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ORIGINAL ARTICLE

Tropheryma whipplei Infection (Whipple Disease) in the USA

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Abstract

Background Whipple disease (WD) is an infection caused by the bacterium *Tropheryma whipplei* (TW). Few cases have been reported in the USA.

Aims To report on the demographics, clinical manifestations, diagnostic findings, treatment, and outcomes of TW infection.

Methods Cases of TW infection diagnosed from 1995 to 2010 were identified in three US referral centers and from 1995 to 2015 in one. Definite classic WD was defined by positive periodic acid-Schiff (PAS) staining and probable WD by specific positive TW polymerase chain reaction (PCR) of intestinal specimens. Localized infections were defined by a positive TW PCR result from samples of other tissues/body fluids.

Results Among the 33 cases of TW infections, 27 (82%) were male. Median age at diagnosis was 53 years (range 11–75). Diagnosis was supported by a positive TW PCR in 29 (88%) and/or a positive PAS in 16 (48%) patients. Classic WD was the most frequent presentation ($n=18$, 55%), with 14 definite and 4 probable cases. Localized infections ($n=15$, 45%) affected the central nervous system ($n=7$), joints ($n=4$), heart ($n=2$), eye ($n=1$), and skeletal muscle ($n=1$). Blood PCR was negative in 9 of 17 (53%) cases at diagnosis. Ceftriaxone intravenously followed by trimethoprim and sulfamethoxazole orally was the most common regimen ($n=23$, 70%). Antibiotic therapy resulted in clinical response in 24 (73%).

Conclusions TW infection can present as intestinal or localized disease. Negative small bowel PAS and PCR do not exclude the diagnosis of TW infection, and blood PCR is insensitive for active infection.

Keywords Whipple disease · Intestinal lipodystrophy · Tropheryma · Malabsorption syndromes

Introduction

Classic Whipple disease (WD) is a rare and difficult-to-diagnose infectious disease that often presents as a chronic systemic illness and has been described as diarrhea, weight loss, abdominal pain, and arthralgias in middle-aged men [1–4]. The cause remained a mystery until the 1950s when responsiveness to antibiotics suggested an infectious etiology [5]. In subsequent decades, the causative agent was identified as bacteria [6], and alternative presentations, termed localized *Tropheryma Whipplei* (TW) infection, were recognized to occur outside the gastrointestinal tract [7].

Development of a polymerase chain reaction (PCR) assay in 1991 [8] allowed expansion of diagnostic testing to a multitude of tissues and body fluids [9–14]. PCR precedes diagnostic histologic changes, is highly sensitive for diagnosis, and is useful in monitoring treatment response [15, 16]. The sensitivity of PCR has been enhanced with the genome

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sequencing of TW, which has improved the diagnostic tools for TW infection [17].

Despite the rarity of TW infection, the bacterium is ubiquitous in the environment and has been identified in the stool of asymptomatic individuals in Europe and Africa, and gastric aspirates in America, suggesting a genetic susceptibility to infection [18–20]. This is supported by the association between chronic TW infection and certain human leukocyte antigen types [21]. Even with advances in diagnostic techniques, and improved understanding of TW infection, the protean nature of symptoms and the rare occurrence create continued challenges in diagnosis and treatment. Several reports and large series describe persistent predominance in middle-aged men, latency of diagnosis, and risk of relapse with development of CNS manifestations [3, 4, 22–26].

With a paucity of recent US series, our aim was to report clinical features, diagnostic profiles, response to therapy and outcomes for a series of both classic WD and localized TW cases from referral centers throughout the USA in the era of PCR.

Methods

Patients

After institutional review board approval, an electronic billing and coding retrieval system at each institution was used to search patient records for International Classification of Diseases, 9th and 10th revision codes for diagnoses of intestinal lipodystrophy or Whipple's disease (040.2 and K90.81, respectively) at three US referral centers from 1995 to 2010: Beth Israel Deaconess in Boston, MA (BI); Columbia University in New York, NY (CU); and University of Chicago Hospital in Chicago, IL (UCh), and at Mayo Clinic in Rochester, MN (MCR) from 1995 to 2015. Potential cases were reviewed by a single examiner at each institution. Cases required a clinical history compatible with TW infection and positive PCR result from at least one site or clear histologic evidence of TW by positive periodic acid-Schiff (PAS) staining macrophages in clinically involved tissue [25–27]. Cases without PCR positivity or clear histologic evidence of WD/TWI in the pathology report were excluded.

Data Abstraction

The medical records were reviewed by a single examiner at each institution. A standardized data collection form was used to record data on patient characteristics, presentation, physical and laboratory examination, and prior diagnoses and treatments. The time from reported onset of symptoms ultimately attributed to TW infection to case defining diagnosis was used to calculate the interval to diagnosis. Following diagnosis, the type of treatment, duration, clinical response, and relapse were recorded. Follow-up was determined by date of last documented face-to-face meeting with a clinician, or notice of death.

