Annals of Gastroenterology (2022) **35,** 333-344

Diagnostic approach to *Helicobacter pylori*-related gastric oncogenesis

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Abstract

Helicobacter pylori (*H. pylori*) is a causative agent of peptic ulcer disease and plays an important role in the development of various other upper and lower gastrointestinal tract and systemic diseases; in addition to carcinogenesis and the development of mucosa-associated lymphoid tissue lymphoma, extragastric manifestations of *H. pylori* are increasingly being unraveled. Therefore, prompt and accurate diagnosis is essential. Within this narrative review we present an overview of the current trend in the diagnosis of *H. pylori* infection and its potential oncogenic sequelae, including gastric mucosa atrophy, intestinal metaplasia, dysplasia and gastric cancer. Signs of *H. pylori*-related gastric cancer risk can be assessed by endoscopy using the Kyoto classification score. New technology, such as optical or digital chromoendoscopy, improves diagnostic accuracy and provides information regarding *H. pylori*-related gastric preneoplastic and malignant lesions. In addition, a rapid urease test or histological examination should be performed, as these offer a high diagnostic sensitivity; both are also useful for the diagnosis of sequelae including gastric and colon neoplasms. Culture is necessary for resistance testing and detecting *H. pylori*-related gastric dysbiosis involved in gastric oncogenesis. Likewise, molecular methods can be utilized for resistance testing and detecting *H. pylori*-related gastric cancer development and progression. Noninvasive tests, such as the urea breath and stool antigen tests, can also be implemented; these are also suitable for monitoring eradication success and possibly for detecting *H. pylori*-related gastric malignancy. Serological tests may help to exclude infection in specific populations and detect gastric and colon cancers. Finally, there are emerging potential diagnostic biomarkers for *H. pylori*-related gastric cancer.

Keywords *Helicobacter pylori*, diagnosis, rapid urease test, urea breath test, histology

Ann Gastroenterol 2022; 35 (4): 333-344

Introduction

Helicobacter pylori (*H. pylori*), is a gram-negative microaerophilic spiral bacterium [1] with an estimated global prevalence of about 58% [2]. Since its discovery in 1982 by the Australian Nobelists Marshall and Warren [1,3,4], *H. pylori* has attracted the attention of the biomedical community with its numerous implications, which surpass the "narrow" anatomical limits of the stomach. This bacterium is present almost in all biological samples, including gastric mucosa samples, its site of residence, as well as blood, saliva, breath, feces, and urine. Apart from its well-established etiologic role in peptic ulcer disease, as well as its substantiated carcinogenetic effect on the stomach via both the Correa cascade and the formation of mucosa-associated lymphoid tissue (MALT) lymphoma [5,6], a plethora of extraintestinal manifestations have been associated with *H. pylori* infection [2,7-9], including the metabolic syndrome with its hepatic component, nonalcoholic fatty liver disease [5,10,11], neurodegenerative entities such as Alzheimer's disease, glaucoma (also commonly known as 'ocular' Alzheimer's disease) [1,12-14], and hematological and cardio-cerebrovascular diseases [15-17]. Therefore, prompt and accurate diagnosis of *H. pylori* infection is of great significance. In this review, we summarize all the current diagnostic modalities used for *H. pylori* infection detection and provide relevant information by highlighting the advantages,

Conflict of Interest: S. Rupp has received a travel grant from Takeda. M. Doulberis and T. Kuntzen have received a travel grant from Gilead Sciences Switzerland Sàrl. S. Rupp and T. Kuntzen have also received a travel grant from Vifor. The rest of the authors declare no conflict of interest.

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Received 02 June 2021; accepted 26 October 2021;
published online 2 June 2022

DOI: https://doi.org/10.20524/aog.2022.0725

and limitations of each method, and its potential application for *H. pylori*-related gastric carcinogenesis.

Invasive methods

Endoscopy

A fundamental aspect of endoscopy is the capability to predict *H. pylori*-induced gastritis by visual assessment of the gastric mucosa to detect patients at high risk for gastric malignancy. Representative findings of *H. pylori*-induced gastritis include mucosal edema, atrophy, diffuse erythema or redness, mosaic pattern with focal area of hyperemia, enlargement of mucosal folds, mucosal nodularity and fundic gland polyps [18,19]; a positive association with *H. pylori* infection is exhibited for antral nodularity in pediatric patients, which also predicts a higher activity grade and moderate to severe chronic inflammation of the gastric mucosa, as illustrated in Fig. 1 [20]. To evaluate the *H. pylori*-related gastric cancer risk, the Kyoto classification score is used: it includes scores for 5 endoscopic findings (gastric atrophy, intestinal metaplasia, enlarged folds, nodularity, and diffuse redness, with or without regular arrangement of collecting venules) with a total that ranges from 0-8. A Kyoto classification score ≥2 indicates the presence of *H. pylori* infection and a score ≥4 may indicate a risk of gastric cancer. Specifically, gastric atrophy, intestinal metaplasia, enlarged folds and nodularity provide evidence of a gastric cancer risk [21]. In this regard, new endoscopic techniques, such as white-light imaging (WLI) and blue-laser imaging (BLI), have been considered to identify *H. pylori* status and gastric tumor lesions [22-24]. For instance, map-like redness by WLI or a cracked shape by BLI have been proposed as features of post-eradicated gastric mucosa polyps [18,19]. However, these endoscopic findings do not have objective indicators, and there is potential for interobserver or intraobserver variability in the optical diagnosis of *H. pylori*-infected mucosa [25]. Beyond WLI and BLI, image-enhanced endoscopy (IEE), such as narrow-band imaging (NBI) or linked color imaging (LCI), with or without magnification, have also been introduced. Recent data have suggested increased diagnostic accuracy in the detection of gastrointestinal tumors with the application of these modalities during endoscopic examination [26,27]: NBI endoscopy has been introduced to improve the diagnosis of *H. pylori*-induced gastritis, preneoplastic lesions and early gastric cancer [28]; and LCI can be used to identify gastric intestinal metaplasia and, moreover, exhibits superiority to WLI for identifying *H. pylori* status and gastric tumors [22,24,29]. It is important to note, however, that IEE requires substantial training and a prolonged procedure time, while there are no uniform features of *H. pylori* infection in IEE [27]. Thus, currently there are no recognized procedures for the optical endoscopic diagnosis of *H. pylori* infection; hence, histologic evaluation by endoscopic biopsy is still required.

