Chronic Fatigue Syndrome and Arthralgia Following Parvovirus B19 Infection

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ABSTRACT. ***Objective.*** To determine the incidence of arthralgia and fatigue complicating B19 infection, along with associated B19 markers and autoantibodies.

Methods. We studied patients with acute B19 infection (n = 51), patients followed from the time of acute B19 infection (mean 22.5 mo) (n = 39), and healthy controls (n = 50). Clinical details were collected using a questionnaire and blood was tested for B19 markers and autoantibodies.

Results. Acute B19 arthralgia occurred in 31 patients and was associated with female sex ($p = 0.007$) and age > 20 years ($p = 0.02$). Acute B19 fatigue occurred in 8 patients and was not significantly associated with any marker. At followup, symptoms consisted of arthralgia (n = 5), arthralgia and fatigue (n = 6), fatigue (n = 7), lymphadenopathy (n = 1), and purpura due to thrombocytopenia (n = 2). Chronic B19 arthralgia was associated with persistent B19 viremia ($p = 0.029$). Comparison of the B19 followup group with the controls revealed a significantly increased prevalence of arthralgia ($p = 0.0002$), fatigue ($p < 0.0001$), and all other markers. Chronic B19 arthralgia was associated with both acute B19 arthralgia ($p = 0.0168$) and positive ANA at acute infection ($p = 0.0043$). Chronic B19 fatigue was associated with acute B19 fatigue ($p = 0.011$). Five patients fulfilled the Centers for Disease Control criteria for a diagnosis of chronic fatigue syndrome (CFS) and one of these was negative for serum anti-B19 IgG at followup by both Western blot and immunofluorescence. However, there was no characteristic pattern of B19 markers/autoantibodies in patients with B19 associated chronic fatigue.

Conclusion. CFS may follow acute parvovirus B19 infection; however, attribution of a case of CFS to B19 infection may be extremely difficult in the absence of serological confirmation of acute infection at fatigue onset. (J Rheumatol 2002;29:595–602)

Key Indexing Terms:
PARVOVIRUS B19 ARTHRALGIA FATIGUE CHRONIC FATIGUE SYNDROME
NS1 ANTIBODIES B19 VIREMIA RHEUMATOID FACTOR ANTINUCLEAR ANTIBODY

Human parvovirus B19, discovered in 1975[1] and first linked with human disease in 1981[2], is a small single stranded DNA virus classified within the family *Parvoviridae*, and genus *Erythrovirus*, having tropism primarily for erythroid precursors. B19 is the only parvovirus that has been clearly linked with disease in humans. B19 replicates only in human cells and is autonomous, not requiring the presence of a helper virus.

Acute B19 virus infection may be asymptomatic in 50% of infected children and in symptomatic persons is classically associated with the childhood rash illness, erythema infectiosum, arthralgia, fetal death, transient aplastic crisis in those with shortened red cell survival, and pure red cell aplasia in immunocompromised persons[3]. The incidence of chronic B19 arthralgia following acute B19 infection appears to vary widely, ranging from 0 of 54 patients after a mean followup of 5 years[4], to 20 of 53 (37.7%) patients after a mean followup of 4.75 years[5].

Fatigue may complicate B19 infection both at the acute phase and during the following years. There are several case reports of CFS associated with acute B19 infection[5-7], and one of these responded to treatment with normal human immunoglobulin[7], the only specific treatment for parvovirus B19 infection. The only study that examined patients with chronic fatigue syndrome (CFS) for B19 markers found only one of 7 cases positive for anti-B19 IgM[8]. Followup of 53 persons with acute B19 infection for a mean of 4.75 years revealed 2 patients fulfilling diagnostic criteria for CFS[9], and one of these had B19 DNA detected in peripheral blood at fol-

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lowup[5]. In addition, B19 DNA has been detected in skeletal muscle in a case of CFS[10].

We investigated the incidence of fatigue and arthralgia following acute B19 infection in the UK, associated additional symptoms, and B19 markers and autoantibodies.

MATERIALS AND METHODS

Patient enrollment, assessment, and serum collection. Fifty-one patients with acute B19 infection were identified by the Department of Virology, Manchester Royal Infirmary, from 1998 to 2000 by detection of serum anti-B19 IgM. After this, patients were contacted and with their consent, visited at home by one of us (JRK) to obtain a detailed history and to draw a blood sample. Thirty-nine patients were successfully followed.

