Brief Report

Role of Seroconversion in Confirming Cure of *Helicobacter pylori* Infection

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Context.—The role of serologic testing to confirm cure of *Helicobacter pylori* infection after antimicrobial therapy is not completely defined.

Objective.—To determine the utility of serologic testing in confirming cure of *H pylori* infection more than 1 year after therapy.

Design.—A prospective, before-after interventional trial.

Setting.—An outpatient clinical research laboratory in an academic, urban Veterans Affairs medical center.

Participants.—Twenty-three otherwise healthy men and women with active *H pylori* infection demonstrated by gastric biopsy and with positive *H pylori* serologic findings.

Intervention.—A 14-day course of bismuth, tetracycline, and metronidazole.

Main Outcome Measures.—Determination of IgG serum antibodies to *H pylori* at baseline, 1 month, 3 months, and approximately 18 months after completion of therapy compared with serial gastric mucosal biopsy specimens with stains for *H pylori* and for histologic examination as the criterion standard.

Results.—Fifteen (65%) of 23 subjects were cured of their *H pylori* infection as assessed by gastric biopsy, with elimination of gastritis; median antibody levels declined from 92.5 U/mL at baseline to undetectable levels at 18 months. The other 8 subjects (35%) were not cured and had persistent gastritis at 18 months; median antibody levels declined from 130.6 U/mL at baseline to 89.7 U/mL at 18 months. Sensitivity and specificity of seroconversion (from a positive to negative test result) in detecting cure of *H pylori* infection were 60% and 100%, respectively.

Conclusion.—Undetectable antibody levels beyond the first year of therapy accurately confirm cure of *H pylori* infection in initially seropositive healthy subjects, with reasonable sensitivity.

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IN 1994, a National Institutes of Health consensus conference recommended that patients with peptic ulcers who are infected with *Helicobacter pylori* should be treated with antimicrobial drugs.[1,2] Moreover, in the past few years more and more individuals infected with *H pylori* without ulcers are being treated.[3-5]

If there is a desire to confirm cure of *H pylori* infection early after therapy, urea breath testing or a gastric biopsy, obtained either endoscopically or nonendoscopically,[6] are required, since *H pylori* serum IgG antibody tests remain positive for many months after cure.[7] However, urea breath tests and gastric biopsy are time-consuming and expensive, and biopsy is invasive. If one waits 6 months after therapy and performs a quantitative serologic test on paired serum samples, a 20% or more reduction in serum IgG may be informative of cure.[7] While the sensitivity and specificity of this paired-quantitative serologic approach for confirming cure have been reported to be between 80% and 90%,[7] serum storage for prolonged periods is not practical. Furthermore, many laboratories do not report quantitative serum antibody levels, but instead report results qualitatively as "positive" or "negative."

Many physicians are interested in confirming cure (or persistence) of *H pylori* infection only on recurrence of symptoms, which may occur a year or more after antimicrobial therapy. Perhaps by then, enough time has elapsed for antibody titers to become undetectable in cured individuals, rendering *seroconversion* (defined in this context as a change from a positive to a negative serologic test result) clinically helpful. In one study, however, less than 50% of subjects who had been cured (as assessed by urea breath tests) seroconverted during the second year of therapy.[8] Nevertheless, only 15 patients were followed beyond 15 months in that study.[8] To further evaluate the role of late serologic testing in confirming cure, we measured *H pylori* serum antibodies and obtained gastric mucosal biopsy specimens in 23 men and women before and for approximately 18 months after completion of therapy with bismuth, tetracycline, and metronidazole. Using tissue stains of *H pylori* as the criterion standard, we calculated the sensitivity and specificity of *H pylori* seroconversion in confirming cure of *H pylori* infection.

