

Whipple's Disease

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Whipple's disease is a rare multisystemic infectious disorder affecting predominantly middle-aged men. Clinical manifestations are very variable with a very long, insidious, prediagnostic course. Weight loss, chronic diarrhea, arthralgias, and low-grade fever are characteristic features in most patients. Although gastrointestinal compromise is very common, atypical clinical forms are being increasingly recognized. Although a bacterial cause was strongly suggested for many years, the infectious agent was elusive until recently. The bacillus that was classified as an actinomycete was named *Tropheryma whipplei* and has singular characteristics. It presents affinity for the periodic acid-Schiff stain, but it is negative for Ziehl-Neelsen staining and has a characteristic trilamellar cell wall. Its genetic material has been recently sequenced, and culture was finally performed on a human fibroblast cell line. Pathological specimens show macrophage infiltration with mostly intracellular invasion of live bacteria. Immunologic factors, such as a subtle defect of cellular immunity possibly specific for the Whipple's bacterium, are believed to play a role in pathogenesis. The diagnosis requires the histologic assessment of diseased tissue, showing the characteristic infiltration, as a first approach, and confirmatory tests such as electron microscopy and/or polymerase chain reaction. Antibiotic treatment is mandatory and leads to a rapid clinical improvement and remission in most patients. Although the rationale for treatment is largely empiric, current recommendations include a 2-week parenteral therapy (third generation cephalosporin) followed by a long-term therapy with trimethoprim-sulfamethoxazole. This approach has been shown to reduce the number of relapses and was effective for prevention and/or treatment of the neurologic compromise.

Almost a century ago, the distinguished physician George Hoyt Whipple¹ performed an exceptional clinical and pathological description of a medical missionary who was affected for more than 5 years with an unknown illness. On the basis of the morphologic finding of vacuole in the cytoplasm of macrophages with an abnormal material inside attributed to fat deposition, Whipple called the unknown disorder "intestinal lipo-

dystrophy." A review of the English language literature showed that 12 years before Whipple's seminal clinical observation, British authors had published a similar case, which they named "lymphangiectasis intestini."²

The so-called Whipple's disease (WD) is an infectious multisystemic disorder affecting the small intestine in a very high proportion of cases and presenting with protean clinical manifestations. Although the disease is rare and probably no more than 1000 cases have been reported since it was first recognized, it seems probable that many other cases have been diagnosed but have not been reported in the international literature.³ Even though most of the published studies did not add substantial information about the disorder, the last decade witnessed a series of significant advances increasing our understanding of the disease and its etiology, pathogenesis, and diagnosis. The most relevant is the recognition of a novel and likely unique bacterium responsible for the disease. However, risk factors or genetic susceptibilities have not yet been recognized. Furthermore, the small number of identified patients and the ubiquitous geographic distribution of cases make it impossible to generate prospective, randomized, controlled trials to determine the optimum treatment.

Epidemiology

Current epidemiologic information about WD is still very limited. In this context, Dobbins³ published an outstanding clinical and epidemiologic analysis based on the largest number of cases ($n = 696$) collected from his own observation, data from cases published in the English language literature (case reports and small series), and a series of nonpublished patients. The substantial information provided by the review clearly showed that the disease predominantly occurs in men (86% of cases),

Abbreviations used in this paper: CNS, central nervous system; IL, interleukin; PAS, periodic acid-Schiff; PCR, polymerase chain reaction; TMS, trimethoprim-sulfamethoxazole; WD, Whipple's disease.

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middle-aged adults, and whites. However, more recent data show that modest but significant epidemiologic changes are occurring. On the basis of a comparative analysis of the last three decades of the 20th century, a German study showed a significant increase in the mean age of patients at diagnosis and a progressive increment in the proportion of female cases.⁴ Some authors have suggested that WD seems to occur more frequently among farmers and carpenters^{3,5}; however, this has not been confirmed by others.⁶ The mode of transmission is still a topic of speculation. Interestingly, the disease was detected in a set of patients within a familial context (siblings and father-daughter pairs) and in a pair of spouses.^{7,8} However, this is not enough evidence to argue either for a contagious pathogenesis or to suggest a genetic predisposition. On the basis of some characteristics of the disorder, an oral infectious route for the bacterium has been suggested.⁹ However, although the gastrointestinal compromise is relevant, there is no clear evidence in favor of this type of disease propagation. Interestingly, polymerase chain reaction (PCR) techniques have detected bacterial genetic material in gastrointestinal biopsy samples, saliva, and gastric juice in the absence of clinical WD.^{6,10,11} Although these observations must be confirmed by other studies before definitive conclusions, it was speculated that the WD bacterium might be present as a commensal nonvirulent microorganism in the digestive tract, which could transform it into a noxious agent producing the active disease.^{12,13}

Clinical Manifestations

The clinical features of WD are protean, involving mainly the gastrointestinal tract, but also producing manifestations in other systems according to their pathological compromise.¹⁴ Although the clinical severity is usually associated with a multiorgan involvement, there is general consensus that more organs are involved than is suggested by clinical symptoms. Table 1 summarizes the most common symptoms and signs at the time of diagnosis of patients. There is a triad characteristic of WD: weight loss, chronic diarrhea, and arthralgia.³ When this triad is associated with prolonged fever and peripheral lymphadenopathy, the presumption of WD should be very high.⁸ The presence of enlarged lymph nodes is a frequent finding, and lymphadenopathies can be either peripheral or abdominal or both, and sometimes they can be detected as tumor masses.

The most prominent manifestations in patients with gastrointestinal disease are chronic watery diarrhea and/or severe fat malabsorption associated with asthenia,

Table 1. The Most Prevalent Clinical Symptoms and Signs in Whipple's Disease Patients

Systemic compromise
Weight loss
Anorexia
Hyporexia
Fever
Fatigue
Edema
Anemia
Peripheral and mesenteric lymphadenopathy
Skin hyperpigmentation
Gastrointestinal system
Watery diarrhea
Steatorrhea
Abdominal pain and cramps
Abdominal bloating
Occult bleeding and hematochezia
Hepatomegaly
Splenomegaly
Ascites
Osteo-skeletal symptoms
Migratory arthralgias
Arthritis
Sacroiliitis
Spondylitis
Myalgias
Cardiovascular system
Cardiac systolic murmurs
Pericardial friction rub
Pericardial effusion
Nonspecific electrocardiogram changes
Congestive heart failure
Endocarditis
Myocarditis
Mitral and aortic valve dysfunction
Pulmonary manifestations
Chronic non-productive cough
Pleuritic chest pain
Pleural effusions
Pulmonary infiltrates
Mediastinal lymphadenopathy
Neurologic manifestations (see Table 2)
Eye manifestations
Uveitis, vitritis, retinitis, retrobulbar neuritis
Loss of vision

weakness, and cachexia. Weight loss is almost invariable, and most patients lose 10%–30% of their original body weight. In some severe cases with a prolonged prediagnosis period, weight loss can be as severe as a 50% reduction.⁸ Pathogenesis of weight loss includes hyporexia or anorexia associated with a chronic malabsorptive state. Hyperphagia can be observed rarely. Abdominal pain is present in almost 50% of patients and usually has varied characteristics.⁸ Some patients can have a chronic dehydration state with electrolyte depletion. In severe cases hypokalemia might produce respiratory and cardiac problems.⁸ Hypocalcemia and hypomagnesemia caused by malabsorption might produce cramps and tetany.¹⁵ Protein-losing enteropathy caused by lym-

phatic hypertension is very common in WD. The most common associated findings are severe hypoalbuminemia and peripheral edema. Anemia is present in 90% of WD patients and is caused by intestinal blood loss, iron deficiency, and vitamin B₁₂ and folic acid malabsorption. Ascites, hepatomegaly, and splenomegaly can be present in a minority of cases. Whereas the first finding can be interpreted as a result of polyserositis (including pleural and pericardial effusions), the others are related to the presence of granulomas or infiltration of organs. The frequent presence of splenomegaly contrasts with the common finding of thrombocytosis during active periods of the disease. Increased platelet count is likely related to spleen hypofunction and can be present both at diagnosis and after successful antibiotic treatment of WD.¹⁶

