Do procalcitonin and C-reactive protein levels have a place in the diagnosis and follow-up of *Helicobacter pylori* infections?

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The aims of this study were to determine the levels of procalcitonin (PCT) and C-reactive protein (CRP) in *Helicobacter pylori*-positive (HP⁺) patients diagnosed with duodenal and gastric ulcer and to evaluate the correlation of PCT and CRP levels with other invasive and non-invasive diagnostic methods for determination of *H. pylori* eradication in post-treatment follow-up. Thirty-five HP⁺ patients with dyspepsia were included in this study. Serum samples (5 ml) were collected at admission and after 24 h. Antimicrobial therapy (omeprazole, amoxycillin and clarithromycin) was given for 1 week to HP⁺ patients who were positive only by culture or by urease test plus pathology. After 1 month, serum samples (5 ml) were collected again and culture, urease and pathology investigations were performed on endoscopic samples. PCT and CRP levels were measured in the collected blood samples. Thirty-five *H. pylori*-negative (HP⁻) cases with dyspepsia, 38 cases with bacteraemia and 35 healthy blood donors were included in this study as control groups. The mean and minimum–maximum levels of PCT were 1.39 (0.25–6.75), 0.35 (0.12–0.71), 7.45 (0.68–51.5) and 0.40 (0.12–0.71) ng ml⁻¹ for the groups of HP⁺, HP⁻ and bacteraemia patients and healthy donors, respectively. Mean CRP levels were 1.00 (<0.5–8.11), 0.62 (<0.5–3.2), 11.5 (3.2–43.5) and 0.63 (<0.5–5.46) mg dl⁻¹ for the same groups. A statistically significant difference was found between HP⁺ patients and both HP⁻ cases and healthy blood donors for PCT levels, and higher PCT levels were found on admission in cases of bacteraemia than in the other groups (*P*, 0.05). PCT levels of HP⁺ cases decreased significantly (from 1.39 to 0.86) between admission and the post-treatment period (30 days); however, PCT levels remained higher than the cut-off value (0.5 ng ml⁻¹). Similar ranges of CRP levels were found over the same time-period. The sensitivity of PCT was found to be higher than that of CRP on admission, but the specificity of PCT was found to be lower than that of CRP on the day of admission (65 and 74 %, respectively). The sensitivity of PCT was the same as that of CRP for the post-treatment period, but specificity of PCT was higher than that of CRP for the post-treatment period (83 and 76 %, respectively). It was concluded that PCT and CRP are not very effective markers for *H. pylori* infection in primary diagnosis or in eradication follow-up after therapy when used in parallel with conventional diagnostic methods, even if there is a difference in PCT and CRP levels between HP⁺ and HP⁻ cases on admission.

**INTRODUCTION**

*Helicobacter pylori* has an important role in the pathogenesis of peptic ulcer disease, and can also have a role in the development of mucosa-associated lymphoid tissue lymphoma and gastric cancer (IARC Working Group, 1994). Invasive diagnostic methods (which require endoscopy) and non-invasive diagnostic methods (which do not) are available for the diagnosis of *H. pylori* infections. Each test has its own advantages in relation to convenience in use, sensitivity and

Abbreviations: CRP, C-reactive protein; HP⁺, *H. pylori*-positive; HP⁻, *H. pylori*-negative; NPV, negative predictive value; PCT, procalcitonin; PPV, positive predictive value.
specificity. For example, the urea breath-test is used for post-treatment follow-up (Logan, 1996) and studies of the detection of *H. pylori* stool antigen are of interest (Vaira et al., 1999). C-reactive protein (CRP) is an acute-phase reagent that originates from the liver. CRP has many clinical and biological effects and can be used for the diagnosis and follow-up of different inflammatory and traumatic processes (Le Moullec et al., 1984). Procalcitonin (PCT), a prohormone of calcitonin, is a polypeptide that consists of 116 aa and is released from the C cells in the thyroid gland of normal hosts during bacterial infections, particularly in sepsis. PCT is therefore useful for determining the diagnosis and prognosis of bacterial infections (Assicot et al., 1993).

The aim of this study was to determine the diagnostic value of PCT and CRP and their benefits in the eradication and post-treatment follow-up of *H. pylori* infections by comparing PCT and CRP levels in patients diagnosed with duodenal and gastric ulcer with clinical findings and laboratory results in gastroenterology clinics.

