Evaluation of IgA and IgG serology for the detection of *Helicobacter pylori* infection

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CA FALLONE, GE WILD, CA GORESKY, AN BARKUN. Evaluation of IgA and IgG serology for the detection of *Helicobacter pylori* infection. Can J Gastroenterol 1995;9(2):105-111.

BACKGROUND: Serology is a safe, simple and noninvasive means of determining the presence of *Helicobacter pylori*. Reported sensitivity and specificity, however, have varied tremendously, and it is not clear whether positive serology reflects previous infection (treated within the past one to two years) or ongoing infection.

OBJECTIVES: To assess the usefulness of two commercial kits to detect both ongoing *H pylori* infection and eradication. The kits, Pyloriset EIA-A and EIA-G, use enzyme immunoassay techniques to estimate immunoglobulin (Ig) A and IgG antibody titres, respectively.

PATIENTS AND METHODS: Consenting adult patients referred for upper endoscopy underwent antral biopsies for histology and a serum sample for serological testing. The serum samples were divided into three groups: group 1 – 17 samples from patients with *H pylori* detected histologically; group 2 – 13 samples from patients with negative histology and no recent (less than three months) eradication therapy; and group 3 – six samples from patients in whom *H pylori* had been successfully eradicated one month before serological testing. The ability of IgA and IgG serology to differentiate between the presence or absence of *H pylori* infection was assessed using contingency table analysis. Ideal titre cut-offs were determined using receiver operating characteristic curve analysis.

RESULTS: The ideal cut-off titres for IgA and IgG were 300 and 900 U, respectively. The sensitivity for IgA was 82% (95% CI: 57-96), specificity 85% (55-98) and diagnostic accuracy 83% (65-94). For IgG, the sensitivity, specificity and diagnostic accuracy were 76% (95% CI: 50-93), 85% (55-98) and 80% (61-92), respectively. IgA levels one month posteradication (group 3) were significantly lower ($P<0.05$) than the titres from the *H pylori* positive patients (group 1), whereas IgG levels were not.

CONCLUSIONS: Serology is a simple, noninvasive method of *H pylori* detection exhibiting good diagnostic accuracy. The Pyloriset EIA-A assay has test performance characteristics similar to the Pyloriset EIA-G. At one month posteradication therapy IgA, but not IgG, detection may be a good method of assessing disappearance of *H pylori*. (*Pour résumé, voir page 106*)

Key Words: *Helicobacter pylori, Immunoglobulin A, Immunoglobulin G, Serology*

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Received for publication July 12, 1994. Accepted November 10, 1994

HELICOBACTER PYLORI HAS, IN A short period of time, become one of the most important gastrointestinal infections. Since its identification in the 1980s this organism has dramatically affected gastroenterology, and it is now felt to play major roles in the recurrence or pathogenesis of duodenal ulcer disease, chronic antral gastritis, gastric ulcer disease and, possibly, nonulcer dyspepsia (1-3). Patients with gastric adenocarcinoma have also been shown to exhibit a higher prevalence of *H pylori* infection (4-6). The importance of *H pylori* makes it imperative to develop a safe, noninvasive and simple method of detection.

Histological staining and microbiological culture of antral tissue remain the gold standards for *H pylori* detection (1,7). Unfortunately, both these methods require gastroscopy, and hence are expensive, time-consuming and not without complications. The biopsy urease or campylobacter-like organism (CLO) test requires less time because the results are available at gastroscopy (8,9). However, endoscopy is still necessary, with its associated disadvantages. Noninvasive methods of *H pylori* detection include serology and breath urea testing. Serological testing requires a few millilitres of serum compared with breath urea testing where ingestion of ^{13}C- or ^{14}C-labelled urea, followed by breath sampling 20 to 30 mins later to trap expired carbon dioxide, is needed. The breath tests also involve use of an expensive mass spectrometer with the ^{13}C method, and ra-

Mesure sérologique de l'IgA et de l'IgG dans le dépistage de l'infection à *Helicobacter pylori*

