Gastrointestinal Diagnosis of Classical Whipple Disease: Clinical, Endoscopic, and Histopathologic Features in 191 Patients

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Abstract: Classic Whipple disease (CWD) is a systemic infection caused by Tropheryma whippelii. Different diagnostic tools have been developed over the last decades: periodic acid-Schiff (PAS) staining, T. whippelii-specific polymerase chain reaction (PCR), and T. whippelii-specific immunohistochemistry (IHC). Despite all these advances, CWD is still difficult to diagnose because of a variety of clinical symptoms and possibly a long time span between first unspecific symptoms and the full-blown clinical picture of the disease.

Herein, we report an observational cohort study summarizing epidemiologic data, clinical manifestations, and diagnostic parameters of 191 patients with CWD collected at our institution. Gastrointestinal manifestations are the most characteristic symptoms of CWD affecting 76% of the cohort. Although the small bowel was macroscopically conspicuous in only 27% of cases, 191 (91%) patients presented with characteristic histological changes in small bowel biopsies (in 2 patients, these changes were only seen within the ileum). However, 18 patients displayed normal small bowel histology without typical PAS staining. In 9 of these patients, alternative tests were positive from their duodenal specimens (ie, T. whippelii-specific PCR and/or IHC). Thus, in 182 patients (95%) a diagnostic hint toward CWD was obtained from small bowel biopsies. Only 9 patients (5%) were diagnosed solely based on positive T. whippelii-specific PCR and/or IHC of extraintestinal fluids (eg, cerebrospinal fluid, synovial fluid) or extraintestinal tissue (eg, lymph node, synovial tissue), respectively.

Thus, despite efforts to diagnose CWD from alternative specimens, gastroscopy with duodenal biopsy and subsequent histological and molecular–biological examination is the most reliable diagnostic tool for CWD.

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Abbreviations: CSF = cerebrospinal fluid, CWD = classical Whipple disease, IHC = immunohistochemistry, IRIS = immune reconstitution inflammatory syndrome, PAS = periodic acid-Schiff, PCR = polymerase chain reaction.

INTRODUCTION

Classical Whipple disease (CWD) is a systemic chronic infection by Tropheryma whippelii that can involve various organ systems such as gastrointestinal tract, joints, and central nervous system (CNS). 1–3 CWD was first described by George H. Whipple in 1907 and in 1949, the typical histological picture of the intestinal mucosa was described displaying foamy macrophages with cytoplasmatic periodic acid-Schiff (PAS) reactivity. 2 The etiology was further clarified in 1961 by detecting bacteria by electron microscopy. 6,7 Not until 30 years later, a first specific polymerase chain reaction (PCR) assay was established targeting T. whippelii 16S ribosomal RNA (rRNA) genes from duodenal lesions of a patient. 8 In 2000, the bacterium could be finally cultured in human fibroblast cells enabling the sequencing of its whole genome and the development of a specific immunohistochemical staining in 2002 and various diagnostic PCR assays. 11–13

Despite all these advances in the field of diagnostic tools, CWD is still difficult to diagnose. First, CWD may present with a variety of rather nonspecific symptoms—such as diarrhea, weight loss, abdominal pain, lymphadenopathy, and fever. 1–3 Second, there might be a long time span between first symptoms and the full-blown clinical picture of the disease. 5,14 In most cases, CWD is characterized by its clinical involvement of the gastrointestinal tract. An infection of the cerebrospinal fluid (CSF) occurs in 10% to 40% of the patients who may present with neurological or psychiatric symptoms but also may remain asymptomatic. 1,15 However, in addition, atypical isolated manifestations of chronic infection with T. whippelii such as T. whippelii-induced endocarditis, neurologic infections, arthritis, uveitis, and pneumonia lacking an involvement of the gastrointestinal tract may occur. 1,2,16–18

Today, the diagnosis of CWD mainly is based on the histological investigation of the small bowel mucosa (ie, positive PAS staining of duodenal biopsies) but atypical cases require a more elaborate diagnostic scheme. 3,19 For the diagnosis of CWD, the conduction of several tests of which at least 2 should be positive to assure the diagnosis of CWD was suggested. However, the value of gastroscopy, histology, and molecular–biological methods for the diagnosis of CWD has
not been systemically evaluated. But despite all advances, histological examination of duodenal biopsies seems the most reliable diagnostic method for the diagnosis of CWD.

