

IMMUNOLOGICAL PATHWAYS IN THE DEVELOPMENT  
OF POSTOPERATIVE HYPERAMYLASEMIA AND  
POSTOPERATIVE PANCREATITIS – PRELIMINARY  
RESULTS

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**Abstract**

Postoperative pancreatitis complications can be separated into two main groups based on literature reviews and clinical and experimental trials. Some authors suggest that postoperative pancreatitis is a severe complication that should be treated conservatively and operatively if needed. Other authors defend an opposite opinion that only postoperative hyperamylasemia exists, which is a transitory condition with no risk of complications.

At “Alexandrovska” University Hospital for Active Treatment, Clinic of General and Liver-Pancreatic Surgery, a cohort of patients with increased amylase concentrations in the postoperative period between January 2017 and December 2018 were studied and followed with immunological and biochemical tests for interleukin (IL)-6. Regardless of the type of surgery to which they were subjected, all patients who presented with an elevated amylase concentration in the postoperative period were selected. The working hypotheses were the following: i) IL-6 is directly relevant in the development of postoperative pancreatitis; ii) the concentration of IL-6 is a prognostic factor in the early postoperative period and marks the boundary at which patients will develop postoperative pancreatitis and its various clinical manifestations; and iii) the postoperative period is accompanied by transient postoperative hyperamylasemia and absence of complications. To test these hypotheses, the values of IL-6 were statistically analyzed using SPSS v23 software.

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The results show that, with a 95% confidence interval (CI), a statistically significant difference is found in the concentration of IL-6 in patients who developed postoperative pancreatitis versus those with transient hyperamylasemia.

The present study shows a direct link between the immunological marker, IL-6, and the course of both postoperative pancreatitis and transient hyperamylasemia, making this marker valuable in elucidating the differences in the development of postoperative pancreatitis and transient hyperamylasemia in the early postoperative period. Thus, IL-6 can be used to reach the correct diagnosis and decide effective treatment.

**Key words:** postoperative pancreatitis, interleukin 6

**Introduction.** Postoperative pancreatitis is a severe complication in the postoperative period. Although postoperative pancreatitis develops in a pathway quite similar to an acute pancreatitis, there are a number of specifics in its etiology and evolution, which make the postoperative pancreatitis a matter of clinical interest and provides it with individual characteristics. The cases of postoperative inflammation of the pancreatic gland represent 10% of the entire amount of all cases of acute pancreatitis. Postoperative inflammation of the pancreatic gland rests upon various reasons dependent on immunological cell diversity of the pancreas, pro- and anti-inflammatory cytokines and biochemical reactions. In the early postoperative period another clinical event can be observed – postoperative hyperamylasemia. This is a transitory condition, connected with higher levels of serum amylase, which develops with no risk of complications. This versatile picture of clinical conditions, that can appear in the early postoperative period, is in direct connection with the great diversity of immune cells in the pancreas. There is a great structural difference in the immune cells in the exocrine and endocrine pancreas [1,2]. LICHANSKA and HUME [3] declared that the endocrine pancreas and the exocrine pancreas have pools of immune cells different in their origin and function. These cells secrete cytokines – interleukins like IL-12 and IL-6, which are pro-inflammatory, and of IL-10, which is anti-inflammatory [4-6].

IL-6 is a pro-inflammatory cytokine that is secreted by T lymphocytes and macrophages during infection, trauma, and tissue damage [7]. SATHYANARAYAN et al. [8] reported the major role of IL-6 in the development of postoperative pancreatitis. IL-6 is a predictor of the inflammatory process in the pancreas and is associated with the severity of inflammation. An increase in IL-6 blood levels can predict developing organ damage and organ insufficiency [7]. A change in the concentration of IL-6 is followed by the development of postoperative pancreatitis [8,9]. The IL-6 concentration increases during inflammation and declines during repair and regeneration.

Serum blood levels of interleukin IL-6 play a major role in the evolution of pancreatitis in the early postoperative period. Pancreatitis can progress as an acute inflammation and develop various clinical manifestations if pro-inflammatory interleukins are abundant [10,11].

Postoperative pancreatitis complications can be separated into two main groups based on literature reviews and clinical and experimental trials. Some authors suggest that postoperative pancreatitis is a severe complication that should be treated conservatively and operatively if needed. Other authors defend an opposite opinion that only postoperative hyperamylasemia exists, which is a transitory condition with no risk of complications. MORRISSEY et al. [12] state that approximately 10% of patients developed postoperative hyperamylasemia with no clinical complications after abdominal surgery. They suggest that this condition does not require active treatment. FRULLONI et al. [13] suggest that postoperative hyperamylasemia should be considered and treated as postoperative pancreatitis due to its ability to evolve.

