Diagnosis of *Helicobacter pylori* infection by specific gastric mucosal IgA and IgG pylori antibodies

R A Veenendaal, J M Götz, V Schroijen, F Kurban, A T Bernards, M Veselic, A S Peña, C B H W Lamers

Abstract

Aims—To investigate the diagnostic value of mucosal IgA and IgG *Helicobacter pylori* antibodies.

Methods—The study population comprised 209 consecutive patients with severe dyspeptic complaints referred for upper gastrointestinal endoscopy. A positive culture or histological identification of *H pylori* in gastric biopsy specimens, or both, were used to confirm infection. Specific IgA and IgG *H pylori* antibodies were determined using a modified ELISA technique.

Results—Of the 209 patients, 137 were infected with *H pylori*. The diagnostic value of systemic IgA and IgG *H pylori* antibodies was confirmed. Systemic IgA antibodies had a sensitivity of 76-6% (95% confidence interval 69-5-83-7) and a specificity of 94-4% (89-1-99-7). The sensitivity and specificity for systemic IgG antibodies were, respectively, 97-1% (94-3-99-9) and 98-6% (95-9-100). A moderate but clinically important correlation was found between local and systemic IgA and IgG. Mucosal IgA *H pylori* antibodies had a sensitivity of 98-5% (96-5-100) and a specificity of 91-7% (85-3-98-1), while for IgG these figures were, respectively, 88-3% (82-9-93-7) and 98-6% (95-9-100). As a diagnostic test mucosal IgA *H pylori* antibodies were comparable with culture and histology. Conclusion—Determination of local IgA and IgG *H pylori* antibody levels is a highly sensitive and specific test for the diagnosis of *H pylori* infection.

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Keywords: ELISA, *Helicobacter pylori*, IgA, IgG, serology.

Ten years after the successful culture of *Helicobacter pylori*, major progress has been made in the understanding of the pathogenic importance of this bacterium. *H pylori* causes chronic active gastritis, is one of the most important pathogenic factors in peptic ulcer disease and may have a role in the pathogenesis of gastric carcinoma.

Several tests for diagnosing *H pylori* infection have been developed. These can be divided into direct tests, for which gastric biopsy specimens are required, and indirect tests which rely on serum or breath (13CO2, 14CO2) samples. Investigators are still polarised as to which test should be used for the diagnosis of *H pylori* infection. Proponents of indirect testing argue that upper gastrointestinal endoscopy is unnecessary and expensive, while for those in favour of direct tests the opportunity to detect macroscopic disease during endoscopy is paramount. In the present study we examined the diagnostic value of detecting local (direct) and systemic (indirect) IgA and IgG antibodies against *H pylori* using a modified in-house enzyme linked immunosorbent assay (ELISA) and compared this with culture and histology.

Methods

The study population comprised 209 consecutive patients (120 men and 89 women) with severe dyspeptic complaints who were referred for upper gastrointestinal endoscopy. Diagnostic tests for *H pylori* related gastritis were requested by the referring physician in all cases. Patients with an upper gastrointestinal malignancy and those taking antibiotics, bismuth preparations or omeprazole in the three months prior to the endoscopy were excluded.

After an overnight fast, an upper gastrointestinal endoscopy was performed in all patients and blood was obtained for serological tests. At endoscopy, biopsy specimens were taken from intact mucosa in the antrum 3-5 cm proximal to the pylorus. One biopsy specimen was set aside for culture of *H pylori* and was transported to the laboratory within two hours in a small sterile glass jar with a screw-cap in 2 ml sterile 0-9% NaCl. The biopsy specimen was cultured on blood agar (Blood Agar Base No 2, Oxoid CM 271, containing 5% sheep's blood) and Skirrow's medium, and cultures were incubated at 37°C in a nitrogen atmosphere containing 8% CO2 and 6% O2 for five days. The bacteria were identified as *H pylori* on the basis of their morphology, and oxidase, catalase, and urease production.

Four biopsy specimens, two from the anterior and two from the posterior wall, were obtained for histological assessment and measurement of local IgA and IgG *H pylori* antibodies. The
two specimens for histological examination were immediately fixed in 10% buffered formalin, embedded in paraffin wax and cut into 4 μm thick serial sections, which were stained with haematoxylin and eosin. The histological detection of Helicobacter-like organisms was based on the identification, by an experienced pathologist, of micro-organisms with appropriate morphology, location, and staining characteristics in the mucosal biopsy specimens. Additional staining techniques (Giemsa, Whartin–Starry) were used where necessary.