Thus, here we report on our experience and the value of gastroscopy, macroscopic appearance of the duodenum, and histological and molecular–biological examination of duodenal biopsies for the diagnosis of Whipple disease in CWD patients with typical gastrointestinal involvement and atypical cases with no explicit hint toward Whipple disease based on intestinal symptoms.

PATIENTS AND METHODS

Patients

In this observational cohort study from 2002 up to now, 603 cases from central Europe suspected to suffer from CWD were analyzed in the course of an European project on Whipple disease (Figure 1). Samples were collected either directly in our clinic or specimens were referred to us as a reference center for the diagnosis of CWD. Among them, 222 patients were reported to have chronic infection with *T. whippeli* whereas 372 cases revealed no positive diagnostic test. Five patients were misdiagnosed because of positive PAS staining of muciphages in colon biopsies20 (4 cases) or PAS-positive germinoma cells in CNS biopsies (1 case) and 4 cases revealed a positive *T. whippeli*-specific PCR from duodenal biopsies. In 7 cases with chronic infection with *T. whippeli*, diagnosis was not assured reliably and they were excluded from this study; 24 patients with isolated *T. whippeli* infection of the heart valve were subject of a previous publication19 and are not reported here in detail. Thus, 191 cases with CWD are presented here (Figure 1); 42 CWD patients were primarily seen at our clinic, 79 patients were monitored in the course of treatment trials,21,22 1 still ongoing, whereas 70 patients CWD were referred to us from other hospitals or medical practices to assure the diagnosis of CWD or monitor treatment success. Epidemiologic data and information on clinical manifestations of each patient were collected.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Clinical Ethics Committee of the Charité, Berlin, Germany, and all adult subjects provided written informed consent.

Diagnosis of CWD: Case Definition

In this observational cohort study, CWD was defined as a systemic chronic infection with *T. whippeli* and diagnosis

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**FIGURE 1.** Details of the described cohort of suspected and excluded cases and 191 patients with CWD. CNS = central nervous system, CWD = classic Whipple disease, IHC = immunohistochemistry, PAS = periodic acid-Schiff, PCR = polymerase chain reaction.
required 2 independent positive tests. Isolated T whipplei-induced endocarditis, patients with only 1 unsecure positive test, or asymptomatic carriage was excluded (Figure 1). Primary diagnosis of CWD was based on histological examination and PAS staining of duodenal biopsies followed by T whipplei-specific PCR or immunohistochemistry (IHC) for confirmation. Two positive tests from the duodenum assured the diagnosis of CWD. In cases with only 1 positive test from the duodenum, PAS staining and T whipplei-specific PCR and/or IHC of extraintestinal tissue or fluids were conducted in addition. T whipplei-specific PCR from CSF was performed independent of neuronal symptoms whenever possible.

Cases without histological diagnosis from the duodenum required 2 independent PCR assays from sterile specimens targeting T whipplei sequences (eg, 16S rRNA gene amplification followed by sequencing and rpoB gene amplification with specific probe hybridization), positive PAS and T whipplei-specific IHC or PCR from affected organs, or the combination of at least 2 independent tests from 2 different locations.

All 191 patients with CWD achieved and maintained clinical and laboratory remission during a median follow-up of 66 months (range 0–324 months).

For the majority of cases and controls, diagnosis was set based on duodenal biopsies. This is a possible bias of the study. However, to minimize this bias, in all cases with extraintestinal symptoms but negative duodenal histology, additional specimens for diagnosis were taken from symptomatic tissues.

**Investigations**

PAS staining with subtyping, PCR, and IHC were performed in 3 to 5 biopsies of normally the duodenal but also gastric, jejunal, or ileal mucosa whenever available. Biopsies were fixed in formalin, embedded in paraffin, and subjected to PAS staining. PAS-positive macrophages in the intestinal mucosa were classified as subtypes 1 to 4 as described by von Herbay et al.12,13,25 The PCR results were assured by sequencing of the 16rDNA gene PCR product and, a second, T whipplei-specific PCR with probes specific for T whipplei.12

T whipplei-specific PCR was performed on fresh biopsies of fluids and in exceptional cases on frozen specimens or paraffin-embedded biopsies. PCR assays were used as previously described.12,13,25 The PCR results were assured by sequencing of the 16S rRNA gene PCR product and, a second, T. whipplei-specific PCR with probes specific for T whipplei.12

Blood for laboratory investigations was drawn at the time of diagnosis and a lumbar puncture was performed to access CSF for T whipplei-specific PCR whenever possible. Baseline laboratory parameters were determined by routine laboratory tests, not available in all patients but only in 108 as they were transferred to us after initiation of antibiotic treatment. For quantitative laboratory parameters of these 108 patients, mean and standard deviation are given.