DONNÉES DE DÉPART : La sérologie est un moyen sûr, simple et non effractif de déterminer la présence de *Helicobacter pylori*. Les rapports sur le degré de sensibilité de spécificité ont toutefois varié considérablement et l'on ignore si une sérologie positive est le reflet d'une infection antérieure (traitée au cours des deux dernières années) ou d'une infection évolutive.
OBJECTIFS : Évaluer l'utilité de trousses commerciales de dépistage de l'infection évolutive à *H. pylori* et de son éradication. Les trousses, Pyloriset EIA-A et EIA-G, utilisent des méthodes immuno-enzymologiques pour mesurer les titres d'immunoglobuline (IgA et IgG respectivement).
PATIENTS ET MÉTHODES : Des patients adultes et consentants adressées pour une endoscopie des voies digestives supérieures ont subi des biopsies antrales à des fins histologiques ainsi que des prélèvements pour analyse sérologique. Les échantillons sériques ont été divisés en trois groupes : groupe 1 – 17 échantillons de patients atteints de *H. pylori* décelé à l'histologie; groupe 2 – 13 échantillons provenant de patients présentant une histologie négative et aucun traitement récent d'éradication (moins de trois mois); et groupe 3 – 6 échantillons de patients chez qui *H. pylori* avait été éradiqué avec succès un mois avant l'épreuve sérologique. La capacité des épreuves sérologiques sur l'IgA et l'IgG à différencier entre la présence ou l'absence de *H. pylori* a été évaluée à l'aide d'analyses par tables de contingence. Les points de clivage des titres idéaux ont été mesurés à l'aide de l'analyse des courbes de caractéristiques opérationnelles.
RÉSULTATS : Les titres de clivage idéaux pour l'IgA et l'IgG ont été estimés à 300 et 900 U respectivement. La sensibilité à l'égard de l'IgA a été de 82 % (IC 95 % : 57-96), la spécificité à 85 % (55-98) et la précision diagnostique à 83 % (65-94). Pour l'IgG, la sensibilité, la spécificité et la précision diagnostique ont été de 76 % (IC 95 % : 50-93), 85 % (55-98), et 80 % (61-92) respectivement. Les taux d'IgA un mois après l'éradication (groupe 3) étaient nettement inférieurs ($P<0,05$) aux titres mesurés chez les patients positifs à l'égard de *H. pylori* (groupe 1) contrairement au taux d'IgG.
CONCLUSIONS : La sérologie est une méthode simple non effractive de dépistage de *H. pylori*, qui s'accompagne d'une bonne précision diagnostique. Les épreuves Pyloriset EIA-A présentent des caractéristiques de rendement similaires à celles du Pyloriset EIA-G. Un mois après le traitement pour éradication, le dépistage de l'IgA, mais non pas de l'IgG, peut être une bonne méthode pour vérifier si *H. pylori* a été éliminé.

diation exposure (180 μGy for helicobacter infected patients, which is approximately equivalent to the exposure from a chest x-ray, and 70 μGy for uninfected patients) with the ^{14}C method (1,7,10).

Serology, however, also has its drawbacks. The sensitivity and specificity of this method of detection vary from 54 to 100% and from 29 to 100%, respectively (11-30). In addition, it is unknown whether a randomly obtained positive serology reflects an infection treated within the previous one to two years or ongoing infection because it is unclear how long the antibodies persist after eradication of the infection (31).

The aims of this study were to evaluate objectively two commercial kits for the detection of *H pylori* antibodies, to compare immunoglobulin (Ig) A versus IgG antibodies in terms of sensitivity and specificity for ongoing *H pylori* infection, to determine the optimal serum threshold values for each and to assess the usefulness of serology testing one month after *H pylori* eradication in patients who have not had pretreatment serology.

PATIENTS AND METHODS

Adult patients presenting for upper gastrointestinal endoscopy at the Montreal General Hospital between November 1992 and May 1993 who were not taking acid suppression medication or antibiotics were asked to participate in this prospective study. Informed consent was obtained in all patients in accordance with the guidelines set forth by the Institution's Ethics Review Board. Initially subjects underwent four antral biopsies for histological detection of *H pylori*. Part way through the study it became clear that two biopsies were sufficient for an accurate diagnosis (32), and all subsequent subjects underwent only two antral biopsies. A serum sample was also drawn for serological testing at the time of endoscopy. There were 27 sets of samples from such patients. Nine additional samples were drawn from patients who had completed treatment for *H pylori* infection one month before serological sampling. These patients had not undergone serological testing before treatment. Eradication was successful in six of these nine patients, all of whom had been positive for *H pylori* on histology in the past, had had duodenal ulcers and had undergone different eradication treatment regimens for *H pylori*. Successful eradication was defined as the absence of *H pylori* organisms on histology three months after termination of treatment.