Specific IgA and IgG antibodies against H pylori were measured in serum and in snap frozen antral biopsy specimens using a modified ELISA.

Antigen was prepared for the ELISA as follows: a mixture of six pooled H pylori strains (whole bacteria) was sonicated for six minutes. The suspension was adjusted to a protein concentration of 3 mg/ml. The optimum concentration of reagents was determined by checker board titration as described elsewhere.12 Each well of a flat bottom polystyrene microtitre plate (Dynatech Laboratories, Chantilly, Virginia, USA; M129A) was coated with 100 μl antigen solution (10 μg suspension/ml carbonate buffer, pH 9-6, for IgA and the local IgA and IgG antibodies, and 1 μg suspension/ml for systemic IgG) and incubated overnight at room temperature. The plates were washed three times with phosphate buffered saline (PBS) (pH 7-5) containing 0-05% Tween 20.

Systemic specific IgA and IgG antibodies directed against H pylori were measured in serum, diluted 1 in 200 in PBS/Tween 20, by a modified ELISA technique using conjugates labelled with immunoperoxidase specific for human IgA and IgG. The following conditions were used to standardise antibody measurement: the mean (SD) values for absorbance of the standard reference serum were 0.5 (0.1) for IgA and 1.0 (0.1) for IgG. These values were used to correct the absorbance given by the serum samples under study.

The absorbance index (AI) was calculated from the mean of two readings of the optical density (OD) of the serum. The results were expressed as follows:

\[ AI = \frac{\text{Patient's OD} - \text{OD of blank reading}}{\text{Reference OD} - \text{OD of blank reading}} \]

Intra- and interassay variabilities were determined as described in detail elsewhere.12

Local specific IgA and IgG antibodies directed against H pylori were also measured using a modified ELISA technique.

Biopsy samples were weighed and homogenised (Braun Potter S) in 300 μl PBS/Tween 20 and then adjusted to 1 mg/100 μl and stored at −20°C. The samples were then diluted 1 in 100 in PBS/Tween 20 and 100 μl added to each well. Two reference samples (one positive and one negative) were also assayed. The plates were incubated at room temperature for 90 minutes and then washed three times in PBS/Tween 20. Diluted goat anti-human peroxidase (100 μl) was added to each well (Pasteur Institute, Paris, France; code no. 75051 diluted 1:1000 in PBS/Tween 20 for IgG and code no. 75041 diluted 1 in 2500 in PBS/Tween 20 for IgA). The plates were then incubated for 90 minutes at room temperature, washed and 100 μl substrate (0-40 mg/ml O-phenylenediaminedihydrochloride (Sigma, St Louis, Missouri, USA) containing 0.012% H2O2) was added. After 30 minutes the reaction was stopped with 50 μl 2.5 M H2SO4. The OD was measured at 492 nm on the Titertek Multispan plate reader (Flow Laboratories, Irvine, Scotland, UK).

Before the data were analysed, the protein concentration (Lowry) of each biopsy homogenate was determined. To standardise the measurement of these antibodies, one positive serum (diluted 1 in 500) was tested more than 10 times in duplicate and the mean OD was used as a standard reference.

The results were expressed as follows:

\[ AI = \frac{\text{mean OD reading (n=2) of biopsy homogenate}}{\text{mean OD of blank reading (n=10)}} \]

\[ \times \left( \frac{\text{protein conc. of the homogenate}}{10\%} \right) \]

Intra-assay variability for specific IgA H pylori antibodies was determined by measuring 17 homogenates (five with a low titre, six with a moderate titre and six with a high titre) six times in one plate, which resulted in a coefficient of variation of 6-9%. Intra-assay variability for specific IgG H pylori antibodies was also determined by measuring 17 homogenates (nine with a low titre, four with a moderate titre and four with a high titre) six times in one plate, which resulted in a coefficient of variation of 10-0%.

Interassay variability was determined by measuring specific IgA and IgG H pylori antibodies in the same biopsy homogenates (in duplicate) over five different days which resulted in coefficients of variation of 12-1% and 15-9%, respectively.