**RESULTS**

**Patients**

From 603 cases suspected to suffer from CWD, 372 cases revealed no positive diagnostic test (Figure 1). In 5 cases, misleading positive PAS staining of muciphages in colon biopsies20 (4 cases) or PAS-positive germinoma cells in CNS biopsies (1 case) initially resulted in misdiagnosed CWD and corresponding treatment. CWD diagnosis later was disproved by more specific tests. Among 222 patients with chronic infection with T whipplei, in 7 cases, diagnosis was not assured reliably and they were excluded from this study. In addition, 24 patients with T whipplei-induced endocarditis did not reveal any signs of systemic infection and thus also were not included in the analyses. Thus, our study included 191 assured cases of CWD (Figure 1) that all revealed a sustained clinical response (median observation period of 66 months, range 0–324 months) after specific antibiotic treatment.21,26,27

**CWD Patients’ Characteristics**

Of 191 patients, 77% (n = 148) were male. The mean age at time of diagnosis was 57 years (ranging from 31 to 84 years). The period of time between onset of first symptoms and diagnosis of CWD averaged 7.5 years (ranging from 0 to 51 years; Table 1). Generally, first manifestations preceding the gastrointestinal symptoms were articul pain.

**Clinical Manifestations**

At time of diagnosis, 146 patients (76%) presented with gastrointestinal symptoms, that is, chronic diarrhea, and 99 patients with weight loss (52%), whereas 129 patients (68%) displayed inflammatory arthritis. Neurological symptoms, such as cognitive changes, ophthalmoplegia, nystagmus, and myoclonia were present in 46 patients (24%). Before initiation of antibiotic treatment, 49 patients (26%) had a raised temperature or fever and 65 patients (34%) received an immunosuppressive treatment before diagnosis of CWD due to the suspicion of a rheumatic disease (Table 1).

**Laboratory Investigations**

Data of 108 patients were available and are presented in Table 2. Anemia was reported in 87 patients (81%), and raised leukocyte and platelet values were found in 52 and 60 patients (48%, 56%), respectively. An increased C-reactive protein was detected in 74 patients (69%).

**TABLE 1.** Baseline Clinical Characteristics of the CWD Patients (n = 191)

| Characteristic | No. |
|---------------|-----|
| Male (%)       | 148 (78) |
| Mean age at diagnosis, y (range) | 55 (31–84) |
| Time between first symptoms and diagnosis, y (range) | 7.1 (0–51) |
| Diarrhea/malabsorption (%) | 146 (76) |
| Weight loss (%) | 99 (52) |
| Joint involvement (%) | 129 (68) |
| Neurologic symptoms (%) | 46 (24) |
| PCR positive of CSF tested before treatment (%) | 56/135 (41) |
| Fever (%) | 49 (26) |
| Immunosuppressive treatment before diagnosis (%) | 65 (34) |

CSF = cerebrospinal fluid, CWD = classic Whipple disease, PCR = polymerase chain reaction.
TABLE 2. Baseline Laboratory Characteristics of the CWD Patients (n = 108)

| Parameter                  | No. (%) | Mean (SD) | Median (range) |
|----------------------------|---------|-----------|----------------|
| Anemia                     | 87 (81) | 10.7 ± 2.3| 10.0 (8.5–12.0)|
| Elevated leukocyte level   | 52 (48) | 13076 ± 9990 | 11586 (9630–20100) |
| Elevated platelet level    | 60 (56) | 389 ± 147 | 354 (230–950) |
| Elevated C-reactive protein | 74 (69) | 36.8 ± 51.4 | 11.4 (–11.5–72.6) |

CWD = classic Whipple disease.

