Enferm Infecc Microbiol Clin. 2020;38(9):407–409

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Editorial

Diagnosis of *Helicobacter pylori* infection: Progress and challenges

Diagnóstico de la infección por *Helicobacter pylori*: progresos y desafíos

The discovery of *Helicobacter pylori* in 1982 was a breakthrough in the field of Gastroenterology. The recognition of this microorganism as the cause of severe gastroduodenal disorders has dramatic change the diagnosis, treatment and prognosis of these maladies. Since then, clinical aspects of *H. pylori* infection have evolved over time and new methods have been incorporated to respond ongoing challenges.[1,2]

In this issue, Miqueleiz-Zapatero et al.[3] reported for first time a national survey about how is tested *H. pylori* infection in the microbiology laboratories of our country. A major strength of the study is the relevant role that new non-invasive methods are acquiring in clinical routine. Nowadays, there is not a gold standard test to diagnosis *H. pylori* infection, and the clinical status of the patient usually addresses the test employed. Traditionally, the detection methods are classified as invasive and non-invasive. All invasive methods rely on gastric biopsy samples and required upper endoscopy. This procedure is performed mainly in subjects in whom alarm symptoms are present or the risk of gastric cancer is high. The invasive tests include histologic examination, rapid urease test, acid nucleic testing and bacterial culture. The latter has the highest specificity but with a moderate-high sensitivity depending on the laboratory skills.

Non-invasive tests were developed shortly after the discovery of the bacterium. In 1988 Marshall et al. described the urea breath test to diagnose *H. pylori*-related gastritis.[4] Currently, urea breath test has the highest accuracy and it is the standard non-invasive method used in subjects with mild symptoms and low risk of gastric cancer. However, this method has several shortcomings as it is not a rapid test and is an indirect assay.[2,5] To overcome these limitations, others non-invasive tests have been developed; in particular, stool antigen tests using enzyme immunoassays or more recently immunochromatography in point-of-care format, as well as molecular methods. Up to now, enzyme immunoassays have provided more accurate and reliable results than immunochromatographic tests. However, enzyme immunoassay tests show still worse performance than urea breath test. Serologic testing for *H. pylori* immunoglobulin G antibody is another diagnostic option, but the fact that serology does not distinguish current from past infections is a serious limitation. Nevertheless, it should be considered for patients with conditions associated with low bacterial load (p. e. bleeding ulcers or gastric cancer) that can produce false negative results in others non-invasive tests.[5,6]

In spite of these limitations, non-invasive methods are key for the implantation of "test and treat strategy" in the management of *H. pylori* infection. In 2005, the Maastricht V/Florence Consensus Conference[5] states that *H. pylori* gastritis is an infectious disease, independent of whether or not presenting with clinical manifestations, and treatment should be offered to all infected individuals. This crucial paradigm shift is consequence of several large studies, which have shown that although in most infected subjects *H. pylori* gastritis causes no symptoms, over the long-term it predisposes the infected person to a variety of eventful complications including gastric cancer. A corollary of this statement is the use of "test and treat strategy" for cancer prevention.

H. pylori is the greatest risk factor for gastric cancer and responsible for nearly 75% of gastric cancers worldwide. Nowadays, gastric cancer represents the fifth most common cancer in the world and remains as the third most frequent cause of cancer-related death, after lung and colorectal cancers. The area with the highest incidence of gastric cancer is East Asia, where approximately two thirds of all gastric cancers worldwide are diagnosed. Others regions with an increased incidence are Middle East, Central and South America and Eastern Europe. In contrast, Western Europe, North America, Australia, and Africa are low incidence areas.[7] Spain has a low incidence of this cancer although figures are remarkable: it is estimated that 7,577 of gastric cancer will be diagnosed in 2020 (in comparison with 44,231 cases of colorectal cancer in the same period).[8] The effectiveness of primary gastric cancer prevention by *H. pylori* eradication has been demonstrated in several clinical trials and cohort studies in high incidence areas and also in Western countries. In some countries with high gastric cancer incidence, national population screening programs for *H. pylori* infection have been implemented.[9] Accordingly, Japan has also launched a program for mass-eradication of this bacterium. Alternatively, no institutionalized screening programs exist for gastric cancer prevention in Western countries. So far, the lack of a target population to screen and economic considerations are the main reasons that interfere with the introduction of such strategies in routine practice. Nevertheless, it is noteworthy that the prevalence of *H. pylori* infection is high in subjects came from high incidence areas of gastric cancer, and they retain the associate risk despite immigration to low incidence countries. In addition, randomized trials have shown that *H.*

DOI of original article: https://doi.org/10.1016/j.eimc.2019.11.008

https://doi.org/10.1016/j.eimce.2020.09.001

pylori screening and treatment reduces dyspepsia costs and may also be cost-effective in areas at low or intermediate risk.[5] These data raise the question about implantation of *H. pylori* screening programs for cancer gastric in developed countries. Obviously, the first approach should be a non-invasive method and stool antigen tests could be a promising screening test. Another alternative could be serological tests against *H. pylori* virulence factors, notably CagA, which are associated to the risk of developing gastric cancer.[10]

