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REVIEW

Clinical Manifestations, Treatment, and Diagnosis of *Tropheryma whipplei* Infections

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SUMMARY Whipple's disease is a rare infectious disease that can be fatal if left untreated. The disease is caused by infection with *Tropheryma whipplei*, a bacterium that may be more common than was initially assumed. Most patients present with nonspecific symptoms, and as routine cultivation of the bacterium is not feasible, it is difficult to diagnose this infection. On the other hand, due to the generic symptoms, infection with this bacterium is actually quite often in the differential diagnosis. The gold standard for diagnosis used to be periodic acid-Schiff (PAS) staining of duodenal biopsy specimens, but PAS staining has a poor specificity and sensitivity. The development of molecular techniques has resulted in more convenient methods for detecting *T. whipplei* infections, and this has greatly improved the diagnosis of this often missed infection. In addition, the molecular detection of *T. whipplei* has resulted in an increase in knowledge about its pathogenicity, and this review gives an overview of the new insights in epidemiology, pathogenesis, clinical manifestations, diagnosis, and treatment of *Tropheryma whipplei* infections.

KEYWORDS Whipple's disease, *Tropheryma whipplei*, PCR, histopathology, clinical manifestations, antibiotics, therapy

INTRODUCTION

Whipple's disease, first described by George Hoyt Whipple in 1907 (1), is a multisystemic chronic infectious disease. Although George Whipple described "silver-stained rod-shaped microorganisms" in the vacuoles of macrophages of affected patients, he did not think of them as the cause for the disease (1). Whipple considered the observed intestinal lipodystrophy to result from a disturbed fat metabolism (1). It was only when the first treatment with antibiotics in 1952 appeared to be successful that it was postulated that a bacterium might be the causative agent of this disease (2). In the 1960s, electron microscopy studies provided additional evidence for this hypothesis (3, 4). In the 1990s, specific segments of the bacterium's 16S rRNA could be PCR amplified, and after confirmation and extension of these data, the bacterium was tentatively named *Tropheryma whippelii* (Greek *trophe* for nourishment and *eryma* for barrier because of the resulting malabsorption) (5, 6). In 2001, the bacterium was officially renamed *Tropheryma whipplei* (7). *T. whipplei* is a rod-shaped, Gram-positive bacterium that was cultured for the first time in 1997 (8, 9). Until then, it was believed to resist culturing, which made it hard to characterize this pathogen (10–13). The human intestine is the only known natural niche of *T. whipplei*. Once in the intestinal mucosa, the bacterium is taken up by macrophages in which it then replicates (14). Although the disease is rare, *T. whipplei* is considered to be a bacterium that is common in the human gut, implying that only a small percentage of carriers actually become symptomatic (15–17). However, the assumption that carriers of *T. whipplei* are truly asymptomatic is based on little evidence and may result from a missed diagnosis of, e.g., gastroenteritis or other aspecific symptoms that are common in the general population. Many of the papers on pathogenesis, diagnostics, and treatment come from a single French group and their collaborators whose research efforts contributed greatly in the understanding of the pathogenesis and led to an increase of new knowledge on diagnosis and treatment (8, 11, 18, 19). This review will give a thorough overview of the clinical manifestations, diagnosis, and treatment of *T. whipplei* infections.

BACTERIOLOGY

Culture

One of the first studies where the bacterium was cultured *in vitro* used deactivated (using interleukin 4 [IL-4] and IL-10) mononuclear phagocytes (9). Although they could maintain the bacterium *in vitro*, this was not regarded as a true continuous culture, as the strain was not established and could thus not be made available to the scientific community (20). Also, to our knowledge, this work has never been reproduced. Later in 2000, the bacterium was successfully cultured in human fibroblast cell lines in the laboratory of Raoult et al. (7, 13). This first cultured strain of *T. whipplei* (named the Twist-Marseille strain) has been firmly established. and its genome has been completely sequenced (GenBank accession no. NC_004572) (8). While it is mentioned in one of the papers by this research group that others are able to reproduce their findings (21), to our knowledge, all peer-reviewed reports on successful continuous culture attempts from clinical isolates are by this French group.

Culture of the bacterium requires that the living eukaryotic host cell is present (13) as a consequence of the lack of specific metabolic pathways (8, 22, 23). In 2003, a synthetic medium (specific axenic medium) that contains amino acids and other essential components that *T. whipplei* is unable to synthetize was successfully used to culture *T. whipplei* in the absence of cells for the first time (22). This specific axenic medium has subsequently allowed the culture and the establishment of several strains of *T. whipplei* (22).

The initial *in vitro* growth experiments suggested a very slow replication of the bacteria with a doubling time of 18 days (13), which is even slower than the 18 to 54 h of phylogenetically closely related, slowly growing *Mycobacterium tuberculosis* (24). Since then, several studies have been performed, and from these studies, it became clear that on average, it takes at least 30 days to detect *T. whipplei* in these cultures (21, 25, 26). This slow replication rate of *T. whipplei* severely impairs routine culture-based diagnostics. Culture of *T. whipplei* is further improved by the use of axenic medium instead of cell culture medium, and this greatly simplifies the isolation of strains from patient samples (21, 25, 26). In addition, disinfection of patient samples by filtration and pretreatment with glutaraldehyde greatly improves the success rate (25). Culture of *T. whipplei* is complicated by the large numbers of commensal bacteria present in saliva and stool samples (7, 13, 27) that would infect and overgrow these bacterial cultures, thus rendering it almost impossible to reliably establish the presence of *T. whipplei* through culture of patient samples.

Microscopy

The initial electron microscopic studies on jejunal biopsy specimen materials from *T. whipplei*-infected patients revealed that the bacterium resides within the macrophages and has a cytoplasmic membrane enclosed by a thin cell wall, which is enclosed by the periplasmic membrane (28). This study wrongfully postulated that *T. whipplei* multiplies extracellularly and that bacteria observed in the macrophages were being degraded. A later study revealed that bacteria could be in human fibroblast cell lines and reported two different forms of *T. whipplei* (7): the first form being intracellular bacteria embedded within the vacuoles of infected cells and the second being extracellular forms where the bacteria lay in massive aggregates in the extracellular matrix. Among the extracellular bacteria, initially dividing cells were reported to be observed (7). This does not imply that *T. whipplei* is able to replicate outside the eukaryotic cell, as experiments with axenic media (13) showed that there is an absolute need for nutrients that are present only inside the eukaryotic cell. Hence, we believe that these extracellular bacteria represent a dormant form of *T. whipplei*. Phylogenetic studies show that the bacterium is Gram positive, but due to its Gram-variable nature, it may appear as Gram negative in Gram stains (7). Intracellular bacteria are best visualized using periodic acid-Schiff (PAS) staining (7). With these stains, individual rod-shaped bacteria will be visible within vacuoles, but large vacuoles with no bacteria will also be present. Alternatively, immunostaining can be used for the visualization of intracellular bacteria

(7). Ziehl-Neelsen staining can discriminate between intracellular *T. whipplei* and *Mycobacterium* spp., as both are positive with PAS staining (29), but only mycobacteria are positive with Ziehl-Neelsen staining. Obviously, in the case of a coinfection, it will be difficult, if not impossible, to distinguish mycobacteria from *T. whipplei* using only a simple microscopic stain.

Genome and Metabolism

The first partial sequences of *T. whipplei* were published in 1991 by Wilson and colleagues (5). In this study, the 16S rRNA gene was amplified by PCR with generic 16S primers on a duodenal specimen obtained from a patient with classic Whipple's disease. The PCR product was then sequenced, and it was found that it did not correspond to the 16S rRNA gene of any bacterial species known at that time (5). Based on the ribosomal DNA sequences, it was predicted that *T. whipplei* would probably belong to the *Actinomycetes*, a group that also contains other known pathogens, such as *Nocardia*, *Rhodococcus*, *Mycobacterium*, and *Corynebacterium* spp. This assumption was later confirmed in 2003 when the genome of the *T. whipplei* strain Twist was partially sequenced, and that of the reference strain TW08/27 was fully sequenced (8, 23). The total genome of *T. whipplei* strain TW08/27 has a GC content of 46% and a genome size of 925,938 bp and is thus much smaller than those of other actinomycetes which have genomes of approximately 2 to 10 Mbp (30). Also the GC content differs, as most other actinomycetes have a higher GC content ranging from 50 to 75% (e.g., *Streptomyces coelicolor* [72%], *Mycobacterium tuberculosis* [66%], and *Mycobacterium leprae* [58%]) (8, 23). The genome contains only a relatively small number of genes associated with energy metabolism; in particular, the biosynthetic pathways for essential amino acids are lacking in the bacterium (8, 23). This suggests that the bacterium is dependent on its host in terms of acquiring specific substrates for energy metabolism (8). It also implies a complex intracellular lifestyle, as the bacterium must acquire these substrates somehow while in the intracellular vacuoles of the macrophage. This complex intracellular lifestyle is further supported by the finding that a large number of genes (around 74%) are involved in the production of surface-associated molecules, suggesting immune evasion strategies (23). The lack of certain biosynthetic pathways and the presence of an abundance of genes involved in immune evasion strategies suggest an intimate interaction with its host. The published *T. whipplei* genomes show regions with a high degree of heterogeneity (31) and contain several systems resulting in antigenic and genetic variability. These systems include both phase variation and mechanisms for inducing specific genetic rearrangements and mutations (23). Moreover, several genes contain repetitive loci which allow the bacterium to rapidly undergo genetic variation of immunologic determinants, e.g., through phase variation and homologous recombination, thereby helping the bacterium to escape host immunity (8, 32).

