Primary Diagnosis of Whipple Disease Manifesting as Lymphadenopathy

Use of Polymerase Chain Reaction for Detection of Tropheryma whippelii

Serhan Alkan, MD,1 Theodore F. Beals, MD,2 and Bertram Schnitzer, MD3

Key Words: Tropheryma whippelii; Whipple disease; Lymph node; Polymerase chain reaction; PCR; 16S ribosomal RNA

Abstract

Whipple disease is a rare, chronic multisystem disease associated with the recently characterized organism Tropheryma whippelii. Extraintestinal manifestation involving the central nervous system, heart, and joints occasionally occurs. Involvement of the abdominal lymph nodes, especially the mesenteric and periaortic nodes, is not uncommon. However, peripheral lymphadenopathy as the sole clinical manifestation of Whipple disease is rare. We describe 2 patients with Whipple disease whose initial manifestation was lymphadenopathy. Lymph nodes from both patients showed infiltration of the sinuses by macrophages containing periodic acid–Schiff–positive, diastase-resistant, sickle-like structures. Electron microscopic evaluation confirmed the presence of rod-like organisms. DNA from each sample was amplified by the polymerase chain reaction using a specific set of oligonucleotide primers developed against the 16S ribosomal RNA coding sequence of T whippelii. The histopathologic features and differential diagnosis of lipogranulomatous lymphadenopathy secondary to Whipple disease, as well as use of molecular-based assays, are discussed.

Whipple disease is a systemic illness involving the gastrointestinal tract and mesentery, as well as the central nervous system, heart, and peripheral lymph nodes. Rarely, peripheral or mesenteric lymph nodes may be the only diagnostic histologic material at clinical manifestation of the disease. Periodic acid–Schiff (PAS)–positive, diastase-resistant, sickle-like particles commonly are demonstrated within the cytoplasm of the histiocytes. Traditionally, the histologic diagnosis had been confirmed by electron microscopic examination to demonstrate the presence of organisms. However, the electron microscopic features of Tropheryma whippelii are not diagnostic by themselves.

The diagnostic use of polymerase chain reaction (PCR) amplification in diagnostic microbiology is expanding. Particularly, the PCR assays combined with DNA sequencing provide the opportunity for phylogenetic analyses of nonculturable bacteria at the ribosomal RNA (rRNA) level. Oligonucleotide primers to amplify selective targets of a ribosomal sequence of 16S rRNA genes have become useful techniques for the identification of organisms that usually are difficult to culture. Sequence analysis of 16S rRNA also is finding wider application in the detection of organisms in routine analysis of microorganisms for infectious disease. The 16S rRNA sequence of the bacillus in tissues with Whipple disease revealed identification of a new organism closely related to actinomycetes, T whippelii. Since the characterization of T whippelii, PCR assays for detection have been increasingly sought for confirmation of histologic or clinical suspicion of Whipple disease.

We describe 2 patients with the initial manifestation of lymphadenopathy due to marked involvement by Whipple disease. The histologic features and differential diagnosis of
Whipple disease manifesting as lymphadenopathy are discussed. In addition to providing a description of the histopathologic features of Whipple disease, we also demonstrate a modification in the PCR amplification of the 16S rRNA sequence of *T. whippelii* by PCR to confirm the lymph node involvement by Whipple disease.

**Materials and Methods**

**Case Reports**

**Case 1**

A 52-year-old man with a 5-year history of neck and shoulder arthralgias sought care. He also had a 3-month history of diarrhea, fatigue, malaise, night sweats, and intermittent confusion. Physical examination revealed no peripheral lymphadenopathy or organomegaly. Laboratory evaluation showed hypoalbuminemia and iron deficiency anemia. A computed tomography scan of the abdomen showed retrocrural, retroperitoneal, and mesenteric lymphadenopathy. An exploratory laparotomy with biopsy of mesenteric lymph nodes was performed.

**Case 2**

A 38-year-old man sought care because of chronic abdominal pain unrelated to meals. He complained of malaise, weight loss, and some degree of joint pain. Physical examination revealed slight inguinal lymphadenopathy and no organomegaly. Laboratory evaluation revealed iron deficiency anemia. A computed tomography scan of the abdomen showed massive intra-abdominal and retroperitoneal lymphadenopathy. An exploratory laparotomy with retroperitoneal lymph node biopsy was performed.