The aim of the present study was to test and determine serum blood concentrations of IL-6 in the postoperative period in patients who received surgery on the abdomen. The study also aimed to determine the accuracy of immunological marker IL-6 to differentiate postoperative hyperamylasemia from postoperative pancreatitis.

**Materials and methods.** At “Alexandrovka” University Hospital for Active Treatment, Clinic of General and Liver-Pancreatic Surgery, a cohort of patients with increased amylase concentrations in the postoperative period between January 2017 and December 2018 were studied and followed with immunological and biochemical tests for interleukin IL-6. Regardless of the type of abdominal surgery to which they were subjected, all patients who presented with an elevated amylase concentration in the postoperative period were selected. The study protocol and ethics approval were postulated by a clinical council of the Department of General and Operative Surgery, Medical University Sofia, which serves as a scientific-ethical committee for the Clinic of General and Liver-Pancreatic Surgery at the “Alexandrovka” University Hospital for Active Treatment. The clinical council stated that immunological and biochemical tests for interleukin IL-6 are standard procedures in the diagnostic and treatment process at the Clinic of General and Liver-Pancreatic Surgery and do not need a specific ethics approval.

Two standard tubes of peripheral venous blood were taken from each patient. The tubes were of the SST type (serum with gel and clot activator) for the separation of serum from blood components. Both tubes were centrifuged at 10 000 rpm using an ultracentrifuge for 15 min. Serum supernatant was dispensed into 2-ml Eppendorf tubes and frozen immediately at  $-82^{\circ}\text{C}$ . Two tubes of serum were frozen from each patient to measure IL-6. Enzyme-linked immunosorbent assay kits were used to perform the IL assay, to evaluate the serum concentrations of IL-6 in a total of 78 patients. Patients were tested for IL-6 following all established protocols, norms and standards for human IL-6 enzyme-linked immunosorbent assay as given by the ELISA kits provider authority – eBioscience.

**Results.** Figure 1 shows the concentration of IL-6 in pg/ml. In most patients, the IL concentration increased above normal. A normal concentration of IL-6

(when measured in serum using an SST tube with a blot activator) ranged from 00.0 to 12.7 pg/ml (Fig. 1).

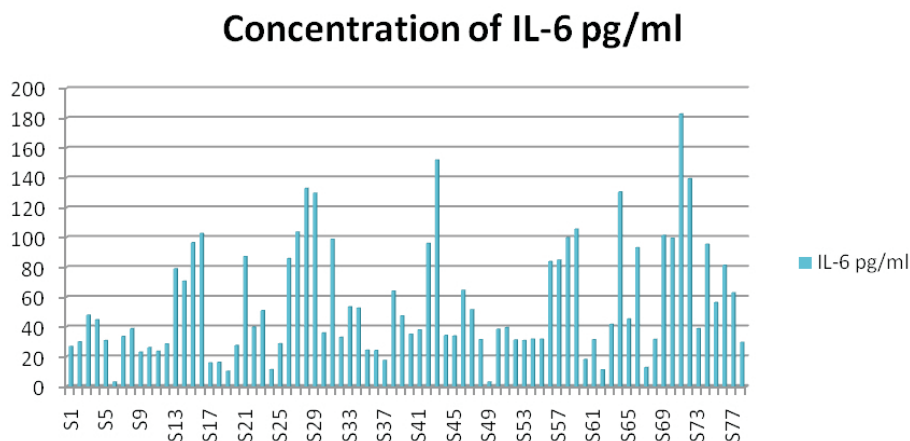


Fig. 1. Concentration of IL-6 pg/ml

In seven patients, the concentration of IL-6 remained within the normal range. In the clinical follow up, these seven patients did not develop acute postoperative pancreatitis, and no complications emerged. The remaining 71 patients developed complications affecting the gastrointestinal, cardiovascular, pulmonary, or excretory systems, all of which are clinical features of postoperative pancreatitis.

In patients, when the absolute concentrations of IL-6 were greater than the upper limit of the nominal, the clinical development of postoperative pancreatitis and subsequent complications with varying severities were observed.

The following working hypotheses were formulated: i) IL-6 is directly relevant in the development of postoperative pancreatitis; ii) the concentration of IL-6 is a prognostic factor in the early postoperative period and marks the boundary at which patients will develop postoperative pancreatitis and its various clinical manifestations; iii) the postoperative period is accompanied by transient postoperative hyperamylasemia and absence of complications. To test these hypotheses, the value of interleukin IL-6 was statistically analyzed using SPSS v23 software.

