

Frequency of recent parvovirus infection in patients examined for acute reactive arthritis. A study with combinatorial parvovirus serodiagnostics

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ABSTRACT

Objective. To determine the causative role of human parvovirus B19 as a preceding infection in patients examined for acute reactive arthritis (ReA).

Methods. Sixty adult patients with acute arthritis were screened for evidence of triggering infections. In all patients, cultures of stool specimens and of *Chlamydia trachomatis* in urethra/cervix, and/or bacterial serology were studied. The timing of primary infection of human parvovirus B19 was determined by measurement in serum of VP2-IgM, VP2-IgG, epitope-type specificity of VP2-IgG, and avidity of VP1-IgG.

Results. Median time from onset of joint symptoms to the rheumatological consultation was five weeks (range 1-62). Of the 60 patients, 35 fulfilled the diagnostic criteria for ReA; in the remaining, the diagnosis was unspecified arthritis (UA). Thirty-six patients had antibodies for the B19 virus. Occurrence of these antibodies did not differ significantly between ReA and UA groups ($P = 0.61$). Of these 36 patients, 34 had a pre-existing immunity to the B19 virus. Of the two other patients, one had rash and self-limiting polyarthritides with serological evidence of B19 primary infection, and the other had arthritis of the lower extremities with serological evidence of a convalescence period after the B19 primary infection. The latter patient also had antibodies to *Yersinia*, with a clinical picture typical for ReA.

Conclusion. In patients examined for acute ReA, the frequency of recent B19 virus infection was 3.3% (2 out of 60). The diagnostic utility of the presented methodology, by using a single serum sample, was evident.

Introduction

Many viral infections including rubella, alphaviruses, hepatitis C, retroviruses, and human parvovirus B19 can induce acute arthropathy or arthritis (1). However, viruses have not classically been considered to cause reactive arthritis (ReA), an acute nonpurulent inflammation, which is known to be triggered by various bacteria such as various *Salmonella* serotypes, *Yersinia*, *Shigella*, *Campylobacter*, *Chlamydia trachomatis*, and *Chlamydia pneumoniae* (2).

Human parvovirus B19, a small single-stranded DNA virus of the *Parvoviridae* family, causes several clinical syndromes, including erythema infectiosum, transient aplastic crisis, hydrops fetalis, bone marrow suppression in immunocompromised hosts, and acute or prolonged arthropathy (3, 4). The joint symptoms of B19 infection occur in up to 80% of infected adults, mostly women. B19 arthropathy is typically of acute onset and symmetric distribution, occurring preferentially in the wrists, hands, knees, and ankles (5).

To determine the etiology of acute ReA, we recently conducted a study in which evidence of a triggering bacterial infection was obtained for nearly 60% of the patients (6). Among the remaining patients, with inflammatory arthritis of unspecified etiology, we here determine the causative role of parvovirus B19 primary infection by use of modern combinatorial serodiagnostics.

Patients and methods

Patients

Sixty consecutive adult patients with acute arthritis and/or inflammatory low back pain with a clinical suspicion of ReA were screened at the Outpatient Rheumatological Department in Helsinki University Central Hospital (HUCH) for evidence of triggering infections. Patients with rheumatoid arthritis (RA), gout, or septic arthritis were excluded. All patients were asked about their recent respiratory, abdominal, urinary, and cutaneous symptoms occurring within a month before the onset of joint symptoms. All patients underwent bacterial stool cultures (*Salmonella*, *Yersinia*, *Campylobacter*, *Shigella*), *Chlamydia trachomatis* culture of the urethra/cervix and/or bacterial serology (antibodies to *Salmonella*, *Yersinia*, *Campylobacter*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, streptolysin O, and staphylolysin). In addition, 5 ml of serum was collected at entry and stored at -20°C for B19 virus serology. During the diagnostic workup, the patients were asked for permission to store an additional serum sample for further analysis of triggering infections.

Diagnostic criteria

Arthritis was defined as the development of synovitis in a previously asymptomatic joint. For synovitis, we accepted either the findings observed or the suggested symptoms verified in an interview by an experienced rheumatologist (M. L-R) at the clinical examination. "Acute" denotes disease condition as opposed to "chronic" (arthritis or back pain), i.e. long-standing disease condition, such as osteoarthritis or ankylosing spondylitis.

ReA was defined as seronegative asymmetric predominantly lower extremity arthropathy with one or more of the following: urethritis/cervicitis, dysentery, inflammatory eye disease, or mucocutaneous disease (balanitis, oral ulceration, keratoderma); with exclusion of ankylosing spondylitis, psoriatic arthropathy, or other rheumatic disease (7, 8). In addition, the patient had to show microbiological/serological evidence of preceding gut, urogenital, or respiratory infection, or to have a history of urethritis or enteritis prior to joint symptoms.

