Clin Rheumatol (2000) 19:281–286

Clinical Rheumatology

Original Article

High Incidence of Parvovirus B19 DNA in Synovial Tissue of Patients with Undifferentiated Mono- and Oligoarthritis

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Abstract: A common problem in rheumatological practice is inflammatory joint disease that cannot be classified. The prognosis of such undifferentiated arthritides is uncertain. The synovial tissue of 41 consecutive patients with various forms of arthritis was tested for the presence of viral DNA in a diagnosis-unaware fashion, using the polymerase chain reaction (PCR). Of all tested viruses, cytomegalovirus and parvovirus B19 were positive (each in 10 patients, two double-positives), whereas herpes simplex virus was positive in two patients. Rubella virus RNA was detected in three specimens. When the positivity for viral material was analysed in terms of distribution among the various diagnostic groups, it became evident that five out of 10 parvovirus B19-positive patients belonged to the undifferentiated arthritis group, whereas cytomegalovirus-positive patients were spread among all diagnostic groups. This indicates the possibility of a new diagnostic category of undifferentiated mono- and oligoarthritis, which can be identified by the presence of parvovirus B19 DNA in synovial tissue.

Keywords: Cytomegalovirus; Diagnostic category; Herpes simplex virus; Monoarthritis; Oligoarthritis; Parvovirus B19; Polymerase chain reaction; Synovial tissue; Undifferentiated arthritis

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Introduction

A common problem in clinical practice relates to inflammatory joint diseases that cannot be diagnosed according to generally accepted criteria [1]. In most cases, not only does the aetiology remain unclear, the prognosis is also uncertain.

Viral infections play a role in some forms of arthritis [2]. For example, parvovirus B19 infection in adults is characterised by a usually self-limited symmetrical polyarthritis of the small joints of the hands, wrist, and knees, accompanied by fever and rash [3]. Also, parvovirus B19 DNA has been detected in the synovial tissue of patients with rheumatoid arthritis [4], although its etiological role has been questioned [5,6]. Finally, several viruses have been reported in association with monoarticular arthritis, for instance human immunodeficiency virus type I [7] or herpes DNA viruses, such as cytomegalovirus (CMV), herpes simplex (HSV), Epstein–Barr virus (EBV) and varicella zoster virus (VZV) [8,9].

Thus, because of the possibility that viral mechanisms may play a more defined role in early, monoarticular or undifferentiated forms of arthritis, we tested the synovial tissue of 41 consecutive patients with various forms of arthritis, but with a referral bias towards undifferentiated mono- and oligoarthritis, for the presence of viral DNA and RNA.

Patients and Methods

In total, 41 patients undergoing diagnostic synovial biopsy or therapeutic synovectomy were studied: 12 with

Table 1. Patient data

Diagnosis[1]	Undifferentiated monoarthritis	Undifferentiated oligoarthritis	Spondylarthropathy	Lyme Disease	Rheumatoid arthritis	Osteoarthritis	Miscellaneous[5]
Number	12	4	6	4	7	6	2
Age (years)	20 (11–43)	30 (18–49)	37 (16–60)	41 (15–61)	57 (30–66)	63 (38–88)	31 (24–38)
Disease duration[2]	26 (2–120)	76 (8–216)	120 (1.5–308)	102 (3–336)	153 (11–326)	111 (18–360)	57 (30–84)
Joint count	1 (1)	2 (2)	4 (1–14)	1 (1–2)	22 (4–30)	1 (1)	1 (1)
ESR 1st hour	20 (2–52)	24 (6–58)	41 (3–39)	16 (3–39)	45 (20–74)	9 (4–20)	11 (11–12)
CRP positivity	3	1	5	1	7	1	1
RF positivity[3]	2	0	0	1	5	0	0
ANA positive[4]	2	0	2	0	3	0	1
HLA-B27 positivity	3	1	4	0	0	2	0

[1]None of the patients had serological evidence of recent infection with *Salmonella*, *Campylobacter*, *Yersinia*, *Clamydia trachomatis* or *Streptococcus*
[2]months before surgery
[3](>10.9 IU/ml)
[4](⩾1:80)
[5]1 septic arthritis, 1 systemic lupus erythematosus.
ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; ANA, antinuclear antibody; HLA, human leukocyte antigen.

undifferentiated monoarthritis, four with undifferentiated oligoarthritis and four with Lyme arthritis. The remaining 21 met defined diagnostic criteria [10–13]: six had spondylarthropathy, seven rheumatoid arthritis, six osteoarthritis, one systemic lupus erythematosus and one septic arthritis (Table 1).

