Evaluation of a New Laboratory Test Measuring Plasma (1→3)-β-D-glucan in the Diagnosis of Candida Deep Mycosis: Comparison with a Serologic Test

Motofumi HIYOSHI*, Shinichi TAGAWA, Shigemi HASHIMOTO, Chikahiko SAKAMOTO and Noriyuki TATSUMI
Department of Laboratory Medicine, Osaka City University Medical School, Osaka, Japan
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Abstract

We evaluated the effectiveness of the newly developed WAKOTM β-glucan test which measures plasma (1→3)-β-D-glucan concentrations in the diagnosis of Candida deep mycosis. This test was compared to the Cand-Tec test. The WAKOTM β-glucan test and Cand-Tec test were performed on 212 plasma specimens which were taken at 212 instances from 62 immunocompromised patients with serious diseases; i.e. hematopoietic malignancy, solid malignant tumor, etc. The sensitivities and specificities for the WAKOTM β-glucan test were 84.8 and 85.9%, respectively, and 60.9 and 80.0% for the Cand-Tec test.

Introduction

The number of patients with opportunistic infections continues to increase as a result of medical therapies, including anti-tumor chemotherapy, long-term glucocorticoid treatment, and broad-spectrum antibiotics. One of the more devastating opportunistic infections is deep mycosis. In the diagnosis of deep mycosis, serologic tests that detect fungus-specific antibodies have been utilized, however, their sensitivity is low (~50%) and therefore, these tests have been of limited clinical use1). Other diagnostic tests that detect antigens from each type of fungus (for example, Cand-Tec™ for candidiasis, and Pastrex™ Aspergillus for aspergillosis) and are about 70% sensitive have been developed2). However, these tests also may lack sufficient sensitivity for clinical use.

(1→3)-β-D-glucan, a cell wall component commonly found in fungi, reacts specifically with coagulation factor G of the horseshoe crab3,4. This observation has been used to develop a new method to diagnose fungal infection by measuring plasma levels of (1→3)-β-D-glucan (β-glucan). This technique cannot differentiate between various fungal pathogens, but this may not be important in clinical medicine where it is often difficult to diagnose deep fungal infections. We evaluated the newly developed WAKOTM β-
Glucan Test (Wako Pure Chemicals, Osaka, Japan) by comparing it to the Cand-Tec test using clinical specimens obtained from immunocompromised patients with *Candida* deep mycosis.

**Materials and Methods**

**Study population**

Two-hundred and twelve whole blood specimens (212 instances) were collected after obtaining informed consent from 62 patients suspected of having *Candida* deep mycosis (24 with hematopoietic malignancies; 8 with solid malignant tumors; 6 with brain tumors or cerebrovascular disease; 5 with serious bacterial infections; 6 with collagen vascular disease; 4 with diabetes mellitus; 3 who were post-cardiovascular surgery patients; 2 with multiple organ failure of unknown cause; 2 with acute pancreatitis; and 1 with Crohn's disease). The WAKO™ β-glucan test and Cand-Tec test were performed on 212 plasma specimens separated from 212 whole blood specimens taken at 212 instances from 62 immunocompromised patients. Patients undergoing hemodialysis and patients receiving anti-tumor polysaccharide agents were excluded from the study population. Aliquots of plasma (EDTA) separated from the 212 whole blood specimens were stored at −80°C prior to testing.

**Disease definition of Candida deep mycosis**

*Candida* deep mycosis was defined by the occurrence of unexplained fever for 7 or more days despite bacterial antibiotic therapy. In addition, at least one of the two following conditions was present on the day a specimen was obtained: (1) *Candida* species was isolated from the peripheral blood or the focus of disease by culture. This category was defined as "definite *Candida* deep mycosis". (2) A radiographic finding suggesting *Candida* deep mycosis (for example, multiple hepatic microabscesses on computed tomography) and a positive laboratory test detecting *Candida* antigenemia; that is, Cand-Tec (Ramco Laboratories, Inc., Houston, TX) or Pastorex Candida (Sanofi Diagnostics Pasteur, Paris, France). This category was defined as "suspected deep mycosis".

This definition of *Candida* deep mycosis was developed considering other disease definitions previously described concerning deep mycosis or other types of infectious diseases.

**WAKO™ β-Glucan test (Wako Pure Chemicals, Osaka, Japan)**

Plasma (0.1 ml) was added to 0.9 ml of a pretreatment solution containing Triton X-100 and polymyxin B, heated at 80°C for 10 min, and cooled on ice. Inhibitors and endotoxin present in the plasma were inactivated during this treatment. The pretreated sample (0.2 ml) was added to the Limulus lysate reagent and the resulting turbidity change of the mixture measured using a Toxinometer MT-358 to obtain the plasma β-glucan concentration.

**Cand-Tec test (Ramco Laboratories, Houston, TX)**

Fifty microliters of plasma were diluted 1:2 with the diluent supplied in the manufacturer’s kit. The diluted specimen (20 µl) was mixed with 20 µl of latex particles coated with rabbit anti-*Candida* antibody. The mixture was rotated at 140 rpm for 10 min in a moist chamber and then examined immediately for agglutination. Specimens with a titer of 1:2 or greater were considered to be set positive according to the manufacturer’s instructions.