A questionnaire was administered by the investigator in each patient's home. The first part enquired as to the state of health prior to B19 infection, any medical history, history of smoking and drug/alcohol abuse, and history of taking medications. The second part of the questionnaire enquired as to symptoms present during acute B19 infection and in the following months and years; for each symptom present, its time of onset, duration, and character were noted. Specific questions related to rashes, joint pain (including specific joints affected), joint swelling (specific joints affected), sore throat, increased tendency to sweat, painful aching muscles, particular fatigue (not due to ongoing exertion, not relieved by rest, and resulting in a substantial reduction in occupational, educational, social, and personal activity), headaches, dizzy spells, blurring of vision, alcohol intolerance, difficulty sleeping, unrefreshing sleep, deterioration in memory, inability to concentrate, enlarged glands in the neck or axillae, and postexertional malaise[9]. Routine blood investigations were also performed, including full blood count, urea and electrolytes, liver function tests, and erythrocyte sedimentation rate (ESR). Patients with chronic fatigue were examined by a clinical rheumatologist (RMB or INB).

Prolonged fatigue was defined as self-reported, persistent fatigue lasting one month or longer. Chronic fatigue was defined as self-reported persistent or relapsing fatigue lasting 6 or more consecutive months. CFS was defined by, first, clinically evaluated, unexplained (by factors other than B19 infection), persistent, or relapsing chronic fatigue that was of new or definite onset (not lifelong); not the result of ongoing exertion; not substantially alleviated by rest; and resulting in substantial reduction in previous activities. Second, the concurrent presence of ≥ 4 of the following symptoms, all of which must have persisted or recurred during 6 or more months of illness and must not have predated the fatigue; self-reported impairment in short term memory or concentration severe enough to cause substantial reduction in previous levels of occupational, educational, social, or personal activities; sore throat; tender cervical or axillary lymph nodes; muscle pain, multijoint pain without joint swelling or redness; headaches of a new type, pattern. or severity; unrefreshing sleep; and postexertional malaise lasting > 24 h.

Fifty healthy control persons were also enrolled, chosen to match the test group in terms of age and sex, geographical location (Northwest England), and sampling time. Controls were employed by Manchester Royal Infirmary and were enrolled with their consent. In an identical manner to that used for the patients, a questionnaire was administered by the investigator (as above, except for details relating to B19 infection). A blood sample was collected for analysis as described below.

All blood samples were collected in pyrogen-free blood collection tubes using the Vacutainer® system (Becton Dickinson, Oxford, UK), separated using centrifugation, and serum stored at –20ºC until analysis. DNA was extracted from EDTA anticoagulated blood by phenol-chloroform extraction. All sera from patients and controls were tested for anti-B19 VP2 IgM, anti-B19 VP1/2 IgG, anti-B19 NS1 IgG, B19 DNA, rheumatoid factor (RF), antinuclear antibody (ANA), C-reactive protein (CRP), and serum amyloid A (SAA). All DNA samples were tested for B19 DNA.

Qualitative parvovirus B19 antibody testing. Serum anti-B19 VP2 IgM was detected by ELISA (Biotrin, Dublin, Ireland) according to the manufacturer's instructions. Serum anti-B19 VP1/2 IgG and NS1 IgG were detected by Western blot (Mikrogen, Martinsreid, Germany) according to manufacturer's instructions. In patients with fatigue at followup, serum was also tested for anti-B19 VP1 IgG by indirect immunofluorescence using recombinant baculovirus infected SF9 insect cells expressing B19 VP1[11].

