UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCTORAL SCHOOL

PHD THESIS
ABSTRACT

DIAGNOSTIC EFFICIENCY OF PARACLINICAL EXPLORATION IN ACUTE PANCREATITIS

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Content

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Introduction

Dieulafoy's "Great Abdominal Drama" - acute pancreatitis is a pathological entity with multiple difficulties to diagnosis, a multifactorial etiology and functional repercussions throughout the body.

In Romania, the incidence of this disease is of 30-50 per 100,000 inhabitants per year, with a proportion of 80% benign and 15-20% necrotic hemorrhagic, and a mortality of 30% - 40%. The pathophysiological mechanisms underpinning the onset of pancreatic autodigestion are incompletely elucidated, although advances in the etiopathogenicity of the disease have led to new therapeutic approaches with direct implications for disease progression.

I chose the aforementioned doctoral research theme because of both the epidemiological data and the use of paraclinical explorations in the diagnosis and follow-up of the evolution of this pathological entity, its importance in medical practice being undeniable. I have structured the scientific paper in two parts, respecting the current requirements in place regarding the writing of a doctoral thesis.

Part I. State of Knowledge

The first part is structured into three major chapters, which present an indepth documentation of the literature.

The first chapter outlines the pancreatic morphophysiological data, detailed in the bibliography (classical and modern). The second chapter focuses on the complex description of the acute pancreatic disease, both in terms of etiology, clinical manifestations, and appropriate therapeutic management. The last chapter is a review of the paraclinical explorations used to outline the positive diagnosis of pancreatic acute disease, using both laboratory, immunohistochemical and imaging determinations.

Part a II-a. Original Contributions

Aim and General Research Objectives

The second part of the thesis presents the contributions resulting from my own research, by structuring the purpose and objectives of the study, the description of the material and the study methods, with ulterior presentation and discussion of the results in the context of specialty literature.

The main purpose of our research was to evaluate the diagnostic efficiency of laboratory, imagistic and immunohistochemical tests in acute pancreatic inflammatory disease.

To achieve this goal, we have proposed the following objectives:

✓ Creation of a database by retrospective analysis of the observation sheets of patients with varying degrees of acute pancreatitis severity and of various etiologies: biliary, alcoholic, postoperative.

✓ Assessing the severity of acute pancreatic inflammatory disease by calculating specific multiparameter scores.

✓ Plasma level testing of procalcitonin in a subgroup of patients with acute pancreatitis as a marker of a grave prognosis and the presence of infection of the necrosis area.

✓ Creation of a reference batch obtained from pancreatic tissue specimens, harvested from patients with acute non-pancreatic acute abdominal surgery and subsequently processed by immunomarking.

✓ Establishing a group for studying changes induced by acute pancreatic inflammation, lymphocytic remodeling, and VEGF-A participation in these processes, as evidenced by immunomarking with specific monoclonal antibodies.

Study groups

Our retrospective research was conducted on a group of 70 patients diagnosed with acute pancreatitis, selected from the archives of the Craiova Emergency Clinical Hospital and the Emergency Military Hospital "Dr. Ștefan Odobleja "Craiova, and the information was collected in accordance with the rules and principles of the ethics committees of the above mentioned hospitals and of the University of Medicine and Pharmacy of Craiova, all of said institutions having agreed on the conduction of this research. The data obtained was recorded in such a manner that identification information (names, other identifying elements) was excluded.

A subgroup of the initial study group was made up of 20 patients, within the 2016-2017 interval, for whom the serum procalcitonin levels were evaluated.

The study groups set up to highlight the immunohistochemical and VEGF-A changes in acute pancreatitis were represented by:

The control group, made up 8 patients, with pancreatic tissue samples being collected from non - acute pancreatitis acute surgical abdomen patients within the Pathological Anatomy Laboratory of the Emergency County Hospital, Craiova.

One study group, with samples of pancreatic tissue taken from a total of 21 patients deceased due to acute pancreatitis. It was divided into two subgroups, depending on the date of death: one subgroup (AP1) made up of patients who died within the first two weeks of the diagnosis,

through multiple organ dysfunction syndrome (MODS), and a second subgroup (AP2), made up of patients who died in the following months, through extensive retroperitoneal necrosis and/or septicemia.

Study Work Method

For each patient involved in the present study, we performed the general objective exam, collected laboratory samples and established a positive diagnosis of the disease by corroborating clinical, laboratory and imaging data.

Laboratory tests performed were: evaluation of hemoleucogram components (leukocyte count, hemoglobin test, hematocrit determination), amylase concentration, glycemia, urea, serum creatinine, hepatic transaminases, total bilirubin, direct and indirect, procalcitonin.

The paraclinical exploration included ultrasound, computer tomography and nuclear magnetic resonance in Iodine allergic patients, the results being integrated into the clinical and laboratory context of each patient.

Multiparameter scores for assessing the severity of the disease and making correlations to predict the evolution of the pancreatic acute disease were also performed.

The data obtained from the tests carried out were analyzed and statistically processed using the appropriate methods.

