Introduction

Acute pancreatitis (AP) is an acute inflammatory disease of pancreas that occurs frequently in emergency medicine; often due to biliary obstruction and alcohol abuse (1). Usually (80%), the disease goes easily and medical treatment is conservative (1–3). Treatment of severe forms of AP (20%), associated with pancreatic necrosis, is still difficult and has a higher mortality rate.

Diagnosis of the disease is based on clinical presentation, ultrasonographic scan and clinical-laboratory studies (2).

The first step in the diagnostic algorithm should be the differentiation between acute biliary pancreatitis (ABP) and nonbiliary forms of AP (AAP and idiopathic AP) (4). ABP should be treated with endoscopic retrograde cholangiopancreatography (ERCP) and/or elective cholecystectomy (5).

An early differentiation between mild AP (edematous AP) and severe forms of the disease (necrotic pancreatitis, associated with focal and systemic complications) is of great importance and requires contrast-augmented computed tomography (3, 4). Therapy of these patients is mainly supportive, nonsurgical, and consists of antibiotic prevention for the sake of reduction of eventual sepsis risk. Surgical intervention is indicated during the first 4 weeks after the beginning of symptoms for the sake of achieving better results in debridement and demarcation of necrotic tissue (3).

Clinical laboratory plays a major role in diagnostic, differential-diagnostic and prognostic evaluation.
processes. Gathering database from serum and urine amylase, lipase, AST, ALT, ALP, and total bilirubin is of great importance for the early assessment of diagnosis.

According to some investigations, serum and urine amylase, AST, ALT, and ALP are initially higher in patients with ABP, while lipase, lipase/amylase ratio, bilirubin and GGT do not present statistically reliable differences between ABP and AAP (6–9). According to other investigations, the best indicator for early determination of AAP is the carbohydrate-deficient transferin, in combination with MCV, and the lipase/amylase ratio (7,10).

It is interesting to mention the importance of the enzymes ALT and AST for the identification of micro- lithiasis (up to 75%) in patients with idiopathic acute pancreatitis (11,12). In these cases, a microscopic study of stimulated duodenal-biliary sediment, simultaneously with evaluation of both enzymes till the 24th and 72nd hour, is performed. Higher levels are achieved till the 24th hour after admission (12).

New scientific achievements in the same field reveal an increased interest in the role of trypsinogen- trypsin cascade for the pathophysiology and etiology of AP. It is now accepted that evaluation of trypsinogen 1 and 2 and trypsin-antitrypsin complex in blood serum till the 12th hour following admission is an important prerequisite for the determination of etiology and prediction of severity of the disease (13, 14). The significance of trypsinogen 2 in urine is almost the same (15).

As a whole, our results confirm data in literature establishing higher initial concentrations of serum amylase, AST, and ALT in patients suffering from ABP, and an early rise in the levels of GGT and MCV in patients with AAP, ALP and total bilirubin, for the time being, do not play a substantial role.

**Material and Methods**

**Patients**

In this trial, 116 patients were enlisted (77 men, 39 women). They were admitted to the Second Surgical Clinic of the N.I.Pirogov MBALSM in between June 2000 and November 2003. Diagnosis was assessed on the basis of medical history data, typical clinical manifestations (epigastrical pain, nausea, vomiting), ultrasonography, and highly raised values of urine amylase on the date of admission (thrice the upper referent normal value, above 1500 U/L). Contrast augmented computed tomography was performed in cases of a suspected severe (necrotic) form of AP.

The severity of the disease was determined by the following criteria: days of stay at the clinic (admission to the ward for active treatment); any surgical intervention carried out; CT data for a pancreatic pseudocyst; pancreatic necrosis and/or formation of a pancreatic abscess; and development of systemic complications like multi-organ failure, sepsis, DIC syndrome and others (16, 17).

The diagnoses of patients having ABP (n=58) were assessed on the database of ultrasonographic visualization of biliary calculi, cholecystectomy in past medical history (18), diagnostic laparotomy and/or histological evaluation of gallbladder. The diagnoses of patients with AAP (n=30) were assessed on the database of heavy alcohol ingestion before the appearance of characteristic symptoms and the lack of biliary calculi on the ultrasonographic scans. Idiopathic etiology of the disease was determined, in cases when no evidence of heavy alcohol ingestion or biliary calculi had been found (Table 1).

Patients transferred from another hospital and those suffering from underlying chronic pancreatitis (chronic relapsing pancreatitis) were discharged from the investigation.

**Materials**

Serum/whole blood indices were analyzed in the first 24 hours after admission to the clinic.

