Diagnostic Marker of Interleukin 6 in Acute Pancreatitis

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Abstract

Cytokines exert their action by binding to specific receptors on the cell surface. The experimental model of acute pancreatitis has conclusively shown that TNF-α (tumor necrosis factor), IL-1 (interleukin) and IL-6 are also produced outside the pancreatic parenchyma. This phenomenon occurs one hour after the induction of acute pancreatitis, being accompanied by appreciable histological changes in the pancreas. IL-1 and TNF-α have as primary inducers the production of IL-6 and IL-8 and both occur systematically in acute pancreatitis. IL-6 is the major cytokine mediator of the acute phase response and is produced by monocytes, macrophages and endothelial cells. IL-6 is a measure of systemically activated pro-inflammatory cytokines. The serum level of IL-6 also reflects the severity of acute pancreatitis, its growth precedes that of C-reactive protein by 24-36 hours. IL-8 is the secondary mediator of TNF-α-induced neutrophil activation. It is a chemotactic factor that attracts neutrophils and plays a significant role in the development of MODS (multiple organ dysfunction), especially in acute pulmonary injury, associated with sepsis.

Keywords: Cytokines, pro-inflammatory, neutrophils, primary inducers

INTRODUCTION

Pathophysiological and molecular research has led to the understanding of the primary events that take place in triggering acute pancreatitis, although early diagnosis in pancreatic diseases, in general, continues to be a source of frustration in modern medicine. It presents the news in pathogenesis (co-localization theory, trypsinogen auto-activation theory), location of early events (acinar pancreatic cells being the "key" elements involved: muscarinic receptors, acinar membrane, the role of ionized calcium, the apoptosis) [1-3], extracellular events in the initiation of acute pancreatitis with a central place to enzyme activation and then to the systemic inflammatory response. Aspects related to early microvascular changes, dysfunctions of the ischemia-reperfusion injury and systemic microvascular abnormalities are so important that they justify the therapeutic concept of microcirculation protection [4,5]. The participation of the monocyte/macrophage system, the excessive activation of leukocytes involving the release and activation of lysosomal enzymes and of oxygen free radicals associated with the mechanism of the ischemia-reperfusion injury are defining for the pathogenesis of acute pancreatitis.

There are three factors that are generally involved in pathogenesis: intrapancreatic activation of the digestive enzymes, excessive stimulation of the inflammatory cells and vascular phenomena. Physiologically, zymogen granules are secreted by exocytosis into the excretory duct of the pancreas, and from here into the duodenal lumen where enterokinase converts trypsinogen into trypsin which, in turn, further activates other zymogens [6,7].

According to the co-localization theory, there is a premature fusion between the lysosomes and the zymogen granules, resulting in the phenomenon of crinophagy that triggers the activation of trypsinogen. Excessive trypsin disrupts the protease-antiprotease balance by consumption of specific (PSTI) and nonspecific trypsin inhibitors (α1-antitrypsin and α2-macroglobulin). This activates other zymogens (chymotrypsinogen, proelastase, phospholipase) as well as various protease systems (complement, kinin, coagulation and fibrinolytic factors) triggering a strong inflammatory reaction. The release of different mediators (platelet activating factor - PAF, cytokines, prostaglandins, leukotriene) stimulates the production of acute-phase proteins (endogenous antiproteases, C-reactive protein - CPR) and the activation of granulocytes and macrophages in the pancreas and peripancreatic level [8,9]. Cellular degradation results in the release of proteolytic and lipolytic enzymes (polymorphonuclear elastase-PMN-e), IL-6 generating free oxygen radicals in excess beyond the natural power of neutralization [10]. These cascade activations triggered in the acinar cells, rapidly encompass the entire pancreatic gland as well as the peripancreatic region. Secondary transport in the systemic circulation of many substances produced during the inflammatory reaction will lead to distant complications: cardiac circulatory failure, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation, acute renal failure, multiple organ dysfunction (MODS). These pathophysiological data enhance our understanding of the interest given in the dosage of biological markers (CPR, interleukins, PMN-e, phospholipase A2). Undoubtedly, the kinetics of the appearance of these markers represents a progress for early diagnosis and severity assessment, prognosis and rapid application of the appropriate therapeutic measures [11,12].

MATERIAL AND METHODS

A prospective study was used in order to achieve the proposed objectives that of determining the importance of the laboratory tests and of defining interleukin-6 as an early predictive marker in acute appendicitis.

In this regard, a group of 90 patients diagnosed with acute pancreatitis was created. All patients were hospitalized during 2021 in the surgical units of the County Emergency Clinical Hospital Oradea.

