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Failure and relapse after treatment with trimethoprim/sulfamethoxazole in classic Whipple's disease

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Objectives: Classic Whipple's disease is a chronic disease caused by *Tropheryma whipplei*. A recent study reported that intravenous treatment with ceftriaxone or meropenem followed by a 1 year treatment with trimethoprim/sulfamethoxazole cured all patients. However, we have previously reported that *T. whipplei* is poorly susceptible to β-lactams and resistant to trimethoprim. Herein, we want to evaluate these antibiotic regimens.

Patients and methods: Since the organism was first cultured in Unité des Rickettsies, Marseille (France), we received samples for the diagnosis of *T. whipplei* infections. Among the 37 patients referred to us for management, 24 patients presented classic Whipple's disease. Among them, 14 patients treated with trimethoprim/sulfamethoxazole were followed up for >3 years.

Results: None of the 14 patients was cured. One patient presented with an adverse side effect necessitating treatment cessation. Two patients developed an immune reconstitution inflammatory syndrome. One patient died 4 weeks after initiation of the treatment. Five patients developed clinical resistance; four of these having mutations on the target gene of sulfamethoxazole (*folP*). Five patients developed a relapse after cessation of trimethoprim/sulfamethoxazole after an average of 30 months. The high relapse rate may be linked to our recruitment. However, discrepancies with other centres could be due to the heterogeneity of diagnosis and cure criteria, different follow-up methods or infections due to *T. whipplei* strains with better susceptibility to antibiotics.

Conclusions: We confirmed, as predicted from prior testing of *T. whipplei* susceptibility, that trimethoprim/sulfamethoxazole is not optimal for classic Whipple's disease. In addition, 1 year treatment may be followed by relapses.

Keywords: *Tropheryma whipplei*, antibiotic treatment, immune reconstitution inflammatory syndrome

Introduction

Whipple's disease (WD) is a chronic disease, first described in 1907,[1] that was fatal before the advent of antibiotics.[2] Since 1952, after the first report of chloramphenicol efficacy,[3] antibiotic treatment has been empirical. In 1966, a therapy was proposed with intravenously administered penicillin and streptomycin for 2 weeks, followed by tetracycline orally for 3–12 months.[4] Thereafter, several cases of effective use of tetracyclines, chloramphenicol, penicillin, streptomycin or trimethoprim/sulfamethoxazole were reported,[2,5] and tetracycline became the drug of choice for long-term therapy for many years. However, in 1985, a study revealed a high rate of CNS relapse.[6] Thus, the decision was taken to switch to an antibiotic that was better at crossing the blood–brain barrier,[7] such as trimethoprim/sulfamethoxazole.[2] Since that report, an introductory treatment of intravenous streptomycin (1 g per day) together with penicillin G (1.2 million U per day) or ceftriaxone (2 g per day) for 2 weeks, followed by long-term therapy with oral trimethoprim/sulfamethoxazole (160 mg/800 mg) twice daily for 1–2 years,[8,9] has been considered.

Thanks to advances in *Tropheryma whipplei* culture (*T. whipplei* is the causative bacterium of WD),[10] full genome sequencing[11,12] and antibiotic susceptibility tests[13,14] have been possible since 2000. *In vitro*, many antibiotics, including penicillin G, amoxicillin, gentamicin and ceftriaxone are active in axenic medium.[14] However, classic WD is mainly a disease associated with intramacrophagic *T. whipplei*. In cell culture, if doxycycline and macrolides are active, cephalosporins are not active and the susceptibility to imipenem is variable.[13]

Genomic analysis suggests that *T. whipplei* lacks the coding sequence for dihydrofolate reductase,[15] which is the trimethoprim target.[16] As *in vitro* tests have confirmed that trimethoprim and ceftriaxone are ineffective against intracellular *T. whipplei*,[13] the current recommendation for WD treatment is sulphonamide monotherapy.[17] Moreover, we previously reported several mutations in the target gene of sulfamethoxazole, *folP*, that lead to *in vitro* resistance and secondary clinical failure or biological failure.[5,17] Because of advances in knowledge[18] with *in vitro* tests, we proposed that an alternative may be doxycycline and hydroxychloroquine, an alkalinizing agent.[2] We supplement these agents with sulfadiazine in patients with neurological involvement.[2] Recently, intravenous treatment with meropenem or ceftriaxone followed by 1 year of oral trimethoprim/sulfamethoxazole has been suggested to cure all patients,[19] although the susceptibility of *T. whipplei* to these antibiotics varies.[13,17] Although we currently have no explanation for the discrepancies between studies, we would like to report our experience of treating classic WD with trimethoprim/sulfamethoxazole. Herein, we report on 14 of our patients treated using this approach for which we have follow-up data for at least 3 years.