More than 50% of patients had prolonged periods with low-grade intermittent fever accompanied by night sweats. Both features have been reported as unique or dominant symptoms for a long time before diagnosis.^{3,8} Skin hyperpigmentation of light-exposed areas is also reported in 50% of patients. This finding is often misdiagnosed as Addison's disease, but the differential diagnosis must rely on the lack of mucosal pigmentation involvement in WD patients.

Arthropathy is a very frequent finding affecting 80%–90% of patients. Joint symptoms often precede the diagnosis by several years.¹⁵ Arthropathy consists of chronic, symmetrical, migratory, nondestructive, usually short-lived (2–4 days), and seronegative joint disease. Joints more often affected are wrist, knees, elbows, fingers, and shoulders. Patients complain of joint pain, but usually there is no objective inflammation or radiologic evidence of pathological involvement.¹⁷ In contrast, swelling with synovitis and local effusions might be present. Periodic acid–Schiff (PAS)–positive material and the Whipple's bacillus have been detected in synovial samples and effusions.¹⁸ Inflammation of the sacroiliac joint is present in some patients. Arthropathy in WD was associated with the presence of HLA-B27.^{19,20} However, this genetic susceptibility has not been found in other populations.^{21,22}

Almost one third of patients develop cardiovascular symptoms associated with cardiac pathological involvement.^{3,8} More prevalent findings are cardiac murmurs, pericardial friction rub, congestive heart failure, mitral and aortic valve insufficiency, and pericarditis.¹⁵ The most relevant pathological finding is blood culture–negative endocarditis as a result of the infectious compromise of the myocardium and pericardium.¹⁴ Necropsy material has shown that the pathological

cardiovascular involvement is more prevalent than clinical manifestations of heart disease associated with WD.³

More than 20% of patients report chronic nonproductive cough as the most prevalent pulmonary manifestation of WD (it was first documented in the patient reported by G. Whipple).³ Other pulmonary symptoms of active disease are pleuritic chest pain and dyspnea. Pleural effusions are also part of the polyserositis, and chest x-ray can show pulmonary infiltrates and mediastinal lymphadenopathies. In some cases, biopsy samples from disease tissues can detect granulomatous inflammatory reaction not staining positive for characteristic stains (see Morphology Observations). Some of these patients were misinterpreted as having sarcoidosis.²³ Ocular symptoms in WD (uveitis, vitritis, retinitis, retrobulbar neuritis) are present in less than 10% of cases.²⁴

Neurologic Compromise

At the most severe end of the clinical spectrum of WD are those features related to the central nervous system (CNS). The frequency of symptoms is not easy to determine and is probably underestimated. Figures vary between very rare, as was reported in our early series,⁸ to more than 40% of cases.^{3,25} For some researchers, the neuropathological involvement of the CNS is more prevalent than their clinical evidence.^{3,22,25} However, the progressive awareness of neurologists has resulted in the detection of cases with scanty general symptoms or only confined to the nervous system without any evidence of gastrointestinal compromise.^{26,27} Neurologic findings might be very variable; they might be the sole clinical manifestation or can be associated with an overt polysymptomatic disease present before diagnosis.^{3,8,28} Furthermore, neurologic symptoms may be the only clinical evidence of relapse after successful treatment with antibiotics.^{27,28} CNS involvement in WD is often irreversible despite otherwise successful antibiotic treatment and result in progressive deterioration.²⁸ Neurologic relapses seem to be more frequent among patients with CNS involvement before treatment, and relapse can occur several years after antibiotics were stopped.^{28,29} There is a clinical triad of the neurologic manifestations, which includes dementia, ophthalmoplegia, and myoclonus.^{3,14,15} For alert neurologists, such a triad is highly suggestive of WD. The WD bacillus can be detected in cerebrospinal fluid of patients without clinical features of neurologic involvement, even after prolonged remission by antibiotics.³⁰

Most common clinical findings are very variable and can be grouped into 4 major phenomena as summarized in Table 2. Mental changes are the most often recognized neurologic abnormalities reported in up to 50% of pa-

Table 2. Neurologic Symptoms and Signs in Whipple's Disease

Mental abnormalities
Dementia
Depression
Cognitive alterations
Memory loss
Confusion
Behavioral abnormalities
Personality change
Movement disorders
Ophthalmoplegia (supranuclear and intranuclear)
Oculomasticatory myorhythmia
Oculofacial-skeletal myorhythmia
Myoclonus
Ataxia
Hypothalamic symptoms
Sleeping abnormalities
Polydipsia
Hyperphagia
Others
Epilepsy
Cerebellar dysfunction
Dysphasia

tients.^{3,15,26,28} However, they are frequently not interpreted as a manifestation of the neuropathological compromise of the CNS. In most cases, the onset and outcome of symptoms are insidious and progressive. Interestingly, the movement disorders are very characteristic of WD. Oculomasticatory myorhythmia and oculofacial-skeletal myorhythmia are pathognomonic findings of WD that have not been described in other disorders.³¹ Hypothalamic symptoms are very infrequently reported. Finally, a myriad of other neurologic symptoms and signs were described in WD patients, most likely associated with the patchy compromise of the CNS or microembolism and microinfarcts.^{3,8,28}

Several researchers have extensively explored the neuropathology of the CNS compromise in WD patients. In general, patients present with generalized cerebral atrophy, and small granulomas are scattered diffusely in the gray matter of the cerebral and cerebellar cortex. Lesions often are patchy, and granulomas are PAS-positive with the characteristic bacillary infiltration.³² The distribution of CNS lesions accounts for the variety of clinical symptoms.^{3,14,15} Neuronal vacuolization, demyelination, and microinfarcts are shown very frequently. Since the intestine is not always affected in patients with predominant CNS compromise, histologic examination of samples from affected CNS tissue might be diagnostic.²⁷ The PCR analysis of these samples is mostly helpful, and the examination of the cerebrospinal fluid for PAS-positive cells or PCR analyses might also be of great value in the diagnosis and follow-up.³⁰

Pathogenesis

A recently identified infectious agent causes WD. There is some evidence suggesting that a series of immunologic and nonimmunologic deficiencies detected in patients seem also to be necessary. Malabsorption is often present in patients with intestinal involvement and has been associated with lymphatic obstruction from lacteal compression in lamina propria and lymph nodes, and with suspected epithelial dysfunction.

