THE ROLE OF C-REACTIVE PROTEIN IN THE EVALUATION OF THE SEVERITY OF ACUTE CHOLECYSTITIS

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ABSTRACT

Introduction: Acute cholecystitis (AC) is the acute inflammation of the gallbladder wall. Tokyo guidelines provide criteria for the diagnosis and classification of AC. We aimed this study to identify the role of C-reactive protein (CRP) in determining the severity of AC and determine cut-off levels for each group.

Materials and methods: A retrospective analysis was made of 236 patients diagnosed with AC between January 2009 and January 2015. The patients were divided into three groups according to the Tokyo classification. CRP values of the patients were analyzed and compared among the groups.

Results: Disease severity was determined as mild in 57.6%, moderate in 28.8% and severe in 13.6% of the patients. The mean age was 53.7±18.3 years and the clinical picture was seen to become more severe with increasing age (p<0.05). CRP values of the patients were determined to rise with increasing grade (p<0.05). There was no relationship between CRP and white blood cell (WBC) (p>0.05). The CRP cut-off values were determined as 0.815 mg/dl (sensitivity 44.9%, specificity 34%) for the Grade 1 patient group, 1.095 mg/dl (sensitivity 57.4%, specificity 57.7%) for the Grade 2 patient group and 2.095 mg/dl (sensitivity 71.9%, specificity 69.6%) for the Grade 3 patient group.

Conclusion: CRP values may be useful in assessing the severity of AC, and may be considered to be a parameter that could be added to the Tokyo classification.

Key words: C-reactive protein, Acute Cholecystitis, Severity of Illness Index.

DOI: 10.19193/0393-6384_2017_3_070

Received November 30, 2016; Accepted February 02, 2017

Introduction

Acute Cholecystitis (AC) is the acute inflammation of the gallbladder wall\(^\text{1-3}\). AC constitutes 3-10% of acute abdominal patients. Inflammatory changes occurring during AC may become a clinical picture ranging from mild inflammation to empyema and gangrene\(^\text{4}\). As patients often present at the Emergency Department (ED) because of sudden abdominal pain, they are sent to surgical clinics after being diagnosed\(^\text{5}\).

The Tokyo guidelines criteria for the diagnosis and classification of AC in three severity grades (mild, moderate, severe) based on physical examination, laboratory and imaging findings\(^\text{5,6}\). Additional examinations may be required in patients considered to have additional organ involvement\(^\text{5-8}\). Since its publication in 2007 (TG 07) and the update in 2013 (TG 13), the Tokyo guidelines for the diagnosis and management of acute cholangitis and AC rapidly gained popularity\(^\text{5,9}\).
Mortality increases significantly with increasing grade of AC.

C-reactive protein (CRP) is a well-known acute phase reactant that increases in cases of inflammation. CRP level starts to increase after 6-8 hours depending on the severity of the inflammation\(^9,10\). While there aren't enough studies to determine the value of CRP levels in grading the severity of AC according to TG, CRP levels are frequently used in the estimation of the severity of inflammation for other intraabdominal inflammatory diseases such as acute appendicitis and acute pancreatitis\(^10,11\).

In this study, we aimed to identify the role of CRP levels in determining the severity of AC and determine cut-off levels for each group.

**Materials and methods**

**Study Population and Protocol**

The study was conducted retrospectively with 236 patients diagnosed with AC between January 2010 and January 2015 with the approval number 0590 of the Local Ethics Committee of Ankara Training and Research Hospital. The study included 236 patients over the age of 18 years, who were diagnosed with AC.

Acute cholecystitis was diagnosed as recommended in the Tokyo guidelines (Table 1).

