JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1996, p. 2014–2016 Vol. 34, No. 8
0095-1137/96/$04.00+0

Efficacy of Immunoglobulin M Serodiagnostic Test for Rapid Diagnosis of Acute Babesiosis

PETER J. KRAUSE,[1]* RAYMOND RYAN,[2] SAM TELFORD III,[3] DAVID PERSING,[4] AND ANDREW SPIELMAN[3]

Division of Infectious Diseases, Department of Pediatrics,[1] and Department of Laboratory Medicine,[2] University of Connecticut School of Medicine, Farmington, Connecticut; Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts[3]; and Department of Medicine and Pathology, Mayo Clinic, Rochester, Minnesota[4]

Received 19 January 1996/Returned for modification 5 March 1996/Accepted 9 May 1996

To evaluate the efficacy of an immunoglobulin M indirect immunofluorescent-antibody procedure for diagnosing acute babesiosis, we tested patients with acute babesiosis from a site in New England where the disease is enzootic. The sensitivity of the test was 91%, the specificity was 99%, the positive predictive value was 86%, and the negative predictive value was 99%. This *B. microti* immunoglobulin M indirect immunofluorescent-antibody procedure is sufficiently sensitive, specific, and reproducible for use in the routine clinical diagnosis of acute babesiosis.

Acute human babesiosis, which is caused by the malaria-like piroplasm *Babesia microti*, often defies diagnosis early in the course of illness because of the absence of pathognomonic clinical findings and the generally sparse nature of the developing parasitemia (2, 12). A recently described indirect immunofluorescent-antibody (IFA) test can usefully aid in the diagnosis of babesiosis late in the course of the illness after demonstration of a fourfold seroconversion between acute- and convalescent-phase sera (3). Although the immunoglobulin M (IgM) IFA test may be suitable for rapid diagnosis, it has not yet been evaluated systematically. Such an evaluation can be performed most effectively with patients with clinically documented cases of infection and with a subject population that is exposed to this tick-borne zoonosis. Accordingly, we conducted a blinded study of the sensitivity, specificity, and reproducibility of the babesial IgM IFA test with patients with babesiosis and a group of asymptomatic residents from a region in southern New England where the disease is enzootic.

Blood samples were obtained from 921 Block Island, R.I., residents (511 females and 410 males; mean age, 51 ± 19 years) who participated in a yearly serological survey for the detection of *B. microti* antibody and from 169 Block Island and southeastern Connecticut residents (83 females and 86 males; mean age, 44 ± 21 years) who were evaluated for possible babesial infection from 1992 to 1994. For each clinically ill subject, a history was obtained and a physical examination and a battery of laboratory tests were performed. Informed consent was obtained from each study participant. The study was carried out in accordance with the guidelines of the Human Subjects Committee of the Harvard School of Public Health. We sought evidence of parasitemia by examining Giemsa-stained blood smears at ×1,000 magnification and by PCR amplification of parasite DNA. Serological testing included IFA tests for IgG and IgM antibodies. A subject was considered to have acute babesial infection if piroplasm DNA was amplified by PCR or a fourfold rise in antipiroplasm IgG antibody was detected.

Antigen for the IFA test was derived from the GI strain of *B. microti* that was maintained in continuous passage in female golden hamsters by monthly intraperitoneal inoculation, alternating with a passage through tick vectors every 4 months (3). When at least 40% of the erythrocytes were parasitized, 1 ml of blood was collected in a heparinized syringe by cardiac puncture following halothane anesthesia. The blood was centrifuged at 500 × *g* for 5 min, the plasma was removed, and the packed cells were diluted in 10 ml of phosphate-buffered sterile saline (PBS; pH 7.6). The cells were washed three times in PBS and were resuspended in PBS so that thick smears contained 100 to 500 piroplasms per field (×400). A 5-μl drop of cell suspension was added to each well of a 12-well glass IFA slide (Cel-Line Associates, Inc., Newfield, N.J.), permitted to air dry at 23°C, fixed in chilled acetone, dated, and stored in a desiccator at 4°C until use. Such slides were used within 3 months of preparation. For IFA testing, slides were permitted to come to room temperature. Test sera were diluted 1:32 in PBS, and 20 μl of the dilution was added to a slide well. Each slide was incubated for 30 min at 37°C, washed three times with agitation in PBS, and allowed to air dry. A 20-μl drop of goat anti-human IgG or IgM immunoglobulin labeled with fluorescein isothiocyanate (Kirkegaard & Perry, Gathersburg, Md.) diluted in PBS and Evans Blue (final concentration, 0.0005%) was added to each well, and the contents of the wells were permitted to incubate for 30 min at 37°C. The slide was again washed three times with agitation in PBS, air dried, mounted with buffered glycerin (pH 9.0), and covered with a coverslip. A Leitz fluorescence microscope with a ×100 water immersion objective and ×10 oculars (for a total magnification of ×1,000) was used to examine the test slides. The controls used with each run included serum from a patient with babesiosis confirmed with a blood smear (positive control), serum from a healthy subject (negative control), and PBS. Reactions were graded on a scale of 0 to 4+. All specimens with 1+ to 4+ reactions were retested at dilutions of 1:16 and 1:32. A positive serum was defined as one reacting at 1:32. The titers of positive specimens were determined to the endpoint.

