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Research Article

Comparing Efficacies of Biopsy and Rapid Urease Testing for *H. Pylori* at a Single Institution

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Helicobacter pylori (*H. pylori*) infects > 50% of the world's population, leading to gastric cancer if left untreated. An estimated 26,000 gastric cancer cases will occur in the US in 2023, with 40% of cases becoming the primary cause of death. Invasive and noninvasive techniques are used to diagnose *H. pylori* infection; however, controversy exists regarding the "gold standard" for diagnosis. We sought to evaluate the efficacy of *H. pylori* invasive detection methods: stained biopsy and rapid urease test (RUT) at a single institution. For the study, 200 patients (100 H. pylori + and 100 H. pylori -) from a single institution that underwent gastric biopsies were selected and retrospectively evaluated for *H. pylori* status. Demographics and clinicopathologic data were collected, including diagnostic tests performed, treatment, and outcomes. Histology and RUT were highly positively and negatively correlative; however, disparate results occurred in 7% of samples which was significant ($p < 0.001$). Of those that were *H. pylori* positive, 60% had a posttreatment test completed. Gastric cancer developed in 3 patients (1.5%), all of whom were *H. pylori* positive. Histology and RUT testing yield similar results; therefore, there is no efficacious reason to run both tests on patients. Since histology has greater sensitivity (>95%) and the ability to identify other gastropathies, it should be considered the "gold standard," for the identification of *H. pylori*.

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium residing in the gastric epithelium that is estimated to infect over 50% of the world's population [1, 2]. Although the majority of infected populations are identified in developing countries, the infections remain prevalent in westernized countries [2]. The United States reports an overall infection rate of 36%, much less than developing countries [2]. *H. pylori* is known to result in chronic gastritis and peptic ulceration, which are known precursors to gastric malignancies such as adenocarcinomas and mucosal-associated lymphoid tissue (MALT) lymphomas [3]. The American Cancer Society (AMS) estimates that over 26,000 gastric cancer cases will occur within the United States in 2023, with over 40% of cases becoming the primary cause of death [4].

Both invasive and noninvasive diagnostic techniques are used to detect *H. pylori* infection in clinical cases. Invasive testing includes histologic analysis (biopsy and stain), rapid urease test (RUT), also known as campylobacter-like organism

(CLO) tests, microbiological cultures, and polymerase chain reaction (PCR). Noninvasive testing includes serology, urea breath testing (UBT), and stool antigen assays. Each test, regardless of invasiveness, detects *H. pylori* via a specific mechanism and therefore contains its own advantages and disadvantages [5]. For example, the RUT test is thought to be highly sensitive and cost-effective but presents the opportunity for false negatives due to factors such as proton pump inhibitor (PPI) use, biopsy location, observational errors, and intestinal metaplasia [5–7].

Clinical organizations determine which diagnostic technique to utilize based on factors such as sensitivity, specificity, and cost-effectiveness of each test. However, there is a controversy within the literature regarding which test should be the "gold standard." Stained biopsy and RUT remain the primary invasive testing methodologies used but are often done simultaneously, which proves redundant and costly [8]. This study serves to evaluate the efficacy of the two primary invasive testing techniques: biopsy and RUT. Additionally, noninvasive testing techniques, primarily UBT and stool antigen assays, are investigated to evaluate any discrepancies between test results. With modern clinical organizations constantly searching for ways to decrease the unnecessary costs of healthcare while improving the health of the population, standardizing a treatment plan that maximizes cost-effectiveness and increases diagnostic accuracy would prove more financially responsible and potentially allow for better care to be provided.

2. Methods

Following IRB approval, all patients that underwent gastric biopsies between 6/1/2016 and 12/31/2017 at a single institution were evaluated for *H. pylori* status. Patients were retrospectively evaluated. Patients for whom complete medical records were unavailable were excluded. A total of 200 patients were selected to be included in the study and stratified by *H. pylori* status: 50% (100) positive and 50% (100) negative *H. pylori* diagnoses. Typical demographics and clinicopathologic data were collected, including age, race, gender, and comorbidities associated with *H. pylori* infection, diagnostic tests performed (biopsy, RUT, and noninvasive tests), treatment, and outcomes. Treatment of *H. pylori* infection was completed based on standard protocol.