Determining interleukin- 6

The major actions of interleukin 6 (IL-6) on lymphoid and non-lymphoid cells are modulatory mechanisms of the body's immune and inflammatory responses. Although many of these functions overlap with those of type 1 interleukins (IL-1), such as the synthesis of acute phase reactants and fever, IL-6 also has anti-inflammatory effects. The IL-6-specific receptor (IL-6R) belongs to the (haematopoietic) cytokine receptor superfamily. IL-6R is a membrane protein complex consisting of two structural and functional subunits: a specific 80-kDa IL-6 binding protein (α chain) and a signal transducer, gp130 (b chain, a component for several types of receptors, such as IL-11, IL-27, IL-31). Cytokine IL-6 is secreted as a polypeptide consisting of 184 amino acids, with a molecular weight of about 21 kDa, depending on the degree of glycosylation. Like IL-1, IL-6 is secreted mainly by macrophages, being also synthesized by T and B lymphocytes, fibroblasts and endothelial cells, keratinocytes, synoviocytes, chondrocytes, epithelial cells. Thus, IL-6 is produced in response to bacterial and viral infections, inflammation or trauma, rapidly reaching detectable plasma levels unlike many other cytokines. Cytokine IL-6 is considered the major mediator for the hepatic production of acute-phase reactants: fibrinogen, serum amyloid A, haptoglobin, C-reactive protein, complement. Following exposure to IL-6, the liver decreases the albumin and transferrin synthesis, initiating processes of hepatocyte regeneration instead. Cytokine IL-6 stimulates humoral and cellular immune responses by acting on both B and T lymphocytes. IL-6 plays an important role in the differentiation and growth of the B cells and stimulates their production of immunoglobulins. It also promotes T cell activation, growth and differentiation. It is involved in the pathogenesis of multiple myeloma, being used as prognostic factor of the disease. IL-6 stimulates haematopoiesis (acts synergistically with IL-3), induces the secretion of ACTH and other pituitary hormones (prolactin, growth hormone, luteinising hormone). In addition to its pro-inflammatory effects, IL-6 also mediates several anti-inflammatory effects: while IL-1 and TNF mutually induce their synthesis as well as that of IL-6, IL-6 completes this inflammatory cascade as it inhibits the synthesis of both IL-1 and TNF while stimulating the synthesis of IL-1RA.

Recommendations for the determination of IL-6

Interleukin titres determined in various biological fluids may be used to diagnose immune disorders and to monitor treatments only when correlated with complementary clinical and paraclinical data.

Preparing the patient - fasting (in a fasting state) or postprandial

Collected sample - venous blood

Collecting tube - vacutainer with no anticoagulants, with/without separator gel4.

Collected quantity - minimum 0.5 mL serum

Causes of sample rejection - intensely hemolyzed, jaundiced, lipemic or bacterially contaminated sample; samples that did not arrive frozen at the laboratory

Processing after collection - the serum is separated by centrifugation as soon as possible after complete coagulation and the sample shall be immediately frozen at -20°C; samples collected outside laboratory points shall be transported in the container for frozen samples.

Sample stability – the serum is stable for one month at -20°C; do not defrost/refreeze.

Method - the immunochemical method with chemiluminescence detection (CLIA)

Reference values - <3.8 pg / mL4.

Interpretation of results

Increased levels of the marker are found in: rheumatoid arthritis; multiple myeloma (prognostic factor); autoimmune diseases (lymphomas); sepsis (AIDS); alcoholic liver disease; viral infections; transplant rejection; severe preeclampsia.

RESULTS

Bioclinical examinations were recorded in 90 patients (33.5%), 45 cases from every stage of severity, 30.2% representing mild acute pancreatitis and 37.5% severe acute pancreatitis (Figure 1).

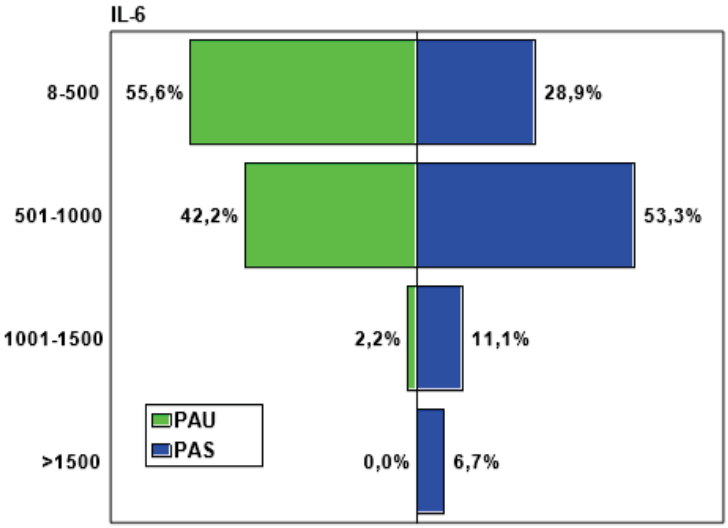

Figure 1. Distribution of cases in terms of severity of acute pancreatitis and IL-6. PUA=Mild acute pancreatitis; PAS=Severe acute pancreatitis