Serology

Salmonella and *Yersinia* antibodies were studied by agglutination test (Widal), *Campylobacter* antibodies by enzyme immunoassay (EIA) (9), and *Chlamydia trachomatis* antibodies by microimmunofluorescence test (MIF) (10). *Chlamydia pneumoniae*-specific antibodies were measured by MIF with purified elementary bodies of the Finnish Kajaani 6 isolate (11).

B19-virus VP2-IgM was determined by the EIA of Biotrin (Dublin, Ireland), and VP2-IgG by the in-house EIA (assay #8 in reference 12) employing the recombinant capsid antigen (13). VP1-IgG avidity was determined as earlier described (14), and epitope-type specificity (conformational dependence) of VP2-IgG as earlier described (13). No viruses other than parvovirus B19 were searched for.

Statistical analysis

Data were analyzed by the BMDP statistical software system. Proportional data were compared with the Chi square test. Statistical significance was set at the 5% level.

Results

Mean (\pm SD) age of the 60 patients was 37 (\pm 12.4) years. Of these, 26 (43%) were female. All patients except one had peripheral arthritis (Table I). Median time from onset of joint symptoms to the rheumatological consultation at HUCH was five weeks (range 1-62). If only patients with disease duration of ≤ 3 months would have been enrolled, all the essential findings would have remained the same (data not shown). Of the 35 patients satisfying our diagnostic criteria for ReA, 10 had ReA triggered by *Yersinia*, eight by *Chlamydia trachomatis*, four by *Chlamydia pneumoniae*, three by *Salmonella*, and two by *Campylobacter*. Furthermore, three patients had enteroarthritis and five patients uroarthritis, but the triggering microbe was not demonstrable.

The remaining 25 patients had mono-, oligo-, or polyarthritis without laboratory evidence of infections associated with ReA or urethritis or enteritis prior to arthritis. This group was designated unspecified arthritis (UA).

In total, 36 out of 60 patients had either IgG or IgM class antibodies for B19 virus infection. The occurrence of these antibodies did not differ sig-

nificantly between the ReA (22 of 35) and UA (14 of 25) groups ($P = 0.61$; Chi square test). Of these 36 patients, nearly all, 34, had merely IgG antibodies referring to pre-existing immunity to the B19 virus. Of the two remaining patients, one showed positive IgM antibodies together with low avidity and low epitope-type specificity of IgG, indicating recent B19 infection, and the other had serological evidence (negative IgM, with low avidity and borderline epitope-type specificity of IgG) compatible with convalescence period (2-4 months) after B19 infection.

The former was the only patient with cutaneous rash, and belonged to the UA group. She was a 32-year-old woman who, after a fever lasting three days, developed a rash on her arms, legs, and chest. Following the rash, she experienced stiffness and swelling of her fingers, wrists, ankles, and knees, which resolved spontaneously. At the rheumatological consultation, seven weeks after onset of her joint symptoms, no signs of synovitis were present anymore. Erythrocyte sedimentation rate (ESR) was 4 mm/h, and hemoglobin 124 g/l. Rheumatoid factor and antinu-

Table I. Characteristics of the study patients.

	ReA	UA
Patients, n	35	25
Age, years, mean (\pm SD)	37.7 (12.4)	35.9 (12.8)
Gender, M/F	21/14	13/12
Triggering microbe demonstrable, n (%)	27 (77)	1 (4)
<i>Yersinia</i>	10* (37)	0 (0)
<i>Chlamydia trachomatis</i>	8 (30)	0 (0)
<i>Chlamydia pneumoniae</i>	4 (15)	0 (0)
<i>Salmonella</i>	3 (11)	0 (0)
<i>Campylobacter</i>	2 (7)	0 (0)
Human parvovirus B19	0 (0)	1 (100)
Triggering microbe not demonstrable, n	8 (23)	24 (96)
Patients with peripheral arthritis, n (%)	34 (97)	25 (100)
Patients with only inflammatory low back pain, n (%)	1 (3)	0 (0)
No. of affected joints, mean, range	7.5 (1-21)	5.4 (1-15)
Monoarthritis, n (%)	4 (12)	6 (24)
Oligo- or polyarthritis, n (%)	30 (88)	19 (76)
Other clinical manifestations, n (%)		
Tenosynovitis	12 (34)	2 (8)
Enthesopathy	22 (63)	11 (44)
Conjunctivitis	8 (23)	0 (0)

ReA: reactive arthritis; UA: unspecified arthritis; M: male; F: female; *One of the patients had also serological evidence compatible with convalescence period after parvovirus B19 infection.

clear antibodies were negative; HLA-B27 antigen was not tested.