EPIDEMIOLOGY

Incidence and Prevalence of Classic Whipple's Disease

Only a few studies on the prevalence and incidence rate of Whipple's disease were performed. It is noteworthy that classic Whipple's disease is a typical Caucasian illness that is very rare in the native Asian and African populations (33), but carriage is common in these latter two continents (34, 35). A recent study in northwestern Italy gives information about the prevalence and estimated it to be 3 of 1,000,000 (95% confidence interval [95% CI], 2.1 to 3.8) (36).

Based on studies on duodenal biopsy specimens, the annual incidence rate of classic Whipple's disease is approximately 12 new cases per year worldwide (33). This estimation was made before the introduction of PCR testing as a tool for diagnosis of Whipple's disease, so the real incidence is probably much higher (10, 37). Nowadays, the incidence rate has been estimated to be between 1 and 6 new cases per 10,000,000 persons per year worldwide (38).

Carriage of *Tropheryma whipplei*

Until 1999, the bacterium was found only in duodenal biopsy specimens sent in for microscopic analysis (39). With the introduction of PCR, it became clear that the bacterium could also be found in other samples (18, 40). Microscopy of biopsy samples is a costly and invasive method and therefore not frequently used for screening purposes and usually performed only to confirm the diagnosis in the case of a serious suspicion of infection. Carriage of *T. whipplei* was first suggested by Dutly and Altwegg (37), and since then *T. whipplei* DNA has been detected in stool, saliva, and biopsy samples from asymptomatic individuals by using PCR (18, 41–45). Upon the initial encounter with *T. whipplei*, most carriers develop a protective immune response that prevents further spread of the bacterium through the body or eliminates the bacterium completely (46–48). In spite of this immune response, carriage can last for several years, and over time, the infecting strain can even be replaced by a novel strain (44). Asymptomatic carriers of *T. whipplei* represent a large reservoir from which other humans might be colonized. The bacterium has been found in various samples, including saliva, urine, blood, cardiac valve, myocardium, synovial fluid, skeletal muscle, stool, skin, lymph node, lung, bronchoalveolar fluid, stomach, spleen, liver, larynx, small bowel, colon, maxillary sinus, cerebrospinal fluid, brain, and aqueous humor samples (18, 42, 43, 45, 49–51).

Human Carriage and Human-to-Human Transmission

While the incidence of classic Whipple's disease is low, *T. whipplei* is now recognized as a widespread bacterium, as in the general populations of Europe and Senegal, respectively, 48% and 72% carried antibodies against the bacterium (16, 17). Recent data suggest that the presence of *T. whipplei* in fecal samples from asymptomatic individuals varies between 1.5 to 4% (14, 18, 41–43) in the general population of Europe but that it can be up to 12 to 25% in specific populations like sewage workers, HIV-infected, and the homeless (43, 52, 53). Also, patients with cirrhosis were found to have a high carriage rate of 12.5% (52). A small proportion of the classic Whipple's disease patients with positive stool samples have positive saliva carriage as well, and viable bacteria have been found in saliva samples from carriers (25). Salival carriage was found to be 0.2% to 2.2% in sewage workers and 3.7% in the homeless. Interestingly, when saliva samples are positive, stool samples are generally positive as well (16–18, 43). This suggests that the bacterial load in stools is higher than in saliva samples and that the gut is the preferred niche of this bacterium. The amount of bacteria in fecal samples is also significantly higher in symptomatic patients than it is in carriers (16–18, 43), indicating that a high load is associated with symptoms of *T. whipplei* infections.

Relatives of chronic Whipple's disease patients have a higher chance of carrying the bacterium (16). It is unclear whether these family members have a higher rate of carriage because of human-to-human transmission or because they are infected by the same environmental source (16). Saliva and stool samples of these relatives have been found to be 8% and 31% positive, respectively (16). Eighty percent of relatives of carriers had positive stool samples, and 20% of relatives had positive saliva samples (16). This suggests that oral-oral and fecal-oral transmission with this bacterium occurs (25, 27). In a study on gastroenteritis in 2- to 4-year-olds, a clonal outbreak of *T. whipplei* was found, which indicates that it is circulating in the population or their direct environment (15). Also, in a study in Senegal, only three different epidemic genotypes were found (17). Finally, outbreaks of two different genotypes in homeless shelters in France have been reported (54). These three studies all suggest that clonal outbreaks of *T. whipplei* do occur.

It is assumed that the bacterium is acquired during childhood (12, 35). In Senegal, up to 75% of children of $<$4 years of age were found to carry the bacterium (12). By using quantitative real-time PCR (qPCR), a study in Laos showed a prevalence of 48% in the feces of children (35). In Ghana, PCR-based prevalence in stool samples of 534 children (aged 2 months to 15 years) was 27.5% using multiple qPCRs (55). Sewage and surface water have been shown to contain *T. whipplei*-specific DNA and are thus a possible environmental source of infection (39, 52). It has been postulated that based

on the results of analysis of the genome sequences, *T. whipplei* might form spores (8). Although these spores have never been observed, they may explain the long-term survival in the environment. Finally, in addition to the oral route of transmission, there is some evidence for a respiratory route, as *T. whipplei* was shown to be present in bronchoalveolar lavage (BAL) fluid samples of patients with pneumonia (26, 56, 57).

Nonhuman Sources of *T. whipplei*

Animals may be carriers of *T. whipplei*, although there is little experimental proof for this (12). Granulomatous colitis of dogs showing microscopic similarity to Whipple's disease has been found, but no specific microbiologic diagnosis was made (58). *T. whipplei* was not found in the stools of 127 monkeys or apes (43). Intestinal biopsy specimens from a small collection of domestic animals (24 pigs, 20 cattle, 19 chickens, 15 sheep, 14 cats, 13 dogs, and 10 horses) were also investigated and showed no positive results after analysis by PCR (37). Furthermore, in Senegal where the bacterium is highly prevalent, only 4 of the 1,002 environmental specimens (including domestic and synanthropic animals) tested positive for *T. whipplei* (34). Although it is commonly believed that there is no significant nonhuman reservoir, this cannot be definitely excluded without proper studies on this subject.

PATHOGENESIS

Predisposing Factors for Classic Whipple's Disease

Only a limited number of carriers develop Whipple's disease, so it is assumed that host, bacterial, and environmental factors may all contribute to the pathogenesis (11, 12). It has been postulated that subjects who do not develop a protective immune response are prone to development of classic Whipple's disease (59–61). Most patients are between 48 and 54 years old when they are first diagnosed (33, 37). The reason why the disease is more common in middle-aged individuals could be due to the fact that there is a substantial delay between the first symptoms of the disease and the final diagnosis (37). The prevalence is higher in men than in women with a ratio of 8:1 according to classic microscopic diagnosis (10, 33, 62). The fact that there are more male than female patients could be related to an X-linked predisposition, although this has not yet been unequivocally proven. It may be that males and females are equally susceptible for the disease but that the disease is less symptomatic in women because of immunologic differences, as is, e.g., seen in Barrett's esophagus (63, 64). Alternatively, males could have a higher exposure to the bacterium than females. Since the introduction of PCR-based diagnostics on *T. whipplei*, several studies in Germany have shown a relative increase in the prevalence in females from 13% to 20% (38, 45), which cannot be solely explained by the increased sensitivity of the detection method, especially as the overall incidence rate of Whipple's disease remained stable over the years (38, 45, 65). If this is a true increase in prevalence in females, it may be caused by a changed lifestyle resulting in an increased exposure of females to the bacterium.

Genetic predisposition. There is evidence for a genetic predisposition to *T. whipplei* infection. Genetic causes for the difference in susceptibility, recurrent infections, and some familial cases have been reported for *T. whipplei* (16, 66–70). Several immune-related genes have been shown to correlate with disease susceptibility. As described above, an X-linked inheritance pattern of regulatory genes involved in the expression of cytokines might be the responsible factor (71, 72). The contribution of this immunological defect is probably specific, as patients with Whipple's disease do not seem to suffer more often from other infections than the general population (33, 73). Human leukocyte alleles (HLA) (which are also important factors in the generation of immune responses) are other contributing risk factors, and in one study, 26 patients were positive for HLA DRB1*13 and/or DQB1*06 (16, 62, 69, 70, 74). The genetic cytokine profiles of 111 German patients and 22 Italian patients were analyzed, and these patients were all low secretors of transforming growth factor β1 (TGF-β1) and high producers of IL-4 (70). IL-4 is known to downregulate IL-12 and gamma interferon (IFN-γ) secretion (61), while TGF-β1 activates the Th17 subset (75). This immunological

background may be responsible for the diminished Th1 and Th17 reactivity, which is suggested to contribute to the transition from the initial infection to classic Whipple's disease (70). There is also evidence for an association with IL-16 polymorphisms and some other Th2 response genes that probably contribute to long-term carriage of *T. whipplei* (69, 70, 76).