STATISTICAL ANALYSIS

The significance of differences between test results in H pylori positive and negative patients was assessed using the Student's t test. To correct for multiplicity a p value <0.01 was chosen as the criterion for statistical significance. The significance of differences between the single diagnostic tests used was assessed using the χ² test.

Results

Of the 209 patients included in the study, 72 (mean age (SD) 47 (15) years) were H pylori negative while 137 (mean age (SD) 51 (13) years) were positive for H pylori on culture and/or histology. The diagnostic findings at upper gastrointestinal endoscopy are summarised in table 1.

In H pylori positive patients specific local (mean (SEM) 140.3 (5.2) v 2.8 (0.5), p<0.01 and systemic (mean (SEM) 81.6 (4.5) v 21.2
Table 1  Diagnoses at upper gastrointestinal endoscopy in H pylori negative and H pylori positive patients with severe dyspeptic complaints

<table>
<thead>
<tr>
<th>Diagnosis at endoscopy</th>
<th>H pylori positive</th>
<th>H pylori negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abnormalities</td>
<td>45 (32-8%)</td>
<td>6 (59-7%)</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>48 (35-0%)</td>
<td>7 (1-4%)</td>
</tr>
<tr>
<td>Gastric ulcer</td>
<td>15 (10-9%)</td>
<td>2 (2-8%)</td>
</tr>
<tr>
<td>Reflux oesophagitis</td>
<td>13 (9-5%)</td>
<td>17 (23-4%)</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td>16 (11-7%)</td>
<td>9 (12-5%)</td>
</tr>
</tbody>
</table>

*p Associated with non-steroidal anti-inflammatory drug use.

Table 2  Differences between local and systemic specific IgA and IgG H pylori antibodies in H pylori positive and H pylori negative patients. Results given as mean (SEM)

<table>
<thead>
<tr>
<th>H pylori antibodies (AI x 100)</th>
<th>H pylori positive</th>
<th>H pylori negative</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local IgA</td>
<td>140 (3-2)</td>
<td>2-0 (5-9)</td>
<td>p&lt;0-01</td>
</tr>
<tr>
<td>Systemic IgA</td>
<td>81 (6-4)</td>
<td>2-1 (3-3)</td>
<td>p&lt;0-01</td>
</tr>
<tr>
<td>Local IgG</td>
<td>32 (3-2)</td>
<td>1-6 (0-3)</td>
<td>p&lt;0-01</td>
</tr>
<tr>
<td>Systemic IgG</td>
<td>76 (2-1)</td>
<td>13-9 (0-9)</td>
<td>p&lt;0-01</td>
</tr>
</tbody>
</table>

*p Student’s t test.

Figure 1  Mucosal IgA H pylori antibodies versus systemic IgA H pylori antibodies in H pylori positive (*) and negative (●) patients. Line: regression (r=0.029; p<0.01) line in H pylori positive patients. The dotted lines indicate the cut off levels used in both assays.

Figure 2  Mucosal IgG H pylori antibodies versus systemic IgG H pylori antibodies in H pylori positive (*) and negative (●) patients. Line: regression line (r=0.052; p<0.01) in H pylori positive patients. The dotted lines indicate the cut off levels used in both assays.

Discussion

Helicobacter pylori induces a specific local13-15 and systemic16,17 immune response with the development of mucosa associated lymphoid tissue,14,18,19 which is absent in normal stomach. The inflammatory changes in the gastric mucosa associated with this specific immune response are described as active chronic or chronic gastritis depending on the presence or

(3-3), p<0-01 IgA H pylori antibody levels (AI x 100) were significantly higher than those in H pylori negative patients (table 2). Specific local (mean (SEM) 32-3 (2-5) vs 1-6 (0-3), p<0-01) and systemic (mean (SEM) 76-2 (1-8) vs 13-9 (0-9), p<0-01) IgG H pylori antibody levels (AI x 100) were also significantly higher in H pylori positive patients (table 2). A weak but significant correlation (r=0-29, p<0-01) between local and systemic IgA H pylori antibody levels was found in H pylori positive patients (fig 1). A moderate but significant correlation between local and systemic antibody levels (r=0-52, p<0-01) was found for IgG H pylori antibodies (fig 2).

