Whipple's Disease

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Whipple's disease is an infectious disease caused by a gram-positive bacterium, *Tropheryma whipplei*. The first case was reported in 1907 by GH Whipple. Its classic symptoms are diarrhea and arthralgias, but symptoms can be various. Cardiac or central nervous system involvement, not always associated with digestive symptoms, may also be observed. For a long time, diagnosis has been based on duodenal biopsy, which is positive using periodic acid-Schiff staining. However, for patients without digestive symptoms, results can be negative, leading to a delay in diagnosis. For 10 years, a tool based on polymerase chain reaction targeting the 16S rDNA sequence has been used. In vitro culture of the bacterium, achieved 3 years ago, has allowed new perspectives for diagnosis and treatment. The natural evolution of the disease without treatment is always fatal. Current treatment is based on administration of trimethoprim–sulfamethoxazole for at least 1 year.

Introduction

Whipple's disease, first described in 1907 by GH Whipple, is a rare chronic infectious disease that is fatal in the absence of adequate treatment [1]. The disorder is caused by a bacterium, *Tropheryma whipplei* [2–6]. It is a multisystem disorder that mainly involves the intestinal tract, causing weight loss, diarrhea, and abdominal pains associated with arthralgias and lymphadenopathy [2–6]. The clinical manifestations can be diverse and nonspecific. Cardiac, ocular, and central nervous system (CNS) involvement has been described, and these manifestations are not systemically associated with digestive symptoms [2–6]. Thus, due to the varied manifestations of the disease, many clinical disorders can be considered as differential diagnoses. Classically, the diagnosis is obtained by a small bowel biopsy (most often duodenal) that shows the presence of positive inclusions using periodic acid-Schiff (PAS) staining [2–6]. Consequently, for patients without digestive symptoms, the diagnosis is often delayed due to negative small bowel biopsy. In the past decade, polymerase chain reaction (PCR) followed by sequencing has emerged as a promising tool for diagnosis [2–6].

For many generations of microbiologists, the culture of the microorganism responsible for Whipple's disease has been an elusive goal. Three years ago, the isolation and establishment of the first strain of *T. whipplei* has led to new perspectives, including bacterium characterization, sequencing of the genome, realization of a new immunohistologic tool for diagnosis, and in vitro antibiotic susceptibilities.

Disease Mechanisms

The epidemiology and physiopathology of Whipple's disease remain obscure. Whipple's disease is considered to be rare, but its actual level of incidence has never been estimated [7]. Racially, the disease primarily affects Middle-European and North American whites. Men with a mean age of 50 years are most frequently affected [7]. Few cases in the same family have been described [7]. No epidemic or interhuman transmission has been reported to date. A recent study suggested that disinfection of gastroscopes with a 2% solution of glutaraldehyde or peracetic acid for 20 minutes may be insufficient to prevent transmission of *T. whipplei* organisms on the instruments [8]. The source of *T. whipplei* and its transmission is not yet determined. *T. whipplei* DNA has been detected in human stool [4,9,10] and in sewage plants [11]. An oral route is suspected [2,12]. The hypothesis that humans are a reservoir for the bacterium cannot be excluded.

Several studies have attempted to evaluate the distribution of the bacterium in humans. *T. whipplei* DNA has been amplified from saliva, gastric fluid, and duodenal biopsy samples of people without Whipple's disease [13,14•,15•]. In addition, *T. whipplei* DNA has been retrieved in 35% of 40 saliva specimens from healthy subjects in England and in 4.8% of duodenal biopsies and 13% of gastric liquids from 105 patients in Switzerland without clinical suspicion of Whipple's disease [14•]. These data suggest that *T. whipplei* may be a commensal bacterium of the gut, but confirmation by other investigators has not been established [12,16]. In reality, the frequency of positive samples for *T. whipplei* DNA in asymptomatic individuals depends on the geographic origin of the patients. This hypothesis is supported by the results of a new study showing the presence of *T. whipplei* DNA in the saliva of healthy Swiss subjects but not in the saliva of Malaysian people without suspicion of Whipple's disease [4]. A new study performed by a Swiss team has shown the presence of *T. whipplei* DNA in