Nested polymerase chain reaction for B19 DNA. DNA was extracted from 100 μl serum samples by phenol-chloroform extraction; 5 μl DNA extract was added to 45 μl PCR mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 200 μM dTTP, 0.5 μM primer 1 (5'-AATACACTGTGGTTTTATGGGCCG-3'), 0.5 μM primer 6 (5'-CCATTGCTGGTTATAACCACAGGT-3'), and 1.25 U Amplitaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). The second reaction utilized the same mix, but with 0.5 μl primer 2 (5'-GAAAACTTTCCATTTAATGATGTAG-3') and 0.5 μl primer 5 (5'-CTAAAATGGCTTTTGCAGCTTCTAC-3') instead of primers 1 and 6[12]. Primers 1, 2, 5, and 6 correspond to nucleotides 1399–1422, 1498–1525, 1576–1600, 1659–1682, respectively, of B19 genomic DNA within the nonstructural gene NS1[13]. For both first and second steps, dsDNA was initially denatured for 6 min at 95°C, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Primers 1 and 6 yielded a product of 284 bp and primers 2 and 5 yielded product of 103 bp[12]. Positive controls were $1:10^3$ and $1:10^6$ dilutions of viremic serum; negative control was distilled water. PCR product was subjected to gel electrophoresis using a molecular weight marker, and the separated DNA molecules stained with 1% ethidium bromide and visualized by ultraviolet transillumination. The sensitivity of this PCR assay has been shown to be of the order of 1–10 genome copies[12]. B19 viremia was defined as serum B19 DNA positivity.

Autoantibody measurement. Serum was tested for the presence and titer of RF using the Serodia-RA latex particle agglutination kit (Fujirebio Inc., Tokyo, Japan) according to the instructions of the manufacturer. ANA were detected using human epithelioma type 2 (HEp-2) cells by standard indirect immunofluorescence.

Serum CRP and SAA measurement. CRP was quantified in serum by ELISA (Eurogenetics NV, Tessenderlo, Belgium) according to the manufacturer's instructions; 5 standards ranged from 0 to 800 μg/ml; normal values were taken to be < 5 μg/ml. SAA was quantified in serum by ELISA (Tridelta, Greystones, Ireland) according to the manufacturer's instructions; 5 standards ranged from 0 to 100 μg/ml and normal values were taken to be < 6 μg/ml.

Statistical analysis. The strength of association between clinical variables and markers was estimated using odds ratios (OR) or relative risks (RR) with 95% confidence intervals (CI). Levels of significance were determined using 2×2 or $2 \times k$ contingency tables by either chi-squared or Fisher's exact analysis. Where appropriate, Yates' (chi-squared) and Haldane (OR) corrections were applied. To identify trends in this pilot study that may be worthy of analysis in future studies, associations with p values ≤ 0.15 were also included and referred to as trends.

RESULTS

Controls. The ages of healthy controls ranged from 22 to 63 years, mean 33.4 years, with a male:female ratio of 1:3. All controls had normal blood hematology, biochemistry, and ESR. Thirty-seven persons were positive for serum anti-B19 IgG and 8 had NS1 antibodies. No control was positive for either serum or leukocyte B19 DNA.

B19 infected patients. In all cases, the state of health of these patients had been good prior to B19 infection. Exceptions were Patient 2, who had a 20 year history of Raynaud's phenomenon, Patient 11, who had had chronic pancreatitis related to alcohol abuse, and Patient 32, who had several previous fractures caused by motorcycle accidents.

Acute B19 infection. Fifty-one patients with acute B19 infec-

tion (serum anti-B19 IgM positive) were examined. These patients had an age range of 4–54 years, mean 28.2 years, and a male:female ratio of 1:4.1. Clinical details at the time of acute B19 infection were rash (n = 15), arthralgia (n = 31), arthralgia and joint swelling (n = 15), fatigue (n = 8), lymphadenopathy (n = 4), fetal hydrops (n = 3), transient aplastic crisis (n = 2), neutropenia (n = 2), myelodysplasia (n = 1), thrombocytopenia (n = 1), and pancytopenia (n = 1). Serum anti-B19 VP1/2 IgG was detected in 42 patients. Serum anti-B19 NS1 IgG was detected in 7 patients. Serum B19 DNA was detected in 42 patients. RF and ANA were detected in 16 and 9 persons, respectively. Serum CRP and SAA were increased in 24 and 15 persons, respectively.

In this group, the presence of arthralgia was associated with female sex (p = 0.007) and age > 20 years (p = 0.02) (Table 1); however, this was not the case for fatigue. There was a trend toward increased prevalence of rash with female sex, increased prevalence of B19 viremia with arthralgia, and increased prevalence of ANA with both arthralgia and fatigue (Table 1). Serum NS1 antibodies did not correlate with particular symptoms. As expected, serum CRP and SAA were associated (p = 0.04). There was a trend toward a negative association between increased serum amyloid A and RF (p = 0.12).