Role of the immune system. Similar to other chronic infections, the immune system significantly contributes to the development of clinical manifestations. Transmission probably occurs via the fecal-oral route, and in the presence of a fully functional immune system, this results in a self-limiting asymptomatic colonization of the gastrointestinal tract. Typically, classic Whipple's disease is accompanied by massive infiltration of the intestinal mucosa with *T. whipplei*-infected macrophages (10, 14). The presence of the bacterium does not trigger an adequate protective intestinal inflammatory response in these patients (77). *In situ* hybridization studies revealed that in these patients, the bacterium is localized mainly in the deeper mucosa and to a lesser extent in the lamina propria near the villous tips of the intestinal wall (78). The bacterium is internalized by mucosal macrophages which then migrate to the deeper mucosa but seem unable to successfully kill the bacteria. This is due in part to a bacterium-induced decreased expression of CD11b by these macrophages leading to inappropriate antigen presentation by the macrophages and the dendritic cells (77, 79). This results in an immunological environment with increased expression of IL-10, CCL-18 (CC chemokine ligand 18), and TGF-β and low expression of IL-12 and IFN-γ (47, 59, 61, 75, 77, 80–82). In this environment, the macrophages that ingested *T. whipplei* suffer from impaired maturation of phagosomes and reduced thioredoxin expression, rendering them unable to kill the bacteria and present their antigens (83–85). As a consequence, there is insufficient differentiation of Th1 cells, which are required for an effective response against *T. whipplei* (47). Among other processes, the bacterium interferes with the differentiation of naive $CD4^+$ cells into Th2 cells, and an ultimate consequence of the bacterial manipulation of the immune system is that it can no longer eliminate the infection but in fact the bacterium uses it to multiply in macrophages and spread throughout the body (47, 61, 77, 81, 85, 86). In addition, there is an enhanced activity of regulatory T cells in both the gut and blood, resulting in a further increase of the local concentrations of TGF-β and IL-10, further augmenting the immunosuppressive environment (75). This is in line with our hypothesis that carriage results in the induction of pathogenic mechanisms even though patients may not experience or display clinical symptoms, and thus, that true asymptomatic carriage may not exist.

With an increased Th2 response, one would expect an increase in antibody production. Paradoxically, the titer of specific immunoglobulin M (IgM) and IgG in classic Whipple's disease patients is very low or almost absent compared to carriers who do not show symptoms (87, 88). This may be due to the bacterial antigen variation and/or masking from immunological detection, thereby allowing them to escape the humoral immune system (89). While IgM and IgG titers are low, the IgA titers in patients are usually much higher than in carriers (48). However, a diagnostic application based on these findings has thus far not been validated (48).

Bacterial Factors

Virulence. *T. whipplei* creates an anti-inflammatory milieu, thereby preventing an effective immunological response (75, 77, 81, 90). This leads to bacterial survival and replication and spread of the bacterium in the mucosa. Bacteria are then thought to travel through the lymphatic system via lymph ducts and lymphatic nodes and eventually end up in the blood circulation system, giving rise to systemic spread of the bacterium (78, 91). Only limited research has been performed on how *T. whipplei* is able to survive after it is phagocytized by macrophages (84, 86, 90, 92, 93). In a study where macrophages from type I IFN receptor-deficient mice were used, it was found that *T. whipplei* causes macrophages to undergo type I IFN-dependent apoptosis (93). This type I IFN response is the initial step of infected macrophages to induce apoptosis and is critical for bacterial replication and systemic spread of *T. whipplei* (93). The results of

another study where isolated human monocytes were used supported this finding, and the study concluded that apoptosis is induced through the extrinsic pathway (84). *T. whipplei* also induces IL-16 secretion by macrophages, mobilizing more macrophages and dendritic cells to the site of infection, thereby facilitating a further spread of the bacterium (83, 85). Furthermore, the bacterium creates a suitable niche for survival by inhibition of the phagolysosome biogenesis (92). It is suggested that this is achieved by acting on Rab5, a GTPase essential for phagolysosomal fusion events. Hereby, *T. whipplei* prevents the maturation from early to late phagosomes (90, 92).

Bacterial variation. Genetic variation within *T. whipplei* might contribute to disease manifestation, but thus far, no clear relation between genotype and clinical symptoms have been reported. Large parts of the genomes of the different strains were highly similar, and overall there was 99% identity at the nucleotide sequence level, but some local regions with high heterogeneity were identified (8, 23). The initial proof for the existence of local outbreaks with genetically closely related strains of *T. whipplei* was provided by several studies using multispacer typing (MST) (37, 94–96), a convenient sequence-based tool for typing strains in various genetic (sub)types (97). The first of these studies was relatively small and included data from only nine Swiss patients, but it provided the first evidence that there were discernible clusters of *T. whipplei* strains (94). When this study population was expanded, the existence of at least six different MSTs became manifest (96, 98). For a brief period, the data obtained from these studies even raised the question whether *T. whipplei* could be considered a single species with variants or whether it was six different, closely related species (37). It is now commonly accepted that *T. whipplei* is a single highly diverse species consisting of several variant subspecies (37). Subsequent studies evaluated the genetic variation of the bacterium based on comparisons of regions with highly variable genome sequences (HVGS) (15, 15, 31). The first of these studies used 4 HVGS from 10 carriers and 39 patients with Whipple's disease. They identified 24 new HVGS, but no correlation was found between the HVGS genotypes and clinical presentation (31). *T. whipplei* strains showed a high genetic diversity that did not correlate with geographical distribution or bacterial pathogenicity (31). However, all the patients tested in this study came from European countries except for one patient from Canada (31). To allow a simple designation of genotypes, Li et al. designed a straightforward genotyping system based on the sequences of four variable genomic sequences (31). They showed the presence of 24 different genotypes among 49 strains studied (31). The heterogeneity of the *T. whipplei* genome was subsequently confirmed by a study of 34 children with gastroenteritis in Marseille, France, as 12 new genotypes were detected (15). While there were many distinct genotypes observed, infections with type 1 and 3 are predominant, as they account for 35% of all European samples and caused small clonal outbreaks in central Europe (15, 54, 99). In a recent study, 72 different genotypes of *T. whipplei* have been found among strains from 191 positive samples from patients from central Europe (99). Studies in Senegal confirmed the heterogeneity of the genome, as 30 new genotypes of *T. whipplei* were found (17). Similar data come from a study in Laos where several distinct genotypes were detected among young children, and 21% of subjects carried genotype 2 strains that were frequently detected in Europe (35).

CLINICAL MANIFESTATIONS

The clinical presentation of Whipple's disease is highly polymorphic. There are four commonly recognized manifestations of infections with *T. whipplei* (Table 1) (15, 16, 26, 100, 101): (i) classic Whipple's disease; (ii) localized chronic infections, predominantly endocarditis; (iii) acute infections, e.g., pneumonia, bacteremia, and gastroenteritis; and (iv) carriage. All of these will be discussed in detail below.

Classic Whipple's Disease

The typical patient with classic Whipple's disease is a Caucasian male (73 to 87% of patients with the disease were male) 48 to 54 years old who has had initial intermittent arthralgia (73 to 80% of patients) or chronic digestive troubles with diarrhea (72 to 81%) and/or is suffering from weight loss (79 to 93% of patients) (11, 14, 102–104). However,

TABLE 1 Clinical manifestations of *Tropheryma whipplei* infection[a]

Classic Whipple's disease (% incidence)	Chronic localized infections[b]	Acute infections[b]
Weight loss (79–99)	Endocarditis	Gastroenteritis
Gastroenteritis (63–85)	Encephalitis	Pneumonia
Abdominal pain (23–60)		Bacteremia
Arthritis (20–83)		
Neurological symptoms (6–63)		

[a]See text for references.
[b]Values for relative incidence are unknown.

before patients display these characteristic signs, they often go through a long period (6 to 8 years) where they have only some aspecific symptoms (Table 2) (105).

Among the most common symptoms of patients with classic Whipple's disease are arthralgia, diarrhea, steatorrhea, weight loss, lymphadenopathy, abdominal pain, hypoalbuminemia, and anemia (Table 3) (10, 14, 62, 65, 102, 106). These symptoms tend to develop in three phases, the early, middle, and late phases. Patients in the early phase of the disease can have symptoms of infection, fever, arthritis, and arthralgia. Patients in the middle phase have symptoms like diarrhea and weight loss (Table 2). Most patients with Whipple's disease had arthritis, arthralgia, or other joint problems years before they were diagnosed with the disease (107). Every organ system can be involved in the late phase, but mostly the eyes, heart, and central nervous system are infected (64).