The Agent

At the beginning of the second half of the 20th century, studies suggested that an infectious agent(s) should be the cause of WD. The finding of bacilliform intracellular structures in macrophages staining positive for PAS stain³³ and the successful response to antibiotics³⁴ were of remarkable importance. Strong support of the infectious etiology was provided by electron microscopy findings suggesting that the abnormal intracellular material is made up of monomorphic bacilliform bodies with structural characteristics resembling bacteria.³⁵⁻³⁷ Attempts to culture the putative infective agent(s) failed. In the early 1990s, two different groups used PCR to identify numerous characteristics of the bacterium. Wilson et al.³⁸ characterized the bacterium molecularly by using universal genomic amplification and PCR. They only sequenced part of the bacterium based on the amplification of a 1321-base bacterial 16S ribosomal RNA obtained from infected tissue (duodenal samples) of 1 patient with active WD. In 1992, Relman et al.³⁹ sequenced the microorganism completely and performed a correct phylogenetic analysis in which they showed that the bacterium is a member of the actinomycete line (class Actinobacteria). Thus, they identified the bacterium as a previously unknown agent and proposed the genus and species designation *Tropheryma whippelii*. Interestingly, most of the phylogenetically related microorganisms are environmental bacteria. PCR with 16S rDNA primers of *T. whippelii* was subsequently used both to confirm WD in patients with classic symptoms and signs and to diagnose the disease in atypical cases.⁴⁰ Recently, variations in the 16S-23S rRNA internal transcribed spacer region have suggested the possibility of at least 6 different, closely related species or subtypes of the single species *T. whippelii*.⁴¹ These variations were associated with a geographic distribution of cases; however, this was not confirmed by others.⁴²

Culture of the bacillus of WD has been frustrating and elusive for decades. The first successful attempt was performed by Schoedon et al.^{43,44} In those studies the bacterium was isolated and grown in human macro-

phages deactivated with interleukin (IL)-4 and IL-10. However, the isolated bacterium could not be subcultured. In 2000, Raoult et al.⁴⁵ were able to culture the bacterium, to detect specific antibodies in the tissue of patients, and to generate specific polyclonal antibodies to be used in the immunodetection of the agent in infected samples. The bacterium was successfully grown and subcultured in human fibroblast cell line (HEL cells), and its amplified 16S rRNA showed identical base sequence to the *T. whippelii* reference sequence identified previously. The study has shown that the agent grows slowly with an estimated doubling time of 17 days. On the basis of recent findings, the definitive name of *Tropheryma whippelii* was officially accepted.⁴⁶ Although cell biological mechanisms of intracellular bacterial survival are unknown, a very recent study detected that the existence of an acidic intracellular environment seems to be critical for the prolonged persistence of the bacillus in host cells.⁴⁷

Habitat of the Bacterium

The natural habitat of *T. whippelii* is still largely unknown. The proposed high prevalence among farmers suggested that it could be a zoonosis.^{3,5} However, up to now, the bacterium has only been found in infected human tissues, with no evidence of human-to-human transmission or a reservoir in domestic animals. The study of Maiwald et al.⁴⁸ reported the detection of DNA specific for the WD bacterium in 25 of 38 wastewater samples from 5 different sewage treatment plants in the area of Heidelberg, Germany. This original finding provided the first evidence showing the environmental presence of *T. whippelii* within a polymicrobial flora and suggested an environmental source for the infection in which the ingestion of contaminated foods or water could be the route of acquisition. This is consistent with the phylogenetic classification of the bacterium. In contrast, some authors suggested that *T. whippelii* might be part of the commensal flora of the intestines.^{49,50} However, because the specificity of PCR techniques for the detection of WD bacterium has been questioned,⁵¹ more studies are necessary to clarify this important epidemiologic aspect of the WD infection.

Host Immunologic and Nonimmunologic Defects

WD disease is a systemic disorder in which the possibility of a defective immune response has been raised.⁵² There are still key questions. Do these abnormalities precede the infection, or are they a consequence of the nutritional compromise? Are these immunologic

defects nonspecific alterations, or are they specifically directed against the *T. whippelii*? Contradictory immunologic findings have been reported. A genetic predisposition was formerly suggested on the basis of the high prevalence of some clinical characteristics (arthropathy), but not on epidemiologic data. The genetic susceptibility was related to the presence of the histocompatibility antigen HLA-B27. Dobbins⁵² reported the presence of HLA-B27 in 28% of 47 patients collected compared to a 10% prevalence in the general population. Feurle et al.²⁰ detected 4 HLA-B27 positive cases among 9 unrelated WD patients. In contrast, we reported a low prevalence of the antigen in a series of 14 patients with WD (8%) compared with 4% in the control population; furthermore, there was no significant association with other class I and class II HLA antigens.^{21,22} Therefore, the potential genetic host predisposition for WD remains unclear and requires new and more extensive genetic studies.

Evidence strongly supports that humoral immunity is not abnormal in patients with active infection, and if present, defective humoral immunity results from malnutrition.¹⁹ Patients show normal immunoglobulin levels, secretory components, serum complement both before and after treatment. On the basis of the identification of the specific antigen, serum IgM and IgG antibodies against *T. whippelii* were recently detected by using immunofluorescence.⁵³ This finding strongly argues against the presence of tolerance to the bacterium which had been suggested by the presence of living bacteria in affected tissues.

Several features of the histopathological response in WD are generally assumed to result from a defective cell-mediated immune function. Abnormal delayed-type hypersensitivity reactions to different antigens have been documented (Table 3). Immunohistologic assessment has shown no lymphocytic and plasma cell infiltration in affected tissues.⁵⁴ In contrast, there is a proliferation of mature T-cell subpopulations (predominantly CD8⁺) and increased cell-activation markers.⁵⁴ Most early studies have shown a reduced proliferative response of peripheral T cells to phytohemagglutinin, concanavalin A, and other mitogens, not only before treatment but also a long time after remission produced by a successful antibiotic treatment.⁵⁵ The presence of bacteria living and multiplying in the cytoplasm of macrophages has suggested that the function of the mononuclear-macrophagic/phagocytic system is abnormal.⁵⁶ This family of cells widely distributed throughout the body has a well-defined function in the defense against microorganisms, especially intracellular ones. Early studies on the nonspe-

Table 3. List of Some of the Most Common Cell-Mediated Immune Defects Found in Patients With Active Whipple's Disease

Impaired cutaneous response to antigens
Tuberculin
<i>Candida</i>
<i>Trichophyton</i>
Histoplasmin
Mumps
Streptokinase-streptodornase
Dinitrochlorobenzene
T-cell-dependent factors
Impaired T-cell proliferative response
Reduced CD4/CD8 ratio
Impaired T-helper1 response (interferon- γ , IL-2)
A shift of T-helper2 response (IL-4)
Macrophage-dependent factors
Reduced production of IL-12
Reduced expression of CD11B
Normal phagocytosis
Impaired killing function
Impaired degradation

sific function of macrophages in WD patients have concluded that these cells express normal phagocytosis but severely impaired degradation of foreign material.⁵⁷ Interestingly, these phenomena were detected in patients with active disease (both before treatment and relapses) and a long time after remission.¹⁶ A dysregulated T-helper1/T-helper2 response has been documented in both peripheral and mucosal T cells.⁵⁸ A low production of IL-12 by macrophages and a low T-helper1 response affecting interferon- γ and IL-2 secretion have been suggested as factors involved in the abnormal immunity.⁵⁸ Other features potentially involved in the immunologic disturbance associated with WD are summarized in Table 3. In general, it is accepted that the suspected defective immune response might be subtle and quite specific for the etiologic agent because patients are not predisposed to other infections both in the acute phases of the disease and in remission.^{55,58} Further studies involving a greater number of patients will add substantial information to the exact mechanism(s) involved in the pathophysiology of the WD infection.

