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DIAGNOSTIC VALUE OF INFLAMMATORY MARKERS IN PATIENTS WITH ACUTE PANCREATITIS

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Introduction: Acute pancreatitis (AP) is a sudden inflammatory reaction that causes autodigestion of the pancreas, edema, bleeding, and can lead to pancreatic necrosis and necrosis of the surrounding tissue. Since the initial symptoms of mild, moderate and severe pancreatitis are the same, doctors often cannot determine the severity of AP with certainty based on the first examination. Aim of the work: Numerous biomarkers have been studied as potential early predictors of the severity of this disease, so that treatment can be optimally adapted to prevent complications. The aim of the paper is to provide an overview of the most important inflammatory markers that are used, or can potentially be used to determine the severity of acute pancreatitis. Inflammatory markers: Markers of inflammation in AP are: the hormone procalcitonin, then reactants of the acute phase such as C-reactive protein, serum amyloid A, pentraxin 3; enzymes: polymorphonuclear elastase, phospholipase A2, myeloperoxidase; cytokines: interleukins (IL-6, IL-8, IL-17) and tumor necrosis factor (TNF-α). Conclusion: The most frequently determined parameter in clinical practice is CRP, as a non-specific marker of inflammatory diseases. The disadvantage in determining this parameter is that the maximum serum value is reached only 72 hours after the onset of AP symptoms. Numerous biomarkers have proven to be more sensitive for determining the severity of AP, of which procalcitonin stands out, which has been widely used in recent years, for the early prognosis of the development of local complications and multiorgan failure in AP. Cytokine determination is increasingly part of clinical practice. The most commonly used IL-6 is a sensitive and specific marker for predicting organ failure in severe AP.

Key words: acute pancreatitis; inflammatory marker; procalcitonin; acute phase reactants; enzymes; cytokines

INTRODUCTION:

According to the recent classification of acute pancreatitis, there is a division into interstitial pancreatitis (with diffuse enlargement of the pancreas and inflammatory edema, without the signs of necrosis) and necrotic pancreatitis, which is further subclassified into sterile and infectious [2]. Pancreatic necrosis during acute pancreatitis is a key factor predicting outcome, and infection of the necrotic tissue is a serious complication in severe acute pancreatitis. Additionally, intestinal barrier dysfunction leads to infected necrosis, bacteremia, and multiorgan failure [3]. Severity of AP can be classified into three types, mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP) and severe acute pancreatitis (SAP) [1]. The severity of AP was observed to be related to the type and degree of cell death: severe AP was associated with extensive necrosis of acinar cells, while mild AP showed extensive apoptotic cell death and a minimal degree of necrosis. Therefore, apoptosis is interpreted as a beneficial cell response to an injury, and inducing apoptosis is an effective strategy to reduce the severity of experimental pancreatitis. A newly discovered modality of cell death is necroptosis, which is characterized by both necrosis and apoptosis, namely, it is actively regulated by special genes, and has typical morphological characteristics of necrosis. Necroptosis is gradually becoming an important topic in the field of inflammatory diseases [4]. A variety of agents can cause injury to pancreatic acinar cells. Activation of mononuclear macrophages leads to the activation of neutrophil leukocytes, which further release large amounts of inflammatory mediators responsible for inflammatory effects, by a cascade mechanism. Loss of local control leads to excessive uncontrolled activation ofinflammatory cells and mediators. Proinflammatory cytokines are released through the portal vein and reach the circulation through

the lymph [5]. Mild forms of inflammation of the pancreas, go away within three to four days with adequate therapy, and usually without any consequences. However, the initial symptoms of mild, moderate and severe inflammation are the same, so doctors cannot determine the form based on the first examination. Typical laboratory findings in AP are increased parameters of inflammation, as well as increased values of pancreatic amylase and lipase. According to most guidelines, lipase is more reliable parameter than amylase [6]. The reliability of determination of amylase also depends on the time when the sample is taken.