The first prodromal sign of classic Whipple's disease in 80 to 90% of the cases is seronegative arthritis and/or arthralgia, frequently accompanied by fever and elevated acute-phase reactants (14, 102, 108). This is why patients are often misdiagnosed with palindromic rheumatism (109). In any middle-aged male with joint manifestation where there is no effect of immunosuppressive treatment, Whipple's disease should be considered, even if there are no other typical symptoms (10, 108, 110). Other associated clinical manifestations like anemia and weight loss increase the likelihood of Whipple's disease, as they are not characteristic for palindromic rheumatism (109). It is important that clinicians are aware of this, because up to 50% of the patients with classic Whipple's disease were initially misdiagnosed and given antirheumatic agents like disease-modifying antirheumatic drugs (DMARDs), anti-necrosis factor alpha, glucocorticoids, or drugs that can enhance the spread of the infection and thus may have fatal consequences (102, 111). In retrospect, physicians should consider occult *T. whipplei* infections in patients treated for unrelated bacterial infections showing a surprising decrease in their previously unexplained arthralgia (112, 113).

Gastrointestinal manifestations. Gastrointestinal manifestations are the most prominent symptoms of Whipple's disease. The duodenum, jejunum, and ileum are affected in most patients with classic Whipple's disease. The liver, esophagus, and stomach can also be affected, but this is less common (114, 115). Most diagnoses are based on at least one symptom related to these organ systems (64). Diarrhea, abdominal pain, and steatorrhea are among the most frequent symptoms in classic Whipple's disease. Apart from these symptoms, hepatosplenomegaly, anorexia, nutritional deficiencies, cachexia, hematochezia, and malabsorption are also common (64). These manifestations can progress to a severe wasting syndrome (with significant weight loss,

TABLE 2 Most common disease course of classic Whipple's disease[a]

Symptom (%) for early phase (<6 yrs)	Symptom (%) for middle phase (6 to 8 yrs)	Symptom (%) for late phase (>8 yrs)
Intermittent arthralgia (73–80)	Chronic obstructive troubles with diarrhea (72–81)	Neurological symptoms (6–63)
Fever (19–54)	Weight loss (79–93)	
	Abdominal pain (23–60)	
	Lymphadenopathy (35–66)	

[a]See text for references.

TABLE 3 Frequency of clinical manifestations in six sets of patients with classic Whipple's disease

Characteristic or clinical manifestation	Value for characteristic or frequency of patients (%) showing the indicated clinical manifestation in the following study[a]:					
	Maizel et al. (166)	Fleming et al. (106)	Durand et al. (65)	Ojeda et al. (230)	Lagier et al. (102)	Gunther et al. (73)
Characteristic						
Publication yr	1970	1988	1997	2010	2010	2015
Study period	1950–1970	1954–1984	1967–1994	1947–2001	2000–2010	2002–2015
Country	USA	USA	France	Spain	France	Germany
No. of patients	114	29	52	91	113	191
Male (%)	88	79	73	88	83	77
Mean age (yrs) at diagnosis [range]	50 [1–83]	54 [34–70]	55 [20–82]	56 [23–79]	57 [33–80]	57 [31–84]
Clinical manifestation						
Weight loss	95	89	85	80	79	99
Diarrhea	78	75	85	63	71	76
Abdominal pain	60	NS	23	27	31	NS
Hyperpigmentation	47	54	15	25	NS	NS
Fever	38	54	19	23	34	26
Joint symptoms	65	82	83	20	78	68
Neurological symptoms	NS	43	21	16	22	24
Adenopathy	52	54	66	35	50	NS
Pleural effusion	NS	7	10	9	14	NS
Prior joint symptoms	NS	NS	67	58	NS	NS
Prior digestive symptoms	NS	NS	15	27	NS	NS
Chronic cough	NS	NS	2	3	NS	NS
Relapse	NS	NS	13	12	NS	NS

[a]NS, not specified.

fatigue, weakness, muscle atrophy, and loss of appetite) and abdominal lymphadenopathy (64). Although gastrointestinal involvement is common, it is not necessarily present, as endocarditis and neurological symptoms without gastrointestinal symptoms have been reported (116, 117).

Bone and joint manifestations. Arthalgia, arthritis, and/or spondylodiscitis are seen in the majority (Table 3) of patients with classic Whipple's disease, and they often present with unexplained polyarthritis (108). This explains the frequent misdiagnosis of Whipple's disease as inflammatory rheumatoid disease (102). G. H. Whipple stated that "the first symptoms were attacks of arthritis coming on in various joints" (1). In the early stage of the disease, the majority (about 75%) of the patients suffer from arthritis for an average of 6.7 years before the disease is diagnosed (108). The presentation of arthritis is usually according to the palindromic rheumatism pattern (108, 109), meaning that attacks come suddenly with acute signs of inflammation (e.g., redness, swelling, pain, and loss of function) common in multiple joints (109). Spondyloarthropathy and chronic destructive polyarthritis have been reported (108). Knees, ankles, and wrists are the most frequently affected joints, and in fewer cases (11 to 27%), elbows, hips, and shoulders may be affected (108). While relatively rare, spondylodiscitis is sometimes an initial symptom of Whipple's disease (110, 118, 119). Bone marrow involvement by *T. whipplei* is rarely looked into and may thus be more common than expected from the few cases described in the literature (120–123).

Neurological manifestations. Symptoms of the central nervous system (CNS) are the third most frequent manifestation of Whipple's disease (124) and range from 6 to 63% (Table 3) (33, 102, 125, 126). Depending on the location of the lesions, symptoms can be both central or peripheral and either isolated or multifocal. Symptoms associated with Whipple's disease range from abnormal movements (myoclonus, choreiform movements, oculomasticatory myorhythmia), hypersomnia, coma, ophthalmoplegia, cognitive impairment, frontal lobe syndrome, cerebellar ataxia, or upper motor neuron and extrapyramidal symptoms (127). It has even been suggested that there is a relation between *T. whipplei* infection and the development of Parkinson's disease (117, 128, 129), but it is unlikely that there is a causative relation. Although most Whipple's disease patients do not present any obvious neurological symptoms, postmortem investigation showed that 90% of brain and

spinal cord specimens from both patients and presumed carriers revealed lesions of the CNS, meaning that CNS involvement is more common than expected from the clinical manifestations (33). Another indication for frequent neurological involvement is the presence of *T. whipplei* DNA in the cerebrospinal fluid (CSF) of patients with classic Whipple's disease even in the absence of evident neurological manifestations (40, 130). The prognosis for patients with symptomatic central nervous system involvement remains poor, as major sequelae are seen in 25% of patients and 4-year survival rates are <75% (131). Early diagnosis and treatment are thus essential in these patients (124).

Cardiovascular involvement. Endocarditis can also occur in the classic form of Whipple's disease and is usually present only in the late phase (113). This manifestation is often preceded by arthralgia or arthritis without gastrointestinal signs (132). A case of predominant pleuropericarditis caused by *T. whipplei* with developing dense pleural fibrosis requiring decortication has recently been reported (133). Pleuropericardial involvement is a frequent pathological finding in patients with Whipple's disease, although it rarely results in clinical symptoms (133).

Less-frequent manifestations. Especially in the early phase of the disease, several less common symptoms of Whipple's disease can become manifest (Table 3) (120, 134–138). Dermatologists should be aware that both cutaneous (10, 139) and subcutaneous (140–143) lesions may be a manifestation of Whipple's disease, as cutaneous biopsy specimens containing PAS-positive macrophages characteristic of Whipple's disease have been reported (136). If lymphadenopathy, anemia, and pancytopenia are present, apart from lymphoma of other nonspecific granulomatous reticuloendothelial disorders, Whipple's disease has to be considered as well (120, 134, 144). Retroperitoneal pseudotumor formation was found in a patient with loss of appetite, weight loss, malnutrition, malaise, abdominal pain, diarrhea, vomiting, and intermittent fever (135). After negative diagnostic results for malignancy, Whipple's disease was confirmed as a diagnosis, and the patient was treated with ceftriaxone, resulting in clinical improvement and weight gain (135). There is an association between thrombocytopenia and *T. whipplei* infection, which probably results from peripheral platelet sequestration. This thrombocytopenia rapidly resolves upon treatment (138). Finally, ocular manifestations have been described (145), and crystalline keratopathy has been reported as a typical characteristic of ocular Whipple's disease (146).

Localized Chronic Infections

T. whipplei can also cause localized infections which do not develop into the classic form of Whipple's disease. In these cases, there is no systemic involvement of *T. whipplei*, and the stool and saliva samples tested by PCR and/or PAS-stained duodenal biopsy specimens may often be negative (18, 73, 147, 148).

