RESEARCH NOTE

Assessment of the cag pathogenicity island status of Helicobacter pylori infections with serology and PCR
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ABSTRACT

The serological characterisation of Helicobacter pylori strains has been questioned, e.g., when the presence or absence of the cag pathogenicity island (PAI) is determined. This study compared CagA-reactive serum antibodies, assessed with immunoblot, with cag PAI status, as determined by PCR. CagA serology results were available for 101 individuals contributing 202 bacterial samples for cag PAI PCR. There was a high degree of correlation between the two methods (kappa coefficient, 0.82; 95% confidence interval, 0.68–0.97). Combined with suggested biological explanations for the discrepancies, this finding supports the application of well-evaluated serological assays in the assessment of the cag PAI status of H. pylori infections in clinical and epidemiological studies.

Keywords CagA, cag pathogenicity island, Helicobacter pylori, PCR, serology

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Helicobacter pylori infection is associated with peptic ulcer and gastric cancer, with the bacterial cag pathogenicity island (PAI) being a virulence factor associated with these conditions. The cag PAI encodes a type IV secretion apparatus that induces a vigorous host response and translocates the PAI-encoded CagA protein into epithelial cells [1]. In epidemiological and microbiological studies, H. pylori cag PAI status is frequently assessed either by the immune response to the immunodominant CagA or by detection of the cagA gene. Available serological tools to characterise infecting H. pylori strains have been questioned because of inadequate sensitivity, specificity and congruence [2–4]. The present study investigated the agreement between the occurrence of CagA-reactive antibodies, as assessed by immunoblot, and cag PAI status, as determined by PCR.

Family members from an epidemiological study were invited to contribute blood and undergo gastroscopy, followed by culture of H. pylori with standard methods from antrum and corpus biopsy specimens [5]. The study subjects were not patients, and hence clinical symptoms were not monitored systematically. The local ethics committee approved the study, and the participants gave informed consent. In 39 families, 104 individuals contributed 208 bacterial isolates that were found by molecular typing to correspond to 80 distinct strains [5].

The serological CagA response was assessed by immunoblot (HelicoBlot 2.0; Genelabs Diagnostics, Singapore). For each biopsy specimen, bacterial DNA was prepared from a pool of all colonies on the culture plate, and the presence or absence of the cag PAI was determined by two PCRs, followed by agarose gel electrophoresis and staining with ethidium bromide (Fig. 1). First, a fragment within the cagA gene was amplified with primers cagAsbraF 5’-ATGATGGCGTGATTTGT-3’ and cagAsbraR 5’-TTTTCAAGGGTCTTGGTTTGC-3’ [6]. Second, an empty-site PCR was performed with primers UpCagF 5’-ACCTTCAAGGGTCTTGGTTTGC-3’ and DownCagR 5’-TTGGCATGCGTTATTATTTTCAC-3’, which flanked the 40-kb cag PAI and yielded a product when the PAI was absent [7]. For the statistical analyses, a data set was created with independent observations, i.e., with each strain and individual being represented only once, by randomisation of isolates for which both serology and PCR results were available. The correlation between the
immunoblot and PCR assays was estimated with the kappa coefficient (SAS program v. 8.02; SAS Inc., Cary, NC, USA). Sensitivity and specificity were calculated with exact binomial 95% confidence intervals (CIs).

Of the 208 isolates, 202 were from individuals with available immunoblot results, of whom 174 also had PCR results. Randomisation yielded 66 independent observations, for which the correlation between the two assays had a kappa coefficient of 0.82 (95% CI 0.68–0.97) (Table 1). Disagreement between serology and PCR results was present in one direction only, namely CagA + serology and cag PAI– PCR. This finding could reflect limited immunoblot specificity (0.78; 95% CI 0.56–0.93) or limited PCR sensitivity (0.90; 95% CI 0.77–0.97) (Table 1). In the complete collection of 174 isolates, the same unidirectional disagreement was observed for all PCR- and serology-discordant isolates, with similar kappa, sensitivity and specificity point estimates as shown in Table 1.

