The continuing story of peptic ulcer bleeding
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Chapter 5

Lack of accuracy of the non-invasive Helicobacter pylori Stool Antigen test in patients with gastroduodenal ulcer bleeding

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Abstract

Objectives: The antigen based stool assay has proven to be accurate in diagnosing *Helicobacter pylori* infection in dyspeptic patients. We evaluated the *H. pylori* antigen-based stool assay (HpSA) in patients with peptic ulcer bleeding.

Methods: Thirty-six patients with peptic ulcer bleeding underwent endoscopy and antral and corpus biopsy specimens were taken for rapid urease test, histology and culture. The first stool sample after admission was collected for the HpSA test. To evaluate cross-reaction with blood constituents, citrated blood samples from 10 healthy volunteers (nine *H. pylori* serology negative and one *H. pylori* serology positive) were assessed by the HpSA test.

Results: A total of 36 consecutive ulcer-bleeding patients (21 male) with a mean age of 69.5 years were included in the study. Using either culture or rapid urease test and histology as the “gold standard”, the sensitivity and specificity of the HpSA test was 100% and 52%, respectively. Citrated blood samples of three *H. pylori* negative and one *H. pylori* positive volunteer gave a positive result in the HpSA test, suggesting cross-reaction with blood constituents.

Conclusions: The HpSA test gave a high number of false positive results in patients with peptic ulcer bleeding, probably due to blood constituents cross reacting in the enzyme immunoassay. The HpSA test is not accurate for testing *H. pylori* infection in patients with peptic ulcer bleeding.
Introduction

Non-steroidal anti-inflammatory drug (NSAID) use and *Helicobacter pylori* (*H. pylori*) infection play an important, probably largely independent role, in the etiology of ulcer disease, and are both significant risk factors for developing ulcer bleeding. Eradication of *H. pylori* prevents ulcer recurrence and reduces the rate of recurrent peptic ulcer bleeding (PUB). Therefore, accurate diagnosis of the infection is essential in the management of PUB.

Combinations of (invasive) tests including rapid urease test (RUT), histology and culture or the urea breath test (UBT) are highly accurate in diagnosing *H. pylori* infection in peptic ulcer disease or dyspeptic patients. However, in patients with PUB the accuracy of invasive and non-invasive *H. pylori* tests is often disappointing. In addition, it might not be possible to take biopsies during emergency endoscopy, when patients are in critical conditions. UBT can be performed when patients resume oral feeding, however, most patients are on acid suppressive medication, which might lead to false negative UBT results. Recently, a non-invasive test to detect *H. pylori* antigen in stool specimens of *H. pylori* infected patients has been evaluated. The test was reliable, easy-to-use and inexpensive for diagnosing *H. pylori* infection, with a pre-treatment sensitivity of 92-94% and specificity of 92-93%.

In this study the *H. pylori* Stool Antigen (HpSA) test was evaluated in patients with PUB. In addition, the effect of the presence of blood on the performance of the antigen-based stool assay was evaluated.

Materials and Methods

Subjects
Consecutive patients, 18 years of age or older, with melena and or hematemesis due to gastroduodenal ulcer bleeding, who underwent an esophagogastroduodenoscopy, were eligible for the study. An ulcer was defined as a disruption in the mucosal integrity of > 3 mm with apparent depth. Endoscopic therapy was given at discretion of the endoscopist. Patients...
previously treated for *H. pylori* infection and patients with antibiotic use in the last four weeks or using proton pump inhibitor or H$_2$ receptor antagonist the last two weeks were excluded. Other exclusion criteria were previous gastric surgery, coagulopathy or other disorders that are contra-indications for endoscopy and/or biopsy sampling, upper gastro-intestinal bleeding caused by other lesions and discharge before collection of a feces sample. Data about age, sex, medical history, distribution of symptoms, drug history, gastric and/or duodenal lesion, and presence or absence of blood in the stomach were recorded. Informed consent was obtained from each patient before inclusion into the study.

**Diagnostic procedures of *H. pylori* infection**

During endoscopy one antral and one corpus biopsy were obtained for RUT (CLO test; *Campylobacter* like organism test, Delta West, Bentley, Australia). The samples were incubated at room temperature and monitored for color change at 1 hour, 24 and 36 hours. A CLO-test was considered positive with the appearance of an appropriate color change from yellow to red. Two antral and two corpus biopsy specimens were taken for routine histological examination, and for culture. Biopsy specimens used for bacterial culture were placed in 2 ml of normal saline at 4°C and then smeared on the surface of a chocolate agar plate (3% hemoglobin in GC agar base, Oxoid CM 367, Unipath, Basingstoke, England) and on a horse blood agar plate (7% defibrinated horse blood in Columbia agar base, Oxoid CM 331, Unipath) containing Skirrow supplement (Unipath). Isolates were identified as *H. pylori* by Gram stain morphology and by urease, oxidase and catalase positivity.