Endocarditis. This form of endocarditis is an intracellular infection rather than a biofilm-like superficial cardiovalvular colonization with the bacterium. In most patients, there are no other manifestations of classic Whipple's disease (113, 149–152), and clinical signs are similar to those of cardiac disease with negative blood cultures (113). In spite of the negative blood cultures, 80% of these patients displayed an increased C-reactive protein level, indicative of an infection and vegetations in 79% of cases (113). The first reported localized *T. whipplei* endocarditis case was diagnosed by broad-range bacterial PCR analysis (153). As there are no evident diagnostic criteria, clinicians should be especially alert for a potential *T. whipplei* endocarditis when dealing with white men who are around 50 years of age with cardiac symptoms like heart failure, large vegetations, and destruction of the heart valve, acute ischemic stroke, and embolic events (103, 113, 154). Of all patients with blood culture-negative endocarditis, PCR revealed the presence of a *T. whipplei* infection in 1.9 to 6.3% of patients (103, 113, 155). These patients include 1,135 endocarditis patients who underwent cardiac surgery in two German university hospitals. In 255/1,135 (22%) of the cases, a bacterial cause could be established by a combination of molecular methods and culture. Surprisingly, 16/255 (6.3%) of the patients with a proven bacterial infection (1.5% of the total population) carried *T. whipplei*, making it the fourth most frequent pathogen identified

from these proven infectious cases (103). Follow-up of these patients revealed that most of these *T. whipplei*-positive patients displayed no symptoms of classic Whipple's disease and had thus neither been diagnosed nor treated properly for their potentially lethal *T. whipplei* infection.

In a study from 2004, the cardiac valves from five patients with *T. whipplei* endocarditis were histologically evaluated and compared to those of patients with endocarditis but without Whipple's disease. The histologic findings were significantly more fibrosis, a lack of calcification, slightly less vegetations, and reduced inflammation and vascularization compared to a control group with endocarditis (156).

Encephalitis. Several studies by a French group reported the clinical implications of isolated encephalitis caused by *T. whipplei* (127, 157, 158). The most common neurological symptoms are cognitive impairment, ataxia, and supranuclear ophthalmoplegia (157). A study from 2011 showed that ataxia and dementia were more severe in patients with encephalitis than in patients with classic Whipple's disease, making early recognition clinically important (157). Recently, a paradoxical association between *T. whipplei* encephalitis (as was documented by brain biopsy specimen) and obesity has been found in several cases (157). These patients suffered from unexplained progressive dementia that was in most cases associated with ataxia and recent obesity. These patients rapidly recovered from their neurological manifestations after antibiotic treatment, and surprisingly, the obesity also rapidly reversed. The combination of cerebellar syndromes, dementia, and obesity should therefore trigger physicians to consider a *T. whipplei* infection. Definite diagnosis is complicated, as it would involve obtaining brain biopsy specimens, but since empirical treatment was reported to rapidly resolve the clinical symptoms, one might consider starting treatment even in the absence of a final diagnosis (157).

Infections in other locations. *T. whipplei* has also been linked to interstitial lung disease, pulmonary hypertension, and other pulmonary manifestations (26, 56, 57, 159–162). Two recent studies of BAL fluid samples from hospitalized patients provided the best evidence that *T. whipplei* is a probable etiologic agent of pneumonia, as they found a total of 10 patients where *T. whipplei* was the only bacterium found (56, 163). While it was postulated that these infections resulted from aspiration (163), an infectious route resulting from translocation through the circulation system or direct *de novo* infection from the environment cannot be excluded at this time. Clinical respiratory symptoms include dry cough, chest pains, and shortness of breath (162). These symptoms often present without gastrointestinal symptoms (162).

Finally, osteoarticular involvement (102), uveitis (102, 164), and lymphadenopathy (102) have also been reported to result from *T. whipplei* infection.

Acute Infections

T. whipplei has also been associated with acute infections, including gastroenteritis, pneumonia, and bacteremia (15, 56, 101). As only limited data exist on these clinical manifestations, further research is required to determine the exact involvement of the bacterium in these acute infections.

Gastroenteritis. A 3-year study on the correlation between *T. whipplei* carriers and gastroenteritis was performed by Raoult et al. (15). PCRs on feces and Western blotting on sera for *T. whipplei* were performed for a cohort of children between 2 and 4 years of age with diarrhea in Marseille, France (15). The presence of *T. whipplei* was established in 36 (15%) of 241 children with gastroenteritis, and in 23 (64%) of these 36 positive samples, no other diarrheal pathogens were found (15). In addition, a study of 534 stool samples from children aged between 0 and 12 years from rural Ghana, it was found that children with diarrhea carried *T. whipplei* in their stool twice as often as controls without diarrhea (55). *T. whipplei*-infected patients usually have watery diarrhea, and periodically, they have colicky abdominal pain (165, 166). In another study, *T. whipplei* was associated with adult traveler's diarrhea in two of nine pilgrims, as *T. whipplei* DNA was found in rectal swabs of travelers suffering from diarrhea (167). These studies indicate that *T. whipplei* infections can cause diarrhea.

Pneumonia. *T. whipplei* was shown to be an etiological pathogen in pneumonia (56, 137). *T. whipplei* was found to be an etiological pathogen in pneumonia in a study where 210 BAL fluid samples obtained from patients in intensive care units were tested using both a generic 16S rRNA gene PCR and a specific quantitative PCR (56). A total of six (3%) of these samples contained *T. whipplei*, and in one of these samples, *T. whipplei* was in fact the only bacterium found (56). In a recent study, *T. whipplei* was shown to be the only bacterium present in BAL fluid samples from a patient with severe pneumonia (26). In addition, an unexpectedly large number of HIV patients carried high loads of *T. whipplei* in BAL fluid samples (137, 168). This provides further support that *T. whipplei* might be a causative agent in lung disease. Macrophages are the main target cell for *T. whipplei* infection, and as macrophages are abundant in the alveolar tissue, they might provide a suitable niche for *T. whipplei*.

Bacteremia. A study in Senegal where blood samples from patients with fever were tested using PCR showed a 6.4% presence of *T. whipplei* (101). Interestingly, cough and sleep disorders were significantly more present in patients with *T. whipplei* than those without *T. whipplei*. There was no correlation between the occurrence of the bacterium in saliva and stool samples and bacteremia (101). This suggests that *T. whipplei* infections can result in acute, self-limiting bacteremia (101).

DIAGNOSIS

Whipple's disease is frequently diagnosed at a late stage, because it is rare and has a broad spectrum of nonspecific clinical presentations, which makes it hard for clinicians to diagnose (10). In the early stage, often some of the typical manifestations may be not be present. The presence of the *T. whipplei* bacterium can be established diagnostically in various tissues and body fluids by several routine methods (40). The different laboratory methods to diagnose *T. whipplei* infections and the advantages and disadvantages of each diagnostic method will be discussed below.

Routine Diagnostic Methods

The most common routine diagnostic methods are histopathology and PCR, as these can be performed in most laboratories while cultivation of *T. whipplei* is still difficult and can be performed in only a few laboratories (Fig. 1) (107).

Duodenal biopsy. For most patients, the definite diagnosis is based on histological observation of *T. whipplei* bacilli in (proximal) small bowel biopsy specimens (10, 73, 169–171). Most patients with classic Whipple's disease usually have large numbers of bacteria in their duodenal mucosa, but this seldom results in an inflamed appearance of the duodenum during endoscopy (10, 73). The mucosa of the duodenum frequently has dilated villi with ectatic lymph vessels and a pale yellow color (10, 171, 172). As the bacterium is not evenly distributed over the duodenum, several samples of the duodenum should be obtained in order to avoid sampling bias in patients who do carry the bacterium in their duodenum. In addition, it is also advisable to obtain samples from the gastric antrum, jejunum, and/or ileum (10, 51, 62, 102, 111, 169).

Histopathology (PAS and hematoxylin-and-eosin stain [H&E]). Classic Whipple's disease is typically accompanied by histological lesions in the duodenum or other parts of the small bowel. Histological detection of the bacterium is mostly performed by using periodic acid-Schiff (PAS) stain (Fig. 2) (40). Although PAS staining is frequently used, it is not a very specific way to diagnose Whipple's disease, because in patients with infections caused by other bacteria, such as *Rhodococcus equi*, *Mycobacterium avium intracellulare*, *Corynebacterium*, *Bacillus cereus*, *Histoplasma*, or fungi, PAS-positive foamy macrophages can also be found (14, 173). Ziehl-Neelsen staining can be used to differentiate the non-acid-fast *T. whipplei* from acid-fast mycobacteria (174). Histologically, duodenal lesions contain foamy macrophages, which are located in the lamina propria (175). These macrophages contain many PAS-positive, Ziehl-Neelsen-negative, diastase-resistant inclusions. Gastrointestinal symptoms are negligible or not at all seen in approximately 10 to 15% of the patients, and duodenal biopsy specimens might be PAS negative in these patients (14, 176). Depending on the clinical manifestations,

