Rheumatoid Factor: a Cause of False Positive Histoplasmin Latex Agglutination

R. W. OXENHANDLER,* E. H. ADELSTEIN, AND W. A. ROGERS

Department of Pathology, University of Missouri, School of Medicine, Columbia, Missouri 65201

Received for publication 23 August 1976

Ten of thirteen patients with positive histolatex agglutination titers of 1:32 or greater had no evidence of acute histoplasmosis. Three of these false positives had rheumatoid arthritis. A fourth had a rising mycoplasma complement fixation titer, and the fifth had a high titer of cold agglutinins. All of these are associated with abnormal immunoglobulin M production. To evaluate the role of rheumatoid factor in producing false positive histolatex agglutination, the histolatex test was performed on sera from 32 patients having rheumatoid factor at a titer of 1:40 or greater. Four of these sera agglutinated the histoplasmin-coated latex particles at titers of 1:32 or greater. Review of clinical records suggests that this reactivity is nonspecific. It is our purpose to call attention to rheumatoid factor as a cause of false positive histolatex agglutination.

Bennett (1), in 1966, suggested that the latex agglutination test for the diagnosis of acute histoplasmosis was “accurate and outstanding.” Clinical pathologists have come to regard titers of 1:32 or greater as strong evidence of active or very recent disease. There is very little information in the literature regarding disorders responsible for false positive latex agglutination reactions. However, rheumatoid factor has been reported to interfere with the complement fixation test for histoplasmosis (3), as well as with the slide latex test for cryptococcal antigen (2).

The purpose of this report is to call attention to rheumatoid factor as a cause of false positive histolatex agglutination.

MATERIALS AND METHODS

Rheumatoid factor was determined by a modification of a method by Singer and Plotz (4) (Hyland Laboratory, Div. of Travenol Lab, Costa Mesa, Calif.). Serum specimens were inactivated at 56°C for 30 min. Latex particles coated with altered immunoglobulin G (IgG) were incubated with inactivated sera for 15 min at 37°C. The tubes were then centrifuged at 2,500 rpm for 1 min. The titer of the specimen was reported as the highest dilution showing definite agglutination.

Histolatex agglutination tests were performed using reagents supplied by Inolox Corp., Glenwood, Ill. Heat-killed, mycelial-phase antigens of Histoplasma capsulatum were coated onto latex particles. The patients’ sera were incubated with this substrate for 2 h at 37°C and then refrigerated overnight at 4°C. The tubes were then centrifuged at 2,500 rpm, with the titer reported as the highest dilution showing a 4+ or 3+ agglutination.

RESULTS

From November 1972 to October 1974, 130 histolatex agglutination tests were performed at the Harry S. Truman Memorial Veterans Administration Hospital. Thirteen patients (10%) had titers of 1:32 or greater. Summaries of these patients, abstracted from their charts, are presented in Table 1. Only 3 of the 13 patients (23%) having titers of 1:32 or greater had probable or proven acute histoplasmosis as defined by clinical course, X-ray, and serological tests. Three of the remaining 10 patients had sero-positive rheumatoid arthritis, one patient had a positive cold agglutinin (1:2,560), and another had a rising mycoplasma complement fixation titer (1:8 to 1:256).

As a result of these findings, 32 patients’ sera having rheumatoid arthritis titers of 1:40 or greater were randomly chosen, and the histolatex test was performed. Seven of these patients had reactive histolatex agglutinations with a titer of 1:16 or greater (Table 2); four of these seven had titers of 1:32 or greater, a range considered highly suggestive of active or recently active disease (1). None of these patients had any clinical or laboratory evidence of active histoplasmosis.

DISCUSSION

Singer and Plotz (4) noted that 11% of sera having high titers of rheumatoid factor produced agglutination of uncoated latex particles. They suggested that the particles nonspecifically became coated with an immunoglobulin fraction to which the rheumatoid factor re-

31
acted, causing agglutination. When we reviewed the cases of the 13 positive histolatex agglutination tests performed during a 2-year period, 10 (77%) false positives were found. One-half of these patients had conditions associated with elevated IgM, rheumatoid arthritis, mycoplasma infection, and cold agglutinins. Furthermore, 7 of 32 patients' sera (22%) with positive rheumatoid factor and no clinical evidence of active histoplasmosis demonstrated reactivity in the latex agglutination test for histoplasmosis.

The result of this study suggests that rheumatoid factor, generally an IgM antibody, may nonspecifically bind and agglutinate latex particles. The elevated cold agglutinin titer in

