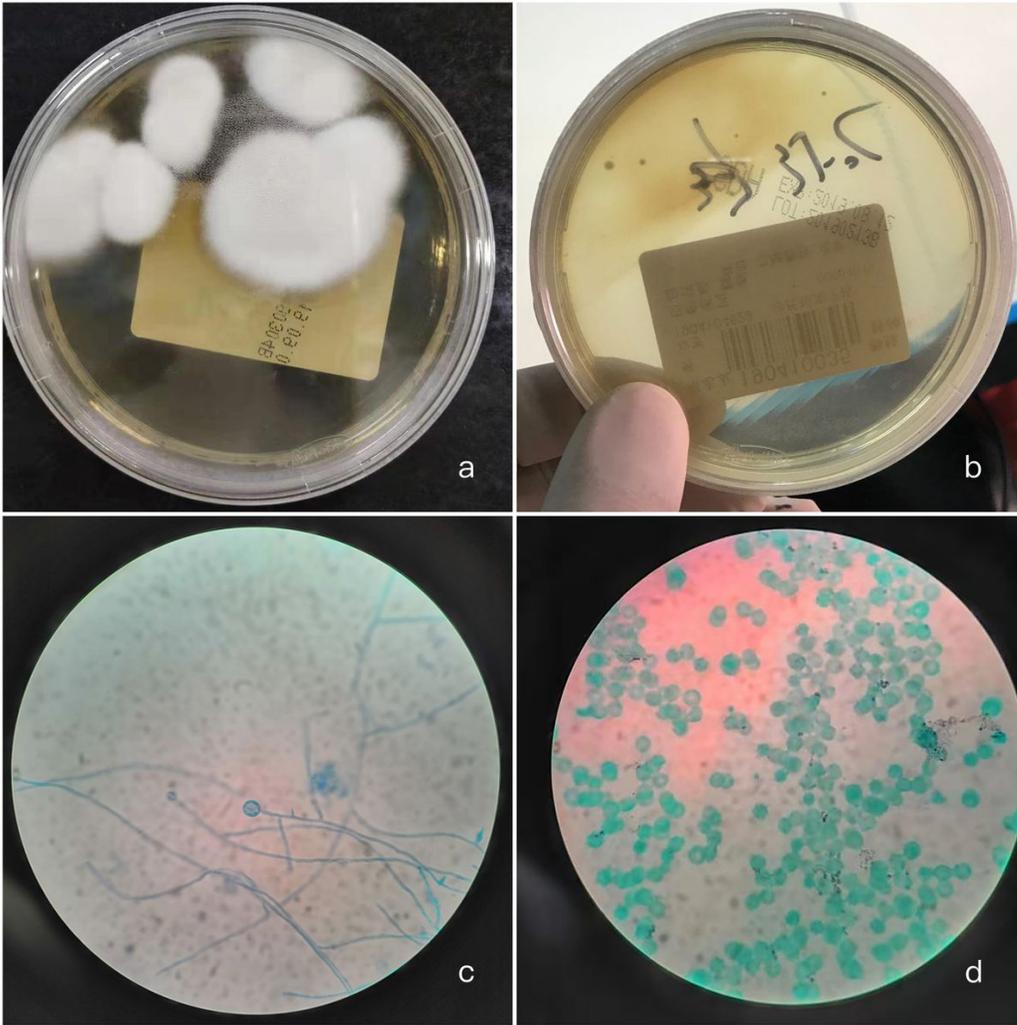


Figure 1
 Fungi growth was found in the BALF and blood culture of No.1 patient, then they were cultured for 37 days on Sabouraud medium in two culture dishes under 25°C and 37°C, respectively. Fungi grew into white cotton-like colonies under 25°C, but into smooth and cheese-like colonies under 37°C. Within colonies, blastospores of 3~5um diameter made the cotton blue staining and hexamine silver staining positive (Figure 1).