The normal range, for both systemic and local IgA and IgG H pylori antibodies, was defined as the 95% confidence interval of the mean absorbance indices (AI) in 49 H pylori negative patients without histological evidence of gastritis. Under the conditions of the ELISA technique described, local IgA H pylori antibodies with an AI>0-06 and systemic IgA H pylori antibodies with an AI>0-37 indicated a positive H pylori infection. Local IgG H pylori antibodies with an AI>0-08 and systemic IgG H pylori antibodies with an AI>0-32 were also regarded as indicative of a positive H pylori infection. Using these criteria, determination of mucosal IgA H pylori antibody levels falsely classified six patients as H pylori positive and two as H pylori negative. Systemic IgA H pylori antibodies falsely classified four patients as H pylori positive and 32 as H pylori negative. These findings resulted in a sensitivity and specificity of, respectively, 98-5% (95% confidence interval 96-5–100) and 91-7% (85-3–98-1) for mucosal IgA H pylori antibodies and, respectively, of 76-6% (69-5–83-7) and 94-4% (89-1–99-7) for systemic IgA H pylori antibodies. Mucosal IgG H pylori antibodies falsely classified one patient as H pylori positive and 16 as H pylori negative. Systemic IgG H pylori antibodies falsely classified one patient as H pylori positive and four as H pylori negative. These findings resulted in a sensitivity and specificity of, respectively, 88-3% (82-9–93-7) and 98-6% (95-9–100) for mucosal IgG H pylori antibodies and, respectively, of 97-1% (94-3–99-9) and 98-5% (95-9–100) for systemic IgG H pylori antibodies.

The results of each individual test, with regard to the H pylori status of the patients, are shown in table 3 and compared with results of culture, histology, and culture and histology. There was no significant difference (p = 0-13) among gastric mucosal IgA antibodies, culture, histology, and culture and histology for the diagnosis of H pylori infection.
absence of leucocytes and lymphocytes infiltrating the gastric mucosa. These inflammatory changes are probably directly or indirectly responsible for functional derangements in the gastric physiology and mucosal defence mechanisms leading to peptic ulcer disease. Although the specific humoral immune response in most patients apparently does not lead to elimination of the bacterium from the gastric mucosa, it has successfully been used to diagnose H pylori infection serologically using systemic circulating specific IgA and IgG H pylori antibodies.12,14 Besides serological detection of H pylori infection H pylori antibodies have also successfully been used for follow up25-24 and in epidemiological studies.9 25 In the present study we confirmed the high sensitivity (97-1%) and specificity (98-6%) of systemic IgG H pylori antibodies which correlated significantly with mucosal IgG antibodies. Although systemic IgA H pylori antibodies were highly specific (94-4%), they were less sensitive for detecting H pylori infection (76-6%), indicating that the predominant local production of IgA does not lead to high systemic IgA antibody levels in all patients (fig 1) rendering this test less suitable for diagnostic or epidemiological studies.

When compared with other more generally used non-invasive tests for diagnosing H pylori infection only the urea breath test26-27 has a sensitivity and specificity comparable with that of the IgG ELISA. The applicability of the breath test is, however, hampered by its requirement for equipment and its cost. With a large number of commercial kits for IgA and IgG H pylori antibodies becoming available it seems most likely that this non-invasive test will be more generally used. Direct tests can have low sensitivity28 and high inter- and intraobserver variation especially when the number of bacteria is reduced by suppressive treatment with bismuth preparations, antibiotics, or proton pump inhibitors.29 The direct test described herein may also facilitate follow up by demonstrating a decrease in the local production of H pylori antibodies. For oral vaccines against H pylori to be successful, the induction of a specific local IgA immune response is necessary. The level of local IgA H pylori antibodies necessary for preventing reinfection (after treatment) or conveying protection (after oral vaccination) has yet to be established. In conclusion, determination of gastric mucosal IgA and IgG H pylori antibody levels is highly sensitive and specific for the diagnosis of H pylori infection.

19 Genta RM, Hammer HW, Graham DY. Gastric lymphoid follicles in Helicobacter pylori infection: frequency, distribution, and response to triple therapy. Hum Pathol 1993;24:577-83.
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