Case Definition

Three classifications for TW infection were used in this study: definite classic WD, probable classic WD, and localized WD. While previously considered pathognomonic, positive PAS staining macrophages from small bowel biopsies are not entirely specific and may have a high false positive rate, as they can be present in other infections, such as *Mycobacterium Avium* [28]. Definite classic WD was therefore defined by both a positive PAS and positive PCR from small bowel biopsies [26]. Probable classic WD was defined by positive PCR with either no or a negative PAS result, or positive PAS and no or a negative PCR result from small bowel biopsies. Cases of positive PCR in the setting of a negative PAS have been attributed to the patchy localization of bacteria in the gut, leading to a sampling error that has a larger negative impact on PAS due to smaller sample size and poorer sensitivity [29]. Both definite and probable classic WD can have extraintestinal involvement.

Localized TW infection was defined as PAS or PCR positivity from a nonintestinal location without intestinal involvement [26]. Extraintestinal infection was defined by either negative intestinal investigation by PCR or PAS, or an absence of bowel investigation. Negative intestinal investigation in localized disease was defined as a small bowel biopsy with negative PCR and/or PAS with ongoing clinical symptoms prior to, or no more than seven days after starting antimicrobial therapy for WD (Table 1).

PCR samples were collected from specific tissues and fluids (small bowel, colonic, gastric, blood, synovial fluid, cerebral spinal fluid, dura, muscle, lymph node, brain tissue, thrombus, and vitreous fluid). Stool and saliva samples were not collected or processed for PCR. The PCR primers used were specific TW primers, and in the case of MCR, were

Table 1 Diagnostic criteria for three classifications for *T. whipplei* infection used in this study

	Small bowel		Extraintestinal tissue	
	PCR	PAS	PCR	PAS
Definite classic WD	+	+	−/+/ND	−/+/ND
Probable classic WD	+	−/ND	−/+/ND	−/+/ND
	−/ND	+		
Localized WD	−/ND	−/ND	+	−
	−/ND	−/ND	−	+

WD Whipple's disease; *ND* biopsy not done; *PCR* polymerase chain reaction; *PAS* periodic acid-Schiff

directed against the heat shock protein 65 gene (LCWHIP Set #121, TIB MolBiol), which has been shown to have a higher sensitivity and equal to slightly lower specificity than the traditional 16S ribosomal DNA primers [30]. The run was performed on the LightCycler 2.0 platform using 45 PCR cycles for amplification; when compared to conventional PCR, the sensitivity and specificity were 98 and 99%, respectively. There was no cross-reaction when tested on 28 genotypically similar organisms, and analytical sensitivity was less than 50 targets per reaction.

Statistical Analysis

Data were summarized with descriptive statistics. For comparison of demographic data between the two groups, classic WD or localized TW infection, differences were assessed by Wilcoxon rank-sum test for continuous variables. Proportions were assessed by Fisher's exact test. P value < 0.05 was considered significant.

Results

We present a total of 33 cases of TW infection: 29 (88%) confirmed by PCR, and four cases diagnosed by clinical symptoms and PAS positivity without PCR testing. No cases diagnosed by PAS had a negative PCR from the same site. Eighteen cases were classified as classic WD (55%) and 15 cases as extraintestinal localized TW infection [CNS ($n=7$), articular ($n=4$), vitreitis ($n=1$), pericarditis ($n=1$), bacteremia ($n=1$), and myositis ($n=1$)].

Demographics

The majority of TW infection (both classic and localized) occurred in males (82%) (Table 2). Median age at diagnosis was 53 years (range 11–75) and was similar between groups. Interval to diagnosis varied but was not significantly longer in localized disease. Race data were available for 20 of 33, and 18 (90%) were white.

Table 2 Baseline characteristics of patients with confirmed *T. whipplei* infection from 1995 to 2015

	All Cases	Classic WD	Localized WD[a]
N	33	18	15
Male	27 (82%)	15 (83%)	12 (80%)
Median age years (range)	53 (11–75)	52 (34–69)	53 (11–75)
History of immunosuppressive therapy (%)	15 (45%)	8 (44%)	7 (47%)

[a]There were no significant differences between classic and localized disease. Classic vs localized WD

Previous Diagnosis and Immunosuppression

Of 33 patients, 19 had a previous diagnosis based on symptoms ultimately attributed to TW infection. Fourteen individuals received immunosuppressives for these prior diagnoses, and one received immunosuppressives for a kidney transplantation, resulting in 15 individuals on immunosuppressives. The majority of individuals (9 of 14, 60%) received multiple medications, which included prednisone, methotrexate, etanercept, azathioprine, anakinra, adalimumab, infliximab, certolizumab, tocilizumab, hydroxychloroquine, and rituximab. Therapy was directed at inflammatory arthropathies in the majority of cases (8 of 14, 57%) and also at pericarditis, Lyme disease, Crohn's disease, and chronic inflammatory pachymeningitis. The indication for prior use of corticosteroids in the 2 remaining cases was not clear.

The 14 patients on immunosuppressives for a prior diagnosis either had a lack of response or worsening of symptoms with these medications. The majority of patients on prednisone experienced improvement on the steroid, specifically with joint pains and ocular dryness, and felt that their symptoms flared during a prednisone taper.