Patients were recruited in two orthopaedic hospitals from February 1995 to November 1997, with a clear bias towards undifferentiated mono- and oligoarthritides (a total of 16 out of 41 cases).

Serological Assays

The referred patients were included as outpatients in a long-term follow-up programme at the Institute of Clinical Immunology of the University of Leipzig. Sera were usually obtained at the first follow-up visit.

The specific anti-viral IgG and IgM responses were tested by standard ELISA according to manufacturers' instructions, using kits for cytomegalovirus (Hoffmann-La Roche AG, Grenzach-Whylen, Germany); Epstein–Barr virus (Dade-Behring Diagnostics, Marburg, Germany); herpes simplex virus (Enzygnost, Dade-Behring Diagnostics); varicella zoster virus (Enzygnost Dade-Behring Diagnostics); adenovirus (Genzyme Virotech GmbH, Rüsselsheim, Germany); and rubella virus (IgG Hoffmann-La Roche AG). Antiparvovirus B19 IgG and IgM antibody titres were tested by means of indirect immmunofluorescence based on recombinant B19 virus VP1 produced in a baculovirus expression system (Biotrin, Dublin, Ireland). Antibodies against picornaviruses were tested by complement-fixing test (Virion, Viron Deutschland GmbH, Planegg/München Germany). Testing for bacterial antibodies (data not shown) in sera was performed according to standard procedures: *Borrelia burgdorferi* (immunofluorescence test, BAG, Lich, Germany), *Mycoplasma* (immunoadherence test, Virion, Viron Deutschland GmbH, Planegg/München Germany), *Yersinia enterocolitica* (KIT YERSINIA, Sanofi Diagnostics, Marnas-Lacoquette, France).

Preparation of Synovial DNA and RNA

Synovial tissue was collected during surgery, snap frozen on dry ice, and stored at –80 °C.

Samples (18–150 mg) were mechanically homogenised (Uniprep Gyrator, InViTec, Berlin, Germany). To enable isolation of both DNA and RNA from a single sample, the RNA isolation kit InViSorb RNA kit II (InViTec) was employed according to the manufacturer's instructions. The DNA adsorbed on the matrix was subsequently purified using a DNA isolation kit (InViSorb Genomic DNA kit II, InViTec).

PCR-based Testing of DNA and RNA in Synovial Tissue

The PCR experiments were performed using the primers listed in Table 2. All amplifications, except for adenovirus, herpes simplex virus and *Mycoplasma*, were carried out by nested PCR. All experiments were carried out using thermal cyclers 2400 (Perkin-Elmer Cetus, Weiterstadt, Germany).

The following samples were used as positive controls: (1) herpes simplex virus type I: Vero cell culture infected with patient material, tested by immunofluorescence; type II: Vero cell culture infected with patient material, tested by immunofluorescence; (2) varicella zoster virus: human fibroblasts infected with OKA strain; (3) cytomegalovirus: human diploid fibroblasts infected with AD 169 strain; (4) adenovirus: HeLa cells infected with APC1 strain; (5) parvovirus B19 (NS1 sequence): positive patient serum; (VP2 gene-segment):