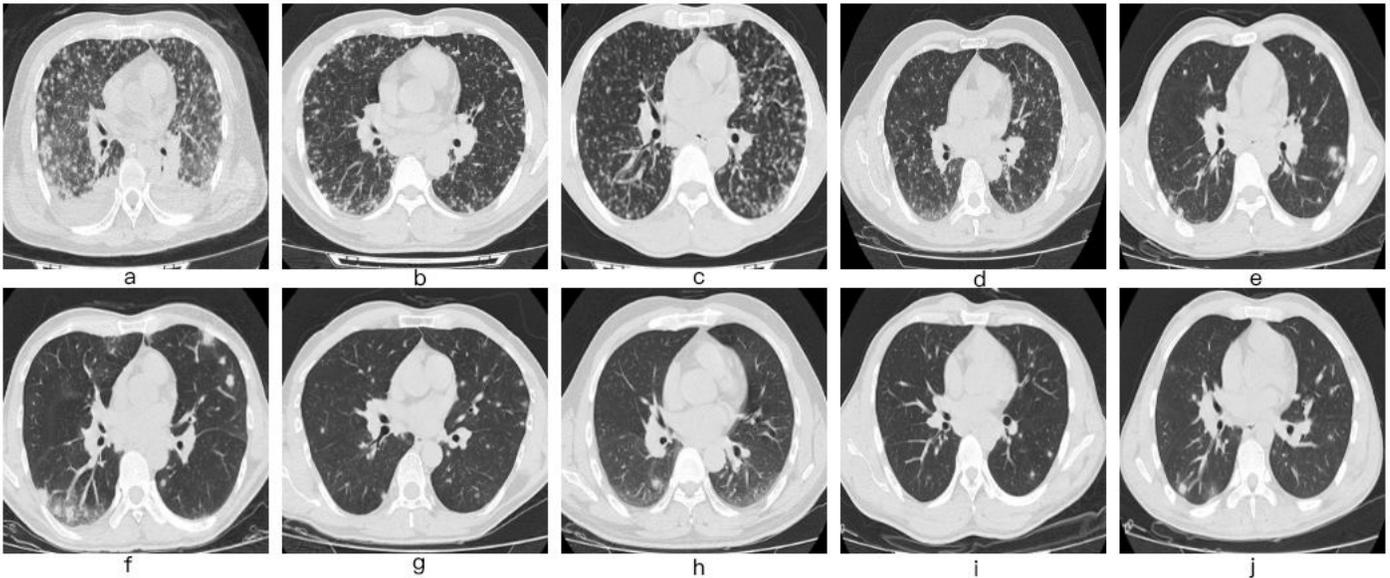


Figure 2
 Chest CT scanning

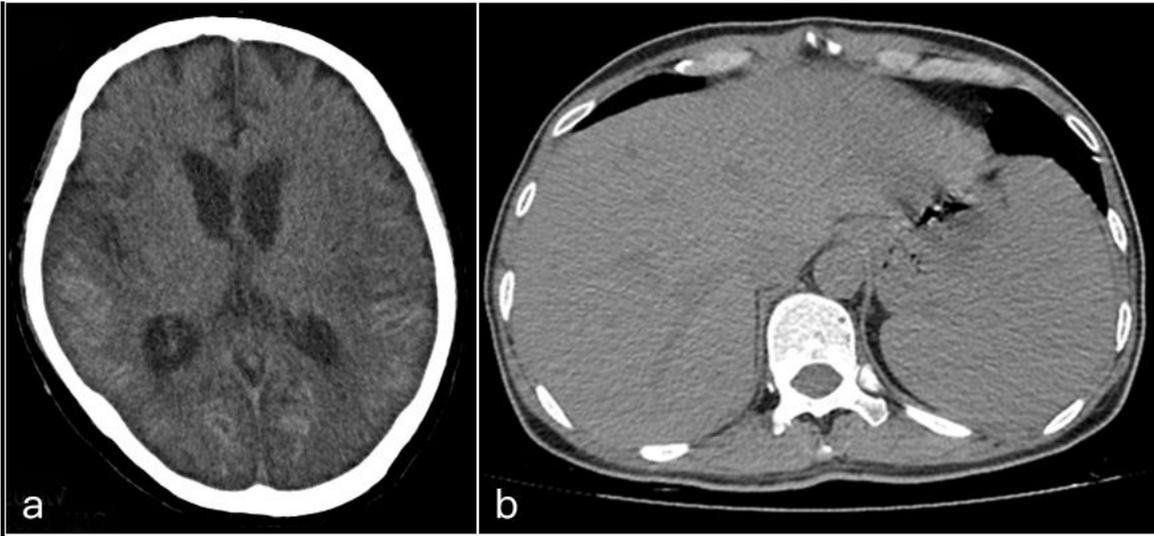


Figure 3

a) Cranial CT scanning b) Abdominal CT scanning