Diagnosis

Clinical Suspicion of Whipple's Disease

In general, the onset of WD is insidious, and it often takes several years until overt disease is present and a definitive diagnosis can be made. Very often, polyarthralgias and low-grade persistent fever are the main or only symptoms detected during the prediagnostic period.^{3,8} On the other hand, a characteristic of the natural history of the disease before treatment is a chronic re-

lapsing course in a high proportion of patients. Even though most patients have severe clinical compromise, physicians must be alert to cases with minimal symptomatology. When gastrointestinal symptoms occur, diagnosis is more accessible; gastrointestinal manifestations were irrelevant or absent in almost 15% of patients reported.¹⁹ A more profound analysis of clinical features is detailed in the clinical manifestation section of this review.

Laboratory Findings

There are no specific laboratory abnormalities in WD. Most findings suggest a chronic inflammatory state and malabsorption. Anemia is present in most patients.⁸ Thrombocytosis with other features associated with hyposplenism was seen in active WD and a long time after successful antibiotic treatment. Other common findings are increased erythrocyte sedimentation rate, hypoalbuminemia, and high C-reactive protein level. Culture of the WD bacterium led to new diagnostic tools such as a serologic test for easier diagnosis. Thus, Raoult et al.⁴⁵ have recently developed an immunofluorescence test for IgG and IgM antibodies on a monolayer cell substrate. They detected IgA antibodies in 100% of WD patients and 75% of control subjects.⁵⁹ In contrast, IgM antibodies had a moderate sensitivity (78%) but a very high specificity (93%). Titers above 1:400 were specific for WD. The low specificity might reflect impurities of methods and the presence of immunologic cross-reactivity by other microorganisms including the ubiquitous distribution of virulent and nonvirulent strains of the WD bacterium. Large-scale studies could help to clarify this topic.

Images

Small bowel contrast radiology can show nonspecific findings characteristic of mucosal and submucosal infiltration⁶⁰ (Figure 1). Abdominal computed tomography might show thickening of small bowel folds, mesenteric and retroperitoneal lymphadenopathies, ascites, hepatomegaly, and splenomegaly. These findings are not specific for WD. Brain computed tomography scan can be normal or reveal cerebral atrophy, hydrocephalus, or focal lesions.

Endoscopic Findings

Because endoscopy is used as a very simple and useful tool for the diagnosis of most malabsorptive disorders, evaluation of the mucosa of the distal duodenum and the collection of mucosal samples for histologic assessment are of major importance in diagnosis of WD. The first description of endoscopic markers of WD was

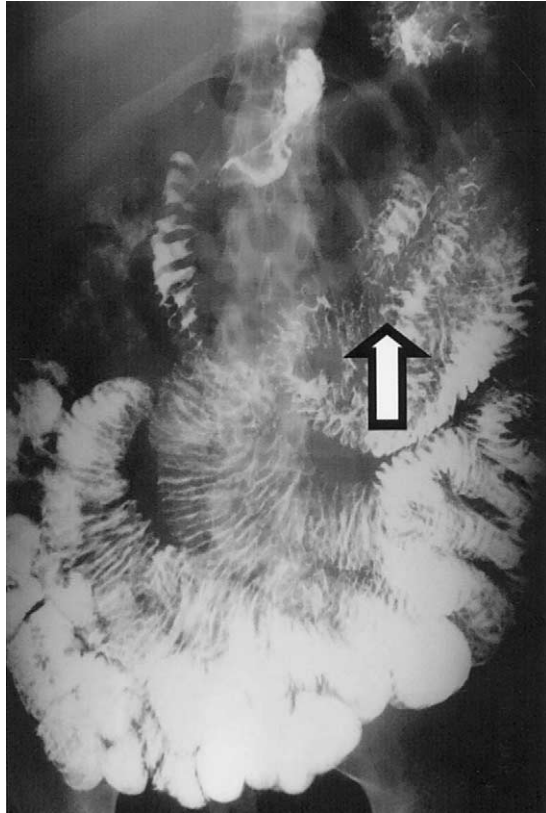


Figure 1. Small bowel follow-through in a patient with active WD showing thickened folds in the jejunum (Kerkring's folds). Some folds show a nodular tip as shown by the arrow. These characteristics are produced by the macrophage infiltration of the lamina propria, lymphangiectasias, and edema.

published by Spanish authors in a non-English language journal almost 3 decades ago.⁶¹ Several endoscopic findings have been described with active mucosal WD. Non-specific features such as thick Kerckring's folds, erosions, and erythematous mucosa are commonly detected in the distal duodenum of WD patients.⁸ Perhaps the most suggestive marker is the presence of multiple whitish-yellow small plaques diffusely distributed in the intestinal mucosa (Figure 2). Sometimes these plaques can have a patchy distribution. Very rarely, similar findings can be detected in the colonic mucosa. This finding is not pathognomonic of WD and can also be detected in other diffuse disorders such as intestinal lymphangiectasia and Waldenström's disease (Mauriño E, Bai JC, unpublished observations, August, 1984). Treated patients recover normal endoscopic appearance usually within 6 months after onset of antibiotics.⁶²

Morphologic Observations

The great majority of patients with WD have involvement of the proximal intestine and lymphatic drainage, and duodenal biopsy is the diagnostic proce-

dure of choice.^{3,8,63,64} Furthermore, atypical patients without gastrointestinal symptoms often have intestinal involvement. The most relevant microscopic finding is the presence of foamy-appearing vacuole macrophages infiltrating the lamina propria and staining PAS-positive^{3,8,14,15,33,65-69} (Figure 3). With optical microscopy, intestinal villi are thickened and clubbed, distorted by the macrophage cell infiltration of the lamina propria. These PAS-positive vacuoles contain collections of fine rod-shaped particles corresponding to intact or partially degraded bacteria. By using the PAS stain, the tinctorial characteristics of the 2 seminal cases were retrospectively studied, confirming the diagnosis of WD.^{67,68} Furthermore, bacterial material and staining can also be shown inside other cells (e.g., intraepithelial lymphocytes, epithelial cells) and in the interstices of macrophages. Such infiltration has been found in every involved tissue and has been shown to be negative for Ziehl-Neelsen staining for acid-resistant microorganisms. Both tinctorial characteristics are suggestive of WD but not pathognomonic. Although PAS-positive staining is also detected with

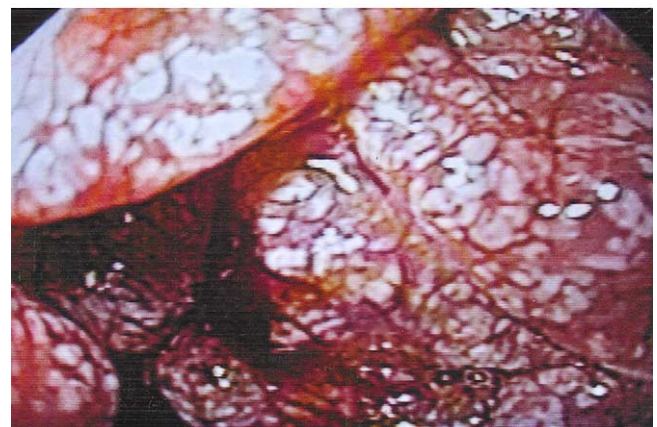
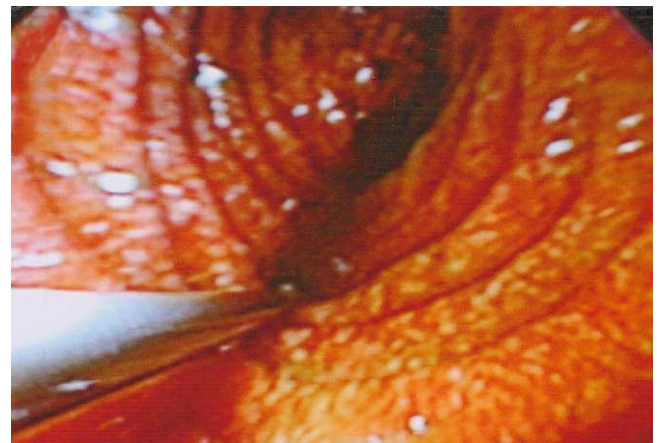


Figure 2. Characteristic endoscopic findings in the distal duodenum showing thick intestinal folds with multiple whitish-yellow small plaques diffusely distributed in the intestinal mucosa.