Methods

Twenty-three adults (10 men and 13 women) with no history of peptic ulcer and no chronic upper gastrointestinal tract symptoms were enrolled in this prospective study during 1993 and 1994. They ranged in age from 25 to 85 years (median, 48 years; mean, 49 years). Thirteen were African American, 8 were white, 1 was Hispanic, and 1 was American Indian. Each had a positive baseline *H pylori* serum antibody test result (>20 optical density units per mL [U/mL]), using a fluorescence immunoassay to detect IgG antibodies to *H pylori* in serum (FIAX test kit, Bio Whittaker, Walkerville, Md; sensitivity and specificity, 99% and 97%, respectively[6]). Each subject also had a baseline gastric mucosal biopsy demonstrating *H pylori* organisms.[6,9] Studies were approved by an institutional review board. Informed written consent was obtained from each subject.

After baseline studies confirmed *H pylori* seropositivity and active infection on gastric biopsy, each subject was treated with a 2-week course of bismuth subsalicylate (524 mg 4 times daily), tetracycline (500 mg 4 times daily), and metronidazole (250 mg 4 times daily). One subject intolerant to tetracycline received amoxicillin (500 mg 4 times daily) instead. No antisecretory therapy was prescribed. Each subject was interviewed by telephone halfway through this 2-week course to encourage compliance and to record adverse effects. They were also interviewed at the end of the 2-week course of therapy.

One month, 3 months, and approximately 18 months (15-23 months) after completion of therapy, subjects returned to the laboratory for remeasurement of *H pylori* serum antibodies, for mucosal biopsies of the gastric body, and for mucosal biopsies of the gastric antrum (month 3 and month 18 only). Gastric mucosal bi-

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Table 1.—Mean (± SEM) Gastritis Severity Scores Before (Baseline) and 1 Month, 3 Months, and Approximately 18 Months After Antimicrobial Therapy in 15 Subjects*

	Baseline	1 Month	3 Months	18 Months
Cured				
Body (n = 15)	1.7 (± 0.3)	0.3 (± 0.2) (*P* = .01)	0.3 (± 0.2) (*P* = .01)	0 (*P* = .002)
Antrum (n = 15)†	2.6 (± 0.2)	Not done	0.2 (± 0.2) (*P* = .009)	0 (*P* = .003)
Not cured				
Body (n = 8)	1.8 (± 0.3)	0.9 (± 0.3)	1.8 (± 0.4)	2.1 (± 0.3)‡
Antrum (n = 8)§	3.0†	Not done	2.0 (± 0.3)	2.8 (± 0.2)

*P values in parentheses refer to comparisons with baseline by the Wilcoxon signed rank test.
†n = 7 at baseline (antral biopsy specimens were not routinely obtained at baseline) and n = 14 at 18 months (antral biopsy specimen revealed duodenal mucosa in 1 subject).
‡*P* = .02 vs month 1.
§n = 3 at baseline (antral biopsy specimens were not routinely obtained at baseline).

opsy specimens were obtained through a modified nasogastric tube under fluoroscopic guidance, a technique that does not require intravenous sedation or endoscopy.[6,9] Biopsy specimens were interpreted by one of us (E.L.) who was provided no clinical information. The presence or absence of stainable *H pylori* organisms using a hematoxylin and eosin stain was reported.[9] If there was a question as to the presence of *H pylori*, a Giemsa stain was also performed.[10] The presence or absence of chronic gastritis, using previously described criteria,[9] was also assessed and gastritis was graded in intensity as absent (score = 0), mild (score = 1), moderate (score = 2), or severe (score = 3). Serum samples were processed in "real time" (ie, they were run in the laboratory immediately after collection, rather than stored). Antibody levels were reported as positive (>20 U/mL), borderline (15-20 U/mL), or negative (undetectable, ie, <15 U/mL). The actual antibody concentration (U/mL) was also recorded for positive and borderline test results. Subjects whose serologic results changed from positive to negative were considered to have seroconverted.