Clinical Features

Weight loss was the most common clinical feature (22 of 33, 67%) and was present in 17 of the 18 classic cases (94%). Quantitative data were available in 14 with a median of 11 kg (interquartile range (IQR) 7–16.5). Weight status was documented in 8 cases of localized disease, and weight loss was present in 5 of 8 (63%) with a median of 15 kg (IQR 11–17). Of the 22 patients with documented weight loss, 13 (59%) had diarrhea: 10 classic WD and 3 localized TW infection.

Fever, diarrhea, asthenia, and adenopathy were found in roughly half of patients with classic WD, and arthritis/arthralgias occurred in nearly two-thirds (64%) of localized disease. Arthritis/arthralgias were present in 9 of 18 (50%) cases of classic WD. Of 18 patients without fever, 8 were classic and 10 were localized TW infection, and 5 had positive blood PCR at diagnosis. Abdominal pain was noted in 10 cases with the majority (7 of 10, 70%) in classic WD. Ascites was a rare finding (2 cases). Melanoderma—hyperpigmentation of the skin—was seen in three cases, all classic WD. Melanoderma is viewed as a classic symptom that is now rarely reported due to earlier diagnosis [25].

Neurologic symptoms (cranial nerve deficits, dementia, memory impairment, encephalopathy, seizures, ataxia, paresthesia, vertigo, diplopia, proximal muscle weakness, oculomasticatory myorhythmia) were present in 21 of 33 (64%). Eleven (52%) had localized disease, and 10 (48%) had classic WD. Of the 11 patients with localized disease

and neurologic symptoms, 7 of 11 (64%) were localized CNS disease. Oculomasticatory myorhythmia was noted in only one case, which was classic WD (Table 3).

Psychiatric signs (depression, or personality changes) were present in 7 of 33 (21%): 5 classic and 2 localized infection, all of whom had neurologic symptoms.

Four patients had the classic combination of fever, diarrhea, abdominal pain, and arthralgias: two males and one female with classic WD and one male with localized disease. An additional 11 cases were noted to have 3 of the 4 classic findings.

Laboratory Studies

The most common laboratory finding was anemia (Table 4). Median anemic hemoglobin value was 10.5 g/dL overall (range 7.4–12.5) and was 9.4 (range 7.4–12.5) and 11.3 (range 9.1–12.3) for classic and localized disease, respectively ($p = 0.18$). Seven patients had microcytosis with median mean corpuscular volume (MCV) of 76.4 fL (range 60.4–78.8). Erythrocyte sedimentation rate values were available in 23 cases and were elevated in 11 (48%). C-reactive protein (CRP) values were available in 11 cases and were elevated in 9 (82%). CRP was elevated in all cases of classic disease with CRP data. Albumin values were available for 26 patients and 14 of 26 (54%) were hypoalbuminemic with a median value of 2.9 g/dL (range 1.1–3.37). Leukocytosis was present in less than 33% of all cases.

Diagnostic Evaluation

The majority of cases were diagnosed by PCR, 29 of 33 (88%); 15 of 18 (83%) and 14 of 15 (93%) of classic and localized cases, respectively (Table 5). Positive PAS was present in 11 of 15 (73%) of cases of WD with positive PCR (Fig. 1). Three cases of classic disease diagnosed by

Table 3 Most common clinical features of patients with confirmed *T. whipplei* infection from 1995 to 2015

	All cases	Classic WD	Localized WD (total)	Localized WD					
				CNS infection	Articular infection	Vitreitis	Pericarditis	Bacteremia	Myositis
Weight loss	22/33 (67%)	17/18 (94%)	5/15 (33%)	2/7 (29%)	1/4 (25%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
Diarrhea	17/33 (52%)	11/18 (61%)	6/15 (40%)	1/7 (14%)	2/4 (50%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Adenopathy	14/33 (42%)	10/18 (56%)	4/15 (21%)	1/7 (14%)	1/4 (25%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	0/1 (0%)
Fever	15/33 (45%)	10/18 (56%)	5/15 (33%)	3/7 (43%)	2/4 (50%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
CNS disturbance[a]	21/33 (64%)	10/18 (56%)	11/15 (73%)	7/7 (100%)	2/4 (50%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)
Arthritis/ arthralgia	19/33 (56%)	9/18 (50%)	10/15 (67%)	3/7 (43%)	4/4 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Asthenia	15/33 (45%)	8/18 (44%)	7/15 (47%)	3/7 (43%)	1/4 (25%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Abdominal pain	10/33 (30%)	7/18 (39%)	3/15 (20%)	1/7 (14%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)

[a]Crainial nerve deficits, dementia, memory impairment, encephalopathy, seizures, ataxia, paresthesia, vertigo, diplopia, proximal muscle weakness, oculomasticatory myorhythmia

CNS central nervous system

Table 4 Most common laboratory findings of patients with confirmed *T. whipplei* infection from 1995 to 2015