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Rapid urease test (RUT)

RUT, formerly known as the *Campylobacter*-like organism (CLO) test [30], provides quick results, enabling treatment initiation without delay (Fig. 2). It is a simple and low-cost invasive method for *H. pylori* detection, where gastric mucosa samples are placed into a commercially available analysis kit. The results, indicated by a change in color, require minutes to hours [31-33]. This test, however, requires an adequate gastric mucosa biopsy sample and its sensitivity varies depending on the site of any existent *H. pylori* organisms: a sufficient number of bacteria must be included in the samples to obtain more accurate results [34,35]. There is thus a greater risk of tissue injury, with subsequent adverse events such as bleeding, which can affect the sensitivity and specificity of the test. Furthermore, its specificity decreases in relation to the storage time of the samples. Recent evidence suggests that, for the best results overall, 2 samples should be obtained from the (if possible, macroscopically normal) corpus and antrum [36]. There is also a risk of false-negative results if the patient is using antibiotics, bismuth-containing agents or proton-pump inhibitors (PPIs), or displays achlorhydria, gastric atrophy, intestinal metaplasia or peptic ulcer bleeding [34,37,38]. In contrast, false-positive results may be triggered by some urease positive bacteria, such as *Staphylococcus capitis ureolyticus* [39]. When compared with the conventional RUT, a recently introduced "sweeping" method, which collects a large quantity of *H. pylori* organisms by absorbing the gastric mucus using swabs, seems to provide higher sensitivity and accuracy in the detection of *H. pylori* organisms, with a faster detection time [40]. The "sweeping" method may provide more accurate diagnosis of patients who require *H. pylori* eradication, thus possibly preventing the progression of adenoma to gastric carcinoma [41] and reducing the development of metachronous gastric malignancy following endoscopic submucosal dissection [42,43]. In addition, RUT has also been used to detect both gastric and colorectal neoplasms [44,45].

Histology

Histology allows not only the detection of active *H. pylori* infection, but also the evaluation of pathologic lesions such as

