

Impact of real-time PCR on Whipple's disease diagnosis: the experience of

Fondazione IRCCS Policlinico San Matteo



Irene Mileto^{1,2}, Cristina Merla¹, Debora De Vitis¹, Marco Ardizzone¹, Marta Corbella¹, Federico Biagi³, Patrizia Cambieri¹



¹SC Microbiology and Virology, IRCCS Policlinico San Matteo, Pavia, Italy;
²School of Specialization in Microbiology and Virology, University of Pavia, Pavia, Italy;
³Clinical Scientific Institutes Maugeri IRCCS



Poster N° P207
i.mileto@smatteo.pv.it

BACKGROUND

Tropheryma whipplei is a Gram-positive or Gram-variable bacterium extensively present in the environment. *T. whipplei* is responsible for Whipple's disease (WD), a rare systemic infection that primarily affects the small intestine. Symptoms are non-specific, long-term and include arthralgia, fever, weight loss, diarrhea, abdominal pain, joint pain, and neurological complications.

Many patients may receive a later diagnosis of WD due to non-specific symptoms and low incidence of the disease.

Small bowel biopsy and an upper tract endoscopy is recommended when WD is suspected. Indeed, the gold standard for WD diagnosis is the histological detection of foamy macrophages containing large amounts of diastase-resistant Periodic Acid Schiff (PAS)-positive particles in the lamina propria of the duodenum. PCR testing should be performed when PAS staining is negative, but WD is clinically suspected. Here we describe our experience with real-time PCR assay for the detection of *T. whipplei*, introduced at Fondazione IRCCS Policlinico San Matteo in January 2021.

