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Evaluation of Serological Assays for Identification of Parvovirus B19 Immunoglobulin M

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Three different enzyme immunoassays (EIAs) (Parvoscan-B19, IBL parvovirus B19, and IDEIA parvovirus B19) and one immunofluorescence assay (Biotrin Parvo B19 IFA) were evaluated for detection of parvovirus B19 immunoglobulin M (IgM) antibodies in 203 clinical serum samples. An IgM antibody capture radioimmunoassay was used as a reference test. Serum specimens obtained from patients with clinical symptoms suggestive of parvovirus B19 infections were used to evaluate the sensitivities of the assays, which were shown to be comparable for the Biotrin IFA and IDEIA (97%) and lower for the other two EIAs (90%). In order to test the specificity of the assays, clinical serum samples with IgM antibodies against other viruses were examined, as well as sera with rheumatoid factor activity and sera from healthy pregnant women. The specificities of B19 IgM antibody detection were 96% for the Biotrin IFA, 96% for IDEIA, 90% for Parvoscan, and 88% for the IBL assay. These results show that all four assays can be recommended for diagnostic purposes, although false-positive results may be seen with other acute viral infections, healthy pregnant women, and rheumatoid factor-positive samples.

Human parvovirus B19 is the infectious agent of erythema infectiosum (fifth disease), a mild influenza-like disease in children (4). However, in certain cases, B19 infections may lead to life-threatening complications, such as severe aplastic crisis in patients with underlying hemolytic disorders (21), intrauterine infection with hydrops fetalis and fetal loss (7), and infections with severe anemia in immunocompromised individuals (17).

The symptoms associated with B19 infection may also be similar to other clinical conditions occurring in children and adults and during pregnancy. Examples of differential diagnoses are scarlet fever, rheumatoid arthritis, and infections caused by rubella virus. To definitively distinguish these infections, the sensitivity and specificity of B19 diagnostic tests are thus extremely important.

The first assay for specific immunoglobulin M (IgM) for diagnosis of B19 infection was described by Anderson et al. (2). In 1983, Cohen et al. (10) described a B19 IgM antibody capture radioimmunoassay (MACRIA). This assay is still considered the reference test. A few years later, an enzyme-linked immunosorbent assay (ELISA) utilizing monoclonal antibodies to B19 was established (1). The limitation for B19 antibody assays is the supply of B19 antigen, which for the MACRIA is prepared from sera of B19 viremic patients. The virus has as yet not been propagated in vitro in sufficient amounts to be used as antigens in diagnostic tests. Alternative strategies to obtain antigens for diagnostic tests include production of empty capsids of B19 virus in a genetically engineered cell line (15) and use of B19 capsids synthesized in a baculovirus expression system (6, 23). Already in 1983, Cohen et al. (10) reported nonspecific reactivity in the B19 MACRIA with sera containing IgM against hepatitis A virus, hepatitis B virus, and cytomegalovirus (CMV) as well as with rheumatoid factor (RF)-positive sera. Subsequent reports have shown various degrees of nonspecific reactivities in different B19 IgM tests, probably depending on the selection of serum samples (8, 14). In the present study, we have evaluated the sensitivity and specificity of some of the commercially available anti-B19 IgM tests with a defined panel of sera representing patients with several conditions that may cause difficulty in differential diagnosis.

MATERIALS AND METHODS

Patients and serum samples. The sera included in this study were sent to the Swedish Institute for Infectious Disease Control (formerly the National Bacteriological Laboratory), Stockholm, Sweden, for viral diagnostic purposes during 1987 to 1994. A total of 203 samples were investigated; 48 serum samples from patients without clinical B19 infection were selected for the presence of IgM antibodies to other viral agents (enteroviruses, herpes simplex virus [HSV], Epstein-Barr virus [EBV], CMV, and rubella virus), and 100 serum samples were from patients with clinical symptoms suggestive of B19 infection (with or without B19 IgG and RF). An additional group of 25 specimens was selected for the presence of B19 IgM antibodies as detected by the MACRIA. In the latter group, all patients presented with rash, fever, and/or arthropathies except for one, who had a hereditary spherocytosis with acute anemia. These 25 patients were between 4 and 50 years old (median = 37 years) and had been symptomatic for 3 to 30 days (median = 8 days). For six of the patients, the duration of symptoms was not known.