Cure of *H pylori* infection was diagnosed when mucosal biopsy specimens from both the gastric body and the antrum no longer showed *H pylori* organisms 18 months after therapy. Persistent infection was diagnosed when *H pylori* organisms were still visible on gastric biopsy specimens 18 months after therapy.

Data were analyzed with Systat 6.0.1 (SPSS Inc, Chicago, Ill, 1996). We used the Wilcoxon signed rank test to compare mean gastritis scores before vs after therapy, and paired *t* tests to compare mean antibody concentrations; because *H pylori* antibody levels were not normally distributed, their square root was used. Two-sided *P* values less than .05 were considered significant.

Results

Helicobacter pylori infection was cured in 15 subjects (65%). The other 8 subjects (35%) still had *H pylori* infection approximately 18 months after antimicrobial therapy. None of these 8 subjects had had elimination of *H pylori* infection on earlier biopsy specimens and then became reinfected. Thus, *H pylori* infection in these 8 subjects was persistent, not recurrent.

Table 1 presents mean (± SEM) gastritis severity scores at baseline (body and, in some cases, antrum), month 1 (body), month 3 (body and antrum), and month 18 (body and antrum) in the 2 groups of subjects (cured vs not cured). Cure of *H pylori* infection was associated with significant declines in gastritis severity scores, with no gastritis in the gastric body or antrum in any of the 15 subjects at 18 months (*P* = .002 and .003 vs baseline, respectively). In the 8 subjects whose *H pylori* infection was not cured, there was nevertheless a 50% decrease in the mean gastritis severity score in the gastric body at month 1 (*P* = .06 vs baseline). However, by month 3, and at month 18, severity of gastritis in the gastric body in these 8 subjects with persistent infection increased to baseline scores (*P* = .06, month 3 vs month 1; *P* = .02, month 18 vs month 1). Antral gastritis severity was similar at baseline and at month 18 in these 8 subjects who were not cured of their infection.

Table 2 displays quantitative *H pylori* serum antibody levels in each of the 23 subjects, separated into those cured of their infection and those not cured, as well as the median antibody levels for the 2 groups. In the cured group, there was no change in serum antibody level at month 1, after which levels declined approximately 50% at month 3 (*P* = .03, month 3 vs month 1). Serum antibody levels at month 18 were significantly lower than at baseline, month 1, and month 3 (*P*<.005). Nine of 15 subjects whose *H pylori* infection had been cured had undetectable *H pylori* serum antibody levels at 18 months (ie, 60% had seroconverted). In the 6 remaining cured subjects, there was a significant decline in *H pylori* serum antibody levels at 18 months (*P*<.01, month 18 vs baseline).

There were no statistically significant changes in *H pylori* serum antibody levels in the 8 subjects whose *H pylori* gastritis had not been cured 18 months after therapy (subjects 16-23, Table 2). None of these 8 subjects seroconverted 18 months after therapy, although one (subject 20) had a borderline antibody titer.

Helicobacter pylori seroconversion from positive to undetectable levels (<15 U/mL) at 18 months had a sensitivity of 60% for diagnosing cure of *H pylori* infection and a specificity of 100% (Table 3). If the serum antibody cutoff was raised, the sensitivity increased but the specificity decreased, and at a cutoff of 100 U/mL the sensitivity was 100% but the specificity was only 37.5%. Serology at earlier times (ie, at 1 month and 3 months) was insensitive at low cutoff levels and nonspecific at high cutoff levels.