Table 2. Primers used for PCR amplification

Pathogen	Primer	Sequence	Amplification	Size (bp)	Ref.
Adenovirus	A1	5′- TAC GCC AAC TCC GCC CAC GCG CTA (forward)	Hexon region	161	15
	A2	5′- GCC GAG AAG GGC CTG CGC AGG TA (reverse)		161	15
Cytomegalovirus	ex1	5′- CCA AGC GGC CTC TGA TAA CCA AGC C (forward)	Major IE antigen region (internal)	435	16
	ex2	5′- CAG CAC CAT CCT CCT CCT CCT CTG G (reverse)		435	16
	in1	5′- AGA GTC TGC TCT CCT AGT GTG (forward)	Major IE antigen region (internal)	280	16*
	in2	5′- AGA CAC TGG CTC AGA CTT GAC (reverse)		280	16*
EBV	E2P1	5′- AGG GAT GCC TGG ACA CAA GA (forward)	EBNA2 gene (external)	596	17
	E2P2	5′- TGG TGC TGC TGG TGG TGG CAA T (reverse)		596	17
	Ap1	5′- TCT TGA TAG GGA TCC GCT AGG ATA (forward)	EBNA2 gene (internal):	497	17
	Ap2	5′- ACC GTG GTT CTG GAC TAT CTG GAT C (reverse)		497	17
HSV	e1	5′- CAG AAC TAC ACG GAG GGC ATC (forward)	Glycoprotein B gene (external)	136	18
	e2	5′- TCC CCA TAA ACT GGG AGT AGC (reverse)		136	18
Parvovirus B19	B19/1	5′- TTC TTT TCA GCT TTT AGG (forward)	VP2 region gene (external)	201	19
	B19/2	5′- TGA ATT GCA TGG TCT TCA TG (reverse)		201	19
	B19/3	5′- TAT AAG TTT CCT CCA GTG CC (forward)	VP2 region gene (internal)	121	19
	B19/4	5′- TTG GGT CAC CTC CTA ATG T (reverse)		121	19
	P1	5′- AAT ACA CTG TGG TTT TAT GGG CCG (foward)	NS1 region (external)	283	20
	P6	5′- CCA TTG CTG GTT ATA ACC ACA GGT (reverse)		283	20
	P2	5′- AAT GAA AAC TTT CCA TTT AAT GAT GTA G	NS1 region (internal)	102	20
	P5	5′- CTA AAA TGG CTT TTG CAG CTT CTA C(reverse)		102	20
VZV	VZV1	5′- ATG TCC GTA CAA CAT CAA CT (forward)	XbaI M region (external)	267	21
	VZV2	5′- CGA TTT TCC AAG AGA GAC GC (reverse)		267	21
	VZV3	5′- GCC CAT GAA TCA CCC TCT TGT (forward)	XbaI M region (internal)	125	21*
	VZV4	5′- CGT GCT ATT GAA GTC GTC TCC (reverse)		125	21*
Mycoplasma spp.	Myk5	5′- GGC GAA TGG GTG AGT AAC ACG (forward)	16S rRNA gene	464	22
	Myk6	5′- CGG ATA ACG CTT GCG ACC TAT G (reverse)		464	22
Enterovirus	E1	5′- CAC CCG ATG GCC AAT CCA (reverse)	CBV non-translated region (external)	208/128	23
	E2	5′- TCC GGC CCC TGA ATG (reverse)		208	23
	E3	5′- ACA CGG ACA CCC AAA GT (reverse)	CBV non-translated region (internal)	128	23
Rubella virus	1	5′- GGC CTC TTA CTT CAA CCC TG (forward)	E1 polypeptide (external)	368	24
	2	5′- CAG TCC CGG TGG GGC CTC GG (reverse)		368	24
	3	5′- GGC GGC AGC TAC TAC AAG CAG (forward)	E1 polypeptide (internal)	282	24
	4	5′- GAG GTC CAG GTC CCG CCC GA (reverse)		282	24

*Modified protocols.

baculovirus recombinant AcB19VP1L expressing VP1 protein of human parvovirus B19 (kindly provided by Professor W. Spaan, Leiden) [14]; (6) Epstein–Barr virus: EBV-immortalized B-lymphocytes; (7) enterovirus: HeLa cells infected with Coxsackie B5 11327/30; (8) rubella virus: rk-cells infected with rubella strain Judith; (9) *Mycoplasma:* infected fibroblast cells.

Negative controls consisted of PCR-mastermix with sterile water (Aqua ad iniectabili Braun, B. Braun Melsungen AG, Melsungen, Germany). PCR results were judged to be valid only in combination with the respective negative and positive controls listed above. All patients were tested for the complete set of DNA and RNA viruses listed above, with the exception of three with osteoarthritis who could not be tested for RNA viruses.

Results

Parvovirus B19 and cytomegalovirus DNA were detected each in the synovial tissue of 10 patients (Figs 1 and 2, respectively). Herpes simplex virus DNA was detected in two specimens only, and rubella virus RNA in three. The samples, in turn, were negative for adenovirus, Epstein–Barr virus, varicella zoster virus, enterovirus and *Mycoplasma.*

In contrast to the more limited positivity in the synovial tissue, the serum antibody response against the viruses was highly prevalent, for example, 36 patients were seropositive for parvovirus B19 antibodies of the IgG class. Only one patient had antibodies against parvovirus B19 of both IgG and IgM classes, indicating recent infection. In addition, the following patients tested seropositive: 23 for cytomegalovirus, 35 for herpes simplex virus, 33 for adenovirus, 37 for Epstein–Barr virus, 38 for varicella zoster virus, 20 for Coxsackie virus and 40 for rubella virus. Also, 13 patients showed serological evidence of contact with *Mycoplasma*. Therefore, only a minority of patients previously exposed to viral pathogens (as indicated by the serum-specific response) appeared to harbour viral DNA in the inflamed synovial tissue, and none of them harboured *Mycoplasma* DNA.