Miqueleiz-Zapatero et al.[3] also reported interesting data on antimicrobial resistance to *H. pylori* at national level. Until now, only local and regional studies had been carried out in our country. The increasing *H. pylori* resistance to previously efficacious antibiotic regimens is one of the major challenges currently. Antibiotic resistance remains as the most critical factor on the effectiveness of therapies to eradicate *H. pylori*. Most treatments are based on a combination of two or more antibiotics, mainly clarithromycin, metronidazole, amoxicillin, levofloxacin and tetracycline.

H. pylori's antimicrobial resistance is mainly acquired by point mutations, which are transmitted vertically by binary fission and not by mobile genetic elements as we observed with many gram-negative bacilli. Clarithromycin, metronidazole and levofloxacin are the antibiotics which more frequently developed antibiotic resistance. Amoxicillin and tetracycline usually remains susceptible to *H. pylori* although it has been reported a high rate of resistance to both of them in Africa, probably due to abusive consumption of these antibiotics in this continent. Clarithromycin resistant strains are due to point mutations in the domain V of the 23S ribosomal ribonucleic acid (rRNA) gene. A2142G, A2142C, or A2143G are the most frequent mutations found in clinical isolates. For levofloxacin, N87I is the most common mutation followed by D91N and N87K, all in the *gyrA* gene. Resistance of *H. pylori* can be detected either by phenotypic or genotypic techniques. For clarithromycin and levofloxacin, there is a good correlation among the genotypic detection of point mutations, susceptibility test in cultured gastric biopsy samples and regimen failure. In contrast, the prediction of metronidazole resistance based on genotypic information remains challenging. Resistance to metronidazole *in vitro* does not correlate with its efficacy *in vivo* possibly because *in vitro* test does not take into account the redox potential inside the cell which is important in reducing metronidazole to its active metabolite.[11]

Phenotypic susceptibility tests are hampered by the need of cultivation *H. pylori* which limits its extensive application. Molecular techniques based on PCR have been developed to detect from gastric biopsy the different mutations that can confer resistance to antibiotics, avoiding the culture of the strain. At the moment, there are a number of molecular assays commercially available for *H. pylori* detection besides the most frequent clarithromycin resistance mutations. Tests based on PCR able to determine both clarithromycin and levofloxacin susceptibility also exist. Another advantage of these methods is to test directly samples obtained by non-invasive procedures, such as gastric juice, saliva and stool. However, except for stool samples where promising results have been proved,[12] the sensitivity reported from rest of specimens is still low. This fact can explain the almost practical absence of molecular methods for the diagnosis and antimicrobial susceptibility determination reported by Miqueleiz-Zapatero et al.[3] Furthermore, although antimicrobial susceptibility tests should be performed whenever possible to guide therapy selection, testing for antimicrobial resistance is only considered if the first two regimens fail according to all recent guidelines.[13] Therefore, it is urgent the development and availability of non-invasive tools, in particular stool-based molecular test, for antibiotic susceptibility to *H. pylori*.

In the near future, it is probable that next generation sequencing (NGS) could be included to the routine.[14–16] Applied microbiological studies have been focus on correlation between phenotypical features and whole-genome sequencing, and gut microbiome. Whole-genome sequencing of *H. pylori* would allow to identify all possible resistance mutations associated to therapeutic failure, characterize the virulence of the strain and its possible clinical outcome, and to perform molecular epidemiological studies. So far, the main drawback is that we need large amount of DNA which has to be obtained from culture *H. pylori* isolates or gastric biopsy specimens. Another limitation is the cost of NGS but it has consistently decreased over the last years.

NGS has also revolutionized our knowledge of the gut microbiota. Undoubtedly, the gut microbiome has emerged as an essential player in both the healthy and diseased stomach. Furthermore, gut microbiota also influences the entire gastrointestinal tract health status. In recent years, many studies have investigated the relationship between altered microbiome with *H. pylori* and gastric cancer. Two large studies have shown that gastric microbiota in gastric cancer is distinct from that of patients with chronic gastritis. These findings suggest the potential involvement of microbes other than *H. pylori* in gastric carcinogenesis. Future prospective cohort studies will determinate their role in the coming years.[17,18] In addition, the complex interplay between microbiome and host remains to be fully elucidated.[19] For example, the effect of *H. pylori* eradication on the gut microbiota is controversial as long as the role of probiotics and prebiotics.[20] All these exciting questions will broaden the field of Clinical Microbiology in a next future.

Conflict of interest

The authors have no conflict of interest.