Primary Gastrointestinal Diagnosis of CWD

Macroscopic Changes at Gastroscopy

Gastroscopy with biopsy was performed on all patients. Macroscopically, the appearance of the duodenum was conspicuous in only 50 of 191 patients (26%) (Table 3): 21 cases (11%) revealed changes in the mucosal appearance as described to be characteristic for CWD with clumsy or dilated villi (13 cases), ecstatic lymph vessels (8 cases), or edema (6 cases) that were very prominent (Figure 2A) or discrete (Figure 2B), whereas 19 (10%) revealed signs of a duodenitis (Figure 2C), versus 10 (5%) where the changes were unspecific and the duodenal mucosa appeared reddened or red and turgid in 5 cases (Figure 2D and E), the villus architecture appeared edematous or clumsy in 6 cases, hemorrhagic in 2 cases, and severe in 1 case. In 10 (5%) cases, the changes were unspecific and the duodenal mucosa was very prominent (Figure 2A) or discrete (Figure 2B), whereas 19 (10%) revealed signs of a duodenitis (Figure 2C), versus 10 (5%) where the changes were unspecific and the duodenal mucosa appeared reddened or red and turgid in 5 cases (Figure 2D and E), the villus architecture appeared edematous or clumsy in 6 cases, hemorrhagic in 2 cases, and severe in 1 case. Polypoid changes, partial villous atrophy, and whitish deposits were described each in 1 case; and 1 case presented with little aphthous ulcerations in the duodenal mucosa (Figure 2F).

Histological Examination

Histologically, villous atrophy was described in 35 (18%) cases and lymphangiecstasia in 37 (19%) cases. Foamy macrophages with a strong positive PAS reaction classified as subtype 1, as described by von Herbay et al., were detected in 168 patients (88%) (Figure 3A), in some cases confirmed by T whipplei-specific IHC (Figure 3B); in 2 patients, these histological changes were only seen within the terminal ileum. Six additional patients probably received antibiotic regimens before the diagnosis of CWD and revealed a positive PAS staining of duodenal biopsies that was classified according to von Herbay et al., as types 2 and 3. Thus, routine PAS staining of small bowel biopsies was a clear diagnostic hint toward CWD in 173 cases (91%). Nine additional patients—without a positive or conclusive PAS reaction within their duodenal biopsies—revealed alternative positive tests from duodenal specimens (see Table 3, patients 5, 9–12, 15–18; Figure 3 C–H). Thus, in 182 patients (95%), a diagnostic hint toward CWD was obtained from small bowel biopsies.

Among 159 cases with signs of malabsorption such as either diarrhea or weight loss or both, only 1 case did not reveal a characteristic PAS staining of duodenal biopsies (Table 3, patient 18; Figure 3G). However, T whipplei-specific IHC was positive in this patient’s duodenal specimen (Figure 3H). Thus, in 99.4% of patients with clinical hints toward gastrointestinal CWD, PAS staining of small bowel biopsies established diagnosis.

In 32 patients, neurologic or rheumatoid symptoms were the only clinical manifestations of CWD. Gastroscopy was performed in 31 of them. However, in 15 among those 31 patients (48%), histology of duodenal biopsies was also indicative of CWD.

TABLE 3. Primary Diagnosis of CWD in Patients Without Distinct Duodenal PAS Staining (n = 18)

| Patient | PAS Duodenum | PCR Duodenum | IHC Duodenum | PCR CSF | Diagnosis Via |
|---------|--------------|--------------|--------------|---------|---------------|
| 1       | –            | Ø            | –            | +       | CSF           |
| 2       | –            | Ø            | –            | +       | CSF           |
| 3       | –            | +            | –            | +       | CSF, PCR duodenum IHC skin |
| 4       | –            | –            | +            | Ø       | IHC duodenum |
| 5       | –            | –            | –            | +       | CSF, PAS/PCR antrum |
| 6       | –            | Ø            | –            | +       | CSF           |
| 7       | –            | Ø            | –            | Ø       | PCR lacrimal fluid |
| 8       | –            | –            | –            | Ø       | PCR synovial fluid |
| 9       | –            | +            | +            | –       | PCR/IHC duodenum |
| 10      | –            | –            | –            | –       | PCR synovia (femoral head) |
| 11      | +            | +            | +            | +       | PCR/IHC duodenum, PAS/IHC synovia (knee) |
| 12      | (+)          | +            | Ø            | –       | PCR duodenum, PCR synovial fluid |
| 13      | –            | +            | Ø            | –       | PCR/IHC duodenum, PAS/IHC synovia (knee) |
| 14      | –            | –            | Ø            | Ø       | PAS/IHC lymph node |
| 15      | –            | Ø            | +            | –       | PAS/IHC lymph node |
| 16      | Ø            | Ø            | Ø            | Ø       | PAS/IHC lymph node |
| 17      | (+)          | +            | +            | –       | PCR/IHC duodenum |
| 18      | (+)          | +            | +            | +       | PCR synovial fluid |

