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Analysis of Clinical Characteristics and Diagnostic Value of Fungal Serology in Patients with Invasive Candidiasis

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Abstract

Objective: To evaluate the diagnostic value of (1 - 3)-β-D glucan and mannan assay for invasive candidiasis. **Methods**: A retrospective study was conducted on 32 cases in the disease group (18 proven invasive candidiasis and 14 probable invasive candidiasis) and 48 cases in the control group. The subjects were recruited from January 2018 to March 2019 in Clinical Laboratory of Hainan General Hospital. All subjects were detected by (1 - 3)-β-D glucan and mannan assay. **Results**: The mean concentration of (1 - 3)-β-D glucan in the disease group was 97.45 (43.23, 224.35) pg/ml and it was significantly higher than the mean concentration of the control group which was 49.85(41.91, 56.07) pg/ml ($P = 0.005$). The mean concentration of mannan in the disease group and the control group were 161.36 (34.96, 224.49) pg/ml and 25.80 (25.00, 29.31) pg/ml, respectively, which were significantly different ($P < 0.001$). The sensitivity, specificity, positive predictive value and negative predictive value of (1 - 3)-β-D glucan assay were 59.38%, 89.58%, 79.17%, 76.79%, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of mannan assay were 65.63%, 95.83%, 91.30%, 80.70%, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of combination of two types of assays were 81.25%, 85.42%, 78.79% and 87.23%, respectively. **Conclusions**: Combination of (1 - 3)-β-D glucan and mannan assay can improve diagnostic specificity and it has essential clinical diagnostic value for invasive candidiasis.

Keywords

Invasive Candidiasis, Mannan, (1 - 3)-β-D Glucan, Combination of Serological Tests

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1. Introduction

Invasive candidiasis usually occurs in immunocompromised patients, with a prevalence of 2.1 - 21/100,000 and a fatality rate of 40% - 60%. Total parenteral nutrition, indwelling central venous catheter (CVC), giving antibiotics and glucocorticoids as well as abdomen surgery are all high risk factors for candidiasis [1]. The usage of antibiotics destroys the balance of gut microbiome, leading to growth advantages of Candida. Gastrointestinal surgery or perforation and/or CVC damage the intestinal barrier. Candida albicans colonized in the intestinal cavity breaks through the intestinal mucosal barrier and invades into the blood, causing Candidaemia and invasive candidiasis. Moreover, neutropenia caused by chemotherapy or corticosteroid treatment undermines the natural immune defense and leads to Candida invasion [2] [3].

It is conducive to the early diagnosis of fungi through identifying and preventing high-risk factors of complicating with fungal infection. Culture and tissue biopsy are evidence of fungal infection. However, detection time of fungus culture is long and positive rate is low. Histopathology is inconvenient for critically ill patients due to invasive procedures [4]. In recent years, serological testing attracted more clinical attention.

(1 - 3)-β-D glucan exists in fungal cell wall [5], using as a detection marker for a variety of pathogenic fungal infections. (1 - 3)-β-D-glucan is a highly immunogenic molecule with a specific pattern recognition receptor dectin-1 on the surface of host immune cells. Dectin-1 can mediate phagocytosis, cytokine and chemokine production, leading to a strong immunological response [6]. Mannan is a glycoprotein exposed on the outermost layer of the cell wall and it may be the first molecule to interact with host dendritic cell. Mannan promotes the maturation and activation of dendritic cell, completes the antigen presentation, and activates immune response. Different structures of mannans bind to different types of receptors for dendritic cells: α-mannan of Candida albicans bind to dectin-2 receptor, while β-mannan recognized by galectin-3 and Toll-like receptor 2 (TLR-2) [7] [8]. Jiang *et al.* demonstrate that compared with the bacterial infection group, the Candida colonization group and the control group, the positive rate of mannan group was significantly higher in the invasive candidiasis [9]. When mannan detection using enzyme-linked immunoassay technology, it can improve the diagnostic performance of invasive fungal diseases [10]. This study explored the clinical diagnostic value of combined detection of (1 - 3)-β-D glucan and Candida mannan in invasive candidiasis through a retrospective study.