**METHODS**

**Patient and control groups**

Eighty patients (53 males and 27 females, aged 23–86) with dyspepsia, who attended gastroenterology clinics of two medical centres and were diagnosed with chronic gastritis and duodenal ulcer (by using four biopsy specimens taken from the antrum and corpus for the detection of *H. pylori*), were included in this study. Patients who had been treated with antibiotics during the 30 days preceding endoscopy or with bismuth compounds or proton pump inhibitors during the 4 months preceding endoscopy were excluded. Additional exclusion criteria were pregnancy or lactation, severe systemic illness, manifest clotting disorders or use of anticoagulants. Antimicrobial therapy (omeprazole, amoxicillin and clarithromycin) was given for 1 week to HP+ patients (n = 40), who were determined to be *H. pylori*-positive by culture only or by urease test plus pathology. After 1 month, serum samples (5 ml) of 35 HP+ patients were collected again and culture, urease and pathology investigations were performed on endoscopic samples. The other five HP+ patients were not followed-up after therapy and were excluded from the study. Thirty-five HP+ patients out of 80 patients with dyspepsia, 38 patients with bacteraemia and 35 healthy blood donors were used as control groups.

**HP+ cases.** *H. pylori* was judged to be present if culture only or histology plus culture were positive (Working Party of the European Helicobacter pylori Study Group, 1997).

**HP− cases.** These cases were those that did not meet the diagnostic criteria of *H. pylori* by culture, urease test or histology.

**Cases with bacteraemia.** Cases were defined as having true bacteraemia according to the criteria of Aranson & Bor (1987): growth in one culture or growth of a different micro-organism in one culture, contamination, growth of the same micro-organism in two of three consecutive cultures, real positivity. They were hospitalized with open-heart surgery in the intensive-care unit (surgical) of the Cardiology Institute or in one of the centres in this study.

**Healthy blood donors.** These were blood donors who had no gastric or duodenal symptoms and did not take any drugs related to these symptoms.

**Aetiology of duodenal and gastric ulcer (*H. pylori* diagnosis)**

**Culture.** Biopsy samples were inoculated on selective Columbia agar (Oxoid) plates, supplemented with sterile horse blood, vancomycin, nalidixic acid and trimethoprim and on non-selective blood and endo agar (Becton Dickinson). Plates were incubated at 37 °C in a microaerophilic atmosphere for 7 days. Identification was established by testing characteristic colonies for catalase, oxidase and urease production and Gram-stain morphology.

**Histopathology.** Staining with haematoxylin and eosin was performed routinely for histopathological diagnosis and biopsy samples were reviewed according to the Sydney classification by a single pathologist.

**Urease test in tissue.** A urease test kit (Pronto Dry) was used for the detection of urease activation caused by *H. pylori* from biopsy samples. A yellow colour that turned to red within 5 min indicated urease positivity. If the result was negative, the test was reassessed after 24 h.

**Collection of blood samples and analytical methods**

Serum samples (5 ml) were collected from 80 patients who complained of dyspeptic symptoms on admission and 24 h after admission. Serum samples (5 ml) were collected from 35 of 40 HP+ patients who were followed-up for 1 month after therapy with invasive test procedures, including endoscopy. Serum samples from 35 HP+ cases were collected only on admission and 24 h after admission. Blood samples of the 38 patients with bacteraemia were collected when definite diagnoses were made from clinical findings and laboratory results. Blood samples (with no anticoagulants) of 35 healthy blood donors were collected on admission. Serum samples were stored in the laboratory at −70 °C until the test was performed. For detection of PCT levels in serum, a monoclonal, immunoluminometric method (Lumitest PCT; BRAHMS Diagnostica) was used. In this method, which is specific to the PCT molecule, two different mAbs that bind to calcitonin and catacalcin are used. This test can detect levels as low as 0.1 ng ml−1, considering that internal evaluation levels are between 0.1 and 500 ng ml−1. The pathological limit is evaluated as >0.5 ng ml−1; this value is <0.5 ng ml−1 for healthy people (Meisner, 1996). For detection of CRP levels in serum, an immunoturbidimetric analyser (BIA; Behring Diagnostics) was utilized and a concentration of >0.5 mg dl−1 was evaluated as a pathological value (Behring Diagnostics, 1997). A Mann–Whitney U-test was used in the comparison of the evaluation of PCT and CRP values between patient and control groups on admission, by statistical analysis using the program STATE 5.0.