As far as the biological markers are concerned, the progress made in the field of prognostic evolution of acute appendicitis is based on the current knowledge of the pathophysiological events that appear during the evolution of the disease. They represent a priority in the current medical research due to their role in human pathology, in general, and due to the fact that these bioactive compounds may become therapeutic targets. The ideal marker should be an objective indicator and non-observer-dependent, simple, fast and cheap, safe and non-invasive, not influenced by comorbidities, with a high positive predictive value and usable in the first 24-48 hours after the onset of the disease.

There are significant differences between the two forms of acute pancreatitis in terms of IL-6 ($p < 0.001$). Thus, the number of normal values of IL-6 is almost two times higher in patients with mild acute pancreatitis than in patients with severe acute pancreatitis (55.6% versus 28.9%) and the mean value at mild acute pancreatitis is within normal limits (425.0 pg/ml), while in the group with severe acute pancreatitis the value is significantly increased (733.0 pg/ml).

DISCUSSIONS

Interleukin 6 (IL-6) is a pro-inflammatory cytokine synthesized by a wide variety of cells, including the periacinar fibroblasts, under the action of TNFα and IL-1β. It is the major mediator of acute-phase protein synthesis. Serum concentration precedes the growth of CRP by 24-36 hours and evolves in parallel with it [13,14]. The experimental evidence showed that IL-6 overexpression induces severe forms of acute pancreatitis and its counteraction with

monoclonal antibodies has protective effects. In human acute pancreatitis, the increased values were correlated with complicated and lethal forms of the disease. Recently, it has been reported that IL-6 is the best prognostic marker of respiratory failure [15-17]. There is an 18- to 48-hr latency, with maximum values in the first two days of illness, a period in which the discrimination between mild and severe forms of acute pancreatitis is maximum, comparable to that of IL-8. However, increased levels in concentration were observed in burns, trauma and elective abdominal surgery [18-20]. Despite all these shortcomings and contradictions, IL-6 remains one of the most widely used biological markers for the early detection of the severity of acute pancreatitis.

It is assumed that cytokines play an important role in the pathogenesis of acute pancreatitis, but little is actually known. Interleukin-6 (IL-6) exerts a wide spectrum of regulatory activities in immune and inflammatory responses [21-23].

Salvatore Cuzzocrea et al. conducted a study that investigated the role of endogenous IL-6 in the inflammatory response associated with acute pancreatitis. Thus, it has been demonstrated that endogenous IL-6 has an anti-inflammatory role in acute pancreatitis, through the activation and adhesion of neutrophils and the generation of cytokines, as well as reactive oxygen and nitrogen species.

In their study, Ohmoto Kenji and Yamamoto Shinichiro showed significant correlation between serum IL-6 level and the markers for predicting severity in acute pancreatitis, suggesting that IL-6 was a useful indicator of the severity of this disease [25].

CONCLUSIONS

The predominant pathological state of the patients with severe acute pancreatitis may be altered from the systemic inflammatory response syndrome to the compensatory anti-inflammatory response syndrome by successful treatment. There are significant differences between the two forms of acute pancreatitis in terms of IL-6 ($p < 0.001$). The number of normal values of IL-6 is almost two times higher in patients with mild acute pancreatitis than in patients with severe acute pancreatitis (55.6% versus 28.9%) and the mean value at mild acute pancreatitis is within normal limits (425.0 pg/ml), while in the group with severe acute pancreatitis the value is significantly increased (733.0 pg/ml).

Inflammatory markers appear in the serum later than the preceding ones. They offer the advantage of being able to monitor the clinical progression and provide complementary opportunities.

These biochemical parameters show promise and need to be evaluated prospectively in large patient cohorts, including a significant proportion of severe cases.

Apart from their diagnostic role, these new markers can become therapeutic targets, with the possibility of preventing or mitigating the severity of acute pancreatitis (AP).

Future hopes are tied to markers of pancreatic injury and inflammation, especially cytokines, provided that a dosing methodology is developed to make them useful in emergency conditions.

Genetic manipulation holds a new promise in identifying markers of severity in AP.

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