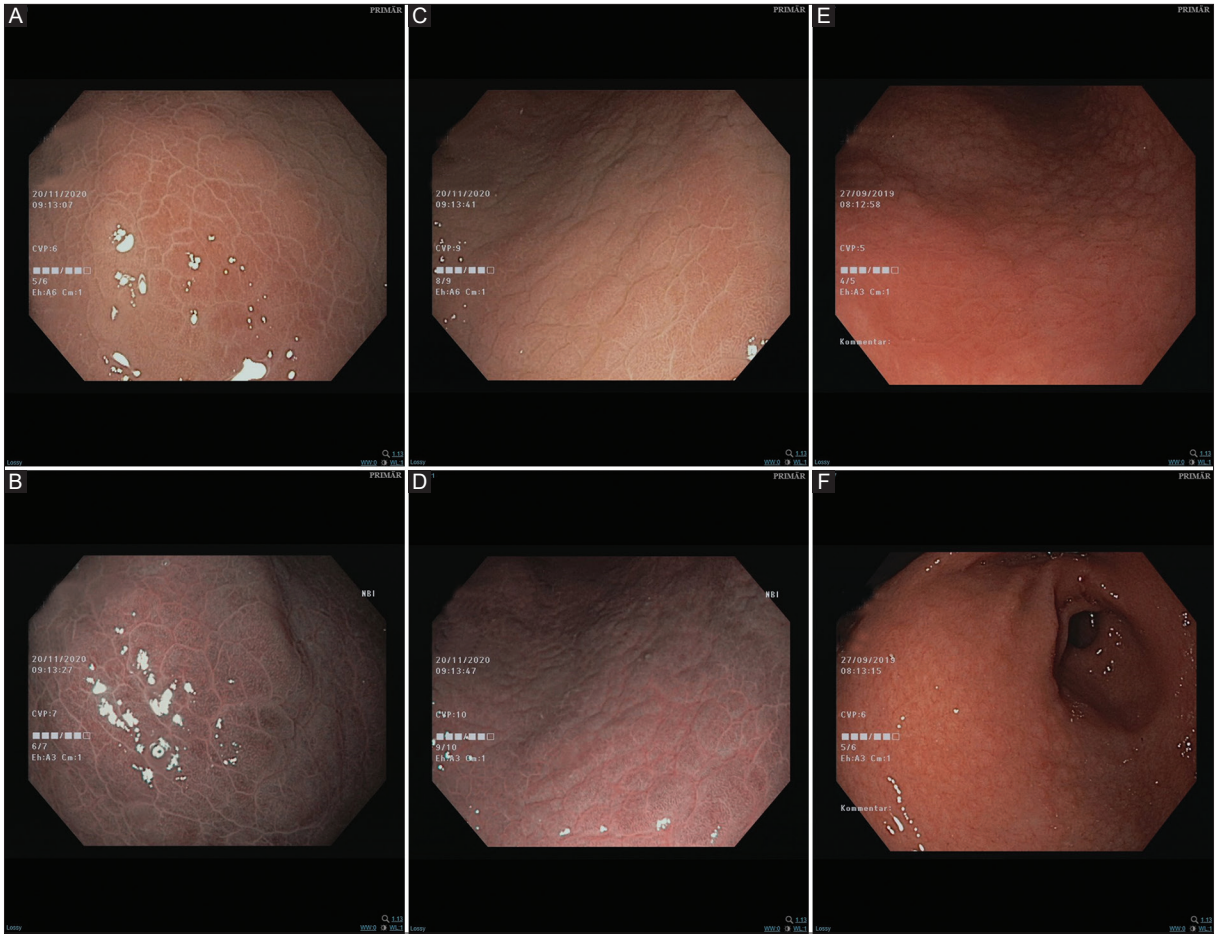

Figure 1 Endoscopic images of patients infected by *Helicobacter pylori*. (A) White light endoscopy demonstrating an antral region with typical inflammatory lesions of gastric mucosa. (B) same region with narrow-band imaging. (C, D) Corpus localization of the same patient depicting inflammatory mucosal changes with white-light and narrow-band imaging, respectively. (E, F) Typical lesions of pediatric patients depicting antral nodularity. Images were captured with a 190 series Olympus Exera III gastroscope (Tokyo, Japan). Pediatric images courtesy of Professor Köhler

gastritis, gastric atrophy, intestinal metaplasia and neoplasia. Factors that may influence *H. pylori* detection include the number and site of biopsies, the staining methods and the pathologist's experience [46]. Histological examination of gastric specimens is considered to be the practical diagnostic "gold standard" [47-49], since it offers the highest sensitivity and specificity for the detection of active *H. pylori* infection (Table 1) and provides additional information regarding the topographic distribution of the bacteria, as well as relevant microscopic lesions. The most commonly used histochemical staining for routine usage is hematoxylin and eosin (H&E), which yields a sensitivity and specificity of 69-93% and 87-90%, respectively [50]. Although the visualization of inflammation is very satisfactory with H&E, in cases with an atrophic epithelium and a low density of *H. pylori*, *H. pylori* detection might become challenging. By utilizing special histochemical staining techniques or immunohistochemistry (IHC), including modified Giemsa, Warthin-Starry silver, Giminez, McMullen, Dieterle and Genta staining (Fig. 3), specificity can be further ameliorated to 90-100%. Dieterle and Genta staining combines silver stain, H&E and Alcian blue, and offers the advantage of both visualization of *H. pylori* and scoring of inflammation [49,50].

As a general rule, 2 different stains should be used for the substantiation of *H. pylori* infection diagnosis. The modified Giemsa stain has become well established and prevalent worldwide as a routine special staining for the detection of *H. pylori*; it combines simplicity, low cost and consistent results [47,48]. The risk of a false-negative result when staining with modified Giemsa was recently demonstrated to be elevated in patients with gastric adenocarcinoma, as well as in those with a compromised gastric secretory ability, defined typically as a low (<7.45 ng/mL) serum level of pepsinogen II, due to *H. pylori* migration from superficial epithelial cells to deeper layers [51]. Approximately 10^5 bacteria must be present in the biopsies for the test to be positive. Otherwise, false-negative tests may occur when risk factors for poor bacterial detection exist, including use of antibiotics, bismuth-containing compounds or PPIs. The 2 most common causes of false-negative results are the abovementioned PPI usage as well as the presence of intestinal metaplasia, a particularly "unfriendly" microenvironment for *H. pylori* colonization. H_2-receptor antagonists do not impact the bacterial density, but are hardly ever used nowadays [52]. False-positive results are much less frequent and are caused mainly by other urease-producing microorganisms, such as

Figure 2 Representative rapid urease test demonstrating the results, typically readable within minutes, of *Helicobacter pylori* status: (A) negative test (B) mild positive test (C) positive test

Proteus mirabilis, Citrobacter freundii, Klebsiella pneumonia, Enterobacter cloacae, Staphylococcus aureus or *Staphylococcus capitis ureolyticus*, typically found only in achlorhydria or hypochlorhydria settings [36]. To increase sensitivity, especially in patients with a history of recent or systematic antibiotic or PPI usage, biopsies should be obtained from both corpus and antrum [53,54].

By means of IHC, morphologically similar-shaped microorganisms can be ruled out, although this is not practical on a daily basis. Therefore, its use should be reserved for special cases: a) no *H. pylori* bacteria are found after H&E and Giemsa staining despite the existence of relevant inflammation; b) after MALT-lymphoma treatment, to substantiate successful *H. pylori* eradication; and c) microorganisms cannot certainly be classified morphologically as *H. pylori* [50,55].