Patients and methods

Patient recruitment

Since the first culture of *T. whipplei*,[10] we have received different samples for analysis. Nearly 215 patients have been diagnosed or confirmed with *T. whipplei* infections in Marseille, France. Thirty-seven patients who contacted us by e-mail or were referred to us by their physician were followed up either after the diagnosis or after failure or relapse. Among them, 24 suffered from classic WD, 5 suffered from endocarditis, 4 suffered from neurological infections, 2 suffered from uveitis, 1 suffered from isolated adenitis and 1 suffered from isolated pulmonary infection.

Among the 24 patients suffering from classic WD, 14 were first treated with trimethoprim/sulfamethoxazole in another centre, 7 were first treated with the combination of doxycycline and hydroxychloroquine and 3 were first treated with doxycycline, hydroxychloroquine and sulfadiazine because of neurological involvement. Only the patients treated with trimethoprim/sulfamethoxazole were included in this study. Indeed, as full eradication of the bacterium should be for us the major criterion for presumed cure of classic WD, it is too early to evaluate this alternative treatment. Currently, all 10 patients treated with doxycycline and hydroxychloroquine ± sulfadiazine are still being treated with this regimen. None of them has died and none has developed an immune reconstitution inflammatory syndrome (IRIS) or clinically acquired resistance. The mean duration of treatment with doxycycline and hydroxychloroquine ± sulfadiazine is currently 30 months (2–48 months).

The patients were directly followed in consultation with one of us (D. R.). Among them, 14 patients with classic WD treated with trimethoprim/sulfamethoxazole and a post-diagnosis follow-up of >3 years were retained. All patients gave informed consent, and the local ethics committee approved this report.

Diagnosis and follow-up

Tools

Tools used for the diagnosis and the follow-up of classic WD were periodic acid-Schiff (PAS) staining[2,20] and immunohistochemistry using antibodies specific for *T. whipplei*, as previously described.[21] Specific PCR assays[22] were also performed on small-bowel specimens and different tissues or body fluids such as blood, CSF, saliva and stool samples from a subset of patients. Various PCR assays targeting specific sequences of *T. whipplei* were applied depending on improvement in the technologies.[22,23]

Inclusion criteria

We considered only definite diagnosis of classic WD and excluded uncertain cases. Our criteria used for establishing a definite diagnosis of classic WD were the presence of positive results of PAS staining and/or specific immunohistochemistry of a small-bowel biopsy specimen. Patients included in this study had been treated with a trimethoprim/sulfamethoxazole regimen (160 mg/800 mg per day) with or without initial intravenous therapy.

Clinical failure or relapse

Immediate failure occurred <3 months after initiation of antibiotics. When the aggravation of the clinical status (fever and erythema nodosum-like lesions) of patients occurs within a few weeks after the initiation of adequate antibiotic therapy, IRIS should be strongly considered.[24,25] These side effects correspond to regained host capacity, defined as the development of several inflammatory disorders linked to a paradoxical worsening of clinical status.[25]

Late failure was observed >3 months after initiation of antibiotics and was due to clinically acquired resistance to trimethoprim/sulfamethoxazole. This was defined by the presence of an initial clinical response to oral trimethoprim/sulfamethoxazole using the adequate dose described in the literature, with complete disappearance of clinical signs followed by the recurrence of the clinical symptoms under the same treatment. That failure was mainly linked to the presence of several mutations in the *folP* gene, which encodes dihydropteroate synthase, the target for sulfamethoxazole.[5]

Finally, 'relapse' was defined by the reappearance of clinical symptoms occurring after antibiotic cessation.

Cure criteria

Patients followed in other centres were frequently treated for 1 year and the decision to cease antibiotic therapy was based on lack of clinical manifestations. Currently, in our centre, we propose to obtain histological pictures showing full clearance of any macrophagic bacteria in the small-bowel biopsy. Before the cessation of antibiotic, we require negative PAS staining and specific immunohistochemistry performed on small-bowel biopsy after at least 18–24 months of treatment.