Patients 1 to 17 without typical gastrointestinal symptoms, and patient 18 with diarrhea. Ø = not done, CSF = cerebrospinal fluid, CWD = classic Whipple disease, IHC = immunohistochemistry, PAS = periodic acid-Schiff, PCR = polymerase chain reaction, + = positive, (+) = positive.
Molecular–Biological Analysis of Duodenal Specimens and CSF

T whipplei-specific PCR was initially performed on duodenal biopsies of 96 CWD patients, of which 87 (91%) showed a positive result. Of 9 patients with a negative PCR from the duodenum at diagnosis of CWD, 3 received previous antimicrobial regimens for other reasons and 4 displayed no signs of gastrointestinal disease.

To assess for neuronal involvement in CWD, T whipplei-specific PCR of CSF was initially performed on 135 patients, thereof. The result was positive for 56 patients (41%). In contrast to 31 of 41 (76%) patients with neurological symptoms, only 25 of 94 (27%) neurologically asymptomatic patients displayed a positive PCR result.

Difficulties in Diagnosis: Whipple Disease Patients With Atypical Features

In 20 patients, duodenal PAS staining was either negative or weak and inconclusive, or not done (Table 3, patient 16). Of these patients, 2 revealed typical PAS-positive macrophages in the terminal ileum, and the other 18 are summarized in Table 3; although patients 1 to 17 did not present with gastrointestinal symptoms, patient 18 suffered from diarrhea. In 9 patients, alternative tests of the duodenum were indicative of CWD; in 2 patients, PAS-positive macrophages were detected solely within the duodenal submucosa and could not be classified according to von Herbay et al (Figure 3C and E); and in 1 case, PAS-positive cells were of type 2 according to von Herbay et al (Table 3, patient 18). In these 3 cases, T whipplei infection was proven via T whipplei-specific IHC and PCR. Five additional patients had a positive T whipplei-specific PCR of their duodenal biopsies (Table 3, patients 3, 9, 11, 13, 18), 3 of them with an additional positive T whipplei-specific IHC in duodenal specimens (Table 3, patients 9, 11, 18). In 2 patients (Table 3, patients 4, 15), diagnosis of CWD was established only on the basis of a positive duodenal T whipplei-specific IHC. In these patients with hints toward CWD from the duodenum, diagnosis was assured by detection of T whipplei in synovial tissue (Table 3, patients 11, 12, 13, 18), the CSF (Table 3, patients 3, 11, 15, 18), the lymph node (Table 3, patient 15), or skin biopsies (Table 3, patient 3).

In the remaining 9 patients, small bowel biopsies did not give any hint on CWD. Only positive T whipplei-specific PCR results of CSF (Table 3, patients 8, 10), positive PAS and IHC of lymph node specimens (Table 3, patients 14, 16), positive PAS, PCR, and IHC of the antrum (Table 3, patient 5), or positive PCR of lacrimal fluid during the course of an uveitis (Table 3, patient 7) established the diagnosis of Whipple disease with atypical features.

Interestingly, in both patients with initially normal duodenal findings (PAS and PCR negative) and solely positive T whipplei-specific PCR results of lacrimal and synovial fluid (Table 3, patients 7, 8), repeated duodenal biopsies for surveillance of their...
treatment showed CWD-like histological changes and positive T whipplei-specific PCR results. Similarly, in the 2 patients in whom diagnosis was first established from the terminal ileum, histological CWD-like changes were seen in the duodenum later during the course of disease.