Regarding the anatomical topography of the biopsies obtained, inclusion of the gastric corpus is necessary to establish the pattern of inflammation. Nevertheless, the highest degrees of gastric atrophy, as well as intestinal metaplasia and dysplasia, are consistently detected at the *incisura angularis*. For the classification of gastritis, the Sydney grading system and its updated Houston version are used [48,50].

Some disadvantages of the histological method should be acknowledged: a) the elapse of time (i.e., at least 2-3 working days) with the associated higher cost; b) its dependence on pathologist expertise and interobserver variability; and c) the intake of PPIs and antibiotics, which cause *H. pylori* to transform from spiral to coccoid, thus rendering it under-detectable by the routine above-mentioned techniques. Nevertheless, the latter problem can be overcome with fluorescent *in situ* hybridization (FISH) [46].

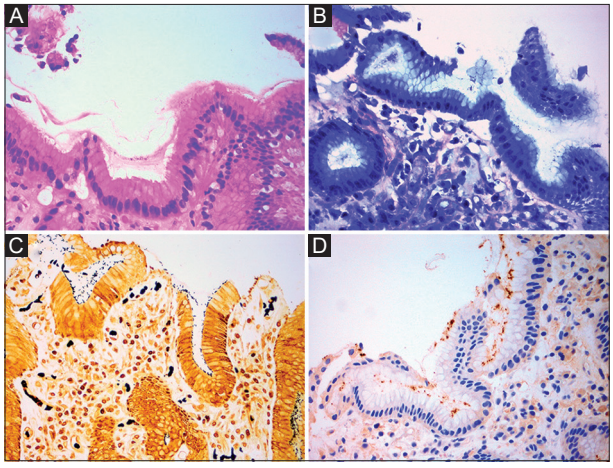

Figure 3 Numerous *Helicobacter pylori* (*H. pylori*) microorganisms within the mucus layer adherent to foveolar epithelium in different stains (400×): (A) hematoxylin and eosin, (B) modified Giemsa staining, (C) Warthin-Starry silver staining, (D) immunohistochemistry for *H. pylori*

H. pylori, ongoing gastric inflammation and its severity are the most critical precursors of gastric oncogenesis. Because both histopathology and polymerase chain reaction (PCR) have very high sensitivity and specificity [50], the degree of chronic gastric inflammation, usually evaluated by the Sydney classification, and the conditions (atrophic gastritis, intestinal metaplasia, dysplasia) that create a susceptibility to stomach cancer development, cannot yet be evaluated with noninvasive tests, and require upper gastrointestinal endoscopic biopsies [56].

Culture

Culture constitutes the reference method for the detection of *H. pylori*, providing a specificity of almost 100% (Table 1) [57]. The sensitivity of the bacterium isolation has been reported to vary greatly among laboratories as a result of the demanding nature of the culture of the microorganism [58]. Specifically, *H. pylori* culture demands highly skilled laboratory personnel and takes up to 7 days until samples can be declared negative, and up to 2 weeks until *H. pylori* has grown and an antibiogram can be offered to the treating physician. The long waiting time for the results of the culture is a drawback of this method, and is due to the abovementioned long incubation time of diagnosis; however, this is usually insignificant, given that the infection is not acute [59]. When performed under optimal settings, *H. pylori* culture from gastric biopsy samples has a sensitivity of more than 90% and a specificity of 100% [59]. Careful transport and storage of

Table 1 The main characteristics of the established diagnostic methods for *Helicobacter pylori* infection

	Examined substrate	Time to diagnosis	Advantages	Limitations
INVASIVE				
Endoscopy	Gastric mucosa	Minutes	Gastritis prediction	Absence of objective indicators
			Evaluation of malignancy risk (Kyoto classification)	Interobserver variability
			obtaining biopsies	Training required
			Multiple adjuvant imaging techiques	
RUT	Gastric mucosal sample	Minutes to hours	Quick result to start treatment	Depends on the site of forceps sampling
			Simple/low-cost, adjuvant techniques ("sweeping" test)	Affected by blood, PPIs, antibiotics, bismuth regimens, achlorhydria, gastric atrophy, IM, presence of urease positive bacteria
				Necessitates endoscopy
Histology	Gastric mucosal sample	Days to weeks	Pathologic evaluation of the mucosa,	Depends on the number and site of biopsies, staining, experience
			The "gold standard"	probability of false negative results with Giemsa staining
			Potentially assisted by IHC and FISH	PPIs, antibiotics and IM affect sensitivity
				Expensive and time elapsing
				Necessitates endoscopy
Culture	Gastric mucosal sample	Days to weeks	Optimal specificity	Expensive and time elapsing
			Offers antibiogram	Lab dependent sensitivity
			DST ability	Necessitates endoscopy
			Potential of non-invasive sample collection	Specific conditions for transport and culture
Molecular methods (rt-/q-PCR, FISH, NGS)	Gastric mucosal, juice sample, stool, saliva	Hours	Invasive (biopsies) and non-invasive (saliva-stool)	False positives from residual genetic material
			genotype and antibiotic resistance identification	expensive
			fast and automated	
			not affected by environmental conditions	Warrants specific education
NONINVASIVE				
UBT	Exhaled air	Days	Safe, readily available, accurate, and cost-effective	False positive by urease producing flora
			highly sensitive	affected by blood, PPIs, antibiotics, bismuth regimens, achlorhydria, gastric atrophy, IM, presence of urease positive bacteria
SAT	Stool	Minutes	Safe, readily available, accurate, and cost-effective	Specific conditions for storage and handling
			potentially diagnostic in GC	affected by blood, PPIs, antibiotics, bismuth regimens
				heterogeneity between kits
Serology	Serum/saliva	Hours	Not affected by environmental conditions	Cannot assess eradication
			the titer could predict activity	heterogeneity between kits
			patient-friendly	
			predictive tools of GC	