2. Materials and Method

Study participants: Participants consisted of the disease group (32 cases of invasive candidiasis, including 18 proven cases and 14 probable cases) and 48 cases of the control group (patients under physical examination) collected from the Clinical Laboratory of Hainan General Hospital from January 2018 to March

2019. The enrolled patients all met the diagnostic criteria in the 2019 EORTC/MSG guideline [11]. The study was approved by the Ethics Committee of Hainan General Hospital.

Instruments and reagents: Fungus (1 - 3)-β-D-Glucan, Candida Mannan Assay (ELISA) provided by Dynamiker Biotechnology (Tianjin) Co., Ltd, enzyme-labeled instrument (SUNRISE, TECAN).

Method: 5 mL venous blood centrifuged at 3000 rpm for 10 - 15 minutes and collected the supernatant. If the test was completed within 24 hours, it could be stored at 4˚C, otherwise it could be stored at −20˚C to avoid repeated freezing and thawing. Serum samples were tested with Fungus (1 - 3)-β-D-Glucan (G test) and Candida Mannan Assay (Mn test), the results were fetched by enzyme-labeled instrument. Thresholds of G test and Mn test were 70 pg/ml and 50 pg/ml, respectively.

Statistical analysis: Mann-Whitney U test was performed using IBM SPSS Statistics 21.0 software to complete concentration level analysis and differences were considered significant at $P < 0.05$. GraphPad Prism 5 was applied to drawing graph of concentration level. Receiver operating characteristic curve (ROC) was established by IBM SPSS Statistics 21.0 software and the area under the curve (AUC) was a 95% confidence interval. $P < 0.05$ indicated statistical significance.

3. Result

1) Patient characteristics: The results showed that the median age of the disease group was 68.00 (58.50, 76.75) and the median age of the control group was 36.00 (31.00, 52.00). The difference between the two groups was statistically significant (Z = 6.058, $P < 0.001$). The underlying disease, imaging characteristics, microbial culture results, antifungal treatment and treatment outcome of 32 patients in the disease group were shown in Table 1. The common underlying disease was pulmonary infection (pneumonia) (31 cases, 96.88%), followed by MODs (9 cases, 28.13%), respiratory failure (8 cases, 25.00%), sepsis/septic shock/pyemia (6 cases, 18.75%) and cancer/tumor (6 cases, 18.75%). The imaging features statistics were as follows: cavity (2 cases, 6.25%), exudative lesion (9 cases, 28.13%), pulmonary bulla (2 cases, 6.25%), miliary shadow (1 case, 3.13%), nodule (2 cases, 6.25%), patchy (1 case, 3.13%), stripe shadow (1 case, 3.13%), emphysema (1 case, 3.13%) and air containing space (1 case, 3.13%), Table 1.

2) Concentration levels of G test and Mn test in disease group and control group: The median concentration of G test in the disease group was 97.45 (43.23, 224.35) pg/ml, which was significantly higher than 49.85 (41.91, 56.07) pg/ml (Z = −2.809, $P = 0.005$) in the control group. The median concentration of Mn test of patients in the disease group was 161.36 (34.96, 224.49) pg/ml and compared with 25.80 (25.00, 29.31) pg/ml in the control group, it was significant difference (Z = −5.547, $P < 0.001$) (Figure 1).

Table 1. Characteristics of patients.