**Microscopic Examination**

Lymph nodes from each case were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were stained with H&E, PAS with and without diastase digestion, Fite, and Ziehl-Neelsen stains. For ultrastructural study, fresh tissue was fixed in 3% buffered glutaraldehyde, postfixed in 1.5% osmium tetroxide, and then embedded in resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

**DNA Isolation**

Paraffin-embedded lymph nodes were cut on a standard microtome, using a new disposable blade for each case. Five 10-µm sections from each case were placed in an Eppendorf tube. These sections were deparaffinized twice with xylene for 5 minutes, followed by an ethanol wash. After a centrifugation at 5,000 rpm for 2 minutes, the precipitate was resuspended in a lysis buffer containing a 10-mmol/L concentration of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 8.3), a 100-mmol/L concentration of potassium chloride, a 2.5-mmol/L concentration of magnesium chloride, 1% polysorbate 20, 1% NP-40, and 10 µg/mL proteinase K. Following overnight proteinase K treatment at 37°C, DNA was extracted with phenol-chloroform treatment, and then ethanol precipitated by standard methods. For comparison, a portion of the tissue lysate was ethanol precipitated directly without a preceding phenol-chloroform extraction. Isolated DNA was quantified by optical densitometry.

**PCR Amplification and Hybridization**

Oligonucleotide primer sequences for the Whipple disease organism were pW3FE (5’-GGA ATT CCA GAG ATA CGC CCC CCG CAA-3’) and pW2RB (5’-CGG GAT CCC ATT CGC TCC ACC TTG CGA-3’). As a procedural control, aliquots of template DNA also were amplified using human beta-hemoglobin gene primers.

One microgram of template DNA was incubated in a 50-µL reaction mixture containing a 2-mmol/L concentration of magnesium chloride, a 10-mmol/L concentration of Tris-hydrochloride (pH 8.3), a 50-mmol/L concentration of potassium chloride, a 200-µmol/L concentration of deoxyribonucleotide triphosphate, a 200-nmol/L concentration of each oligonucleotide primer, and 1.5 U of *Taq* polymerase. After an initial 4 minutes of denaturation at 95°C, the samples were subjected to 30 cycles of amplification in a thermocycler (Perkin-Elmer Cetus, Norwalk, CT). Each cycle consisted of denaturation at 94°C (1 minute), annealing at 60°C (1 minute), and extension at 72°C (2 minutes). A 15-µL aliquot of PCR product was analyzed by electrophoresis on an ethidium bromide–stained 1.2% agarose gel. Water, human placental DNA, 2 lymphoid tissue samples with reactive hyperplasia, and DNA from a *Mycobacterium avium-intracellulare* (MAI)–infected lymph node were used as negative controls. In addition, DNA samples of benign lymph nodes from 8 colon resection specimen were used for PCR evaluation. A biopsy of involved small intestine from a patient with previously diagnosed Whipple disease was used as a positive control.

Small aliquots (5 µL) from each PCR amplification were spotted onto a nylon membrane for subsequent hybridization. The membranes were then hybridized with a T4 kinase end-labeled *T. whippelii* specific probe (5’-ATA CCG ACC TTG CGG GGG GCG TAT CTC TAC GCC TTT CCG GTA TAT A-3’). The membranes then were exposed to X-ray film for detection of PCR amplification products specific for *T. whippelii*. 
Results

Lymph nodes from 2 patients showed similar yet slightly different histopathologic changes. The common findings were marked distention of sinuses with a diffuse histiocytic infiltrate. The paracortex and medullary areas also were largely replaced by the histiocytic infiltrates, and a few smaller residual germinal centers were present within the cortex. In case 1, there also were many variably sized lipid vacuoles within the sinuses Image 1 and rare foreign body–type giant cells admixed with the histiocytic infiltrate. Overall, histologic features were reminiscent of a lymphadenopathy secondary to postlymphangiogram-related changes; however, this possibility was excluded by the clinical history and the presence of a histiocytic infiltrate that reacted with a PAS stain (Image 1). There was no evidence of well-defined granuloma formation or necrosis in either patient.