When the cases of synovial specimens positive for viral DNA (18 out of 41) were assigned to their respective diagnostic groups, it became evident that as many as eight of the 10 parvovirus B19-positive patients belonged to the undifferentiated monoarthritis (five patients) or oligoarthritis (three patients) groups. In contrast, the 10 patients positive for cytomegalovirus

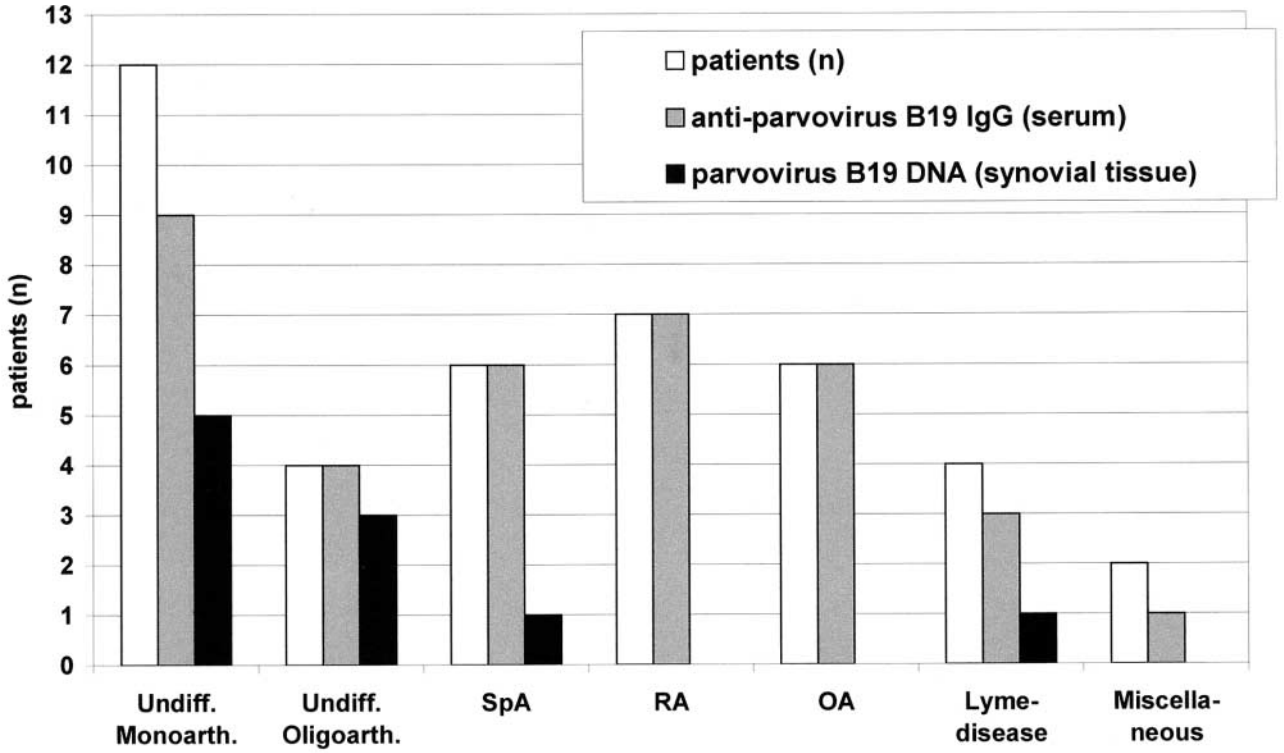

Fig. 1. Frequency of parvovirus B19 positivity in serum and synovial tissue of patients with various forms of rheumatic diseases (Undiff. Monoarth, undifferentiated monoarthritis; Undiff. Oligoarth, undifferentiated oligoarthritis; SpA, spondylarthropathy; RA, rheumatoid arthritis; OA, osteoarthritis).