**FIGURE 3.** Histopathological appearance of the duodenal mucosa of CWD patients following PAS staining (A, C, E, G) or *Tropheryma whippelii*-specific IHC (B, D, F, H). (A) Classical histopathological appearance of the duodenal mucosa of untreated CWD with flattened and clumsy villi, lymphangiectasia, and numerous PAS-positive macrophages of type 1 that can be visualized more intensively and specifically with (B) *T whipplei*-specific IHC. (C) Atypical PAS staining of only the submucosa (faint PAS-positive cells in the inset that can be specified with (D) IHC. (E) Villus lamina propria with negative PAS staining with few (F) *T whipplei*-containing cells visualized by IHC (marked by black arrows and with a close-up in the inset). (G) PAS-negative duodenal submucosa containing positive cells following (H) *T whipplei*-specific IHC. CWD = classic Whipple disease, IHC = immunohistochemistry, PAS = periodic acid-Schiff.

**Treatment and Outcome**

Most of the patients were treated with a 14 days regime of intravenous ceftriaxone (n = 91) or meropenem (n = 18) followed by 12 months of oral trimethoprim/sulfamethoxazole (TMP/SFX). Other patients received a 14 days regime of
intravenous ceftriaxone followed by 3 months of oral TMP/SFX (n = 35) or doxycycline in combination with hydroxychloroquine (n = 11) or solely doxycycline (n = 7). The most serious complications during follow-up were an immune reconstitution inflammatory syndrome (IRIS) (n = 28), fatal outcome (n = 15), and persistent infection or relapse (n = 6), respectively. For 5 of the 15 deaths, no direct association with CWD was obvious, although we cannot exclude that chronic immune activation or tissue damage finally resulted in a fatal outcome. However, in 10 of the patients, death seemed to be directly associated with the chronic infection with T. whipplei. Four patients acquired severe IRIS after initiation of antimicrobial treatment, 4 patients suffered from irreversible severe neuronal damage, 1 patient died immediately at diagnosis before initiation of treatment, and 1 patient passed away shortly after diagnosis due to pneumonia.

**DISCUSSION**

Here we report on our experience in diagnosis of CWD. The cohort that was monitored was consistent with previous studies concerning their mean age of diagnosis (57 years), the mean time span between first symptoms and diagnosis (7.5 years), and the variety of clinical symptoms.\(^1\)–\(^3\),\(^2\)\(^1\)–\(^2\)\(^2\)\(^3\)\(^1\) The age at diagnosis and the proportion of women affected (23%) did not change in comparison with the previous data on patients diagnosed between 1985 and 1996.\(^2\)\(^8\) In this cohort, arthritis was not the major symptom as described recently,\(^2\)\(^2\) but diarrhea.

In our cohort, diagnosis of CWD was based on a positive PAS staining of small bowel biopsies in 173 of the 191 patients (91%). However, PAS positive may not be of subtype 1 or only detected within the ileum. PAS-positive macrophages may be only later detected within duodenal biopsies subsequently taken for histological examination with 1.) PAS-staining and 2.) PCR and/or T. whipplei-specific immunohistochemistry (IHC). Two test positive PCR from fluids

**FIGURE 4.** Three-stage schema for the diagnosis of CWD. First-line diagnosis should be performed for all cases suspected to suffer from Whipple disease. Even in the absence of gastrointestinal symptoms, the second step only when diagnosis from the small intestine is doubtful, and the third step for all patients with the assured diagnosis of Whipple disease.\(^4\) A possible antimicrobial pretreatment, also for other causes than Whipple disease, should be checked carefully in cases with PAS-positive cells of types 2 to 4 or only a positive IHC.\(^7\) PAS-positive cells of type 1 in the presence of the typical clinical picture of CWD that are evaluated by an experienced pathologist assure the diagnosis, PCR or IHC should be added whenever possible.\(^5\)\(^5\) For patients with gastrointestinal symptoms, negative histological results are very rare, so further diagnostic steps are only recommended for patients with additional extraintestinal symptoms. CWD = classic Whipple disease, IHC = immunohistochemistry, PAS = periodic acid-Schiff, PCR = polymerase chain reaction.
for the surveillance of antibiotic treatment, indicating that the duodenal affection might be very patchy and multiple biopsies need to be assessed for CWD diagnosis. We experienced that light microscopy with PAS staining of gastrointestinal biopsies is still the most reliable tool for both diagnosis of CWD and monitoring of treatment success. In doubtful cases, T whipplei-specific PCR and/or T whipplei-specific IHC might enhance a questionable PAS staining. For the assured diagnosis of CWD, at least 2 different tests should be positive to avoid misdiagnosis and subsequent ineffective treatment.