	All cases	Classic WD	Localized WD* (total)	Localized WD					
				CNS infection	Articular infection	Vitreitis	Pericarditis	Bacteremia	Myositis
Hypoalbuminemia	14/26 (54%)	7/14 (50%)	7/12 (58%)	3/6 (50%)	3/3 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	NA
Anemia	18/30 (60%)	13/17 (76%)	5/13 (38%)	2/6 (33%)	2/4 (50%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	NA
Elevated ESR	11/23 (48%)	7/12 (58%)	4/11 (36%)	2/5 (40%)	2/3 (67%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	NA
Leukocytosis	10/31 (32%)	6/17 (35%)	4/14 (29%)	1/7 (14%)	2/4 (50%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	NA
Elevated CRP	9/11 (82%)	6/6 (100%)	3/5 (60%)		2/3 (67%)	NA	1/1 (100%)	0/1 (0%)	NA

There were no significant differences between classic and localized disease for any of the laboratory findings

CRP C-reactive protein; *ESR* erythrocyte sedimentation rate; *NA* not available

Table 5 Results of pathologic examination and PCR testing for 33 patients with Whipple disease (WD)

No. of patients/sample type	No. of tested PAS	PAS positive	No. of tested PCR	PCR positive
18 classic Whipple disease				
Small bowel—duodenum	16	11	13	13
Small bowel—jejunum	2	2	1	1
Gastric	2	2	0	–
Colon	5	2	0	–
CSF	0	–	4	1
Blood	0	–	10	6
Lymph node	2	2	0	–
Arterial thrombus	0	–	1	1
7 CNS infection				
Small bowel—duodenum	2	0	3	0
Small bowel—jejunum	1	0	2	0
CSF	0	–	5	5
Blood	0	–	3	1
Dura	0	–	1	1
Cerebellum/brain tissue	1	1	2	2
4 Articular infection				
Small bowel—duodenum	1	0	1	0
Synovial fluid	0	–	4	4
1 Vitreitis				
Blood	0	–	1	1
Vitreous humor	0	–	1	1
1 Pericarditis				
Small bowel—duodenum	1	0	0	–
Blood*	0	–	2	0
Pericardium	1	1	1	1
1 Bacteremia				
Small bowel—duodenum	1	0	1	0
CSF	0	–	1	0
Blood	0	–	1	1
1 Myositis				
Muscle	1	1	0	–

* Single patient with two blood PCR tests three days apart

PAS without PCR were from small bowel ($n=2$), and gastric ($n=1$) mucosa. There were 4 cases of probable WD and 14 cases of definite WD. Twenty-three of 33 (70%) had multiple sites tested at the time of diagnosis, and 11 of 33 (33%) had multiple sites with positive findings for TW infection. The only case of localized disease not defined by PCR was myositis with robust PAS positivity on a quadriceps biopsy. While PAS positivity of muscle can be seen in acid maltase deficiency, this condition typically does not present with the inflammatory changes that would be seen in myositis.

Of 15 localized cases, 6 (43%) had negative intestinal evaluation: articular infection ($n=1$), bacteremia ($n=1$), pericarditis ($n=1$), CNS infection ($n=3$). The remaining 9 cases had no bowel investigation within 4 weeks of diagnosis.

Blood PCR was performed in 17 cases at the time of case defining testing and was negative in 9 of 17 (53%) overall, 5 of 11 (40%) classic WD, 2 of 3 (67%) CNS, 0 of 1 (0%) vitreitis, 1 of 1 (0%) articular infection, and 2 of 3 (100%) pericarditis (2 cases of pericarditis occurred in the setting of classic WD).

Of 3 patients with localized disease, and clinical symptoms of diarrhea and weight loss, 1 had no small bowel investigation (myositis), 1 had a negative small bowel PAS prior to starting antimicrobial therapy (pericarditis), and 1 had negative PAS and PCR from the jejunum.