IgG and IgA antibody levels were measured from the serum samples by following the directions provided in two commercial kits: Pyloriset EIA-G and Pyloriset EIA-A (Orion Diagnostica, Espoo, Finland). These kits use enzyme immunoassay techniques to identify antibodies directed at the acid glycine extract, which comprises a number of protein antigens, including the species-specific 120 kDa protein, the presumptive flagellar 61 to 62 kDa protein and the outer membrane 29 to 31 kDa protein, but only reduced amounts of the major cross-reactive 54 kDa protein (33). Briefly the ELISA involves dilution of the patient's serum (1 to 201 for IgG and 1 to 101 for IgA) with the provided serum dilution buffer, placement of this diluted solution into the provided *H pylori* antigen-coated wells and incubation for 60 mins. The wells are then washed, leaving only the serum antibodies that had bound to the antigen-coated wells. The provided enzyme conjugate, which binds to the antibody, is placed into the

wells, followed by another incubation period of 60 mins. The wells are washed again and incubated with the provided substrate solution, which reacts with the conjugate until the addition of the provided stopping solution 30 mins later. This reaction causes a colour change in the solution within the well, depending on the amount of conjugate present, and reflects the concentration of antibody within the serum. The amount of colour change is then determined objectively by measuring the absorbance of the well with an ELISA reader (EMAX Precision Microplate Reader, Molecular Devices, California) set at 405 nm. Control serum solutions of known *H pylori* titre, which are provided with the kit, are also run in an identical manner alongside the patient serum samples each time the ELISA reader is used. The serum sample titre is obtained by comparing the measured absorbance from a curve constructed using the control samples. It is suggested by the manufacturer that any titre 500 U or more be considered positive for the presence of *H pylori* antibodies, and less than 500 U be considered negative. The investigator performing the serology testing was blinded to the histological results.

Antral biopsies were interpreted by an experienced pathologist blinded to the serology results using hematoxylin-eosin stains with or without silver stains for *H pylori* detection. In one patient, the tissue biopsy was insufficient for diagnosis but culture happened to have been done and was positive; hence, this patient was included in the group of positive biopsy results.

Statistical analysis: All results are expressed as actual values or as mean ± SEM. Independent *t* tests, with Bonferroni adjustments, were used to assess statistical significance between groups. Test performances were analyzed with 2x2 tables. 95% CI were calculated for proportions reflecting test performance using the standard normal approximation of the binomial distribution. The serology threshold value able to differentiate between the presence or absence of *H pylori* infection was assessed by using receiver operating characteristic (ROC) curve analysis (34-36). A ROC curve displays the false positive rate on the x axis (1 – specificity), and the true positive rate on the y axis (sensitivity) for the varying test thresholds, thus plotting the performance of a diagnostic test (34,36). For both IgG and IgA serology, five possible cut-offs were chosen: 300, 500, 700 and 900 U for both IgA and IgG as well as 200 U for IgA and 1100 U for IgG. The ideal cut-off values for IgA and IgG titre were chosen by determining the point lying geometrically closest to an ideal test with 100% specificity and sensitivity (the upper left corner of the graph) (35,36). A modified maximum likelihood program was used to compare the area under the fitted curve for the IgA serological test, which represents test performance, with the area beneath the curve for the IgG test (37,38). These comparisons were performed (where appropriate) with matched analysis.

TABLE 1
Endoscopic diagnosis of patients used in this study

Diagnosis	n	Histology	
		Positive (%)	Negative (%)
Duodenal ulcer disease			
No eradication therapy in three months preceding serology	14	13 (93)	1 (7)
Eradication therapy one month before serology	9	3 (33)	6 (67)†
Normal endoscopy (indications)			
Dyspepsia/pain	3	1 (33)	2 (67)
Pregastric bypass for obesity	1	0 (0)	1 (100)
Obstructive jaundice (endoscopic retrograde cholangiopancreatography)	2	0 (0)	2 (100)
Chronic diarrhea	2	0 (0)	2 (100)
Follow-up of duodenal ulcer treated previously (more than three months) with eradication therapy	3	0 (0)	3 (100)
Nonsteroidal anti-inflammatory drug gastropathy	1	0 (0)	1 (100)
Acetylsalicylic acid versus reflux gastropathy	1	0 (0)	1 (100)
Total	36	17 (47)*	19 (53)

*These 17 patients comprise group 1; †These six patients comprise group 3 (the 13 remaining negative patients comprise group 2)

RESULTS

Patient population: Thirty-six biopsies and serum samples were analyzed. The endoscopic diagnoses of these patients are shown in Table 1. The majority of the patients included in the study had duodenal ulcer disease. Ninety-three per cent of duodenal ulcer patients who had not received eradication therapy within the preceding three months were positive for *H pylori* on histology. Successful eradication of *H pylori* was noted in 67% of patients who had undergone eradication therapy with different regimens. Eleven subjects had a normal upper gastrointestinal endoscopy which had been performed for indications listed in Table 1. Two patients underwent endoscopic retrograde cholangiopancreatography (ERCP) for obstructive jaundice. The stomach and duodenum of these patients were carefully examined prospectively with the duodenoscope, and biopsies of the antrum were taken during the ERCP.