<table>
<thead>
<tr>
<th>Grade III (severe) acute cholecystitis</th>
<th>Grade II (moderate) acute cholecystitis</th>
<th>Grade I (mild) acute cholecystitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated with dysfunction of anyone of the following organs/systems:</td>
<td>Associated with anyone of the following conditions:</td>
<td>Does not meet the criteria of “Grade III” or “Grade II” acute cholecystitis. Grade I can also be defined as acute cholecystitis in a healthy patient with no organ dysfunction and mild inflammatory changes in the gallbladder, making cholecystectomy a safe and low-risk operative procedure</td>
</tr>
<tr>
<td>1. Cardiovascular dysfunction: Hypotension requiring treatment with dopamine (\geq 5 \mu g/kg permin), or any dose of norepinephrine</td>
<td>1. Elevated white blood cell count (&gt;18,000/mm(^3))</td>
<td></td>
</tr>
<tr>
<td>2. Neurological dysfunction: Decreased level of consciousness</td>
<td>2. Palpable tender mass in the right upper abdominal quadrant</td>
<td></td>
</tr>
<tr>
<td>3. Respiratory dysfunction: (\text{PaO}_2/\text{FiO}_2) ratio &lt;300</td>
<td>3. Duration of complaints &gt;72 h</td>
<td></td>
</tr>
<tr>
<td>4. Renal dysfunction: Oliguria, creatinine &gt;2.0 mg/dl</td>
<td>4. Marked local inflammation (gangrenous cholecystitis, pericholecystic abscess, hepatic abscess, biliary peritonitis, emphysematous cholecystitis)</td>
<td></td>
</tr>
<tr>
<td>5. Hepatic dysfunction: PT-INR &gt;1.5</td>
<td>6. Hematological dysfunction: Platelet count &lt;100,000/mm(^3)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: TG13 diagnostic criteria for acute cholecystitis\(^6\).

<table>
<thead>
<tr>
<th>(1) Local signs of inflammation etc.</th>
<th>(1) Fever, (2) elevated CRP, (3) elevated WBC count</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Local signs of inflammation etc.</td>
<td>C. Imaging findings</td>
</tr>
<tr>
<td>Murphy’s sign, RUQ mass/pain/tenderness</td>
<td>Imaging findings characteristic of acute cholecystitis</td>
</tr>
<tr>
<td>(1) Systemic signs of inflammation etc.</td>
<td>Suspected diagnosis: One item in A + one item in B</td>
</tr>
<tr>
<td>(1) Fever</td>
<td>Definite diagnosis: One item in A + one item in B + C</td>
</tr>
</tbody>
</table>

Table 2: TG13 severity grading for acute cholecystitis\(^6\). TG13: Tokyo guidelines 2013, \(\text{PaO}_2\): partial pressure of oxygen, \(\text{FiO}_2\): fraction of inspired oxygen, PT: prothrombin time, INR: international normalised ratio.

**Exclusion criteria were:** patients aged <18 years, other acute abdomen patients (acute cholangitis, acute appendicitis, acute pancreatitis, etc.), chronic cholecystitis patients and those with inflammatory bone diseases, malignancy, lung infections, soft tissue infections, burns or trauma were not included in the study.

**Laboratory analysis**

Daily calibrated automatic hemocytometry (LH-780, Beckman Coulter, USA) was used for the WBC determination. The number of WBC (Reference Values: 4.5-13.5x 10\(^9\)/L) was measured from 2 ml of venous blood withdrawn into standard tubes (Beckton Dickinson, USA) containing 0.072 mL 7.5% K3-ethylenediaminetetraacetic acid solution. CRP was determined using a Beckman Coulter IMMAGE 800 immunochemistry system neph-
elometerautoanalyzer (USA) with the scale of mg/dL, using reagents from Beckman Coulter (USA). Extended analytical range of the test was from 0.1 to 96 mg/dl (Reference range 0-0.8 mg / dl). The intra-assay and inter-assay precisions of test was observed at 3 level. Mean ± standard deviation (SD) of level 1, 2 and 3 was 0.35±0.039 mg/dl, 4.95±0.129 mg/dl and 6.69±0.266 mg/dl, respectively. Intra-assay coefficient of variations (CV) of level 1, 2 and 3 were 11.1%, 2.6% and 3.5%, respectively. inter-assay CV of level 1, 2 and 3 were 12.1%, 3.0% and 4.0%, respectively.