Statistical analysis

EPI info software, version 3.5.1 (CDC, Atlanta, GA, USA) was used for analysis. Statistical significance was defined as $P < 0.05$.

Results

Patient characteristics

Among the patients followed in Marseille for classic WD, we reported 14 patients treated with trimethoprim/sulfamethoxazole with a follow-up of >3 years. All patients were addressed to one of us (D. R.) for failure or relapse. Among them, 11 were male (79%). The age range at the time of diagnosis was 26–78 years (mean 54.57 years). The mean time of follow-up since diagnosis was 6 years and 5 months (range 36–240 months). The characteristics of the population at diagnosis and the clinical and biological features are summarized in Table 1. The average

duration of trimethoprim/sulfamethoxazole treatment was 14.4 ± 15.5 months (5 days–60 months).

Patient follow-up

Initial intravenous therapy

Among 14 patients, 7 had been previously treated with an intravenous antibiotic for 14 days (Table 2). Among patients receiving intravenous treatment, four patients were treated with ceftriaxone (2 g once daily), one patient was treated with ceftriaxone (2 g once daily) and gentamicin (3 mg/kg once daily), one patient was treated with amoxicillin (4 g three times per day) and gentamicin (3 mg/kg once daily), and one patient was treated with piperacillin/tazobactam (4 g three times per day). Concerning failure ($P=1$) and relapses ($P=0.59$), we noted no significant difference between patients who did not receive initial therapy and patients treated with intravenous initial therapy.

Table 1. Population characteristics, diagnosis delay and clinical and biological data of the 14 patients treated with trimethoprim/sulfamethoxazole for classic WD at the time of diagnosis

	Number (%)
Population characteristics	
male	11 (78.6)
mean age, years (range)	54.57 (26–78)
previous immunosuppressive treatments	6 (42.9)
Diagnosis delay, years (range)	6.6 ± 2.78 (0.5–12)
Clinical signs at diagnosis	
diarrhoea	12 (85.7)
arthralgia	11 (78.6)
weight loss	11 (78.6)
adenopathy involvement	6 (42.9)
neurological involvement	2 (14.3)
memory impairment	1
psychiatric signs	1
pulmonary involvement (pleuritis)	2 (14.3)
cardiac involvement (pericarditis)	1 (7.1)
Biological characteristics	
positive PAS staining on small-bowel biopsy	14 (100)
positive specific IHC on small-bowel biopsy	11/11 (100)
positive PCR performed on small-bowel biopsy	3/4 (75)
positive PCR performed on saliva or stool	3/3 (100)
positive PCR performed on stool	2/3 (66.7)
positive PCR performed on saliva	1/2 (50)
positive PCR performed on CSF	1/3 (33.3)

IHC, immunohistochemistry.

Follow-up at 3 months after the beginning of treatment

Three months after initiation of treatment, among the 14 patients, 4 showed no response to trimethoprim/sulfamethoxazole (data are summarized in Table 3). One patient, who had received initial intravenous treatment, presented with major toxidermia 5 days after beginning trimethoprim/sulfamethoxazole, necessitating cessation of treatment. Two patients presented with fever and erythema nodosum leprosum-like lesions, at 4 and 6 weeks, respectively, after beginning trimethoprim/sulfamethoxazole. These developments have been considered an IRIS and one of them was previously reported.[25] One of these patients had previously been treated for 14 days with ceftriaxone. The fourth patient died 4 weeks after introduction of oral trimethoprim/sulfamethoxazole associated with doxycycline (200 mg per day) and rifampicin (900 mg per day). This patient developed respiratory distress and, retrospectively he was suspected to have developed an IRIS.

In the other 10 patients, we noted a quick improvement, with an average of 8.87 ± 3.31 days (5–15 days) for arthralgia and 12 ± 4.52 days (7–30 days) for diarrhoea. After these 10 patients were treated for 3 months, PCR assay results were negative for four out of five saliva specimens (80%) and three out of three stool specimens (100%).