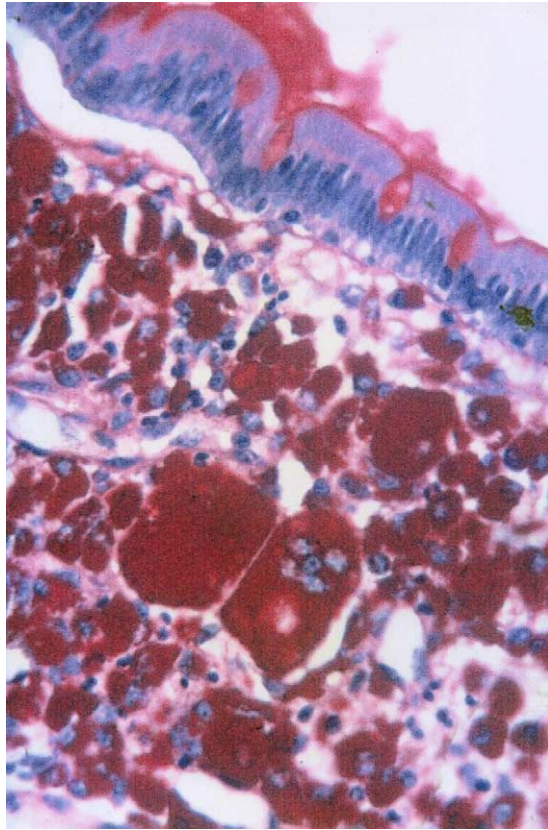


Figure 3. PAS-positive staining of a duodenal biopsy showing part of a villous structure exhibiting the lamina propria infiltrated by macrophages (histiocytes) filled with PAS-positive inclusions from a patient with WD. The intestinal epithelium shows as preserved. Original magnification 1000 \times .

other predominant intracellular infectious agents such as *Rhodococcus equi*, *Histoplasma capsulatum*, *Bacillus cereus*, and others,^{8,14} only *Mycobacterium avium-intracellulare* in patients with acquired immunodeficiency syndrome can present a practical problem in the differentiation from WD bacterium. In this context, Ziehl-Neelsen staining is very useful in discriminating WD bacterium (negative staining) from *M. avium-intracellulare*, which is acid-fast positive.⁷⁰ PAS-positive staining is also detected in some rare noninfectious disorders such as intestinal histiocytosis, sarcoidosis, Gaucher's disease, and berylliosis.⁸ The intensity of infiltration by macrophages is variable, with patients with malabsorption presenting most dense staining located in more superficial mucosal areas. On the other hand, infiltration of the lamina propria can be scanty infiltration and is sometimes limited to the submucosal layer.^{3,8,71} This is often associated with a lack of gastrointestinal symptoms. Dilated lymphatic vessels are very commonly associated with moderate to severe intestinal involvement and lymphatic obstruction. Both the presence of superficial dilated lymphatic vessels and the deposition of lipid droplets in the interstitium at the tip

of villi seem to be responsible for the characteristic endoscopic appearance.⁷⁰

Electron microscopy showed that the PAS-positive material has very typical characteristics considered specific of the bacterium. The material consist of live and partially degraded microorganisms presenting a characteristic trilamellar wall appearance (the plasma membrane, a thin homogeneous wall, and a plasma membrane-like structure)³⁵⁻³⁷ (Figure 4). The size of bacteria varies between 1–2.5 μm by 0.25 μm , and bacteria can be located both intracellularly (in the cytoplasm of cells from the intestinal epithelium, blood cells, intraepithelial lymphocytes, plasma cells, mast cells) and extracellularly. Detailed observation of infected samples can detect bacteria in the process of binary division, which suggests intracellular viability of the agent.³

Granulomas with epithelioid cells can be detected in the small bowel mucosa, spleen, liver, and lymph nodes, which in some circumstances might not stain positive for PAS.^{19,72} The development of anti-*T. whippelli* antibodies led to immunohistochemical diagnosis of the bacillus a highly specific, sensitive, and very useful method both during active infection or in a retrospective assessment.⁵⁹ Furthermore, by using a polyclonal rabbit anti-*T. whippelli* antibody the same group of researchers was able to diagnose WD in circulating blood monocytes.⁷³

Polymerase Chain Reaction Analysis for Diagnosis

The advent of PCR gene amplification has aided diagnosis of WD,^{38,39} with very high sensitivity and specificity.^{6,9,40} However, some laboratories have reported a number of potential false-positive results from control individuals without clinical evidence of WD or PAS-positive staining in samples.^{6,10-13,48-50} Several

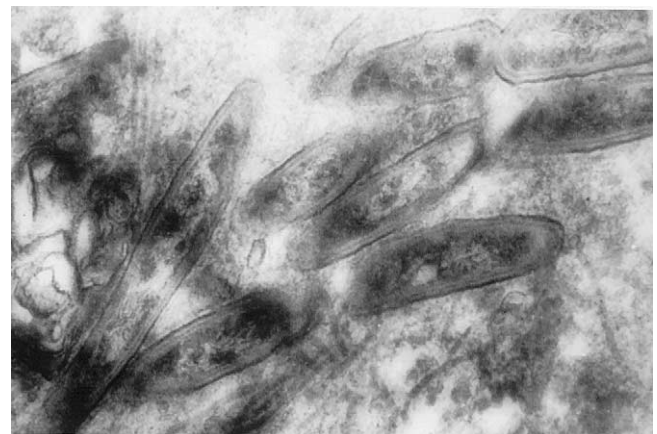


Figure 4. Electron micrograph of a tissue sample (duodenal biopsy) of a patient with untreated WD illustrating a macrophage showing the characteristic trilamellar wall appearance of *T. whippelli*.

gene sequences are available, and it was recommended that a definitive diagnosis in atypical cases requires the use of at least 2 PCR tests based on primers obtained from 2 different genes to avoid false-positive results.¹⁴ PCR-based diagnosis still requires more experience; meanwhile, results must be interpreted with caution.

Guidance for Diagnosis

In classic WD, the diagnosis must be established on the basis of clinical features (symptoms and signs, images, and endoscopic markers) and histologic findings. In most cases with classic clinical features, the presence of foamy-appearing vacuoles in the cytoplasm of macrophages infiltrating the small intestinal lamina propria or samples from infected tissues makes it mandatory to proceed to both PAS and Ziehl-Neelsen stains. If samples depict the characteristic PAS-positive Ziehl-Neelsen-negative staining, there is a very strong suspicion of WD. Confirmatory tests depend on the availability of more sophisticated tools. The ideal test is electron microscopy of samples with the identification of the characteristic trilamellar cell wall appearance. PCR-based tissue assay has been shown to be more sensitive than other studies. However, PCR test is expensive, has the potential risk of DNA cross-contamination, and is technically demanding, requiring experienced clinical microbiology laboratories and use of more than 1 primer. In the future, application of immunohistochemistry by using specific antibodies should be of great value. Although not yet widely available, serology tests could also offer useful tools for screening.