Comment

If a patient returns with recurrent symptoms after an attempt at eradication of *H pylori* infection, it is reasonable to ask whether or not the therapy has been successful. If therapy has been successful, other causes for symptoms will need to be considered, while if therapy has not been successful, retreatment may be appropriate. Largely from the work of Cutler et al,[7] it has been concluded that serology can be a sensitive method for detecting cure of *H pylori* infection, but only beyond the first 6 months after therapy and only if a baseline (before-therapy) serum sample can be stored and then sent to the laboratory along with an after-therapy sample. Using this "paired serum sample" approach, a 20% or greater decline in IgG antibody level has more than 80% sensitivity and specificity for detecting cure. However, this approach is not practical. Furthermore, many laboratories do not routinely report quantitative antibody levels, but instead report serologic results qualitatively as "positive" or "negative." In a follow-up study in the same subjects, Cutler and Prasad[8] reported that only 29% to 45% of cured patients (as assessed by urea breath tests) seroconverted from positive to negative test results during the second year after therapy. However, only 15 patients were followed beyond 15 months. Moreover, they did not report any patients with persistent gastritis beyond the first year, so that the specificity of seroconversion could not be determined.

Our study design differed from the studies of Cutler et al.[7,8] First, we treated healthy subjects, while they treated a more heterogeneous group of patients with duodenal ulcer, gastric ulcer, and nonulcer dyspepsia. Second, we used gastric histology with tissue stains for *H pylori* organisms to assess cure, while Cutler et al[13] used the C-urea breath test. While both techniques have high sensitivity for detecting cure, our use of biopsies allowed us to also confirm directly that cure was associated with elimination of gastritis (Table 1). Third, we studied not only individuals who were cured, but also

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Table 2.—Quantitative *Helicobacter pylori* Serum Antibody Levels (U/mL) in 23 Subjects

Subject No.	Baseline	1 Month	3 Months	18 Months
Cured				
1	106.1	257.0	166.7	Undetectable
2	92.5	78.6	56.9	24.8
3	129.1	149.8	42.0	Undetectable
4	69.5	57.0	54.5	20.9
5	171.9	146.8	39.2	Undetectable
6	184.1	99.5	49.8	Undetectable
7	657.2	286.0	275.3	40.5
8	50.0	66.1	25.8	Undetectable
9	438.2	156.7	143.1	51.0
10	84.8	470.8	413.7	50.1
11	84.6	60.6	56.5	Undetectable
12	250.1	195.8	253.6	98.9
13	50.7	29.3	26.2	Undetectable
14	40.7	30.2	22.9	Undetectable
15	27.1	24.3	39.4	Undetectable
Median	92.5	99.5	54.5	Undetectable
Not cured				
16	3741.0	2888.3	2584.0	85.5
17	80.6	32.7	487.8	175.3
18	1960.0	2018.0	815.0	567.2
19	72.9	126.6	93.2	58.4
20	27.2	50.1	38.9	15.0
21	110.1	81.4	154.9	195.0
22	151.0	240.1	113.7	93.9
23	153.4	112.6	121.3	49.0
Median	130.6	119.6	138.1	89.7

Table 3.—Sensitivity and Specificity of *Helicobacter pylori* Serum Antibody Levels 1 Month, 3 Months, and Approximately 18 Months After Therapy for Confirming Cure of *Helicobacter pylori* Infection

	1 Month		3 Months		18 Months	
Cutoff, U/mL	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %	Sensitivity, %	Specificity, %
<15 (undetectable)	0	100	0	100	60	100
<20 (undetectable or borderline)	0	100	0	100	60	87
<25	7	100	7	100	73	87
<55	20	75	53	88	93	75
<100	53	63	67	75	100	38

individuals with persistent gastritis after therapy, allowing us to calculate both sensitivity *and* specificity of seroconversion. Finally, we performed serologic tests in real time (as serum samples were collected) to simulate clinical practice, while Cutler et al stored serum samples and ran them together in a single assay. Both studies had a 2-week regimen using bismuth, tetracycline, and metronidazole and both used an IgG serologic test from the same manufacturer. Likewise, *H pylori* eradication rates in the 2 studies were similar (65% in ours and 57% in the study by Cutler et al[7,8]).