The lymph node from case 2 showed an extensive histiocytic infiltrate but no lipid vacuolation Image 2. There were foamy macrophages, but no lipid vacuoles and no granulomas, a picture reminiscent of a lymphadenopathy due to involvement by a histiocyte-storage disorder. Because these histologic features were atypical, they initiated cytochemical and electron microscopic studies. The PAS stain with diastase showed strong positivity due to sickle-shaped particles filling the cytoplasm of the histiocytes, while Fite and Ziehl-Neelsen stains for mycobacteria were negative. Electron micrographic evaluation showed macrophages with cytoplasmic phagocytic vacuoles filled with short, fusiform bacilli measuring up to 2 µm in length and up to 0.5 µm in diameter and typical trilamellar cell wall Image 3. Based on the histopathologic features, the patient underwent endoscopic biopsy of the small bowel, which showed an abundant histiocytic infiltrate containing PAS-positive particles within the lamina propria, thus confirming the involvement of the gastrointestinal system.

To demonstrate the presence of T whippelii, we isolated DNA from paraffin sections for both cases. For negative controls, DNA was extracted from a tonsillary tissue with lymphoid hyperplasia and a cervical lymph node with reactive hyperplasia. In addition, 8 benign mesenteric lymph nodes isolated as part of colon resection for colon cancer were used for PCR screening. DNA from a patient with AIDS with lymphadenopathy due to marked involvement by MAI infection also was used for comparison. Amplification of the formalin-fixed DNA from both patients yielded a PCR product of the size expected (284 base pairs) for T whippelii Image 4. This product was detected easily by visual inspection under UV light in the ethidium bromide–stained agarose gel analysis. DNA from a colonic biopsy with the clinical and pathologic diagnosis of Whipple disease was used as a positive control. The amplification products from both lymph nodes with Whipple disease and colonic biopsy revealed an identical molecular size. Although phenol-chloroform treatment provided highly pure DNA, omission of the phenol-chloroform purification

**Image 1** (Case 1) Histologic features showing classic findings of Whipple disease in a lymph node. A, Lipid vacuoles, histiocytic infiltration of sinuses (low power, ×40). B, A histiocytic infiltrate and rare giant cells (high power, ×400). Inset reveals histiocytes laden with periodic acid–Schiff–positive organisms consistent with Tropheryma whippelii.

**Image 2** (Case 2) Whipple disease. A, Histologic features of lymph node showing sinusoidal pure histiocytic infiltrate without evidence of lipid vacuoles or granuloma. B, Enlarged histiocytes reminiscent of a histiocyte-storage disorder (high power, ×400; inset, oil immersion, ×1,000).
did not affect the PCR amplification results. There were no nonspecific bands observed by visual inspection. All negative control samples, including DNA from tonsillar tissue and a lymph node with reactive lymphoid hyperplasia and an MAI-infected lymph node, yielded no PCR amplification product (Image 4). There was no amplification product detected in mesenteric lymph nodes (data not shown). The presence of amplifiable DNA was confirmed by the amplification of beta-globin in all these samples (data not shown). To further confirm that the PCR amplification products belonged to \( T. \) whippelii, amplification products were spotted onto a nylon membrane by a slot-blot assay. The

![Image 3](image3.png) Electron microscopic examination demonstrates typical curvilinear \( T. \) whippelii organisms (A) and typical trilamellar cell wall (B), a characteristic feature of this organism.

![Image 4](image4.png) A, Results of polymerase chain reaction (PCR) amplifications using primers specific for \( T. \) whippelii. B, Slot-blot hybridization of PCR products with a \( T. \) whippelii–specific probe. Lane MW, molecular size markers (→ indicates 310-base-pair [bp] molecular size); lane 1, water without template; lane 2, tonsil with reactive lymphoid hyperplasia; lane 3, cervical lymph node with reactive hyperplasia; lane 4, \( Mycobacterium \) avium-intracellulare–infected lymph node from a patient with AIDS; lanes 5 and 6, lymph nodes from 2 patients with Whipple disease described in the text; lane 7, gastrointestinal biopsy specimen from a patient with known Whipple disease. Note that the band (284 bp) represents the Whipple disease amplification product.
hybridization analysis demonstrated that the PCR products represented Whipple disease organism because they reacted with the \textit{T whippelii}–specific probe (Image 4).