2.1. Histology. Specifically, biopsy samples were collected from various gastric locations (antrum, corpus, fundus, duodenal bulb, and EG junction) and submerged in 10% formalin solution before being sent to the laboratory for analysis. Paraffin-embedded, 8 μm sections were then collected for clinical microscopic analyses by pathology consultants affiliated with the institution.

2.2. Rapid Urease Test. The rapid urease test was performed via the hpFast® (Mechanicsburg, Pennsylvania) commercial test and executed under the company instructions. A gastric mucosal endoscopic biopsy is placed in an agar gel containing urea. The urease produced by *H. pylori* catalyzes urea into ammonia, raising the pH. Two pH dye indicators, bromthymol-blue and methyl-red, display any change in acidity. The recommended reading times are 15, 30, and 60 minutes; 4 hours; and 24 hours from biopsy collection. All biopsies were from the same locations as the histologic analysis.

2.3. Stool Antigen Test. Stool samples were tested using Premier Platinum HpSA PLUS® (Cincinnati, Ohio), a commercial microwell enzyme immunoassay performed by a national laboratory service. Patient stool samples are diluted and combined with peroxide conjugate and a monoclonal antibody mixture before added to the specified wells for a one-hour incubation period. The wells are washed to remove any unwanted particles before substrate is added and incubated for 10 minutes at room temperature. Bound enzymes produce color, and results are interpreted via photospectrometer at OD 450 or 450/630 nm.

2.4. Urea Breath Test. A commercial breath test, BreathTek® (Rockville, Maryland), was administered by a healthcare professional as a qualitative detection of urease associated with *H. pylori.* A synthetic ^{13}C-urea component is ingested by the patient via a Pranactin-Citrin® solution. In the presence of urease associated with *H. pylori*, ^{13}C-urea is decomposed to $^{13}CO_2$ and NH_4^+. The carbon dioxide is absorbed through the bloodstream and exhaled in the breath. The postdose sample ratio of $^{13}CO_2$ to $^{12}CO_2$ is recorded and compared to the baseline ratio recorded before the ingestion of the Pranactin-Citrin® solution. Breath samples are analyzed via IR300 infrared spectrophotometer in the institutional clinical laboratory.

Data were analyzed using Fisher's *t*-test or chi-squared test for association as appropriate. Continuous variables were compared using an ANOVA analysis. All statistical analyses were performed using R version 3.6.2. The alpha level was set at <0.05.

3. Results

Overall, the demographics and clinicopathological data of 100 *H. pylori* positive and 100 *H. pylori* negative patients were evaluated (Table 1). The mean age of diagnosis for the total cohort was 52 years (range 7-89; positive mean 55; negative mean 49; $p = 0.0236$). For the entire cohort and the *H. pylori* status stratifications, more females were represented than males (total 62%; positive 56%; negative 68%; $p = 0.0919$; Table 1); but this was not significantly different between groups. However, a higher percentage of males were infected with *H. pylori* when stratifying by gender. As has also been noted in the literature, race was noted to be significantly different between stratifications ($p < 0.001$), with Black patients presenting more as positive than negative (67% positive; 33% negative), likewise, for those of Hispanic race (63% positive; 37% negative; Table 1). None of the comorbidities evaluated between the stratifications, including PPI usage, were significantly different (Table 1).

White blood cells (WBC) did not show any significant correlation with diagnosis ($p = 0.5957$); however, neutrophil levels were significantly associated with patients that tested negative for *H. pylori* ($p = 0.0249$; Table 1).

Table 1: Demographics.