DST, drug susceptibility testing; FISH, fluorescence in situ hybridization; GC, gastric cancer; IHC, immunohistochemistry; IM, intestinal metaplasia; NGS, next generation sequencing; PCR, polymerase chain reaction; PPI, proton-pump inhibitor; RUT, rapid urease test; SAT, stool antigen test

biopsy specimens under microaerophilic conditions could increase the sensitivity [60]. A commonly used medium for transportation is saline solution, if the duration of transport is less than 4 h. Better results for recovering *H. pylori* have been obtained using a cysteine and 20% glycerol containing medium [60]. Another well described liquid transport medium is 20% glucose. Commercially available media include Portagerm pylori (bioMérieux), Brucella broth (Oxoid CM 169; BBL 11088, Becton Dickinson; Difco 0495) containing 0.5% bovine serum albumin, and Stuart's semi-solid transport medium [61]. Apart from the 100% specificity, this culture also allows the performance of resistance testing for a number of antimicrobial agents (antibiogram), which is important considering the constantly growing resistance of microbes to antibiotics. With the worldwide rise of antibiotic resistant *H. pylori* isolates and consequently progressively failing empiric first-line regimens, bacterial culture and phenotypic drug susceptibility testing remains a critical diagnostic mean for antibiotic resistance surveillance and management of antibiotic treatment failures. A variety of potential clinical specimens have been used, including gastric biopsies, feces, vomitus and saliva [61]. *H. pylori* culture from specimens obtained by noninvasive methods, such as the abovementioned gastric juice, saliva or stool, is challenging and hampered by low sensitivity [62-64]; thus, it is not recommended in routine clinical practice [65]. Culture of gastric biopsy specimens provides the most reliable results [66]. Some authors [65] have reported that obtaining a single biopsy specimen from the gastric antrum is not sufficient for reliable diagnosis, and therefore suggested that at least 3 specimens should be obtained from the antrum, along with 1 additional specimen each from the anterior and posterior corpus. The gastric corpus constitutes an ideal site for obtaining specimens, as after the consumption of PPIs it may be the only *H. pylori*-positive site [67].

Notably, *H. pylori* infection has been associated with gastric dysbiosis, and alterations in gastric microbiota can be related with the development of gastric malignancy beyond *H. pylori* infection [68,69]. *H. pylori*-induced hypochlorhydria leads to changes in gastric bacterial abundance that may play a role in the development of gastric cancer [70]. *Campylobacter* is among the most influential genera in *H. pylori*-induced atrophic gastritis specimens, and gastric atrophy-associated gastric microbiota dysbiosis may be an important contributor to gastric tumorigenesis [71]. Therefore, further research is needed to evaluate in depth the potential role of *H. pylori* plus its related altered gastric microbiota positive cultures in the pathophysiology of gastric pathologies, including gastric neoplasms.

Molecular methods

Based on real-time PCR, molecular testing is an infrequently used screening method that utilizes new technology to reveal the occurrence of bacterial DNA in the case of low bacterial loads. This test can be made invasively (gastric biopsies) and noninvasively (saliva or stool) and does not require specialized transport [72]. It might be useful in epidemiological studies, genotyping, and estimation of antibiotic resistance trends [72,73]. Several target genes, such as *ureA, glmM, ureC, 16SrRNA, 23SrRNA, hsp60* and *vacA*, have been used for the recognition of *H. pylori* [70]. An important limitation is the possibility that false positives might result as a consequence of residual genetic material following antibiotic treatment. As a screening test it is not usually available and it is not currently used in clinical practice [74]. Moreover, PCR can detect DNA from both live and dead bacteria, which may yield false-positive results. Specifically, it is suitable for examination of resistance to macrolides, which might be helpful for the choice of the eradication regimen in regions with high antibiotic resistance and/or eradication failure [75]. An advantage of the molecular test is that it is less susceptible to unfavorable conditions compared with the culture of bacteria for resistance testing [75]. It is also a relatively simple, fast and automated procedure that can detect *H. pylori* better in acute bleeding conditions compared to other diagnostic modalities [76]. A recently introduced test (real time multiplex ARMS-PCR assay) was able to detect *H. pylori* with high analytical sensitivity (50 plasmid copies) and to detect mutations associated with resistance to clarithromycin and levofloxacin. In a relevant study (n=192), diagnostic sensitivity and specificity both reached 100% for single clarithromycin resistance, 98% and 95% for levofloxacin resistance and 100% and 96.9% for clarithromycin-levofloxacin double resistance, respectively. The test was also reported to be fast; results were provided in less than 2 h after receipt of the samples [77]. On the other hand, it is a relatively expensive diagnostic modality, requires some expertise, while false-positive results may occur, as previously mentioned [76]. Another molecular method being implemented for *H. pylori* infection diagnostics is FISH. This test is based on the detection of fluorescently labeled oligonucleotides that bind to DNA fragments of *H. pylori* (16S rDNA or 23S rDNA sequences) containing specific point mutations that are responsible for clarithromycin resistance. The method is independent of the culture of bacteria and can also be used for testing for clarithromycin resistance on formalin-fixed and paraffin-embedded gastric biopsies. Several commercially available test systems are available. Like PCR, however, the procedure is expensive and requires expertise and technical equipment [78]. In a large study comparing Giemsa staining with IHC and FISH, FISH and IHC were superior to Giemsa staining. The sensitivity of the latter was 83.3% compared to 98.8% for IHC and 98.0% for FISH; notably, the diagnostic performance of FISH and IHC was barely affected by mucosal inflammation and structural lesions [75]. Next generation sequencing (NGS) is a completely new and promising method. The great advantage of NGS is that entire genomes can be decoded within a short time. Especially with regard to the increasing resistance of bacteria to antibiotics, it might be worth considering abandoning the current "test-and-treat" strategy in favor of a primarily resistance-based treatment. Nevertheless, it would be rather premature to apply this modality in clinical practice. Recently, an improved quantitative PCR (qPCR) with an impressive detection