B19 infected persons at followup. Thirty-nine of the 51 patients with acute B19 infection were contacted after a followup period of 2–37 months (mean 22.5 mo) (in 37 of these persons the followup period was at least 7 mo). At this time, 19 patients were found to have symptoms that began at the time of acute infection and that persisted throughout the followup period. These symptoms were arthralgia (n = 5), arthralgia and fatigue (n = 6), fatigue (n = 7), lymphadenopathy (n = 1), and purpura known to be due to thrombocytopenia (n = 2). Except for the 2 patients with thrombocytopenia, all patients had normal hematology. All patients had normal ESR and blood biochemistry, and only one patient had joint swelling at followup. All B19 infected persons at followup were negative for serum anti-B19 VP2 IgM, and all except for Patient 32 were positive for serum anti-B19 VP1/2 IgG; Patient 32 tested negative for anti-B19 IgG by both Western blot and fluorescent antibody test. Serum anti-B19 NS1 IgG was detected in 16 persons. Serum B19 DNA was detected in 10 persons and leukocyte B19 DNA was detected in 4 persons. RF and ANA were detected in 14 and 6 persons, respectively. Serum CRP and SAA were increased in 5 and 6 persons, respectively (Table 2).

At followup, B19 arthralgia was associated with persistent B19 viremia (p = 0.029). There was a trend toward increased prevalence of chronic B19 arthralgia with age > 20 years (p = 0.11) and detection of B19 genome in leukocyte DNA (p = 0.11), respectively. There was a trend toward an increased prevalence of B19 viremia with detection of leucocyte B19 DNA (p = 0.075) (Table 2). Neither chronic B19 arthralgia nor fatigue was significantly increased in women. RF and ANA did not correlate with arthralgia, fatigue, or any B19 marker. As expected, increased serum CRP correlated with increased SAA (p = 0.022) (Table 2).

Clinical symptoms and laboratory test results in these patients were markedly different from those of the controls, with significantly increased prevalence of arthralgia, fatigue, VP1/2 antibodies, NS1 antibodies, B19 viremia, leukocyte B19 DNA, RF, ANA, raised CRP, and raised SAA (Table 3).

Predictors of outcome of B19 infection. To assess the value of each symptom and marker present at onset in predicting the outcome of B19 infection and markers present at followup, relative risks were determined (Table 4). This identified that chronic B19 arthralgia was associated with both acute B19 arthralgia (p = 0.0168) and positive ANA at acute B19 infection (p = 0.0043). Chronic B19 fatigue was associated with acute B19 fatigue (p = 0.011). There was a trend toward increased prevalence of chronic B19 fatigue with decreased prevalence of NS1 antibodies at acute infection (p = 0.13), and increased prevalence of both RF (p = 0.078) and ANA (p = 0.12) at acute infection (Table 4).

Table 1. Acute B19 infection; results of chi-square analysis of the relationships between clinical manifestations, B19 markers, autoantibodies, and markers of inflammation. Data are OR (95% CI)

	Age ≥ 20 yrs, n = 39	Female Sex, n = 41	Serum B19 DNA, n = 42	Rheumatoid Factor, n = 16	Antinuclear Antibody, n = 9	Increased CRP, n = 24
Rash, n = 15		OR 4.8 (0.92–25.97) p = 0.1				
Arthralgia, n = 31	OR 6.02 (1.28–40.49) p = 0.02	OR 10.55 (1.93–57.60) p = 0.007	OR 13.33 (0.76–57.72) p = 0.095		OR 6.26 (0.72–54.75) p = 0.15	
Fatigue, n = 8					OR 4.31 (0.93–19.98) p = 0.11	
Increased serum amyloid A, n = 15				OR 0.26 (0.06–1.12) p = 0.12		OR 4.65 (1.23–17.67) p = 0.04

Table 2. Convalescent B19 infection; results of chi-square analysis of the relationships between clinical manifestations, B19 markers, and autoantibodies. Data are OR (95% CI).