Checking whether there is a statistically significant difference between the concentrations of IL-6 in patients who developed postoperative pancreatitis and those with transient hyperamylasemia was necessary. In essence, this is a statistical hypothesis test that can be confirmed or rejected with a reasonable certainty.

H0: No difference exists in the mean concentrations of IL-6 in patients with postoperative pancreatitis versus those with transient hyperamylasemia.

H1: A difference is present in the mean concentrations of IL-6 in sick versus healthy patients.

As already stated, the risk of error with which the hypothesis was tested was 5%. In order to determine the hypothesis test method, it is necessary to verify

that the two conditions for applying a parametric test method are fulfilled. Since IL-6 is quantified, only the second condition (i.e., normality of distribution) was verified. Two hypotheses for normality of distribution were stated:

H0: The distribution of IL-6 is normal.

H1: The distribution of IL-6 is different from normal.

The level of significance in both tests was less than the error of 0.05 (Table 1).

T a b l e 1

Statistical test for normality: Kolmogorov–Smirnov<sup>a</sup> and Shapiro–Wilk tests

Tests of Normality						
	Kolmogorov–Smirnov <sup>a</sup>			Shapiro–Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
IL-6	.182	78	.000	.892	78	<b>.000</b>

<sup>a</sup> Lilliefors Significance Correction

Therefore, the alternative hypothesis, which states that the distribution of IL is not normal, was accepted. This conclusion can be argued with a 95% probability. Since one of the two conditions for applying a parametric method was not fulfilled, a non-parametric method was used for verification. The applied statistical method is a check of the difference between two averages of two independent groups (1st, patients who developed postoperative pancreatitis; 2nd, patients with transient hyperamylasemia), the method used was the Mann–Whitney U test.

Test results for the difference between patients who developed postoperative pancreatitis and those with transient hyperamylasemia are presented in Table 2.

T a b l e 2

Statistics with the Mann–Whitney U test

Group Statistics					
	Clinical Status	N	Mean	Std. Deviation	Std. Error Mean
IL-6	postoperative pancreatitis	71	58.3009	38.85991	4.61182
	postoperative hyperamylasemia	7	25.8449	17.19951	6.50080

The results of the hypothesis test show that the level of significance was  $< 0.05$ , which means that both of the ILs are considered to be valid alternative hypotheses.

With a 95% confidence interval, a statistically significant difference exists in IL-6 concentration between patients who developed postoperative pancreatitis and those with transient hyperamylasemia.

Subsequently, hypotheses were designed to test for a correlation between the immunological marker IL-6 and its value in predicting the pattern of postoperative pancreatitis and transient hyperamylasemia.

As was statistically proven, the clinical course of the postoperative period was smooth with the manifestation of transient hyperamylasemia only in patients with low IL-6. Contrary to the above, in patients with a high concentration of IL-6, the postoperative period was clinically mediated by postoperative pancreatitis and its various clinical manifestations.

**Discussion.** The present study shows a direct link between the immunological marker IL-6 serum levels and the pathway of development of postoperative pancreatitis. Statistically proven IL-6 serum levels predict postoperative pancreatitis progression as an acute inflammation and development of various clinical manifestations, or progression into a transient postoperative hyperamylasemia with absence of complications. Results show that, with a very high confidence interval (CI) statistically, IL-6 is a predictor of the inflammatory process in the pancreas and is associated with the severity of inflammation. An increase in IL-6 blood levels can predict developing organ damage [7-9]. The results from that clinical trial confirm other authors' results, and also elucidate the direct connection between clinical evolution of postoperative pancreatitis and IL-6 serum levels. Postoperative pancreatitis is a complex process with many cytokines involved in its regulation. IL-6 plays a major role in pancreatitis development, but as every cell transduction mechanism it is a sophisticated process with a lot of cytokines involved in it. So this is only a preliminary research and more studies should follow to elucidate the great variety of cytokines on which evolution of postoperative pancreatitis is dependent. Acquired results will elucidate a better understanding of postoperative pancreatitis evolution, and also help in optimizing treatment process by early prediction of development of various clinical manifestations and complications.

**Conclusion.** The present study shows a direct link between the immunological marker IL-6 and the evolution of postoperative pancreatitis and transient hyperamylasemia. With a 95% confidence interval, a statistically significant difference was found between patients who developed postoperative pancreatitis and those with transient hyperamylasemia in the IL-6 study. These results show that IL-6 can be used to clinically elucidate differences in the development of postoperative pancreatitis and transient hyperamylasemia in the early postoperative period; thus, these markers can aid correct diagnosis and effective treatment.

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