It is unlikely that the observed discrepancy between the two methods resulted from a lack of PCR sensitivity, as the empty-site PCR yielded products of the expected size. The immunoblot used has been reported to have a specificity of 80–90% in determining the CagA status of an infection [2–4], although either bacterial cagA or CagA status was used as the reference, and the imperfections of these comparisons should be considered. The discordance could result from additional cag PAI+ clones that remained unsampled in the stomach. An H. pylori infection usually involves a single predominant strain, but strain variants that differ in cag PAI status may be relatively common [6–8]. Furthermore, CagA seropositivity could reflect a lingering immune response towards a CagA + infection that has been replaced by a CagA – infection [9,10].

In previous studies, individuals with cagA + or CagA + strains but CagA – serology have been described, corresponding to a sensitivity of 71–90% for the present immunoblot [2–4]. The cagA gene is linked closely to expression of the CagA protein [11], but mutation may render the secretion apparatus non-functional, thereby limiting exposure of CagA to the immune system. For example, a recent study found partial deletions in the cag PAI in ten of 66 isolates, but these often left an intact cagA gene [6]. It has also been proposed that a detectable CagA immune response could be hampered by host factors and CagA variability [2,8,12].

The anticipated result in the PCR assay was an amplified product in one reaction, with a negative result in the other (Fig. 1). Of the 208 isolates, 28 from individuals with CagA + immunoblots gave ambiguous PCR results, with amplification in both or neither of the reactions. Twenty-four isolates from 15 individuals yielded bands in both PCRs, suggesting that these samples contained a mixture of clones with and without the cag PAI [6–8]. Four isolates, from two individuals, yielded

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**Table 1. Correlation between serological CagA status and cag PAI PCR**

<table>
<thead>
<tr>
<th>Serology</th>
<th>PCR</th>
<th>Serology (with PCR as standard)</th>
<th>PCR (with serology as standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cag PAI</td>
<td>cag PAI</td>
<td>Kappa</td>
</tr>
<tr>
<td>CagA−</td>
<td>18</td>
<td>0</td>
<td>0.82 (0.68–0.97)</td>
</tr>
<tr>
<td>CagA+</td>
<td>5</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

95% confidence intervals are given in parentheses.
no PCR products in either reaction, indicating that the target sequences of the \textit{cagA} primers were mutated or deleted. The 28 isolates with ambiguous PCR results were excluded from the evaluation in Table 1. However, if they were included and regarded as \textit{cag PAI\textsuperscript{+}}, in accordance with the interpretations made above, the kappa coefficient was estimated as 0.80 (95\% CI 0.64–0.95), compared with 0.57 (95\% CI 0.40–0.75) if they were regarded as \textit{cag PAI\textsuperscript{−}}. The former value is essentially identical to that in Table 1, thereby supporting the original interpretations. The occurrence of such PCR ambiguities could be reduced by examining single-colony isolates, but this increases the risk of overlooking strain variants unless numerous colonies are included.

In conclusion, agreement between the results of tests to determine the serological CagA status and the \textit{cag PAI} PCR results of \textit{H. pylori} infections is satisfactory, considering the biological prerequisites. Genotypic approaches are required when the characteristics of individual bacterial clones are being studied. However, in the clinical and epidemiological settings, current and past infections of the whole gastric mucosa are typically of interest. Genotypic methods are then limited by the availability of biological specimens from sometimes asymptomatic individuals, and by the need to assess strain variants dispersed throughout the stomach. Despite previous advice against serology [4], the present data support the application of well-evaluated serological assays in clinical and epidemiological studies.

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REFERENCES


RESEARCH NOTE

A seroprevalence study of poliovirus antibody in the population of northern Greece

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ABSTRACT

This study assessed immunity to poliomyelitis in a representative sample of 1064 persons living in

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