In the case of the testing of lymphocytic remodeling and VEGF-A in pancreatic tissue, monoclonal anti-human mouse antibodies against CD20, CD3 and VEGF were utilized. Images were subsequently acquired with a Nikon 55i microscope and we captured a series of images for each case studied. The images were further processed with Image ProPlus ANS7 software, and with respect to the immunohistochemical expression analysis of VEGF-A, the images were analyzed from both the signal's point of view as well as the integrated optical density point of view (IOD). For the immunohistochemical expression of lymphocytes labeled with anti-CD3 and anti-CD20 antibodies, the percentage of cells immunized with the above-mentioned antibodies using the ProPlus AMS 7 software was calculated, by determining their percentage from the total pancreatic stroma or pancreatic parenchyma or both.

Test Results for Initial Study Group of 70 Acute Pancreatitis Patients

Our research aimed at and achieved the goal of assessing the diagnostic effectiveness of paraclinical tests in acute pancreatitis.

We tested most laboratory and imaging tests used in medical practice to determine the proportion and sensitivity of their changes within our studied group.

In summary, we found that the number of leukocytes exceeded normal limits in all patients in the group (100%), hemoglobin values were below 12mg / dl in the subgroup of cholecystectomy patients (12.857% of the group). The changes to hematocrit were similar, with low values (29.33%) also for the the cholecystectomy patients.

Amylase dosing, a test considered of major importance in the diagnosis of acute pancreatitis, revealed serum levels of the enzyme that exceeded the upper normal range by at least three times in all the study group patients (100%).

Glycemia, although in relation with the endocrine pancreas component, can indicate disorders in insulin secretion, involvement in tissue trophicity and even in digestive enzyme secretion alteration. For all these reasons we included glucose determination in the laboratory tests, performed when suspecting acute pancreatitis. In our group, abnormal levels were recorded in 47 patients (67.1%) on admission, of whom 32 were previously diagnosed with type II diabetes.

Taking into account the presence of a biliary disease as associated pathology, determination of liver transaminases - GOT and GPT - was performed in 40 patients, who at admission showed levels exceeding normal, those also included in the chronic microlithiasis cholecystitis subgroup showing the highest levels.

Gallbladder pathology, associated with acute pancreatic inflammation, includes within its laboratory testing range the determination of transaminase levels, along with serum determination of bile pigments. The laboratory tests range also includes the measurement of urea and serum creatinine, to highlight possible multiple organ failure, which requires the establishment of a complex therapeutic approach.

Patients included in the study group were also subjected to ultrasound and CT tomography, with 62 cases presenting pancreatic image alterations with varying degrees of complexity, 32 of which presented extrapancreatic extensions, demonstrating the presence of complications.

The existence of an extremely extensive range of tests for establishing the diagnosis of acute pancreatitis and its degree of severity, has led to the creation of multiparameter scores, the most useful of which being the Ranson score, modified by Imre, Marshall, Balthazar and the EPIC score, all of which use both the results of laboratory tests, initial and in evolution, as well as those provided by imaging exploration.

In our research, all four of the above mentioned scores were calculated, classifying patients in degrees severity for each score.

Procalcitonin dosed study group test results

Determination of procalcitonin plasma levels in 20 of the patients in the study group (8 with intermediate severe acute pancreatitis and 12 with the severe form) showed high values in 13 patients (65%), which corroborates with the presence of infected necrosis.

We analyzed the relationships between procalcitonin levels and multiparameteter scores calculated in acute pancreatitis to investigate organ dysfunction (Marshall, EPIC), or evolution type (Ranson), by using Pearson's r correlation coefficient. All values of correlation coefficients were highly significant ($p < 0.001$), the most important correlation being between Ranson score and procalcitonin ($r = 0.918$). The correlation with the EPIC score recorded a r value of 0.797, and a value of r of 0.736 with the Marshall score, respectively.

Immunohistochemical Test Results on Acute Pancreatitis Group in Comparison with Control Group

The percentage of T lymphocytes for control group patients was higher ($27.53 \pm 7.60\%$ for pancreatic stroma and $3.03 \pm 0.83\%$ for pancreatic parenchyma) than for patients in the AP1 subgroup ($18.4 \pm 7.22\%$ for pancreatic stroma and $2.67 \pm 0.70\%$ for pancreatic parenchyma) or patients in the AP2 subgroup ($19.02 \pm 6.51\%$ for pancreatic stroma and $2.48 \pm 0.54\%$ for pancreatic parenchyma).

The percentage of B lymphocytes in control patients was higher ($15.66 \pm 6.51\%$ for pancreatic stroma and $2.66 \pm 0.74\%$ for pancreatic parenchyma) than for patients in the AP1 subgroup ($8.52 \pm 7.06\%$ for pancreatic stroma and $2.08 \pm 0.89\%$ for pancreatic parenchyma) or in the case of patients in the AP2 subgroup ($8.15 \pm 6.22\%$ for pancreatic stroma and $2.23 \pm 0.58\%$ for pancreatic parenchyma).