two saliva specimens among 215 (1%) healthy Swiss subjects [17]. This study also showed the presence of *T. whipplei* DNA in five saliva specimens among 46 (10.9%) patients with gastroesophageal reflux disease, suggesting that the stomach may be a habitat for the bacterium [17]. Another Swiss study showed the presence of *T. whipplei* DNA in the oral cavity of four of 10 healthy Swiss subjects and in three of nine Swiss patients with periodontitis but in none of 10 subjects from China. All positive samples were derived from bacterial plaques of the gingival region, whereas saliva smooth-surface plaque and samples from the tongue were negative. These data suggest that bacterial plaques of the gingival crevice and the gingival pocket may also serve as a habitat for *T. whipplei*.

The role of risk factors or different strains of *T. whipplei* is still discussed. Disturbances in the human immune system lead to symptomatic infection. Recent studies have demonstrated in several patients a diminution of the production of interleukin (IL)-12 and interferon (IFN)-γ by monocytes in acute Whipple's disease and in its remission phase [18].

After concurrent giardiasis was found in two patients with Whipple's disease, a recent investigation allowed diagnosis of giardiasis in four of 25 patients with Whipple's disease and in only two of 150 control subjects [19]. These data suggest a possible link between these two chronic intestinal diseases. The two diseases may be promoted by a common immune defect or source of infection, or infection with one may predispose to infection with the other.

The Agent of Whipple's disease

T. whipplei is a rod-shaped bacterium measuring about 0.2 to 2.0 μm. In the initial description by Whipple, microorganisms were detected using silver staining in tissues, but no link was made with the disease [1]. In 1949, PAS staining showed the presence of abnormal material in the intestine and lymphatic glands of patients with Whipple's disease [4,6]. This material was thought to correspond with mucopolysaccharides. In 1960, structures resembling bacteria were observed in intestinal biopsies using transmission electron microscopy, and these data were confirmed a year later [6]. The bacterial cell wall presented a trilaminar appearance that was not typical of gram-positive or gram-negative bacteria. In an effort to stain this bacterium, antibodies directed against *Shigella* and *Streptococcus* species were tested against intestinal biopsies from patients with Whipple's disease and shown to present a cross reactivity against *T. whipplei* [6]. In 1991, based on 16S rDNA PCR and phylogenetic analyses, the bacterium was placed within the gram-positive bacteria with high gram-positive cocci [20]. *T. whipplei* is grouped within bacteria found mainly in the environment, but it is also found within human-associated bacteria such as *Rothia dentocariosa*. Bacteria of the *T. whipplei* species have shown genetic heterogeneity based on the sequence of the 16S–23S rDNA spacer and the 23S rDNA [21–23]. The genomic variants have not yet been linked to different clinical manifestations or to a particular geographic distribution. *T. whipplei* has a single circular chromosome and a small genome size (926 kb). Full genomes from two strains of *T. whipplei* have been sequenced [24••,25••]. In vitro, *T. whipplei* can multiply in human fibroblasts, monocytes, or HeLa cells in an acidic vacuole [26••,27••,28]. The bacterium grows slowly, and its initial doubling time has been estimated at 17 days.

Clinical Findings of Whipple's Disease

Historically, Whipple's disease has been considered mainly a disease of the digestive tract. In reality, it may affect many different organs, and 15% of diagnosed patients have no digestive symptoms [29]. The clinical manifestations of Whipple's disease are various and nonspecific. Arthralgias have been reported in 75% of patients, involving peripheral joints. These arthralgias are migratory and neither destructive nor deforming [7,30]. Thus, the diagnosis of rheumatic disease is frequently suggested before diagnosis with Whipple's disease. At diagnosis, weight loss, diarrhea, and abdominal pains are the most frequent symptoms [6]. Constipation instead of diarrhea is rare [31]. Hepatomegaly and splenomegaly are sometimes present [4,7]. Very rare cases of hepatitis and ascites have been observed [4,32]. Neurologic involvement has been reported in 6% to 43% of patients [2–7]. Some authors speculate that neurologic involvement is present in almost all patients with Whipple's disease.