Erythrocyte sedimentation rate (ESR) tests were performed using an automatic ESR measuring device (Test 1 THL, Alifax, Italy), which was calibrated daily. Venous blood samples for the ESR test were collected into tubes containing 0.072 ml of 7.5% K3-ethylenediaminetetraacetic acid solution (Beckton Dickinson, USA). Ultrasonic evaluations of the gallbladder were performed using the ultrasound device model Toshiba SSA-660A with a 3.5 to 5 MHz convex probe.

### Results

According to the Tokyo classification, mild disease was determined in 57.6%, moderate in 28.8% and severe in 13.6% of the 236 patients included in the study. The mean age of the patients was determined as 53.7±18.3 years and there was a positive relationship between the clinical picture and age (p<0.05). Of the total patients, 30.9% were male and no difference was determined between the groups in terms of gender (p>0.05). The mean value of CRP was 0.7 mg/dl (0.1-12) in grade 1 patients, 1.5 mg /dl (0.1-16) in grade 2 patients, 5.3 mg/dl (0.5- 34.6) in grade 3 patients. CRP was in normal limits in 32% of grade 2 patients and in 18% of grade 3 patients. The WBC, CRP and ESR values of the patients were determined to rise in parallel with increasing grade (p<0.05). There were USI findings in 124 (52.5%) patients and the most frequent USI findings were in the Grade 2 group (p<0.05) (Table 3).

### Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (Version 19.0; IBM, Chicago, IL, USA) was used for the statistical analysis. Whether the distribution of continuous and discrete numeric variables was close to normal or not was investigated via the Kolmogorov Smirnov test.

Descriptive statistical data was shown as mean ± SD or median (minimum - maximum) for continuous and discrete numerical variables and as the number of cases and percentage (%) for categorical variables. The significance of difference between the groups in terms of mean values for parametric data was analyzed through the ANOVA test, while the significance of the difference of non-parametric data in terms of median values was evaluated through the Kruskal Wallis test. Categorical variables were analyzed with the chi-Square test. The appropriate cutoff value for the availability of the test, sensitivity and specificity values were determined through ROC analysis. Unless otherwise specified, results of p<0.05 were accepted as statistically significant.

### Table 3: Comparison of the Ages, Genders, USI, WBC, CRP and ESR Levels of the Groups.

<table>
<thead>
<tr>
<th></th>
<th>Grade 1 (n:136)</th>
<th>Grade 2 (n:68)</th>
<th>Grade 3 (n:32)</th>
<th>Toplam Mean±SD/n (%)/ Median (Min-Max)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>49.9±17.6</td>
<td>56.5±17.5</td>
<td>64.1±18.5</td>
<td>53.7±18.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Gender Male</td>
<td>37 (27.2)</td>
<td>25 (36.8)</td>
<td>11 (65.6)</td>
<td>73 (30.9)</td>
<td>0.342**</td>
</tr>
<tr>
<td>Female</td>
<td>99 (72.8)</td>
<td>43 (63.3)</td>
<td>21 (65.6)</td>
<td>163 (69.1)</td>
<td></td>
</tr>
<tr>
<td>USI Present</td>
<td>43 (31.6)</td>
<td>59 (86.8)</td>
<td>22 (68.8)</td>
<td>124 (52.5)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Absent</td>
<td>93 (68.4)</td>
<td>9 (13.2)</td>
<td>10 (31.3)</td>
<td>112 (47.5)</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>9500</td>
<td>12200</td>
<td>12100</td>
<td>10400</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>(cells/mm3)</td>
<td>(3600-17200)</td>
<td>(3000-32700)</td>
<td>(1700-34000)</td>
<td>(1700-34000)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.7 (0.10-12)</td>
<td>1.5 (0.1-16)</td>
<td>5.3 (0.5-34.6)</td>
<td>0.95 (0.1-34.6)</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>24 (2-120)</td>
<td>32 (2-114)</td>
<td>38 (2-92)</td>
<td>28 (2-120)</td>
<td>0.001***</td>
</tr>
</tbody>
</table>

WBC: White blood cell, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, USI: Ultrasonographic imaging
While there was no relationship between CRP and WBC (p>0.05), there was a positive correlation between CRP and ESR (p<0.05) (Table 4).