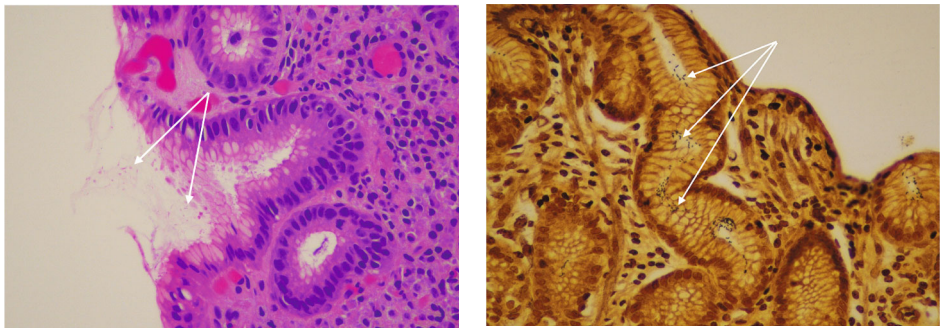
	Total $n = 200$	H. Pylori pos $n = 100$	H. Pylori neg $n = 100$	p value
Age at diagnosis				**0.0236**
Mean	52	55	49	
Range	7-89	15-84	7-89	
Gender				0.0919
Male	76 (38%)	44 (44%)	32 (32%)	
Female	124 (62%)	56 (56%)	68 (68%)	
Race				**<0.001**
White	123 (61.5%)	47 (47%)	76 (76%)	
Black	51 (25.5%)	34 (34%)	17 (17%)	
Hispanic	19 (9.5%)	12 (12%)	7 (7%)	
Other	7 (3.5%)	7 (7%)	0 (0%)	
BMI				0.5611
Mean	28.5	29	28	
Range	15-46	18-44	15-46	
Hypertension				0.4788
Positive	91 (45.5%)	48 (48%)	43 (43%)	
Negative	109 (54.5%)	52 (52%)	57 (57%)	
Diabetes	$n = 40$	$n = 21$	$n = 19$	
Type 1	2 (1%)	1 (1%)	1 (1%)	1
Type 2	38 (19%)	20 (20%)	18 (18%)	
Alcohol				0.3284
Yes	50 (25%)	22 (22%)	28 (28%)	
No	150 (75%)	78 (78%)	72 (72%)	
Drug use				0.0972
Yes	14 (7%)	4 (4%)	10 (10%)	
No	186 (93%)	96 (96%)	90 (90%)	
Tobacco use				0.771
Current	43 (21.5%)	23 (23%)	20 (20%)	
Former	58 (29%)	27 (27%)	31 (31%)	
Never used	99 (49.5%)	50 (50%)	49 (49%)	
PPI history*	$n = 123$			0.0614
>2 weeks	11 (5.5%)	9 (9%)	2 (2%)	
<2 weeks	112 (56%)	57 (57%)	55 (55%)	

*PPI history was self-reported. Bold values are significant based on the alpha value of 0.05.

Per protocol, all tissue samples obtained from biopsy were stained with H&E (Figure 1(a)); of those, a subset was stained with a Warthin-Starry silver stain (Figure 1(b)), which significantly correlated with a positive diagnosis ($p < 0.001$).

The identification of inflammation on biopsy was not significantly different between *H. pylori* positive and negative samples ($p = 0.0817$); as expected, intestinal metaplasia was noted significantly more often in *H. pylori* positive samples (0.0376). Serology and UBT were also used to identify *H. pylori* infection; however, these tests were rarely used (Table 2). The majority of patients with *H. pylori* infection (84%) were treated with a regimen containing antibiotics and a PPI. Interestingly, 16% of *H. pylori* positive patients ($n = 16$) did not seek treatment. Most people did not undergo postdiagnostic testing to evaluate the definitive eradication of the organism. For those that did, 26 people got histology testing, 10 got RU testing (Table 2), 40 got SA, and 12 got UBT. Of the invasive postdiagnostic tests (histology and RU), neither was found to be statistically significant between groups ($p = 0.0748$ and 0.1288, respectively). Noninvasive postdiagnostic tests (SA and UBT) were not statistically different between the two groups; however, the numbers were small ($n = 40$, $n = 12$, respectively; data not shown). Overall, three patients developed gastric cancer, with all patients having definitive *H. pylori* infection.