Atypical forms of WD are considered difficult to diagnose because of unusual clinical presentation and no notable gastrointestinal symptoms. Sampling of infected tissues (e.g., CNS, endocardium, mediastinal nodules) is mandatory but in some circumstances impractical. Despite absence of macroscopic or microscopic involvement, intestinal biopsy is an easy form of access to a potential infected tissue, allowing a PCR analysis to detect genomic material of *T. whipplei*.¹⁹ When all these confirmatory tests are not available, a clinical trial with antibiotics might be very useful.

Treatment

The therapeutic goal in all patients with WD is to eradicate the infection and to avoid relapses. Until the 1950s, the disease was incurable, and most patients died of the disease.⁸ Paulley³⁴ was the first to report the successful treatment of WD with the use of chloramphenicol and opened a new era in the understanding of the pathogenesis and treatment of the disease. Since then,

Table 4. Estimation of Effectiveness and Relapses in the Most Recommended Antibiotic Combinations for Treatment of Whipple's Disease During the Last 2 Decades

Antibiotic combinations	Effectiveness	Relapses	
		Systemic	CNS
PCN + STM + TMP-SMX	+++	+ / ++	0
TMP-SMX	+++	+ / ++	0
CPP + TMP-SMX ^a	+++ ^a	? ^a	? ^a
CBP + TMP-SMX ^a	+++ ^a	? ^a	? ^a

PCN, penicillin; STM, streptomycin; TMP, trimethoprim; SMX, sulfamethoxazole; CPP, third-generation cephalosporin (ceftriaxone); CBP, carbapenem (imipenem or meropenem); +++, very effective in the active phase; + / ++, low rate of relapses but still significant; ?, still not known.

^aEuropean Project on Whipple's Disease: No final data were reported¹⁴, <http://www.whipplesdisease.info>.

a variety of antibiotics and different schedules were successfully used. Thus, patients have shown rapid improvement in their clinical status within a few days of starting treatment.

The selection of antibiotics and duration of treatment have been a matter of debate, and remain largely empiric due to the lack of scientifically randomized, double-blind studies comparing different antibiotic regimens. The ideal antibiotic requires proven effectiveness, very low primary resistance, administration for a period long enough to destroy surviving bacteria, and adequate penetration of the blood-brain barrier. The first and more frequently used antibiotics were tetracycline and penicillin, either alone or in combination schedules.^{74,75} Due to the very high prevalence of relapses with such antibiotics, each used alone was considered inadequate therapy for WD. Until the 1980s, many patients were treated with a 2-week course of IV penicillin plus streptomycin followed by oral tetracycline,^{3,8,75} but there was a high rate of relapses (35%) and CNS compromise (14%) or relapses.⁷⁵ A retrospective analysis from many case reports and several patient series has shown that the most commonly used schedule associated with clinical success and low risk for relapses is the combination of penicillin G (1.2 million units/day IM) and streptomycin (1 g/day IM) and/or a third-generation cephalosporin for 2 weeks followed by the administration of trimethoprim-sulfamethoxazole (TMS, 160 mg/800 mg, 2 times a day) for at least 1 year^{14,15} (Table 4).

Many investigators claim that the duration of the treatment correlates with the length of remission.^{3,75,76} This topic remains controversial.^{77,78} Given the potentially dangerous CNS relapse and the evidence of silent neurologic compromise, longer and more adequate ther-

apy is necessary until further evidence defines the optimal treatment strategy.⁷⁶ Several antibiotics have been proposed as second line regimens in cases of primary resistance or intolerance to some drugs. It is evident that the availability of culture will allow exploration of the susceptibility of the bacterium and, thus, to attempt more adequate regimens. Resistant cases have been treated with cephalosporins or fluoroquinolones, and the concomitant use of interferon- γ was also proposed.⁷⁹ Supportive measures should be similar to those of other malabsorptive disorders.

Recent insights on the pathophysiology may impact the future of treatment of WD. The fact that intracellular survival of *T. whipplei* requires acidic environment has suggested that manipulating the intravacuolar pH might constitute a potential approach for the treatment of WD.⁴⁷ In the experimental context, the treatment of HeLa cells with ammonium chloride, chloroquine, or bafilomycin A1 increased the vacuolar pH and promoted the killing of *T. whipplei*.⁴⁷ At present, TMS is the preferred antibiotic because of the very low rate of relapses and the effect beyond the blood-brain barrier. A very recent report, based on the genomic analyses of *T. whipplei*, questioned the rationale of the use of TMS because it does not target tetrahydrofolate biosynthesis in the bacterium.⁸⁰

Strategies in the Follow-up of Patients

It has been suggested that treatment should be followed until histologic or PCR evidence of WD bacterium is not present.^{14,15} However, this might take months or even years. Thus, for those cases followed with PAS staining on biopsies, the tinctorial characteristic might remain in macrophages for several years, despite the fact that the bacterium is not viable (because of the abnormal degradation by phages).^{37,77} This finding is clear evidence of defective degradation by the macrophages.^{15,56,57} The former recommendation contrasts with reports of cases treated for a short period of time in which persistence of PAS-positive material in post-treatment biopsies was the rule, but relapses were not present in excess.^{77,78} Further studies are needed to provide new avenues and alternatives for treatment of WD.

Conclusions

WD is an extremely interesting infectious systemic disorder. The increased attention to this condition in recent years has led to a greater understanding and awareness of the disease by the medical community. Classic patients have predominantly gastrointestinal manifestations. Extraintestinal manifestations are pro-

tean, and when not associated with intestinal symptoms, the diagnosis might be difficult. The diagnosis of WD often requires endoscopic biopsy of the duodenal mucosa showing the characteristic PAS-positive macrophage infiltration. Electron microscopy and PCR assay are confirmatory tests especially useful in atypical cases. Use of antibiotics has a major impact on outcome with clinical response being more dramatic than the histologic response. More studies are required to better define the habitat of the bacterium, mode of transmission, and factors associated with host predisposition. Furthermore, it is also necessary to improve diagnostic tests, to elucidate pathogenesis, and to establish the best treatments for CNS involvement and avoid relapses.