**Discussion**

Whipple disease is a multisystem disorder that usually involves the gastrointestinal tract and also may affect the mesenteric, abdominal, and peripheral lymph nodes, as well as other tissues. The diagnosis is established most commonly by a biopsy of the small intestine, which commonly shows a marked histiocytic infiltration of the lamina propria.\textsuperscript{2,3,9} Histochemical stains show PAS-positive, diastase-resistant sickle-form particles representing the causative bacillus that can be easily demonstrated by electron microscopy. However, the electron microscopic features of the bacteria are not sufficiently distinctive to differentiate it from other organisms.

Targeting specific sequences of the 16S rRNA has been used for identification of organisms difficult to culture and for identification of novel organisms. This technique also has been useful for classification of bacteria.\textsuperscript{17,18} Since all bacterial organisms have the 16S rRNA gene consisting of approximately 1,500 nucleotides and include highly conserved sequences, universal or broad-range primers can be designed to amplify the conserved region of 16S rRNA gene sequences. The use of species-specific PCR amplification with subsequent probe hybridization has been used for identification of different pathogens. Since PCR amplification and analysis of bacterial DNA in clinical specimens could be completed in a short time, it is considered advantageous compared with other classic methods, including bacterial culture. Since the regions of the 16S rRNA gene sequences are highly conserved among all bacteria, PCR amplification will allow identification of various pathogens. It is expected that the rRNA-based PCR amplification will find larger use in the near future for pathogen identification.

Even though many different organisms have been proposed previously as the etiologic agent,\textsuperscript{12,16,19-22} the nature of this bacillus had been poorly understood until recently, because attempts for bacterial cultures had been unsuccessful. Sequence analysis in highly variable parts of 16S rRNA was essential in initial identification of \textit{T whippelii}.\textsuperscript{14} Relman et al\textsuperscript{14} analyzed the putative organism in tissue affected by Whipple disease by using primers against the gene coding for bacterial 16S rRNA. Phylogenetic sequence analysis suggested that the bacillus causing Whipple disease belonged to the subdivision of gram-positive bacteria called actinomycetes and known as \textit{T whippelii}. Since then, analysis of rRNA has progressed, and it is predicted that completion of rRNA analysis of \textit{T whippelii} will provide new opportunities to develop diagnostic molecular assays.\textsuperscript{23} Recently, the growing body of evidence for \textit{T whippelii} being the causative agent for Whipple disease has been further supported by fulfilling the Koch postulate when this organism was cultured from a patient with this disorder.\textsuperscript{24}

The abdominal lymph nodes commonly are involved, and peripheral lymphadenopathy is seen in half of the patients with Whipple disease. However, owing to the atypical clinical manifestations of patients similar to the patients described herein, Whipple disease often is not clinically considered. Such cases were reported by Southern et al\textsuperscript{25} and Wilcox et al,\textsuperscript{26} who described patients with multiple nonnecrotizing granulomas in a lymph node biopsy specimens that originally were diagnosed as atypical sarcoidosis. Interestingly, all of the histochemical stains in both patients, including PAS, were negative. A third patient described recently by Turkington et al\textsuperscript{27} also had lipogranulomatous lymphadenopathy; however, the diagnosis of Whipple disease was not considered clinically or pathologically. Subsequent review of the original mesenteric lymph nodes at postmortem examination revealed PAS-reactive histiocytes, which led to a diagnosis of Whipple disease.

Lymph nodes may be the initial and/or the only material available for microscopic examination from patients with Whipple disease. Involved lymph nodes generally show numerous lipid vacuoles, an increased number of foamy macrophages with finely granular appearance, and granulomatous inflammation with or without foreign body–type giant cell formation. Both of the cases described herein demonstrate the importance of systematic and careful review of histologic features. However, it should be kept in mind that PAS stain analysis is not sufficient to exclude the diagnosis of Whipple disease, as discussed, and PAS staining may not react in rare cases, potentially leading to misdiagnosis or underdiagnosis.\textsuperscript{25,26}