Serial number	Diagnostics result	Underlying disease	Image feature	Culture	Antifungal treatment	Treatment outcome
1	Proven	Sepsis, septic shock, MODs, Severe pneumonia	None	Candida albicans by blood culture	Meropenem + voriconazole	Death
2	Proven	Hospital acquired pneumonia	Bilateral Lung exudative lesion	Candida albicans by blood culture	Fluconazole + linezolid + Meropenem	Discharged
3	Proven	Left middle cerebral artery aneurysm, right vertebral artery dissecting aneurysm, pulmonary infection	Exudative lesion of inferior lobe of Bilateral Lung	Candida tropicalis and Candida albicans by blood culture	Cefoperazone/Sulbactam	Discharged
4	Proven	Aspiration pneumonia, type II respiratory failure	Bilateral Lung exudative lesion	Candida tropicalis by blood culture	Caspofungin + Piperacillin Sodium and Sulbactam Sodium + Levofloxacin Lactate	Discharged
5	Proven	Pulmonary infection, right lung carcinoma	None	Candida albicans and Candida dubliniens by blood culture	Caspofungin	Was critically ill
6	Proven	Pulmonary infection	None	Candida albicans by blood culture	Meropenem + Levofloxacin	Discharged
7	Proven	Type II respiratory failure, pulmonary infection	Lesser tubercle of superior segment of inferior lobe of right lung, emphysema and pulmonary bullae of bilateral Lung	Candida albicans by blood culture	Meropenem + Etimicin + Cefopergone Tazobactam	Was consciousness disorder and discharged
8	Proven	Pulmonary infection	Bilateral Lung exudative lesion, cavitation of superior lobe of right lung	Candida parapsilosis by blood culture	Caspofungin	Discharged
9	Proven	MODs, pyemia, septic shock, Pulmonary infection	None	Candida albicans by blood culture	Piperacillin Sodium and Sulbactam Sodium	Discharged
10	Proven	Pulmonary infection	None	Candida tropicalis by blood culture	None	/
11	Proven	Bacterial pneumonia	Air containing space of superior lobe of right lung	Candida albicans by blood culture	Cefoperagone Sodium and Tazobactam Sodium + imipenem-cilas-tatin	/
12	Proven	MODs, pyemia, Pulmonary infection	None	Candida albicans by blood culture	Doxycycline + fluconazole	Death
13	Proven	Pyemia, septic shock, Severe pneumonia, MODs	None	Candida glabrata by blood	/	/
14	Proven	Respiratory failure, Pulmonary infection	None	Candida lusitaniae by blood	/	/
15	Proven	Pulmonary infection	None	Candida tropicalis by blood	/	/

Continued

16	Proven	None	None	Candida albicans by blood culture	/	/
17	Proven	Malignant Neoplasm of Larynx, Pulmonary infection, Type II respiratory failure	Emphysema of superior lobe of Bilateral Lung, nodule of anterior segment of superior lobe of left lung	Candida albicans by blood culture	/	/
18	Proven	Community acquired pneumonia, MODs	Bilateral Lung exudative lesion	Candida albicans by blood culture	/	/
19#	Probable	Pulmonary infection, right lung squamous cell carcinoma	Lesser tubercle of apical segment of superior lobe of right lung, pulmonary bullae of bilateral Lung	Candida albicans by sputum smear	None	Discharged
20	Probable	Pulmonary infection, nasopharyngeal carcinoma after radiotherapy and chemotherapy, Type I respiratory failure	Bilateral Lung exudative lesion	Candida albicans by sputum smear	Piperacillin Sodium and Sulbactam Sodium	Discharged
21	Probable	Pulmonary adenocarcinoma, Pulmonary infection, Type II respiratory failure	Bilateral Lung patchy shadow	Candida albicans by sputum smear	Piperacillin Sodium and Sulbactam Sodium	Was critically ill and discharged
22	Probable	Pulmonary infection	Bilateral Lung exudative lesion	Candida albicans by sputum smear	Fluconazole + Caspofungin	Discharged
23	Probable	Bacterial pneumonia	Bilateral lung lesions with enlarged cavities	Candida albicans by sputum smear	Using Cefoperagone Sodium and Tazobactam Sodium + fluconazole to fight infection, but ineffective, instead of imipenem	/
24	Probable	Aspiration pneumonia, Type I respiratory failure	Exudative lesion of inferior lobe of left lung	Candida albicans by sputum smear	Piperacillin Sodium and Sulbactam Sodium	Was critically ill and discharged
25	Probable	Pulmonary infection, Type I respiratory failure	None	Candida albicans by sputum smear	Piperacillin Sodium and Sulbactam Sodium	Was uncontrolled infection and discharged
26	Probable	Pulmonary infection	None	Candida albicans by sputum smear	Cefoperagone Sodium and Tazobactam Sodium	Discharge
27	Probable	MODs, septic shock, Pulmonary infection	None	Candida albicans by sputum smear	Meropenem + voriconazole + Cefoperazone/Sulbactam + fluconazole	Was respiratory failure and discharged
28	Probable	MODs, Severe pneumonia	Bilateral lung diffuse miliary shadow	Candida albicans by sputum smear	/	/

Continued

29	Probable	Pyemia, septic shock, MODs, Severe pneumonia	Nodule, patchy and stripe shadow of bilateral Lung	Candida albicans by sputum smear	/	/
30	Probable	Pulmonary infection	Exudative lesion of inferior lobe of Bilateral Lung	Candida albicans by sputum smear	/	/
31	Probable	Acute interstitial pneumonia, Severe pneumonia, MODs	None	Candida albicans by sputum smear	/	/
32	Probable	Pulmonary infection	None	Candida albicans by sputum smear	/	/

[Φ]MODs, Multiple organ dysfunction syndrome; #, This patient carried out pulmonary tumor resection and lymphadenectomy over 14 months ago in other hospital; /, Situation unknown; +, Combined application.