Neurologic localization may correspond to a sanctuary of the bacterium responsible for relapse of the disease. Dementia, external ophthalmoplegia, and facial myoclonus correspond to the three classic signs [7,33]. The neurologic manifestations are in fact various [33]. Cephalagia, ataxia, personality changes, seizures, memory loss, somnolence, dysarthria, pyramidal signs, hypothalamic involvement, and hypothalamopituitary involvement have also been described [7,33]. More rarely, myopathies, myoclonus, meningitis, and parkinsonian syndrome have been observed in patients with Whipple's disease [33].

Cardiac involvement is observed in 35% of patients [34]. Cardiac manifestations are various and include hypotension, pericardic friction rub, cardiac murmur, nonspecific electrocardiogram manifestations, congestive heart failure, valvular insufficiency, coronary arteritis, myocardial fibrosis, myocarditis, pericarditis, and negative blood culture [34,35].

Ocular involvement has been estimated to occur in 2% to 3% of patients with Whipple's disease. The ocular manifestations have rarely been described in patients without digestive symptoms [7]. Blurred vision, uveitis, hemorrhagic retinopathy, papillar edema, optical atrophy, and keratitis have been observed [2,3,36]. Recently, *T. whipplei* organisms were isolated from the aqueous humor of a patient [37]. Neuro-ocular manifestations may include

ophthalmoplegia, supranuclear paralysis, nystagmus myoclonus, and ptosis [36]. An abnormal movement called oculofacial cervical myorhythmia has also been characterized. Some authors consider this symptom to be pathognomonic of Whipple's disease, but this possibility should be viewed with caution.

The presence of a cough has been described in approximately 50% of patients with Whipple's disease [4,6]. Peripheral lymphadenopathies are also present in approximately half of patients [4,6]. Cutaneous involvement is rare. Hyperpigmentation was often observed with late diagnosis of Whipple's disease [7], but it is rarely observed today. The presence of systemic granulomatous inflammation in adenopathy, liver, or spleen among patients with Whipple's disease (9%) may lead to confusion with the diagnosis of sarcoidosis [7]. Kidney involvement, such as glomerulonephritis and interstitial or granulomatous nephritis, is very rare, as is endocrine system involvement [7]. Despite improvement in diagnostic tools, most new case reports reflect the difficulty in diagnosis of this disease.

Diagnostic Strategies

The tools available for diagnosis of Whipple's disease are summarized in Table 1.

Nonspecific diagnosis

Blood

The hemogram may show hypochromic microcytic anemia and inconstant hyperleukocytosis [7]. Hypereosinophilia is sometimes observed [7]. An inflammatory syndrome may be seen [2,4,7]. Abnormalities of immunoglobulin levels are sometimes present [7].

Endoscopy

Pale yellow shaggy mucosa alternating with erosive, erythematous, or mildly friable mucosa are classically described on endoscopy of the postbulbar region of the duodenum or in the jejunum of patients with Whipple's disease. Patchy whitish-yellow plaques are sometimes observed [6]. Thus, biopsy samples should be taken from the proximal and distal duodenum or the jejunum.

Histologic study by specific staining

Histologic study by duodenal (or jejunal) biopsy using PAS staining was the first available tool used for diagnosis of Whipple's disease, and it is still considered the gold standard. The diagnosis is based on observation in the mucosa of spumous macrophages with purple material in PAS staining and lymphatic dilation. If involvement is sparse, several biopsies may be needed for diagnosis. Deep localization may be observed in the submucosa, and PAS staining may be considered negative if biopsies are too superficial. Depending on the observed clinical manifestations, other samples may be available for PAS staining to establish the diagnosis, including adenopathies, synovial biopsies, synovial liquid, bone marrow, cerebrospinal fluid (CSF), cardiac valves, muscle, lung, liver, and spleen [2,4,6].