References

1. Crowe SE. *Helicobacter pylori* infection. N Engl J Med. 2019;380:1158–65, http://dx.doi.org/10.1056/NEJMcp1710945.
2. Sabbagh P, Mohammadnia-Afrouzi M, Javanian M, Babazadeh A, Koppolu V, Vasigala VR, et al. Diagnostic methods for *Helicobacter pylori* infection: ideals, options, and limitations. Eur J Clin Microbiol Infect Dis. 2019;38:55–66, http://dx.doi.org/10.1007/s10096-018-3414-4.
3. Miqueleiz-Zapatero A, Alba-Rubio C, Domingo-García D, Cantón R, Gómez-García de la Pedrosa E, Aznar-Cano E, et al. Primera encuesta nacional sobre el diagnóstico de la infección por *Helicobacter pylori* en los laboratorios de microbiología clínica en España. Enferm Infecc Microbiol Clin. 38;410–6.
4. Marshall BJ, Surveyor I. Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. J Nucl Med. 1988;29:11–6.
5. Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, et al. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66:6–30, http://dx.doi.org/10.1136/gutjnl-2016-312288.
6. Makristathis A, Hirschl AM, Mégraud F, Bessède E. Review: diagnosis of *Helicobacter pylori* infection. Helicobacter. 2019;24 Suppl. 1:e12641, http://dx.doi.org/10.1111/hel.12641.
7. Eusebi LH, Telese A, Marasco G, Bazzoli F, Zagari RM. Gastric cáncer prevention strategies: a global perspective. J Gastroenterol Hepatol. 2020, http://dx.doi.org/10.1111/jgh.15037.
8. Sociedad Española de Oncología Médica (SEOM). Las cifras del cáncer en España 2020. Available from: https://seom.org/seomcms/images/stories/recursos/Cifras_del_cancer_2020.pdf.
9. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on *Helicobacter pylori* gastritis. Gut. 2015;64:1353–67, http://dx.doi.org/10.1136/gutjnl-2015-309252. Epub 2015 Jul 17.
10. Kishikawa H, Kimura K, Takarabe S, Kaida S, Nishida J. *Helicobacter pylori* antibody titer and gastric cancer screening. Dis Markers. 2015;2015:156719, http://dx.doi.org/10.1155/2015/156719.
11. Alba C, Blanco A, Alarcón T. Antibiotic resistance in *Helicobacter pylori*. Curr Opin Infect Dis. 2017;30:489–97, http://dx.doi.org/10.1097/QCO.0396.
12. Pichon M, Pichard B, Barrioz T, Plouzeau C, Croquet V, Fotsing G, et al. Diagnostic accuracy of a noninvasive test for detection of *Helicobacter pylori* and resistance to clarithromycin in stool by the amplidiag *H. pylori*+ClariR real-time PCR assay. J Clin Microbiol. 2020;58:e01787–1819, http://dx.doi.org/10.1128/JCM.01787-19.
13. Fallone CA, Moss SF, Malfertheiner P. Reconciliation of recent *Helicobacter pylori* treatment guidelines in a time of increasing resistance to antibiotics. Gastroenterology. 2019;157:44–53, http://dx.doi.org/10.1053/j.gastro.2019.04.011.
14. Pohl D, Keller PM, Bordier V, Wagner K. Review of current diagnostic methods and advances in *Helicobacter pylori* diagnostics in the era of next generation sequencing. World J Gastroenterol. 2019;25:4629–60, http://dx.doi.org/10.3748/wjg.v25.i32.4629.

15. Lauener FN, Imkamp F, Lehours P, Buissonnière A, Benejat L, Zbinden R, et al. Genetic determinants and prediction of antibiotic resistance phenotypes in *Helicobacter pylori*. J Clin Med. 2019;8:53, http://dx.doi.org/10.3390/jcm8010053.
16. Imkamp F, Lauener FN, Pohl D, Lehours P, Vale FF, Jehanne Q, et al. Rapid characterization of virulence determinants in *Helicobacter pylori* isolated from non-atrophic gastritis patients by next-generation sequencing. J Clin Med. 2019;8:1030, http://dx.doi.org/10.3390/jcm8071030.
17. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018;67:226–36, http://dx.doi.org/10.1136/gutjnl-2017-314205.
18. Coker OO, Dai Z, Nie Y, Zhao G, Cao L, Nakatsu G, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut. 2018;67:1024–32, http://dx.doi.org/10.1136/gutjnl-2017-314281.
19. Rajilic-Stojanovic M, Figueiredo C, Smet A, Hansen R, Kupcinskas J, Rokkas T, et al. Systematic review: gastric microbiota in health and disease. Aliment Pharmacol Ther. 2020;51:582–602, http://dx.doi.org/10.1111/apt.15650.
20. Ye Q, Shao X, Shen R, Chen D, Shen J. Changes in the human gut microbiota composition caused by *Helicobacter pylori* eradication therapy: a systematic review and meta-analysis. Helicobacter. 2020;25:e12713, http://dx.doi.org/10.1111/hel.12713.

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