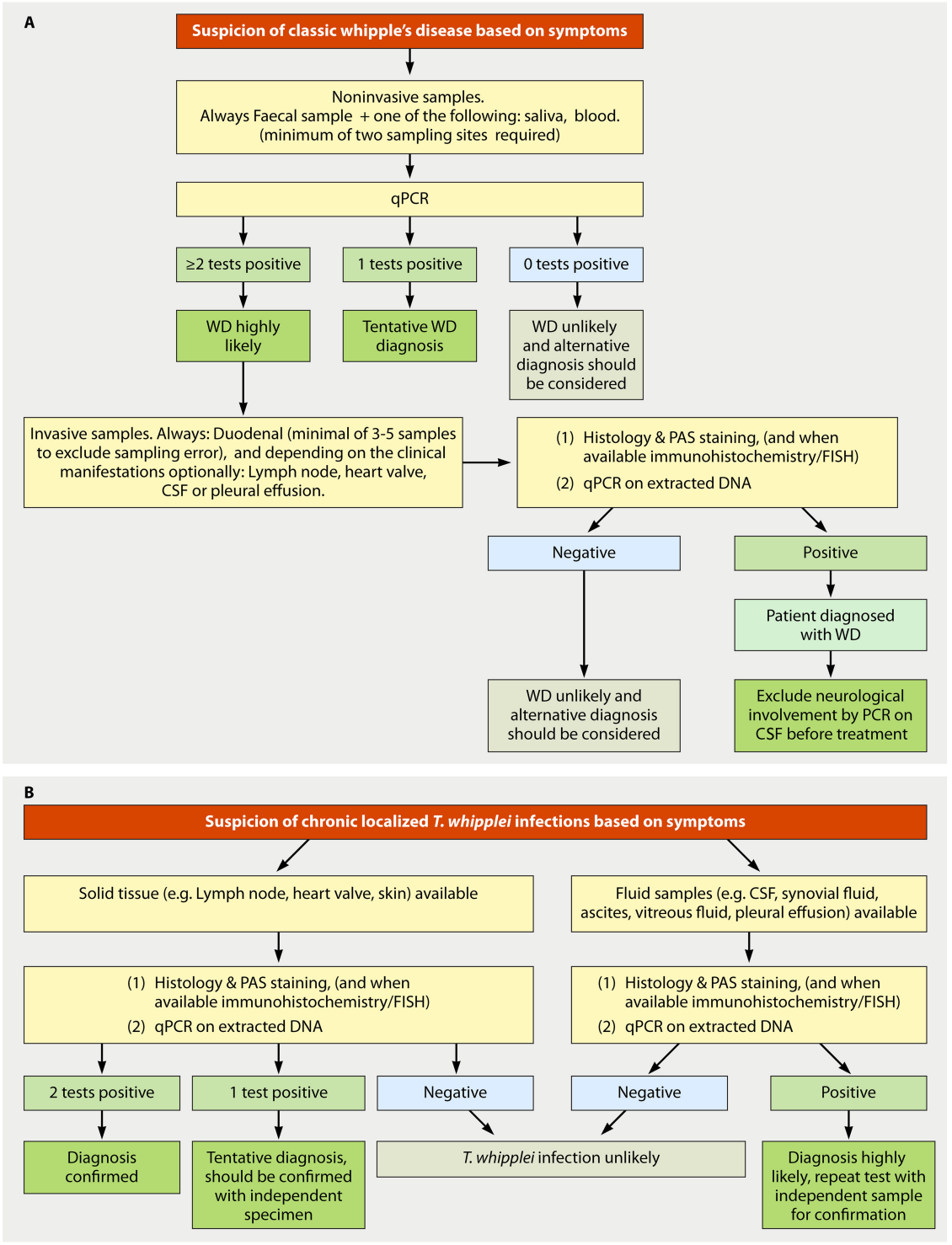

FIG 1 Schematic representation of the diagnostic algorithm. (A) Diagnostic strategy for classic Whipple's disease (WD). (B) Diagnostic strategy for chronic localized *T. whipplei* infection.

PAS-positive cells may not only be observed in gastrointestinal biopsy specimens but they may also be present in samples from, e.g., the cerebrospinal fluid or brain biopsy specimens of patients with CNS disease or cardiac valves of patients with culture-negative endocarditis. However, one should be aware that PAS staining has a poor specificity in brain biopsy samples (130, 177). Depending on the localization of the infection, *T. whipplei* can also be found in bone marrow, lymph nodes, skin, liver,

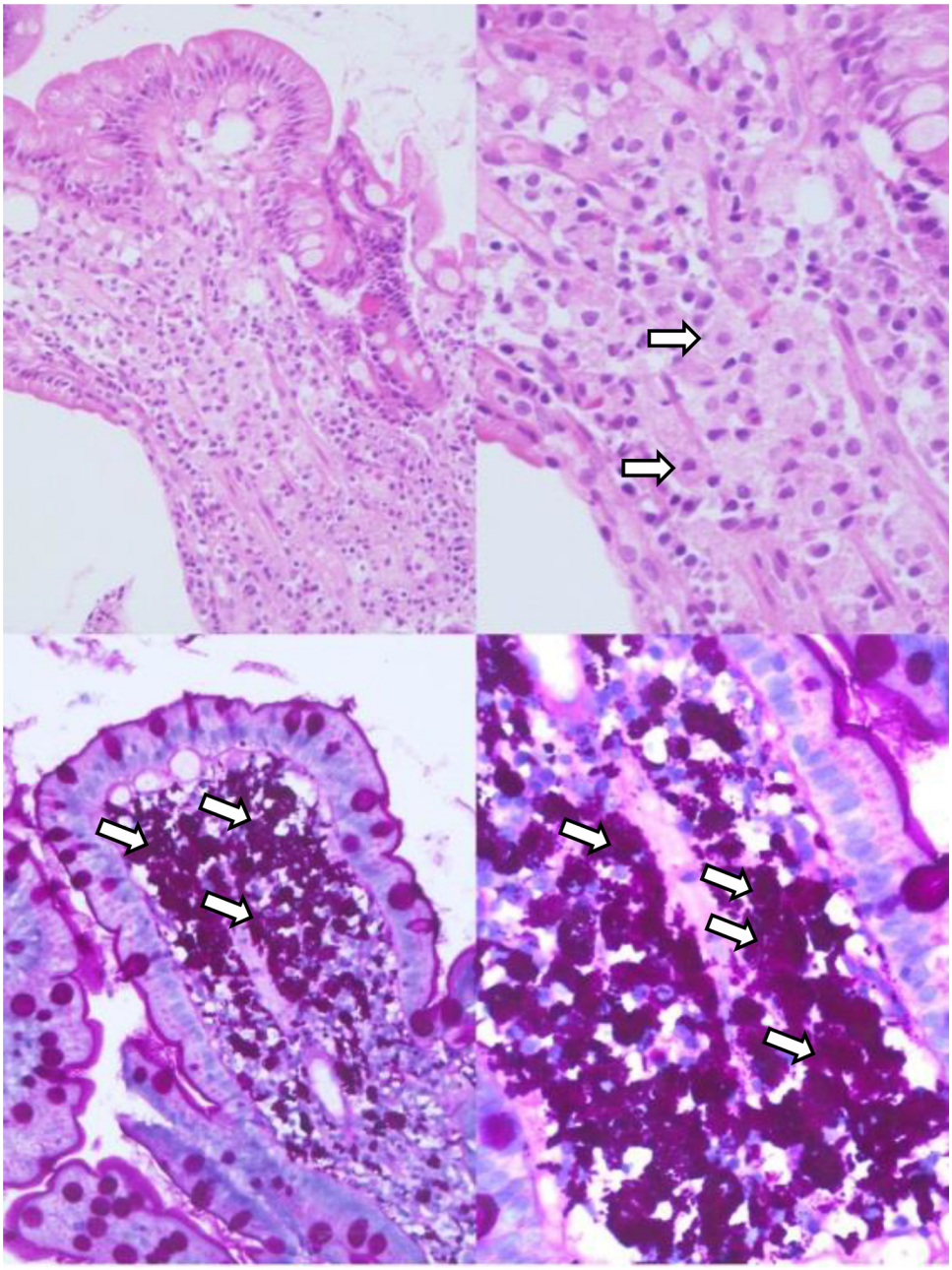

FIG 2 Microscopic detection of *T. whipplei*-infected duodenal mucosa. (Top) Hematoxylin-and-eosin-stained duodenal biopsy specimens with foamy macrophages in the lamina propria (arrows). The specimens were photographed with a 20× (left) and 40× (right) lens objective. (Bottom) Periodic acid-Schiff-diastase (PAS-D)-stained duodenal biopsy specimens with PAS-D-positive granules in the foamy macrophages (arrows). The same duodenal biopsy specimens as those used in the top panels were used here. The specimens were photographed with a 20× (left) and 40× (right) lens objective.

muscle, eye, and lung (10, 62, 103, 113, 120, 137, 178). It is important to realize that in chronic localized infections, PAS-stained duodenal biopsy specimens may be negative (102, 130, 162, 179, 180).

PCR. PCR is becoming a popular technique for diagnosing Whipple's disease, as it is thought to be more specific and sensitive than other methods (40, 181). Recently, a patient in Japan was presumptively diagnosed with Whipple's disease based solely on clinical suspicion followed by a positive PCR result on duodenal biopsy specimens (182). The corresponding small bowel biopsy samples from this patient were negative by PAS staining, and if PCR had not been performed, this patient would have been misdiag-

nosed (182). The presumptive diagnosis was supported by clinical symptoms and fast remission of symptoms after the start of *T. whipplei*-specific antibiotic treatment, but it lacks confirmation on a second independent specimen (182). An explanation for the discrepancy between histology and PCR may be an uneven distribution of the bacterium within the gut. Due to its higher sensitivity and larger sample size (that is homogenized during the DNA isolation step), PCR may not suffer as much from these sampling errors caused by a patchy distribution of the bacterium (51, 111).