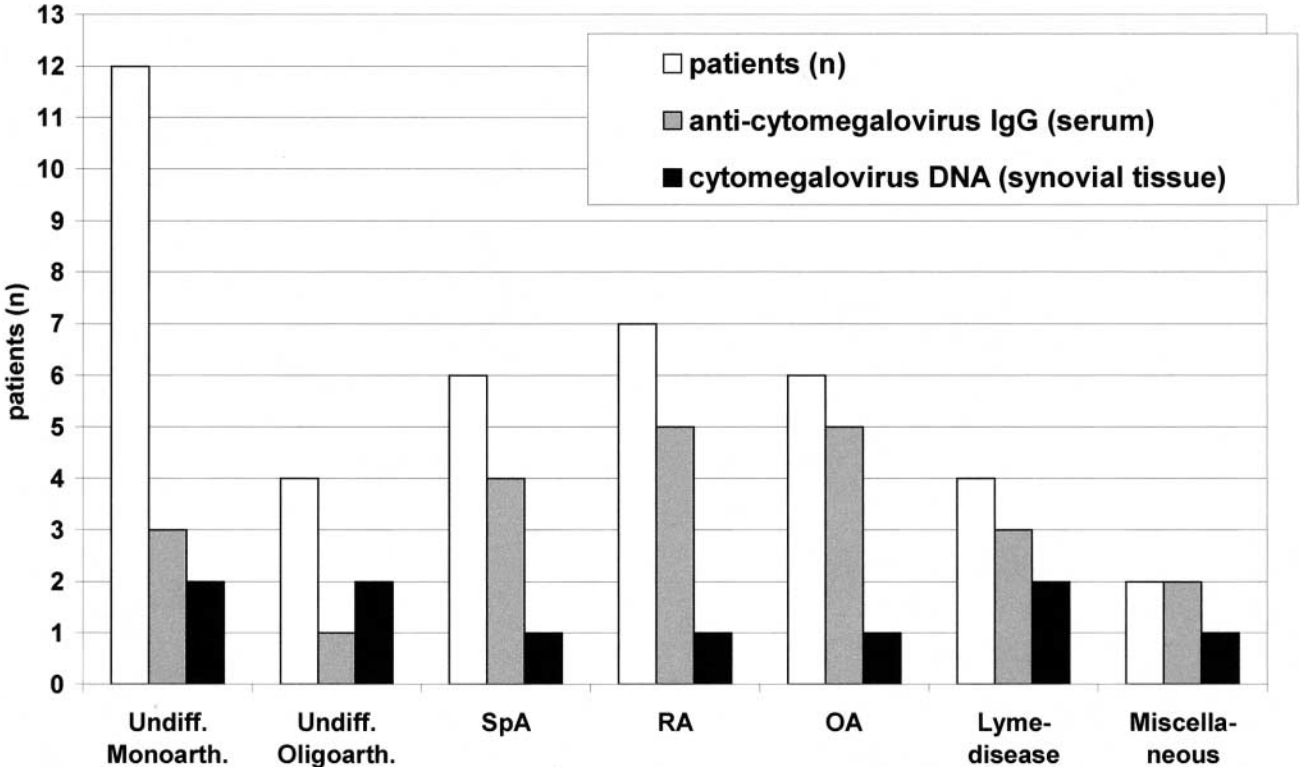

Fig. 2. Frequency of cytomegalovirus positivity in serum and synovial tissue of patients with various forms of rheumatic diseases (Undiff. Monoarth, undifferentiated monoarthritis; Undiff. Oligoarth, undifferentiated oligoarthritis; SpA, spondylarthropathy; RA, rheumatoid arthritis; OA, osteoarthritis).

DNA were evenly distributed among the seven diagnostic groups (one to two each).

The clinical outcome of patients with synovial tissue positive for parvovirus B19 DNA was as follows: two patients with monoarthritis (10 and 13 years of age; of the five patients with monoarthritis the mean age was 18, range 10–31) were free of symptoms within 6–7 months. One patient (age 31) of the three with oligoarthritis (mean age 24 years, range 18–31) became symptom-free within 7 months. In contrast, the remaining three of the five monoarthritis patients (mean age 22 years, range 16–31), as well as two of three patients with oligoarthritis (ages 18 and 23 years) still suffered from persistent arthritis after an average of 15 months follow-up (range 7–19 months). Notably, at retrospective questioning none of these patients had had a history of a viral prodromal illness.

