Correlation of Severity of Cholecystitis and Cholangitis with Inflammatory Mediators

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Summary

The main goal of our prospective study was to point toward certain inflammatory mediators as potential biomarkers for severity of acute biliary pathologies. Our study had two equally designed branches one for acute cholecystitis and a second for acute cholangitis. Also, three mediators were chosen: a) tumor necrosis factor (TNF-α), b) interleukin 6 (IL-6) and c) procalcitonin (PCT) and possible correlation of these factors with acute cholecystitis and acute cholangitis were rigorously explored. Both TNF-α and IL-6 blood levels were recorded, across both branches, and were significantly increased while PCT levels were increased only to acute cholangitis group.

Further statistical analysis concluded that TNF-α had a strong correlation to cholangitis and weaker with cholecystitis group. As a result, this marker could pose a strong indicator of disease severity across both clinical entities. On the other hand, IL-6 showed conflicting findings. Although a trend exists between IL-6 and disease severity statistically significant correlation was noted possibly due to the small sample size of our study. Furthermore, we showed that PCT couldn't pose a credible biomarker in both groups.

Consequently, TNF-α presents the most prominent candidate as biomarker of disease severity while IL-6 usefulness as well as PCT remain to be elucidated with larger studies.

Abstract

Background

Cholecystitis and cholangitis are amongst the most common pathologies in clinical practice. A great number of studies have focused on the various pathophysiologic mechanisms responsible for the severity of the disease, merely bacterial burden, stone formation and inflammatory mediators. In this prospective study we examine three mediators, tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and procalcitonin (PCT) as predictors of disease severity to identify if they could be used as markers for biliary disease severity.

Materials and Methods

We prospectively studied two groups of patients suffering from cholecystitis and cholangitis respectively, measuring TNF-α, IL-6 and PCT values. Plasma levels of those mediators were measured before and after treatment were implicated. We also recorded gallbladder wall characteristics, bile duct and blood cultures as well as histopathologic findings in patients with severe disease. Statistical analysis was performed using the SPSS software.

Results

There is a statistically significant correlation between TNF-α factor and disease severity across both sexes. PCT and IL-6 showed a trend to increase during both acute cholecystitis and cholangitis but not significantly, probably due to the small sample size in our study. In addition, it seems that age and gender do not affect the level of inflammatory mediators.

Conclusion

Tumor necrosis factor is a sensitive biomarker for biliary disease severity. Its correlation with PCT and IL-6 however remains unclear and needs to be tested in larger prospective studies.

Key Words: TNF-α (Tumor Necrosis Factor α); IL-6 (Interleukin-6); PCT (Procalcitonin); RUQ (Right Upper Quadrant Abdomen); Biliary Disease

Introduction

Acute cholecystitis and cholangitis are amongst the most frequently encountered clinical pathologies. Although mortality is quite low,
it is important to note that the rate of gallstone burden is as high as 15% in epidemiology studies [1]. Tokyo guidelines [2] define acute cholecystitis as an acute inflammatory disease of the gallbladder, often attributed to gallstones: however, many other factors, such as ischemia, motility disorders, direct chemical injury, infections due to microorganisms, protozoa, and parasites, collagen disease and allergic reaction are also involved. Acute cholangitis is defined as an acute inflammation and infection of the bile duct, a condition with severe morbidity and mortality.

There is a wide spectrum of clinical manifestations, from mild symptoms to most severe disease, leading to sepsis and septic shock [2]. As a result, many factors have been studied as potential predictors of disease severity in order to elucidate disease course and treatment options. Recent studies show that elderly patients are more prone to sepsis as well as septic shock leading to higher mortality, while younger patients present with a more intense disease course with less complications [3]. Also gram negative bacteria, virulence factors and gallstones consistency are amongst the causative agents with the strongest correlation to acute cholecystitis and cholangitis severity [4,5,6,7,8].

Latest studies focus on inflammatory mediators that could be more sensitive in predicting intensity of cholecystitis and cholangitis. Many inflammatory factors are implicated with both clinical entities. TNF-α and IL-6 are well established proinflammatory biomarkers while procalcitonin (PCT) is an acute phase reactant for monitoring infectious diseases. Recent articles underscore that PCT is a relatively sensitive and specific biomarker of bacterial infections [9,10,11]. An older inflammatory factor, C reactive protein (CRP), seems less reliable to assess sepsis compared to PCT [12,13,14]. Although CRP remains a sensitive mediator for a wide range of inflammatory processes [15] other than bacterial infections its implication to our protocol was excluded. Furthermore, Serum Amyloid A (SAA) presents another critical factor of acute inflammatory phase that its production is simulated by cytokines such as interleukin-1 (IL-1) and IL-6 [16]. However, PCT and IL-6 pose more reliable predictors of bacterial infections in febrile patients compared to SAA [14,16,17,18]. Also, superiority of PCT and IL-6 markers is noted among patients with febrile neutropenia and bacterial infection in relation to CRP [19,20]. Consequently, tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and procalcitonin (PCT) have been selected for our study protocol and in relation to clinical studies and animal models [21,22].

