THE AMERICAN JOURNAL OF GASTROENTEROLOGY
© 1999 by Am. Coll. of Gastroenterology
Published by Elsevier Science Inc.

Vol. 94, No. 8, 1999
ISSN 0002-9270/99/$20.00
PII S0002-9270(99)00341-X

Usefulness of Serological IgG Antibody Determinations for Confirming Eradication of *Helicobacter pylori* Infection

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OBJECTIVE: Prior studies have suggested that IgG antibody titers may be useful to confirm successful treatment of *Helicobacter pylori* (*H. pylori*) infection. However, the diagnostic value of decreasing IgG titers is limited by the necessity to perform pre and posttreatment tests in parallel which requires stored sera. Our objective was to assess the accuracy of IgG antibody titers using the HM-CAP IgG EIA kit (Enteric Products) in monitoring treatment of *H. pylori* infection and to compare the relative accuracy of parallel *versus* serial determinations.

METHODS: The ^{14}C urea breath test (UBT) was used to confirm *H. pylori* infection in 83 dyspeptic patients and eradication of the organism at 4 wk and 6 months posttreatment. IgG titers pretherapy and 6 months posttherapy were determined either serially (separate EIA plates) or in parallel (same EIA plate), and the relative percent decline in antibody titer was calculated.

RESULTS: When a decline of ≥25% at 6 months was used as the cut-off for *H. pylori* eradication, mean sensitivities of serial and parallel determinations were 87.5% and 86.8%, respectively, and mean specificities of both were 100%. In 68 of 75 patients in whom the organism was eradicated, the mean decrease in IgG titer at 6 months was 41.1% for serial determinations and 41.5% for parallel determinations.

CONCLUSIONS: Serial or parallel IgG titers offer equivalent diagnostic accuracy for confirming *H. pylori* eradication after therapy. A ≥25% decline in titer 6 months after therapy is a sensitive and specific marker for eradication of the infection. Serial evaluation of IgG titers does not require serum storage, and is a cost-effective and accurate alternative to the UBT or endoscopy-based methods. (Am J Gastroenterol 1999;94:2105–2108. © 1999 by Am. Coll. of Gastroenterology)

INTRODUCTION

Helicobacter pylori (*H. pylori*) has been established as the causative agent in type B gastritis and peptic ulcer disease (1–3). Infection with the bacterium is also associated with gastric carcinoma and primary gastric B cell lymphoma and the organism is classified by the World Health Organization as a Class I human carcinogen (4, 5). Suggested indications for *H. pylori* eradication therapy include peptic ulcer disease (active or inactive), MALT lymphoma, gastric cancer, family history of gastric cancer, and dyspepsia. It has been recommended that eradication of *H. pylori* be confirmed, at least, in complicated peptic ulcer disease (*e.g.*, bleeding), MALT lymphoma, and in cases of uncomplicated duodenal ulcer and nonulcer dyspepsia when symptoms persist (6).

The diagnostic accuracy of serological detection of antibodies to *H. pylori* has been well established and there are several commercial kits available for this purpose (7–11). Serology is less expensive and is quicker and easier to perform than the urea breath test (UBT) and endoscopy-based procedures. Serology is noninvasive; it does not involve exposure to radioisotopes, and does not require participants to discontinue use of proton pump inhibitors, H2-blockers, or other medications before screening patients for initial infection. The advantages inherent in serology make this an ideal method to monitor treatment. Studies have demonstrated the clinical utility of decreasing titers of antibodies to *H. pylori* in confirming successful treatment (12–19). Most of these studies have reported a drop in IgG titer at 6 months of approximately 40–50% from pretreatment levels in patients in whom the bacteria was eradicated. However, Cutler *et al*. reported that a 20% reduction in IgG titer at 6 months was associated with successful treatment. Many of these studies relied on antibody assays developed for research use only, and for which a firm body of validation data demonstrating overall diagnostic accuracy was not available. Furthermore, in these studies, the diagnostic value of decreasing IgG antibody titers to confirm eradication of *H. pylori* was limited by the necessity to perform parallel testing on stored sera. Serial serological testing would be a more practical means of monitoring antibody titers in response to eradication of the organism, as it does not require storage of pretreatment serum samples.