	Age ≥ 20 yrs, n = 34	NS1 Antibodies, n = 16	Serum B19 DNA, n = 10	Leukocyte B19 DNA, n = 4	Increased CRP, n = 5
Arthralgia, n = 11	OR 10.03 (0.69–47.89) p = 0.11	OR 0.22 (CI 0.04–1.22) p = 0.14	OR 7.2 (1.47–35.32) p = 0.029	OR 10.13 (0.92–111.25) p = 0.11	
Serum B19 DNA, n = 10				OR 12.0 (1.08–133.6) p = 0.075	
Increased serum amyloid A, n = 6					OR 15.5 (1.81–132.54) p = 0.22

Table 3. Comparison of prevalence of arthralgia, fatigue, B19 markers, and autoantibodies in B19 infected persons at followup versus healthy controls.

	B19 Infection at Followup, n = 39	Controls, n = 50	OR (95% CI)	p
Arthralgia	11	0	40.75 (3.46–209.04)	0.0002
Fatigue	13	0	45.91 (3.94–236.84)	< 0.0001
VP 1/2 antibodies	38	37	28.44 (2.46–146.40)	0.0017
NS1 antibodies	16	8	3.65 (1.36–9.82)	0.016
Serum B19 DNA	10	0	35.95 (3.01–183.15)	0.0005
Leukocyte B19 DNA	4	0	12.8 (0.87–50.62)	0.072
RF	14	0	57.43 (5.0–299.04)	< 0.0001
ANA	9	0	31.46 (2.59–158.96)	0.0012
Increased serum CRP	5	0	16.10 (1.17–76.35)	0.032
Increased serum amyloid A	6	0	19.60 (1.49–95.14)	0.014

Table 4. Acute and comvalescent B19 infection; relative risks (RR) of various clinical manifestations and B19 markers at the time of acute infection for the development of chronic arthralgia and chronic fatigue.

Acute B19 Infection	Followup of B19 Infection		
	Arthralgia, n = 11	Fatigue, n = 13	NS1 Antibodies, n = 16
Arthralgia, n = 31	RR 1.87 (1.19–2.70) p = 0.0168		
Fatigue, n = 8		RR 2.5 (1.37–4.63) p = 0.011	
NS1 antibodies, n = 7		RR 0.27 (0.05–1.35) p = 0.13	RR 10.5 (1.93–62.35) p = 0.0021
RF, n = 16		RR 2.53 (1.08–5.84) p = 0.078	
ANA, n = 9	RR 4.65 (1.86–11.23) p = 0.0043	RR 2.42 (1.0–5.16) p = 0.12	

Table 5. Symptoms, tender points B19 markers, and autoantibodies in 13 patients with fatigue (not relieved by rest and resulting in significantly reduced activities) that persisted since the time of acute B19 infection. All patients had fatigue for the entire followup period, onset of which coincided with onset of acute B19 infection.

	Patients												
	1	2	6	7	8	9	11	20	22	29	31	32	37
Sex	M	F	F	F	F	F	F	F	F	F	F	M	F
Age at onset of B19 infection/ fatigue, (yrs)	26	42	44	25	34	35	40	27	46	46	36	46	34
Time since acute B19 infection, (mo)	4	19i	23	24	26	22	7	30	27	26	25	30	27
Deterioration in memory/ concentration	–	+	–	–	+	–	+	+	+	–	–	+	–
Sore throat	–	+	–	–	+	–	–	–	–	–	–	–	–
Tender cervical/ axillary lymph nodes	–	–	–	+	–	–	–	–	–	–	–	–	–
Painful aching muscles	–	+	–	–	+	+	+	+	–	–	–	+	–
Arthralgia	–	+	+	–	+	+	+	+	+	+	–	+	–
Arthralgia duration, (mo)[2]	–	19	23i	–	26	2	7	2	1	26	3	30	1
Joints affected[3]	–	HFAW	WF	–	EKBN FW	EWFK AN	FWES	WFAK	WFK	WFKS AH	Gen	WFKH A	WFK
Joint swelling/ redness	–	–	–	–	–	–	+	–	–	–	–	–	–
New headaches	+	+	+	+	+	+	+	+	+	–	–	+	–
Difficulty sleeping	+	+	–	–	+	–	+	+	–	–	–	+	–
Unrefreshing sleep	–	+	–	–	+	–	+	+	–	–	–	+	–
Postextertional malaise	–	+	+	–	+	–	+	+	+	+	+	+	–
Increased tendency to sweat	–	+	–	–	+	–	–	–	–	–	–	+	–
Dizzy spells	–	+	–	–	+	–	–	+	–	–	–	–	–
Blurred vision	–	+	–	–	+	–	–	–	–	–	–	–	–
Other[4]	–	RDA	CH	–	Th	–	C	–	–	–	–	–	–
Tender Points[5]	NP	7	2	NP	7	NP	12	NP	NP	7	4	4	NP
Diagnosis of fatigue[6]	PF	CFS	PF	PF	CFS	PF	CFS	CFS	PF	PF	PF	CFS	PF
Serum B19 DNA	+	+	–	–	+	–	+	–	+	–	–	+	–
Leukocyte B19 DNA	+	–	–	–	–	–	+	–	–	–	–	–	–
Serum anti-B19 VP1 1/2 IgG	+	+	+	+	+	+	+	+	+	+	+	–	+
Serum anti-B19 NS1 IgG	+	+	–	–	–	+	–	+	–	–	+	–	+
RF	–	+	+	–	+	+	–	–	–	+	–	–	+
ANA[7]	–	–	–	–	H300	–	–	–	–	–	–	–	–
CRP (normal, < 5 μg/ml)	1.14	0.17	0.10	44.0	0.36	0.99	1.83	4.8	0.77	10.3	0.44	1.47	1.92
Serum amyloid A (normal, < 6 μg/ml)	–	–	–	61.78	–	4.18	–	–	–	63.54	12.95	–	–