However, PAS staining, considered for many years to be pathognomonic of Whipple's disease, is not fully specific. Patients infected with *Actinomyces*, *Bacillus cereus*, *Corynebacterium*, *Histoplasma*, *Mycobacterium avium-intracellulare*, and *Rhodococcus equi* species may also present with PAS-positive macrophages [2,3,7]. For alcohol-acid resistant microorganisms, the diagnosis of Whipple's disease is usually excluded by positive Ziehl-Neelsen staining. Patients suffering from histiocytosis, macroglobulinemia, or melanosis coli may also present with PAS-positive macrophages in their samples [2–4,6,7]. Results from colonic biopsies of patients with Crohn's disease may be confused, in rare cases, with Whipple's disease [6]. However, gastric or rectal biopsies are not adapted for diagnosis of Whipple's disease. Weakly PAS-positive macrophages may also be observed in the stomach or rectum of healthy people [4]. Granulomas composed of noncaseous epithelioid cells may be observed in the lymphoid tissue, gastrointestinal tract, liver, or lung in patients with Whipple's disease, but these granulomas may be PAS negative [2,4,6].

Other tools, such as immunochemistry or PCR, are useful for diagnosis of Whipple's disease. Diagnosis of Whipple's disease with PAS staining has several limitations. Diagnosis should not be based exclusively on the presence of PAS-positive macrophages, nor should it be completely excluded on the basis of negative PAS staining.

Electron microscopy

Electron microscopy may reveal the presence of bacterium in the macrophages in various biopsies and procedures involving aspirate fluid, including CSF, pleural effusion, synovial fluid, bone marrow, vitreous fluid, or gastric fluid [2]. The main limitations of this technique are its lack of sensitivity in comparison with histology or PCR and the absence of specialized apparatus in most laboratories. Electron microscopy is not a practical tool for diagnosis of Whipple's disease.

Specific diagnosis

Immunohistochemical diagnosis

With the culture of *T. whipplei*, rabbit polyclonal antibodies and mouse polyclonal antibodies directed against the bacterium have been synthesized, demonstrating good results [38,39]. Immunohistochemical diagnosis has been performed on various biopsies (duodenum, adenopathy, brain, and cardiac valve), on liquid punctures such as the aqueous humor, and on blood monocytes [26,27,37,39–41,42•]. This technique has several advantages. It can be performed retrospectively in previously fixed samples [42•]. This technique was able to detect *T. whipplei* in tissues of the first patient described by Whipple [42•]. It is a highly sensitive and specific tool.

Table 1. Tools for the diagnosis of Whipple's disease

Technique	Sample	Advantage	Inconvenience
Electron microscopy	Biopsy, aspirate fluid	None	Not available in all laboratories
PAS staining	Biopsy, aspirate fluid	Available in all laboratories Retrospective diagnosis	Bad specificity
Genomic detection	Biopsy, aspirate fluid Blood	Available in most laboratories	Specificity depends on sample
Immunodetection	Biopsy, aspirate fluid Blood	Good sensitivity Good specificity Retrospective diagnosis	No commercialized antibodies
Culture	Biopsy, aspirate fluid	New strain isolation	Specific technology
Serology	Serum	Noninvasive	Experimental

PAS—periodic acid-Schiff.