The biopsy specimens for histological examinations were fixed in 10% buffered formalin and routinely processed. Paraffin sections (5 μm) were cut and stained by hematoxylin and eosin and were blind and at random examined.

**HpSA test**

The first stool sample after admission was collected and stored at −20°C until the HpSA test was performed. Analysis of stool samples was carried out using a commercial available enzyme immunoassay, Premier Platinum HpSA™ (Meridian Diagnostics, Inc., Cincinnati, USA) and performed according to the manufacturer’s protocol. Using optical density measured at single wavelength of 450 nm (OD$_{450}$), the cut-off levels of the HpSA assay were OD$_{450} < 0.140$ for a negative result, $0.140 \leq$ OD$_{450} < 0.160$ OD as equivocal, and OD$_{450} \geq$
0.160 for a positive result, as recommended by the manufacturer. The HpSA test was performed without knowledge of the other test results.

**Definition of H. pylori infection**

A patient was considered to be infected with *H. pylori* if either culture was positive or if both histology and CLO test were positive.\(^6\)

**Healthy volunteers**

To study the effect of blood on the HpSA test, citrated blood was collected from 10 healthy volunteers after informed consent. To mimic the presence of blood in liquid stool samples often seen in patients with PUB, HpSA was performed on solely citrated blood (100 µl). In addition, one gram of a solid feces sample, which had previously been shown to be *H. pylori* negative by HpSA (OD\(_{450}\) = 0.059), was liquefied by mixing with 3.5 ml phosphate buffered saline (PBS). The HpSA test was performed on the liquefied stool sample (125 µl) mixed with an equal volume citrated blood. As controls 100 µl of citrated PBS and liquefied HpSA tested negative feces mixed with an equal volume of citrated PBS were tested by the HpSA.

**Serology**

IgG antibodies against *H. pylori* in the sera of ten volunteers were detected by enzyme linked immunosorbent assay (HM-CAP\(^\text{TM}\) ELISA Enteric Products, Inc. Stony Brook, NY). A cut off level of 1.8 U/l was used according to the manufacturer’s instructions.

**Statistical analyses**

Specificity, sensitivity, positive and negative predictive values and positive and negative likelihood ratios were calculated using the invasive tests as reference value (positive culture or positive histology and CLO-test). The Mann-Whitney test was used to compare groups.

**Results**

**Comparison of diagnostic tools for H. pylori infection in patients with PUB**

A total of 36 consecutive PUB patients (21 male, 15 female) with a mean age of 69.5 years (range 18-100) were included in the study. Duodenal ulcers were found in 56\(^{\circ}\)n and gastric ulcers in 44\(^{\circ}\)n as the origin of gastrointestinal bleeding. Fifty-six percent was using NSAIDs.
The CLO-test was missing in three patients (one with positive culture, one with both positive culture and histology and one with both negative culture and histology). In one patient the CLO-test became positive between 24 and 36 hours. Clear-cut HpSA results were obtained from 35 (97%) patients. One patient had an OD₄₅₀ of 0.147 at initial testing, re-testing showed a positive result. OD₄₅₀ 0.167.

Of 36 patients, 15 (42%) were *H. pylori* positive by culture or histology and CLO-test. With HpSA test the median OD₄₅₀ was 1.36 (range 0.18-3.50) and 0.16 (range 0.06-1.55) in the patients positive and negative for *H. pylori* respectively (p<0.01). Of the 21 *H. pylori* negative patients, the feces samples of 10 patients yielded a false positive HpSA test result with a median OD₄₅₀ of 0.21 (range 0.16-1.55). The performance of the different tests is shown in table 1 and 2.

**Table 1.** Comparison of different diagnostic test to assess *H. pylori* infection in 36 patients with peptic ulcer bleeding

<table>
<thead>
<tr>
<th>No. Patients</th>
<th>culture</th>
<th>histology</th>
<th>RUT</th>
<th>HpSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>NA</td>
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<tr>
<td>11</td>
<td>-</td>
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</tr>
</tbody>
</table>


Sensitivity of the HpSA test was high, 100%, whereas specificity was only 52%. Positive likelihood ratio was 2.1 and negative likelihood ratio was 0. There was no relationship between presence or absence of blood in the stomach and the result of the HpSA test. From the 25 patients with a positive HpSA test, 8 patients (of whom 5 were *H. pylori* positive according to the ‘gold standard’) had blood in the stomach and 17 patients (of whom 10 were
H. pylori positive according to the ‘gold standard’) did not have blood in the stomach (p=0.22).

Table 2. Performance of the Helicobacter pylori Stool antigen (HpSA) test using positive culture or both positive histology and rapid urease test as gold standard, in 36 patients with peptic ulcer bleeding.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(95% CI)</td>
<td>(95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HpSA</td>
<td>100</td>
<td>52</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>(100-100)</td>
<td>(31-73)</td>
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</tr>
</tbody>
</table>

PPV: Positive predictive value. NPV: negative predicting value. 95% CI: 95% Confidence Interval.