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>r 0.097</td>
</tr>
<tr>
<td></td>
<td>p 0.136*</td>
</tr>
<tr>
<td>ESR</td>
<td>r 0.402</td>
</tr>
<tr>
<td></td>
<td>p &lt;0.001*</td>
</tr>
</tbody>
</table>

Table 4: Relationships of CRP with WBC and ESR.
*Pearson Correlation

In our patients, considering the availability of CRP, the AUC, cut-off value, sensitivity and specificity values were determined as 0.297 [Confidence Interval (CI): 0.229-0.366], 0.815 mg/dl, 44.9% and 34%, respectively for the Grade 1 patients group, 0.586 (CI: 0.503-0.688), 1.095 mg/dl, 57.4% and 57.7%, respectively for the Grade 2 patients group, and 0.772 (CI: 0.683-0.860), 2.095 mg/dl, 71.9% and 69.6%, respectively for the Grade 3 patients group (Figure 1).

In addition, WBC and CRP levels reveal the inflammatory process and can be used in the patient follow-up(16). In a study by Schafer et al, it was stated that it was possible to determine the severity of gallbladder inflammation with CRP levels and leukocytosis(13). Although WBC values are using in severity assessment of AC, there isn’t enough study to determine the predictive value of CRP and whether located in severity assessment of AC. Thus, in this study we aimed to determine whether CRP has a predictive cut-off level for the severity of AC.

Fig. 1: Roc Analyses of the Groups.

**Discussion**

The management of patients with AC could be associated with high rates of morbidity and mortality. In Tokyo guidelines, diagnose and severity of the disease is graded based on physical examination, laboratory and imaging findings. It suggests a management strategy for the disease following the assessment of severity. Tokyo guidelines recommend early laparoscopic cholecystectomy for grade 1 AC and elective cholecystectomy for grade 2 patients as the first-line of treatment. If a patient with grade 2 AC does not respond to initial medical treatment, urgent or early gallbladder drainage is required.

For patients with grade 3 AC, urgent or early gallbladder drainage is essential in addition to organ support and medical treatment(14). An assessment by multicenter analysis of TG13 diagnostic criteria shows that sensitivity (91.2%) and specificity (96.9%) are favorable and that diagnostic capacity is almost the same as that in TG07[8]. There is a report suggesting that the assessment criteria have contributed to a decrease in the period of hospital stay(15).

In addition, WBC and CRP levels reveal the inflammatory process and can be used in the patient follow-up(16). In a study by Schafer et al, it was stated that it was possible to determine the severity of gallbladder inflammation with CRP levels and leukocytosis(13). Although WBC values are using in severity assessment of AC, there isn’t enough study to determine the predictive value of CRP and whether located in severity assessment of AC. Thus, in this study we aimed to determine whether CRP has a predictive cut-off level for the severity of AC.

Studies have shown that 39.3%-68.5% of patients with AC are at grade 1, 25.5%-59.5% at grade 2 and 1.2%-6% are at grade 3(8,15,17). In the current study, 57.6% of the patients were grade 1, 28.8% were grade 2 and 13.6% were grade 3. The main reason for the higher number of grade 3 patients in this study, compared to the previous reports, could be the referral of grade 3 AC patients to our hospital, which is a tertiary level hospital, while grade 1 and 2 patients are treated in peripheral hospitals.

It is known that in complications such as AC and the sepsis arising from it, rates of gall bladder...
perforation increase together with the increasing rate of cholecystitis with age. In the current study, it was seen that the grade increased in parallel with the increasing age of the patients.

