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### Diagnosis and therapy monitoring of Whipple's arthritis by polymerase chain reaction

SIR, Whipple's disease is a multisystem bacterial infection usually characterized by malabsorption syndrome with diarrhoea and weight loss, low-grade fever and lymphadenopathy. Arthritis is often the first sign of Whipple's disease and may begin years before typical intestinal manifestations occur. Examination of joint fluid and tissue might therefore provide an opportunity for an early diagnosis. Diagnosis of Whipple's disease is usually established by the presence of periodic acid–Schiff (PAS)-positive, rod-shaped inclusions in macrophages of biopsies of the small bowel and other involved tissues and/or the identification of bacteria by electron microscopy. The non-cultivable *Tropheryma whippelii* has been identified by the polymerase chain reaction (PCR) based on the 16S-rRNA gene sequence [1]. Recently, positive PCR tests either of synovial fluid (two patients) or membrane (one patient) were demonstrated in Whipple's disease patients with arthritis [2, 3]. Only in one patient with chronic and erosive arthritis PAS-positive macrophages were demonstrated in small bowel biopsies. There was no evidence of *T. whippelii* by PAS staining or by PCR testing in the upper gastrointestinal tract in the other two cases. No post-treatment examinations of joint specimens have been reported. Similarly, patients with Whipple's endocarditis negative in histopathology and by PCR in duodenal biopsies have been described recently [4].

We describe two middle-aged Caucasian males presenting with arthritis who were admitted for diagnostic needle arthroscopy. Synovial fluid and tissue as well as small bowel biopsies were *T. whippelii*-positive by PCR and became negative after 1 yr of treatment with

trimethoprim-sulphamethoxazole. Specific detection of *T. whippelii* DNA was performed using primer combinations TW-1/TW-3, resulting in a 141-base pair (bp) fragment, or TW-1/TW-2 followed by seminested reamplification with TW-4/TW-2, resulting in an amplicon of 229 bp [5]. Both patients became asymptomatic and no relapse occurred over a period of 22 and 18 months respectively. Neither by histology nor by electron microscopy were Whipple bacilli detected in joint and small bowel specimens. For PCR results see Table 1.

Patient 1 presented with an 8-yr history of arthralgia and intermittent arthritis of the small and large joints, which responded well to anti-inflammatory drugs. He had no gastrointestinal or neurological manifestations. Radiography revealed joint space narrowing of the knees but no erosions. Laboratory findings included an erythrocyte sedimentation rate (ESR) of 16 mm/h, C-reactive protein (CRP) of 37 mg/l, a normal blood cell count and a negative test for rheumatoid factor. Synovial fluid from the left knee showed 2100 leukocytes/mm<sup>3</sup>, mainly mononuclear cells; there were no crystals. Histologically, mononuclear infiltration was seen in the synovial biopsy, and stains for microorganisms, including PAS-positive organisms, were negative. All conventional cultures for bacteria remained negative. Because of *T. whippelii*-positive PCR results of joint specimens, a small bowel biopsy was taken by upper gastrointestinal endoscopy. No PAS-positive macrophages were demonstrated by light and electron microscopy but PCR was positive. Ten weeks after the onset of antibiotic therapy, the patient was asymptomatic and remained without relapse for at least 22 months after 1 yr of treatment with trimethoprim-sulphamethoxazole. In the follow-up examination after 1 yr of treatment, no synovitis was found and PCRs of synovial fluid, synovial tissue and small intestine biopsy were negative.

Patient 2 reported episodes of abdominal pain and diarrhoea without weight loss over the previous 6 yr. During the 8 months before admission for needle arthroscopy, he suffered from arthralgia and intermittent arthritis of the shoulder, wrist, knee and ankle joints. There was normal radiography of the joints, and

TABLE 1. Pre- and post-treatment results of PCR for *T. whippelii*

| Source of specimen     | Patient 1     |                | Patient 2     |                |
|------------------------|---------------|----------------|---------------|----------------|
|                        | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment |
| Joint                  |               |                |               |                |
| Synovial fluid         | +             | –              | +             | –              |
| Synovial tissue        | +             | –              | +             | –              |
| Gastrointestinal tract |               |                |               |                |
| Stomach                | n.a.          | n.a.           | +             | –              |
| Duodenum               | +             | –              | +             | –              |

Post-treatment, after 12 months of trimethoprim-sulphamethoxazole therapy; n.a., not available.

laboratory findings were normal, including ESR, CRP, blood cell count, stool cultures for bacteria and fungi, and special tests for parasitic infection. Synovial fluid contained 12 200 leukocytes/mm<sup>3</sup> with 75% polymorphonuclear cells, and the synovial tissue showed monocytic inflammation without PAS-positivity. Bacterial DNA was found by amplification in synovial fluid, biopsy material and duodenal biopsies. Its sequence was identical to that of *T. whippelii*. This result was confirmed by *T. whippelii*-specific PCR. The histology of the mucosa revealed non-specific inflammation without demonstration of *T. whippelii* by PAS staining or electron microscopy. Antimicrobial therapy with trimethoprim-sulfamethoxazole was given for 1 yr, with improvement of the gastrointestinal symptoms. PCR results of joint and intestinal samples were all negative after this time.

The availability of the DNA sequence of the 16S rRNA gene from *T. whippelii* has facilitated the development of relatively specific and sensitive diagnostic tests for Whipple's disease [1, 6]. This report underlines the usefulness of PCR in Whipple's patients presenting with arthritis. In the absence of convincing histological findings, PCR of joint samples or intestinal biopsies may be an important diagnostic tool. The presence of the pathogen in small intestine biopsies without histological confirmation was demonstrated by PCR in patients with a strongly considered diagnosis of Whipple's disease [4–7]. All except one presented with intestinal symptoms. Positive PCR results of duodenal samples and gastric juice, however, were found in about 5–12% of patients without clinical signs of Whipple's disease who were referred for elective gastroscopy [7]. The relationship of *T. whippelii* genetic material detected in the gastrointestinal tract to the pathogenesis and manifestation of disease remains to be investigated.

The arthritic presentation of Whipple's disease can be associated with the presence of *T. whippelii* DNA in synovial tissue and fluid. In our patients, no erosions were demonstrated by radiography despite an arthritic history over several years. The question whether the identification of Whipple's bacillus by histology or electron microscopy in synovial tissue relates to erosive changes remains open. Whether PCR of synovial fluid and tissue reveals equivalent results is still to be elucidated. In addition, PCR may be useful for monitoring the response to treatment. Post-treatment small bowel biopsies of five patients with no clinical gastrointestinal relapse were negative, whereas in the remaining seven of 12 patients with persistently positive PCR results, relapses or no response to treatment occurred [6]. Our results suggest that negative post-treatment PCR of synovial specimens may indicate cured disease. PCR may be the only positive diagnostic test in patients with Whipple's disease and it may be helpful in the follow-up.

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