Review Article

Serum YKL-40 in Patients with Acute Pancreatitis

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Abstract

Background: YKL-40 is secreted by macrophages and neutrophils. Serum YKL-40 is elevated in patients with diseases characterized by inflammation, tissue remodeling, and fibrosis. We tested the hypothesis that high serum YKL-40 levels can be used to identify patients with severe disease during the early course of acute pancreatitis.

Methods: Serum YKL-40 was determined by ELISA in 75 patients with acute pancreatitis (mild n=63; severe n=12) during the early phase of the disease and in 121 controls.

Results: Serum YKL-40 at admission was higher in patients with acute pancreatitis than in controls (p <0.0001). Much higher serum YKL-40 levels were found in patients with severe disease (median 2110 µg/l, range 204-9650) compared to mild disease (140 µg/l, 20-252l, p <0.0001). Sixty-seven percent of the patients with mild disease and all patients with severe disease had elevated serum YKL-40 (compared to normal levels). Serum YKL-40 correlated with serum CRP (admission: r=0.70; 48 hours: r=0.66) (p <0.0001), APACHE II score (admission: r=0.63; 48 hours: r=0.55) (p <0.0001), and Ranson score (48 hours: r=0.38 (p=0.002). At admission, serum YKL-40 yields the best separation between patients with severe and those with mild pancreatitis (Prob (X >Y): 0.91) versus APACHE II score (0.78), Ranson score (48 hours: 0.78), and serum CRP (0.73). During hospitalization, serum YKL-40 decreased in both patient groups, but reached normal levels within a week only in patients with mild disease.

Conclusions: Serum YKL-40 may be a novel biomarker to identify patients with acute severe pancreatitis at admission.

INTRODUCTION

Acute pancreatitis is a common acute inflammatory disease of the pancreas with multiple etiologies and characterized by a sudden onset of upper abdominal pain, nausea, vomiting, fever, leukocytosis, and elevated serum levels of pancreatic enzymes [1-3]. Acute pancreatitis varies according to severity, and most patients have an edematous pancreatitis, which is associated with an uneventful recovery within a few days with simple supportive therapy. However, 20% of patients with acute pancreatitis develop a rapidly progressive severe necrotizing pancreatitis, which is associated with organ failure or local complications, including necrosis, infection/sepsis, or pseudo cyst formation. Patients who have persistent organ failure have pancreatic necrosis and a mortality of at least 30% [4] and need multidisciplinary medical and surgical critical care support [1-4].

The management of patients with acute pancreatitis is complicated by the difficulty to differentiate patients with mild from patients with severe disease at time of admission. This is important in order to facilitate immediate treatment in an intensive care unit [1-4]. Prognostic multiple factor systems using clinical criteria have been developed to determine severity in patients with acute pancreatitis like the Ranson score and the acute physiology and chronic health evaluation (APACHE) score. These systems are cumbersome, requiring multiple measurements, and the Ranson score is first accurate 48 hours after presentation of the disease [1-4].

The inflammatory process in acute pancreatitis is initiated by an activation of pancreatic zymogens, resulting in pancreatic auto digestion and an inflammatory response mediated by the immune system. The inflammatory reaction is initiated at the site of injury and, if marked, can lead to the systemic inflammatory response syndrome [1-5]. The inability of current biochemical markers or multiple factor scoring systems to predict severe disease

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at the time of admission strengthens the need for simple tests like serum biomarkers of inflammation and tissue remodeling to identify patients who develop severe disease and identify patients who have a poor prognosis already at admission.

YKL-40 (chitinase 3-like 1, CHI3L1) is a highly conserved glycoprotein and a member of “mammalian chitinase-like proteins” [6,7]. YKL-40 is primarily produced by macrophages [8], neutrophils [9], and cancer cells [7,10]. YKL-40 regulates vascular endothelial growth factor (VEGF) and plays a major role in inflammation and angiogenesis [10-13], remodeling of the extracellular matrix [6], and in the development of fibrosis [14-16]. YKL-40 regulates cellular and tissue responses via the IL-13 receptor α2 [17]. It has been suggested that YKL-40 may be a potential target for inhibition of progression in both cancer and inflammatory diseases [10-12].