performance can be used for quantitative *H. pylori* recognition and testing for the virulence genes *vacA s1, vacA m1, cagA and babA2* simultaneously; compared with RUT, qPCR exhibits better consistency with the classic gold standard of *H. pylori* culture [79].

PCR is also an important method for detecting and distinguishing different pathogenic *H. pylori* strains, which could play a role in the development of gastric cancer [80]. In this respect, for instance, the vacAs1m1 genotypes increase the gastric cancer risk 2.8-fold [81]. The s1m1/cagA+/babA2+ strains of *H. pylori* predominate in the gastric malignant and surrounding tissues, and their occurrence may be linked with the probability of invasion and metastasis [82]. The expression of CYP3A4 genotype may be related with the potential oncogenic transformation of *H. pylori*-induced chronic atrophic gastritis to gastric cancer development and progression [83]. Furthermore, *H. pylori* upregulates the orphan nuclear receptor Nurr1, which correlates with gastric cancer and a poor prognosis. Therefore, it may represent a new target for the diagnosis and treatment of gastric cancer [84].

Noninvasive methods

Urea breath test (UBT)

The principal noninvasive testing method in current use is UBT, a safe, readily available, accurate, and cost-effective method for *H. pylori* testing with the highest sensitivity (up to 94%) [75] (Table 1). Furthermore, like all noninvasive methods, it is suitable for patients who have contraindications for conventional endoscopy and subsequent biopsy specimens [31,32]. The patients are given a test meal with enriched carbon (^{13}C or ^{14}C), supplemented with substances such as citric acid or dietary supplements, which inhibit gastric emptying to extend the time in the stomach. The concentration of CO_2 is then measured in the exhaled air [85]. The exhaled $^{13}CO_2$ is estimated by mass spectrometry that yields results quickly, in-office, while $^{14}CO_2$ must be processed by a nuclear medicine laboratory [86,87]. ^{13}C is preferred for children and pregnant women because it is harmless, even though the radiation exposure of ^{14}C is comparable to a person's daily radiation exposure [86]. False-positive results can occur in the setting of a microbiome that is also capable of producing urease, such as *Helicobacter heilmannii*, due to urease activity, contamination with oral flora, and/or in achlorhydria due to the lack of inhibition of bacterial growth other than *H. pylori* species (e.g., *Proteus mirabilis, Citrobacter freundii, Staphylococcus aureus*). False-negative test results can occur through reduction in *H. pylori* gastric diversity, reported for antibiotics, bismuth compounds and PPIs. Specifically, decreased sensitivity occurs in the setting of active gastrointestinal bleeding and recent usage of the mentioned bismuth-containing compounds, antibiotics, or antisecretory drugs [88,89]. Therefore, it is recommended to terminate antibiotics and bismuth-containing compounds at least 4 weeks before testing. Likewise, PPIs and H_2-receptor antagonists should be discontinued at least 2 weeks before testing. Antacids that do not include bismuth, such as aluminum hydroxide, do not appear to influence test results [88]. Even for patients with *H. pylori* infection predominantly in the gastric corpus, a higher proportion of false-negative results can occur when testing with ^{13}C-UBT [90]. Recent data indicate that the ^{13}C UBT diagnostic test appears to be more sensitive and accurate than the stool antigen test (SAT), and moreover displays a comparable outcome to the SAT in evaluating the success of the eradication regimen [91].

It is important to note that conflicting evidence exists regarding the potential usefulness of UBT to detect *H. pylori*-related gastric malignancy. Some studies indicate that the UBT value is not a sensitive predictor of gastric cancer and low values are related with risk of gastric malignancy; compared with gastritis and peptic ulcer, UBT values are significantly lower in patients with gastric cancer [92,93]. Nevertheless, other studies indicate that the ^{14}C-UBT is highly sensitive for detecting the occurrence of *H. pylori* even in gastric cancer, regardless of its stage; *H. pylori* is present in 98% of patients with gastric cancer (positive by UBT), and active *H. pylori* infection occurs in early and advanced gastric cancer as estimated by UBT [94]. Therefore, since *H. pylori* eradication significantly decreases the incidence of gastric cancer without concomitant adverse events [95], UBT may offer clinicians the ability to detect this high-risk group of patients indirectly by this readily available and noninvasive test. Moreover, UBT, apart from other gastroduodenal pathologies, might also be considered as a pre-endoscopy screening test for gastric cancer. Thus, in view of the conflicting data, further studies are needed to clarify this important issue.