Follow-up at 1–2 years after the beginning of treatment

Among the 10 patients evaluated, 5 patients had a reappearance of clinical signs during trimethoprim/sulfamethoxazole treatment between 8 and 19 months (average of 11.2 ± 7.54 months) after therapy initiation. We noted the reappearance of arthralgia in two patients and of diarrhoea in two patients. The fifth patient presented with anorexia and weight loss. Small-bowel biopsies for all patients were positive for PAS staining. PCR analyses were positive on duodenal biopsy for three out of four, on stool specimens for three out of three and on CSF for one out of four patients tested. For four of these five patients, clinical resistance was linked with

Table 2. Induction treatment before oral treatment with trimethoprim/sulfamethoxazole

	Induction treatment					
	ceftriaxone ($n=4$)	ceftriaxone + gentamicin ($n=1$)	amoxicillin + gentamicin ($n=1$)	piperacillin + tazobactam ($n=1$)	No induction treatment ($n=7$)	P
Immediate failure	1	0	0	0	2	0.53
Late failure	1	0	1	1	2	0.59
Relapses	2	1	0	0	2	0.59
Death	0	0	0	0	1	0.31

Table 3. Follow-up after 3 months and 1–2 years of treatment, and 3 years after cessation of trimethoprim/sulfamethoxazole

Follow-up after 3 months of treatment	
number of patients evaluated	14
mean time of clinical signs resolving, days ± SD	
arthralgia	8.87 ± 3.31
diarrhoea	12 ± 4.52
adverse side effects necessitating cessation of treatment, *n* (%)	1 (7)
immediate failure, *n* (%)	
IRIS	2 (14)
death	1 (7)[a]
negativity of molecular analysis for patient without failure, *n* (%)	
saliva	4/5 (80)
stools	3/3 (100)
Follow-up after 1–2 years of treatment	
number of patients evaluated	10
late failure	
reappearance of clinical signs, *n*	5 (average delay of 11.2 months)
negativity of molecular analysis for patients without failure, *n* (%)	
PCR saliva	4/4 (100)
PCR stools	3/3 (100)
small-bowel biopsy for patients without failure, *n* (%)	
positive PAS staining	3/3 (100)
positive immunohistochemistry	1/2 (50)
positive PCR	2/3 (66)
Clinical relapse after cessation of treatment	5/5
average time of relapse, months (range)	30 ± 29.05 (2–60)
average duration of treatment, months (range)	14.4 ± 15.5 (5 days–60)
small-bowel biopsy, *n* (%)	
positive PAS staining	5/5 (100)
positive immunohistochemistry	3/3 (100)
molecular data, *n* (%)	
positive PCR saliva	2/3 (66)
positive PCR stools	2/3 (66)

[a]This patient was retrospectively suspected to have an IRIS.

several mutations in the target gene of sulfamethoxazole (*folP*).[5] No mutations were detected for the fifth patient.

Among the five patients remaining symptom free after 12 months of treatment with trimethoprim/sulfamethoxazole, a PCR assay was performed on saliva and stool samples for four and three patients, respectively. All results were negative. Small-bowel biopsy was performed for three patients and showed the persistence of infected macrophages using PAS staining and immunohistochemistry, such as in Figure 1.

Follow-up after the cessation of treatment

Five patients were evaluated after cessation of trimethoprim/sulfamethoxazole and these data are summarized in Table 3. For all patients, treatment was stopped in the absence of clinical symptoms. Among them, all patients experienced a relapse 2–60 months after cessation of the treatment (average of 30 ± 29.05 months). Three patients presented with the reappearance of arthralgia, one patient with the reappearance of diarrhoea and the last patient with an isolated cough. Small-bowel biopsies for all patients were positive for PAS staining. PCR analyses performed on saliva and stool samples were positive for two out of three patients.

Discussion

One century after the first description[1] and 10 years after the first culture of the causative bacterium *T. whipplei*,[10] classic WD remains difficult to treat and manage. The same bacterium causes asymptomatic carriage[26,27] and a wide spectrum of clinical symptoms.[2,28,29] A specific, yet underlined, host genetic immune defect has been strongly suspected[2,30] and probably contributes to relapse.[31] Among the 215 *T. whipplei* infections diagnosed or confirmed in Marseille, using new diagnosis tools[21,22] developed after the first culture, we confirmed 113 definite diagnoses of classic WD.[32] We know the approximate follow-up for 50 patients, but we exclusively reported here on the 14 patients treated with trimethoprim/sulfamethoxazole who were followed up for >3 years. All patients described were