The efficacy of the invasive diagnostic tests was analyzed among *H. pylori* status stratifications (Table 3). When histology positively identified *H. pylori*, RUT also was positive (92.5%); likewise, when histology was negative, RUT was also negative (93.9%. Disparate results occurred in 7% of samples: 6.1% ($n = 2$) of histology was positive when RUT was negative, and 7.5% ($n = 3$) of histology was



(a) (b)

Figure 1: *H. pylori* specific staining. (a) Stomach biopsy (H&E, 400x) demonstrating a chronic, active inflammatory infiltrate of the lamina propria with *Helicobacter pylori* organisms visible along the surface of the epithelium as minute pink corkscrews (arrows). (b) Stomach biopsy (400x) with a Warthin-Starry silver stain greatly enhances the ability to visualize the *Helicobacter pylori* organisms along the surface of the epithelium.

negative when the RUT test was positive ($p < 0.001$). Overall, the sensitivity of RUT compared to histology was 94.87%, and the specificity was 91.18%, for an accuracy of 93.15%.

4. Discussion

Helicobacter pylori infection is the most common human infection in the world [9, 10] and is most commonly transmitted through direct contact between human subjects, the oral-oral or fecal-oral route, or through contaminated food or water [9, 10]. The identification of *H. pylori* infection is especially important because of its association with the development of gastric cancer when left untreated, making early diagnosis and identification of *H. pylori* infection key [11, 12].

Historically, the gold standard of diagnosis for *H. pylori* is histology (sensitivity and specificity >95%) with H&E [13, 14] and Warthin-Starry staining. However, biopsies are invasive, and the opportunity for sampling error persists [15–17]. The RUT (sensitivity > 92% and specificity > 95%) was developed to avoid the time and expense of histologic analysis; however, it is still invasive, and sampling error can still result in false negative results. The sensitivity of both histology and RUT varies based on gastric location [18, 19], with higher organismal densities in the upper body and greater curve of the stomach [19]; increasing biopsy sites to overcome the sampling error is possible [6]. Intestinal metaplasia and atrophy can also decrease sensitivity, with some suggesting a second confirmatory test necessary to verify results [19].

PPI use can also affect the sensitivity of RUT [7, 13, 14, 18, 20] as PPIs act by suppressing *H. pylori* infection or causing migration of the bacteria from the antrum to the fundus of the stomach [21]. Given this, studies have recommended against the exclusive use of RUT for those with a history of PPI use [22]. This is especially important as these medications are available over-the-counter, and their use is self-reported. For patients taking PPIs, multiple biopsies from the corpus and fundus are suggested, but, as such, a negative RUT should be interpreted with caution [21]. RUT can also generate a false positive result if other urease-producing organisms are present, or the specimen and media are in contact for over 24 hours [7].

The comparison of histology and RUT in our cohort noted a RUT false negative rate of 6.1% and a false positive rate of 7.5% for those *H. pylori* positive by histology, which was significant ($p = 0.001$; Table 3); two tests did have a similar concordance (92.5% positive and 93.9% negative) and specificity (95%). Thus, given these data, and that histology has a greater sensitivity (>95%), histology should be considered the "gold standard," as some literature suggests. This is contrary to other studies that have suggested that RUT should be used initially instead of histology, with these reports noting the sensitivity of RUT up to 94% (ranges as low as 73% for some tests) with no significant difference in sensitivity when compared to histologic analysis [23, 24]. The rationale appears to be the cost differential between the two tests and the "time needed for histologic analysis," citing the importance of early diagnosis and treatment [23, 24]. One study even noted that RUT should be used as a confirmatory test for *H. pylori* infection [23]. Given that both RUT and histology are invasive, and that our institution has the benefit of a well-organized, efficient pathology process that allows for histologic analysis to include the Warthin-Starry stain within 24 hours, it does not appear efficacious to run both tests. Additionally, histology has the added benefit of being able to evaluate for other gastropathies, not simply determining the presence or absence of *H. pylori* infections.