**Case description**

The study population included several patients who provided sequential specimens during their dis-
Results

Clinical usefulness of the WAKO™ β-glucan test

According to the disease definition described above, it was decided whether each of 212 instances provided true positives or true negatives for Candida deep mycosis. In order to obtain an optimal cutoff value for the WAKO™ β-glucan test, a receiver operating characteristic (ROC) curve was constructed (Fig. 2). That is, various provisional cutoff values were set from 7 pg/ml to 20 pg/ml (every 0.5 pg/ml). The provisional sensitivities and specificities were calculated at each provisional cutoff value. The optimal cutoff value for the WAKO™ β-glucan test was calculated using this ROC curve as previously described and determined to be 10.6 pg/ml. With this final cutoff value, it was decided whether
Motofumi HIYOSHI et al

Table 1 Comparison of the WAKOTM $\beta$-Glucan and Cand-Tec tests

<table>
<thead>
<tr>
<th></th>
<th>No. of specimens using results for Candida deep mycosis</th>
<th>sensitivity (%)</th>
<th>specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>definite deep mycosis</td>
<td>suspected deep mycosis</td>
<td>negative</td>
</tr>
<tr>
<td>WAKOTM $\beta$-Glucan test</td>
<td>39</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>positive</td>
<td>7</td>
<td>2</td>
<td>116</td>
</tr>
<tr>
<td>negative</td>
<td>28</td>
<td>21</td>
<td>27</td>
</tr>
<tr>
<td>Cand-Tec test</td>
<td>18</td>
<td>10</td>
<td>108</td>
</tr>
</tbody>
</table>

a. calculated using results for definite deep mycosis

each of the 212 instances provided positive or negative specimens for the WAKOTM $\beta$-glucan test. It was then decided whether each of the 212 instances were positive or negative for Cand-Tec. Table 1 summarizes the data. According to the disease definition, 46 of 212 specimens were assessed as being definite Candida deep mycosis and 31 as being suspected Candida deep mycosis. The sensitivities and specificities were 84.8 and 85.9%, respectively, for the WAKOTM $\beta$-glucan test and 60.9 and 80.0%, for the Cand-Tec test.

Discussion

Currently, there are only two commercially available tests that measure plasma levels of $\beta$-glucan: the G-test$^{6,13,}$ and the WAKOTM $\beta$-glucan, both of which were evaluated in this study. These two tests are based on a similar principle, namely that coagulation factor G of the horseshoe crab reacts specifically with $\beta$-glucan$^{3,4}$. However, each test uses a different detection system to determine plasma $\beta$-glucan concentrations. The G-test uses the end point chromogenic method$^{14,15}$, whereas the WAKOTM $\beta$-glucan test uses the kinetic turbidimetric method$^{16}$. The G-test requires a total of 10 procedure steps and 9 pipetting steps$^{14,15}$. While the WAKOTM $\beta$-glucan test requires only two pipetting steps and procedure steps$^{15}$. In general, procedural deviations and laboratory errors are more likely to occur in procedures with more steps. Therefore, the WAKOTM $\beta$-glucan test may be more suitable for laboratory use than the G-test.

The sensitivities of laboratory tests designed to diagnose Candida deep mycosis have improved. The WAKOTM $\beta$-glucan test was 84.8% sensitive (Table 1) while the Cand-Tec test was only 60.9% sensitive (Table 1), which may not be sufficient for clinical use. Despite a negative Cand-Tec test result, a physician may feel uncomfortable withholding antifungal agents.

The specificity of the WAKOTM $\beta$-glucan test (85.9%) appears to be lower than the 100% specificity reported for the G-test$^{6}$. Our lower specificity may have been caused by including $\beta$-glucan test positive specimens (Table 1) collected from patients who were suspected of having Candida deep mycosis, but who could not be diagnosed definitively at that instance. These specimens were counted as false positives, resulting in a lower specificity for our test. The specificity of the G-test was determined using specimens collected from healthy volunteers or patients with known bacterial infections as true negative test results. The apparent high specificity was obtained because these patients never suffered from Candida deep mycosis. The paper about the G-test$^{6}$ also described the number of specimens collected from the patients who were suspected of having Candida deep mycosis, but who could not be diagnosed definitely at
that instance. Using the number, the specificity of the G-test would be calculated as 83.8%, which compares with the 85.9% specificity for the WAKO™ β-glucan test evaluated in this study (Table 1).

The WAKO™ β-glucan test shows high sensitivity and specificity for the diagnosis of Candida deep mycosis and is less labor intensive, therefore being suitable for laboratory use. In fact, the β-glucan test reflected the spread of Candidiasis in a typical case with definite Candida deep mycosis (Fig.1). The WAKO™ β-glucan test may become a new standard for measuring plasma β-glucan levels in the diagnosis of Candida deep mycosis.

Acknowledgment

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References

新しい血漿 (1→3)-β-D-グルカン測定キットのカンジダ深在性真菌症の
臨床診断における有用性の評価：血清検査との比較

大阪市立大学医学部臨床検査医学
日吉 基文  田川 進一  橋本 卯巳
阪本 親彦  異 典之

要 旨
新しく開発されたβ-グルカンテストワロー |血
漿中の (1→3)-β-D-グルカン濃度を測定するキッ
ト| のカンジダ深在性真菌症の臨床診断における
有用性を検討した。β-グルカンテストワローはカ
ンジテックの結果と比較された。β-グルカンテス
トワローとカンジテックは造血器悪性腫瘍、固形
癌などの重症疾患を患っている日和見患者 62 人
から 212 の検体に採取された 212 検体の血漿につ
いて行われた。感度、特異度はβ-グルカンテスト
がそれぞれ 84.8, 85.9%，カンジテックがそれぞれ
60.9, 80.0% だった。