**Laboratory methods**

Evaluation of serum amylase, AST, ALT, GGT, ALP, total bilirubin and MCV was carried out by routine clinical laboratory methods. Serum amylase was evaluated by an enzymatic method, ALT and AST—kinetically; GGT and ALP—using a kinetic-colourimetric method, and total bilirubin—colourimetrically. Normal values for serum amylase were estimated at levels from 15 to 110 U/L, for AST between 11 and 36 U/L for women and 11 and 42 U/L for men. Normal ALT values were 11 to 45 U/L for women and 13 to 53 U/L for men. GGT—9 to 60 U/L, ALP—60 to 216 U/L, total bilirubin—3.4 to 21 μmol/L, and MCV from 82 to 92 fl. Till May 2003, biochemical values were estimated using reagents from Merck company (Merck KGaA,
Germany), applied on the Kobas Mira S analyzer (Roche Diagnostics, Switzerland). From June 2003, they were analyzed by original reagents of Beckman Coulter (Beckman Coulter, Inc., USA) applied on the system Synchron CX (Beckman Coulter, Inc., USA). MCV was evaluated using a hematological counter Pentra 60 (ABX Diagnostics, France) and CELL-DYN 1700 (Abbott Diagnostics, USA).

### Statistical analysis

Processing of the results was carried out using the following instruments of statistical analysis.

The Mann-Whitney U test was used for comparison of values between the two major diagnostic groups (AAP and ABP).

The diagnostic accuracy of values was carried out by ROC analysis. The area under the curve (AUC) of 1.0 designated 100% sensitivity and specificity of the test, and that of ≤0.5 designated the absence of discriminative power for the respective parameter (19).

After a logarithmic computation of the records, a correlation of Pearson was used for assessing the correlation between continuous variables, and logistic-regression analysis was carried out for the purpose of evaluating discrimination capabilities of different parameters between AAP and ABP (19, 20).

The Chi Square test was used in order to determine correlation between discrete variables.

Values of $P<0.05$ were accepted as statistically reliable.

### Results

Our results revealed higher values of serum amylase ($P=0.047$), AST ($P=0.019$), ALT ($P=0.001$) and the total bilirubin ($P=0.512$) in patients suffering from ABP, while higher levels of GGT ($P=0.128$), ALP ($P=0.721$) and MCV ($P=0.015$) were found in the group of patients with AAP (Table II). We established a correlation (under Pearson) between amylase and ALT ($r=0.29$, $P=0.041$) which is of moderate statistical significance, and a better correlation between ALT and GGT ($r=0.77$, $P=0.001$), ALT and ALP ($r=0.59$, $P=0.042$) and between ALT and total bilirubin ($r=0.41$, $P=0.002$).

We found correlation between sex and etiology ($P<0.0001$, Chi Square test) and no correlation between days of stay at the clinic and severity of the disease (number of deaths standing for severity) ($P=0.072$, Mann-Whitney U test).

After a logistic-regression and analytic check for the determination capability of values, AST ($P=0.013$) and particularly ALT ($P<0.0001$) revealed differentiating value as independent variables. Other parameters did not reveal the expected discriminative power for the assessment of diagnosis. A compilation of a logistic-regression model in the future, consisting of a combination of several parameters, would improve their importance in assessment of precise diagnosis of the disease.

We also performed ROC analysis in order to check the diagnostic accuracy of the different parameters in ABP and AAP (Table III). The analysis revealed higher values of AUC for MCV (AUC=0.744) and ALT (AUC=0.741), followed by AUC values for GGT (AUC=0.709) and AST (AUC=0.659). AUC for amylase also showed good results (AUC=0.643).

Cut-off values of MCV above 94 fl with a sensitivity of 0.46 and a specificity of 0.90 demarcated AAP from nonalcoholic forms of AP.

### Discussion

As a whole, the best capability for any discrimination between AAP and ABP, for the time being, is demonstrated by the enzyme ALT which is typically elevated in patients with ABP. Cytometric parameter MCV may be used to differentiate AAP at an early stage from other forms of AP. GGT requires additional research work in order to be enlisted in the model as a secure indicator. Results from total bilirubin and ALP were not satisfactory.

### Table II Medians/range of different laboratory parameters from patients with AAP, ABP and idiopathic AP are presented

<table>
<thead>
<tr>
<th></th>
<th>Amylase, U/L</th>
<th>ALT, U/L</th>
<th>AST, U/L</th>
<th>GGT, U/L</th>
<th>ALP, U/L</th>
<th>Total bilirubin, μmol/L</th>
<th>MCV, fl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP (n=58)</td>
<td>476/16–2058</td>
<td>60/7–485</td>
<td>69/8–520</td>
<td>123/13–1642</td>
<td>253/39–658</td>
<td>21/6.2–151</td>
<td>86/69–113</td>
</tr>
<tr>
<td>Idiopathic (n=28)</td>
<td>113/33–2755</td>
<td>24/24–582</td>
<td>24/9–594</td>
<td>48/12–566</td>
<td>169/91–225</td>
<td>20/5.8–139.87</td>
<td>88/73–113</td>
</tr>
</tbody>
</table>

### Table III AUC values±SE and statistical reliability of different parameters capable of discriminating ABP from AAP are presented

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>SE</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>0.643</td>
<td>0.065</td>
<td>0.064</td>
</tr>
<tr>
<td>ALT</td>
<td>0.741</td>
<td>0.054</td>
<td>0.001</td>
</tr>
<tr>
<td>AST</td>
<td>0.659</td>
<td>0.062</td>
<td>0.022</td>
</tr>
<tr>
<td>GGT</td>
<td>0.709</td>
<td>0.120</td>
<td>0.126</td>
</tr>
<tr>
<td>ALP</td>
<td>0.433</td>
<td>0.138</td>
<td>0.637</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>0.549</td>
<td>0.069</td>
<td>0.488</td>
</tr>
<tr>
<td>MCV</td>
<td>0.744</td>
<td>0.096</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Jordanov et al.: cytological investigations reveal direct influence of amylase and cholecystokinin (22). Biochemical and may induce a transitory production of pancreatic pathogenesis of AP.