After sequencing of the 16S rRNA of this bacterium (5–7), PCR assays could be developed using species-specific regions of the 16S-23S intergenic regions and the 16S rRNA gene of *T. whipplei* (37). In these PCR assays, a positive result required that the presence of the bacterium was confirmed by DNA sequencing of the PCR fragment in order to avoid false-positive results (18, 183). Later, quantitative real-time PCR (qPCR) was developed. Quantitative real-time PCR combines rapid cycling with fluorescence-based detection in a closed tube, thereby eliminating false-positive results due to cross contamination with PCR products (184, 185). Also, the PCR target was altered from 16S rRNA to repetitive sequences of *T. whipplei*, which has resulted in increased sensitivity and specificity of the test (186). This increased diagnostic reliability resulted in a shift from biopsy specimen-based diagnostics to noninvasive qPCR tests on saliva and stool samples as a complement to the initial screening (40). When a positive PCR result is found in saliva samples, stool samples are almost always positive as well, while the opposite is not true (18). In 2012, a retrospective study on the value of PCR for diagnosing Whipple's disease was performed by Edouard and colleagues using histology as the gold standard (40). They concluded that qPCR on stool and saliva samples is a good initial screening method (40). However, it is critical to validate the specificity of the PCR, as false-positive PCR results on saliva has been reported, especially when using 16S rRNA gene-based primers as the *T. whipplei* 16S rRNA genes are very similar to those of other bacteria that can be present in the oral cavity (183, 187). Whole-genome sequencing of *T. whipplei* identified more-specific PCR targets like the repetitive sequences, which when used in addition to other targets helped to rule out false-positive results (186, 188).

The main risk of PCR techniques is contamination, which can occur during several steps of the PCR process, including collection of the samples, isolation of DNA, and performing the PCR amplifications (111, 189). It is advisable that in atypical cases of *T. whipplei*, two different specific target genes are tested and that the results for both target genes should be positive in order to rule out potential false-positive results (51, 183). The occurrence of false-positive PCR results has been reported, e.g., in cerebrospinal fluid samples and duodenal biopsy specimens (187). In order to minimize the chance of false-positive results caused by contamination or due to a nonspecific PCR, qPCR should preferably be performed on more than one sample and whenever possible also include invasive samples, for instance, blood or biopsy specimen (40). In cases of arthralgia and arthritis, qPCR on joint fluids has become the preferred method of diagnosis (190). In addition, it is good practice to test the cerebrospinal fluid by PCR, as even without clinical signs of neurological involvement in many patients, *T. whipplei* is present in the CNS (73, 102, 147, 148). If localized endocarditis is suspected in a patient, a blood sample should be taken. The positive predictive value of PCR of blood samples was found to be 100%. However, the sensitivity (and negative predictive value) on blood samples is low (40).

PCR on various noninvasive samples can indeed serve as a relatively easy, highly sensitive and specific, cost-efficient prescreening prior to biopsy specimen-based diagnostics, but one has to be aware of carriage when analyzing these PCR-based data (40, 51, 111, 182). However, a positive PCR on a second invasive sample should be performed to confirm the initial diagnosis.

Alternative Diagnostic Methods

Apart from the routinely used methods to diagnose Whipple's disease, several

experimental diagnostic methods have been described; these methods are used in only a few specialized laboratories.

Immunohistochemistry. The presence of *T. whipplei* can be detected by using immunohistochemistry (191–194). With this method, antibodies are used to specifically detect *T. whipplei* in fixed specimens (192, 194). *In vitro* cultivation of *T. whipplei* has allowed generation of these highly specific antibodies in rabbits (193), enabling clinicians to detect *T. whipplei* in formalin-fixed, paraffin-embedded biopsy specimens.

In a study performed in 2003 by Lepidi and colleagues, the diagnostic value of *T. whipplei*-specific immunohistochemistry on duodenal biopsy samples was evaluated (193). Immunohistochemistry was highly specific and also more sensitive than PAS staining, as some of the PAS-negative duodenal biopsy samples were positive by immunohistochemistry (193). These patients later relapsed. After treatment (varying from 6 to 160 months), immunodetection signals of the bacterium were lower compared to PAS stains (193). The reason for this might be that dead bacteria are no longer well detected by immunohistochemistry, whereas PAS can still detect them (193). In another study, Lepidi and colleagues successfully used immunohistochemistry to detect *T. whipplei* in the lymph nodes of two Whipple's disease patients suffering from lymphadenopathy, without gastrointestinal signs (191).

Serology. After the development of a reliable culture method for *T. whipplei*, several serological methods to diagnose classic Whipple's disease could be developed (13). The development of serology is difficult, because of the paradoxically higher specific immunoglobulin M (IgM) titers in carriers compared to patients with Whipple's disease (48). The initial antigens were used for the specific detection of IgM class antibodies but had a low specificity (13). As with immunohistochemistry, this technique is employed by only a few specialized laboratories due to the lack of a readily available (commercial) source of antigen and/or antibodies. When there are only saliva or stool samples with positive PCR results for patients, Western blot serology could be used to differentiate between carriers and patients based on the higher antibody titers in carriers compared to patients in these studies (48, 87). It is unclear whether this finding also applies to a larger population and thus has any practical application for routine diagnostic use.

Fluorescence *in situ* hybridization. The fluorescence *in situ* hybridization (FISH) technique is useful to detect and confirm the presence of *T. whipplei* in extraintestinal tissues and in PAS-positive small bowel biopsy specimens (78, 103, 195). The target for these probes is the ribosomes, which degrade more rapidly in dead bacteria, in contrast to the target of PAS staining that stays positive for a longer period under treatment (196).

Electron microscopy. Electron microscopy can be a useful method for diagnosis of *T. whipplei* infections (3), but only a few laboratories have the means to perform electron microscopy. Due to sampling error, the negative predictive value of electron microscopy is low. Also, electron microscopy is rather laborious and therefore more suited for experimental studies on the pathology of *T. whipplei* (4, 197, 198).

TREATMENT

Without adequate antibiotic treatment, Whipple's disease can be fatal, although exact numbers on the mortality rate are unknown (10, 33, 62). While antibiotic treatment of *T. whipplei* infections usually leads to rapid improvement of clinical conditions, the eradication of *T. whipplei* requires prolonged treatment. Symptoms like diarrhea, joint pain, and fever usually disappear within a week, while other symptoms may take several weeks to disappear (33, 62, 102, 106). It is difficult to cure patients with late symptoms like eye, heart, and CNS involvement, and these patients tend to have high relapse and mortality rates (33, 62, 125, 127, 130). Sometimes patients with early symptoms are also hard to treat, as they seem to suffer from lifetime infection either due to permanent carriage of the bacteria in a niche in the patient where it is difficult for the antibiotics to eradicate the bacteria, the development of resistance against these antibiotics, or by reinfection (41, 199). Whatever the reason, it is a common belief that there are frequent late relapses after antibiotic treatment (131, 199–205). The

TABLE 4 Value of different antibiotics in treatment of Whipple's disease

Antibiotic(s)	Success rate	Dose per day	Reference(s)
Streptomycin	Bad	1.0 g	205
Penicillin	Fair	1.2 million units	205
Tetracycline	Fair	600 mg	205, 231
Trimethoprim-sulfamethoxazole	Fair	160 mg/800 mg	201–204, 207
Ceftriaxone–trimethoprim-sulfamethoxazole	Fair	2 g/160 mg/800 mg	147
Meropenem–trimethoprim-sulfamethoxazole	Fair	3 g/160 mg/800 mg	147
Doxycycline-hydroxychloroquine	Good	200 mg/600 mg	207

usefulness of supplementing antibiotic therapy with IFN-γ in order to enhance the antibacterial effect has been proposed and may be effective to overcome antibiotic resistance and/or relapses (206). Furthermore, as there is currently no universally recognized noninvasive method to monitor patients, it is difficult to evaluate the effectiveness of therapy. The intracellular concentration of antibiotics should be sufficient and in the case of CNS involvement, the drug must be able to efficiently cross the blood-brain barrier. There is no evidence for different treatment regimens for chronic localized infections and classic Whipple's disease, and therefore, patients should be treated similarly (103, 113, 125, 127).

Antibiotics

Several combinations of antibiotics have been used since the first successful treatment of *T. whipplei* infection in 1952 (2). These antibiotics include penicillin, streptomycin, tetracycline (205), ceftriaxone (147), meropenem (147), co-trimoxazole (207), doxycycline (104, 207), and hydroxychloroquine (104, 207) (Table 4). However, all these studies were performed with relatively small groups of patients, and larger studies are not available. Furthermore, relapses were not uncommon in patients who were treated with these antibiotics (131, 200–205).