In addition, 10 serum specimens were selected for the presence of high serum RF titers (Tinaquant RF; Boehringer-Mannheim) without indication of active viral infection and were kindly supplied by Calab Medical Laboratory, Stockholm, Sweden. Serum samples from 20 healthy pregnant women were kindly supplied by the Department of Gynecology and Obstetrics, Danderyds Hospital, Danderyd, Sweden. All serum specimens were stored at −20°C until used.

Assays for detection of IgM directed against other viral agents. IgM antibodies against enterovirus were detected by a solid-phase reverse immunosorbent test (19). The presence of IgM antibodies to EBV was detected by published methods (18). IgM antibodies to HSV, CMV, and rubella virus were detected by in-house indirect enzyme immunoassays (EIAs). One of the nine initially tested rubella IgM-positive samples was excluded from the study since it was B19 IgM positive in all four assays as well as in the MACRIA. It has not been possible to retrospectively establish whether this sample represents a B19 or rubella virus infection.

Assays for IgG and IgM to B19. An immunofluorescence assay (IFA) was performed with a Biotrin Parvo B19 kit (Biotrin International Ltd., Dublin, Ireland) according to the procedure specified by the manufacturer. The assay is an indirect immunofluorescence test using recombinant VP1 antigen expressed in an insect cell line. The samples were pretreated with RF absorbent (Behringwerke AG, Marburg, Germany), as recommended by the manufacturer, to avoid false-positive IgM results due to the presence of RF and to prevent competition by IgG antibodies. Positive and negative controls were included on each slide.

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TABLE 1. Results of testing for the presence of parvovirus B19-specific IgM antibody in different serological assays

Serum sample type or source	Total no. tested	No. of patients with the indicated result as determined by[a]:									
		Parvoscan		IDEIA		IBL		Biotrin IFA		MACRIA	
		Pos.	Eq.	Pos.	Eq.	Pos.	Eq.	Pos.	Eq.	Pos.	Eq.
B19 IgM positive											
Group A[b]	25	24	1	25	0	23	2	25	0	25	0
Group B[c]	5	3	1	4	0	4	1	4	0	5	0
Positive for other viral infection											
Enterovirus	10	0	0	0	0	0	0	0	0		
HSV	10	1	0	1	0	3	1	0	0		
CMV	10	4	2	0	0	7	0	0	0		
EBV	10	3	0	0	0	3	3	1[d]	0		
Rubella virus	8	3	0	0	0	0	0	0	0		
Other control groups											
RF positive	10	0	1	1	0	0	0	0	0		
Healthy pregnant women	20	1	2	0	0	1	0	0	0		
False positive		12	5	2	0	14	4	1	0		
With clinically suspected B19 infection[e]	100	5	1	5	1	8	2	6	0		

[a] Pos., positive result; eq., equivocal result. From these results, sensitivities of 90, 97, 90, and 97%, respectively, were determined for Parvoscan, IDEIA, IBL, and Biotrin IFA (MACRIA, 100%). Specificities for these assays were 90, 96, 88, and 96%, respectively.

[b] A total of 25 clinical serum samples were submitted for diagnosis of B19 infection and found positive by the MACRIA.

[c] These serum samples are the same samples as described in Table 2 (B19 IgM positive by MACRIA).

[d] This sample was negative by MACRIA. The remaining samples representing other viral infections, RF-positive patients, and healthy pregnant women were not tested by MACRIA.

[e] Details presented in Table 2.

Although the definition of positive and negative results is described by the manufacturer, we found borderline results despite repeated testing, and these are presented as "equivocal."