THE AIM OF THE WORK

Numerous biomarkers have been studied as potential early predictors of the severity of this disease, so that treatment can be optimally adapted to prevent complications. Early identification of patients who could potentially developing severe acute pancreatitis would allow selection of patients for early intensive treatment. Accordingly, the aim of the paper is to provide an overview of the most important inflammatory markers that are used, or can potentially be used to determine the severity of acute pancreatitis.

INFLAMMATORY MARKERS

Aninflammatory reaction is triggered at the site of pancreatic damage and can lead to systemic inflammatory response syndrome (SIRS), which is ultimately responsible for most morbidity and mortality [7]. It is known that extensive damage and necrosis of the pancreas lead to the activation of enzymes - proteases that can cause damage to blood vessels, resulting in hypovolemia, hypotension, increased intra-abdominal pressure and kidney damage. Damage of pancreatic acinar cells stimulates the release of cytokines and the generation of free radicals [8]. Accordingly, it is necessary to detect and determine inflammatory markers whose serum levels are correlated with the degree of pancreatic damage. This article gives a review of some of the most important inflammatory markers of AP.

Procalcitonin (PCT) is a prohormone of calcitonin, and under physiological conditions it is created only in C-cells of the thyroid gland. In pathological conditions, it is also produced by extrathyroidal tissues, such as the liver, lungs, monocyte-macrophage system [9]. In healthy individuals, the level of PCT in the plasma is very low, practically unmeasurable, since active calcitonin is secreted into the blood after its proteolytic breakdown. Elevated PCT values may indicate the presence of a bacterial infection. Serum PCT values increase already 2-4 hours after the onset of the infection, which makes it a potential biomarker for monitoring pathological conditions caused by bacteria - pneumonia, lower respiratory tract infections, abdominal sepsis, urosepsis, myocardial infarction [10]. It has been shown that the development of infectious necrosis of the pancreas in patients with acute pancreatitis can be predicted by PCT values, and accordingly, antibiotic therapy can be applied [11]. A serum PCT value of 3.8 ng/ml or more, within 96 h of symptom-onset indicates pancreatic necrosis with a sensitivity and specificity of 93% and 79%, respectively [12]. The determination of serum procalcitonin has been widely used in recent years, for the early prognosis of the development of local complications and multiorgan failure in AP.

C-reactive protein (CRP) is an acute phase protein. It is a non-specific and most commonly used marker of inflammatory diseases. It is used routinely in clinical practice to assess the severity of acute pancreatitis [1]. Determination of CRP concentration has several advantages, such as accuracy, simplicity, accessibility and relatively low cost. The main limitation of determining this parameter is reflected in the time required for the serum concentration to be optimal - 72 hours from the onset of symptoms [13]. CRP values above 210 mg/ml were used to determine moderate and severe AP, with a sensitivity of 83% and a specificity of 85% [14]. CRP is considered a significant individual indicator of pancreatic necrosis due to the availability of determination of this parameter in clinical practice.

Serum amyloid A is a family of acute-phase proteins synthesized in the liver as response to trauma and inflammation of the tissue. They participate as mediators in cellular communication, within the immune response, acting as propagators of the initiated acute immune response [15]. Research has shown that it can be a more sensitive marker of inflammation than CRP [16]. However, the results of a study of a German center, using a different immune assay in a population that also included healthy subjects and patients with chronic pancreatitis and malignancy, did not support these findings [16].

Phospholipase A2 (PLA2) belongs to a family of enzymes that hydrolyze phospholipids. Apart from the digestive function in the intestinal tract, phospholipase A2 participates in the metabolism of cell membrane phospholipids, including prostaglandin synthesis, transmission of cell signals and metabolism of serum lipoproteins. It has been assumed that activation and release of PLA2 in acute pancreatitis is not only responsible for tissue necrosis associated with pancreatic autodigestion, but is also associated with the development of pulmonary complications [17]. Animal studies have shown that PLA2 can damage dipalmitoyl phosphatidylcholine, a phospholipid that is part of lung surfactant, thus causing alveolar collapse [18].