Molecular biology

Molecular diagnosis based on DNA amplification can be performed on liquid puncture (*eg*, CSF, articular liquid, or aqueous humor), on biopsy (*eg*, duodenum, adenopathy, cardiac valve, kidney, liver, spleen, brain, synovial), or with blood, saliva, or stool using primers targeting different genes of the bacterium [43]. Fresh samples are better than fixed samples because of partially degraded DNA with the use of formaldehyde [44]. DNA amplification of *T. whipplei* is usually done with 16S rDNA, the intergenic region 16S–23S, 23S rDNA, the gene *RpoB*, or the gene *hsp65* [43]. The advantage of PCR targeting 16S rDNA is that physicians do not always consider Whipple's disease in their differential diagnosis. The availability of the full genome of two strains of *T. whipplei* should allow optimization of PCR diagnosis [24••,25••]. If an amplified product is obtained with PCR, identification of the bacterium must be confirmed with sequencing techniques. Recent results indicate that PCR performed in intestinal samples or other specimens is more sensitive than histologic study [43].

As a technique, PCR is considered to be full of promise. Its main drawbacks are related to specificity. The first problem is inherent to the technique and concerns the possibility of contamination. Another problem is the divergent results observed in different treatment teams among patients without suspicion of Whipple's disease, depending on the tested samples, including saliva, gastric fluids, and duodenal biopsies [12,13,14•,15•,16]. Nevertheless, PCR has simplified diagnosis, mainly for patients with exclusively neurologic involvement, allowing diagnosis based on a CSF sample instead of cerebral biopsy. In addition, the number of reported patients with atypical Whipple's disease is increasing, and PCR may be the determining factor for diagnosis [4]. The use of two pairs of primers targeting two different genes is recommended for a definitive molecular diagnosis.

Culture

The culture of *T. whipplei* from various samples, biopsies, puncture fluids, or blood can be achieved with cell cultures on human fibroblasts with a minimal essential medium of 10% fetal calf serum and 2 mmol/L of glutamine incubated at 37° C in a 5% CO_2 atmosphere [27••,45,46]. Using this technique, nine isolates from various samples (four cardiac valves, two duodenal biopsies, one blood sample, one aqueous humor, and one synovial fluid) were obtained [46]. Three isolates (one from a duodenal biopsy, one from a cardiac valve, and one from a blood sample) were subsequently established for more than four passages. In addition, another strain of *T. whipplei* was isolated from CSF by another team using our protocol [24••]. The mean delay for the primary detection of *T. whipplei* from human samples is 30 days [46]. Also, culture of *T. whipplei* from samples that naturally contain bacteria, such as duodenal biopsies, requires addition of antibiotics before the cell culture. Decontamination of digestive samples using a mix containing colistin, amphotericin B, cephalotin, and ciprofloxacin, which targets the intestinal flora, did not impair isolation of *T. whipplei*. However, vancomycin should be excluded.

Culture is not now a practical tool for diagnosis of Whipple's disease, but obtaining several more strains may lead to a better understanding of the disease. The one great disadvantage of this method is that culture is only performed in specialized laboratories, making it necessary to improve the culturing technique for more efficient isolation and propagation of the bacterium.

Indirect diagnosis

Recent culture of the bacterium has allowed the first attempts toward serologic diagnosis in Whipple's disease. Two approaches have been used. The first approach, based on indirect immunofluorescence, has brought promising results [26••]. However, after several subcultures, antigenic modification of the bacterium has led to loss of specificity [6]. A second approach used the recombinant protein hsp65 with enzyme-linked immunosorbent assay, but no difference was seen between patients and control subjects in this study [47]. Whipple's disease serology remains experimental at this time.