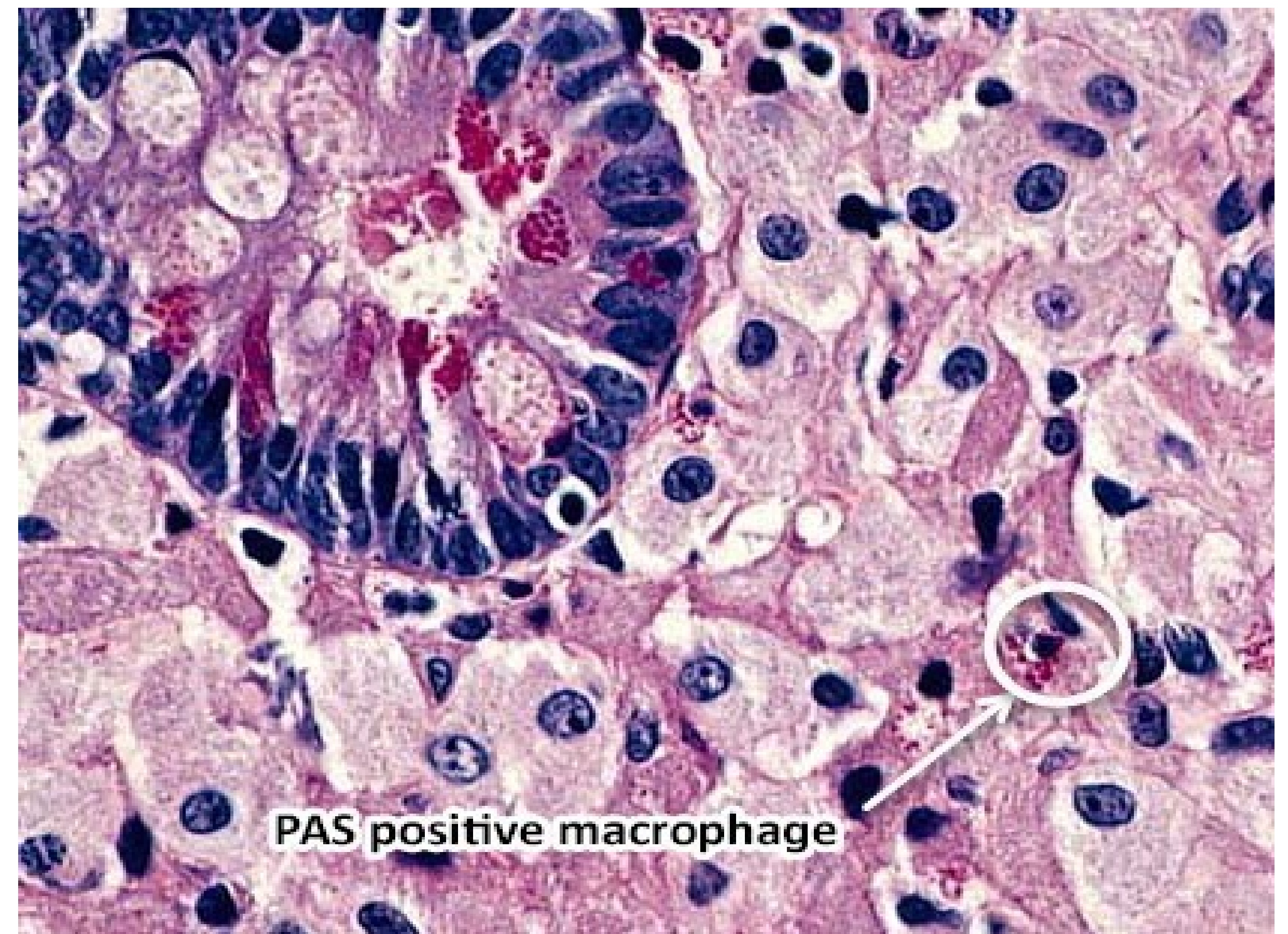


Figura 1. PAS positive macrophage in a small bowel biopsy.

MATERIALS AND METHODS

From January 2021 to May 2024 139 samples including small bowel biopsies (SBB), cerebrospinal fluid (CSF), synovial fluid (SF), urine, stool, and saliva, were tested by the microbiology lab of the Fondazione IRCCS Policlinico San Matteo of Pavia. The 139 samples were collected from 80 patients with clinical suspicion of WD. For each sample genomic DNA automatic extraction was performed on ELITE InGenius (ELITech Group). On the same instrument a real time PCR reaction targeted the *groEL* gene of the bacterium was set up (BactoReal® Kit Tw-Ingenetix).

RESULTS

T. whipplei presence was detected in 14 samples from 10 out of 80 patients (12,5%). Seven/10 were male, median age was 57 (range: 40-76 years-old). Eight/10 patients underwent duodenal biopsy, and for one of them stool and saliva were also positive. The remaining two positive samples were CSF and synovial fluid. Clinical conditions and histology response confirmed WD for 8 out of 10 patients.