Discussion

The highly sensitive PCR technique [20] revealed that 10 of 41 patients with different forms of arthritis carried parvovirus B19 DNA in the inflamed synovial membrane: five of 12 with undifferentiated monoarthritis, three of four with undifferentiated oligoarthritis, one of six with spondylarthropathy, and one of four with Lyme disease. Notably, the synovial tissue of other diagnostic groups, including seven patients with rheumatoid arthritis and six with osteoarthritis, proved negative for the parvovirus B19 genome (see Fig. 1). In the present study, therefore, patients with synovial positivity for parvovirus B19 DNA seem to cluster between undifferentiated mono- or oligoarthritis. Such clustering does not appear to result from epidemics of this particular virus in the referral area, as the patients were recruited continuously over a 2-year timespan.

Interestingly, although the overall positivity for parvovirus B19 material in the synovial tissue was fairly limited (10/41 cases), the serological tests showed that as many as 88% of all patients had been exposed to this virus. All patients with rheumatoid arthritis, osteoarthritis and spondylarthropathy had specific IgG responses against parvovirus B19, whereas only 81% of those with undifferentiated arthritis were seropositive.

Cytomegalovirus DNA was also found in 10 of 41 rheumatic patients, but distributed evenly among all diagnostic groups (see Fig. 2). Also, only two patients were positive for herpes simplex virus DNA and three for rubella virus RNA (data not shown). Finally, all other pathogens tested for, including adenovirus and *Mycoplasma*, were negative. Thus, in contrast to the apparent clustering of parvovirus positivity in undifferentiated arthritis, the positivity for other viruses displays a rather random distribution. The discrepant results with regard to mycoplasmal DNA as reported by Schaeverbeke et al. [25] may be explained by different clinical groups of patients, as well as experimental procedures, especially the use of different primers for PCR analysis.

The frequency of positivity for parvovirus B19 observed in the present study seems to differ from that reported in other studies. For example, parvovirus B19 infection has been frequently reported in association with transient chronic symmetrical polyarthritis [3,26,27], but in only one case of monoarthritis (out of 16 patients with undifferentiated mono- and oligoarthritis) [28]. Another study, focusing on the prevalence of parvovirus B19 DNA in synovial samples of children with juvenile arthritis (but considerably younger than those in the present study), reported that the frequency of tissue positivity for parvovirus B19 DNA is even lower than in age-matched controls with joint trauma [29]. Recent reports found no association between the presence of parvovirus B19 DNA in synovial tissue and inflammatory joint disease [30,31]. However, Takahashi et al. [32] detected parvovirus B19 DNA in synovial tissue in 30 out of 39 patients with RA, suggesting an involvement of parvovirus B19 in the initiation and perpetuation of RA synovitis. Likely reasons for the discrepancies may be technical (for example, the use of different primers for PCR analysis) or demographic (e.g. the age and the diagnostic grouping of the patients).

Although in our study no parvovirus B19 DNA could be detected in the synovial tissue of rheumatoid arthritis or osteoarthritis cases, this was detected in one sample of *Yersinia enterocolitica*-reactive arthritis (assigned to the spondylarthropathy group) and in one patient with Lyme monoarthritis. Both patients had typical serological features and improved within 4 months of appropriate treatment. The significance of parvovirus B19 DNA positivity in these two cases is unclear.

Whether the presence of parvovirus B19 DNA in the synovial tissue has pathogenetic relevance is also unclear. Our data indicate that there may be an association between undifferentiated mono- and oligoarthritis and parvovirus positivity in the tissue. This association may have some prognostic value, as in a fraction of patients undifferentiated arthritis persists for many months. In contrast, in the two patients selectively positive for cytomegalovirus in the synovial tissue, arthritis resolved after synovectomy. It is of note that patients double-positive for cytomegalovirus and parvovirus B19 DNA still had persistent inflammatory disease after 11 and 25 months.

The present study indicates that among forms of undifferentiated arthritis there may be a diagnostic category of mono- and oligoarthritis that can be identified by the presence of parvovirus B19 DNA in the synovial tissue. This generally affects younger patients and may lead to prolonged inflammatory disease.

Acknowledgements. We thank S. Arnold and L. Seydlitz for referring patients. This work was supported by the Bundesministerium für Bildung, Forschung und Technologie (BMB+F), Interdisziplinaeres Zentrum für Klinische Forschung (IZKF) at the University of Leipzig (Project A1), and a HSP3 grant by the Ministerium für Wissenschaft und Kunst of Saxony (SMWK). The study was approved by the Ethics Committee of the University of Leipzig.

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Received for publication 28 June 1999
Accepted in revised form 6 January 2000