Polymorphonuclear elastases (PMN-elastases) are enzymes released from polymorphonuclear leukocytes (neutrophils, basophils, eosinophils). It is a sensitive marker of inflammatory diseases, considering that during inflammation their excessive release occurs. In acute pancreatitis, the maximum concentration of this parameter is reached on the first day of the disease, earlier than CRP. One study, showed the importance of values of PMN-elastase levels in plasma for early prognosis of AP severity in clinical practice, with a sensitivity of 92% and a specificity of 91% for the value of 110 mg/L, in the period from 24 to 72 hours from the onset of the disease [19]. Although PMN-elastase could be relevant for assessing the severity of AP, determination of this parameter has not been introduced into routine laboratory use due to assay-related problems, with non-reproducible test results.

Pentraxin 3 (PTX3) is an acute phase protein. It is synthesized and released by macrophages, monocytes and dendritic cells, in response to stimulation by lipopolysaccharide or proinflammatory cytokines. Some studies [20,21] have shown that elevated values of PTX3 correlate with the severity of AP, that the values of this parameter increase in the early phase of AP, and correlate with the values of interleukin-6 (IL-6), a marker whose importance will be explained further. The determination of PTX3 is not yet suitable for clinical use because its concentration can currently only be measured by enzyme-linked immunosorbent assay (ELISA), a relatively expensive method.

Myeloperoxidase (MPO) is an enzyme primarily released by activated neutrophils and is thought to be involved in the body's immune response during inflammation. Excessive release of this enzyme leads to tissue damage, as demonstrated in studies of experimentally induced AP. It is believed that this enzyme has a role in the development of complications on the lungs, considering that the activity of the enzyme has been identified in the lung parenchyma in patients with AP [20].

Cytokines. As previously explained, acute pancreatitis results in excessive activation of leukocytes and increased migration of neutrophils to the inflammatory area with consequent release of pro-inflammatory cytokines. As mediators, they are thought to be involved in the progression of pancreatic infection to necrosis, which subsequently leads to SIRS and multiorgan dysfunction.

Interleukin-6 (IL-6) is an important inflammatory mediator of the acute phase response that may also be significant in assessing the severity of acute pancreatitis. Experimental studies have shown that interleukin-6 induces the production of major acute phase proteins in the liver, including C-reactive protein, serum amyloid A (SAA), haptoglobin, antichymotrypsin, fibrinogen and hepcidin, while inhibiting albumin production [22]. One study showed that elevated levels of IL-6 were detected in 93% of patients on days 3 and 7 of AP. Serum levels of IL-6 were significantly higher in severe pancreatitis compared to mild pancreatitis at day 3 but not at day 7 [23]. One study showed that a serum levels of IL-6 on day 3 of AP are higher than 160 pg/ml indicates a persistent SIRS and potential organ failure [24]. The prediction of severe pancreatitis is very useful for the prognosis of the disease and the decision to transfer patients with suspected severe pancreatitis to the intensive care unit. Accordingly, IL-6 is a sensitive and specific marker for predicting organ failure in severe AP.

Interleukin-8 (IL-8) is a proinflammatory cytokine, released by activated macrophages or endothelial cells. It belongs to the family of chemokines, molecules involved in chemotaxis, activation and degranulation of neutrophils. In a 2009 meta-analysis, IL-6 was shown to have a sensitivity of 83.6% and a specificity of 75.6%, in contrast to a sensitivity of 65.8% and a specificity of 66.5% shown by IL -8. These values suggest that IL-6 is of greater diagnostic value on the first day. However, IL-6 sensitivity appeared to decline slightly over time with

values of 72.1% on day 2 and 81.0% on day 3, although this decline is not statistically significant. The positive likelihood ratio of IL-8 is significantly higher on the second day compared to the values calculated on the first day. This may be of importance in clinical practice, as it showed that this relationship suggests that patients with higher levels of IL-8 on the second day are about 8 times as likely to have a severe course compared to patients with lower levels of IL-8 [25].