Three different EIA serological kits were also compared for the detection of B19-specific IgM antibodies: Parvoscan-B19 (EuroDiagnostica, Malmö, Sweden), IBL parvovirus B19 IgM ELISA (Gesellschaft für Immunchemie und Immunbiologie MBH, Hamburg, Germany), and IDEIA parvovirus B19 IgM (DAKO A/S, Copenhagen, Denmark). The Parvoscan assay is an indirect EIA with a B19-specific synthetic peptide used as an antigen corresponding to parts of the parvovirus structural proteins VP1 and VP2. The IBL assay is also an indirect EIA but uses the recombinant structural protein VP2 of B19 as an antigen. IDEIA is based on the antibody capture technique using empty B19 viral capsids as antigens which are produced in insect cells infected with recombinant baculovirus encoding VP2.

All assays were performed according to the manufacturers' advice. Tests giving borderline results were repeated. The IgM-positive patient sera were retested after pretreatment with RF absorbent for Parvoscan and the RF absorbent included in the IBL kit. Results before pretreatment are not presented. In the IDEIA, using RF absorbent was advised against, and therefore the sera were not pretreated before being tested. Results were interpreted according to the specifications of each assay. The MACRIA was performed at the Virus Reference Center, Public Health Laboratory Service, London, England (10).

For detection of IgG, the Parvoscreen B19 IgG EIA (EuroDiagnostica) was used according to the recommendations of the manufacturer.

Calculations. Equivocal results were counted as negative when sensitivities and specificities were calculated. Sensitivities were calculated by using the MACRIA as a reference test. The sensitivity (percent) was calculated as [positives in test/true positives (MACRIA)] $\times$ 100. The specificity (percent) was calculated as [negatives in test/true negatives (MACRIA)] $\times$ 100. The samples evaluated for specificity were considered true negative when they scored negative in all four B19 IgM assays. If the samples representing clinically suspected B19 infection scored positive in at least one of the assays, they were tested by the MACRIA.

RESULTS

Sensitivity of B19 IgM antibody assays. The first group of 25 serum samples evaluated for sensitivity was selected for the presence of B19 IgM antibodies in the MACRIA (Table 1), and the patients are described in detail in Materials and Methods. When these samples were tested in the other assays, B19 IgM was detected in all samples, with the following exceptions. One serum specimen gave only an equivocal result by the Parvoscan assay and was derived from an 11-year-old boy with a 4-day history of fever and rash. Specimens from two other patients, a 15-year-old boy with a 4-day history of fever and rash and a 24-year-old woman with similar symptoms of unknown duration, were initially positive in the IBL assay but gave equivocal results after the recommended RF absorption.

The sensitivity was further evaluated by testing all 100 consecutive samples sent to our laboratory for B19 antibody testing from May to November 1994 (non-epidemic season) (Table 1). The samples represented patients with clinically suspected B19 infection. In this group, 3 serum specimens were B19 IgM positive in all five assays (including MACRIA), 9 specimens gave discrepant results, and 88 specimens were B19 IgM negative in all assays. All serum samples giving positive or discrepant results were tested by MACRIA. Five of these 100 serum samples could thus be confirmed as IgM positive by MACRIA and were included in Table 1 for calculation of sensitivity, which thus ranged from 90% (Parvoscan and IBL) to 97% (IDEIA and Biotrin IFA).

Specificity of B19 IgM antibody assays. The specificity of the B19 IgM tests was evaluated by testing 48 serum samples selected for the presence of IgM antibodies directed to either enterovirus, HSV, CMV, EBV, or rubella virus (Table 1). Three different B19 IgM EIAs (Parvoscan, IDEIA, and IBL) and one B19 IgM IFA (Biotrin) were compared. Some of the serum samples with either HSV-, CMV-, EBV-, or rubella virus-specific IgM present yielded false-positive results in the B19 IgM EIAs. Of these serum specimens, one was also false positive in the Biotrin IFA. This patient had symptoms and laboratory findings indicating EBV infection, and the serum was B19 IgM negative in the MACRIA.