The latter patient was a 43-year-old woman who, following diarrhea, had arthritis of her metatarsophalangeal (MTP) joints, Achilles bursitis, and plantar fasciitis. She also had erythema nodosum in her lower extremities. At the rheumatological consultation, three months after onset of the joint symptoms, signs of arthritis and Achilles bursitis were still evident. ESR was 32 mm/h, and hemoglobin 122 g/l. *Yersinia* antibodies as well as antigen HLA-B27 were positive. She had a prolonged course, with arthritis in MTP joints and persisting plantar fasciitis for several months, but made a full recovery in 10 months. Clinically, her diagnosis was ReA attributable to *Yersinia* infection. This patient was the only one with evidence of double infection.

Discussion

Originally, after examination of conventional triggering microbes associated with ReA, our series comprised 35 ReA patients and 25 patients classified as UA patients (6). Among these 60 patients, after the current parvovirus serodiagnostics, one showed unequivocal serological evidence of recent human B19 infection, and another serological evidence compatible with the convalescence period after B19 infection. Before the currently performed serodiagnostics, the former patient was placed in the UA group and the latter in the ReA group. The combinatorial parvovirus serodiagnostics thus explained the etiology of arthritis in 1 out of 25 (4%) patients with UA, and in a broader sense, it contributed to the diagnosis of acute arthritis in 2 out of 60 (3.3%) patients.

In population-based studies of early arthritis, the frequency of serological evidence of recent B19 infection has ranged from 2.7% in England (15) to 3% in Sweden (16). Other studies with unspecified inflammatory arthritis from early arthritis clinics had reported higher frequencies varying between 11% and 18% (17, 18, 19, 20). These studies, however, had also included patients with suspected or certain RA, which were excluded from our study.

This could explain in part our lower frequency. In contrast, a recent study found positive B19 IgM antibodies in only 0.6% of patients with recent-onset inflammatory arthritides, including RA (21). The epidemiologic or seasonal situation, or the serological methodology in use may have contributed to these differences.

In our patient with serological evidence of recent B19 infection, causality between the infection and the joint disease is difficult to determine, but it is highly reasonable, because other triggering microbes were appropriately excluded, and the clinical picture was consistent with arthropathy associated with parvovirus B19 infection (5). Furthermore, although arthralgia or arthritis may occur without the rash associated with B19 infection, this patient was the only one reporting this cutaneous symptom preceding joint complaints. Our other patient with B19 serology compatible with the convalescence period after B19 infection had diarrhea prior to arthritis with other musculoskeletal manifestations typical for ReA and HLA-B27-positive spondylarthropathy. After parvovirus serodiagnostics, she apparently had had a coincident B19 infection, as well. Using our combinatorial B19 serodiagnostics, the diagnosis of B19 arthritis was convincingly verified in the former, while, without use of it, the convalescence period of recent B19 infection would have been missed in the latter.

Time from onset of symptoms to the rheumatological consultation was relatively long in some of our patients, making it possible that the frequency of positive serology for recent B19 infection would have been higher if the patients had been examined earlier. It must be noted, however, that we could not influence this delay, which, on the other hand, reflects the actual conditions in a tertiary referral hospital. More cases might also have been positive for recent B19 infection had an epidemic taken place.

In B19 serodiagnostics, the detection of virus-specific IgM or rising titers of IgG are the classical signs of active or recent infection. The classical ap-

proach not infrequently necessitates serial sampling. Our diagnosis of B19 infection was accomplished by combinatorial analysis from a single serum sample. We measured not only B19-specific IgM and IgG antibodies, but also VP1-IgG avidity and VP2-IgG epitope-type specificity. The former is a protein-denaturing immunoassay that measures the antigen-binding avidity (functional affinity) of antimicrobial IgG antibodies (14). Serodiagnosis by such avidity assays makes use of the gradually increasing binding force of IgG as the antibody response matures, permitting accurate timing of the primary infection. The latter is an even more novel approach, i.e. analysis of the epitope-type specificity of VP2-IgG (13). In this assay, linear epitopes of VP2 are recognized exclusively by acute-phase IgG. Both these two new techniques can distinguish between recent primary infection and past immunity by using a single serum sample, and these two parameters of the quality of B19-IgG have been shown to improve the timing of B19 primary infection in several studies (12-14, 22).

In conclusion, among patients examined for acute ReA at a rheumatological out-patient department, 3.3% (2 of 60) showed serological evidence of recent B19 virus infection. Of these two patients, one had not ReA but had typical rash and self-limited arthritis compatible with B19 infection, and the other had ReA attributable to *Yersinia* infection but apparently a coincident infection with B19, too. Our results suggest that the measurement of B19 serology is not necessary in a typical patient with ReA in routine clinical practise, but should be considered in patients with acute undefined arthritis, especially if there is a recent history of rash. The diagnostic utility of the presented methodology, where the diagnosis of recent parvovirus infection can be made of one serum sample making the waiting of a paired serum sample unnecessary, is evident. The application of the methodology in routine diagnostics should be evaluated in larger patient series with acute unspecified arthritis.

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