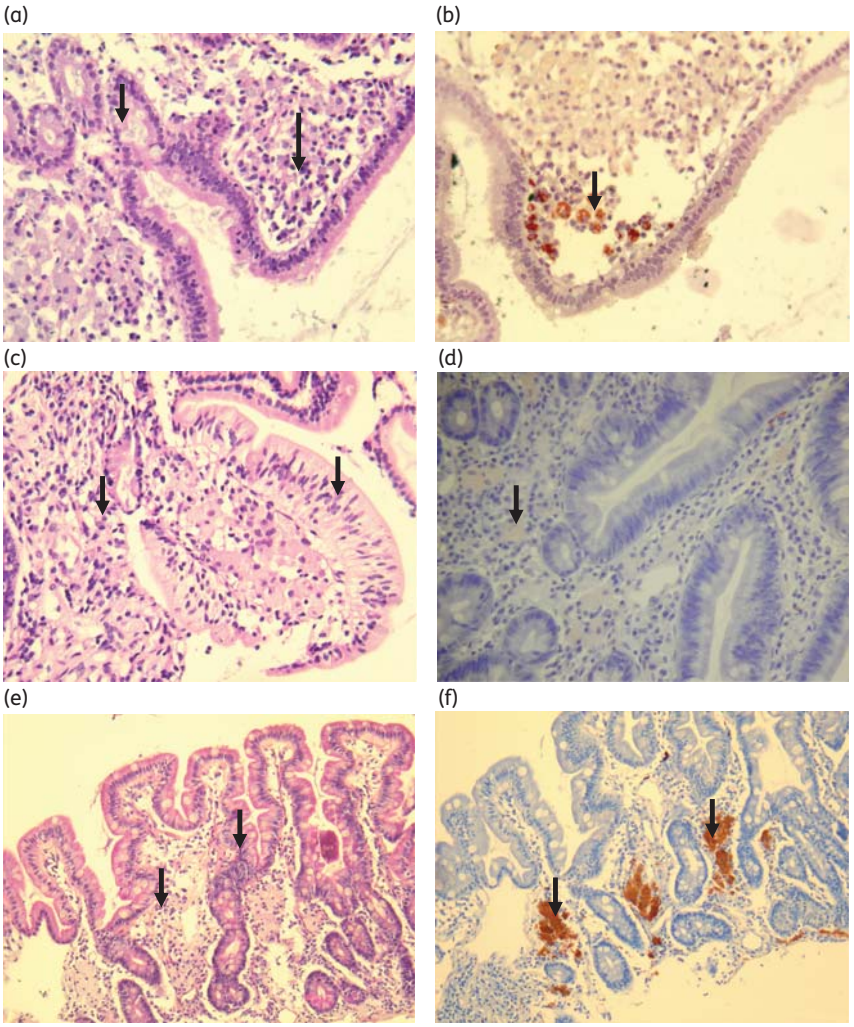

Figure 1. Histological analysis of one patient, performed using a small-bowel biopsy, at the time of diagnosis (a and b), at the time of cessation of treatment after 1 year of trimethoprim/sulfamethoxazole (c and d) and at the time of relapse (e and f), 1 year after cessation of antibiotics. (a, c and e) Positive PAS staining of a duodenal biopsy specimen (see arrows; ×200 for a and c, ×100 for e). (b, d and f) Immunohistochemical staining with polyclonal rabbit anti-*T. whipplei* antibody and Mayer's haemalum counterstain shows *T. whipplei* in a specimen small-bowel biopsy (see arrows; magnification ×200 for b and d, ×100 for f).

addressed by other physicians. Sometimes, patients or physicians directly contacted one of us (D. R.) by email, for second-line treatment. Our high rate of failure and relapse after trimethoprim/sulfamethoxazole can probably be attributed to our existence as a reference centre for WD patients since the first culture of *T. whipplei*.[10]

Since the recent report on the efficacy of trimethoprim/sulfamethoxazole for curing all patients,[19] we have also been contacted four times (by colleagues in Canada, the USA, Switzerland and France) regarding treatment failure with ceftriaxone and trimethoprim/sulfamethoxazole. In fact, in previous series, we have already reported that ~9.1%–15% of patients with classic WD developed a failure or a relapse during or after treatment with trimethoprim/sulfamethoxazole.[5,17]

