Phospholipase A₂, C-Reactive Protein, and White Blood Cell Count in the Diagnosis of Acute Appendicitis

Juha M. Gröroos,¹,⁵ Jari J. Forsström,² Kerttu Irjala,³ and Timo J. Nevalainen⁴

We compared the predictive value of determining group II phospholipase A₂ (PLA₂) in serum for diagnosing acute appendicitis with the predictive values of white blood cell count (WBC) and measurement of C-reactive protein (CRP). In this prospective study, we included 186 patients who were undergoing appendectomy after clinical diagnoses of acute appendicitis. The performance of each test was measured by receiver-operating characteristic curves. WBC was the test of choice in diagnosing uncomplicated acute appendicitis. However, in contrast to CRP and PLA₂, which increased in patients with protracted inflammation, there was not a concomitant increase in WBC. Therefore, especially CRP, but also PLA₂, were better indicators of appendiceal perforation or abscess formation than was WBC. Increased WBC, CRP, and PLA₂ values did not unequivocally corroborate the clinical suspicion of appendicitis, but if all three values were within normal limits, acute appendicitis could be excluded with a 100% predictive value. PLA₂ values showed a highly significant correlation with CRP but not with WBC values, which supports the view that PLA₂ represents an acute-phase reactant.

Indexing Terms: acute-phase reaction/infection/leukocytes/enzymes/immunoassays

Despite intensive research and discussion, the diagnosis of acute appendicitis is still difficult and remains perhaps the most common problem in clinical surgery. On the one hand, a normal appendix at appendectomy represents a misdiagnosis; on the other hand, a delayed diagnosis of appendicitis may lead to perforation and peritonitis. The accuracy of diagnosis of acute appendicitis has improved only marginally in recent decades. In spite of careful clinical, laboratory, and ultrasound examinations, the rates of removing nondiseased appendices and of appendiceal perforations remain at ~20% of all cases subjected to appendectomy after a clinical diagnosis of acute appendicitis (1, 2).

Phospholipase A₂ (PLA₂) is a lipolytic enzyme that hydrolyzes phospholipids into corresponding lysocompounds.⁹ Secretory PLA₂s are divided into two groups on the basis of the amino acid sequence of the enzyme (3). Recent developments in the field of PLA₂ determinations have been essential for understanding the roles of these two types of PLA₂s in various diseases. Group I PLA₂ originates from the pancreas and serves as a digestive enzyme; the cellular source of group II PLA₂ is unknown (4). PLA₂ is the rate-limiting enzyme in the synthesis of eicosanoids, and as such is considered to play a key role in the pathology of various inflammatory diseases, such as acute pancreatitis, septic shock, and multiple injuries involving infection, tissue damage, and inflammation (5–9). PLA₂ is regarded as a central mediator of intestinal injury in inflammatory (10) and ischemic (11) bowel diseases, and group II PLA₂ has been proposed to be an acute-phase reactant (12, 13). Statistically, there is a highly significant correlation between group II PLA₂ and C-reactive protein (CRP) concentrations in serum samples from critically ill surgical patients (9) and from patients with acute pancreatitis (8). In acute pancreatitis, the concentration of group II PLA₂ in serum was the most effective measure, even better than the CRP value, in separating patients with pulmonary and (or) renal insufficiency from those without these complications (14). In the present work we studied the predictive value of measuring the concentration of group II PLA₂ in diagnosing acute appendicitis and compared the result with the predictive values of white blood cell counts (WBC) and measurement of CRP.

Materials and Methods

In this prospective study we included 186 consecutive patients (98 males and 88 females, mean age 35 years, range 15–77 years) undergoing appendectomy after a clinical diagnosis of acute appendicitis at the University Central Hospital of Turku. Blood samples were obtained from the patients on admission to hospital. WBC was determined by an electronic cell counter (Coulter Counter T 890; Coulter Electronics, Hialeah, FL). The concentration of CRP in serum was measured by immuno-turbidimetry (Hitachi® 717; Hitachi, Tokyo, Japan). The concentration of group II PLA₂ in serum was measured by a time-resolved fluoroimmunoassay (15). The upper limits of the reference intervals for WBC, CRP, and PLA₂ were 9 × 10⁹/L, 10 mg/L, and 11 μg/L, respectively.