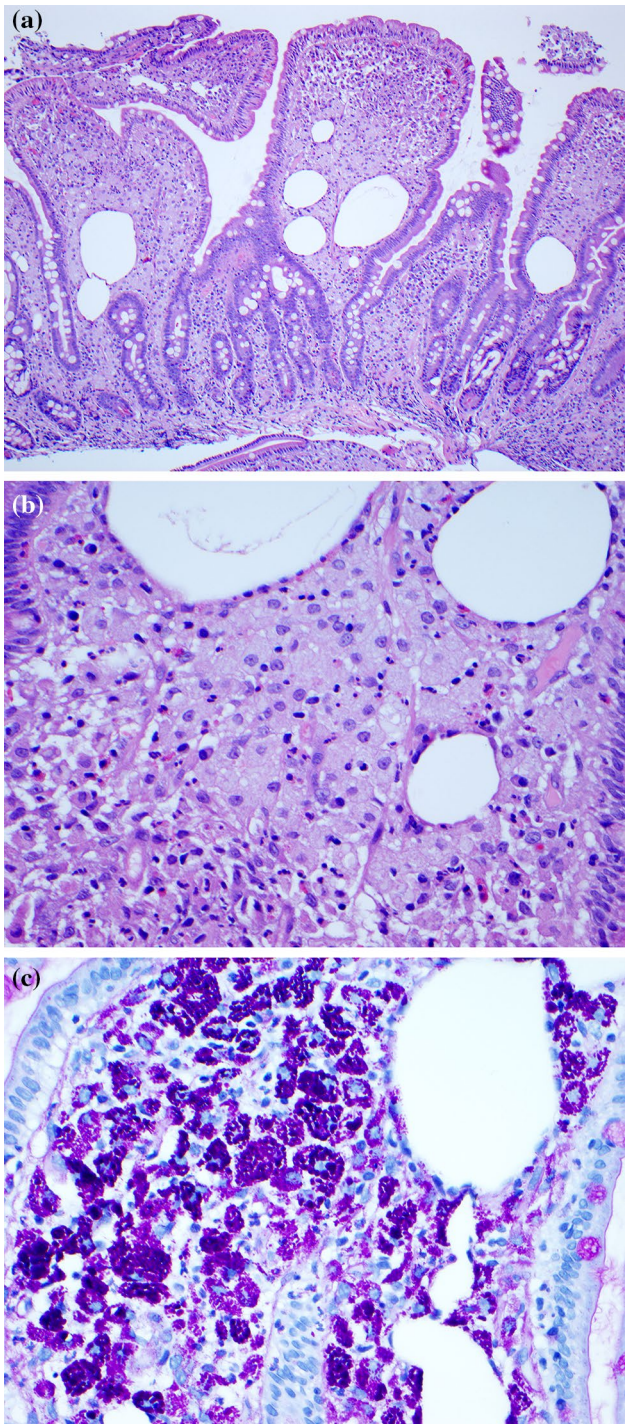

Fig. 1 Classic Whipple disease involved small bowel. Duodenal biopsy showing villous blunting and expansion with foamy histiocytes and large fat vacuoles (**a**, H&E stain, 100×). Higher-power view of the foamy histiocytes (**b**, H&E stain 400×) is positive by PAS stain (**c**, PAS stain, 400×)

Treatment and Response

Treatment data were available in 32 of 33 (97%) cases, and clinical response was documented in 30. Of 30, 24 (80%) showed a clear clinical response to antibiotic therapy, 2 of 30 (7%) no clinical response or further deterioration, and 4 of 30 (13%) equivocal clinical response after median follow-up of 14 months (IQR 2–39). Two of the four cases with equivocal clinical response were CNS disease treated with intravenous (IV) ceftriaxone and either oral trimethoprim–sulfamethoxazole (TMP-SMX) or ceftibuten, and the two other cases were classic WD treated with ceftriaxone and TMP-SMX. The majority, 23 of 32 (72%), received a combination of IV ceftriaxone and oral TMP-SMX. TMP-SMX monotherapy was used in 7 of 32 (25%), minocycline monotherapy in 1 of 32 (4%), and 1 patient deferred therapy. Of the MCR cohort, 4 of 28 cases had serum levels of antibiotics followed.

Lack of response to therapy or further deterioration occurred in two patients, one with classic WD and the other in the patient that deferred therapy. The lack of response in the classic WD case may represent immune reconstitution inflammatory syndrome (IRIS) as opposed to treatment failure, due to the initial response to prednisone; however, there was no confirmation of successful treatment through a negative tissue PCR, which is required for diagnosis of IRIS [31].

All cases treated with TMP-SMX monotherapy showed clear clinical response to therapy including classic disease ($n=4$), CNS infection ($n=1$), articular infection ($n=1$), and bacteremia ($n=1$). Minocycline was used to treat one case of articular infection with good clinical response.

Follow-Up Testing

The follow-up duration in the cohort from MCR ranged from 0 to 211 months (mean 39 months). Follow-up testing data from diagnostic sites that were previously positive were available in 13 cases: 8 classic and 5 localized. All follow-up testing data recorded were greater than 4 weeks after initial diagnosis. One PCR study remained positive on follow-up, a blood PCR 1 month after initiation of therapy in classic disease complicated by PCR positive arterial thrombi. Otherwise, all other blood, bowel, or CSF PCR studies were negative at follow-up (range 1–66 months). The earliest documented negative blood PCR in a case with previous positivity was 1 month and occurred in 3 cases. PAS positivity in the bowel persisted in 3 cases (12, 20, and 66 months), with 2 having a positive PCR at diagnosis but negative at follow-up.

Survival

Five deaths were documented: 2 in classic WD (Creutzfeldt–Jakob disease, unknown cause) and three in localized (aortic dissection, aortic valve stenosis, unknown cause). The patient who died of Creutzfeldt–Jakob disease had negative CSF PCR at the time of WD diagnosis. Deaths occurred 2–157 months after WD diagnosis.

Discussion

The frequency of TW infection is difficult to estimate, but remains rare in the USA. Four major referral centers in the eastern half of the USA combined for 33 cases over the 15–20-year review period. Classic disease remains the most common presentation, but nearly half of the cases in this study were extraintestinal. While we confirmed the predilection for middle-aged white males similar to previous reports, the disease is not limited to this demographic. Exposure to immunosuppressing agents was common, but it is not clear whether exposure to these agents represents an independent risk factor or is merely a clinical misstep before diagnosis.