The plasma concentration of YKL-40 is emerging as a new biomarker in patients with different types of neoplastic diseases and diseases characterized by inflammation and fibrosis [6,7,10,12,18]. High plasma YKL-40 levels in subjects from the general population are associated with an increased risk of liver fibrosis and pancreatitis (both acute and chronic) [15], and a high plasma YKL-40 level in patients with pancreatic cancer is a prognostic biomarker for short overall survival [19]. A small study of 16 patients has shown that patients with chronic pancreatitis have an elevated plasma YKL-40 [20].

In the present study, we tested the hypothesis that high serum YKL-40 levels can distinguish patients with severe disease from those with mild disease during the early course of acute pancreatitis.

PATIENTS AND METHODS

Patients

The study population consisted of 75 (36 males and 39 females; median age of 57 years, range 18-86 years) consecutive patients with acute pancreatitis admitted to Aalborg Hospital, Denmark, from 1998 to 2000 [21]. The diagnosis of acute pancreatitis was based on the presence of acute upper abdominal pain associated with a raised serum amylase concentration and/or radiological evidence compatible with acute pancreatitis based on the then used Atlanta classification from 1992 [22]. The clinical course of the patients was followed prospectively until discharge, withdrawal of consent, or death, including classification of patients with mild and severe disease according to the Atlanta classification of 1992 [22]. In all patients, ultrasonography (UL) was performed within 24 hours of admission. The clinical diagnosis of biliary pancreatitis was based on the detection of gallstones by UL and/or elevated laboratory parameters indicating cholestasis (alkaline phosphatase and bilirubin). Early ERCP was performed in 30 cases with suspected cholestasis. In all patients the APACHE II score was calculated from variables on admission and 48 hours thereafter, and the Ranson score was calculated from variables after 48 hours. A contrast-enhanced computed tomographic (CT) scan of the abdomen was generally performed in patients with an APACHE II score > 9 and/or when local pancreatic complications were suspected clinically. CT scans were therefore performed in 17 patients (23%). Three patients with an APACHE II score > 9 were not examined by CT scan because they demonstrated clinical signs of recovery, and their high scores were largely attributable to advanced age and chronic disease. Necrotizing pancreatitis was verified at CT scan by decreased enhancement of the pancreas in 10 patients [13%]. Nine of the patients had been diagnosed with chronic pancreatitis prior to admission with acute onset of acute pancreatitis. The study protocol was approved by the local ethics committee. Written informed consent was obtained from each subject.

Healthy subjects

The normal range of serum YKL-40 was determined in 121 healthy subjects (41 males and 80 females, median age of 66 years, range 18-88 years). They were all healthy, were not on any medication, and had no clinical signs or symptoms of cancer, joint, liver, kidney, and metabolic or hormonal disease. Written informed consent was obtained from each subject.

Biochemical analysis

Blood samples for serum YKL-40 measurement were available from all patients at admission and from 64 of the patients after 48 hours. During hospitalization, the patients had blood samples collected twice weekly. Serum was separated from cellular elements by centrifugation within 3 hours after blood sampling. All serum samples were stored at -80°C until analysis. Serum concentrations of CRP and amylase were determined by routine methods. Serum YKL-40 was determined by a two-site, sandwich-type enzyme-linked immunosorbent assay (Quidel, Santa Clara, CA, USA) using streptavidin-coated microparticle wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase-labeled polyclonal detection antibody. The sensitivity of the ELISA was 20 µg/l. The intra-assay and inter-assay coefficients of variation of this assay are <3.6% and <3.7%, respectively. Samples from each patient were analyzed on the same ELISA plate. Samples from patients and controls were determined blindly in duplicate. This assay takes <4 hours.

Statistical analysis

The SAS (software package (version 9.1; SAS Institute, Cary, NC, USA) was used to manage the patient data and to perform all statistical analyses. A normal reference region was calculated as described by Royston [23] on the log transformed serum YKL-40 values of the healthy controls after adjusting for age, and the 95% percentile was chosen as the upper limit. The serum YKL-40 levels in the patients were scored as normal or elevated by the normal age-adjusted serum YKL-40 level. Rank statistics were used to calculate correlation coefficients. Comparisons of marker levels were done using the Mann-Whitney two sample test including the estimation, based on the U statistic, of the probability that a randomly selected patient from the severe pancreatitis group (X) has a higher marker level than one from the mild pancreatitis group (Y), here denoted Prob(X>Y). This is exactly the same as the area under the receiver operating characteristic curve. Descriptive statistics was used to characterize follow-up data. The significance level was set to 5%.

