Cryptococcal Antigen Test Revisited: Significance for Cryptococcal Meningitis Therapy Monitoring in a Tertiary Chinese Hospital

Hongzou Lu,1,2* Yingjie Zhou,1 Youkuan Yin,1 Xiaozhang Pan,1 and Xinhua Weng1

Department of Infectious Diseases, Fudan University Huashan Hospital, Shanghai 200040,1 and Shanghai Public Health Center, Shanghai 201508,2 People’s Republic of China

Received 26 November 2004/Returned for modification 4 January 2005/Accepted 7 February 2005

For a total of 29 non-human immunodeficiency virus 1 cryptococcal meningitis cases, titer changes in the latex agglutination test before and after therapy were reviewed along with clinical manifestations, laboratory findings, and therapy regimens. The cryptococcal antigen titer decreased for every case after therapy and was correlated to fungal clearance as defined by fungus smear and/or culture. However, cryptococcal antigen can remain at low titers for long periods of time after therapy, even when fungus smears and/or cultures become negative.

The incidence of fungal meningitis, especially cryptococcal meningitis, has increased in recent years (3). A number of factors have contributed to this increase; these include the increasing prevalence of AIDS, the use of corticosteroids in autoimmune diseases, the use of radio- and chemotherapy for cancer therapy, and the long-term use of immunosuppressants after organ transplantation. Cryptococcal meningitis is still the most common fungal meningitis seen (3, 8), and the cryptococcal antigen is widely recognized as a valuable diagnostic tool in such cases (2). This study therefore reviewed 107 cases of cryptococcal meningitis in our Huashan Hospital over the past 4 years, and from these 107 cases, 29 cases were selected in which there was documentation of the titration changes in the latex agglutination test before and after therapy. These 29 cases were analyzed to evaluate the significance of latex agglutination testing for cryptococcal antigen in the diagnosis of cryptococcal meningitis and therapy monitoring.

The cases were diagnosed as cryptococcal meningitis according to the case histories, the clinical manifestations, the physical signs, and the laboratory evaluations. The laboratory diagnosis criteria were a positive cerebrospinal fluid (CSF) India ink stain or a positive culture for the organism in addition to a positive latex agglutination test for the cryptococcal capsular antigen. The fungal culture was performed on Sabouraud’s medium at 30°C for 4 weeks. Of the 29 cases, 20 were men and nine were women, with an age distribution from 9 to 57 years. The Latex-Crypto antigen detection system (Immuno Mycologics, Inc., Norman, OK) was used for the qualitative and semiquantitative detection of the capsular polysaccharide antigens of Cryptococcus neoformans in CSF according to the manufacturer’s instructions.

A questionnaire was completed which included (i) past underlying diseases, such as tuberculosis, hepatitis, diabetes, etc., and exposure history to pigeon droppings; (ii) clinical manifestations, i.e., fever, headache, nausea, vomiting, and coma; (iii) therapeutic regimens; and (iv) laboratory findings in CSF including leukocyte count, total protein and chloride concentrations, fungal smear and fungal culture counts, and cryptococcal antigen before and after therapy. Seventeen (58.6%) cases had more than one CSF specimen collected after therapy, and only one lower cryptococcal antigen titer was used for analysis.

Among the 29 cases, 5 (17.2%) had a recent exposure history to pigeon droppings and 13 (44.8%) had underlying diseases (diabetes, 5; systemic lupus erythematosus, 2; chronic hepatitis B, 2; nephrotic syndrome, 1; adult-onset Still’s disease, 1; tuberculosis meningitis, 1; and idiopathic thrombocytopenic purpura, 1). Twenty-five cases presented with fevers over 38°C. All 29 cases were human immunodeficiency virus 1 antibody negative and presented with headache, and 20 (69.0%) also experienced nausea and vomiting. Four cases initially were comatose and unconscious.

Amphotericin B and 5-flucytosine (5-FC) were used in 26 cases. Amphotericin B and fluconazole were used in two cases, and liposomal amphotericin B and 5-FC were used in one case. Intrathecal injections with amphotericin B during the intravenous therapy were used in 18 cases. Nineteen cases received fluconazole and 5-FC orally as sequential therapy after intravenous treatment. Patients were diagnosed and started therapy 10 to 260 (mean ± standard deviation, 49.1 ± 54.0) days after the onset of disease.