The classic constellation of diarrhea, weight loss, abdominal pain, and arthralgias occurred in a minority of cases, but 3 of the 4 were found in 33%, most commonly in classic disease. Arthritis and arthralgias were more common in extraintestinal disease than in classic WD. Oculomasticatory myorhythmia (OMMR), the pathognomonic finding for WD which involves ocular nystagmus and synchronous contractions of the masticatory and proximal and distal skeletal muscles, was rare, being noted in only one case. It is not clear whether the finding is uncommon, or simply goes unrecognized or documented by clinicians. Neuropsychiatric signs, when aggregated, occurred in more patients than any other clinical findings. Proximal muscle weakness, alterations in memory, gait, personality, and level of consciousness may be underappreciated findings and do not reliably predict positive diagnostic testing from CSF.

Laboratory findings were variable, and the most common findings of anemia and hypoalbuminemia were present in both classic and localized disease. Nonspecific inflammatory markers were frequently elevated, but in the context of these patients' clinical presentations are unlikely to be helpful. Endoscopic findings in TW infection are nonspecific and can range from a normal appearance to the presence of dilated villi, ecstatic lymph vessels, edema, and duodenitis. Villous atrophy can also be seen, and the mucosa has been described as pale yellow in some cases [32] (Figure 2).

While there were many similarities in the clinical presentation of TW infection in this US cohort as compared to prior European cohorts, there were also two notable differences, specifically the frequency of neurologic symptoms and the apparent rarity of the condition. Weight loss, diarrhea, adenopathy, fever, asthenia, and abdominal pain had similar prevalence when comparing our cohort to two recent, large European cohorts [26, 32]. Arthritis/arthropathy was slightly lower in our cohort—50% in classic WD as compared to 68 and 78% in the European cohorts—however, whether this difference is significant is not clear. Strikingly, neurologic symptoms were present in 64% of total cases in the US cohort as compared to 22 and 24% in the European cohorts. This discrepancy is likely due to a difference in definition of neurologic symptoms. While our study evaluated for the same neurologic symptoms as the two European cohorts, we also included unique symptoms such as vertigo, diplopia, and ataxia. When looking just at the symptoms reported in the European cohorts (dementia, personality changes, myoclonus, oculomasticatory myorhythmia, and hypothalamic changes), the frequency of neurologic symptoms in our study fell to 33.33%.

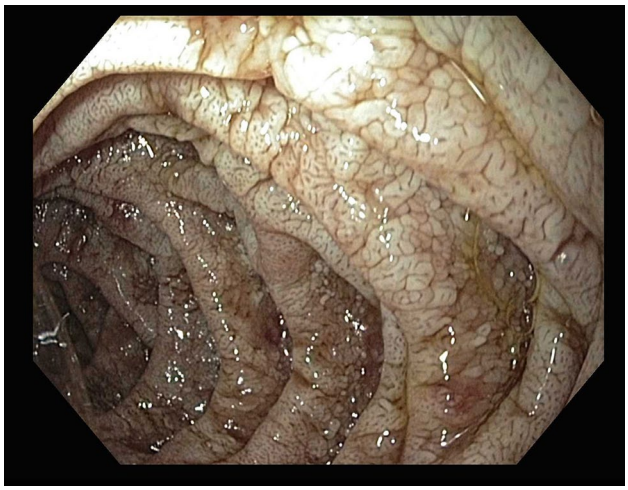

Fig. 2 Endoscopic view of the duodenum in Whipple's disease shows nonspecific edematous mucosa, enlarged white villi, scalloping of circular folds, and fissures

Another notable difference from the European cohorts is the apparent rarity of TW infection in the USA. Our study covered a 20-year time period and identified only 33 cases of TW infection, while the two European cohorts spanned 10 and 13 years and identified 142 and 191 cases, respectively. This difference is unlikely to be due to the lack of noninvasive testing in our cohort, as these techniques were not used in the larger of the two European studies [32]. We postulate that this difference may be due to both the under-recognition of TW infection in the USA, and these two prior studies being done at WD referral centers.

A previous study showed excellent sensitivity and specificity of PCR [15], and the false positive rate of PCR in the gastrointestinal tract has been reported at 6% (95% confidence interval 5–8%) [33], but in our cohort, blood

PCR showed a sensitivity of only 47% (Table 5) and is likely unhelpful in excluding the diagnosis. Therefore, there is a need for aggressive clinically focused diagnostic evaluation. Diagnosis of localized TW infection can prove difficult, and TW immunohistochemistry is a sensitive and specific method that can be used to confirm extraintestinal PAS positive tissue, which is not specific to TW infection [34]. Additionally, recent developments in PCR, such as utilizing primers targeted against repetitive sequences and combining rapid cycling with fluorescence-based detection in a closed tube, have led to increased sensitivity and specificity of PCR. This improved diagnostic accuracy has led to the proposed use of noninvasive PCR testing on saliva and stool samples as an initial screening test [35]. Salivary PCR, however, has been noted to have false positives. These have been attributed to similarities between TW and oral bacteria and the TW gene targeted by the primer [36]. This can be potentially minimized with the use of several or more specific primers, and the requirement to test both stool and saliva. Additionally, there are asymptomatic carriers of TW, both in the saliva and stool, which can lead to false positives. This effect is mitigated by the low prevalence of carriers in the general population, and the sensitivity of high bacterial load in the stool for infection [37]. The positive predictive value of having both a positive stool and saliva PCR has been reported as 95.2% and the negative predictive value of either test being negative as 99.2% [37]. Salivary and stool samples