Morphologic features in a typical case usually are suggestive of Whipple disease; however, these findings are not pathognomonic. A review of previous reports as discussed and the 2 cases presented herein suggests that the histologic findings of Whipple disease in lymph nodes vary. In classic cases, histologic examination typically shows lipid vacuoles and a histiocytic infiltrate with or without granuloma formation. The suggestive features of sinusoidal large lipid droplets and foamy macrophages may be minimal to consider the possibility of involvement by Whipple disease, as shown in our case 2. Atypical histopathologic presentation may include nonnecrotizing granulomas. Furthermore, MAI infection\textsuperscript{28} or other histiocytic infiltrates, including silicone lymphadenopathy,\textsuperscript{29} lipogranulomatosis, lymphangiogram effect, and storage disorders, can morphologically mimic Whipple disease. The mycobacteria in MAI infection are acid-fast and PAS positive, while \textit{T whippelii} is acid-fast.
negative. Rarely, histologic features of Whipple disease may mask features of sarcoidosis including lymph nodes in that well-formed nonnecrotizing granulomas are noted.\textsuperscript{25,26,30} PAS-positive macrophages also can be seen in healthy people, eg, secondary to mineral oil ingestion.\textsuperscript{31} Electron microscopic studies for evaluation of bacilli had been considered to be valuable but not absolutely specific for clinical confirmation. Furthermore, recent changes in the practice of pathology made electron microscopic analysis impractical for routine evaluation.

Although historically the diagnosis of Whipple disease was based on the histologic findings and electron microscopic demonstration of the bacterium, an increasing number of physicians are demanding confirmation of the diagnosis by molecular assays. Considering the long-term antibiotic microscopic demonstration of the bacterium, an increasing number of physicians are demanding confirmation of the diagnosis by molecular assays. Considering the long-term antibiotic administration needed for treatment of this disorder and the possible application for assessment of treatment response, molecular detection has become the choice for confirmation for the diagnosis of Whipple disease.\textsuperscript{9} Although other ancillary tests have been developed to detect the causative organism in Whipple disease, all of these assays lack specificity and sensitivity; thus, PCR seems to be becoming the test of choice for confirmation.\textsuperscript{9,16,32} Indeed, the use of molecular studies for detection of \emph{T whippelii} was essential for the diagnosis of Whipple disease in some patients with atypical clinical manifestations who lacked gastrointestinal involvement.\textsuperscript{35-35} These patients were diagnosed by molecular studies and responded to antibiotic treatments, which further supported the clinical diagnosis. However, the usefulness of a PCR-based assay without the demonstration of PAS positivity in an intestinal biopsy specimen remains controversial because \emph{T whippelii} also may be present in the gastrointestinal tract without the patient having clinical evidence of Whipple disease.\textsuperscript{36}

The specificity of PCR amplification detection for Whipple disease in gastric biopsy specimens has been reported with different results based on the PCR amplification method used. Many investigators have reported 100% specificity.\textsuperscript{32,37,38} Some reports demonstrated presence of PCR-positive material in the gastric biopsy samples in patients without clinical evidence of Whipple disease.\textsuperscript{36} However, contamination of DNA as a false-positive result has not been eliminated completely in these studies. Our experience suggests that it is unlikely that the Whipple organism is present in the lymph nodes hitherto considered to be highly specific for molecular detection of Whipple disease in lymph nodes with suspicion of this disorder.

By using a set of oligonucleotide primers specific for the Whipple disease bacillus, we successfully amplified \emph{T whippelii} in both lymph nodes affected by Whipple disease. Based on the PAS stain analysis, the lymph nodes in both patients contained a large burden of organisms. Data on the sensitivity of PCR detection of \emph{T whippelii} are scarce since the bacteria is difficult to culture; however, it is suggested that PCR-based assays can detect as few as 10 copies of the 16S rRNA gene.\textsuperscript{32,38} The PCR products were visualized easily in ethidium bromide–stained gels without the necessity of performing a hybridization step. Our study confirms the report of Relman et al\textsuperscript{14} and indicates that this technique is applicable to archival formalin-fixed tissues. In contrast with the original method, we directly used the pw3FE and pw2RB primers for specific PCR amplification of \emph{T whippelii} DNA and eliminated the use of initial amplification with consensus primers as originally described.\textsuperscript{14} We believe that the use of 1 set of specific primers usually is sufficient for evaluation by PCR when PAS shows marked presence of the bacillus. This also helps in decreasing the possibility of PCR contamination.

\textit{From the Departments of Pathology, 1Loyola University Medical Center, Maywood, IL; 2Veterans Administration Hospital, Ann Arbor, MI; and 3University of Michigan, Ann Arbor.}

Address reprint requests to Dr Alkan: Dept of Pathology, Loyola University Medical Center, 2160 S First Ave, Building 110, Maywood, IL 60153.

\textbf{References}