Interleukin-17 (IL-17) is a proinflammatory cytokine secreted by activated T-lymphocytes. The most important representative of the IL-17 family is IL-17A, which is produced by activated memory T lymphocytes. It plays a role in stimulating innate immune response. During AP, cellular damage caused by pancreatic autodigestion can cause the activation and aggregation of IL-17-producing CD4+ T helper lymphocytes and stimulate the inflammatory response that is characteristic of this disease. Some studies have shown that IL-17A regulates the transcription of proinflammatory cytokines or chemokines that mobilize neutrophils in acute inflammatory diseases [26]. Compared to healthy controls, AP patients had a significant increase in IL-17 during the first 24 hours, with a positive predictive value of 85.3% [27]. Given its potential prognostic value, IL-17 is considered a promising inflammatory marker of AP.

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine produced by macrophages and which plays one of the main roles in multiple pathophysiological responses to injury and damage [7]. It is a key regulator of other proinflammatory cytokines and leukocyte adhesion molecules, and is a primary activator of immune cells. Additionally, it affects the reduction of T lymphocyte reactivity, which is of large importance for immune homeostasis. Tumor necrosis factor exerts its effect through two receptors, TNFR-1 and TNFR-2 [28]. Tumor necrosis factor alpha also plays a role in the pathogenesis of AP, which is why biological drugs that block TNF-α are being investigated for the treatment of AP[29]. The latest research from 2023 showed that elevated levels of TNF-α correlate with elevated levels of IL-6 and IL-8, and that all three markers are elevated in patients with severe AP [30].

CONCLUSION

In acute pancreatitis, a series of complex chain reactions which lead to damage to pancreatic acinar cells are triggered. Initiation of local and systemic inflammatory response is associated with complications and damage to other tissues and organs. The conventional clinical approach in predicting the severity of AP has limitations and seems to have reached its maximum potential. Given that early identification of patients who can potentially develop severe acute pancreatitis is necessary, various inflammatory markers have been tested to enable early selection of patients with a potentially severe form of AP. The most frequently determined parameter in clinical practice is CRP, as a non-specific marker of inflammatory diseases. Numerous biomarkers have proven to be more sensitive for determining the severity of AP, of which procalcitonin, which has been widely used in recent years, stands out for the early prognosis of the development of local complications and multiorgan failure in AP. Cytokines as mediators in cellular communication play a significant role in all inflammatory processes. In recent years, determination of cytokine has increasingly become a part of clinical practice. The most commonly used IL-6 is a sensitive and specific marker for predicting organ failure in severe AP. The determination of many inflammatory markers that would be used to evaluate AP has both technical and financial limitations; however, with the improvement of molecular methods, it could be expected in the future that their determination will become a part of routine clinical practice.

REFERENCES:

1. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis - 2012: Revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102–11.
2. Garber A, Frakes C, Arora Z, Chahal P. Mechanisms and Management of Acute Pancreatitis. Gastroenterol Res Pract. 2018;2018:6218798.
3. Jabłońska B, Mrowiec S. Nutritional Support in Patients with Severe Acute Pancreatitis-Current Standards. Nutrients. 2021;13(5):1498.
4. Wang G, Qu FZ, Li L, Lv JC, Sun B. Necroptosis: a potential, promising target and switch in acute pancreatitis. Apoptosis. 2016;21(2):121-9.
5. Surbatovic M, Radakovic S. Tumor necrosis factor-α levels early in severe acute pancreatitis: is there predictive value regarding severity and outcome? J ClinGastroenterol. 2013;47(7):637-43.