Laboratory findings in CSF before and after therapy are listed in Table 1. The cryptococcal antigen latex agglutination tests were positive before therapy, with the titers ranging from 1:80 to 1:5,210. Every case studied showed cryptococcal antigen titer decrease after antifungal therapy; within the cases, 9 (31.0%), 12 (41.3%), 5 (17.2%), and 3 (10.3%) decreased in cryptococcal antigens of one, two, three, and more than three dilutions, respectively. There was a remarkable decrease in cryptococcal antigen titers before and after therapy, which was significantly related to the CSF leukocyte count and glucose, chloride, fungal smear, and fungal culture measurements. The total protein level in CSF was decreased after therapy, but the difference was not statistically significant (Table 1). However, cryptococcal antigens remained detectable after therapy when...
both fungal stain and culture turned negative (Fig. 1). These data suggested that a decrease in cryptococcal antigen titers can be used to monitor the antifungal therapy efficacy but cannot be used as an index of cure.

It is estimated that the incidence of cryptococcosis is 0.15% in normal hosts, whereas it is as high as 6.5% in patients with AIDS (1, 5, 7). The principle of the cryptococcal antigen latex agglutination test is based upon the principle that latex particles, sensitized with high-titered, purified immunoglobulin against cryptococcal polysaccharide antigens, will agglutinate with specimens (serum or CSF) containing the appropriate cryptococcal capsular antigens. Since the clinical manifestations and the results of CSF routine and biochemistry examinations for cryptococcal meningitis are similar to those of tuberculous meningitis, viral meningitis, and atypical purulent meningitis, the diagnosis of cryptococcal meningitis depends on CSF India ink smear, fungal cultures, and the cryptococcal antigen latex agglutination test.

Our patients were diagnosed and started antifungal therapy on an average of 49 days after the onset of disease, and all of them presented with high titers of cryptococcal antigens in CSF. This is not unusual, since the onset of cryptococcal meningitis is chronic, and its clinical manifestations and laboratory findings are similar to those of aseptic meningitis caused by other organisms. Since progressive disease is usually accompanied by increasing antigen titers while declining titers are usually associated with clinical improvement, the cryptococcal antigen latex agglutination test appears to have both diagnostic and prognostic value (2, 4). Inadequate therapy and relapse are usually indicated by stationary or rising titers on sequential specimens. On the other hand, after effective antifungal therapy, the antigen titration declines with the negative results of the India ink smears and cultures. This may be because of the continuous release of capsular polysaccharide antigens from dead Cryptococcus neoformans cells, which are slowly eliminated from CSF (1, 6). Our data demonstrate that the cryptococcal antigens can remain detectable even months following successful therapy, suggesting that the cryptococcal antigen test may not be used as an index of cure. A larger patient population and a longer follow-up are needed to confirm this finding.

Our research shows that the cryptococcal antigen latex agglutination test is highly specific to the diagnosis of cryptococcal meningitis; however, its significance for monitoring therapy is limited. Since our goal of therapy for cryptococcal meningitis is to eradicate the pathogenic fungi from the patients’ bodies, the therapy protocols should be based on symptoms and physical signs and on examination for the organism from CSF. Based on our study, intravenous antifungal therapy should be discontinued and oral fluconazole and 5-FC, as a sequential therapy, should be initiated when the CSF glucose and chloride levels and leukocyte count become normal. Sequential therapy was eventually stopped when the above CSF parameters remained normal for two months.

### REFERENCES


### TABLE 1. Laboratory findings in CSF before and after antifungal therapy

<table>
<thead>
<tr>
<th>Time of findings</th>
<th>Leukocyte (10^9/liter)</th>
<th>Protein (mg/liter)</th>
<th>Glucose (mM/liter)</th>
<th>Chloride (mM/liter)</th>
<th>No. of positive fungal smears (%)</th>
<th>No. of positive fungal cultures (%)</th>
<th>Cryptococcal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before therapy</td>
<td>132.4 ± 228.5</td>
<td>801.6 ± 917.1</td>
<td>1.9 ± 1.2</td>
<td>114.8 ± 6.1</td>
<td>27 (93.1)</td>
<td>20 (69.0)</td>
<td>1,049.1 ± 4.1</td>
</tr>
<tr>
<td>After therapy</td>
<td>12.9 ± 11.2</td>
<td>500.3 ± 339.1</td>
<td>3.4 ± 1.8</td>
<td>123.0 ± 5.2</td>
<td>12 (41.4)</td>
<td>0 (0.0)</td>
<td>238.4 ± 4.4</td>
</tr>
</tbody>
</table>

* Data presented as means ± standard deviations. For leukocytes, glucose, chloride, fungal smears, fungal cultures, and cryptococcal titer, differences in pre- and posttherapy findings were statistically significant.