However, in 18 patients, diagnosis could not be based on characteristic positive PAS staining of small bowel biopsies, of which in 9 patients, alternative positive tests from their duodenal specimens (eg, T whipplei-specific PCR and/or IHC) could detect T whipplei. Particularly, T whipplei-specific IHC is a diagnostic test with very high specificity and sensitivity and allows the identification of T whipplei in PAS-negative specimens and should be considered. Thus, in 182 patients (95%), a clear hint toward CWD was obtained from small bowel biopsies. Gastrointestinal symptoms are not a prerequisite for an identification of chronic infection with T whipplei from the small bowel, as in 48% of cases without any gastrointestinal symptoms, PAS staining nevertheless was positive.

However, in 9 patients (4.7%), small bowel biopsies did not give any hint on CWD. This is a proportion similar to previous reports on CWD.11,13 In such cases, a reliable diagnosis requires a positive T whipplei-specific PCR of extraintestinal fluids (CSF, synovial fluids, etc) or PCR, IHC, or PAS staining of extraintestinal tissue (synovial tissue, skin, etc).13,14 Therefore, in patients with clinically suspected CWD and normal duodenal histology, sampling of extraintestinal tissue and fluids for PAS staining, T whipplei-specific PCR or IHC is required and may be essential for the diagnosis of Whipple disease with atypical features of the clinical and/or antibiotic treatment. Particularly, in patients with seronegative rheumatic disease, the possibility of CWD should be considered and diagnosis broadened to extraintestinal specimens. A suggested schema for the diagnosis of CWD based on our previous experience and the data presented here is displayed in Figure 4.

A possible limitation of our study is the investigation of only gastrointestinal specimens in the majority of cases. This bias was minimized, as in all cases with extraintestinal symptoms and negative duodenal histology, additional specimens for diagnosis were taken from symptomatic tissues. Nevertheless, we cannot definitively exclude that we missed patients with asymptomatic infection of extraintestinal tissues.

Recently, the diagnostic value of T whipplei-specific quantitative PCR on saliva and stool specimens as first-line screening for CWD has been described.15 However, this approach is not yet generally applicable. More importantly, asymptomatic carriers with positive T whipplei-specific PCR results of saliva, duodenal biopsies, and stool have been described with varying prevalence. Among the French population, T whipplei DNA was detected in 0.6% of saliva specimens and 4% of stool samples of healthy persons.29,30 Among sewage plant workers in Austria, the prevalence of T whipplei DNA in stool samples was as high as 25%.31 In addition to the asymptomatic carrier status, T whipplei can be detected during self-limiting infections such as acute gastroenteritis of French children.32 Knowing all these pitfalls, Fenollar et al quoted that diagnosis of CWD is highly likely when quantitative PCR of saliva and stool were positive and small bowel biopsies should be looked at to confirm the diagnosis via histology.13 Thus, screening of noninvasive specimen may point at persons with a high risk to suffer from CWD in the future and facilitate the subsequent classical diagnostic approach from tissue specimens.

At time of diagnosis, in our study, overall 41% of patients displayed a positive T whipplei-specific PCR of their CSF. T whipplei was detected by PCR in the CSF of not only in 75% of neurological symptomatic patients but also in 27% of all asymptomatic patients tested. Neurological involvement, even without any symptoms, leads to the most dramatic courses of CWD with a high percentage of fatal outcomes. Hence, at the time of diagnosis, PCR of CSF should be performed on all patients and follow-up PCR after 6 to 12 months under antibiotic treatment is absolutely recommended to ensure the clearance of the CNS infection. However, the optimal treatment for persistent CNS infection has not been evaluated yet and the consequences of a persistently asymptomatic positive CSF for T whipplei are still obscure.

In conclusion, histology with PAS staining of duodenal biopsies still can be considered as the most reliable diagnostic tool for CWD. Tissue should be sampled in any case of suspected CWD as macroscopic appearance of the mucosa is usually unsuspicious and a negative histology in patients with gastrointestinal symptoms dissents CWD. T whipplei-specific PCR and IHC are important to confirm the diagnosis especially in doubtful cases and assess for CNS involvement.

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