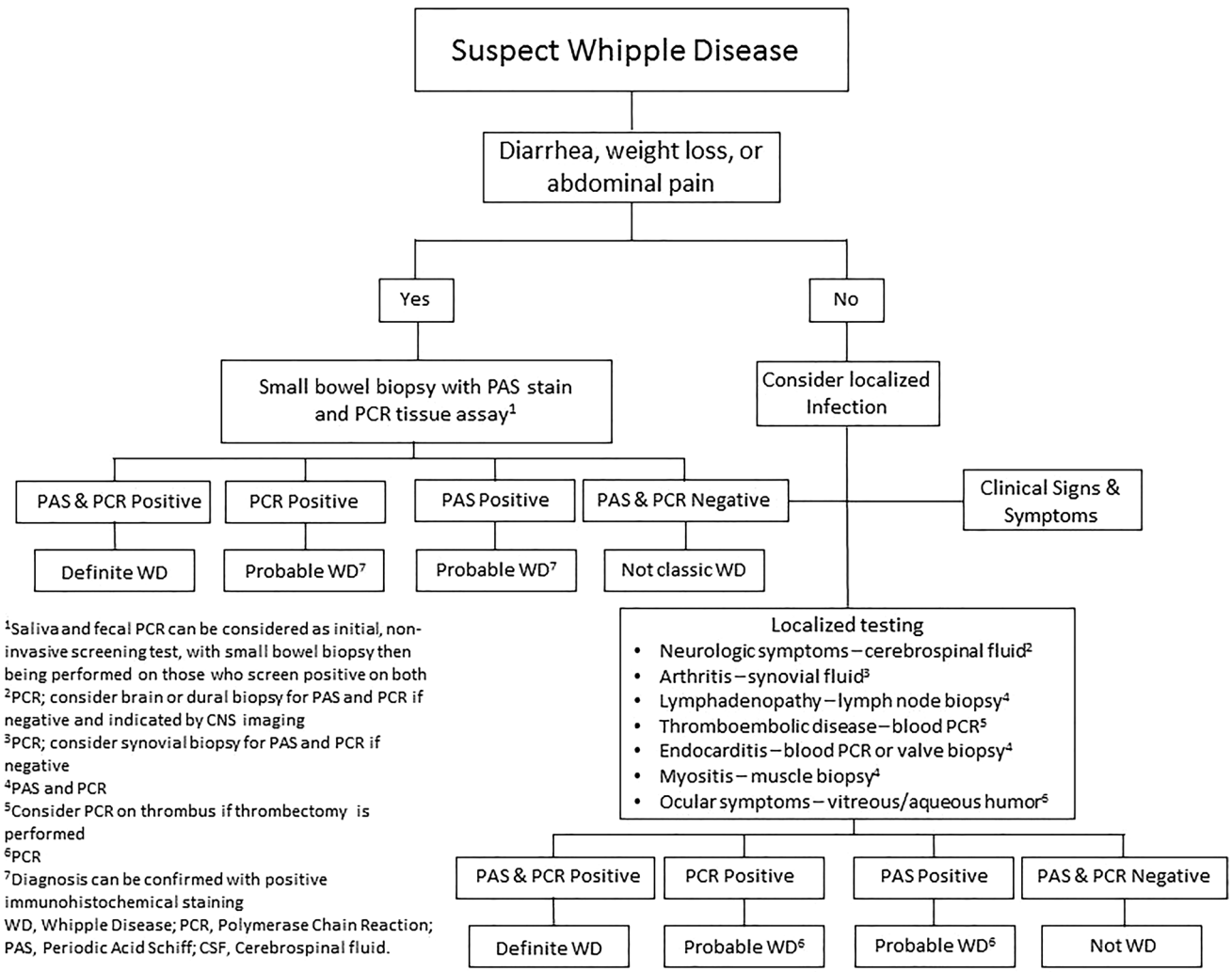

Fig. 3 Recommended approach to possible cases of *T. whipplei* infection based on clinical symptoms. The approach to diagnostic testing should be guided by clinical manifestations and availability of histopathology and PCR assay. Patients with gastroenterologic symptoms should have a small bowel evaluation as the initial diagnostic step. Positive PAS staining and PCR assay confirms the diagnosis of WD. A negative small bowel evaluation should be followed by clinically directed extraintestinal testing. Positive testing indicative of probable TW infection from multiple sites may be helpful in securing the diagnosis. Immunohistochemical staining can be used to confirm diagnosis in cases of discordance between PCR and PAS

are therefore promising noninvasive cost-effective options for screening (Fig. 3).

While neurologic symptoms are a classic feature in classic WD and attributed to CNS involvement, which has been identified in 90% of brain and spinal cord specimens from infected patients and carriers, isolated neurologic infection is less common [29]. This study identified a higher proportion of isolated neurologic infection than previous studies, which may be due to referral bias [26]. Five of the 7 individuals with isolated neurologic infection underwent CSF PCR, and all 5 were positive. Additionally, the single cerebral biopsy performed was PAS positive. This is consistent with a prior study [38].