Overall, the presence of *H pylori* was histologically detected in 17 biopsies (group 1). Nineteen samples were negative for this organism including six samples obtained from patients who had been successfully treated out of the nine who took anti-*H pylori* medication. Because the serum samples for serology were obtained only one month after termination of treatment, and it is not known whether the antibody titre would have dropped by that time, these six samples were analyzed as a group separate from the remaining 13 negative samples. Hence, there are three groups of serum samples for serology: the first group consists of 17 samples from patients with positive biopsies, including the three who failed a recent

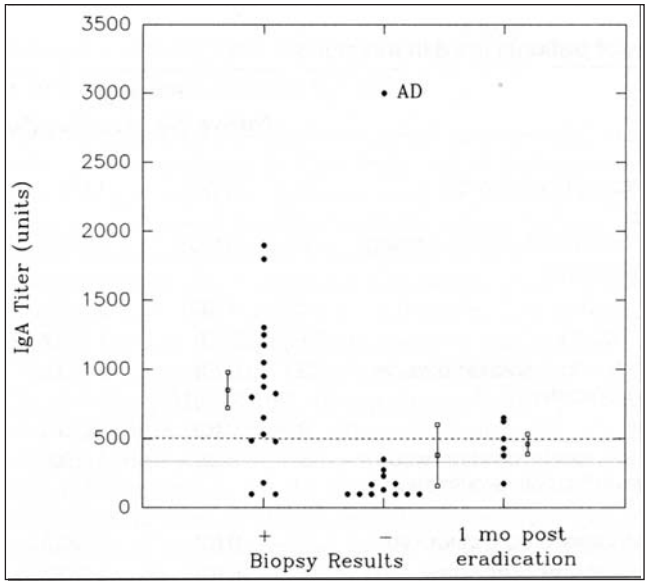

Figure 1) *Individual serology titres (filled circles) for immunoglobulin (Ig) A antibodies in patients with positive histology for Helicobacter pylori, negative histology for H pylori and one month after successful H pylori eradication. The mean titre for IgA antibodies in each group± SEM (□–□–□) is shown next to the group of individual titres. The dashed line represents the titre above which the manufacturer recommends the serology be considered positive for H pylori antibodies, ie, 500 U. The dotted line represents the 300 U cut-off. AD refers to the patient with the highest titre in the histologically negative group. The mean IgA titre was significantly lower (P<0.05) in the treated group compared with the positive group*

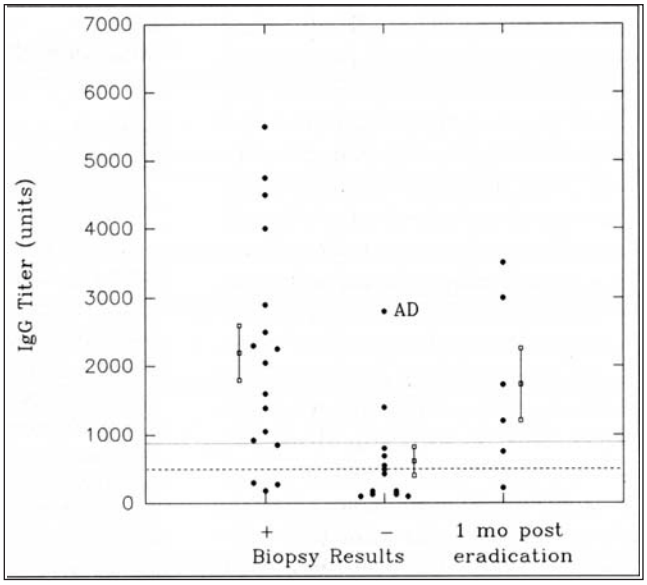

Figure 2) *Individual serology titres (filled circles) for immunoglobulin (Ig) G antibodies in patients with positive histology for Helicobacter pylori, negative histology for H pylori and one month after successful H pylori eradication. The mean titre for IgG antibodies in each group ± SEM (□–□–□) is shown next to the group of individual titres. The dashed line represents the titre above which the manufacturer recommends the serology be considered positive for H pylori antibodies, ie, 500 U. The dotted line represents the 900 U cut-off. AD refers to the patient with the highest titre in the histologically negative group. The mean IgG titre was significantly lower (P<0.01) in the histologically negative group compared with the positive group*

attempt at eradication therapy; the second comprises 13 samples from patients with negative histology and no recent (three months or less) eradication treatment; and the third group is six samples taken one month after successful eradication of *H pylori*.