Therapeutic Strategies

Before the use of antibiotic therapy, Whipple's disease was lethal. When treated, the disease can be cured. Treatment of Whipple's disease is based on observations in small patient groups and anecdotal experience. Several antibiotic agents have been used successfully against Whipple's disease, including penicillin, penicillin and streptomycin, chloramphenicol, tetracycline, and trimethoprim–sulfamethoxazole [7]. Until 1970, protocols were mainly based on tetracyclines. However, several cases of relapse involving the CNS despite prolonged treatment have shown the limits of tetracyclines [4]. These relapses and the hypothesis of asymptomatic involvement of the CNS have led to reconsideration of this treatment strategy and the use of antibiotics with good penetration into the brain [4]. Trimethoprim–sulfamethoxazole, which can reach a therapeutic concentration in the CNS even in the absence of meningeal inflammation, was subsequently chosen. The current relapse rate is estimated to be from 2% to 33% depending on the treatment [4]. Most often, the relapse is localized in the CNS, and its prognosis is poor. Several regimens start with intravenous antibiotic therapy for 2 weeks, followed by oral administration for up to a year with different antibiotics. The current recommendation is an oral regimen of trimethoprim (160 mg) and sulfamethoxazole (800 mg) twice a day, eventually preceded by intravenous administration for 14 days of streptomycin (1 g/d) and benzylpenicillin (penicillin G: 1.2 million U/d). The length of the treatment is from 1 to 2 years [48]. Short-term treatment has been proposed by some authors [4]. Treatment of longer duration is based on prudence in the absence of an indicator of the end of treatment and the presence of neurologic relapse. For patients who are allergic to trimethoprim–sulfamethoxazole or who do not respond to the first treatment, ceftriaxone, cefixime, and chloramphenicol have been successfully prescribed [2,4,49]. With the culture of *T. whipplei*, in vitro antibiotic susceptibility is possible. The bacteriostatic effects of fluoroquinolones against two *T. whipplei* isolates, and the DNA gyrase (GyrA) and intravenous topoisomerase (ParC) sequences, have been studied [50]. High ciprofloxacin minimal inhibitory concentration (4 μg/mL) and low levofloxacin minimal inhibitory concentration (0.25 μg/mL) were observed. The presence in *T. whipplei* GyrA and ParC sequences of mutations previously correlated with fluoroquinolone resistance led to speculation that *T. whipplei* may be relatively resistant to fluoroquinolones. Some patients present with a refractory evolution to antibiotics. The presence of a possible defect of cellular immunity in Whipple's disease has allowed new therapeutic perspectives for the treatment of such patients. IFN-γ has been used successfully in a patient with Whipple's disease that was resistant to antibiotics [51]. The efficacy of this strategy needs to be confirmed.

Follow-up

The follow-up for patients with Whipple's disease is not clearly established. With the initiation of adequate antibiotic therapy, patients improve rapidly. Diarrhea disappears in less than 1 week, whereas arthralgia and other symptoms abate in several weeks [6]. The neurologic manifestations may take longer. The intestinal mucosa recovers during the first weeks to months of antibiotic treatment. Duodenal biopsy 6 months and 1 year after diagnosis is sufficient if the clinical response is good. Antibiotic treatment can be stopped if PAS staining is negative. When PAS material is still present, treatment should be continued. A return to increased PAS-positive material indicates a relapse. PCR analysis quickly becomes negative after initiation of treatment. The use of PCR results to decrease follow-up should be viewed with caution. Even if PCR becomes negative a few weeks after treatment is started, this is not significant evidence that the bacterium has been eradicated.

Conclusions

The recent culture of the microorganism responsible for Whipple's disease has allowed new perspectives. However, the bacterium and the disease are still mysterious. The precise reservoir of *T. whipplei* needs to be identified. Characterization of precise risk factors and host predisposition is also necessary for a better understanding of the disease. The complete clinical range of the disease needs to be characterized as well. Atypical manifestations and benign forms may exist. In the future, immunochemistry of circulating monocytes, a noninvasive procedure, may facilitate diagnosis. Immunochemistry of embedded tissues is an important new tool that allows not only sensitivity and specificity but also retrospective diagnosis. With the availability of the full genomes of two different strains of *T. whipplei*, new PCR targets can be designed based on a rational approach and allowing better sensitivity and specificity. Development of serology should would also be helpful. In the future, data on in vitro antibiotic susceptibility will enhance therapy. Another hypothesis that should be tested is the possible effectiveness of a lysomotropic agent to alkalinize the macrophage vacuole in which *T. whipplei* resides.

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