Aim

The aim of this prospective study was to identify a) any correlation between TNF-α, IL-6 and PCT with the severity of cholecystitis, cholangitis and patients age and b) their sufficiency in predicting clinical severity. Plasma level measurements were conducted for these mediators before and after treatment initiation.

Materials and Methods

Study design included enrolment of 50 patients that were equally distributed in two branches, 25 with acute cholecystitis and 25 with acute cholangitis, and prospectively examined. Eligibility criteria were set for the participants that constituted by clinical manifestations (acute onset of fever, RUQ pain, jaundice and fatigue), blood chemistries (more than 3-fold increase of aspartate and alanine aminotransferases and G-glutamine transerase as well as bilirubin above 3mg/dl) and ultrasonography imaging studies (acute cholecystitis: gallbladder wall thickness > 5mm, bile stones and peri cystic fluid and acute cholangitis: distended common and hepatic bile ducts, presence of bile stones and periductal fluid without gallbladder findings) in order to meet the purposes of this protocol. Patients’ age (with the cut-off point being the 70 years), gender and clinical manifestations of the disease (fever, jaundice, RUQ pain, fatigue, anorexia and weight loss) were recorded. We also measured the plasma levels of TNF-α, IL-6 and PCT before treatment was initiated and a follow-up measurement was conducted 7 days later. TNF-α, IL-6 and PCT levels were normalized after seven days of medical or surgical management. Measurement of plasma PCT levels was conducted with chemiluminescence assay technology while TNF-α and IL-6 were calculated with enzyme immunoassay test (ELISA). Furthermore, certain biochemical markers were investigated (bilirubin, G-glutamine transerase, alkaline phosphatase), hematocrit and white blood cells count. In addition, patients underwent ultrasound imaging studies of the abdomen in order to identify signs of inflammation, i.e. fluid around the gallbladder, wall thickening and biliary duct distension. We collected blood samples from patients with fever (Temperature >38) before antibiotic treatment onset. Furthermore, bile was collected and cultured following Endoscopic Retrograde Cholangio-Pancreatography (ERCP) or cholecystectomy. All gallbladder cysts were removed and examined histologically and color of gallstones was recorded in order to assess disease severity. Statistical analysis was conducted with the SPSS program 20 using descriptive statistics, one-way anova test and correlation.

Results

Although patients were equally distributed between the two genders (women 26/50 and men 24/50), in the acute cholecystitis group we had more women (15/26 vs. 11/26) while in acute cholangitis group this relationship was reversed (male 13/24 vs. female 11/24). This shows a trend between disease manifestation and gender. Also the presence of clinical symptoms was slightly higher in elderly patients, as compared with younger ones (54% vs. 46% respectively), a finding that is consistent with previous studies that showed greater numbers of symptomatic disease presentation in elderly patients [1]. Furthermore, we noticed that the distribution of cases for younger patients was slightly higher in the acute cholangitis group, (52,2% vs. 44,4%) while acute cholecystitis was more frequent in older patients (55,6% older vs. 47,8% younger). According to our findings elderly female patients are prone to acute cholecystitis (80% older females vs. 20% older males) while males in the same age group present more frequently with acute cholangitis (66,6% older males vs. 33,3% older females). On the other hand, we observed a reversed relationship for acute cholecystitis and cholangitis that was more frequent in younger patients (acute cholecystitis: 72,7% males vs. 27,3% females and
We then compared the levels of acute inflammatory mediators’ TNF-α, IL-6 and PCT in relation with clinical and laboratory results. We found that TNF-α values and PCT levels were 4 and 10 times higher respectively in the cholangitis group. Furthermore, IL-6 values were higher by almost a 2-fold in cholecystitis group (Table 3). When we analyzed these results based on gender and disease entity we noted that TNF-α levels were higher across all subjects in the cholangitis group, as compared with the cholecystitis group (males 92.52 ng/ml vs. 12.02 ng/ml and females 103.91 ng/ml vs. 25.68 ng/ml, Table 4). Subsequently, we compared patients’ gender, age and type of disease in relation with TNF-α levels in order to reveal any correlations amongst those factors. Indeed, we found a statistically significant correlation between TNF-α and disease (0.03 male versus 0.01 in female patients, 95% confidence interval (CI), Table 6). We also examined the relation between TNF-α levels with age and gender and found that there is no statistically significant correlation (0.243 in males versus 0.220 in females, 95% CI, Table 5 and 0.156 in the cholecystitis group versus 0.713 in cholangitis group, 95% CI, Table 6 respectively). When we used bivariate correlation, a strong connection was seen at the 0.01 level between TNF-α levels and disease (0.288 in cholecystitis group and 0.001 in cholangitis group). In addition, there was no significant correlation between the aforementioned biomarker and age (0.652 in younger patients and 0.552 in older patients, Table 3) while there was a significant correlation between TNF-α and gender (0.287 in cholecystitis group and 0.079 in cholangitis group). Based on these findings we conclude that this mediator correlates strongly with both acute cholangitis and cholecystitis and is independent of all other factors in our study. Finally, the disease clinical severity was followed by analog changes of TNF-α in both groups: as a result, disease intensity is consistent with mediator up regulation.