The specificity of the assays was further evaluated by testing 10 serum samples containing RF which often yields false-positive results in IgM assays. The instructions for three assays (Parvoscan, IBL, and Biotrin IFA) recommend pretreatment

TABLE 2. Analysis of results determined by five assays for parvovirus B19 IgM in clinically suspected B19 infection[a]

Serum sample code and group	Result by:					Time after onset	Age/sex[b]	Clinical data[c]
	Parvoscan	IDEIA	IBL	Biotrin IFA	MACRIA			
Positive								
130	+	+	+	+	+	Not known	48/M	Erythema infectiosum
138	+	+	+	+	+	8 days	38/F	Arthralgia; rash; fever
139	+	+	+	+	+	15 days	50/M	Arthralgia; rash; fever
Discrepant								
116	−	−	+/−	−	+	4 days	2/M	URTI; rash
119	+/−	+	+	+	+	4 days	16/F	URTI; rash
120	−	−	+	−	−	Months	18/F	Arthritis
122	−	−	−	+	−	Years	31/F	Rheumatoid arthritis
123	+	+	+	+	−	4 mo	58/M	CLL; anemia since 4 mo
142	−	+/−	−	−	−	Not known	66/F	Not known
162	−	−	+/−	−	−	8 mo	39/F	Convalescent-phase serum; intrauterine infection
172	+	−	+	−	−	7 days	5/M	Arthritis
207	−	−	+	−	−	Years	34/F	Rheumatoid arthritis

[a] A total of 100 serum samples with suspected B19 infection were submitted from different parts of Sweden from May to November 1994 and tested for parvovirus B19 IgM. The samples showing positive or discrepant results in the different assays were further tested in the MACRIA. Among these 100 patients, 88 were negative for B19 IgM in all assays (data not shown). All samples for which results are shown here were positive for B19 IgG.

[b] M, male; F, female.

[c] Patient 116 had an upper respiratory tract infection (URTI) and bilateral rash on cheeks initially which later spread to the trunk and limbs. Patient 123 had chronic lymphatic leukemia (CLL); two earlier serum samples (1 and 2 months after onset of acute anemia) were IgM negative by the Biotrin IFA and MACRIA and B19 DNA negative by PCR, and the three consecutive samples had a nonsignificant change in IgG titer (titers, 1:100, 1:130, and 1:150, respectively). Patient 162 had an intrauterine death; two earlier maternal serum samples (0 and 4 months after intrauterine death) were IgM negative as determined by the Biotrin IFA and MACRIA.

of sera with RF absorbent before evaluating a positive IgM reaction. The results in this study are consequently based on this recommendation. However, despite pretreatment with RF absorbent, one serum sample still gave an equivocal result in the Parvoscan assay. In the instructions to IDEIA, the use of RF absorbent is advised against. Following these instructions, we found one false-positive result with the RF-positive group of sera.

Serum specimens from a group of 20 pregnant women with no clinical signs or symptoms of viral disease were also tested in the B19 IgM assays. All samples were negative as determined by Biotrin IFA and IDEIA, whereas some false-positive results were noted in the other EIAs.

The group of 100 serum samples from patients with clinically suspected B19 infection (as described above) was also included to evaluate specificity. Taken together, all these serum samples gave specificities of 96% for Biotrin IFA, 96% for IDEIA, 90% for the Parvoscan assay, and 88% for the IBL assay.

The positive or discrepant B19 IgM reactivities in the group of 100 patients with clinically suspected B19 infection are further presented in Table 2. Some of the patients giving discrepant results presented with chronic arthropathies as the only symptoms and were B19 IgM positive as determined by the MACRIA. In the cases of rheumatoid arthritis, RF activity may have given rise to false-positive results in spite of pretreatment with RF absorbent. Consecutive serum samples, available for patients 123 and 162, showed no IgG titer increase (data not shown), indicating that the IgM reactivities may have been false positive. The results for patient 123 were intriguing, since all assays except the MACRIA scored IgM positive.