[1]Severe enough to result in significant reduction in occupational, educational, social, or personal activities.
[2] intermittent: remitting and relapsing
[3] F, fingers;W, wrists; E, elbows; S, shoulders; H, hips; K, knees; A, ankles; B back; N, neck; Gen, generalized.
[4] R, prior Raynaud's syndrome (20 yrs); A, abdominal pain; D, diarrhea; C, carpal tunnel syndrome; H, Heberden's nodes; Th, hyperthyroidism.
[5] NP, examination not performed.
[6] PF, prolonged fatigue (< 1 month); CFS, chronic fatigue syndrome.
[7] H, homogenous; 300, titer of 300.

Patients with prolonged/chronic B19 fatigue. Of the 39 cases of acute B19 infection assessed at followup, 13 had persistent or relapsing fatigue for greater than one month (mean 21 mo, range 7–30) (Table 5). Of these 13 cases, 5 fulfilled diagnostic criteria for CFS — Patients 2 (woman aged 42 yrs at onset), 8 (woman 34 yrs at onset), 11 (woman 40 yrs at onset), 20 (woman 27 yrs at onset), and 32 (man 46 yrs at onset). B19 associated CFS had resulted in significantly reduced activity (occupational, social, educational, and personal) in these 5 patients, and symptoms included deterioration in memory/ concentration (5/5), sore throat (2/5), tender cervical/axillary lymph nodes (0/5), painful muscles (5/5), arthralgia (without swelling/redness) (4/5), new headaches (5/5), difficulty sleeping (5/5), unrefreshing sleep (5/5), postexertional malaise (5/5), increased tendency to sweat (3/5), dizzy spells (3/5), and blurred vision (3/5).

Regarding additional symptoms associated with chronic B19 fatigue, Patient 2 had had intermittent abdominal pain and diarrhea since the onset of acute B19 infection. This patient also complained of a sensation of heat in the soles of her feet and hot/dry eyes, although a Schirmer test at followup was normal. Patient 8 had a 26 month history of fatigue that

was complicated in the last 4 months by the development of hyperthyroidism, for which she had initially been prescribed propranolol and carbimazole; she was then maintained with carbimazole 60 mg/day. Patient 11 also had carpal tunnel syndrome. Carpal tunnel syndrome also occurred in Patient 6, with prolonged fatigue.

B19 markers, autoantibodies, and inflammatory markers associated with B19 associated CFS included B19 viremia (4/5), leukocyte B19 DNA (1/5), anti-B19 VP1/2 IgG (4/5), NS1 antibodies (2/5), RF (2/5), ANA (1/5), CRP (0/5), SAA (0/5). Although Patient 32 was positive for serum IgG to both VP1/2 and NS1 at acute infection, he was negative for both these antibodies at followup.

Table 6 shows the various clinical and laboratory markers for B19 infected patients at followup with no fatigue, prolonged fatigue, and CFS in comparison with controls. These data show that the prevalence of these clinical and laboratory markers (except leukocyte B19 DNA, for only 4 persons were positive) differs significantly between these groups.