6. Rau CS, Wu SC, Chien PC, Kuo PJ, Chen YC, Hsieh HY, et al. Identification of Pancreatic Injury in Patients with Elevated Amylase or Lipase Level Using a Decision Tree Classifier: A Cross-Sectional Retrospective Analysis in a Level I Trauma Center. Int J Environ Res Public Health. 2018;15(2):277
7. Ge P, Luo Y, Okoye CS, Chen H, Liu J, Zhang G, Xu C, Chen H. Intestinal barrier damage, systemic inflammatory response syndrome, and acute lung injury: A troublesome trio for acute pancreatitis. Biomed Pharmacother. 2020;132:110770.
8. Nassar TI, Qunibi WY. AKI Associated with Acute Pancreatitis. Clin J Am SocNephrol. 2019;14(7):1106-1115.
9. Mustafić S, Brkić S, Prnjavorac B, Sinanović A, PorobićJahić H, Salkić S. Diagnostic and prognostic value of procalcitonin in patients with sepsis. Med Glas (Zenica). 2018;15(2):93-100
10. Mihajlovski M, Perišić Z, Raspopović M, Petrović N. Serum biomarkers of sepsis. Medical Bulletin of the Special Hospital for Thyroid and Metabolic Diseases'Zlatibor'. 2020;25(77):19-27
11. Kasian VV, Sheiko VD, Mamontova TV, Vesnina LE, Shlykova OA. Procalcitonin in early prediction of acute severe pancreatitis. WiadLek. 2020;73(7):1370-1372.
12. Rau BM, Kemppainen EA, Gumbs AA, Bu¨chler MW, Wegscheider K, Bassi C, et al. Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): a prospective international multicenter study. Ann Surg 2007;245:745-54.
13. Kylänpää-Bäck ML, Takala A, Kemppainen E, Puolakkainen P, Haapiainen R, Repo H. Procalcitonin strip test in the early detection of severe acute pancreatitis. Br J Surg. 2001;88(2):222-7.
14. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol. 2018;9:754.
15. Sack GH Jr. Serum amyloid A - a review. Mol Med. 2018;24(1):46
16. Zhang Y, Zhang J, Sheng H, Li H, Wang R. Acute phase reactant serum amyloid A in inflammation and other diseases. AdvClin Chem. 2019;90:25-80.
17. Büchler M, M, Uhl W, Nevalainen TJ. Phospholipase A2. Handbook of Mediators in Septic Shock. CRC Press, 2019; p.363-380
18. Iyer H, Elhence A, Mittal S, Madan K, Garg PK. Pulmonary complications of acute pancreatitis. Expert Rev Respir Med. 2020;14(2):209-217.
19. Domínguez-Muñoz JE, Villanueva A, Lariño J, Mora T, Barreiro M, Iglesias-Canle J et al. Accuracy of plasma levels of polymorphonuclear elastase as early prognostic marker of acute pancreatitis in routine clinical conditions. Eur J GastroenterolHepatol 2006;18:79–83.
20. Simsek O, Kocael A, Kocael P, Orhan A, Cengiz M, Balcı H, Ulualp K, Uzun H. Inflammatory mediators in the diagnosis and treatment of acute pancreatitis: pentraxin-3, procalcitonin and myeloperoxidase. Arch Med Sci. 2018;14(2):288-296.
21. Kusnierz-Cabala B, Gurda-Duda A, Dumnicka P, et al. Plasma pentraxin 3 concentrations in patients with acute pancreatitis. Clin Lab 2013;59:1003–8.
22. Tanaka T, Narazaki M, Kishimoto T. Interleukin (IL-6) Immunotherapy. Cold Spring HarbPerspect Biol. 2018 Aug 1;10(8):a028456.
23. Sathyanarayan G, Garg PK, Prasad H, Tandon RK. Elevated level of interleukin-6 predicts organ failure and severe disease in patients with acute pancreatitis. J GastroenterolHepatol. 2007 Apr;22(4):550-4.
24. Jain S, Midha S, Mahapatra SJ, Gupta S, Sharma MK, Nayak B, Jacob TG, Shalimar, Garg PK. Interleukin-6 significantly improves predictive value of systemic inflammatory response syndrome for predicting severe acute pancreatitis. Pancreatology. 2018;18(5):500-506.
25. Aoun E, Chen J, Reighard D, Gleeson FC, Whitcomb DC, Papachristou GI. Diagnostic accuracy of interleukin-6 and interleukin-8 in predicting severe acute pancreatitis: a meta-analysis. Pancreatology. 2009;9(6):777-85.
26. Li G, