CASE REPORT

Whipple's disease: comparison of histology with diagnosis based on polymerase chain reaction in four consecutive cases

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Abstract
Background—Polymerase chain reaction (PCR) based detection of species specific sequences of the 16S rRNA gene of *Tropheryma whippelii* is a recently described method for diagnosis of Whipple's disease.
Aims—Comparison of histology with PCR in mucosal samples of patients with Whipple's disease before, during, and after treatment. Detection of *T whippelii* in peripheral blood mononuclear cells as a non-invasive test for infection.
Methods—Four consecutive patients with histologically proven Whipple's disease were studied prospectively.
Results—In untreated patients biopsy specimens taken from regions with PAS positive macrophages gave a positive result with PCR for *T whippelii;* however, a PCR signal was also found in tissue biopsy specimens from mucosal regions with negative histology. In two of the patients the PCR performed with nucleic acids extracted from peripheral blood mononuclear cells was positive. After treatment with sulfamethoxazole/trimethoprim the PCR became negative after one month in two patients and after two months in the third patient treated, whereas PAS positive macrophages were found throughout the treatment period in two patients and disappeared in only one of them thereafter.
Conclusions—Detection of *T whippelii* specific sequences based on the PCR is useful to confirm the diagnosis, is able to detect a positive signal in samples taken from histologically negative mucosal areas, and can be used to monitor treatment. The PCR can sometimes be positive in peripheral blood mononuclear cells, but this cellular compartment cannot be taken as a substitute for duodenal biopsy specimens in the diagnosis of Whipple's disease.
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Keywords: Whipple's disease, intestinal lipodystrophy, PAS positive macrophages, *Tropheryma whippelii*, polymerase chain reaction diagnosis.

Whipple's disease is a multisystem bacterial infection associated with malabsorption, diarrhoea, weight loss, fever, adenopathy and polyarthritis.[1 2] Biopsy specimens from small bowel or lymph nodes disclose Gram positive bacteria in macrophages resistant to diastase treatment on periodic acid-Schiff staining. Both electron microscopy[3 4] or the detection of species specific bacterial ribosomal RNA (rRNA) gene by polymerase chain reaction (PCR)[5 6] can be used to confirm the diagnosis. Sequence analysis of the species specific gene of 16S rRNA of *Tropheryma whippelii* allowed it to be grouped as a member of actinomycetes.

Although the diagnosis is usually made by the demonstration of PAS positive macrophages in the lamina propria of the jejunum or duodenum, extraintestinal manifestation of the disease can be present even in the absence of histologically established involvement of the duodenum. The PCR, as a much more sensitive method than histology, should allow a diagnosis of Whipple's disease to be made in PAS negative biopsy samples. We report four prospectively studied consecutive patients with histologically proven Whipple's disease in whom we systematically biopsied duodenal and gastric mucosa at different locations for both histology and PCR based diagnosis. In addition, we performed follow up biopsies in these patients at regular intervals before and after initiation of antibiotic treatment. As the presence of *T whippelii* in blood cells has been described,[7 8] we also analysed peripheral blood mononuclear cells by PCR.

Methods

Patients
Four patients with histologically established Whipple's disease were investigated. Table I gives the clinical details. Three of the patients had PAS positive macrophages in the duodenal mucosa, whereas in one patient (patient 2) PAS positive macrophages and granuloma formation suggesting Whipple's disease was found in a biopsy sample of a mesenteric lymph node. Endoscopic duodenal biopsy did not show PAS positive macrophages in this patient, but PCR for *T whippelii* from this specimen was positive, confirming the diagnosis. Some of the clinical details and PCR findings of patient 1[7] and patient 2[9] have been reported previously.

TABLE I   *Clinical details of patients with Whipple's disease*

Patient	Sex	Age (y)	Symptoms	Clinical findings	PAS+macrophages in	Treatment	Outcome
1	M	58	Anorexia, malaise, recurrent athritis, night sweats	Lymphadenopathy, pleural effusion	Duodenal mucosa	Gentamycin+penicillin G for 2 weeks (iv) SMX-TMP	Complete recovery
2	M	50	Recurrent fever, neurological symptoms	Cachexia, hyperpigmentation, dyskinetic eye movements, jerks	Mesenteric lymph node, (duodenal mucosa negative)	Penicillin G+amikacin for 10 days (iv) ceftriaxone, SMX-TMP	Severe neurological sequelae
3	F	42	Fever, arthritic pain, neurological symptoms	Buccofacial dyskinesia, ophthalmoplegia	Duodenal mucosa	SMX-TMP	Slight neurological improvement
4	M	65	Weight loss, diarrhoea	Cachexia	Duodenal mucosa	SMX-TMP	Lost to follow up

SMX-TMP=sulfametoxazole (800 mg)/trimethoprim (160 mg) twice daily for one year; PAS$^+$=T whippelii specific DNA sequences of 16S rRNA gene detected in biopsy specimen.

Methods

Tissue samples – Four biopsy specimens were taken by grasp forceps during gastroscopy from each of the following sites: descending duodenum, superior duodenum, pyloric antrum, and body region of the stomach. Two biopsy specimens each were fixed in formalin, embedded, and stained with routine hematoxilin and eosin and periodic acid-Schiff stain. Two other specimens from the same locations were stored at –80°C until further processing. Peripheral blood was drawn before endoscopy and peripheral blood mononuclear cells were harvested using density gradient centrifugation and stored at –80°C. Follow up biopsies from the inferior duodenum were performed one, two, four, and nine months after start of antibiotic treatment, at 12 months (after end of therapy), and at 15 months. Duodenal tissues from two control patients, examined with the same washed and desinfected gastroscope immediately after a patient with Whipple's disease, gave negative PCR results for *T whippelii*. In 75 duodenal biopsy samples analysed for the presence of *T whippelii* by PCR no false positive finding was found.

PCR analysis – PCR was performed according to the method described by Relman *et al*[6] with some modifications. Two biopsy samples from the same location were always processed in parallel. Tissues or peripheral blood mononuclear cells was lysed in 4 M guanidium isothiocyanate containing 1% mercaptoethanol, dialysed overnight, and extracted by the standard phenol/chloroform/ethanol method. For PCR amplification a nested PCR protocol was used with confirmation of the specific product of 211 bp length by hybridisation with an internal digoxigenin labelled oligonucleotide.

Results

Diagnostic results before treatment

In the descending and superior duodenum PCR was positive from biopsy material in all four patients, whereas PAS positive macrophages were found in three of those patients only. One patient (patient 2) had no PAS positive macrophages in the lamina propria; in this patient Whipple's disease was histologically verified by PAS positive macrophages in a mesenteric lymph node. In biopsy specimens taken from the pyloric antrum both histology and PCR results were positive in patients 3 and 4, whereas patient 1 had a negative histology but a positive PCR. In patient 2 histology and PCR were negative. In the body region patient 4 still had a positive PCR but a negative histology; the other three patients were negative histologically and with PCR. The PCR for *T whippelii* was positive in peripheral blood mononuclear cells from patients 1 and 4, but negative in patients 2 and 3 (Table II).

Follow up

Three patients could be followed up for a year during antibiotic treatment. In patients 1 and 2 PCR from duodenal samples became negative after one month of therapy, in patient 3 after two months. Histology, however, continued to show PAS positive macrophages in two patients (1 and 3) over the treatment period. Patient 2, in whom PAS positive macrophages were found in a lymph node but not in the duodenal mucosa, was histologically negative throughout the study period. Three months after the end of treatment histology was positive in patient 1, but PCR remained negative. Patient 2 continued to have negative histology and PCR results. Patient 3 was tested after the end of the treatment period at 12 months and was negative by both histology and PCR. Patient 1 was positive for *T whippelii* in peripheral blood mononuclear cells by PCR at the time of diagnosis, but became negative after one month of antibiotic therapy. The other patient with *T whippelii* in peripheral blood mononuclear cells was lost to follow up during treatment.

Discussion

The extremely sensitive PCR method promises detection of microorganisms in tissues at a density below the detection limit of conventional methods such as histology. This was found in our patients with Whipple's disease, with positive PCR results in biopsy samples from PAS negative regions. Specimens taken systematically from the descending duodenum towards the stomach disclosed a declining

TABLE II   *Histology and PCR results from duodenum and stomach, and PCR from peripheral blood mononuclear cells (PBMC) at the time of diagnosis before treatment*

Patient	Descending duodenum		Superior duodenum		Pyloric antrum		Body region of stomach		PBMC
	Histology	PCR	Histology	PCR	Histology	PCR	Histology	PCR	PCR
1	+	+	+	+	–	+	–	–	+
2*	–	+	–	+	–	–	–	–	–
3	+	+	+	+	+	+	–	+	–
4	+	+	+	+	+	+	–	+	+

*Diagnosis was confirmed histologically by PAS positive lymph node. Histology+=PAS positive macrophages found in biopsy specimen; PCR+=T whippelii specific DNA sequences of 16S rRNA gene detected in biopsy specimen.

positivity rate for both histology and PCR; however, in three of four patients PCR gave a positive reaction at a location more proximal than the last PAS positive sample. We approached the question of contamination of samples with *T whippelii* via the forceps used by taking consecutive biopsy specimens from the duodenum and the fundus region of the stomach in one patient; the first was PCR positive, the second PCR negative suggesting that carry over by the endoscopical forceps was not influencing the results. In one of the patients PAS loaded macrophages were found in a surgically removed mesenteric lymph node only, with duodenal mucosal samples being histologically negative; in this patient the duodenal tissue was positive by PCR. This finding shows the clinical usefulness of PCR for diagnosis of *T whippelii* infection and confirms a similar finding by others.[10] Extraintestinal manifestation of Whipple's disease without histologically demonstrable PAS positive macrophages in the intestinal mucosa is well known.[11-14] The discrepancy between histology and PCR results could be explained either by a low number of bacteria in the intestinal mucosa, by a weak inflammatory response of the host not leading to a histologically discernible process, by a patchy pattern of the pathological lesions,[15] or by a predominant involvement of the submucosa and not the lamina mucosa.[16]

In two of our four patients *T whippelii* was found in peripheral blood mononuclear cells by PCR before treatment. One of these patients has been reported previously.[7] The occurrence of *T whippelii* in peripheral blood cells has also recently been shown in two splenectomised patients[8] using DNA extracted from whole blood and stored slides of peripheral blood. It is noteworthy, however, that only two of our patients with Whipple's disease had a positive PCR in peripheral blood mononuclear cells; this makes it clear that peripheral blood mononuclear cells cannot be taken as a substitute for mucosal biopsy specimens from small intestine in the diagnosis of Whipple's disease.

Follow up studies under therapy with sulfamethoxazole/trimethoprim disclosed that duodenal mucosa becomes negative by PCR within a short time period. In contrast to this rapid response PAS positivity in the mucosa persisted for at least nine months and was demonstrable in one patient three months after end of the one year therapy. Earlier reports using electron microscopy have also shown that the bacteria disappear soon after initiation of treatment, whereas PAS positive macrophages remain in the mucosa for a long time.[17 18] In one reported patient using PCR the reaction was found to be negative after 15 months of therapy while PAS staining of mucosal biopsy specimens was still positive.[19] The PCR might be a simpler and now more readily available technique for monitoring treatment effects than electron microscopy. However, it is unclear at present whether a negative PCR from mucosal samples of the small intestine should be used as a guide to end treatment.

Detection of *T whippelii* based on PCR is useful to confirm the diagnosis, is able to detect infection with *T whippelii* in histologically negative mucosal regions, and can be used to monitor therapy. The PCR is sometimes, but not always positive in peripheral blood mononuclear cells; testing this cellular compartment is therefore no substitute for the more invasive duodenal biopsy in diagnosing Whipple's disease.

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