DISCUSSION

B19 viremia is characteristic of acute B19 infection[14], and following acute infection, low titer B19 viremia may persist for months to several years[15,16]. In this study, B19 viremia was found more frequently in patients with arthropathy at both acute B19 infection and at followup, although this was significant only in the latter case. To our knowledge, this is the first documentation of such an association with B19 viremia and chronic B19 arthropathy. We have confirmed that acute B19 arthralgia is more common in women and in older age groups.

B19 infections are thought to be controlled predominantly by neutralizing antibodies and it is interesting that Patient 32 with B19 associated CFS was negative for anti-B19 VP1/2 IgG using 2 different test methods; this phenomenon has been documented with persistent B19 infection[17]. NS1 antibodies occur in 22% of healthy individuals with past B19 infection, and up to 80% in persons with persistent or prolonged B19 infection[18], and have been associated with acute[19] and chronic[20] B19 arthropathy, and persistent B19 infection associated with recurrent granulocytic aplasia, autoimmune hemolytic anemia associated with B cell leukemia, and pancytopenia, respectively[21]. In our study, NS1 antibodies were detected during the acute phase in 7 patients, but these were not associated with particular symptoms or markers. At followup, NS1 antibodies were detected in 16 persons and these were associated with chronic B19 arthralgia, as reported[20], although in the present study this did not reach significance. NS1 antibodies present at acute infection predicted NS1 antibodies at followup ($p = 0.0021$). There was a trend among those who were NS1 antibody positive at followup that those with NS1 antibodies at acute infection had a reduced incidence of arthralgia and/or fatigue at followup ($p = 0.11$), suggesting that these antibodies, if present early in the disease, may protect against development of arthralgia and/or fatigue at followup.

We examined extracted DNA from the EDTA anticoagulated blood cells from these patients at followup. Four patients were positive, 3 of which were also positive for serum B19 DNA and 3 of which had chronic B19 arthralgia. B19 virus is known to persist in the peripheral blood mononuclear cells, and sequence analysis of inverse PCR products from these cells has revealed highly recombined viral DNA, which was rarely juxtaposed with human chromosome, mechanisms that may contribute to *in vivo* viral persistence[22].

ANA at the time of acute infection predicted the occurrence of arthralgia at followup; however, it is unclear what possible role these antibodies may play in the pathogenesis of B19 arthralgia. It is interesting that anti-DNA antibodies are associated with systemic lupus erythematosus and vasculitis, both of which have been increasingly associated with B19 infection[3], and their detection in patients with early rheumatoid arthritis may predict increased severity of pain and higher risk of vasculitis[23].

Although this is a small study selected for severity of B19 infection (since these patients were symptomatic at the time of acute B19 infection), and including no subclinical infections and only one person aged < 10 years, our findings provide valuable insights into this infection and particularly into events during the years following acute infection. For example, after a mean of 22.5 months followup, this group of patients showed significant differences in symptoms, B19

Table 6. Clinical and laboratory findings in patients following B19 infection with and without fatigue and in healthy controls.

	Followup, No Fatigue, n = 26 (%)	Followup, Prolonged Fatigue n = 8 (%)	Followup, Chronic Fatigue Syndrome n = 5 (%)	Controls, n = 50 (%)	p, chi-square
Arthralgia	4 (15)	3 (38)	4 (80)	0	< 0.0001
Serum anti-B19 VP 1/2 IgG	26 (100)	8 (100)	4 (80)	37 (74)	0.0155
Serum anti-B19 NS1 IgG	10 (39)	4 (50)	2 (40)	8 (16)	0.0606
Serum B19 DNA	5 (19)	2 (25)	3 (60)	0	< 0.0001
Leukocyte B19 DNA	2 (8)	1 (13)	1 (20)	0	0.0729
RF	10 (39)	2 (25)	2 (40)	0	< 0.0001
ANA	5 (19)	0	1 (20)	1 (2)	0.0334
Increased CRP	3 (12)	2 (25)	0	0	0.0137
Increased SAA	3 (12)	3 (38)	0	0	0.0007

markers, autoantibodies, and markers of inflammation in comparison with 50 healthy controls (Table 3).