Ultrasonography is an appropriate imaging method that can be used in the diagnosis of AC, as it is cheap and easy to implement. Although the rate of diagnosis in this method depends on personal conditions, the general rate has been reported to be 77-95%. Other system pathologies developing in the Grade 3 patient group can be considered to start to take priority over the AC, thereby causing physicians evaluating USI not to consider the gall bladder objectively.

WBC measurement, which is a method used in the diagnosis of AC, has low diagnostic value due to low sensitivity and specificity. Leukocytosis may not be seen in elderly, diabetic and immuno-suppressive patients, although such patients may have leucopenia. The WBC count has been stated to be over 18,000 in Grade 2 of the Tokyo classification. Yazici et al. stated that the WBC count of 43% of the patients in their study were normal. CRP has a better discriminative power than WBC in most forms of AC and is a useful diagnostic marker of AC.

In the current study, the WBC count was seen to be low in Grade 1 patients and high in Grades 2-3 patients. WBC count can be considered not to respond sufficiently depending on the increase in age in parallel with the grade. It must also be remembered that leukopenia could have developed in the patient group with bad clinical symptoms due to the additional system pathologies or immune suppression.

Lee et al. found CRP positivity in 55.1% of their patients. Yazici et al. stated that CRP increased in 65.3% of patients. Mok et al. and Nikfarjam et al. reported that CRP was significantly higher in gangrenous cholecystis patients. Asai et al. reported that the CRP levels were high in patients on whom open surgery was applied during cholecystectomy and this was explained by higher inflammation in the group with a higher level of CRP. In another study designed by same author, determined a difference in the levels of infection between moderate and severe disease groups and reported a relationship between this amount of infection and CRP levels.

In the current study, CRP levels were determined to increase significantly in parallel with the grade. Therefore, as the clinical picture becomes more severe, the CRP levels are seen to increase significantly.

According the Tokyo guidelines, when ultrasonography shows findings that suggest AC and a CRP level exceeding 3 mg/dl, a diagnosis of AC can be made with 97% sensitivity, 76% specificity, and 95% positive predictive value. Teckchandani et al. defined that serum CRP level ≥3.6 mg/dl at admission was found to be strongly related to failure of early laparoscopic cholecystectomy in AC (sensitivity 84%, specificity 79%). According to histopathological severity grades they found mean CRP level 1.44 mg/dl in grade 1, 2.331 mg/dl in grade 2, 7.467 mg/dl in grade 3 patients.

In our study, according to Tokyo guidelines’ severity grades we found mean CRP level 0.7 mg/dl in grade 1 patients, 1.5 mg /dl in grade 2 patients, 5.3 mg/dl in grade 3 patients.

Beliaev et al. stated that CRP measurement does not influence management of patients with AC and physiologically fit patients with more advanced forms of AC and higher values of CRP should have their operation performed earlier than patients with mild AC and a lower concentration of CRP.

Kabul et al. found that patients with CRP level > 70.65 mg/L may be considered as grade 2 AC (75.5% sensitivity, 96.6% specificity) while patients with CRP level > 198.95 mg/L may be considered as grade 3 cholecystitis (73.9% sensitivity, 75.5% specificity). In our study, cut-off value of CRP for grade 2 was determined to be 1.095 mg/dl, with a sensitivity 57.4% and specificity 57.7%. And we found cut-off value of CRP as 2.095 mg/ml with a sensitivity 71.9% and specificity 69.6% in grade 3 patients. We couldn't compare the CRP values because the measurement techniques and units are different. Kabul et al. stated that all patients in grade 2 and 3 showed CRP elevation. In the current study, 32% of grade 2 and 18% of grade 3 patients didn't show CRP elevation.

Tokyo guidelines were first determined in 2007 and last update was at 2013. These guidelines are still being improved and we suggest that our findings can contribute to improving Tokyo guidelines with further studies.

**Conclusion**

According to our results, CRP may be useful in assessing the severity of AC and may be considered to be a parameter that could be added to the Tokyo classification.
References


Acknowledgements
We would like to thank Dr. Cihat YEL for assistance with statistical analysis.

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