IgA and IgG sensitivities and specificities: The IgA and IgG titre for each serum sample in the three groups are shown in Figures 1 and 2, respectively. Sensitivities and specificities of both serological tests, using histology as the gold standard, were then calculated by using the aforementioned five different cut-off limits. These values are plotted on the ROC curves shown in Figure 3. The aim in using these curves is to maximize the number of true positives and simultaneously to minimize the number of false positives. The ideal cut-off values for IgA and IgG titre were 300 and 900 U, respectively. As shown in Table 2, when an IgA titre cut-off of 300 U was used the sensitivity was 82% (95% CI: 57 to 96), specificity was 85% (55 to 98), negative predictive value was 79% (49 to 95), positive predictive value was 88% (62 to 98) and the diagnostic accuracy was 83% (65 to 94). If the 500 U cut-off was used, as suggested by the manufacturer, the sensitivity, specificity, negative predictive value, positive predictive value and diagnostic accuracy were 71% (95% CI: 44 to 90), 92% (64 to 99), 71% (44 to 90), 92% (64 to 99) and 80% (61 to 92), respectively. For IgG serology (Table 2), using the 900 U cut-off yielded a sensitivity of 76% (50 to 93), specificity of 85% (55 to 98), negative predictive value of 73% (45 to 92), positive predictive value of 87% (60 to 98) and diagnostic accuracy of 80% (61 to 92). If the 500 U cut-off was used, as suggested by the manufacturer, the sensitivity, specificity, negative predictive value, positive predictive value and diagnostic accuracy were 82% (95% CI: 57 to 96), 62% (32 to 86), 73% (39 to 94), 74% (49 to 91) and 73% (54 to 88), respectively. The performance of the IgG test was not significantly different from that of the IgA test (area under ROC curve was 0.85 versus 0.82 respectively, P=0.70) (Figure 3).

The mean IgA titre for the positive histology group was 850±129 U. The negative histology group had a lower IgA titre (379±220 U), but this trend did not achieve statistical significance (P=0.06, Figure 1). In this latter group there was a very high serological titre in one patient (labelled AD in Figures 1 and 2) found to be negative histologically. This was the only false positive IgA titre when the 500 U cut-off was used, and one of two false positives if the 300 U cut-off was used. The IgG titre had more false positives (five with the 500 U cut-off and two with the 900 U cut-off, Figure 2). The IgG titre from the negative histology group was significantly different from the positive histology group, with respective means of 612±210 and 2195±401 U (P<0.01).

Use of serology in assessing eradication one month post-treatment: Group 3 had a mean IgA titre of 459±72 U. This was significantly (P<0.05) lower than the positive histology group

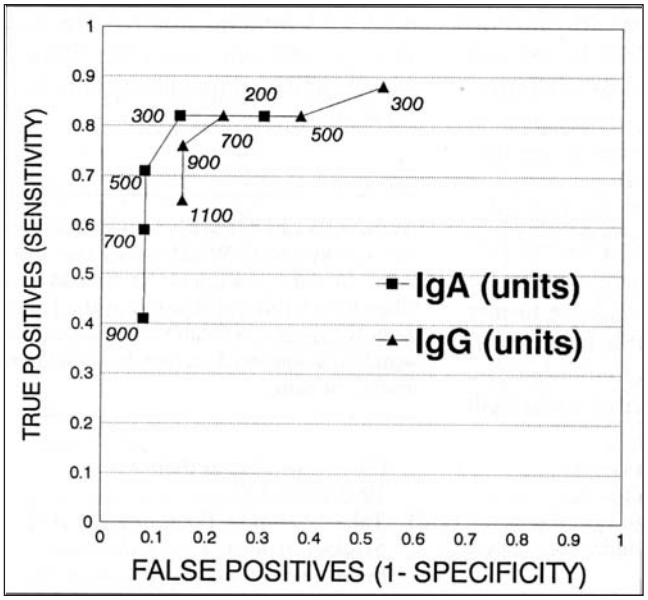

Figure 3) *Receiver operating characteristics (ROC) curve for immunoglobulin (Ig) A and IgG serology. The sensitivity and 1 – specificity of both IgA and IgG tests are plotted for five different cut-off values. The cut-off values are indicated with each point*