This study documents a 13% incidence of CFS following symptomatic, laboratory confirmed acute B19 infection, and the incidence of this complication is significantly increased from a previous estimate of 4%[5]. This may be due to inclusion of a questionnaire on fatigue and related symptoms in the present study. Although B19 associated CFS has been documented in case reports[5-7], we are aware of no other studies on B19 and CFS in which patients were followed from the time of detection of serum anti-B19 IgM. One report describes 3 patients with the related syndrome fibromyalgia, the onset of which coincided with acute B19 infection[24]. The only other study on B19 and CFS examined 7 CFS patients with mild hematological abnormalities (leukopenia, thrombocytopenia, anemia) for evidence of B19 infection; all bone marrow samples were negative for B19 DNA and one patient was positive for serum anti-B19 IgM[8]. In our study, no patient with fatigue at followup had any hematological abnormality.

Various additional syndromes occurred in patients with B19 associated fatigue. Arthralgia occurred in 6 of 13 patients with fatigue at followup and 4 of 5 patients with CFS, which is reminiscent of fibromyalgia. Carpal tunnel syndrome, which occurred in Patient 11 (with CFS) and Patient 6 (with prolonged fatigue) has been documented as a complication of B19 infection[25]. Patient 8 had a 4 month history of hyperthyroidism occurring 22 months after acute B19 infection and the onset of CFS. Although hyperthyroidism has not been reported in association with B19 infection to our knowledge, increased thyroid-stimulating hormone (TSH) levels have been documented in the CFS[26,27]. In addition, the NS1 of minute virus of mice (MVMp), a related autonomous parvovirus, activates the promoter of the human c-erbA1 gene, encoding the thyroid hormone (T3) receptor alpha. This promoter is a target for induction upon MVMp infection, and T3 increases cell sensitivity to parvovirus attack, suggesting an interconnection between T3 signaling and NS cytotoxic pathways[28].

We attempted to identify particular markers that might be useful to indicate that a particular case of CFS is associated with B19 infection. However, patients with B19 associated CFS did not have a characteristic marker profile; serum anti-B19 VP1 IgG (4/5), serum anti-B19 IgM (0/5), B19 viremia (4/5), leukocyte B19 DNA (1/5), NS1 antibodies (2/5), RF (2/5), ANA (1/5), CRP (0/5), SAA (0/5). In each case these markers were not specific for B19 associated CFS, and each of the 5 cases had widely varying profiles (Table 5). Therefore, at present, the only means to diagnose B19 associated CFS is to have a history of fatigue onset coincident with detection of serum anti-B19 IgM. Clearly, this is problematic, as the CFS case definition requires that symptoms be present for a minimum of 6 months, and unless serum taken at the beginning of the illness has been stored and is available for testing or the patient was tested and found positive for serum anti-B19 IgM at onset, it will not be possible to determine the possible involvement of B19 virus in the pathogenesis. It appears that it is not possible even to exclude a B19 viral etiology with antibody testing, as one patient with B19 associated CFS was serum anti-B19 IgG negative.

An infectious etiology in CFS is suggested by a frequently reported flu-like prodrome, chronic immune activation[29], and the fact that infection by several infectious agents has been shown to lead to a syndrome indistinguishable from CFS; these include Epstein-Barr virus[30,31] and *Coxiella burnetii*[32], the cause of Q fever. However, the essential predisposing factors to the development of CFS remain unknown[29]. We have identified an opportunity to study the host immune response to a common virus that is frequently asymptomatic and normally self-limiting, but which in certain cases is associated with chronic fatigue, in order to provide insights into the pathogenesis of the CFS.

In conclusion, a syndrome characterized by fatigue, arthralgia, sore throat, unrefreshing sleep, new headache, postexertional malaise, increased tendency to sweat, dizzy spells, and blurred vision may occur following B19 infection and may persist for several years. As prolonged B19 associated fatigue is associated with a varying B19 marker profile, it is not clear what the pathogenesis of this syndrome may be. Possible disease mechanisms include prolonged cytokine dysregulation as in the CFS[29], infection of central and/or peripheral nervous tissue, and a subclinical cardiomyopathy; B19 infection has been complicated by markedly abnormal cytokine profiles[33,34], meningoencephalitis[35], and myocarditis following *in utero*, childhood and adult B19 infections[36].

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