Despite their intermediate character, these records bring attention to the role of alcohol in the pathogenesis of AP.

According to last reports, ingestion of ethanol may induce a transitory production of pancreatic amylase and cholecystokinin (22). Biochemical and cytological investigations reveal direct influence of ethanol on intrapancreatic activation of digestive enzymes through a sensitization of the acinus and liberation of cholecystokinin from the duodenal cells type I (23, 24). The impact of trypsin on this cascade is related rather to processes of degradation, than to activation of numerous proteases, including trypsin itself. Investigations on the same topic bring attention to the importance of mutagenic trypsin and its ability to induce hereditary forms of pancreatitis. Probably this enzyme plays a protective rather than a key role for the function of other proteases (23).

Mechanisms of the raise of serum amylase in patients with ABP are not so elucidated. It is accepted that the obstruction of the pancreatic duct and not biliary reflux is the important factor for the initiation of the disease (25). The impact of calcium ions for preliminary activation of digestive enzymes is already established in experimental conditions. This activation may be avoided by introduction of intracellular calcium chelator (26).

The pathogenesis of AP in general is a complex mixture of inherited and acquired circumstances that happen in the acinar cell and lay the foundations of organic dysfunction. According to new studies, various pro-inflammatory mediators are responsible for the acute destruction of pancreatic tissue and for the progression of focal and system reactions.

Disclosure from this character may initiate an alternative method of approach to the treatment of the disease (27). It is presumed that mild forms of AP are associated with a process of acino-cellular apoptosis, while severe forms are associated with acinar necrosis (28).

In conclusion, serum amylase continues to play a major role in the assessment of diagnosis and differential diagnosis. Early evaluation of MCV is important for the early elucidation of the etiology of AP. Future inclusion of ALT in a combination or as a ratio to other parameters would make it possible to identify microlithiasis in patients suffering from AP where it is impossible to assess etiology of the disease by usual imaging diagnostic procedures.

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**KLINIČKO-LABORATORIJSKI KRITERIJUMI ZA UPOREDNU PROCENU PACIJENATA KOJI BOLUJU OD AKUTNOG PANKREATITISA**

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**Kratka sadržaj:** Slovočka studija obuhvata 116 pacijenata (77 muškaraca, 39 žena) koji su primljeni na Drugu hiruršku kliniku Pirogov MBALSM bolnice (Višenamenska bolnica za aktivni tretman i urgentnu medicinu) u periodu od juna 2000. godine do novembra 2003. godine. Pacijenti su podeljeni u tri grupe: pacijenti sa prisutnim alkoholom izazvanim akutnim pankreatitismom (AAP) (n=30); pacijenti koji su patili od akutnog pankreatitisa biljarnog porekla (ABP) (n=58); i pacijenti koji su imali idiopatsku formu akutnog pankreatitisa (n=28). U prvi 24 sata nakon prijema na kliniku, u serumu su određivani amilaza, aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), alkalna fosfataza (ALP), gama-glutamil transferaza (GGT), ukupan bilirubin, srednji korpuskularni volumen (MCV). Ovi laboratorijski nalazi analizirani su uz upotrebu softverskih programa SPSS. Nn niosali su početnom atorskih metoda, a rezultati su bili obrađeni sa softverskim programom SPSS.

Kod pacijenata koji su patili od AAP, u serumu su određivane amilaza, aspartat aminotransferaza (AST), alanin aminotransferaza (ALT), alkalna fosfataza (ALP), gama-glutamil transferaza (GGT), ukupan bilirubin, i citometrijski indikator – srednji korpuskularni volumen (MCV). Ovo laboratorijski nalazi analizirani su uz pomoć rutinskih laboratorijskih metoda, a rezultati su bili obrađeni sa softverskim programom SPSS.

Kod pacijenata koji su patili od ABP nađene su više medijalne vrednosti amilaze (P=0,047), AST (P=0,019), i ALT (P=0,001) u serumu, u poređenju sa grupom koja je patila od AAP, u kojoj su bili prisutni viši nivoi GGT (P=0,015). U celery, amilaza, AST i naročito ALT u serumu nastavljaju da imaju glavnu ulogu u dijagnostičkoj i diferencijalno-dijagnostičkoj proceni AP. Uloga hematomakologskog parametra MCV je istovetna. Enzim akutnog idiopatskog pankreatitisa gde je nemoguće identifikovati osnovni bilijarni uzrok rutinskih imaginarnih dijagnostičkih procedura.

**Ključne reči:** akutni pankreatitis, enzimi, serum amilaza, klinička laboratorija
References


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