TABLE 2
Serology test performance

IgA

		Histology		
	units	+	–	
IgA serology	≥300	14	2	16
	<300	3	11	14
		17	13	30

IgG

		Histology		
	units	+	–	
IgG serology	≥900	13	2	15
	<900	4	11	15
		17	13	30

IgA serology: sensitivity = 14/17 = 0.82; specificity = 11/13 = 0.85; negative predictive value = 11/14 = 0.79; positive predictive value = 14/16 = 0.88; diagnostic accuracy = (14+11)/30 = 0.83. IgG serology: sensitivity = 13/17 = 0.76; specificity = 11/13 = 0.85; negative predictive value = 11/15 = 0.73; positive predictive value = 13/15 = 0.87; diagnostic accuracy = (13+11)/ 30 = 0.80

(mean IgA titre of 850±129 U), suggesting that this antibody might be helpful for follow-up of *H pylori* status as soon as one month after cessation of therapy (Figure 1). IgG titre of this group, in contrast, still remained high, with a mean of 1732±525 U (Figure 2), not significantly different from the positive histology group (mean IgG titre 2195±401 U).

DISCUSSION

The clinical importance of *H pylori* infection with regard to peptic ulcer disease is no longer doubted (1-3). It is important that a safe, simple, noninvasive and effective method of identifying this organism be easily available to clinicians. Serology is certainly safe, simple and noninvasive, and we have shown that it can discriminate between *H pylori* infected and noninfected patients. IgG titre was significantly lower in patients who were negative for *H pylori* on histology, and IgA titre just barely failed to show significance because of a small sample size with one outlying patient, who had the highest IgA and IgG titres in group 2. Perhaps this was a false negative histological study rather than two false positive serological studies. This patient had cirrhosis and nonulcer dyspepsia. If this patient is excluded, the difference in the IgA titre means between the histologically negative and positive groups become highly significant (P=0.00006). Despite this patient's results, the test performances were good, with IgA and IgG serology displaying similar sensitivity and specificity (82% and 76%, 85% and 85%, respectively). Also, although the data analyzed using mean IgA titres did not achieve statistical significance, the strength of the association in the 2x2 table (P=0.001, χ^2=10.72, df=1, odds ratio = 25.7 [95% CI: 2.8-308]) is strong enough to justify consideration of the use of IgA serology in the detection of *H pylori*.

We found that the best cut-off values in our population, which consisted of patients referred for upper endoscopy, were 300 U for IgA and 900 U for IgG. This is in contrast to the manufacturer's recommended cut-off of 500 U for both these tests. The reason for this discrepancy may result from the fact that the population studied by the manufacturer was different in age, ethnicity and endoscopic diagnosis (16).

One of the most useful indications for a noninvasive test of *H pylori* detection is to assess eradication after attempted treatment. The use of serology has always been questioned in this setting because of the uncertainty of antibody persistence after eradication. We have shown that the IgG titre remains elevated one month after successful eradication and is thus not useful to assess eradication one month after cessation of treatment (Figure 2). IgA titre, on the other hand, decreased significantly one month after cessation of successful treatment (Figure 1). Thus, the drop in antibody level may be an adequate indication of successful treatment. These findings are consistent with Gobert and colleagues' (17) study where IgM and IgA levels were found to have significantly decreased after six weeks of therapy. Vaira et al (39) and Veenendaal et al (40) also demonstrated significant declines in IgA titre after eradication. However, Kosunen et al (41) found that, although significant, the decrease in IgA titres was not as consistent as, and did not occur more rapidly than, that of IgG. The reason for this discrepancy is not entirely clear, but may be related to the differing diagnostic tests used in the studies. In all of these studies, the IgG titre showed a trend towards declining levels, but the decrease was not significant until 18 to 24 weeks after treatment. Thus, IgG levels only became a reliable indicator of successful treatment four to six months after treatment (18,41,42). IgA could therefore be a

more clinically useful test because seroconversion would be detected early on, and hence obviate the need for prolonged follow-up. Further study with a larger number of patients, followed pre- and posteradication, is required to determine the optimal IgA titre cut-off or percentage drop in titre necessary to identify successful eradication one month after treatment.