RESULTS

The clinical characteristics of the patients are shown in Table
The median serum YKL-40 concentration in the patients with acute pancreatitis was 212 µg/l (range 20-9650 µg/l) and was significantly elevated (p<0.0001) compared with the level in the healthy controls (median 38 µg/l, range 20-215 µg/l). Fifty-four (72%) of the patients had a serum YKL-40 level above the upper 95th percentile (age-corrected) of serum YKL-40 in the controls. Figure 1 illustrates the individual serum YKL-40 in the patients and controls in relation to age. Sixty-three patients were classified with mild pancreatitis and 12 patients with severe pancreatitis. Six of the patients with severe pancreatitis had organ complications and 3 of these died within 65 days after admission. These patients had very high levels of serum YKL-40 at admission (3500 µg/l, 4460 µg/l, and 9650 µg/l). The patient with the highest serum YKL-40 at admission died after 10 days.

Table 2 shows the associations between disease severity and serum YKL-40, serum CRP, APACHE II score, and Ranson score. Patients with severe disease activity had higher serum YKL-40 levels (p <0.0001) at admission compared to patients with mild disease, and both patient groups had elevated serum YKL-40 compared to controls (severe: p <0.0001; mild: p <0.0001). All patients with severe disease activity had elevated serum YKL-40 levels (i.e. above the 95th percentile (age-corrected) of the healthy controls, p=0.016) and 67% (42/63) of the patients with mild disease had elevated serum YKL-40. Serum CRP, APACHE II, and Ranson score (after 48 hours) were also highest in the patients with acute severe pancreatitis (Table 2). There was no significant difference in age, sex ratio, and serum amylase between patients with mild or severe acute pancreatitis.

Table 1: Characteristics at admission of patients with mild and severe acute pancreatitis.

<table>
<thead>
<tr>
<th></th>
<th>Mild (n=63)</th>
<th>Severe (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years#</td>
<td>57 (18-86)</td>
<td>66 (34-78)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>34/29</td>
<td>5/7</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary</td>
<td>35 (56%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>11 (17%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>9 (14%)</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>8 (13%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Pancreatic necrosis</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Died</td>
<td>0</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Later chronic pancreatitis</td>
<td>5 (8%)</td>
<td>4 (33%)</td>
</tr>
</tbody>
</table>

# Values are means (range). For the other parameters, the numbers (% of patients) are given.

Figure 1 Individual serum YKL-40 in patients with acute pancreatitis. Mild disease (N=63): read triangles; severe disease alive (N=9): green stars; died due to severe disease (N=3): green circles; and healthy controls (N=121): open circles. The Y-axis is a logarithmic scale.

Figure 2 Changes in serum YKL-40 given as box plots in patients with severe acute pancreatitis (green) or mild disease (red) during the first week of hospitalization. The box plots display median concentration (lines inside boxes), lower and upper quartiles (limits of the boxes), 10th and 90th percentiles (whiskers), and outliers (circles). The Y-axis is a logarithmic scale. The number of patients in each group at the different time points are: Baseline (mild: 63; severe: 12), day 2 (mild: 50; severe: 12), day 6 (mild: 11; severe: 6), day 7 (mild: 7; severe: 5) and day 8 (mild: 6; severe: 6).

At admission, patients with an APACHE score ≥8 had higher serum YKL-40 levels compared to patients with an APACHE score < 8 (median 1532 µg/l, range 82-9650 (n=16) vs. 140 µg/l, 14-2520 (n=51), p=0.0001). After 48 hours, patients with a Ranson score ≥3 had higher serum YKL-40 compared to patients with a Ranson score < 3 (460 µg/l, 20-9650 (n=27)) vs. 154 µg/l, 20-3240 (n=38), p=0.02).