DISCUSSION

The severity of some of the B19-associated diseases emphasizes the importance of specific diagnostic assay systems for B19 infection. In the present study, we have therefore compared the sensitivities and specificities of four assays for detection of anti-B19 IgM antibodies and related the results to the reference test MACRIA. The study was performed by using clinical serum samples from patients with other acute viral infections, healthy pregnant women, RF-positive individuals, and patients with confirmed B19 infections. The sensitivity was higher in an indirect IFA (Biotrin) and in an antibody capture EIA (IDEIA) (97%) than in two indirect EIAs (Parvoscan and IBL) (90%). A high specificity, 96%, was seen for the Biotrin IFA and IDEIA, whereas the Parvoscan and IBL EIAs had lower specificities, 90 and 88%, respectively. False-positive results were seen for sera that were IgM positive against the herpesvirus group (HSV, CMV, and EBV) and against rubella virus. This may create diagnostic problems in children presenting with fever and rash. The false-positive B19 IgM reactions seen in healthy pregnant women may also create problems in prenatal consulting, since as much as 9% of early pregnancies with acute B19 infections end in abortion or intrauterine death, and there is a risk of hydrops fetalis (22).

The explanation for the relatively high false-positive results observed in the EIAs remains to be found, but these reactions are common in assays for specific IgM. Polyclonal stimulation, resulting in rises in nonspecific-IgM titers, has been seen in the acute phase of other infections such as measles, rubella, and EBV. False-positive results due to RF are also a well-known problem in indirect EIAs and have, for example, been described for rubella virus IgM-positive sera (16). The risk of false-positive results is generally reduced in antibody capture EIAs, although a loss of sensitivity cannot be excluded. Furthermore, it cannot be excluded that some of the sera representing other viral infections than B19 were true positive in the B19 EIAs, reflecting concomitant B19 infections. However, only one of these serum samples was reactive in the Biotrin IFA, and this sample was negative in the MACRIA, thus reducing the probability of dual infection. For the indirect B19 EIAs (Parvoscan and IBL), it was recommended that all serum samples initially IgM positive be retested after pretreatment with RF absorbent to remove RF. Although this reduced the numbers of false-positive results significantly (data not shown),

one RF-positive patient still gave an equivocal result in one of the EIAs.

Several studies have shown that IFAs give fewer false reactivities (11, 14) than EIAs. However, the advantages of using EIAs versus IFAs include the ability to process large numbers of samples and a straightforward objective way of determining cutoff levels and gray zones. The use of an IgM EIA for sensitivity and an IgM IFA for specificity and confirmation may be a useful and practical diagnostic combination for recent B19 infection in laboratories processing a large number of samples. The sensitivities of the different assays were $\geq 90\%$ when tested with the panel of B19 IgM-positive samples.

B19-specific IgM in serum usually appears shortly after the onset of illness and declines over the next 2 to 3 months (10). As in other virus infections, a significant IgG antibody titer rise in paired samples can also be seen. In general, it is believed that B19 virus is rapidly cleared from the body following a primary infection and that long-lasting immunity is conferred by the persistence of anti-B19 IgG antibodies (3). Recent reports, however, have suggested that secondary and/or chronic infections can occur, e.g., in immunocompromised patients after transplantation (24) and in human immunodeficiency virus-infected patients (12). This complicates the interpretation of B19 IgG and IgM results. Fortunately, the use of a B19-specific PCR in diagnosis of an ongoing infection has proven useful, especially for immunodeficient patients, who may not be able to mount an adequate antibody response to the infection, and during pregnancy, for which inconsistencies in the detection of anti-B19 IgM in context with B19 infection in utero have been observed (5, 9, 20). It has also been reported that testing for the avidity of B19-specific IgG allowed a more precise estimation of the time of infection than is possible from the presence of IgM antibody (13).

Further refinement of the test reagents for B19 parvovirus diagnosis is still required, and anti-B19 IgM and IgG standards must also be developed for comparisons of sensitivities of different tests.

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