This study was not designed to determine the exact clinical role of serological testing compared with other diagnostic techniques in the detection of *H pylori*. However, serology is an effective method of making the diagnosis of ongoing *H pylori* infection if one uses the optimal cut-off values of 300 U for IgA titre and 900 U for IgG titre. The Pyloriset EIA-A assay has test performance characteristics similar to the Pyloriset EIA-G, and the former may be useful in establishing eradication as soon as one month after cessation of treatment. Further studies will need to determine whether serology alone is sufficient in certain clinical settings to determine subsequent clinical strategy.

ACKNOWLEDGEMENTS: The authors are indebted to C Wickham for her assistance in data collection. Dr Barkun is a Chercheur Clinicien Boursier of the Fonds de la Recherche en Santé du Québec. Research was supported, in part, by Kabi Pharmacia, Sweden.

REFERENCES

1. Peterson WL. *Helicobacter pylori* and peptic ulcer disease. N Engl J Med 1991;324:1043-8.
2. Graham DY, Go MF. *Helicobacter pylori*: current status. Gastroenterology 1993;105:279-82.
3. Soll AH. Pathogenesis of peptic ulcer and implications for therapy. N Engl J Med 1990;322:909-17.
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. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 1991;325:1132-6.
6. Hansson LE, Engstrand L, Nyren O, et al. *Helicobacter pylori* infection: independent risk indicator of gastric adenocarcinoma. Gastroenterology 1993;105:1098-103.
7. Goodwin CS, Blincow ED, Warren JR, Waters TE, Sanderson CR, Easton L. Evaluation of cultural techniques for isolating *Campylobacter pyloridis* from endoscopic biopsies of gastric mucosa. J Clin Pathol 1985;38:1127-31.
8. McNulty CAM, Dent JC, Uff JS, Gear MWL, Wilkinson SP. Detection of *Campylobacter pylori* by the biopsy urease test: an assessment in 1445 patients. Gut 1989;30:1058-62.
9. Thillainayagam AV, Arvind AS, Cook RS, et al. Diagnostic efficiency of an ultrarapid endoscopy room test for *Helicobacter pylori*. Gut 1991;32:467-9.
10. Marshall BJ, Surveyor I. Carbon-14 urea breath test for the diagnosis of *Campylobacter pylori* associated gastritis. J Nucl Med 1988;29:11-6.
11. Lin SK, Lambert JR, Schembri M, et al. A comparison of diagnostic tests to determine *Helicobacter pylori* infection. J Gastroenterol Hepatol 1992;7:203-9.
12. Logan RP, Polson RJ, Misiewicz JJ, et al. Simplified single sample 13-carbon urea breath test for *Helicobacter pylori*: Comparison with histology, culture and ELISA serology. Gut 1991;32:1461-4.
13. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JI. Evaluation of a commercial ELISA for serodiagnosis of *Helicobacter pylori* infection. J Clin Pathol 1991;44:326-8.
14. Taha AS, Boothman P, Nakshabendi I, et al. Diagnostic tests for *Helicobacter pylori*: Comparison and influence of non-steroidal anti-inflammatory drugs. J Clin Pathol 1992;45:709-12.
15. Aguirre PM, Pascual CY, Merino FJ, Velasco AC. Evaluation of two commercial enzyme immunoassays for the diagnosis of *Helicobacter pylori* infection. Eur J Clin Microbiol Infect Dis 1992;11:634-9.
16. Granberg C, Mansikka A, Lehtonen O, et al. Diagnosis of *Helicobacter pylori* infection by using Pyloriset EIA-G and EIA-A for detection of serum immunoglobulin G (IgG) and IgA antibodies. J Clin Microbiol 1993;31:1450-3.
17. Gobert B, Bene MC, deKorwin JD, Faure G. Isotype evolution in the follow up of patients with *Campylobacter pylori* associated gastritis. Gastroenterol Clin Biol 1989;13:880-3.
18. Thomas JE, Whatmore AM, Barer MR, Eastham EJ, Kehoe MA. Serodiagnosis of *Helicobacter pylori* infection in childhood. J Clin Microbiol 1990;28:2641-6.
19. Talley NJ, Kost L, Haddad A, Zinsmeister AR. Comparison of commercial serological tests for detection of *Helicobacter pylori* antibodies. J Clin Microbiol 1992;30:3146-50.
20. Debongnie JC, Durez P, Luyasu V. Validation et usage clinique et epidemiologique d'un test serologique commercialise dans le diagnostic de l'infection a *Helicobacter pylori*. Gastroenterol Clin Biol 1993;17:98-102.
21. Taha AS, Reid J, Boothmann P, et al. Serological diagnosis of *Helicobacter pylori* – evaluation of four tests in the presence or absence of non-steroidal anti-inflammatory drugs. Gut 1993;34:461-5.
22. Hoek FJ, Noach LA, Raws EAJ, Tytgat GNJ. Evaluation of the performance of commercial test kits for detection of *Helicobacter pylori* antibodies in serum. J Clin Microbiol 1992;30:1525-8.
23. Van den Oever HLA, Loffeld RJLF, Stobberingh ER. Usefulness of a new serological test (Bio-Rad) to diagnose *Helicobacter pylori* associated gastritis. J Clin Microbiol 1991;29:283-6.
24. Westblom TU, Madan E, Gudipati S, Midkiff BR, Czinn SJ. Diagnosis of *Helicobacter pylori* infection in adult and pediatric patients by using pylori set, a rapid latex agglutination test. J Clin Microbiol 1992;30:96-8.
25. Talley NJ, Newell DG, Ormand JE, et al. Serodiagnosis of *Helicobacter pylori*: comparison of enzyme linked immunosorbent assays. J Clin Microbiol 1991;29:1635-9.
26. Goosens H, Glupczynchi Y, Burette A, Van den Borre C, Butzler JP. Evaluation of a commercially available second generation immunoglobin G enzyme immunoassay for detection of *Helicobacter pylori* infection. J Clin Microbiol 1992;30:176-80.
27. Marchildon P, Ciota L, Smith J, Zamaniyan F, Peacoch J. Evaluation of three commercial EIAs for detection of *Helicobacter pylori* infection when compared to C13 urea breath test. Am J Gastroenterol 1994;89:A226.
28. Goodwin CS, Blincow E, Peterson G, et al. Enzyme-linked immunosorbent assay for *Campylobacter pyloridis*: correlation with presence of *C pyloridis* in the gastric mucosa. J Infect Dis 1987;155:488-94.
29. Befrits R, Granstrom M, Rylander M, Rubio C. *Helicobacter pylori* in 205 consecutive endoscopy patients. Scand J Infect Dis 1993;25:185-91.
30. Best LM, Veldhuyzen van Zanten SJ, Bezanson GS, Haldane DJ, Malatjalian