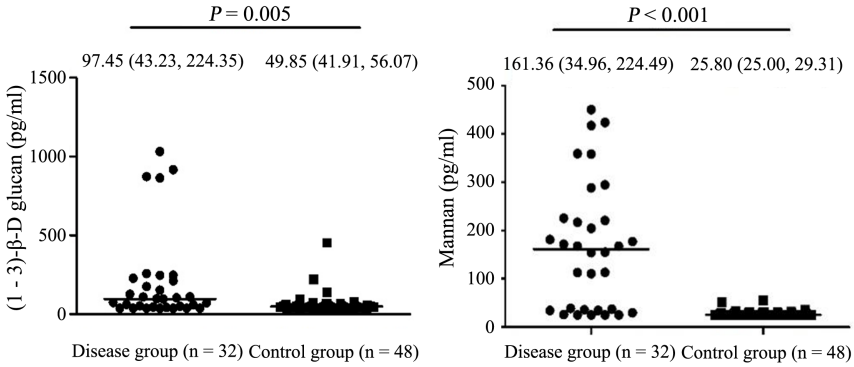

Figure 1. Comparison of concentration levels of G test and Mn test in the disease group and the control group.

3) Diagnostic performance of G test, Mn test and combined detection: The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the G test were 59.38%, 89.58%, 79.17%, and 76.79%, respectively. The above values of Mn test were 65.63%, 95.83%, 91.30%, 80.70% respectively. The sensitivity, specificity, PPV and NPV of the combined detection of two tests were 81.25%, 85.42%, 78.79%, and 87.23%, respectively (Table 2).

4) ROC of G test and Mn test: The AUC of G test and Mn test in the diagnosis of invasive Candida were 0.686 and 0.865, respectively. The optimal cut off of G test was 72.17 pg/ml, corresponding, the sensitivity and specificity were 59.40% and 89.60%. The optimal cut off of Mn test was 34.09 pg/ml, the sensitivity and specificity were 81.3% and 93.7% (Figure 2).

5) Classification of species of candida: Among 32 cases in disease group, 81.25% of patients were infected with Candida albicans, which was the main pathogen. Non-Albicans infections were as follows: four cases of Candida tropicalis (12.50%), 1 case of Candida glabrata (3.13%), 1 case of Candida parapsilosis (3.13%), 1 case of Candida dubliniensis (3.13%) and 1 case of Candida lusitaniae (3.13%).

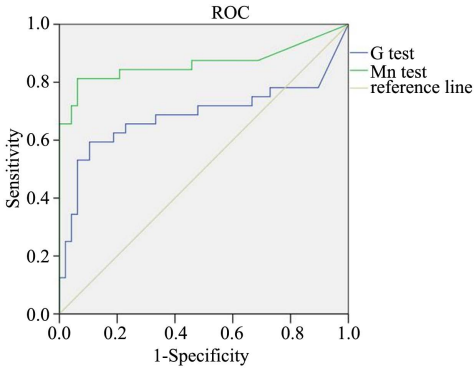

Figure 2. ROC of invasive candidiasis by G test and Mn test.

Table 2. Diagnostic value of G test and Mn test.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
G test	59.38%	89.58%	79.17%	76.79%
Mn test	65.63%	95.83%	91.30%	80.70%
G/Mn test	81.25%	85.42%	78.79%	87.23%

/, if one of the two detection methods was positive, the result was judged as positive, otherwise it was negative.