While acute TW infection can manifest with bacteremia, we identified one case of chronic TW infection and bacteremia, which has not previously been described [13]. This young female had one positive blood PCR in the setting of prolonged exposure to human sewage and dramatic clinical improvement on TMP-SMX with relapses when the medication was stopped. She underwent MRI, abdominal ultrasound, PAS of her small bowel, and PCR of her CSF and small bowel, all of which were negative. She did not undergo echocardiogram, and therefore, a concurrent endocarditis cannot be excluded.

There are few North American reports regarding choice of antibiotic therapy, efficacy, and relapse, and the existing studies are complicated by small sample sizes. Early treatment consisted of chloramphenicol, tetracycline, penicillin, and streptomycin with variable efficacy [5, 39]. Later, medications with blood–brain barrier penetrance, namely TMP-SMX, appeared to reduce relapse. [25, 40, 41]. The use of TMP-SMX was further supported by a 2010 randomized controlled trial on 40 individuals that found that two weeks of IV meropenem or IV ceftriaxone as initial therapy followed by oral TMP-SMX was equally efficacious in achieving clinical resolution and remission for a minimum of almost 6 years [42]. However, there has been increasing in vitro evidence that raises concerns on the continued use of TMP-SMX. French data revealed that the molecular target for TMP is absent in TW, and in vitro studies revealed mutations in TW causing resistance to sulfa agents [43–46]. These in vitro results were supported by a retrospective review of 29 patients with TW infection which found no treatment failures in 13 individuals treated with doxycycline and hydroxychloroquine, and 100% treatment failures in the 14 individuals treated first line with TMP-SMX [43]. The potential toxicity of TMP-SMX, including hematologic effects and Steven-Johnson syndrome, is another consideration in treatment selection. Thus, the most recent European literature suggests a doxycycline and hydroxychloroquine-based regimen, which is the only treatment shown to be bactericidal in vitro [27, 43–45, 47]. While the in vitro data are supportive of this regimen, the in vivo evidence is limited, and further prospective trials are needed.

Clinical response in our series was good with a regimen of IV ceftriaxone and oral TMP-SMX. These agents cross the blood–brain barrier and resulted in equal rates of clinical improvement for classic and localized disease in our series. We did not observe clinical relapse or primary treatment failures sufficient to recommend a doxycycline and hydroxychloroquine-based regimen over a TMP-SMX-based regimen. This raises the question of whether TW in the USA is genetically different from TW in Europe. At present, we recommend initial therapy with IV ceftriaxone, followed by either a TMP-SMX-based regimen, or the European regimen of doxycycline and hydroxychloroquine while monitoring for signs of failure or relapse.

Lifetime susceptibility to relapse despite successful treatment has been described and is likely possible given the underlying genetic alterations of the immune system that predispose individuals to infection. CNS relapse can be severe and lead to death. Given this, a course of 1 year of doxycycline and hydroxychloroquine followed by a lifetime of doxycycline has been proposed [48]. There are no studies examining the optimal duration of antibiotic therapy, and in our study, we had limited follow-up, precluding new input into this clinical question. Future studies into the ideal duration of antibiotic therapy are needed.

Follow-up testing demonstrated PCR response in nearly all cases where available. Prior investigation showed that residual histologic changes can persist for months after clinical response to treatment [15]. A report from MCR using both PCR and PAS evaluation throughout the treatment course found variability of histologic findings which did not predict durability of clinical response. However, patients were more likely to relapse if PCR positivity persisted after clinical response [15].

The limited follow-up data in our series indicate that PCR becomes negative between 1 and 4 weeks after initiation of therapy. This is unlikely to be relevant unless clinical response to therapy is equivocal or absent, and repeat testing would alter management. It may, however, be relevant if considering a patient with recent antibiotic exposure for a new diagnosis of TW infection. Based on our data, we would consider 1 week or less of antibiotic exposure with ongoing clinical symptoms as good predictors for accurate diagnostics. With recent antibiotic exposure for more than 1 week in duration less than 4 weeks in the past, histopathologic examination may be preferred over PCR.

Few deaths occurred during the follow-up period and were insufficient to ascertain a mortality difference between groups. No deaths were directly attributable to WD.

We acknowledge this series is limited and lacks complete data for diagnostic follow-up or clinical relapse; though it represents the largest cohort of TW-infected patients

described in the USA after the introduction of PCR. Additionally, all cases were initially identified from coding and billing records and potential cases may have been missed. These limitations should be balanced against strict inclusion criteria and detailed clinical information.

In conclusion, TW infection remains a challenging-to-diagnose infectious syndrome rarely encountered in the USA. The textbook presentation of classic WD was infrequent in this case series, and the majority of affected individuals may not present with the classic combination of diarrhea, weight loss, abdominal pain, and arthralgias. Additionally, extraintestinal disease is prevalent and can present without any gastrointestinal signs. Latency of diagnosis and previous exposure to immunosuppressive medications are common. A careful clinical history and examination should focus diagnostic testing utilizing both histopathology and PCR, recognizing that blood PCR is insensitive. Because a negative PAS does not rule out classic or localized WD, diagnosis of TW infection requires a high degree of suspicion, targeted testing, and possibly multiple upper endoscopies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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