**RESULTS**

On endoscopy, 37 patients showed normal mucosa, 11 had active or scarred gastric or duodenal ulcer and 12 had gastric and duodenal erosions. *H. pylori* was detected in 40 of 80 cases by culture only or by urease test and histology. Forty cases were determined to be HP+. Thirty-five HP+ and 35 HP− cases (out of the total of 80) were included in this study. The remaining HP+ (5) and HP− (5) cases were excluded. The PCT levels of 35 HP+ cases on admission, given as mean (minimum–maximum), were 1.39 (0.25–6.75) ng ml−1. The pathological limit is evaluated as >0.5 ng ml−1; this value is <0.5 ng ml−1 for healthy people (Meisner, 1996). For detection of CRP levels in serum, an immunoturbidimetric analyser (BIA; Behring Diagnostics) was utilized and a concentration of >0.5 mg dl−1 was evaluated as a pathological value (Behring Diagnostics, 1997). A Mann–Whitney U-test was used in the comparison of the evaluation of PCT and CRP values between patient and control groups on admission, by statistical analysis using the program STATE 5.0.
(P < 0·05) was detected between the PCT levels of HP\(^+\) cases on admission, compared to HP\(^-\) cases and healthy blood donors. When PCT and CRP levels were compared in HP\(^+\) and bacteraemia patients, values in patients with bacteraemia were much higher than those in HP\(^+\) cases (P < 0·05). No significant difference (P > 0·05) was detected between the other groups.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of PCT were 57, 65, 76 and 66 %, respectively, on admission. The same parameters for CRP on admission were 30, 74, 75 and 44 %, respectively. The sensitivity, specificity, PPV and NPV of PCT and CRP (evaluated together) were 50, 60, 62 and 55 %, respectively. The same parameters were 60, 70, 72 and 75 %, respectively, when the two parameters were evaluated separately. PCT levels of HP\(^+\) cases increased slightly during the 24 h following admission, but decreased significantly after therapy. However, they remained higher than the cut-off value (0·5 ng ml\(^{-1}\)). Approximate CRP levels in each of the three periods (admission, after 24 h and post-therapy) were 1·00, 1·15 and 0·95 mg dl\(^{-1}\) (Table 1, Fig. 1).

Five HP\(^+\) cases continued to be positive, according to invasive diagnostic methods, after therapy. The sensitivity, specificity, PPV and NPV of PCT were 60, 83, 62 and 92 %, respectively, after therapy and the same parameters for CRP were 60, 76, 30 and 92 %, respectively (Table 2).

**DISCUSSION**

There has been interest in using PCT as a highly efficient, easy-to-use, less expensive marker for diagnostic and prognostic purposes in various bacterial infections, particularly in septicemia, as evidenced by the growing number of publications (Gramm  et  al.,  1995; Ugarte  et  al.,  1999; Kocazeybek et al., 2003). The amount of PCT induced depends on the infected organ, whether invasion is systemic or localized and the extent of inflammation. PCT levels can be as high as 1000 ng ml\(^{-1}\) in septicemia and can decrease to <5 ng ml\(^{-1}\) in localized and viral infections, as well as in non-infectious inflammation. CRP, an acute-phase reactant, increases during any inflammatory period, whether it is infectious or traumatic. It is also detected in high concentrations in cases where no bacterial infections exist. High concentrations of CRP were also found in polytraumatic cases. CRP levels remain high after recovery (Gramm et al., 1995; Ugarte et al., 1999; Kocazeybek et al., 2003). In this study, the serum levels of PCT and CRP in HP\(^+\) patients were found to be 1·39 ng ml\(^{-1}\) and 1·00 mg dl\(^{-1}\), respectively, on their admission to hospital. The PCT and CRP levels in serum obtained from these patients increased slightly over 24 h. However, PCT and CRP levels in serum obtained from HP\(^-\) patients were lower than those in HP\(^+\) cases. There was a significant difference between HP\(^+\) and HP\(^-\) cases in PCT levels.

**Table 1. PCT and CRP levels of patient and control groups**

PCT sensitivity for *H. pylori* diagnosis on admission, 57 %; specificity, 65 %; PPV, 76 %; NPV, 66 %. CRP parameters: 30, 74, 75 and 44 %, respectively. Abbreviations: min, minimum; max, maximum.