We followed a similar pattern in correlating IL-6: our first observation was that the levels of this factor were higher across cholecystitis group, as compared with TNF-α levels in both sexes (males 46.34 ng/ml vs. 12.02 ng/ml and females 59.48 ng/ml vs. 25.68 ng/ml) but this relation was reversed when comparing patients with cholangitis (males 32.76 ng/ml vs. 92.52 ng/ml and females 113.90 ng/ml vs. 215 ng/ml, Table 4). In addition, it is noteworthy that across the cholecystitis group IL-6 levels were higher, as compared with those in the cholangitis group (53.92 ng/ml vs. 34.32 ng/ml, Table 3). Further analysis revealed no statistical significance between disease and IL-6 levels (0.489 in males versus 0.200 in female patients, 95% CI, Table 6.) Also no relation was found between IL-6 levels and age (0.290 in males versus 0.319 in females, 95% CI) and also IL-6 levels and gender (0.553 in cholecystitis group versus 0.805 in cholangitis group, 95% CI, Tables 5 and 6).

Finally, we recorded the levels of PCT in both cholecystitis and cholangitis group (Table 4). We observed that this factor did not show any changes across all patients of the first group and was independent of age and gender (PCT 0.10 ng/ml). Findings however were completely different for patients with cholangitis, as we noted significant increase (by a 2-fold to 10-fold) when compared to the cholecystitis group, while in two cases measurements showed a

### Table 2: Patients with cholangitis

<table>
<thead>
<tr>
<th>Gender/age</th>
<th>PCT ng/ml</th>
<th>TNF-α ng/ml</th>
<th>IL-6 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/&gt;70</td>
<td>10.5</td>
<td>90.3</td>
<td>66.0</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.47</td>
<td>45.7</td>
<td>94.6</td>
</tr>
<tr>
<td>F/&gt;70</td>
<td>0.12</td>
<td>114.4</td>
<td>176.4</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.37</td>
<td>51.9</td>
<td>96.8</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.25</td>
<td>15.8</td>
<td>150.7</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.50</td>
<td>44.1</td>
<td>89.8</td>
</tr>
<tr>
<td>F/&gt;70</td>
<td>1.35</td>
<td>70.6</td>
<td>220.3</td>
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<tr>
<td>M/&gt;70</td>
<td>1.32</td>
<td>43.6</td>
<td>262.5</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.40</td>
<td>34.4</td>
<td>99.8</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>10.84</td>
<td>78.5</td>
<td>51.0</td>
</tr>
<tr>
<td>F/&gt;70</td>
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<td>33.3</td>
<td>151.3</td>
</tr>
<tr>
<td>M/&gt;70</td>
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<td>46.7</td>
<td>90.6</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>1.33</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.15</td>
<td>0.9</td>
<td>200</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.16</td>
<td>1.1</td>
<td>57.3</td>
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<td>1.8</td>
<td>5.3</td>
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<tr>
<td>F/&gt;70</td>
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<td>0.5</td>
<td>79.5</td>
</tr>
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<td>0.17</td>
<td>1.4</td>
<td>43.9</td>
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<tr>
<td>F/&gt;70</td>
<td>0.24</td>
<td>1.2</td>
<td>200</td>
</tr>
<tr>
<td>F/&gt;70</td>
<td>0.16</td>
<td>2.3</td>
<td>13.5</td>
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<td>47.6</td>
<td>87.1</td>
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<td>0.95</td>
<td>62.5</td>
<td>87.5</td>
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<tr>
<td>M/&gt;70</td>
<td>0.10</td>
<td>18.7</td>
<td>10.2</td>
</tr>
<tr>
<td>M/&gt;70</td>
<td>0.10</td>
<td>15.9</td>
<td>10.4</td>
</tr>
</tbody>
</table>