Effect of human blood on the performance of the HpSA test
Since 48% of the H. pylori negative patients were positive by the HpSA test, cross-reactivity with blood constituents in the antigen-based stool assay was anticipated and subsequently evaluated. Citrated blood from 10 healthy volunteers, of whom one was H. pylori positive by serology, was assessed by the HpSA. Of 10 blood samples, 4 were positive by HpSA, including the sample from the subject with a positive H. pylori serology. The OD$_{450}$ for all 4 samples was well above the cut-off level.

The OD$_{450}$ of the mixture of citrated blood and an equal volume of H. pylori antigen negative feces was significantly higher than that of the H. pylori antigen negative feces sample without citrated blood (mean OD$_{450}$ 0.091 vs 0.059, p<0.001). Citrated PBS without blood, as well as citrated PBS mixed with an equal volume of HpSA negative feces, remained negative with a similar mean OD$_{450}$.

Discussion

The results of this study show the limited accuracy of the antigen-based stool immunoassay to assess H. pylori infection in PUB patients. Taking the invasive tests as reference value, 48% of H. pylori negative patients had a positive HpSA test result (sensitivity 100% and specificity 52%). The unexpectedly high number of positive results can either be explained by improved
sensitivity (true positive) or false positive results. The latter hypothesis is supported by the results of experiments, in which feces samples mixed with blood were assessed by the HpSA.

The ‘gold standard’ for *H. pylori* infection, as suggested by the Maastricht Consensus Report 1997\(^{16}\), being positive culture or both a positive histological examination and a positive RUT test, might not be applicable in PUB patients.

The sensitivity of the RUT in ulcer bleeding is often rather low, ranging from 41% to 75%,\(^{11,17}\) which is usually explained by the buffering effects of blood and especially of albumin\(^{18}\). In our study only one patient had a positive histology with both negative RUT and culture. Considering this patient *H. pylori* positive, the specificity of the HpSA will be 55%. Several studies have also reported a low sensitivity of culture, ranging between 34-80%, and of histology, ranging between 33-77% in PUB patients.\(^{11-13}\) In one study, the sensitivity and specificity of the combination of invasive tests (RUT, histology and culture) were 49% (95% CI: 40-57) and 91% (95% CI: 83-99) respectively.\(^{13}\) In our study, there was a high agreement rate between histology and culture, suggesting a good performance of these tests.

The accuracy of the UBT is claimed to be high.\(^{9}\) This test can be done as soon as the patient resumes oral feeding. However, the use of antisecretory agents often leads to false negative UBT results.\(^{14,19}\) Furthermore, the UBT is expensive and requires specialized equipment. With sensitivity > 95%, serology seemed more sensitive than invasive tests for PUB patients.\(^{11}\) One of the problems of serology is that discrimination between actual or past infection is impossible.

The *H. pylori* Stool Antigen test could be an alternative to assess *H. pylori* infection. In patients with peptic ulcer disease or dyspeptic symptoms, the pre-treatment HpSA test seems accurate.\(^{4,15}\) The current recommendation of the Maastricht Consensus Report 2-2000 is to use the HpSA test for both pre- and post-treatment *H. pylori* testing as an alternative if endoscopy-based tests are not clinically indicated and the UBT is not available.\(^{20}\)

Up to now there is only little literature about the performance of the HpSA in PUB. In an article, published in Spanish, the HpSA test was evaluated in 32 patients with upper gastrointestinal bleeding. In this study the sensitivity and specificity of the HpSA were
respectively 95.6% and 33.3%. In agreement with our results a high number of false positive HpSA test results were found. However, the authors did not find an association between the test results and the presence or absence of melena and concluded, in contrast to our results, that the HpSA test was not influenced by the presence of blood.

Part of the false positive results found in our study could be due to a response to cross-reacting antigens in blood, as demonstrated in the experiment evaluating the effect of blood on the HpSA testing. Blood group antigens may cause this cross-reaction with blood constituents. It is known that *H. pylori* exhibit mimicry by expressing Lewis antigens. In faeces samples, cross-reaction of the enzyme immunoassay has also been described with antigens of other *Helicobacter* species such as *Helicobacter felis* and *Helicobacter acinonyx*.

In the present study the HpSA test was evaluated in only a limited number of PUB patients. Larger studies comparing the HpSA test with histology, culture, RUT, UBT and serology in PUB patients are necessary to confirm these data. Based on current findings we conclude that in patients with PUB, blood in the stool reduces the specificity of the HpSA probably due to constituents cross reacting in the enzyme immunoassay. It is obvious that in contrast to the advice for patients with peptic ulcer disease, the use of the HpSA for the assessment of *H. pylori* infection in patients with ulcer bleeding cannot be recommended. Use of HpSA to assess *H. pylori* infection in PUB patients will lead to frequent over-treatment in these patients.

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References