For the babesial PCR assay, whole-blood samples were analyzed and processed by personnel blinded to the clinical status of the donor, as described previously (8, 10). A 238-bp portion of the *B. microti* nuclear small-subunit ribosomal gene was

* Corresponding author. Mailing address: Department of Pediatrics, University of Connecticut School of Medicine, Farmington, CT 06030. Phone: (860) 545-9494. Fax: (860) 545-9371.

TABLE 1. Indirect fluorescent-antibody test reactivity of the IgM fraction of sera from babesiosis patients and residents of a zoonotic site against *B. microti* antigen

Subject	Subjects with babesial infection[a]			
	Present		Absent	
	No.	% IgM positive	No.	% IgM positive
Acute illness	38	87	130	2
Serological survey	17	100	899	0.6
Total	55	91	1,029	0.8

[a] Babesial infection was determined by a fourfold IgG antibody rise or the presence of parasite DNA. Reaction of a serum sample at a dilution of 1:32 or greater was interpreted as evidence of prior infection.

targeted for amplification by a PCR protocol described previously, except that the volume of blood analyzed was 0.5 ml rather than 0.2 ml (8, 10). The control samples used with each amplification assay included three blank control samples with 5 μl of water substituted for DNA and a positive control sample with 60 pg of total genomic DNA extracted from a hamster with 10% *B. microti* parasitemia. Because amplification products were rarely seen on ethidium bromide-stained gels, samples were considered reactive on the basis of signal detection after hybridization with a radiolabeled or chemiluminescent internal oligonucleotide probe. Barrier-filtered pipette tips and a dedicated set of pipettors were used to prepare all samples. Isopsoralen sterilization was used routinely, and rigorous precautions were used to prevent amplification product contamination.

We compared the IgM seroreactivities of the clinically ill, parasitemic patients with those of the apparently asymptomatic residents of the site where the disease is enzootic. *B. microti* IgM antibody was detectable in 91% (50 of 55) of those with acute babesial infection and was absent from 99% (1,021 of 1,029) of those without infection (Table 1). Twenty-eight of the 50 subjects with *B. microti* IgM antibodies had detectable *B. microti* DNA in their blood; 17 of the 28 also had a fourfold rise in *B. microti* IgG antibody titers in acute- and convalescent-phase sera, while 11 had *B. microti* IgG antibodies, but early serum samples from these subjects for the detection of a fourfold rise in antibody were lacking. Seventeen *B. microti* IgM-positive subjects had no detectable *B. microti* DNA, and the *B. microti* PCR test was not performed with samples from 5 subjects; all 22 of these subjects had a fourfold rise in *B. microti* IgG antibody titers. The characteristics of the *B. microti* IgM antibody test by using titers of 1:32 or 1:64 as positive cutoff values were as follows: sensitivities 91 and 89%, respectively; specificities, 99 and 99%, respectively; positive predictive values, 86 and 88% respectively; negative predictive values, 99 and 99%, respectively.

The need for rapid and reliable diagnostic testing for babesiosis is increasing because of the rising incidence of the disease in the northeastern United States and its emergence in new sites in the Midwest and far west (1, 4, 5, 7, 9, 11). Conventional laboratory diagnosis of acute babesiosis relies mainly on the discovery of parasites in stained smears of peripheral blood (2, 6, 12). This technique is limited, however, because so few erythrocytes are infected early in the course of the illness. Although the presence of babesial IgG antibody in convalescent-phase serum is useful in the confirmation of the diagnosis, IgG appears only late in the infection, after the acute phase of the illness has run its course.

Serodiagnosis of *B. microti* infection depends on fluorescent-antibody testing; no enzyme-linked immunosorbent assay (ELISA) or immunoblot procedure has yet become available. The IFA technique described here is readily established in conventional diagnostic laboratories for IgM and IgG testing. Experienced microscopists generally derive reproducible results with this test, even in the absence of previous experience with human babesiosis. Subjectivity may be introduced in the determination of the serum dilution used as the diagnostic cutoff value, and we have adopted an IgM antibody titer of 1:32 for that purpose. Each laboratory that uses such a test should derive its own cutoff value on the basis of a series of specimens from patients with defined cases of babesiosis, as well as specimens from patients without disease. These values may vary from laboratory to laboratory.