By analyzing the expression of VEGF-A in both the pancreatic tissue of patients in the control group and in the group of patients deceased due to acute pancreatitis, we can state that it tends to emerge in the periphery of the acini, at the boundaries with the stromal connective tissue, while the islets show very little immunomarking for VEGF-A.

In regards to the signal area for VEGF-A, we found that it was smaller in the control group (4140.63 ± 943 μm^2) than in the AP patients group (4524.94 ± 745 μm^2), by a statistically significant difference ($p = 0.042$).

On the other hand, with regard to integrated optical density (IOD) for VEGF-A, it was found that it was lower in the control group (589937.2 ± 65469.47) than in the AP group (639784.9 ± 63208.13), a statistically significant difference being recorded ($p = 0.035$). On the other hand, in regards to the integrated optical density (IOD) for VEGF-A, we found that it

was smaller for the control group (589937.2 ± 65469.47) than in the AP group (639784.9 ± 63208.13), a statistically significant difference (p = 0.035) being recorded.

Conclusions

The interpretation of data, resulting from the corroboration of clinical and laboratory information, imaging and immunohistochemical tests, led to the following conclusions.

 - all patients included in the study group, 73 patients initially, out of which 3 were excluded for leaving the hospital unit, presented gallbladder associated pathology of chronic cholecystitis (30), chronic microlithiasis cholecystitis (31), and nine of them underwent cholecystectomy. Thus, bile disorders can be considered a predisposing factor for inflammation extension to the pancreas.

- of the complete blood count, the leukocyte count, the hematocrit and the hemoglobin concentration were determined within the first three days of admission. Evolutionary to notice their significant decrease.

- glycemia level variations marked a reduction on the second day of admission, for the entire patient group, in comparison to levels on admission.

- of particular importance for establishing the diagnosis of acute pancreatitis were the values of pancreatic amylase, which recorded increases above 3 times the normal limits, with statistically significant decreases compared to levels on admission, with a descending trend on both day two and day three.

- abdominal ultrasound represents an indispensable routine test in assessing patients with both biliary and pancreatic disorders; in our study it was performed on all patients, establishing microlithiasis etiology in a number of 31 cases, out of the 70 included in the study.

- the computer tomographic examination, of performed both on admission and in evolution, for the early confirmation of the inflammatory pancreatic reaction and the prediction of the emergence of complications, revealed a number of 38 cases of acute pancreatic disease, those from Balthazar grades C, D and E.

- corroborating the results of the laboratory tests, we calculated Imre's modified Ranson multiparameter score to assess the severity of acute pancreatitis and the following categories were obtained: acute pancreatitis with benign evolution, favorable prognosis (21.13%), intermediate-severe acute pancreatitis with risk of complications (50.70%), severe acute pancreatitis (25.13%) and 2.82% deceased patients. This score correlated with the hematocrit

value, with a statistically significant inverse correlation - Pearson coefficient r $=-0.339$, p <0.05.

Another score which defined the severity of acute pancreatitis was the Marshall score, which indicated organ dysfunction, which accounted for 73.24% of the cases with organ (or multiorgan) failure.

- the Balthazar and EPIC scores were calculated using imaging data as well. The following severity grades were obtained by calculating the Balthazar score: A (11.43%), B (34.29), C (24.29%), D (17.14%) and E (14.29%). The computer tomography severity index (EPIC) comprised of the following classes: mild (15.49%), 36.62% moderate, and 47.89% severe with complications.

- determination of plasma procalcitonin level in a sample of 20 patients of the study group showed that the normal parameter limit was exceeded in 65% of those tested, indicating the presence of an infected pancreatic necrosis and an unfavorable prognosis.

- the study of 21 pancreatic tissue samples collected from patients deceased due to acute pancreatitis (multiple organ failure, retroperitoneal extended necrosis and / or septicemia) by immunomarking with anti-CD3 monoclonal antibodies for T lymphocytes and anti CD20 for B lymphocytes showed a decrease in the number of T and B lymphocytes in the pancreatic stroma relative to the reference group (which included 8 pancreatic tissue specimens from patients deceased due to non-acute pancreatitis acute surgical abdomen), with a statistically significant difference of p = 0.013 and 0.016, respectively.

- VEGF-A immunomarking showed an absence of its expression in the endocrine pancreas islets and higher levels of immunomarking, compared to the reference group, in those with acute pancreatitis (p = 0.042). The increased immunohistochemical expression of VEGF-A may represent a target in the pathogenesis and evolution of acute pancreatitis and can be predictably associated with the pancreatic inflammation process.

- the original features of our study include: **the tracking and processing of laboratory and imaging data in evolution, as well as the immunohistochemical highlighting of lymphocytic remodelation and VEGF-A participation in changes to pancreatic components during the acute episodes.**

When establishing the diagnosis of acute pancreatic inflammatory disease, as well as the degree of severity of pancreatitis, it is not possible to only take a single parameter into account, but rather it is necessary to corroborate the multiple test parameters, whether grouped into scores or not, for a certainty diagnosis and grading in severity classes, allowing the

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establishment of appropriate therapeutical plans for every category and the development of prognosis.

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