Figure 1 Changes in SLEDAI, proteinuria, and creatinine clearance after leflunomide treatment. Data are expressed as mean (SD). (A) The mean SLEDAI declined from 11.9 (4.8) to 5.4 (4.5) ($p=0.002$). (B) Proteinuria reduced significantly from 3.5 (1.3) g/day to 1.7 (1.5) g/day ($p=0.002$) for the whole group. A comparison of patients with proliferative and patients with membranous nephritis showed that proteinuria decreased significantly only in patients with membranous nephritis (proliferative nephritis: 3.3 (1.4) g/day to 1.9 (1.1) g/day; membranous nephritis: 3.7 (1.1) g/day to 1.3 (0.9) g/day, $p=0.000$). (C) The creatinine clearance rate remained stable and within the normal range (96 (37) ml/min at baseline v 96 (40) ml/min at the end of the study).

in 6/7 patients. Steroid dosages were reduced in 9/12 (75%) patients, with a significant reduction from a mean (SD) of 20.2 (9.4) mg/day to 11.6 (5.5) mg/day ($p=0.03$).

The obvious weakness in this study includes the relatively small numbers of patients who completed the trial, and the follow up was too short to examine the efficacy of leflunomide for long term renal preservation. In addition, our patients were a heterogeneous group with different renal histological classes who had been treated previously with multiple immunosuppressive agents for variable lengths of time. For these reasons, the possibility that the observed improvement, was due in part to corticosteroid or other immunosuppressant drugs given previously cannot be excluded in the absence of a control group. Another limitation includes the correlation of the clinical response with renal histological features that were obtained at a mean of 16 months before leflunomide treatment. Furthermore, repeat renal histology was not available after treatment. In conclusion, leflunomide is a safe and probably efficacious treatment in patients with lupus nephritis who do not respond or cannot tolerate conventional treatments.

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No evidence of parvovirus B19 in tissue samples from patients with polyarteritis nodosa and microscopic polyangiitis as assessed by the polymerase chain reaction

Z Sachetto, S C B Costa, P D Andrade, R A Conde, E M I Amstalden, A M Samara, S R M Fernandes

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Parvovirus B19 (B19) has been implicated in the aetiopathogenesis of some cases of systemic vasculitis, mainly giant cell arteritis.¹ A positive serological test for B19 has been reported in a few patients with polyarteritis nodosa (PAN).²⁻⁴

As far as we know no studies of B19 DNA in tissue samples of patients with PAN have been published, so we used a nested polymerase chain reaction (PCR) approach to screen for B19 DNA in fixed paraffin embedded tissue samples from

patients with PAN and microscopic polyangiitis (MPA). The diagnosis was based on the Chapel-Hill Consensus Conference guidelines.⁵ Only tissue samples from patients with vasculitis were used for DNA preparations.

DNA was extracted using a commercial kit (DNeasy tissue kit, Qiagen). A nested PCR for B19 was carried out using a previously published set of PCR primers.⁶ Positive and negative controls were included in every PCR run. A negative control consisted of a master mix without B19 DNA. DNA

Table 1 Demographic and clinical characteristics of the patients

Characteristics	PAN (n = 10)	MPA (n = 7)
Age at onset (years), mean (SD)	29.3 (15.1)	41.9 (18.5)
Sex ratio (M/F)	1.5/1	1/1.3
Weight loss	8 (80)	5 (71)
Arthritis	4 (40)	4 (57)
Myalgia	6 (60)	3 (43)
Fever	6 (60)	3 (43)
Skin lesions	8 (80)	3 (43)
Purpura	0	2 (29)
Nodules	8 (80)	1 (14)
Livedo	4 (40)	2 (29)
Peripheral neuropathy	5 (50)	4 (57)
Kidney	4 (40)	4 (57)
Renal failure	2 (20)	3 (43)
GI tract	4 (40)	3 (43)
Unexplained pain	2 (20)	1 (14)
Hemorrhage/bowel perforation	1 (10)	2 (29)
Mesenteric artery microaneurysms	2 (20)	0
Cholecystitis	1 (10)	0
Cardiovascular system	4 (40)	4 (57)
Hypertension	4 (40)	4 (57)
AMI	0	1 (14)
Lung	0	4 (57)
Infiltrate		2 (29)
Pleural effusion		2 (29)
Haemorrhage		1 (14)
CNS	2 (20)	0
BVAS, mean (SD)	15.7 (7.7)	20.7 (7.1)

Results are shown as No (%) unless stated otherwise.