4. Discussion

The results of this study showed that the median age of the disease group was 68.00 (58.50, 76.75) and the median age of the control group was 36.00 (31.00, 52.00). Our results indicated that elderly patients may be prone to invasive Candidiasis. It is previously reported that Candida is the most common type of fungus infection in elderly patients [12], with an incidence rate of 45.3%, which may be related to reduced immune function in the elderly [13]. In addition, 28 patients in the disease group gave details of their ward types, showing that patients were mainly concentrated in the emergency intensive care unit and the respiratory and critical care unit (75.00%, 21/28), which was consistent with the report [14]. At the same time, most patients with Candida infection had lung infections (96.85%, 31/32), of which 8 patients (25.00%, 8/32) had respiratory failure. The underlying factors of Candida infection are related to the dysbiosis caused by broad-spectrum antibiotic treatment, mechanical ventilation and tracheal intubation [15] and other respiratory support interventions.

Clinical indicators such as C-reactive protein (CRP) and white blood cell count (WBC) can be used to determine the presence of infectious diseases and the types of pathogens. In this study, 32 patients in the disease group had elevated CRP (25 cases, 78.13%), elevated WBC (19 cases, 59.38%), and elevated neutrophils (21 cases, 65.63%). The increase in CRP and WBC is closely related to various types of infections. For example, CRP has an increasing trend in fungal and bacterial infections [16] [17]. Therefore, when clinical presentations of both are high and bacterial cultures are negative, deep fungal infections, including Candida [18], should be considered. Another result of this study showed that

most patients with Candida infection had elevated neutrophils, which was consistent with the results of Liu Haibo *et al.* [19].

In this study, the median concentrations of G test and Mn test of the disease group were significantly higher than those of the control group, and the difference was statistically significant, confirming that G test and Mn test have clinical diagnostic significance for invasive candidiasis. Among 18 patients in the proven group, 16 patients were divided into Candida albicans group and non-albicans Candida (NAC) group (exclude 2 patients with both Candida albicans and NAC infection). The median concentrations of G test in the Candida albicans group and the NAC group were 118.35 (86.95, 220.60) pg/mL and 75.85 (37.50, 912.53) pg/mL, respectively, and the difference was not statistically significant ($Z = -0.544$, $P = 0.586$). At the same time, the median concentration of Mn test in Candida albicans group and NAC group was 163.15 (28.52, 207.93) pg/ mL and 196.97 (32.27, 419.21) pg/mL, and there was no statistical difference between the groups ($Z = -0.868$, $P = 0.385$). These results indicated that the two detection methods were not interfered by the species of Candida, which was consistent with the report by Vindana Chibabhai *et al.* [20]. A study enrolling 188 patients with Candida infection shows that the majority of cases are Candida albicans infection, up to 93 cases (49.50%) [21]. Similarly, the proportion of patients with Candida albicans infection was relatively large (81.25%) in this study. Nevertheless, the number of cases of NAC infection has been on the rise in recent years [22] [23]. In this study, we also noticed NAC infections, such as Candida glabrata, Candida tropicalis, etc.

Among the 32 patients with invasive candidiasis, 11 patients had negative serum mannan tests. This may be because mannan activates the body's immune cells, resulting in the formation of antigen-antibody complexes, which reduces the blood concentration of mannan below the minimum detection limit; or the low concentration of Candida invading the human blood system makes the test false Negative. It has been reported that only 31% to 90% of patients with candidemia will have high concentrations of mannan, which may be related to the rapid clearance or degradation of mannan in the blood [9] [24]. Among the 32 cases of invasive candidiasis, 13 had a negative serum G test. It may be because (1 - 3)-β-D glucan is not distributed on the surface of the fungus. The glucan can only be released after being phagocytosed by phagocytes. In the early stage of infection, the G test detects the substance 1,3-β-D-glucan which has not reached a positive level; it is also possible that patients with neutrophil deficiency and neutrophil phagocytic dysfunction cannot release 1,3-β-D-glucan from fungi. These will cause false negatives in the G test [25] [26].

In summary, the G test and the Mn test have certain value in the clinical diagnosis of invasive candidiasis. The combined detection of the pan-fungal G test and the specific Mn test can improve the sensitivity and specificity of detection [27]. The study shows that the G test was positive 5 days earlier than the first fever, 10.7 days earlier than respiratory symptoms, and 9.3 days earlier than lung imaging changes. The Mn test report results 4 days earlier than blood culture

which results in candidaemia [20] [26]. It is reported that a negative G test can stop the empirical antifungal treatment of patients with high risk of invasive candidiasis in the ICU [28] [29]. Therefore, it is best to use a combination of fungal culture and serological testing in clinical diagnosis to make up for the shortcomings of traditional testing methods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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