Knowing that *H. pylori* can lead to the development of gastric cancer, treatment and eradication of *H. pylori* infection are important. We examined the follow-up period from 2016 to 2021 to determine if any patients that were *H. pylori* positive developed gastric cancer. Three patients (3%) in our cohort that were *H. pylori* positive developed gastric cancer, similar to the 2.9% gastric cancer rate cited in the literature [25–27]. Two patients were diagnosed with *H. pylori* and gastric cancer concurrently, while the third patient in our cohort, who was immunocompromised, was diagnosed with gastric cancer three months after a positive *H. pylori* test. All patients were over the age of 64, and above the mean, we noted for positive patients (55; range 15-84).

When *H. pylori* is identified, treated, and confirmed to be eradicated, gastric cancer risk decreases by approximately

Table 2: Clinical indicators, treatment, and outcomes.

	Total $n = 200$	*H. pylori* pos $n = 100$	*H. pylori* neg $n = 100$	p value
WBC (TH/mm^3)				0.5957
<4	6 (3%)	3 (3%)	3 (3%)	
4-11.5	145 (72.5%)	70 (70%)	75 (75%)	
>11.5	16 (8%)	7 (7%)	9 (9%)	
N/A	33 (16.5%)	20 (20%)	13 (13%)	
Neutrophils	$n = 147$			0.0249
Low (<50%)	15 (7.5%)	2 (12%)	13 (13%)	
Normal (50-70%)	111 (55.5%)	58 (58%)	53 (53%)	
High (>70%)	21 (10.5%)	10 (10%)	11 (11%)	
Histologic analysis	$n = 200$			<0.001
H&E only	73 (36.5%)	20(20%)	53(53%)	
Warthin-Starry	127 (63.5%)	80(80%)	47(47%)	
Rapid urease	$n = 73$			0.001
Positive	40 (20%)	37 (37%)	3 (3%)	
Negative	33 (16.5%)	2 (2%)	31 (31%)	
Inflammation*	$n = 200$			0.0817
Yes	197 (98.5%)	100 (100%)	97 (97%)	
No	3 (1.5%)	0 (0%)	3 (3%)	
Intest metaplasia*	$n = 122$	$n = 57$	$n = 65$	0.0376
Positive	42 (21%)	25 (44%)	17 (26%)	
Negative	80 (40%)	32 (56%)	48 (74%)	
Serology	$n = 1$			1
Positive	1 (0.5%)	1 (1%)	0 (0%)	
Negative	0 (0%)	0 (0%)	0 (0%)	
UBT	$n = 3$			0.3768
Positive	2 (1%)	2 (2%)	0 (0%)	
Negative	1 (0.5%)	0 (0%)	1 (1%)	
Treatment				<0.001
Anti-B + PPI	86 (43%)	84 (84%)	2 (2%)	
Anti-B only	2 (1%)	0 (0%)	2(2%)	
Treatment N/A	112 (56%)	16 (16%)	96(96%)	
Posttreatment test				
Histology	$n = 26$	$n = 9$	$n = 17$	0.0748
Positive	4 (15%)	3 (33%)	1 (6%)	
Negative	22 (85%)	6 (67%)	16 (94%)	
RU	$n = 10$	$n = 3$	$n = 7$	0.1288
Positive	1 (10%)	1 (33%)	0 (0%)	
Negative	9 (90%)	2 (67%)	7 (100%)	
Gastric cancer				0.0817
Yes	3(1.5%)	3 (3%)	0 (0%)	
No	197 (97.5%)	97 (97%)	100 (100%)	

*Inflammation and intestinal metaplasia were noted on biopsy specimens pretherapy.

Table 3: Efficacy of biopsy versus RUT.