References

- Whipple GH. A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues. *Bull Johns Hopkins Hosp* 1907;18:382-391.
- Allchin WH, Hebb RG. *Lymphangiectasis intestini*. *Trans Pathol Soc London* 1895;46:221-223.
- Dobbins WO III. Whipple's disease. Springfield, IL: Charles C. Thomas, 1987.
- von Herbay A, Otto HF, Stolte M, Borchard F, Kirchner T, Ditton HJ, Maiwald M. Epidemiology of Whipple's disease in Germany: analysis of 110 patients diagnosed 1965-1995. *Scand J Gastroenterol* 1997;32:52-57.
- Durand DV, Lecomte C, Cathebras P, Rousset H, Godeau P. Whipple's disease: clinical review of 52 cases. *Medicine* 1997;76:170-184.
- Kelly P. The PCR for *Tropheryma whippelii*. *Lancet* 1999;354:1476.
- Puite RH, Tesluk H. Whipple's disease. *Am J Med* 1955;19:383-400.
- Crosetti E, Mauriño E, Boerr L. Enfermedad de Whipple. In: Crosetti E, Boerr L, Bai JC, eds. *Patología del intestino delgado*. Buenos Aires: Editorial Científica Interamericana, 1987:258-271.
- Swartz M. Whipple's disease: past, present, and future. *N Engl J Med* 2000;342:648-650.
- Dutly F, Hinrikson HP, Seidel T, Morgeneegg S, Altwegg M, Bauerfeind P. *Tropheryma whippelii* DNA in saliva of patients with Whipple's disease. *Infection* 2000;28:219-222.
- Ehrbar HU, Bauerfeind P, Dutly F, Koelz HR, Altwegg M. PCR-positive test for *Tropheryma whippelii* in patients without Whipple's disease. *Lancet* 1999;353:2214.
- Street S, Donoghue HD, Neild GH. *Tropheryma whippelii* in saliva of healthy people. *Lancet* 1999;354:1178-1179.
- Gross M, Jung C, Zoller WG. Detection of *Tropheryma whippelii* (Whipple's disease) in faeces. *Ital J Gastroenterol Hepatol* 1999;31:70-72.
- Marth T, Raoult D. Whipple's disease. *Lancet* 2003;361:239-246.
- Dutly F, Altwegg M. Whipple's disease and "*Tropheryma whippelii*." *Clin Microbiol Rev* 2001;14:561-583.
- Bai JC, Sen L, Diez R, Niveloni S, Maurino E, Estévez ME, Boerr LA. Impaired monocyte function in patients successfully treated for Whipple's disease. *Acta Gastroenterol Latinoam* 1996;26:85-89.
- LeVine ME, Dobbins WO III. Joint changes in Whipple's disease. *Semin Arthritis Rheum* 1973;3:79-93.
- O'Duffy JD, Griffing WI, Li CY, Abdelmalek MF, Persing DH. Whip-

- ple's arthritis: direct detection of *Tropheryma whippelii* in synovial fluid and tissue. *Arthritis Rheum* 1999;42:812–817.
19. Dobbins WO III. HLA antigens in Whipple's disease. *Arthritis Rheum* 1987;30:102–105.
 20. Feurle GE, Dorken B, Schopf E, Lenhard V. HLA-B27 and defects in the T-cell system in Whipple's disease. *Eur J Clin Invest* 1979;9:385–389.
 21. Bai JC, Mota AH, Mauriño E, Niveloni S, Grossman F, Boerr LA, Fainboim L. Class I and class II HLA antigens in a homogeneous Argentinian population with Whipple's disease: lack of association with HLA-B 27. *Am J Gastroenterol* 1991;86:992–994.
 22. Olivieri I, Brandi G, Padula A, DiStefano M, Mantovani W, Calabrese C, Saccoccio G, DiFebo G, Corazza GR, Miglioli M, Biasco G. Lack of association with spondyloarthritis and HLA-B27 in Italian patients with Whipple's disease. *J Rheumatol* 2001;28:1294–1297.
 23. Dobbins WO II. The diagnosis of Whipple's disease. *N Engl J Med* 1995;332:390–392.
 24. Williams JG, Edward DP, Tessler HH, Persing DH, Mitchel PS, Goldstein DA. Ocular manifestations of Whipple's disease: an atypical presentation. *Arch Ophthalmol* 1998;116:1232–1234.
 25. Adams M, Rhyner PA, Day J, DeArmond S, Smucker EA. Whipple's disease confined to the central nervous system. *Ann Neurol* 1987;21:104–108.
 26. Ryser RJ, Locksley RM, Eng SC, Dobbins WO III, Schoenkecht FD, Rubin C. Reversal of dementia associated with Whipple's disease by trimethoprim-sulfamethoxazole, drugs that penetrate the blood-brain barrier. *Gastroenterology* 1984;86:745–752.
 27. Feurle GE, Volk B, Waldherr R. Cerebral Whipple's disease with negative jejunal histology. *N Engl J Med* 1979;300:907–908.
 28. Sieracki JC, Fine G, Horn RC, Bebin J. Central nervous system involvement in Whipple's disease. *J Neuropathol Exp Neurol* 1960;19:70–75.
 29. Feldman M, Handler RS, Morrison EB. Acute meningoencephalitis after withdrawal of antibiotic on Whipple's disease. *Ann Intern Med* 1980;93:709–711.
 30. Mainwald M, von Herbay A, Fredricks DN, Ouverney CC, Kosek JC, Relman DA. Cultivation of *Tropheryma whippelii* from cerebrospinal fluid. *J Infect Dis* 2003;188:801–808.
 31. Adler CH, Galetta SL. Oculo-facial-skeletal myorhythmia in Whipple disease: treatment with ceftriaxone. *Ann Intern Med* 1990;112:467–469.
 32. Silbert SW, Parker E, Horenstein S. Whipple's disease of the central nervous system. *Acta Neuropathol* 1976;36:31–38.
 33. Black-Schaffer B. The tinctorial demonstration of a glycoprotein in Whipple's disease. *Proc Soc Exp Biol Med* 1949;72:225–227.
 34. Poulley JW. A case of Whipple's disease (intestinal lipoditrophy). *Gastroenterology* 1952;22:128–133.
 35. Cohen AS, Schimmel M, Holt PR, Isselbacher KJ. Ultrastructural abnormalities in Whipple's disease. *Proc Soc Exp Biol Med* 1960;105:411–414.
 36. Yardley JH, Hendrix TR. Combined electron and light microscopy in Whipple's disease. *Bull Johns Hopkins Hosp* 1961;109:80–98.
 37. Perez V, Tafuri W, Schapira A, Crosetti E, De Larrechea I. Light and electron-microscope findings on jejunal biopsy in Whipple's disease: studies before and after antibiotic therapy. *Am J Dig Dis* 1963;8:718–728.
 38. Wilson KH, Blitchington R, Frothingham R, Wilson JA. Phylogenia of the Whipple's disease-associated bacterium. *Lancet* 1991;338:474–475.
 39. Relman DA, Schmidt TM, MacDermont RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992;327:293–301.
 40. Ramzan NN, Loftus E Jr, Burgart LJ, Rooney M, Batts KP, Weisner RH, Fredricks DN, Relman DA, Persing DH. Diagnosis and monitoring of Whipple's disease by polymerase chain reaction. *Ann Intern Med* 1997;126:520–527.
 41. Harmsen D, Hesseemann J, Brablet T, Kirshner T, Muller-Hermelink HK. Heterogeneity among Whipple's disease-associated bacteria. *Lancet* 1994;343:1288.
 42. Hinrikson HP, Dutly F, Altwegg M. Homogeneity of 16S-23S ribosomal intergenic spacer regions of *Tropheryma whippelii* in Swiss patients with Whipple's disease. *J Clin Microbiol* 2000;38:595–599.
 43. Schoedon G, Goldenber D, Forrer R, Gunz A, Dutly F, Hochli M, Altwegg M, Schaffner A. Cultivation of the bacillus of Whipple's disease. *N Engl J Med* 2000;342:620–625.
 44. Schoedon G, Goldenber D, Forrer R, Gunz A, Dutly F, Hochli M, Altwegg M, Schaffner A. Deactivation of macrophages with interleukin-4 is the key to the isolation of *Tropheryma whippelii*. *J Infect Dis* 1997;176:672–677.
 45. Raoult D, Birg MI, La Scola B, Fournier PE, Enea M, Lepidi H, Roux V, Piette JC, Vandenesch F, Vital-Durand D, Marrie TJ. Cultivation of bacillus of Whipple's disease. *N Engl J Med* 2000;342:620–625.
 46. La Scola B, Fenollar F, Fournier PE, Altwegg M, Mallet MN, Raoult D. Description of *Tropheryma whippelii* gen, nov, sp nov, the Whipple's disease bacillus. *Int J Syst Evol Microbiol* 2001;51:1471–1479.
 47. Ghigo E, Capo C, Aurouze M, Tung CH, Gorvel JP, Raoult D, Mege JL. Survival of *Tropheryma whippelii*, the agent of Whipple's disease, requires phagosomal acidification. *Infect Immun* 2002;70:1501–1506.
 48. Maiwald M, Schumacher F, Ditton HJ, von Herbay A. Environmental occurrence of the Whipple's disease bacterium (*T. whippelii*). *Appl Environ Microbiol* 1998;64:760–762.
 49. Ehrbahr U, Bauernfeind P, Dutly F, Koelz HR, Altwegg M. PCR-positive tests for *Tropheryma whippelii* in patients without Whipple's disease. *Lancet* 1999;353:2214.
 50. Street S, Donoghue HD, Neild GH. *Tropheryma whippelii* DNA in saliva of healthy people. *Lancet* 1999;354:1178–1179.
 51. Flemmer M, Esterowitz T. Detecting Whipple's disease: man versus machine. *Am J Gastroenterol* 2002;97:2675–2676.
 52. Dobbins WO III. Is there an immune deficit in Whipple's disease? *Dig Dis Sci* 1981;26:247–252.
 53. Raoult D, Birg ML, La Scola B, Fournier PE, Enea M, Lepidi H, Roux V, Piette JP, Vandenesch F, Vital-Durand D, Marrie TJ. Cultivation of the bacillus of Whipple's disease. *N Engl J Med* 2000;342:620–625.
 54. Ectors N, Goebes K, De Vos R, Heidebuche H, Rutgeers P, Desmet V, Vantrappen G. Whipple's disease: a histological, immunological and electromicroscopic study of the immune response in the small intestinal mucosa. *Histopathology* 1992;21:1–12.
 55. Cerf M, Hurez D, Narche CL, Debray C. Etude des plasmocytes de l'intestine grele au cours de la maladie de Whipple. *Press Med* 1970;48:2127–2129.
 56. Bjerknes R, Odegaard S, Bjerkvig R, Borkje B, Laerum OD. Whipple's disease: demonstration of a persisting monocyte and macrophage dysfunction. *Scand J Gastroenterol* 1988; 23:611–619.
 57. Bjerknes R, Laerum OD, Odegaard S. Impaired bacterial degradation by monocytes and macrophages from a patient with treated Whipple's disease. *Gastroenterology* 1985;89:1139–1146.
 58. Marth T, Kleen N, Stallmach A, Ring S, Aziz S, Schmidt C, Strober W, Zeitz M, Schneider T. Dysregulated peripheral and mucosal Th1/Th2 response in Whipple's disease. *Gastroenterology* 2002;123:1468–1477.
 59. Lepidi H, Fenollar F, Gerolami R, Mege JL, Bonzi MF, Sahel J, Raoult D. Whipple's disease: immunospecific and quantitative immunohistochemical study of intestinal biopsy specimens. *Hum Pathol* 2003;34:589–596.
 60. Clemett AR, Marshak RH. Whipple's disease: roentgen features and differential diagnosis. *Radiol Clin North Am* 1969;7:105–122.

