Inactivated Pronase as the Cause of False-Positive Results of Serum Cryptococcal Antigen Tests

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Four patients who had acquired immunodeficiency syndrome and who were evaluated for headache within a 3-week period had false-positive results of serum cryptococcal antigen tests. This cluster of false-positive test results appeared to be due to inactivation of the pronase vial in the test kit, a cause that has not been reported previously.

Meningitis caused by Cryptococcus neoformans is a common, potentially fatal opportunistic illness among patients with AIDS [1]. Therefore, accurate and rapid diagnosis is of great importance. The widely used latex agglutination test for serum cryptococcal antigen is both sensitive and specific [2]. However, there have been reports of several situations that have led to false-positive test results, including infection with Trichosporon beigeli [3] or Capnocytophaga canimorsus [4], presence of a malignancy [2] or rheumatoid factors [5], and contamination with agar, agarose, or syneresis fluid during laboratory pipetting [6]. Some soaps may also cause false-positive reactions if they are used to wash slides that are to be reused [7].

We describe 4 patients who had false-positive results of a serum cryptococcal antigen latex agglutination test, results that were most likely due to the presence of inactivated pronase in the test kit. All 4 patients had AIDS and had sought medical care for headache during a 3-week period in 1999 (table 1). On examination, no fever, meningism, altered consciousness, or focal neurological abnormalities were found.

To test for early cryptococcal meningitis as a cause of headache, blood samples were submitted for cryptococcal serum antigen testing done with the use of a latex agglutination test (CALAS; Meridian Diagnostics). A positive test result, which was found for all 4 patients, resulted in each patient having to undergo lumbar puncture and also led to hospitalization. Samples of the CSF were clear and did not show relevant abnormalities in all 4 patients. None of the patients had signs of cryptococcal pulmonary disease. Treatment with IV amphotericin B was initiated, but it was discontinued when the negative results of the CSF cryptococcal antigen tests became available. The results of bacterial and fungal stains and of cultures of CSF samples were negative as well.

None of the 4 patients had a chronic infection other than HIV infection or a malignancy. Rheumatoid factors had not been tested. Follow-up of these patients, who did not receive sustained antifungal therapy, occurred up to 1 year later and revealed no evidence for cryptococcal disease (1 patient received intermittent courses of azole antifungal therapy for mucosal candidiasis).

Testing of all 4 patients was done with use of the same kit. The tests were performed according to the instructions provided by the manufacturer. The control latex (latex particles that are coated with rabbit immunoglobulin to rule out non-specific agglutination) showed negative test results for all 4 patients. The tests were performed by 3 different technicians. Neither the kit nor the pronase solution was used after the date of expiration. The pronase solution did not look contaminated or cloudy, but a sample of the solution was not cultured for bacteria.

The serum cryptococcal antigen tests were repeated with use of an EIA antigen capture test (PREMIER EIA; Meridian Diagnostics). For patients 1–3, the initial serum specimen was used, whereas an additional serum specimen was obtained for

Table 1. Results of cryptococcal antigen tests of serum and CSF samples obtained from patients with AIDS who were evaluated for headache during a 3-week period.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Last CD4⁺ cell count, cells/mm³</th>
<th>Results of latex agglutination test performed on serum</th>
<th>Results of EIA performed on CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>+ Titer, ND</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>148</td>
<td>+ Titer, 1:32</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>+ Titer, ND</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>104</td>
<td>+ Titer, 1:32</td>
<td>−</td>
</tr>
</tbody>
</table>

**NOTE.** ND, not done; +, positive result; −, negative result.

a Tested at Denver Health Medical Center.
b Tested at University Hospital, Denver.
patient 4. The results of all 4 repeated cryptococcal antigen tests were negative. In addition, we submitted patient 1’s specimen to Meridian Diagnostics for retesting. The test result was positive when testing was done with the use of the same vial of pronase that was used in the initial test, but it was negative when testing was done with a different vial of pronase obtained from a kit with the same lot number.

We concluded that the results of these serum cryptococcal antigen tests were false positive on the basis of the clinical course of disease in the patients, the negative CSF findings, and the negative results caused by repetition of the tests with use of a different testing system. The fact that the result for the control latex was negative in all 4 patients confirms the limited ability of internal controls to detect false-positive test results, as was suggested by Sachs et al. [8]. Moreover, the specimens for which titers of serum cryptococcal antigen were evaluated had relatively low titers (1:32). Low titers have been reported to be characteristic of false-positive test results [9].

Pretreatment of serum samples with pronase, a proteolytic enzyme, reduces the number of false-positive test results by eliminating nonspecific interference with macroglobulins, such as rheumatoid factors, as well as other unknown factors [10]. In contrast to their presence in serum, interfering factors are virtually absent in the CSF [2]. CSF specimens, therefore, do not require pronase treatment when the latex agglutination test from Meridian Diagnostics is used.

Both the discrepancy between results of serum and CSF tests and the negative test result for patient 1 (which occurred when the serum cryptococcal antigen test was repeated with use of a pronase vial obtained from a different kit) highly suggest that inactivated pronase was the cause of the false-positive test results. Responsible mechanisms could include inappropriate storage or contamination of the pronase vial.

To our knowledge, a temporal cluster of false-positive cryptococcal antigen test results has not been reported previously. Its initial presentation was suggestive of a small outbreak and led to unnecessary hospitalization of these patients. Physicians are therefore well advised to interpret results of serum cryptococcal antigen tests with caution, especially those for patients with a low clinical suspicion for cryptococcal disease and with low titers for results of serum cryptococcal antigen tests.

References