		Histology +	Histology -	p value
RUT result $n = 73$	Positive	37 (92.5%)	3 (7.5%)	<0.0001
	Negative	2 (6.1%)	31 (93.9%)	

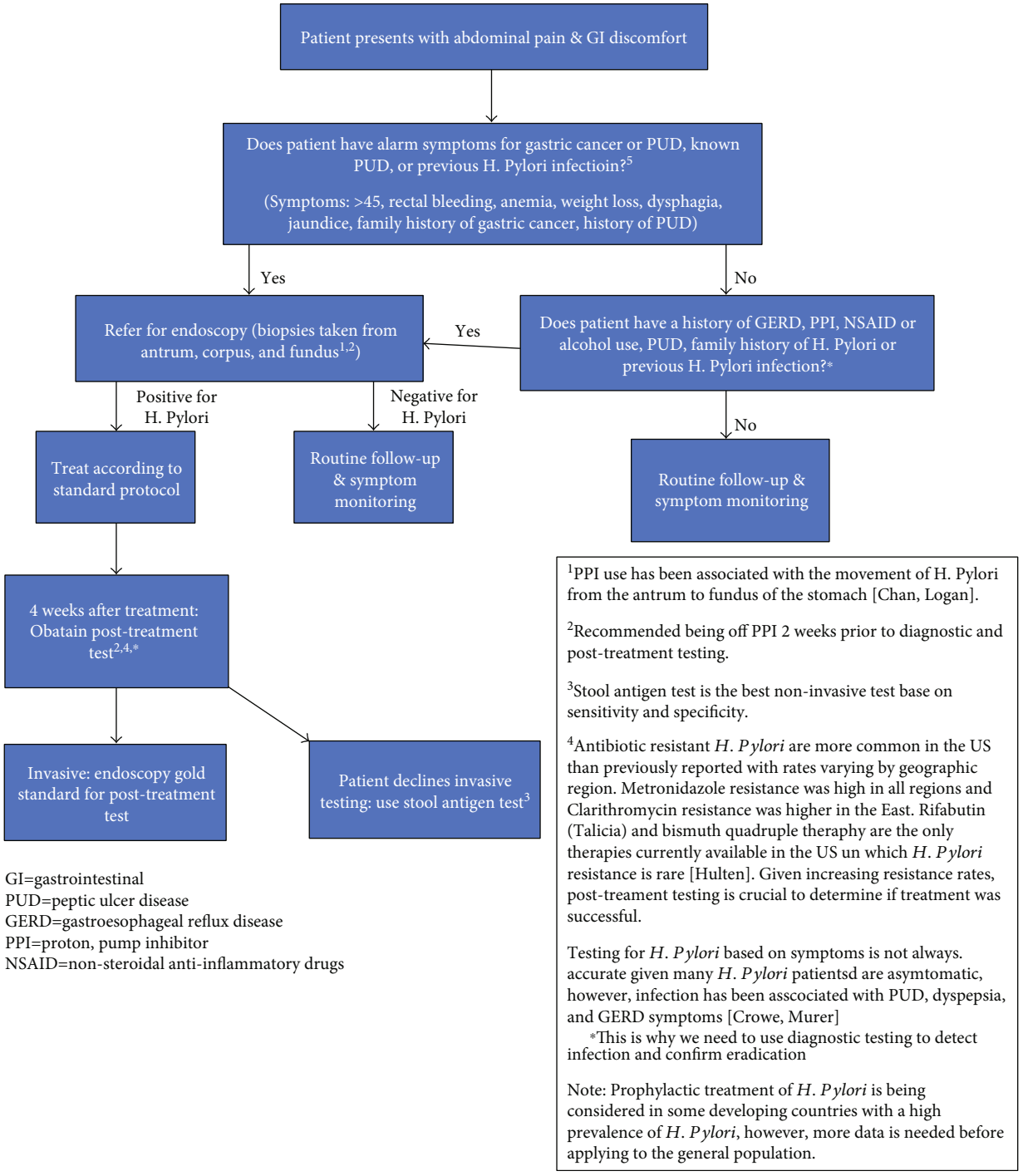


Figure 2: *H. pylori* diagnosis and follow-up algorithm.