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histoplasmin skin</th>
<th>Histoplasmin latex agglutination</th>
<th>Histoplasmin complement fixation</th>
<th>Rheumatoid factor</th>
<th>Diagnosis</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>DK</td>
<td>Negative 12/20/73</td>
<td>1/24/73, 1:32</td>
<td>1/28/73, negative 1/15/74, negative</td>
<td>ND</td>
<td>Seizure disorder Prostatic CA</td>
<td>Sputum culture for fungi negative</td>
</tr>
<tr>
<td>DR</td>
<td>Positive 9/12/73</td>
<td>9/14/73, 1:32</td>
<td>9/11/73, negative</td>
<td>ND</td>
<td>Probable acute histoplasmosis</td>
<td>No fungal culture</td>
</tr>
<tr>
<td>EC</td>
<td>ND</td>
<td>6/21/73, 1:32</td>
<td>6/21/73, negative</td>
<td>Negative</td>
<td>Retroperitoneal abscess and pyelonephritis with E. coli sepsis</td>
<td>Abscess grew E. coli; culture negative for fungi</td>
</tr>
<tr>
<td>GH</td>
<td>Positive 10/1/73</td>
<td>10/1/73, 1:32</td>
<td>10/1/73, negative</td>
<td>ND</td>
<td>Mediastinal cyst</td>
<td>Culture of cyst negative for fungi</td>
</tr>
<tr>
<td>TR</td>
<td>ND</td>
<td>8/14/73, 1:32</td>
<td>8/10/73, GW1 1:128 Positive VG2 1:256</td>
<td>1:32</td>
<td>Acute histoplasmosis</td>
<td>Sputum and bone marrow cultures negative for fungi bone marrow had granulomas</td>
</tr>
<tr>
<td>DN</td>
<td>Positive 10/29/73</td>
<td>10/29/73, 1:32</td>
<td>10/29/73, negative</td>
<td>ND</td>
<td>Inactive histoplasmosis</td>
<td>No fungal culture</td>
</tr>
<tr>
<td>JE</td>
<td>ND</td>
<td>11/1/73, 1:128</td>
<td>11/1/73, 1:54 12/4/73, negative 2/5/74, negative</td>
<td>12/3/73, GW1 8 VG 1:64</td>
<td>Acute histoplasmosis</td>
<td>Sputum and liver cultures were negative for fungi Hepatic granulomas were present</td>
</tr>
<tr>
<td>JT</td>
<td>ND</td>
<td>9/27/74, negative</td>
<td>10/6/74, 1:32</td>
<td>Negative</td>
<td>Died of H. influenzae pneumonia</td>
<td>Mycoplasma complement fixation 1:8 to 1:256; fungal cultures negative</td>
</tr>
<tr>
<td>JW</td>
<td>ND</td>
<td>4/27/73, 1:32</td>
<td>ND</td>
<td>ND</td>
<td>Syphilis</td>
<td>Died of gram-negative pneumonia; fungal cultures negative</td>
</tr>
<tr>
<td>EW</td>
<td>ND</td>
<td>11/8/72, negative</td>
<td>11/20/72, 1:32 12/1/72, negative</td>
<td>11/10/72, negative 11/22/72, negative</td>
<td>Congenital heart disease with fever</td>
<td>Cold agglutinins 1:2,560; B.M. and sputum cultures negative for histoplasmosis</td>
</tr>
<tr>
<td>RC</td>
<td>ND</td>
<td>11/10/74, 1:32</td>
<td>1/28/74, negative</td>
<td>1:20</td>
<td>Classic RA</td>
<td>Polyclonal gamopathy; cold agglutinins 1:40; sputum, blood, and bone marrow cultures negative for fungi</td>
</tr>
<tr>
<td>EF</td>
<td>Done after latex</td>
<td>3/6/73, 1:64</td>
<td>3/5/73, negative 3/28/73, negative</td>
<td>Reactive</td>
<td>Classic RA, recurrent pneumonia</td>
<td>Rheumatoid factor not titered No fungal cultures</td>
</tr>
<tr>
<td>CP</td>
<td>ND</td>
<td>3/15/74, 1:64 3/21/74, 1:128</td>
<td>ND</td>
<td>1:5,120</td>
<td>Classic RA</td>
<td>No fungal cultures</td>
</tr>
</tbody>
</table>

* ND, Not done.
* GW and VG, yeast antigens.
* RA, Rheumatoid arthritis.
TABLE 2. *Rheumatoid factor-positive sera: effect on the histoplasmin latex agglutination test*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Rheumatoid factor Titer</th>
<th>Histolatex Titer</th>
<th>Diagnosis and Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:640</td>
<td>1:128</td>
<td>RA</td>
</tr>
<tr>
<td>2</td>
<td>1:640</td>
<td>1:16</td>
<td>RA, congestive heart failure, pulmonary emboli</td>
</tr>
<tr>
<td>3</td>
<td>1:5,120</td>
<td>1:64</td>
<td>RA</td>
</tr>
<tr>
<td>4</td>
<td>1:5,120</td>
<td>1:16</td>
<td>Subacute bacterial endocarditis, secondary to group D Streptococcus</td>
</tr>
<tr>
<td>5</td>
<td>1:5,120</td>
<td>1:64</td>
<td>RA</td>
</tr>
<tr>
<td>6</td>
<td>1:5,120</td>
<td>1:32</td>
<td>RA</td>
</tr>
<tr>
<td>7</td>
<td>1:2,560</td>
<td>1:16</td>
<td>RA</td>
</tr>
</tbody>
</table>

* None of these patients had any clinical evidence of histoplasmosis. Skin tests were not done on these patients, and only patient 2 had a complement fixation test for histoplasmosis performed (which was negative). RA, Rheumatoid arthritis.

one patient and the rising mycoplasma titer in another patient also suggest that cold-reactive IgM antibodies may cause nonspecific histolatex agglutination.

Since cultural proof of active histoplasmosis is difficult and may take a long time, the diagnosis of acute histoplasmosis is made primarily on the clinical presentation and serological studies. The histoplasmin latex agglutination test, which measures primarily IgM antibodies, often gives significant titers early in the course of the disease, in contrast to the slower rising complement fixation titers. Identification of sera containing rheumatoid factor or cold agglutinins as potential causes of false positive histoplasmin latex agglutination should increase the specificity of this test for the early diagnosis of acute histoplasmosis.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Dinah Scearce, Connie Macedo, Beverly Birdsong, and Donna Rodgers.

LITERATURE CITED