Detection of *Helicobacter pylori* DNA sequences in gastric biopsy samples to refine the diagnosis and therapy

*Helicobacter pylori* (*H. pylori*) is a gastric pathogen responsible for gastritis, peptic ulcer disease and malignant gastric disorders (Ierardi et al., 2014). The most common tools used for detection are the urea breath test (UBT), stool antigen test (HSpA) and histological examination of gastric endoscopic biopsy specimens, defined as the diagnostic gold standard by current guidelines (Ricci et al., 2007; Malfertheiner et al., 2012). Actually, real-time PCR (RT-PCR) represents a widely used technique for the diagnosis of several bacterial and viral infections, being able to amplify small quantities of specific gene sequences with high sensitivity. The application of this technique to diagnose *H. pylori* infection has been described in the literature; amplification of the 23S rRNA gene sequence enables detection of mutations that result in resistance to clarithromycin, the key antibiotic in first-line *H. pylori* treatment (Patel et al., 2014). We investigated the diagnostic accuracy of RT-PCR for *H. pylori* infection in gastric tissue compared to conventional tests (HSpA, UBT and histology) as well as its ability to address therapy by identifying clarithromycin resistance.

Fifty consecutive patients at the first diagnosis of *H. pylori* infection were included in this study and underwent UBT, HSpA and upper endoscopy/histology. The diagnosis was confirmed if at least two tests were concordant. Enrolled patients had a mean age of 52±12 years (range 22–83 years), the female to male ratio was 33:17, 40 subjects had non-ulcer dyspepsia and ten peptic ulcer disease. A control group of 42 dyspeptic subjects with similar demographic characteristics (mean age 56±15 years, range 20–84 years; female to male ratio 27:15) was retrospectively recruited among *H. pylori*-negative dyspeptic patients undergoing endoscopy/histology in the same period. Tissue for molecular analysis was retrieved and UBT and HpsA were performed, when not performed before. All subjects tested negative in histology for *H. pylori*, confirmed by negative HSpA and UBT.

The sample size was calculated according to Schulz & Grimes (2005). A minimum of 42 subjects per group (patients and controls) was required. To evaluate the concordance between RT-PCR and the other tools, we calculated Cohen’s $\kappa$ coefficient and related 95% confidence interval (CI). The coefficient was interpreted in accordance with the benchmarks proposed by Landis & Koch (1977): a value below 0.4 indicated poor agreement, a value between 0.4 and 0.8 moderate to good agreement and a value of more than 0.8 excellent agreement.

All enrolled subjects gave written informed consent to molecular analysis of their biopsy samples according to the local Ethics Committee approval protocol (AOU Policlinico, Bari, n. 4275). RT-PCR was performed in order to achieve two goals: (i) detection of the bacterial gene encoding the 23S rDNA subunit for diagnosis and (ii) the search for the A2143G mutation, which confers clarithromycin resistance in *vitro* and shows a failure rate of more than 50% in *vivo* for all first line therapy regimens (De Francesco et al., 2006a, 2009a, b). The RT-PCR technique used was described in a previous study by our group and then extensively employed and validated (De Francesco et al., 2006b).

RT-PCR was positive in all infected patients. It showed a very good concordance with histology (Cohen’s $\kappa=0.806$; CI 0.598–1). In detail, histology was positive in 47 out of 50 patients (94%) and RT-PCR allowed identification of bacterial DNA in three histology-negative cases, which were HSpA/UBT positive. UBT was positive in 43 infected patients (86%). The agreement between RT-PCR and UBT was moderate to good, with a Cohen’s $\kappa$ coefficient of 0.674 (CI 0.454–0.894). Ultimately, HSpA displayed a less effective performance, since it was positive in 41 infected subjects (82%) and the concordance with RT-PCR was poor (Cohen’s $\kappa=0.21$; CI 0–0.498). Finally, RT-PCR found the A2143G mutation in 11 cases, with a prediction of treatment failure of 22%, a value that is comparable to clarithromycin resistance reports in some areas of Italy (De Francesco et al., 2011), thus confirming the increasing trend of antibiotic resistances towards clarithromycin in Italy (Ierardi et al., 2013). In the control group, RT-PCR did not detect *H. pylori* genetic sequences in any sample. Therefore, RT-PCR had a 0% rate of false positives.

In conclusion, the present report supports other studies that have applied RT-PCR in the diagnosis of *H. pylori* infection and for the detection of antibiotic resistances (Monno et al., 2012). RT-PCR showed an excellent concordance with histology, which is currently the diagnostic gold standard, and moderate to good concordance with UBT, while HSpA disappointing failed to achieve the diagnosis in a relevant number of cases. A possible explanation for this finding is that the stool test used in this study may not have been validated in *laco*, as recommended (Malfertheiner et al., 2012). RT-PCR is a highly sensitive technique, since it is able to diagnose the infection even with few bacteria populating the stomach, as demonstrated by the fact that the germ was found in three histology-negative cases, due probably to a patchy distribution. For these reasons, RT-PCR could be a key tool for refining *H. pylori* detection in the near future, since it might help to clarify the diagnosis in doubtful cases and, simultaneously, to orient a successful therapeutic regimen.

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Abbreviations: CI, confidence interval; RT-PCR, real-time PCR; UBT, urea breath test.


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