### Table 3: Correlation between TNF-α and Disease

<table>
<thead>
<tr>
<th>Age</th>
<th>TNF-α</th>
<th>Disease</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;70</td>
<td></td>
<td></td>
<td>Pearson Correlation</td>
<td>.652**</td>
<td>23</td>
<td>.001</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td></td>
<td></td>
<td>Pearson Correlation</td>
<td>.550**</td>
<td>27</td>
<td>.003</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
100-fold increase. PCT levels also showed no relation with disease (0.519 in male patients versus 0.116 in female patients, 95% CI) and gender (0.412 in males versus 0.373 in females, 95% CI) but TNF-a was different (78,00 in males versus 32,7692 in females, 95% CI) and gender (75,65078 in males versus 73,56094 in females, 95% CI). These differences in inflammatory mediators were further analyzed using ANOVA, which showed significant differences between groups for IL-6, PCT, and TNF-a levels in males and females. The results indicated that IL-6 levels were significantly different between the two groups (F = 11.488, p < 0.003) and TNF-a levels were also significantly different (F = 15.013, p < 0.001). However, PCT levels did not show significant differences between the two groups (F = 2.653, p = 0.116).
group, 95% CI, Tables 5 and 6). As a result, disease, age and gender had no statistically significant effect on PCT up regulation during inflammation.

We also cultured bile fluid and blood samples to isolate certain pathogens responsible for disease severity. Although the number of samples was small to lead us to certain statistical conclusions, there were certain indications about infective bacteria frequency with the most frequently encountered microbe being E. coli followed by Klebsiella pneumonie and Proteus mirabilis. Those samples were isolated from patients with the most severe clinical manifestations, merely gangrenous cholecystitis and acute cholangitis. Most of the strains cultured were sensitive to empirical antibiotic therapy but there were two Klebsiella pneumoniae samples with multiple drug resistance. Finally, gallbladder pathology examination revealed a wide range of findings, from mild changes of chronic cholecystitis to gangrenous necrosis of the gallbladder wall.

**Discussion**

Our study revealed a number of issues that are noteworthy. Firstly, age did not represent an independent risk factor, as elderly patients presented with acute cholangitis at the same frequency as those with acute cholecystitis (Table 1). The small sample size, less than 30 patients in each group, could be responsible for these findings, as other studies showed a strong relationship between age and disease severity [3] relevant to greater virulence of biliary bacteria in patients older than 70 years (Filament 1, slime production and cholangiovenous reflux) as well as the type of these microbes (sensitive vs. resistant and gram negative vs. gram positive) [3,4,5,23-27]. In addition, we observed that serum levels of TNF-α were higher (by a 2-fold) in females, as compared to the male population of this study (Table 4). However, the levels of TNF-α were lower in both cholecystitis and cholangitis groups, as compared to the findings of other studies [3,4,5]. Based on our findings, TNF-α appears to be a strong disease severity biomarker candidate, as a strong correlation exists between these two factors. However, the correlation between IL-6, PCT and biliary infections gave conflicting results. Indeed, the first mediator showed higher levels in all series when compared with TNF-α, having a 2-fold increase in cholecystitis vs. cholangitis group. On the other hand, PCT did not increase in the cholecystitis group, but its levels were higher (by a 5-fold to a 100-fold) in cholangitis group. As a result, IL-6 gathers higher credibility as a sensitive biomarker of disease severity, while PCT may play a role in distinguishing between more critical and benign clinical presentations.

Consequently, our study presents certain strengths and limitations. Strengths include a) study consists of two equalized side branches for acute cholecystitis and acute cholangitis and b) our findings underscore a statistically significant correlation of TNF-α and severity of disease while only increasing trends were observed for IL-6 and PCT. On the other hand, limitations include a) small sample size due to low enrolment of patients, b) One follow-up measurement of inflammatory mediators after the clinical entity
was managed and c) heterogeneity of the infectious etiologic factors that might influence levels of tested biomarkers. As a result, this paper should be considered preliminary. Therefore, future prospective studies of larger scale should be conducted with better defined infectious factors and more follow-up measurements to further confirm our results.

Also, there is debate about other factors that may contribute to disease severity, not included in our study. Body Mass Index (BMI) seems to correlate with less intense infection [7, 28] and the same is true between gallstones type and the inflammatory process, as various studies revealed that disease severity correlates with the burden of bacteria and their location at the surface or the core of the gallstones [1, 7, 8, 20]. Furthermore, there was no assessment of basic levels across TNF-α, IL-6 and PCT in order to compare the impact of biliary infection on these mediators or their polymorphic analogs [2, 29]. Although there were indications in our study that gram negative bacteria are responsible for the clinical manifestations in most of the cases, we were not able to assess their impact on inflammatory mediators or the TNF-α, PCT and IL-6 levels after the implication of certain medical or surgical treatment protocols [30-36].

In conclusion, tumor necrosis factor is a sensitive biomarker for biliary disease severity. Its correlation with PCT and IL-6 however remains unclear and needs to be tested in larger prospective studies.

References