We evaluated the utility of following both serial and parallel serum IgG antibody titers to *H. pylori* using the

commercially available HM-CAP EIA (Enteric Products, Stony Brook, NY). The HM-CAP IgG assay for antibodies to *H. pylori* has been demonstrated to have a diagnostic accuracy of 97.4% based on a study evaluating 473 ^{13}C UBT–characterized patients (11). It was the aim of our study to compare the diagnostic accuracy of a decrease in serial *versus* parallel IgG titers in response to treatment evaluated by the ^{14}C UBT.

MATERIALS AND METHODS

Study Population Treatment Regimen

The patients in this study were part of a larger population of patients evaluated for inclusion in a separate study evaluating treatment strategies for eradication of *H. pylori*. Specifically, a total of 221 dyspeptic patients presenting to the outpatient clinic at the University of Guyana Medical School, Georgetown, Guyana, South America, were screened serologically for infection with *H. pylori* using the FlexSure rapid test (Beckman Coulter Primary Care Diagnostics, Palo Alto, CA). Male and female patients, aged 18 to 75 yr, were eligible for inclusion in the study. Patients were excluded if they had taken medications such as nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, anticoagulants, anticonvulsants, antacids, proton pump inhibitors, or other antiulcer medications (including bismuth-containing compounds) within the preceding month. Patients who had taken an investigational drug during the preceding month or had a documented allergy to clarithromycin, metronidazole, amoxicillin, or omeprazole or who required astemizole or terfenadine were also excluded. Patients with prior gastric resection were excluded. Women who were pregnant, lactating, or who might become pregnant during the study were also excluded.

Infection was confirmed in patients demonstrating a positive serology *via* ^{14}C UBT. A total of 85 patients demonstrated a positive confirmatory ^{14}C UBT. These patients were randomized to receive one of two therapeutic regimens for 10 days: metronidazole 500 mg *b.i.d.*, omeprazole 20 mg *b.i.d.*, and clarithromycin 500 mg *b.i.d.*, (MOC), or amoxicillin 1 g *b.i.d.*, omeprazole 20 mg *b.i.d.*, and clarithromycin 500 mg *b.i.d.*, (AOC). Of 85 patients, 83 completed treatment and all follow-up and were evaluated for the purposes of this study.

Urea Breath Test (UBT)

The ^{14}C UBT (Pytest, Ballard Medical Products, Draper, UT), was performed on all patients initially to screen for infection, and at 4 wk and 6 months posttherapy. The cut-off used was as recommended by the manufacturer for the purpose of screening for infection for all testing performed. Eradication of the bacteria was defined by a negative UBT at 4 wk and 6 months posttherapy. Failure to clear the bacteria was defined by a positive UBT at both 4 wk and 6 months posttherapy. Those patients with a positive UBT at 4 wk and a negative UBT at 6 months, as well as patients with a negative UBT at 4 wk and a positive UBT at 6 months, were excluded from statistical analysis.

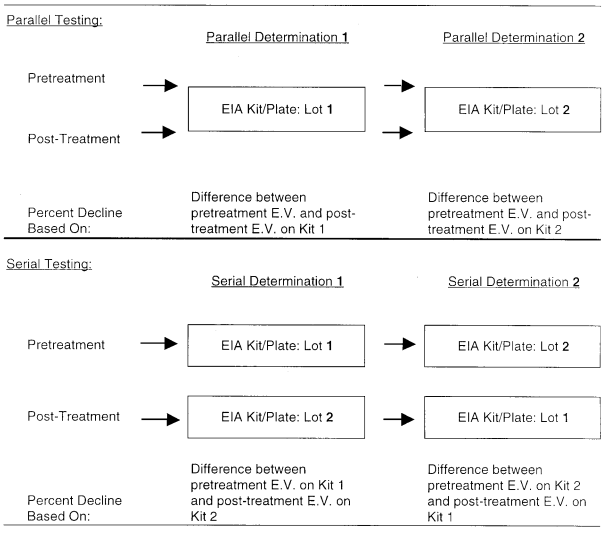

Figure 1. Format for parallel and serial testing of pretreatment and posttreatment serum samples.