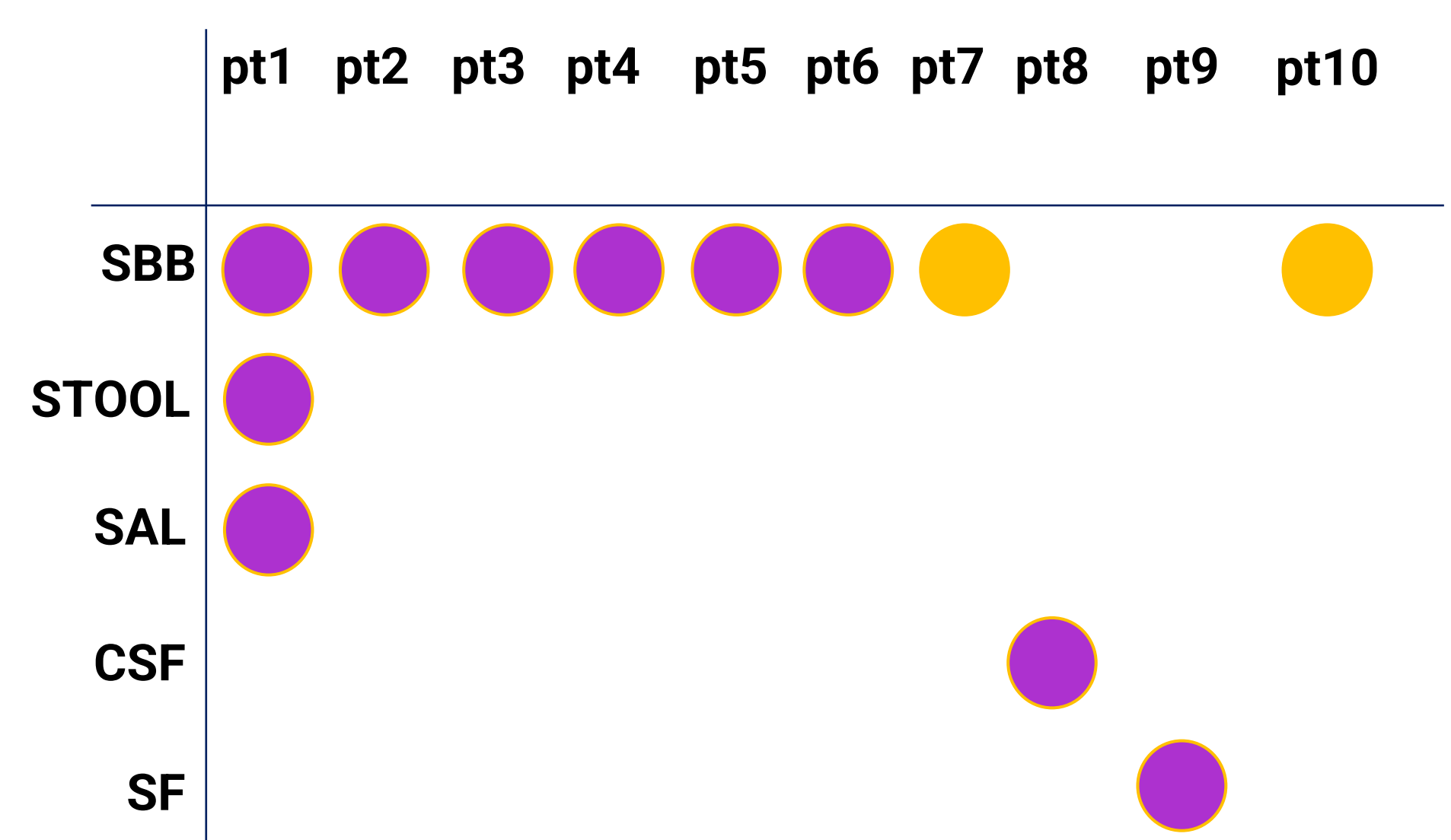


Figure 2. Patients and samples positive in PCR. In yellow, non-confirmed diagnosis with the gold standard method

CONCLUSION

The non-specific symptoms, the presence of carriers, and a negative PAS staining in almost half of the cases, make WD diagnosis still difficult. *T. whipplei* detection via PCR in duodenal biopsies has been proved in our experience as a reliable and valid support in diagnosis. PCR detection of *T. whipplei* from other clinical specimens such as stool, urine, saliva must be contextualized with the clinical status to avoid false positives due to colonization. Thus, the patients can be spared an unneeded antibiotic treatment, which is particularly intense in the case of Whipple's disease. According to guidelines, treatment should begin with intravenous Meropenem or Ceftriaxone for 14 days, and then continue for one to two years with Trimethoprim-Sulfamethoxazole. In geographic regions where resistance to Trimethoprim-Sulfamethoxazole has been found, like in France, Hydroxychloroquine and Doxycycline therapy are given. A lifetime Doxycycline prophylaxis therapy will follow for these patients.

REFERENCES

- 1.Periodic acid-Schiff stain of a small intestinal lesion of Whipple's disease by Mrwick.
- 2.Cappellini A, Minerba P, Maimaris S, Biagi F. Whipple's disease: A Rare disease that can be spotted by many doctors. Eur J Intern Med. 2023
- 3.Dolmans RA, Boel CH, Lacle MM, Kusters JG. Clinical Manifestations, Treatment, and Diagnosis of Tropheryma whipplei infections. Clin Microbiol Rev. 2017