All the appendices removed were sent for histological examination, after which the patients were divided into the following groups according to the clinical, surgical, and histopathological findings: group 1 (n = 51; 19 females, 12 males), patients without appendicitis or other manifest inflammatory disease; group 2 (n = 116; 53 females, 63 males), patients with edematous appendici-
tis; group 3 (n = 29; 8 females, 21 males), patients with perforated appendix or appendiceal abscess; and group 4 (n = 10; 8 females, 2 males), patients with other than appendiceal inflammatory disease (urinary tract infection, diverticulitis, mesenteric lymphadenitis, Crohn disease, pelvic inflammatory disease).

The Spearman rank-order correlation was calculated between WBC, CRP, and PLA2. Because reporting only one value for sensitivity and specificity may result in misleading oversimplification of accuracy, we prefer to measure the clinical performance of the tests by using receiver-operating characteristic (ROC) curves (16). We used the quantitative ROC analysis method described by Beck and Schultz (17). The area under the curve is a measure that shows the probability that a randomly chosen patient in the test group and a randomly chosen patient in the control group are classified correctly. An optimal test gives a value of 1 and a useless test gives a value of 0.5.

This study was approved by the Ethical Committee of the Turku University Central Hospital.

Results
The mean (±SEM) values for WBC, CRP, and PLA2 in each group are shown in Table 1. The correlation between PLA2 and CRP values was statistically highly significant (r = 0.66, P = 0.0001), whereas no correlation was found between PLA2 and WBC (r = 0.09, P = 0.176) or between CRP and WBC (r = 0.02, P = 0.756) values.

The performance of the tests in different clinical situations is shown in Table 2. WBC had a good diagnostic value in differentiating between groups 1 and 2 (ROC 0.726, P < 0.0001) and between groups 1 and 3 (ROC 0.772, P < 0.0001). WBC had no value in differentiating between groups 2 and 3 (ROC 0.580, P = 0.405) or between groups 1 and 4 (ROC 0.605, P = 0.175). WBC showed a good diagnostic value between patients without appendicitis (groups 1 + 4) and patients with appendicitis (groups 2 + 3) (Fig. 1). The area under the curve was 0.730 (P < 0.0001) for all subjects and 0.750 (P < 0.0001) and 0.712 (P < 0.0001) for males and females, respectively.

The performance of CRP was good in differentiating between groups 1 and 3 (ROC 0.909, P < 0.0001), groups 1 and 4 (ROC 0.766, P = 0.0003), groups 2 and 3 (ROC 0.855, P < 0.0001), and groups 2 and 4 (ROC 0.710, P = 0.0006). However, CRP could not indicate a statistically significant difference between nonappendicitis and appendicitis, i.e., between groups 1 + 4 and groups 2 + 3 (ROC 0.591, P = 0.068) (Fig. 1).

PLA2 differentiated well between groups 1 and 3 (ROC 0.830, P < 0.0001), groups 1 and 4 (ROC 0.739, P = 0.020), and between groups 2 and 3 (ROC 0.745, P < 0.0001). However, PLA2 could not differentiate between nonappendicitis and appendicitis, i.e., between groups 1 + 4 and 2 + 3 (ROC 0.583, P = 0.110) (Fig. 1).

When the tests were compared with each other, WBC had significantly better diagnostic value than CRP (P = 0.025) or PLA2 (P = 0.015) in distinguishing patients with appendicitis from those without appendicitis. However, WBC was significantly less effective than CRP (P < 0.0001) and PLA2 (P = 0.023) in identifying patients with complicated appendicitis among all the patients with appendicitis. In this respect, CRP was also superior when compared with PLA2 (P = 0.006).

Table 1. WBC and concentrations of CRP and group II PLA2 in serum of patients undergoing appendectomy.

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC, ×10⁶/L</th>
<th>CRP, mg/L</th>
<th>PLA2, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>1</td>
<td>10.8 ± 0.8</td>
<td>22 ± 4</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>14.2 ± 0.3</td>
<td>33 ± 3</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>15.8 ± 0.9</td>
<td>109 ± 12</td>
<td>134 ± 25</td>
</tr>
<tr>
<td>4</td>
<td>12.1 ± 3.8</td>
<td>86 ± 27</td>
<td>92 ± 29</td>
</tr>
</tbody>
</table>

* See text for makeup of groups.

Table 2. Diagnostic values of WBC and concentrations of CRP and group II PLA2 in serum of patients undergoing appendectomy.

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC</th>
<th>CRP</th>
<th>PLA2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROC</td>
<td>SEM</td>
<td>ROC</td>
</tr>
<tr>
<td>1 vs 2</td>
<td>0.726</td>
<td>0.046</td>
<td>0.607</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>0.772</td>
<td>0.061</td>
<td>0.909</td>
</tr>
<tr>
<td>1 vs 4</td>
<td>0.605</td>
<td>0.126</td>
<td>0.766</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>0.580</td>
<td>0.059</td>
<td>0.855</td>
</tr>
<tr>
<td>2 vs 4</td>
<td>0.706</td>
<td>0.089</td>
<td>0.710</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>0.748</td>
<td>0.098</td>
<td>0.602</td>
</tr>
</tbody>
</table>

Results are expressed as the area under the ROC curve and respective SEM. Groups are as defined in text.

