

# 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 histol latex 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 histol latex agglutination, the histol latex 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 histol latex 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 histol latex 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.

Histol latex agglutination tests were performed using reagents supplied by Inolex 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 histol latex 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 histol latex test was performed. Seven of these patients had reactive histol latex 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-

TABLE 1. Clinical summary of 13 patients with histolates agglutination titers of 1:32 or greater

Patient	Titer				Diagnosis	Comments
	Histoplasmin skin	Histoplasmin latex agglutination	Histoplasmin complement fixation	Rheumatoid factor		
DK	Negative 12/28/73	1/2/74, 1:32 1/16/74, 1:32	12/28/73, negative 1/15/74, negative	ND <sup>a</sup>	Seizure disorder Prostatic CA	Sputum culture for fungi negative
DR	Positive 9/12/73	9/14/73, 1:32	9/11/73, negative	ND	Probable acute histoplasmosis	No fungal culture
EC	ND	6/21/73, 1:32	6/21/73, negative	Negative	Retroperitoneal abscess and pyelonephritis with <i>E. coli</i> sepsis	Abscess grew <i>E. coli</i> ; culture negative for fungi
GH	Positive 10/1/73	10/1/73, 1:32	10/1/73, negative	ND	Mediastinal cyst	Culture of cyst negative for fungi
TR	ND	8/14/73, 1:32	8/10/73, GW <sup>b</sup> 1:128 Positive VG <sup>c</sup> 1:256	1:32	Acute histoplasmosis	Sputum and bone marrow cultures negative for fungi bone marrow had granulomas
DN	Positive 10/29/73	10/29/73, 1:32	10/29/73, negative	ND	Inactive histoplasmosis	No fungal culture
JE	ND	11/1/73, 1:128 11/13/73, 1:64 12/4/73, negative 2/5/74, negative	12/3/73, GW 1:8 VG 1:64	ND	Acute histoplasmosis	Sputum and liver cultures were negative for fungi Hepatic granulomas were present
JT	ND	9/27/74, negative 10/8/74, 1:32	Negative	ND	Died of <i>H. influenzae</i> pneumonia	Mycoplasma complement fixation 1:8 to 1:256; fungal cultures negative
JW	ND	4/27/73, 1:32	ND	ND	Syphilis	Died of gram-negative pneumonia; fungal cultures negative
EW	ND	11/8/72, negative 11/20/72, 1:32 12/1/72, negative	11/10/72, negative 11/22/72, negative	ND	Congenital heart disease with fever	Cold agglutinins 1:2,560; B.M. and sputum cultures negative for histoplasmosis
RC	ND	11/10/74, 1:32	1/10/74, negative 1/28/74, negative	1:20	Classic RA <sup>c</sup>	Polyclonal gamopathy; cold agglutinins 1:40; sputum, blood, and bone marrow cultures negative for fungi
EF	Done after latex	3/6/73, 1:64	3/5/73, negative 3/28/73, negative	Reactive	Classic RA, recurrent pneumonia	Rheumatoid factor not titered No fungal cultures
CP	ND	3/15/74, 1:64 3/21/74, 1:128	ND	1:5,120	Classic RA	No fungal cultures

<sup>a</sup> ND, Not done.<sup>b</sup> GW and VG, yeast antigens.<sup>c</sup> RA, Rheumatoid arthritis.

acted, causing agglutination. When we reviewed the cases of the 13 positive histolates 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 2. *Rheumatoid factor-positive sera: effect on the histoplasmin latex agglutination test*<sup>a</sup>

Pa- tient	Titer		Diagnosis and comment
	Rheu- matoid factor	Histo- latex	
1	1:640	1:128	RA
2	1:640	1:16	RA, congestive heart failure, pulmonary emboli
3	1:5,120	1:64	RA
4	1:5,120	1:16	Subacute bacterial en- docarditis, second- ary to group D <i>Streptococcus</i>
5	1:5,120	1:64	RA
6	1:5,120	1:32	RA
7	1:2,560	1:16	RA

<sup>a</sup> 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 histol-  
tex agglutination.

Since cultural proof of active histoplasmosis is difficult and may take a long time, the diag-

nosis 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 Searce, Connie Macedo, Beverly Birdsong, and Donna Rodgers.

#### LITERATURE CITED

1. Bennett, D. E. 1966. The histoplasmin latex agglutination test: clinical evaluation and a review of the literature. *Am. J. Med. Sci.* 251:175-183.
2. Gordon, M. A., and E. W. Lapa. 1974. Elimination of rheumatoid factor in the latex test for cryptococcosis. *Am. J. Clin. Pathol.* 60:488-494.
3. Johnson, J. E., and G. D. Roberts. 1976. Blocking effect of rheumatoid factor and cold agglutinins on complement fixation tests for histoplasmosis. *J. Clin. Microbiol.* 3:157-160.
4. Singer, J. M., and C. M. Plotz. 1956. The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med.* 21:888-892.