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

CARLO A FALLONE MD, GARY E WILD MD PHD, CARL A GORESKY MD PHD, ALAN N BARKUN MD

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 post eradication (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 post eradication 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
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.


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 effractif 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.
TABLE 1
Endoscopic diagnosis of patients used in this study

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

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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 \textit{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 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 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.
(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 historically 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, \( \chi^2=10.72, df=1, \text{odds ratio} = 25.7 \) [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

**TABLE 2** Serology test performance

<table>
<thead>
<tr>
<th>IgA</th>
<th>Histology</th>
<th>IgG</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td>+</td>
<td>–</td>
<td>units</td>
</tr>
<tr>
<td>≥300</td>
<td>14</td>
<td>2</td>
<td>≥900</td>
</tr>
<tr>
<td>&lt;300</td>
<td>3</td>
<td>11</td>
<td>&lt;900</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

IgA serology: sensitivity = 14/17 = 0.82; specificity = 11/13 = 0.85; positive predictive value = 11/14 = 0.79; 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.
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 post eradication, 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.

REFERENCES
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