As previously described in the literature, classic WD is an infectious disease with a spectacular clinical improvement within a few weeks after beginning antibiotic therapy.[2,33] The typical evolution of treated classic WD is improvement of classical symptoms (such as arthralgia and diarrhoea) in the first 2 weeks after appropriate therapy is initiated.[2] As for trimethoprim/sulfamethoxazole, some adverse effects can occur, such as toxidermia (observed in one of our cases). To the best of our knowledge, spontaneous trimethoprim/sulfamethoxazole resistance has never been described until now. In cases of failure in <3 months of treatment, IRIS should be strongly suspected and several cases[8,24,25] have now been described. A recent report showed that combination of initial intravenous ceftriaxone (2 g once daily) or meropenem (1 g three times daily) and oral maintenance therapy with trimethoprim/sulfamethoxazole (160/800 mg twice daily) for 12 months induced remission in all 40 patients.[19] Intravenous therapy was suspected to have a dramatic clinical effect in classic WD.[34] In our study, one patient previously treated with intravenous ceftriaxone (2 g per day) for 14 days developed an IRIS 1 month after beginning trimethoprim/sulfamethoxazole

treatment.[25] Initial intravenous therapy with ceftriaxone, an antibiotic to which *T. whipplei* is susceptible only in axenic medium,[13,14] does not seem to avoid the development of IRIS.

Moreover, since the early use of sulphonamides, rapid development of resistance to bacterial infections has been established.[35] For classic WD, clinically acquired resistance to trimethoprim/sulfamethoxazole has been reported since 2000.[36] Thus, genomic analysis has indicated that *T. whipplei* lacks the coding sequence for dihydrofolate reductase, the target of trimethoprim. *In vitro* tests have confirmed that trimethoprim is not active.[17] In fact, trimethoprim/sulfamethoxazole is a sulphonamide monotherapy against *T. whipplei*,[17] and secondary clinical failure in response to this compound has been reported.[5] The resistance and pharmacokinetic data concerning serum and CSF concentrations of sulfamethoxazole[37] suggest that a higher dose of sulfadiazine should be used to facilitate crossing of the blood–brain barrier.[32,38] Additionally, the presence of several mutations in the *folP* gene that encodes dihydropteroate synthase, the target for sulfamethoxazole, was also responsible for clinical failure.[5]

The duration of oral therapy is also unclear.[2,19,39] Very late relapses have been previously reported in classic WD[6,40] and in other chronic bacterial infections, such as leprosy,[41] Q fever[42] or prosthetic joint infection.[43] Moreover, neurological failure[44]