No differences in serum YKL-40 at admission were found between patients with an alcoholic disease etiology and those with a non-alcoholic etiology (median 212 µg/l (range 22-4460) vs. 217 µg/l (20-9650), p=0.8) or between patients with a biliary etiology vs. non-biliary etiology (285 µg/l (20-9650) vs. 196 µg/l (20-5000), p=0.18).

At admission, significant correlations were found between serum YKL-40 and serum CRP (Spearman’s r=0.60, p <0.0001), APACHE II score (0.64, p <0.0001), days of hospitalization (0.63,
p <0.0001), and age (0.58, p <0.0001). After 48 hours, serum YKL-40 correlated with serum CRP (0.66, p <0.0001), APACHE score (0.65, p <0.0001), and Ranson score (0.55, p <0.0001). No correlations were found between serum YKL-40 and amylase at admission (0.19, p=0.11) and after 48 hours (0.07, p=0.58).

Comparing serum YKL-40 levels determined at admission with those after 48 hours showed a significant decrease in patients with mild pancreatitis (median decrease 92 µg/l (range -124 to -2088), n=52, p <0.0001) and in patients with severe pancreatitis (median decrease 1478 µg/l (-520 to -9250), n=12, p=0.009). Figure 2 illustrates the changes in serum YKL-40 during the first week of hospitalization. Most patients with mild acute pancreatitis had normalization of serum YKL-40 within 1 week, whereas patients with severe acute pancreatitis had elevated levels (compared to controls) also after 1 week of hospitalization. Six of the 12 patients with severe pancreatitis developed necrosis of the pancreas, but there was no difference in serum YKL-40 levels between these two groups of patients (p=0.49).

Table 3 provides data of the median and range of serum YKL-40 at time of hospitalization and stratified by recovery period (i.e. days in hospital) and age. Serum YKL-40 was high in patients hospitalized for more than 3 days.

**DISCUSSION**

Patients with severe acute pancreatitis had significantly higher serum YKL-40 levels than patients with mild disease at admission. Furthermore, patients with severe pancreatitis had a median level of serum YKL-40 that was >10-fold higher than the upper limit in healthy subjects, and much higher than patients with chronic inflammation [12,15]. According to the literature the overall mortality of patients with severe acute pancreatitis was 25% [1-4]. The patient who died within 14 days had the highest serum YKL-40 at admission (45-fold higher than the upper limit reported in healthy subjects).

It is crucial to identify patients with severe acute pancreatitis at admission. However, clinical assessment alone can only identify less than 50% of patients at risk for severe acute pancreatitis [1-4]. Until now, serum CRP is the best established and most used serological biomarker to determine disease activity in patients with acute pancreatitis. However, serum CRP has only been shown to be valid 48 hours after admission [1-4]. We therefore compared serum CRP and APACHE II score to serum YKL-40 at admission, and our results indicate that serum YKL-40 was a better diagnostic tool at admission. However, forty-eight hours after admission, serum YKL-40 discriminated just as well as serum CRP, and better than the APACHE and Ranson scores.

Follow-up analysis of serum YKL-40 during hospitalization showed that the level decreased in both patient groups, but reached normal levels within a week only in patients with mild disease. Six patients developed necrosis of the pancreas, but the study had not enough power to determine whether serum YKL-40 was a valuable biomarker to detect necrosis. Our study is also too small to recommend a cut-off value for serum YKL-40 for use in patients with acute pancreatitis at high risk of severe disease.

YKL-40 can be regarded as an acute phase protein, and serum YKL-40 may provide new information regarding severe inflammatory disease. YKL-40 is different from hepatocyte-

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**Table 2:** Scoring systems and biomarkers at admission and after 48 hours.