SAT

SAT is an additional frequently used noninvasive method. Like UBT, SAT is also a safe, readily available, accurate, and cost-effective method for *H. pylori* testing, with high sensitivities and specificities exceeding 90% for both [96]. SATs are enzyme immunoassays that identify *H. pylori* antigens in stool specimens using poly- or monoclonal anti-*H. pylori* antibodies [74]. Assays based on monoclonal antibodies are superior in terms of diagnostic accuracy than the older polyclonal-based assays [97]. Issues that may influence their use include the logistics of handling and storage of stool specimens, variability of reimbursements by region, and test availability [74]. Specifically, stool samples can be stored at room temperature for 24 h. For longer storage (up to 72 h) the temperature should not exceed 4°C, otherwise sensitivity will be diminished. In addition, gastrointestinal diseases, including bleeding ulcers and PPI treatment, may reduce the sensitivity of the assay [98]. The test should therefore be deferred for at least 2 weeks. Bismuth-containing drugs or antibiotics that reduce the number of bacteria can also lead to false-negative results, as has been mentioned for UBT [99]. Recent studies have reported good results for the automated chemiluminescence assay LIAISON® (Meridian) compared to histology, culture and RUT. This test uses a monoclonal antibody sandwich method

and chemiluminescent immunoassay technology. A sensitivity of 95.5% and a specificity of 97.6% were obtained for LIAISON, in comparison to a sensitivity and specificity exceeding 80% in previously used monoclonal antibody-based tests [100]. In a recent comparison of LIAISON® with an ELISA test procedure (RIDASCREEN®, R-Biopharm, Darmstadt, Germany) and an immunochromatography test from the same company (RIDAQUICK®), very comparable results were demonstrated for the diagnostic accuracy of the mentioned tests [101]. New tests with alternative techniques are also being developed. In a new approach, *H. pylori* is detected by immunomagnetic beads containing monoclonal antibodies that bind to *H. pylori* with high sensitivity and are conjugated to a polyclonal antibody-conjugating quantum dot probe. Detection is performed using a fluorescence spectrometer [102]. Further studies of the procedure's diagnostic accuracy and comparison with currently used test strategies are necessary.

Regarding gastric malignancy, screening and treatment of *H. pylori* in high-risk individuals has been recommended as a cost-effective strategy in order to decrease the burden of gastric cancer and peptic ulcer disease [103,104]. In this respect, the use of SAT may represent the most cost-effective screening approach [105]. Moreover, SAT might be the most reliable noninvasive approach for the diagnosis of *H. pylori* infection in patients who have undergone distal gastrectomy owing to gastric cancer [106]. It should be noted that gastric cancer patients display a 6-fold *H. pylori* stool load compared to those without gastric malignancy [107]. Thus, further comparative studies including SAT and other noninvasive methods are needed to determine the most cost-effective screening approach for optimal management of *H. pylori*-related gastric cancer.

Serology

Serology by estimation of immunoglobulin G (IgG) *H. pylori*-antibodies shares the same high diagnostic accuracy as biopsy-based and noninvasive tests, though it does not discriminate between current and past *H. pylori* infection. As a possible exception, high anti-*H. pylori* IgG antibody titers are related with the degree of gastritis and mucosal *H. pylori* load. Therefore, high serum anti-*H. pylori* antibody titer may be an index of *H. pylori* load in patients with active infection [2,108]. In addition, serological tests of gastric functional parameters (i.e., pepsinogens, gastrin) may permit an estimate of gastric mucosa alterations, particularly the presence of severe atrophy [109]. The isolation of anti-*H. pylori* antibodies is performed using ELISA or immunoblotting; a plethora of kits are commercially available [110] that recognize different epitopic targets, with anti-CagA being the most common, followed by anti -VacA, -UreB, -UreC, -HspB, -FlaA, -FlaB, -CagII and -CagC [111,112]. Besides the convenience of venipuncture compared to the stool collection and UBT procedures, current kits yield high diagnostic rates, with a sensitivity and specificity of 97.6% and 96.2%, respectively, at least in specific populations [113].

The heterogeneity among kits, combined with the regional differences in *H. pylori* antigen sequences, could compromise the performance of serologic tests, especially when population-based validation has not been performed. In this regard, current ongoing migratory flows could create a significant burden in antibody based *H. pylori* diagnostics, thus necessitating periodic revalidations of population-based techniques. The main disadvantage of serology is the inability to evaluate the eradication treatment results. Nevertheless, early data indicated that a 20-25% decrease in serum antibody titers 6-21 months after *H. pylori* treatment could predict eradication success quite sensitively (93%), albeit needing further confirmation [114,115]. On the other hand, circulating monocyte subpopulations seem to be associated with the treatment outcome, as $CD14^+CD163^+CD206^+$ and $CD14^+CD163^+CD209^+$, expressed in intense *H. pylori* infection-related inflammation, are significantly reduced after *H. pylori* eradication, thus providing, despite relevant costs, a rather promising serological index of successful therapy [116]. Moreover, serology could indirectly assess the risk of *H. pylori* infection-related gastric and extra-gastric complications such as glaucoma [111].