<table>
<thead>
<tr>
<th>Patient and control groups (n)</th>
<th>Time of blood sample</th>
<th>Mean [PCT] (min–max) (ng ml(^{-1}))</th>
<th>Mean [CRP] (min–max) (mg dl(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP(^+) (35)</td>
<td>Admission</td>
<td>1·39 (0·25–6·75)</td>
<td>1·00 (&lt;0·5–8·11)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>1·41 (0·14–7·71)</td>
<td>1·15 (&lt;0·5–7·5)</td>
</tr>
<tr>
<td></td>
<td>AT*</td>
<td>0·86 (0·19–4·85)</td>
<td>0·95 (&lt;0·5–4·2)</td>
</tr>
<tr>
<td>HP(^-) (35)</td>
<td>Admission</td>
<td>0·35 (0·12–0·71)</td>
<td>0·62 (&lt;0·5–3·2)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>0·38 (0·16–0·88)</td>
<td>0·59 (&lt;0·5–3·1)</td>
</tr>
<tr>
<td>Cases with bacteraemia (38)</td>
<td>On diagnosis</td>
<td>7·45 (6·8–51·5)</td>
<td>11·5 (3·2–43·5)</td>
</tr>
<tr>
<td>Healthy group (35)</td>
<td>On admission</td>
<td>0·40 (0·12–0·71)</td>
<td>0·63 (&lt;0·5–4·6)</td>
</tr>
</tbody>
</table>

Significance testing:
- HP\(^+\) × H:\(\times\) H\(^-\)  \(P < 0·05\)
- Bacteraemia × HP\(^+\)  \(P < 0·05\)
- HP\(^+\) × Healthy group  \(P < 0·05\)

\*AT, 30 days (after 1 week therapy).
Table 2. H. pylori-positive and -negative cases after therapy

Five positive cases were detected after therapy, according to the criteria for diagnosis of H. pylori. The pathological borderline was taken to be 0.5 mg d l−1.

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>HP+</th>
<th>HP−</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&gt;0.5</td>
<td>3</td>
<td>5</td>
<td>60</td>
<td>83</td>
<td>62</td>
<td>92</td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>2</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP:</td>
<td></td>
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<tr>
<td>&gt;0.5</td>
<td>3</td>
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<td>&lt;0.5</td>
<td>2</td>
<td>23</td>
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</table>

whereas no significant difference in CRP levels was detected between HP+ and HP− cases. There was also a significant difference in PCT levels between healthy blood donors and HP+ cases. Similarly, a significant difference was also found in PCT levels between the healthy control group and HP+ cases. Gastric and duodenal infection with H. pylori invasion gives a local organ pathology that originates from urease, cytotoxins, adhesins, heat-shock proteins, gastric immune response and environmental conditions (D’Elios et al., 1998). To date, no study has reported PCT and CRP levels in acute or generally chronic, non-bacteraemic clinical cases. Gendrel & Bohuon (2000) have shown that, in serial studies, serum PCT levels were lower in localized bacterial infections with negative blood cultures than in systemic bacterial infections with positive blood cultures. A study from France has demonstrated that a cut-off value of 1 ng ml−1 was detectable in localized infections. This was a useful parameter for indicating the severity of infection (Gendrel et al., 1999).

A similar result was also reported by investigators from Czechoslovakia (Jarešová et al., 1999). Soderquist et al. (1998) have shown that PCT was detectable in moderate levels in cases with closed, focused infections, such as infectious arthritis in adults. Kocazeybek et al. (2003) reported that PCT levels were 1.34 and 1.71 ng ml−1 in two out of 40 infective endocarditis cases, whereas PCT levels averaged 4.15 ng ml−1 in a non-infective endocarditis control group. Several reports have indicated that PCT levels vary between 0.5 and 2 ng ml−1 in localized, non-bacteraemic, bacterial infections in adults and children (Soderquist et al., 1998; Gendrel et al., 1999; Jarešová et al., 1999). PCT levels in serum from acute or chronic cases of H. pylori infection and cases of local organ invasion are in accord with the results of other studies. Conversely, CRP, which is considered to be a marker of acute, systemic inflammation, can be detected at a slightly higher level than the cut-off value in H. pylori infections; this is responsible for localized tissue damage. Therefore, we think that this result can be related to the suggestion that elevated CRP levels are proportional to the extent of tissue damage in general. However, Choussat et al. (2000) suggested that markers of inflammation, such as CRP, were not sufficient to detect infection after a study that determined a correlation between chronic H. pylori infection and coronary heart disease. These authors also emphasized that the characteristics of the micro-organism play an important role in inflammation that originates from chronic infection. In fact, unlike other Gram-negative bacteria (such as Escherichia coli and Salmonella), lipid A molecules in H. pylori strains do not have phosphate groups and they carry four-carbon fatty acids instead of six-carbon ones, increasing the number of carbon atoms. These differences cause a weak induction in proinflammatory cytokine release, particularly IL6 and IL8 from monocytes and neutrophils (Crabtree et al., 1994; Moran, 1998; Strömberg et al., 2003). Although PCT and CRP differ in terms of their release mechanisms from organelles, biosynthesis and stability in serum, their levels were detected to be low (near the cut-off value) in HP+ cases on admission. This result can be related to some known factors (such as characteristics of LPS in the H. pylori cell wall, the level of immune response in the gastroduodenal region and local characteristics of inflammation) and some other, currently unknown factors that may have a role in the bacteria–host relationship.