<table>
<thead>
<tr>
<th>At admission</th>
<th>Mild disease</th>
<th>Severe disease</th>
<th>p-value</th>
<th>Prob (X&gt;Y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum YKL-40 µg/l</td>
<td>140 (20 - 2520)42</td>
<td>2110 (204-9650)12</td>
<td>&lt; 0.0001</td>
<td>0.91</td>
</tr>
<tr>
<td>Serum CRP mg/l</td>
<td>58 (10 - 360)62</td>
<td>171 (14 -360)12</td>
<td>0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>5 (0 - 15)62</td>
<td>10 (0 - 17)31</td>
<td>0.004</td>
<td>0.78</td>
</tr>
<tr>
<td>After 48 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum YKL-40 µg/l</td>
<td>70 (20-870)12</td>
<td>397 (104-3350)12</td>
<td>&lt; 0.0001</td>
<td>0.93</td>
</tr>
<tr>
<td>Serum CRP mg/l</td>
<td>66 (10-360)44</td>
<td>360 (168-360)12</td>
<td>&lt; 0.0001</td>
<td>0.94</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>5 (0-14)10</td>
<td>8 (3-17)12</td>
<td>0.006</td>
<td>0.76</td>
</tr>
<tr>
<td>Ranson score</td>
<td>2 (0-5)51</td>
<td>4 (0-5)12</td>
<td>0.0007</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Values are median (range) unless otherwise stated. Comparisons of marker levels were done using the Mann-Whitney two sample test including the estimation of the probability of a randomly selected patient from the severe pancreatitis group (X) has a higher marker level than one from the mild pancreatitis group (Y), here denoted Prob(X>Y).

**Table 3:** Serum YKL-40 at time of hospitalization and stratified by recovery period (i.e. days of hospitalization) and age.

<table>
<thead>
<tr>
<th>Days of Hospitalization</th>
<th>Numbers</th>
<th>Serum YKL-40 µg/l median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3 days</td>
<td>20</td>
<td>52 (20–464)</td>
</tr>
<tr>
<td>4–5 days</td>
<td>21</td>
<td>152 (20–2520)</td>
</tr>
<tr>
<td>6–10 days</td>
<td>18</td>
<td>285 (22–9650)</td>
</tr>
<tr>
<td>&gt; 10 days</td>
<td>16</td>
<td>1545 (48–5000)</td>
</tr>
<tr>
<td>Patient age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 38 years</td>
<td>19</td>
<td>60 (20–1700)</td>
</tr>
<tr>
<td>38–57 years</td>
<td>19</td>
<td>138 (20–5000)</td>
</tr>
<tr>
<td>58–71 years</td>
<td>18</td>
<td>404 (44–9650)</td>
</tr>
<tr>
<td>&gt; 71 years</td>
<td>18</td>
<td>518 (38–3240)</td>
</tr>
</tbody>
</table>
produced CRP that increases in response to high circulating IL-6 concentrations. In patients with endotoxemia, which is followed by increased plasma TNF- and IL-6 levels, plasma YKL-40 increased earlier than serum CRP [24]. Macrophages and neutrophils are most likely the major source of the high serum YKL-40 levels seen in patients in the early phase of acute pancreatitis. IL-6 stimulates YKL-40 production [25], and YKL-40 is secreted in vitro by activated monocytes and neutrophils [8,9,26,27]. In vitro studies have shown a correlation between granulocyte macrophage colony stimulating factor (GM-CSF) and YKL-40 expression in mouse tracheal epithelial cells after treatment with ovalbumin [28]. Disease activity in some patients with acute pancreatitis is accompanied by chronic inflammation, eventually resulting in the formation of fibrosis [1-4]. Even in the early phase of acute pancreatitis, a fibrotic response may be observed, and in some patients the fibrotic response can produce substantial and irreversible organ dysfunction and remodeling. YKL-40 is a growth factor for fibroblasts and plays a role in fibrogenesis [14-16,29]. Furthermore, high serum YKL-40 levels in patients with liver fibrosis and cirrhosis are associated with a poor prognosis [18]. However, whether YKL-40 is involved in the progression of organ fibrosis in patients with acute pancreatitis remains to be clarified.

Our result suggests that serum YKL-40 may be a valid early prognostic biomarker for severe acute pancreatitis. However, in this prospective study from 2000 we used the Atlanta Classification from 1992 and newer classification system provide both three and four grades of severity, allowing a more specific and differentiated prediction of complication to acute pancreatitis [30,31]. Another limitation of our study is the rather small sample size of patients with acute pancreatitis.

Therefore we suggest larger prospective longitudinal studies based on both Atlanta 2012 and determinant-based classification with the use of serum YKL-40 at admission of patients with acute pancreatitis.

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