Fig. 1. ROC curves for PLA2, CRP, and WBC (Leuk) in distinguishing between patients in groups 1 + 4 (nonappendicitis) and those in groups 2 + 3 (appendicitis).

TPR, true positive rate; FPR, false positive rate.
When the performances of the three tests were combined, the most important finding was that all the patients with normal WBC, CRP, and PLA2 values (n = 10) belonged to the nonappendicitis group. Thus, although increased WBC, CRP, and PLA2 values could not effectively corroborate the clinical suspicion of acute appendicitis, values within normal limits excluded acute appendicitis with a 100% predictive value.

We calculated a multivariate logistic regression model, using PLA2, CRP, and WBC as independent variables. For this model, at the sensitivity level of 95%, the specificity was 44%. Using only WBC and CRP, we found the specificity to be 41% at the same sensitivity; however, this difference was not statistically significant.

Discussion

The diagnosis of acute appendicitis is a common problem in clinical surgery. The classic triad of a history compatible with appendicitis, pain at McBurney’s point, and leukocytosis has a diagnostic accuracy rate <60% and, even when the triad is combined with new radiologic techniques, the accuracy is no better than 90% (1, 2, 18). In females, the diagnostic accuracy may be as low as 60% in large series (1). In the present study, the diagnostic accuracy was 79% for the whole patient population, 87% for males, and 70% for females. These numbers are in good accordance with those reported earlier (1, 2, 18).

In our patient population, 10 patients had WBC, CRP, and PLA2 values all within normal limits. Interestingly, all 10 patients belonged to the nonappendicitis group (n = 41). In other words, at least one of the three laboratory values was above normal limits in all patients with appendicitis (n = 145). Thus, although increased WBC, CRP, and PLA2 values cannot effectively establish the diagnosis of acute appendicitis, values within normal limits may exclude it with a 100% predictive value. In our patients, 25% (10 of 41) of laparotomies would have been avoided by measuring WBC, CRP, and PLA2.

In this study, WBC was the best laboratory method for diagnosing uncomplicated acute appendicitis, supporting earlier results (2, 19). Previous and current results suggest that increased WBC may be the earliest laboratory test to indicate appendiceal inflammation. However, during protracted inflammation, WBC does not show a concomitant increase, in contrast to CRP and PLA2. Therefore, CRP and PLA2 proved to reflect better than WBC the degree of inflammation in and around the appendix. However, both CRP and PLA2 were usually within the normal range in patients with mild uncomplicated acute appendicitis and, thus, these values cannot be used for ruling out early acute appendicitis. A novel finding of the present study is that PLA2 increases in serum in acute appendicitis. However, like the increase in CRP, the increase in PLA2 was marked only after the inflammation was well established in and around the appendix. Thus, PLA2 was superior to WBC in measuring the degree of appendiceal inflammation but could not be used to rule out early acute appendicitis.

Recent advances in the methodology for determining different PLA2s in serum provide new opportunities to study the role of these enzymes in human diseases. Group II PLA2 participates in the inflammatory reaction, but its detailed role and its cellular source(s) are unknown (20). The amount of group II PLA2 is markedly increased in serum during infection and inflammation (9, 20), and the enzyme may function in defense against bacteria (21). In addition, PLA2 regulates eicosanoid production in inflammation (22). Crowl et al. (12) found that hepatoma cells in culture secrete group II PLA2 into the culture medium when stimulated by cytokines; subsequently, they proposed that PLA2 may represent an acute-phase reactant. In the current study, PLA2 values changed in concert and had a statistically highly significant correlation with CRP, the best-characterized marker of the acute-phase reaction, but not with WBC values.

In conclusion, WBC remains the best laboratory method for diagnosing uncomplicated acute appendicitis and seems to be a very early marker of appendiceal inflammation. CRP especially, but also PLA2, was a better indicator of perforation and formation of appendiceal abscess than WBC, given that CRP and PLA2 values increased markedly usually only after appendiceal perforation or abscess formation. Acute appendicitis seems to be very unlikely if WBC, CRP, and PLA2 values are all within normal limits. The present results support the view that group II PLA2 is an acute-phase reactant (12, 13).

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References