PCT sensitivity and specificity of dyspeptic HP+ cases were higher and lower, respectively, than CRP sensitivity and specificity, on the basis of 0.5 cut-off value for each of the two markers.

Our results differ from those of other studies, which demonstrated higher specificity and lower sensitivity for PCT than for CRP (Assicot et al., 1993; Ugarte et al., 1999; Lorrot et al., 2000; Blijlevens et al., 2000). In our study, sensitivity and specificity rose slightly when we considered the two indicators together (PCT and CRP) and decreased when we considered them separately in the diagnosis of H. pylori infections of dyspeptic patients. However, Ugarte et al. (1999) suggested that specificity was particularly raised when PCT and CRP were considered together in prediagnosis of bacterial infections of patients that were hospitalized in intensive-care units. In a similar study, Rothenburger et al. (1999) suggested that specificity was particularly raised when PCT and CRP were considered together in the determination of local and systemic infections after cardiac surgery in Germany. Kocazeybek et al. (2003) suggested that sensitivity and specificity for the diagnosis of infective endocarditis were raised when PCT and CRP were considered separately in a study of 50 patients with infective endocarditis. Tuncbilek et al. (2000) suggested that sensitivity and specificity were raised when PCT and CRP were considered separately in...
the diagnosis of 33 patients with serious bacterial infections and sepsis. Our results were in concordance with the results of Kocazeybek et al. (2003) and Tunçbilek et al. (2000); however, they were different from those reported by Ugarte et al. (1999) and Rothenburger et al. (1999).

The mean PCT level was detected to be higher than the cut-off value of 0.5 ng ml⁻¹; even so, the mean PCT level of HP⁺ cases after therapy decreased significantly when compared with PCT levels on admission. CRP levels on admission and after therapy were similar to each other. However, PCT declined to normal levels significantly faster than CRP in serious infections such as pneumonia, meningitis, pericarditis and infection-complicated cases after operation. It has been reported that this decrease in PCT levels can be an indicator of recovery. Conversely, CRP levels remained high during therapy and decreased gradually over a long period of time. In our study, PCT levels decreased with therapy, although they did not reach normal levels. There may be several reasons for this result. As we have detected in our work, it was thought that continuous PCT induction may occur in H. pylori-infected regions and this may be caused by the continuous presence of H. pylori in the infected regions, as a consequence of factors such as insufficient epithelial cytokine response in the gastroduodenal region and unresponsiveness to therapy in some cases (Vaira et al., 1999; Strömberg et al., 2003).

In some severe, systemic bacterial infections, PCT levels decrease with clinical recovery (Gendrel et al., 2000). Conversely to this result, in our study, PCT levels were detected to be approximately equal to or slightly higher than the cut-off value in some HP⁺ cases after therapy. It is probable that PCT levels are still detected in serum at around the cut-off level, despite eradication after therapy. This observation may be related to specific characteristics of H. pylori infections (local, duration of chronic inflammation, development of an immunopathogenesis base as a result of interactions in the host immune response with H. pylori virulence factors) in the gastroduodenal region.

It was concluded that PCT and CRP were not very effective markers for primary diagnosis, as well as eradication follow-up after therapy, when they were used in parallel with conventional diagnostic methods, even if there was difference in PCT levels as opposed to CRP levels between HP⁺ and HP⁻ cases on admission.

REFERENCES