The currently recommended treatment by UpToDate (208) is based on a single randomized controlled trial where 40 patients were successfully treated with ceftriaxone (one dose of 2 g/day) or meropenem (three doses of 1 g/day) for 14 days followed by oral co-trimoxazole (the combination of trimethoprim and sulfanomides) for 12 months (147). While clinically effective, there is solid *in vitro* evidence that the target for trimethoprim is missing in the bacterium (see below). This regimen results in good antibiotic levels in the brain, which is important, as even without specific symptoms, Whipple's disease often affects the CNS (10, 33, 124, 147). In patients who are intolerant to ceftriaxone, meropenem can be used as an alternative, and for patients intolerant to co-trimoxazole, doxycycline can be used (147, 148, 209). Whole-genome sequence analysis and the successful culturing of *T. whipplei* enabled both sequence-based analysis and *in vitro* susceptibility testing of the bacterium (210, 211). This resulted in some serious discussion on the effectiveness of the first-choice regimen, as *in vitro* data suggest an intrinsic resistance of *T. whipplei* to trimethoprim (204, 207, 212, 213), which was confirmed by sequence analysis revealing that the target for trimethoprim (dihydrofolate reductase) is missing (207, 213). In addition, mutations in the gene encoding dihydropteroate synthase (*folP*), the target of sulfanomide, were reported to result in resistance to sulfamethoxazole and sulfadiazine (202). This hypothetical resistance was confirmed by a recent retrospective analysis where all 14 patients who were first treated with co-trimoxazole failed treatment (207). Also, co-trimoxazole is associated with significant toxicity, and it is thus remarkable that co-trimoxazole is currently still a component of the first-choice treatment (208), and therefore, replacement of co-trimoxazole by an alternative antibiotic is probably more appropriate.

A more rational alternative approach to ceftriaxone followed by co-trimoxazole is the combined use of hydroxychloroquine and doxycycline which is the only *in vitro* bactericidal treatment against cultured *T. whipplei* (Table 4) (207, 210, 211). This treatment algorithm (Fig. 3) for classic Whipple's disease involves doxycycline (200 mg/day) and hydroxychloroquine (600 mg/day) for 12 months (104, 199, 207). For

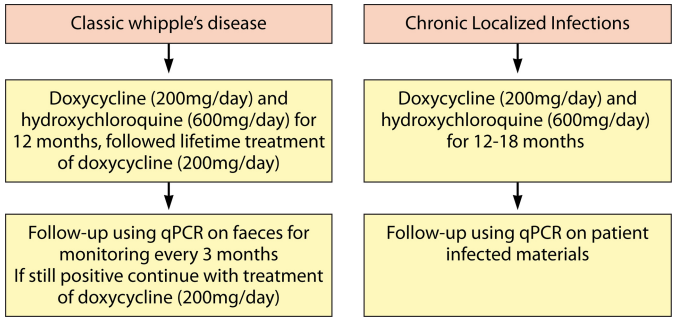

FIG 3 Latest proposed therapeutic strategy to treat *T. whipplei* infections. See the text for explanation.

localized *T. whipplei* infection, treatment with doxycycline (200 mg/day) and hydroxychloroquine (600 mg/day) for 12 to 18 months, followed by a lifetime follow-up, has been proposed (104, 113, 207). These antibiotics are effective against intracellular pathogens and are relatively safe when used for short-term treatment, for example, in Q-fever patients (207, 214). While *in vitro* susceptibility data support this regimen (207), thus far, there is only limited *in vivo* evidence supporting the choice for this combination of antibiotics, as only a handful of prospective trials have been performed (147, 148).

Other Treatment Considerations

One has to be aware that patients receiving immunosuppressive therapy or patients who suffer from immune compromising conditions have a more severe outcome of *T. whipplei* infection. In patients with Whipple's disease, there is a clear association between the onset of diarrhea and immunosuppressive therapy (88). Furthermore, exacerbation of Whipple's disease is associated with the use of corticosteroids and tumor necrosis factor inhibitors, and these drugs should thus be avoided whenever possible (88, 215, 216). Tumor necrosis factor alpha (TNF-α) plays a role in activation of the immune system by influencing the maturation of inflammatory cells and inducing apoptosis of infected cells (215). Biological treatment with TNF-α inhibitors has been shown to be fatal due to exacerbation with a mean time of 26 months after the start of treatment (107, 217). Previous immunosuppressive treatment is a major risk factor for the development of immune reconstitution inflammatory syndrome (IRIS) in classic Whipple's disease (218, 219).

IRIS is a serious complication during antimicrobial treatment observed in approximately 10% of patients with classic Whipple's disease (218, 220). A study done by Moos and colleagues concluded that *T. whipplei* must have an immunosuppressive effect on $CD4^+$ T cells because of the frequent emergence of IRIS after antibiotic treatment of Whipple's disease (220). Activated $CD4^+$ cells escape the peripheral blood and home onto affected tissues, causing the clinical symptoms seen in IRIS patients (220). Increased peripheral and local $CD4^+$ T cell counts are early prognostic markers for IRIS in these patients (220). In patients in whom IRIS develops, adequate therapy is essential, as untreated IRIS can be fatal (218). Oral corticosteroids are the first-choice treatment, and patients usually respond rapidly. If the inflammation does not subside within 24 h, additional or alternative immunosuppressive agents should be prescribed (218, 219). Thalomidine seems to be a good alternative treatment in cases of corticosteroid resistance (221, 222).

Treatment Follow-Up

It is generally advised to obtain duodenal biopsy specimens at 6-month intervals, and therapy needs to be continued as long as these biopsy specimens remain positive. However, this advice might be outdated for the following reasons. (i) Obtaining biopsy samples is both costly and not without risk for complications. (ii) The procedure can be stressful for the patient. (iii) It is known that Whipple's disease is associated with a lifetime susceptibility to *T. whipplei* infections (199). (iv) Macrophages can remain in the lamina propria for years after successful treatment, and thus, the detection of PAS-

positive foamy macrophages cannot be considered definitive evidence for incomplete bacterial remission (223). There is an apparent discrepancy in the therapeutic monitoring between PCR and FISH on one side versus immunohistochemistry and microscopy on the other side (224). This could explain the relapses in patients with PCR-negative duodenal biopsy samples. Recent work showed that both PCR and FISH can give false-negative results due to a biofilm that prevents detection of a low bacterial load after antimicrobial treatment (224). While in general PCR is more sensitive, PAS staining and immunohistochemistry are not influenced by the masking effect of the biofilm and are therefore more suitable to detect *T. whipplei* after treatment. Enzymatic disruption of the biofilm during the isolation of DNA prior to PCR was shown to solve this problem. Therefore, new and noninvasive PCR-based diagnostic methods for *T. whipplei* infection are being improved and tested for their usefulness to monitor patients. If the sensitivity and specificity of PCR for *T. whipplei* detection in feces or saliva is proven to be high enough, and a better correlation with remission is achieved, they might eventually completely replace biopsy specimen-based monitoring.

There is evidence for a genetic predisposition of the disease or increased risk of reinfection (see above). This translates to an increased risk of developing relapses even after complete clinical remission (199). Relapse rates of Whipple's disease were initially reported to be 30% (33, 62, 106, 131, 225) but have seriously declined over the past decades (147, 148). There is some discrepancy between the reported relapse rates when treating with beta-lactam and co-trimoxazole versus doxycycline and hydroxychloroquine (148, 199, 202, 203, 226). Some of these differences can be explained by differences in the length of time of therapeutic monitoring, as relapses usually occur after several years, and thus, good estimates of these relapse rates can be reported only when long-term monitoring is performed. Late relapses can occur even after several years, and it is argued that patients should be monitored for life (62, 227). Although there is evidence that relapses can be treated successfully using the same antibiotics that were used in the initial treatment (205, 209), it may be prudent to change the antibiotic regimen when treating a patient with a relapse.

These frequent relapses led to the recent proposal that the alternative therapy (doxycycline [200 mg/day] and hydroxychloroquine [600 mg/day] for 12 months) should be followed by a lifetime treatment with doxycycline to avoid reinfection (104, 199, 207). However, lifetime treatment with doxycycline may lead to development of resistance (205), and it is not clear whether this treatment is sufficient for every clinical manifestation of *T. whipplei* infection (199). Both doxycycline and hydroxychloroquine are known to have adverse effects when used for a longer period (228, 229). For hydroxychloroquine, the main complication is retinal toxicity and to a lesser extent, cardiotoxicity and neuromyotoxicity (228). Long-term side effects of doxycycline use include mainly gastrointestinal and skin manifestations (229). Although not scientifically proven, resistance to tetracyclines has been suggested to exist, as the use of this antibiotic is associated with frequent relapses (205).

EPILOGUE

Over the last decade, knowledge on *T. whipplei* infections has received a major boost due to the possibility of culturing this bacterium *in vitro* combined with the developments in the molecular methods. Molecular techniques like PCR revealed that infection and carriage are more common than was initially realized. There are, however, still many questions to be answered, as many of the current diagnostic and treatment options are based on studies with relatively small numbers of patients. Also, the pathogenesis and role of the host's immune system in both clearing the infection and developing clinical manifestations are still largely unresolved. We need to resolve these issues, as *T. whipplei* infections, especially with CNS involvement, still lead to substantial morbidity and mortality.

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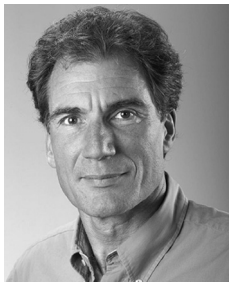

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