44% [28, 29]; however, posttreatment evaluation to confirm eradication is an area for improvement both at our institution and in others [28, 29]. Only 57% of the *H. pylori* positive patients had a posttreatment test carried out to confirm eradication. The American College of Gastroenterology recommends a posttreatment UBT, fecal antigen test, or biopsy-based testing to confirm eradication in patients at least four weeks after completing antibiotic treatment and two weeks without PPIs to limit false negative results [30].

UBT has been shown to have a sensitivity and specificity of approximately 90%; however, reliability is limited because of significant heterogeneity between studies [18, 31]. Additionally, the UBT is expensive and is still affected by prior PPI or antibiotic use [21, 31]. The UBT may also produce a false positive if there are other urea-producing organisms in the stomach [18]. The stool antigen test has been found to have a sensitivity and specificity of approximately 92% [17]. The stool antigen test is low-cost and easy to perform; however, the antigen may degrade in the intestine as it

travels, and antigen shedding may vary over time [18]. If the concentration of the antigen becomes too low to detect, the test could give a false negative even though the organism is still present [18]. Additionally, the stool antigen test lacks accuracy in the presence of antibiotics, PPIs, and N-acetyl cysteine and is influenced by bowel movements and upper gastrointestinal bleeding [32]. The stool antigen test is also patient-administered, allowing for errors in the collection to confound results. Despite these concerns and given the ease of testing for the patient, the stool antigen test is the most frequently used means to confirm eradication post-treatment at our institution. Given these data, we developed an algorithm that summarizes these findings and other published reports to guide proper diagnoses and follow-up for *H. pylori* infection (Figure 2).

Overall, given these data, the efficacy and the ability of histologic analysis to identify a range of associated gastropathies suggest that histologic analysis alone should be considered the gold standard for the diagnosis of *H. pylori* when compared to RUT [33]. Testing for eradication of *H. pylori* after antibiotic treatment should be a priority, with the urease breath test or stool antigen test as appropriate options given that an additional invasive test would not be well-tolerated by most patients. However, repeat posttreatment endoscopic evaluation with histologic analysis might be appropriate for those patients at the highest risk of untoward outcomes from persistent *H. pylori* infection or for those patients with persistent symptoms despite negative noninvasive testing.

5. Limitations

The disadvantages of this study are that we had a small patient population at a single institution which made some data points too small for statistical analysis and increases the likelihood of type 1 errors. The UBT and serology diagnostic tests data did not retain a population high enough for reliable statistical analysis. Additionally, the follow-up period may not have been enough time to see if patients developed gastric cancer or not. If this study were to be repeated or supplemented, we recommend using a larger population and following patient prognosis over a greater period of time.

Data Availability

Data would be available with a data-use-agreement with the institution in which the research was completed upon request.

Additional Points

Presentation. This work was presented in summary at the institutional meeting "Prisma Health Research Showcase" virtual meeting in October 2021, Greenville SC (https://cdn.fourwaves.com/static/media/filecontent/2b088ef7-f89d-48be-9490-807d86686eef/9ecba5f7-666b-4c89-8b9c-b2b171ce0fad.pdf; pg 155).

Ethical Approval

The study was completed only after IRB approval.

Consent

Patient consent was through universal consent (protocol number IRBNet 2042685-1).

Conflicts of Interest

All authors report no conflicts of interest or financial disclosures to declare.

Authors' Contributions

K Hill collected the data and wrote the manuscript (KLHill@email.sc.edu). AV Ohning collected the data and wrote the manuscript (Ohninga@musc.edu). JP Mallory collected the data and wrote the manuscript (Mallojon@musc.edu). S Self conducted the statistical analysis (SCwatson@mailbox.sc.edu). CMG Schammel designed the study, is the primary editor, and conducted the manuscript generation. J Meredith edited the manuscript and with a microbiology expertise (jenny.meredith@prismahealth.org). J Reddic edited the manuscript and with a clinical testing expertise (john.reddic@abbott.com). P Kent edited the manuscript and with an infectious disease expertise (Patrick.kent@prismahealth.org). JB Knight edited the manuscript and with a pathology expertise (histology and testing) (jenny.knight@prismahealth.org).

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