61. Dominguez Macias A, Fernandez Pascual J, Perez Gomez B, Gonzales del Castillo J, Caballero Otero A. Nuevos aspectos clínico-morfológicos de la enfermedad de Whipple. *Rev Clin Esp* 1976;143:253–264.
62. Geboes K, Ectors N, Heibuchel H, Rutgeerts P, Desmet V, Vantrappen G. Whipple's disease: the value of upper gastrointestinal endoscopy for the diagnosis and follow-up. *Acta Gastroenterol Belg* 1992;55:209–219.
63. Bolt RJ, Pollard HM, Standaert L. Transoral small bowel biopsy as an aid in the diagnosis of malabsorption states. *N Engl J Med* 1958;259:32–34.
64. Crane S, Schlipper W. Duodenoscopic findings in Whipple's disease. *Gastrointest Endosc* 1978;24:248–249.
65. Hendrix JP, Black-Schaffer B, Withers RW, Handler P. Whipple's intestinal lipodistrophy: report of 4 cases. *Arch Intern Med* 1950;85:91–131.
66. Comer GM, Brandt LJ, Abissi CJ. Whipple's disease: a review. *Am J Gastroenterol* 1983;78:107–114.
67. Morgan AD. The first recorded case of Whipple's disease? *Gut* 1961;2:370–372.
68. Yardley JH, Fleming WH. Whipple's disease: a note regarding PAS-positive granules in the original case. *Bull J Hopkins Hosp* 1961;109:76–79.
69. Haubrich WS, Watson JS, Sieracki JC. Unique morphologic features of Whipple's disease. *Gastroenterology* 1960;39:454–468.
70. Strom RL, Gruninger RP. AIDS with *Mycobacterium avium* intracellular lesions resembling those in Whipple's disease. *N Engl J Med* 1983;309:1323–1324.
71. De Larrechea I, Rapaport M, Schapira A, Ramos Mejía M, Crosetti E. La biopsia seriada del yeyuno en la enfermedad de Whipple. *Pren Méd Arg* 1961;48:2875–2879.
72. Barbaryka I, Thorn L, Langer E. Epithelioid cell granulomata in the mucosa of the small intestine in Whipple's disease. *Virchows Archiv (Pathol Anat)* 1979;382:227–235.
73. Raoult D, Lepidi H, Harle JR. Tropheryma whipplei circulating in blood monocytes. *N Eng J Med* 2001;345:548.
74. Davis TD, Mc Bee JW, Borland JL, Kurtz M, Ruffin JM. The effect of antibiotic and steroid therapy in Whipple's disease. *Gastroenterology* 1963;44:112–116.
75. Keinath RD, Merrell DE, Vlietstra R, Dobbins WO III. Antibiotic treatment and relapse in Whipple's disease: long term follow-up of 88 patients. *Gastroenterology* 1985;88:1867–1873.
76. Feurle GE, Marth T. An evaluation of antimicrobial treatment for Whipple's disease: tetracycline versus trimethoprim-sulfamethoxazole. *Dig Dis Sci* 1993;39:1642–1648.
77. Bai JC, Crosetti EE, Mauriño EC, Martinez CA, Sambuelli A, Boerr LA. Short-term antibiotic treatment in Whipple's disease. *J Clin Gastroenterol* 1991;13:303–307.
78. Fleming JL, Wiesner RH, Shorter RG. Whipple's disease: clinical, biochemical, and histopathologic features and assessment of treatment in 29 patients. *Mayo Clin Proc* 1988;63:539–551.
79. Schneider T, Stallmach A, von Herbay A, Marth T, Strober W, Zeitz M. Treatment of refractory Whipple's disease with interferon- γ . *Ann Intern Med* 1998;129:875–877.
80. Cannon WR. Whipple's disease, genomics, and drug therapy. *Lancet* 2003;361:1916.

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