DA. Serological detection of *Helicobacter pylori* by a flow microsphere immunoflorescence assay. J Clin Microbiol 1992;30:2311-7.
31. Marshall BJ. *Helicobacter pylori*. Am J Gastroenterol 1994;89:S116-28.
32. Fallone CA, Mitchell A, Paterson WG. Minimizing the cost and discomfort of *Helicobacter pylori* (Hp) detection while maintaining diagnostic accuracy. Am J Gastroenterol 1994;89:A89.
33. Newell DG, Stacey A. Antigens for the serodiagnosis of *Campylobacter pylori* infections. Gastroenterol Clin Biol 1989;13:37B-41B.
34. McNeil BJ, Keeler E, Adelstein SJ. Primer on certain elements of medical decision making. N Engl J Med 1975;293:211-5.
35. Swets JA, Pickett RM. Evaluation of Diagnostic Systems: Methods From Signal Detection Theory. New York: Academic Press, 1982:64-5.
36. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983;148:839-43.
37. Metz CE, Kronman HB. Statistical significance tests for binomial ROC curves. J Math Psychol 1980;22:218-43.
38. Dorfman DD, Alf E. Maximum-likelihood estimation of parameters of signal-detection theory and determination of confidence intervals – rating-method data. J Math Psychol 1969;6:487-96.
39. Vaira D, Holton J, Cairns SR, et al. Antibody titers to *Campylobacter pylori* after treatment for gastritis. BMJ 1988;297:397.
40. Veenendaal RA, Pena AS, Meijer JL, et al. Long term serological surveillance after treatment of *Helicobacter pylori* infection. Gut 1991;32:1291-4.
41. Kosunen TU, Seppala K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titers after eradication of *Helicobacter pylori*. Lancet 1992;339:893-5.
42. Cutler A, Schubert A, Schubert T. Role of *Helicobacter pylori* serology in evaluating treatment success. Dig Dis Sci 1993;38:2262-6.