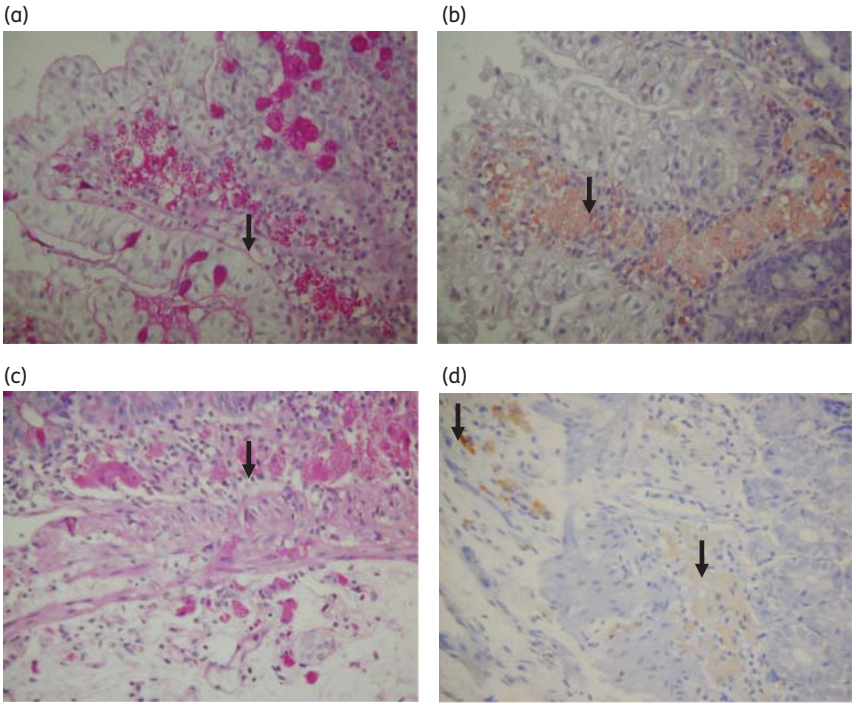

Figure 2. (a and b) Histological analysis of one patient previously treated for 1 year with trimethoprim/sulfamethoxazole, performed using a small-bowel biopsy at the time of clinical relapse. Positive PAS staining (a). Immunohistochemical staining (b) with polyclonal rabbit anti-*T. whipplei* antibody and Mayer's haemalum counterstain shows *T. whipplei* (see arrows; ×200). (c and d) Histological analysis of one patient, performed using a small-bowel biopsy, after 1 year of antibiotics. Persistence of positive PAS staining (c). Immunohistochemical staining with polyclonal rabbit anti-*T. whipplei* antibody and Mayer's haemalum counterstain shows *T. whipplei* in a few macrophages (d) (see arrows; magnification ×200).

Table 4. Antibiotic activity against *T. whipplei* as determined by Light Cycler assay in cell culture[13]

	MICs (mg/L)		
Antibiotic	Twist[a]	Endo-5[a]	Slow[a]
Chloramphenicol	1	1	2
Doxycycline	1	2	2
Trimethoprim/sulfamethoxazole	0.5/2	1/4	1/4
Imipenem	0.5	10	10
Ceftriaxone	10	10	10

[a]Twist, Endo-5 and Slow are three *T. whipplei* isolates obtained in our laboratory and cultured in MRC5 fibroblast cells.

or relapses frequently resulting from insufficient initial treatment[6] have a poor prognosis[45] because of irreversible tissue damage.[8] In our opinion, the cessation of treatment should not be based on empirical data because of the possible consequence of neurological relapse.[46,47] The full eradication of the bacterium should be the major criterion for presumed curing of classic WD. Our criteria for ceasing treatment is an absence of infected macrophages detected using PAS staining or immunohistochemistry. Importantly, PAS staining has a lower sensitivity than that of specific immunohistochemistry staining.[31] The persistence of a few infected macrophages, whatever their subtype, detected by PAS staining, is frequently linked to a late relapse (F. Fenollar, H. Lepidi and D. Raoult, unpublished data); see Figures 1 and 2. Three of our patients treated for only 1 year with trimethoprim/sulfamethoxazole developed a relapse (Figure 2). Twelve months of trimethoprim/sulfamethoxazole seems to be much too short a treatment for many patients.[31] The time needed to evaluate the number of relapses is very long.[31] In our opinion, the absence of failure or relapse in a cohort of patients may be due to a too-short follow-up period. One of our patients developed a relapse 5 years after the cessation of treatment. As classic WD is a chronic infectious disease, follow-up should continue throughout the patient's lifetime. We propose a follow-up visit every 6 months with PCR monitoring of saliva, stool and blood samples. We also performed yearly small-bowel biopsies for histological analysis and specific PCR assays, as well as PCR on CSF samples.

Finally, for the treatment of classic WD, the culture of *T. whipplei* allows us to forgo an empirical approach for one based on hard evidence. In fact, these advances in knowledge have shown that, *in vitro*, ceftriaxone is not active and the carbapenem imipenem is only active against one strain, with two strains being resistant (Table 4).[13] Based on these results, the empirical approach[19] leading to treatment with meropenem or ceftriaxone is probably ineffective for intramacrophagic *T. whipplei*. The optimal treatment of classic WD has not yet been determined. However, based on previous reports of patient failures and relapses as well as *in vitro* studies, we recommend bactericidal treatment with a combination of doxycycline and hydroxychloroquine,[13] associated with sulfadiazine in cases of neurological involvement. None of the 10 patients suffering from classic WD, first treated with this strategy and managed in Marseille, has developed an IRIS or clinically acquired resistance; they are currently still being treated. After the failure of trimethoprim/sulfamethoxazole, all our patients were treated with this alternative regimen. Only one of them presented with a relapse after the cessation of doxycycline and hydroxychloroquine. This antibiotic strategy requires a longer follow-up before its efficacy can be determined conclusively.

Discrepancies with recent reports may be due to our criteria of cure, a short follow-up in other centres or geographical heterogeneity of antibiotic susceptibilities of *T. whipplei*. In conclusion, for us, trimethoprim/sulfamethoxazole alone is not an optimal treatment for classic WD.

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The study was carried out as part of our routine work.

Transparency declarations

None to declare.

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