The combined investigation of anti-*H. pylori* antibodies with serum pepsinogen (PG), which interprets gastric atrophy, provides an additional diagnostic tool, called the "ABC method" [117]; the PG plus gastrin combined with *H. pylori* test (UBT) appears to play a significant role in evaluating gastric atrophy [109]. To overcome the obstacle of isolated false-negative cases from PG, this method classifies patients into 4 groups: Group A [*H. pylori* (–) PG (–)], Group B [*H. pylori* (+) PG (–)], Group C [*H. pylori* (+) PG (+)], and Group D [*H. pylori* (–) PG (+)]; PG(+) is defined when PGI≤70 ng/mL and PGI/II≤3, indicating atrophy [118]. When compared to group A, patients classified into the groups B, C or D were 4.2, 11.2 and 14.8 times more prone to developing gastric cancer, thus necessitating triennial, biennial or annual endoscopic surveillance, respectively [119]. The background of this ABC scale is based on the rationale that, upon atrophy progression, the low-positive anti-*H. pylori* titer is associated with increased risk for gastric cancer, although no definite cutoffs have yet been established [120]. Post-eradication low anti-*H. pylori* titers could represent a reservoir of false-negative cases with a high risk of intestinal type gastric cancer, especially when combined with increased PG I/II, though some investigators proposed 2 subgroups of high- and low-negative anti-*H. pylori* titers to stratify the risk of cancer after eradication [120]. On the other hand, high positive anti-*H. pylori* titers, especially against specific antigens such as CagA and/or FlaA, without atrophy (Group B), have been associated with diffuse type gastric cancer [121-123]. Furthermore, one study evaluated the possible role of anti-*H. pylori* antibodies in the development of gastric cancer by using the abovementioned Kyoto classification endoscopic score. A multivariate analysis disclosed that nodularity, atrophy and age between 40-59 years were associated with a high anti-*H. pylori* titer in *H. pylori*-infected patients. Thus, anti-*H. pylori* titer alterations with age may reflect inflammation of gastric mucosa, and could help predict the risk of gastric malignancy [109]. Finally, in a large cohort, the detection of VacA specific antibodies was prospectively

associated with an 11% higher risk of colorectal cancer (CRC), being higher in Afro-Americans and Asian-Americans (up to 45%) [124]. Therefore, further studies comparing *H. pylori* serology with other invasive and/or noninvasive methods are required to detect the most cost-effective screening approach for optimal management of *H. pylori*-related gastrointestinal cancer.

Emerging diagnostic methods

H. pylori secretes large amounts of urease, a substantial virulence factor that promotes colonization by bacteria. In recent years, efforts have been focused on targeting urease. In this regard, Yang *et al* developed a series of novel oxoindoline derivatives with low cytotoxicity, which seem promising for inhibiting the urease from *H. pylori* [125].

Tucci *et al* developed and validated EndoFaster 21-42 (synonym: Mt 21-42; NISO Biomed S.r.l, Turin; Italy), a new promising device interposed between the endoscope and the suction system, which allows the analysis of gastric juice samples aspirated during upper endoscopy within 30-90 sec [126,127]. The diagnosis of *H. pylori* through Mt 21-42 is based on the ammonium concentration of gastric juice. Its fully automated nature, in combination with low maintenance costs, may make this device valuable and reliable for the detection of *H. pylori* infection [126].

A large number of methods have also been developed for the noninvasive detection of *H. pylori* infection through spotting of anti-*H. pylori* IgG or IgA antibodies in blood, serum, saliva and urine [128]. Regarding the detection of *H. pylori* infection in urine, a large meta-analysis, including 23 studies and 4963 patients, reported that testing for anti-*H. pylori* antibodies in urine could be a valuable marker in the diagnosis of *H. pylori* infection [129]. However, tests for IgG in urine may remain positive over a long period of time after the therapy of the *H. pylori* infection, an acknowledged drawback of the method [128]. Interestingly, recent evidence indicates that, apart from *H. pylori* status, urinary levels of Trefoil factor 1 (TFF1, uTFF1) and metalloprotease 12 (ADAM12, uADAM12) are independent diagnostic biomarkers for gastric cancer; the urinary biomarker panel uTFF1, uADAM12 and *H. pylori* status appears to distinguish gastric cancer patients from healthy controls [130]. Therefore, further studies comparing *H. pylori* urinary testing with the aforementioned additional noninvasive methods are also required to detect the most cost-effective screening approach for optimal control of *H. pylori*-related gastrointestinal cancer.

Concluding remarks

The plethora of diagnostic options for *H. pylori* infection is still growing. Esophagogastroduodenoscopy with biopsy and histopathological examination remains the practical gold standard for diagnosis [47-49] and assessment of long-term effects [57]. Chemical or virtual chromoendoscopy can further enhance the predictive accuracy, but technological equipment is required. Before proceeding to eradication therapy, however, it is still recommended to confirm *H. pylori* infection by RUT, histopathology or a molecular detection method. In patients younger than 60 years with dyspeptic symptoms, the American College of Gastroenterology and the Canadian Association of Gastroenterology primarily recommend a noninvasive test procedure to search for *H. pylori* as part of a "test-and-treat" strategy [56]. UBT and SAT are suitable for this purpose [131], and further procedures with excellent sensitivity and specificity are in the pipeline. NGS will probably set new standards in the future, especially with regard to resistance testing. Ultimately, an individualized approach is advised.

Acknowledgment

We thank Prof. Dr. H. Köhler (Chief physician of the Department of Pediatrics, Kantonsspital Aarau, Aarau 5001, Switzerland) for kindly providing the pediatric endoscopic images.

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