Enzyme Immunoassay (EIA)

All patients had blood drawn for serological testing at the time of UBT. The serum was separated, aliquoted, and frozen at −70°C until the time of testing. The samples were forwarded to a central testing site. All samples were coded so that the technicians performing the testing were blinded as to patient identification, sample date, and UBT status. Serum IgG antibody titers to *H. pylori* were determined using the HM-CAP IgG EIA assay (Enteric Products, Stony Brook, NY). Patient samples were evaluated on two different kit lots. Quantitative ELISA values (EV) were extrapolated and interpreted for each sample according to manufacturer's instructions. The percent decline in IgG antibody values was calculated as follows: 100 − (posttreatment EV/pretreatment EV × 100). Parallel and serial antibody determinations were performed as illustrated in Figure 1. Percent decline for parallel determinations was calculated based on evaluation of pre- and posttreatment samples on the same EIA plate (same kit lot). Percent decline for serial determinations was calculated based on evaluation of pre- and posttreatment samples on different EIA plates (different kit lots). Therefore, two values for parallel decline in IgG titer were determined: one based on the difference in pretreatment and posttreatment antibody titers observed when both samples were evaluated in tandem on one lot of EIA kits (parallel determination 1), and the other based on the difference in pretreatment and posttreatment antibody titers observed when both samples were evaluated in tandem on a second lot of EIA kits (parallel determination 2). Likewise, two values for serial decline in IgG titer were observed: one based on the difference in antibody titer observed based on evaluation of the pretreatment sample on lot 1 and evalua-

Table 1. Patient Treatment Results Relative to the ^{14}C Urea Breath Test (UBT) Results

	^{14}C UBT: No. of Patients (%)
UBT negative at 4 wk and 6 months (eradicated)	70 (84.3)
UBT positive at 4 wk and 6 months (noneradicated)	7 (8.4)
UBT positive at 4 wk and UBT negative at 6 months	4 (4.8)
UBT negative at 4 wk and UBT positive at 6 months	2 (2.4)

tion of the posttreatment sample on lot 2 of kits (serial determination 1), and the other based on the difference in antibody titer observed based on evaluation of the pretreatment sample on lot 2 and evaluation of the posttreatment sample on lot 1 (serial determination 2).

RESULTS

Patient treatment results relative to UBT results at 4 wk and 6 months are shown in Table 1. Ninety-one percent (70 of 77) of this group was successfully treated, as indicated by a negative UBT at 4 wk and 6 months. Eradication rates were similar for the two treatment regimens. The four subjects who were UBT-positive at 4 wk and UBT-negative at 6 months, as well as two patients who were UBT-negative at 4 wk and UBT-positive at 6 months, were excluded from statistical analysis.

The sensitivity of the serologic assay *versus* the ^{14}C UBT for identifying initial infection was 96.3% for kit lot 1 and 97.5% for kit lot 2. Three of 83 subjects, including one subject who was UBT-positive at 4 wk and negative at 6 months, demonstrated negative pretreatment IgG antibody results on at least one kit lot, despite a positive UBT. These subjects demonstrated variable decline in antibody titers upon treatment. This was most likely due to the fact that the initial IgG antibody titer was already low. These subjects were subsequently excluded from further analysis.

The sensitivity and specificity of a ≥25% decline in IgG serial and parallel antibody titers for each kit lot is illustrated in Table 2. The sensitivities of parallel and serial determinations were comparable, with an overall mean of 87.1% and a standard deviation of 5%. Regardless of whether serial or parallel determinations were performed, subjects who were noneradicators were correctly identified 100% of the time.