The major finding of our study was that cure of *H pylori* infection led not only to elimination of gastritis, but also to seroconversion in 60% of subjects approximately 18 months after completion of therapy. None of the 8 subjects whose *H pylori* infection persisted after antimicrobial therapy seroconverted or had resolution of gastritis. These data suggest that seroconversion beyond the first year of therapy may reliably reflect cure, although 40% of the subjects who were cured still had positive test results (ie, they had not seroconverted). In these subjects, who were cured but remained seropositive 18 months after therapy, IgG serum antibody levels nevertheless decreased by 41% to 94% from baseline (median decrease, 72%), raising the possibility that a greater than 40% late fall in serum antibody from baseline may be useful in assessing eradication, even if serum samples are run in real time. However, this possibility is unlikely to be the case, since 4 of our 8 subjects who were not cured nevertheless had greater than 40% decreases in serum IgG antibody levels from baseline after antimicrobial therapy (Table 2).

It is not clear why we found that seroconversion occurred in 60% of cured subjects, while Cutler and Prasad[8] found that seroconversion occurred in less than 50%. This difference could have been due to the markedly different patient populations or to chance. Our studies suggest that serology, the simplest of all tests to diagnose *H pylori* infection, could also be used to assess adequacy of therapy beyond the first year after treatment. During the first 3 months after treatment, however, serology was insensitive for confirming cure in this study, as in the earlier study by Cutler et al.[7]

For an individual more than 1 year beyond antimicrobial therapy, seroconversion is a reliable indicator of cure of *H pylori* infection and should probably be the test of first choice. A negative test result would preclude the need for a urea breath test or gastric biopsy in the majority of patients who are actually cured. Failure to seroconvert is nonspecific, necessitating a urea breath test or gastric biopsy to confirm cure or persistent infection. Because current regimens for eradicating *H pylori* infection are so effective (80%-90% success),[11,12] an even higher percentage of subjects will probably seroconvert beyond the first year than we observed in this study. Additional long-term studies of seroconversion rates with these newer regimens are needed.

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References

1. NIH Consensus Development Panel. *Helicobacter pylori* in peptic ulcer disease. *JAMA.* 1994;272:65-69.
2. Feldman M. The acid test: making clinical sense of the consensus conference on *Helicobacter pylori. JAMA.* 1994;272:70-71.
3. Lee J, O'Morain C. Who should be treated for *Helicobacter pylori* infection? *Gastroenterology.* 1997;113:S99-S106.
4. Howden CW. For what conditions is there evidence-based justification for treatment of *Helicobacter pylori* infection? *Gastroenterology.* 1997;113:S107-S112.
5. Graham DY. Can therapy ever be denied for *Helicobacter pylori* infection? *Gastroenterology.* 1997;113:S113-S117.
6. Cryer B, Lee E, Feldman M. Gastric mucosal biopsy via a nasogastric tube. *Gastrointest Endosc.* 1996;44:317-323.
7. Cutler A, Schubert A, Schubert T. Role of *Helicobacter pylori* serology in evaluating treatment success. *Dig Dis Sci.* 1993;38:2262-2266.
8. Cutler AF, Prasad VM. Long-term follow-up of *Helicobacter pylori* serology after successful eradication. *Am J Gastroenterol.* 1996;91:85-88.
9. Feldman M, Cryer B, McArthur KE, Huet BA, Lee E. Effects of aging and gastritis on gastric acid and pepsin secretion in humans: a prospective study. *Gastroenterology.* 1996;110:1043-1052.
10. Peterson WL, Lee E, Feldman M. Relationship between *Campylobacter pylori* and gastritis in healthy humans after administration of placebo or indomethacin. *Gastroenterology.* 1988;95:1185-1197.
11. Hopkins RJ. Current FDA-approved treatments for *Helicobacter pylori* and the FDA approval process. *Gastroenterology.* 1997;113:S126-S130.
12. Unge P. What other regimens are under investigation to treat *Helicobacter pylori* infection? *Gastroenterology.* 1997;113:S131-S148.

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