Serological cross-reactivity with the Lyme disease spirochete or *Ehrlichia* species is unlikely because of the dissimilarity of these microorganisms. No cross-reactivity has been reported previously, and we have found no evidence of such a reaction. Of the 55 subjects whose sera reacted to *B. microti* antigen in the present study, 48 were nonresponsive to *Borrelia burgdorferi* antigen by either ELISA and Western blot (immunoblot) testing. The remaining seven subjects had strong evidence of concurrent illness with babesiosis and Lyme disease. All seven of these patients had a fourfold rise in *B. burgdorferi*-specific antibody titers, three were *B. burgdorferi* PCR positive, and three had erythema migrans rash. Thus, there was no evidence of serologic cross-reactivity among our study participants.

Our series of tests with numerous babesia-infected as well as apparently uninfected subjects provides reasonable assurance of the usefulness of the IgM diagnostic procedure. An otherwise undetectable parasitemia also may be detected by PCR amplification of single-stranded RNA gene sequences with *B. microti*-specific primers. The PCR technique remains available at only a few research and commercial laboratories (8, 10). We conclude that IgM IFA testing appears to provide a reliable method for diagnosing acute human babesiosis when parasites cannot be seen in peripheral blood.

We thank the following people for their kind assistance: T. V. Rajan, Peter Brassard, Diane Christianson, Linda Closter, Betty Fitzpatrick, Nancy Greenaway, Doris A. Ware, Georgine Burke, and members of the Block Island Residents Association.

This study was supported in part by Public Health Service grants AI32403 and AR41497 from the National Institutes of Health.

REFERENCES

1. **Dammin, G. J., A. Spielman, J. L. Benach, and J. Piesman.** 1981. The rising incidence of clinical *Babesia microti* infection. Hum. Pathol. **12:**398–400.
2. **Krause, P. J.** 1992. Babesiosis, p. 2010–2016. *In* R. Feigin and J. D. Cherry (ed.), Textbook of pediatric infectious diseases. The W. B. Saunders Co., Philadelphia.
3. **Krause, P. J., S. R. Telford, R. Ryan, P. A. Conrad, M. Wilson, J. W. Thomford, and A. Spielman.** 1994. Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. J. Infect. Dis. **169:**923–926.
4. **Krause, P. J., S. R. Telford, R. Ryan, A. Hurta, I. Kwasnik, S. Luger, J. Niederman, M. Gerber, and A. Spielman.** 1991. Geographical and temporal distribution of babesiosis in Connecticut. J. Clin. Microbiol. **29:**1–4.
5. **Krause, P. J., S. R. Telford, A. Spielman, V. J. Sikand, R. Ryan, D. Christianson, P. Brassard, R. Pollack, J. Peck, G. Burke, and D. H. Persing.** 1996. Concurrent Lyme disease and Babesiosis: Evidence for increased severity and duration of illness. JAMA **275:**1657–1660.
6. **Mattia, A. R., M. A. Waldron, and L. S. Sierra.** 1993. Use of the quantitative buffy coat system for detection of parasitemia in patients with babesiosis. J. Clin. Microbiol. **31:**2816–2818.
7. **Meldrum, S. C., G. S. Birkhead, D. J. White, J. L. Benach, and D. L. Morse.** 1992. Human babesiosis in New York State: an epidemiological description of 136 cases. Clin. Infect. Dis. **15:**1019–1023.
8. **Persing, D. H.** 1993. PCR detection of *Babesia microti*, p. 475–479. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
9. **Persing, D. H., B. L. Herwaldt, C. Glaser, R. S. Lane, J. W. Thomford, D.**

Mathiesen, P. J. Krause, D. F. Phillip, and P. A. Conrad. 1995. Human piroplasmosis in the western United States: molecular characterization of the agent and seroprevalence studies. N. Engl. J. Med. **332:**298–303.

10. **Persing, D. H., D. Mathiesen, W. F. Marshall, S. R. Telford, A. Spielman, J. W. Thomford, and P. A. Conrad.** 1992. Detection of *Babesia microti* by polymerase chain reaction. J. Clin. Microbiol. **30:**2097–2103.
11. **Steketee, R. W., M. R. Eckman, E. C. Burgess, J. N. Kuritsky, J. Dickerson, W. L. Schell, M. S. Godsey, and J. P. Davis.** 1985. Babesiosis in Wisconsin: a new focus of disease transmission. JAMA **253:**2675–2678.
12. **Telford, S. R., A. Gorenflot, P. Brasseur, and A. Spielman.** 1993. Babesial infections in man and wildlife, p. 1–47. *In* J. P. Kreier and J. R. Baker (ed.), Parasitic protozoa, vol 5, 2nd ed. Academic Press, Inc., New York.