The mean percent decrease in IgG titers in these subjects

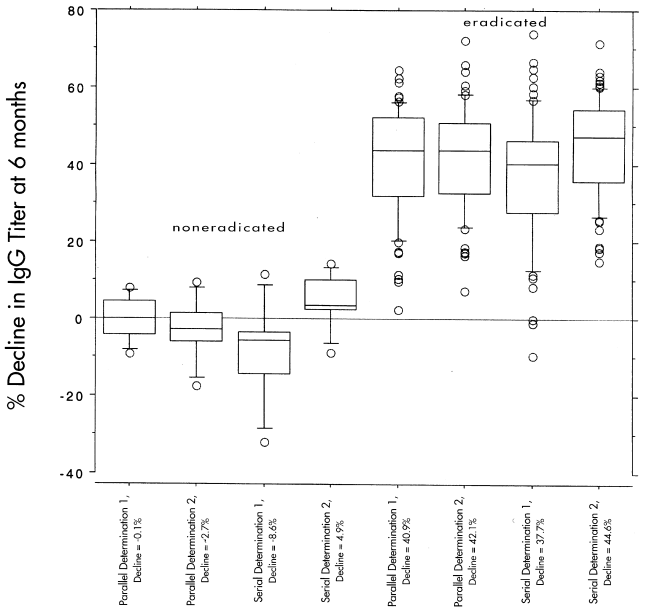

Figure 2. Decline in HM-CAP IgG titer in *H. pylori*-eradicated and -noneradicated groups.

is illustrated in Figure 2. There was no significant difference in mean decline in serial and parallel antibody determinations in the 68 evaluable subjects in whom *H. pylori* was eradicated ($p < 0.05$). In addition, the Pearson product-moment correlation of the mean decrease in antibody titer for serial compared to parallel antibody determinations in these subjects was 0.999.

Of the two subjects who were UBT-negative at 4 wk and positive at 6 months, one demonstrated a decline in antibody titer sufficient to indicate eradication of the organism, whereas the other demonstrated insufficient decline in antibody titer, indicating that the organism had not been eradicated. Two of three subjects who were UBT-positive at 4 wk and negative at 6 months demonstrated a decline in IgG antibody titer (serial or parallel) that was sufficient to confirm eradication of the infection.

DISCUSSION

Our results show that decreases in IgG titers 6 months after treatment is an accurate method of confirming eradication of *H. pylori* infection. Furthermore, this is independent of whether parallel or serial determinations on the HM-CAP EIA are performed.

The eradication success rate of 91% is consistent with

Table 2. Accuracy of a ≥25% Decline in HM-CAP IgG Antibody Titers by Kit Lot as Compared to the ^{14}C Urea Breath Test (UBT)

	Parallel Determination 1	Parallel Determination 2	Serial Determination 1	Serial Determination 2	Mean Overall
Sensitivity	86.8%	88.2%	80.9%	92.6%	87.1%
Specificity	100.0%	100.0%	100.0%	100.0%	100.0%
Accuracy	88.0%	89.3%	82.7%	93.3%	88.3%

previous reports for similar antibiotic regimens (20). In the 68 subjects who were clear eradicators by UBT, the mean sensitivity of a ≥25% decline in antibody titer for confirmation of eradication of infection was 87.1%. This sensitivity is comparable to that reported for serologies as diagnostic tests for primary infection and demonstrates that the HM-CAP assay is a sufficiently sensitive method for confirmation of successful eradication. Furthermore, the minimal difference in sensitivity observed for serial and parallel determinations demonstrates that determination of serial antibody titers is a practical alternative to parallel determinations, thus eliminating the need for storage and reassay of pretreatment samples.

In the group of seven subjects in whom therapy failed, the antibody titer at 6 months increased an average of 6.5%. Decline in IgG titers at 6 months was 100% specific in identifying those subjects in whom the infection persisted. This was true regardless of whether parallel or serial determinations were performed.

The variable decline in antibody titer observed in two subjects who were UBT-negative at 4 wk and positive at 6 months may reflect the variable rate of bacterial regrowth after incomplete clearance.