PAN, polyarteritis nodosa; MPA, microscopic polyangiitis; GI, gastrointestinal; CNS, central nervous system; AMI, acute myocardial infarct; BVAS, Birmingham Vasculitis Activity Score.

extracted from the serum of a patient known to be infected with B19 was used as a positive control. Amplification of a sequence of the human β -globin gene was performed to ensure that amplifiable DNA was present in each specimen.⁷

Histopathological data of 17 patients (10 with PAN and seven with MPA) were evaluated. The specimens were

obtained from skin (11), bowel (three), kidney (two), and muscle, spleen and gallbladder from one patient each. In two patients two tissue specimens were analysed: skin and muscle in one and two skin specimens in the other. Table 1 shows the demographic and clinical features of the patients.

Skin lesions, peripheral neuropathy, and general symptoms, such as fever and weight loss, were the most common features. Renal disease was found in eight patients; five of them developed renal failure. Seven patients had gastrointestinal tract complications, such as haemorrhage and/or bowel perforation in three, cholecystitis in one, and unexplained abdominal pain in another three. Mesenteric arteriography performed in two cases disclosed multiple microaneurysms. One patient with MPA had lung haemorrhage. Renal arteriography done in six cases showed typical changes in three. At disease onset, the mean (SD) of the Birmingham Vasculitis Activity Score shows variable levels of severity of the disease. B19 DNA was not found in any of the samples evaluated (fig 1).

The detection of B19 DNA in biopsy specimens of temporal arteries suggested that B19 may have a role in GCA.¹ In addition, antineutrophil cytoplasmic antibodies have been found in patients with acute B19 infection, which suggests that this virus may be related to vasculitis.⁸ Our negative results, however, indicated that B19 is unlikely to play a part in the development of PAN and MPA.

The evaluation of a possible link between these conditions is important because it may change the treatment and the prognosis of many patients. Reports have been published showing that patients with the diagnosis of PAN and B19 acute infection only achieved remission of the disease after the treatment with endovenous immunoglobulin.⁹ We did not evaluate IgG and IgM anti-parvovirus antibodies in our patients, but Leruez-Ville *et al* found no B19 DNA in the sera of patients with PAN, even in those with serological evidence of B19 infection.¹⁰ The presence of specific IgM anti-parvovirus antibodies is not sufficient to prove that parvovirus B19 infection is a causal factor of PAN. Detection of viral DNA directly at the affected tissue is necessary.

Figure 1 Results of the polymerase chain reaction assay for parvovirus B19. M, molecular weight markers; C+, positive control (B19 DNA); 1, 2, 3, 4 and 5, patients' specimens and C-, negative control (mix without DNA).

Although finding B19 DNA in the arterial tissue would not be enough to establish a causal link between this infection and PAN, this search is an important step in clarifying a possible role of B19 in the aetiopathogenesis of PAN. Hence, the hypothesis that a chronically persistent B19 infection in the endothelium can trigger vasculitis is very unlikely to apply to PAN and MPA.

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CORRECTION

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Altered influenza virus haemagglutinin peptides inhibit T cell responses to type II collagen in rheumatoid arthritis (Xia L, Ru L, Zhanguo L. *Ann Rheum Dis* 2005;**64**:1790–1.)

We regret that the author list for this article was incorrect. The author names should be “Li X, Li R, Li Z”.

Corrections printed in the journal also appear on the *Annals website*
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