CONCLUSIONS

In summary, a ≥25% decline in IgG titer 6 months after therapy is a sufficiently sensitive and specific marker for eradication of *H. pylori* infection. In addition, serial and parallel determinations gave equivalent diagnostic accuracy. Follow-up endoscopy posttreatment is necessary in those patients with complicated ulcer disease in whom visual confirmation of ulcer healing is required, or in cases of gastric cancer or MALT lymphoma. In addition, those patients who remain symptomatic may require either an endoscopy or UBT testing before 6 months. Serological monitoring would not be useful in these patients. However, noninvasive tests to document eradication are more widely applicable. The UBT can be relatively expensive as compared with serology. In addition, the UBT may involve minimal exposure to radioactivity, making it inherently less acceptable to some patients, despite the documented safety of the test. The accuracy of the UBT is also affected by medications taken in the weeks immediately before testing (7–9). We suggest that serial determination of IgG decline in antibody titer at 6 months posttherapy is an accurate, practical, and cost-effective alternative to both UBT and endoscopy for confirmation of *H. pylori* eradication.

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Received Oct. 21, 1998; accepted Mar. 25, 1999.

REFERENCES

1. Blaser MJ. *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. J Infect Dis 1991;161:626–33.
2. National Institutes of Health Consensus Development Panel on *Helicobacter pylori* in Peptic Ulcer Disease. *Helicobacter pylori* in peptic ulcer disease. JAMA 1994;272:65–9.
3. Graham DY, Go MF. *Helicobacter pylori*: Current status. Gastroenterology 1993;105:279–82.
4. Parsonnet J, Friedman GD, Vandersteen DP, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–31.
5. Tompkins L, Falkow S. The new path to preventing ulcers. Science 1995;267:1621–2.
6. Malfertheiner P, O'Morain C, Michetti P. The Maastricht guidelines and other innovations. Curr Opin Gastroenterol 1997;13:1–7.
7. Atherton JC. Non-endoscopic tests in the diagnosis of *Helicobacter pylori* infection. Ailment Pharmacol Ther 1997;11: 11–20.
8. Cutler AF, Havstad S, Ma CK, et al. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 1995;109:136–41.
9. Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clin Microbiol Rev 1997;10:720–41.
10. Glupczynski Y, Goossens H, Burette A, et al. Serology in *Helicobacter pylori* infection. Zentralbl Bakteriol 1993;280: 150–4.
11. Marchildon PA, Ciota LM, Zamaniyan FZ, et al. Evaluation of three commercial enzyme immunoassays compared with the ^{13}C urea breath test for detection of *Helicobacter pylori* infection. J Clin Microbiol 1996;34:1147–52.
12. Chong SKF, Lou Q, Asnicar MA, et al. *Helicobacter pylori* infection in recurrent abdominal pain in childhood: Comparison of diagnostic tests and therapy. Pediatrics 1995;96:211–5.
13. Cutler AF, Prasad VM. Long-term follow-up of *Helicobacter pylori* serology after successful eradication. Am J Gastroenterol 1996;91:85–8.
14. Cutler A, Schubert A, Schubert T. Role of *Helicobacter pylori* serology in evaluating treatment success. Dig Dis Sci 1993; 38:2262–6.
15. Hirschl AM, Brandstatter G, Dragosics B, et al. Kinetics of specific IgG antibodies for monitoring the effect of anti-*Helicobacter pylori* chemotherapy. J Infect Dis 1993;168:763–6.
16. Kosunen TU, Seppala K, Sarna S, et al. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of *Helicobacter pylori*. Lancet 1992;339:893–5.
17. Morris AJ, Ali MR, Nicholson GI, et al. Long-term follow-up of voluntary ingestion of *Helicobacter pylori*. Ann Intern Med 1991;114:662–3.
18. Newell DG, Bell GD, Weil J, et al. The effect of treatment on circulating anti-*Helicobacter pylori* antibodies—A two-year follow-up study. In: Malfertheiner P, Ditchuneit H, eds. *Helicobacter pylori*, gastritis and peptic ulcer. Berlin: Springer-Verlag, 1990:172–5.
19. Veenendaal RA, Pena AS, Meijer JL, et al. Long term serological surveillance after treatment of *Helicobacter pylori* infection. Gut 1991;32:1291–4.
20. Unge P, Berstad A. Pooled analysis of anti-*Helicobacter pylori* treatment regimens. Scand J Gastroenterol 1996;220 (suppl):27–40.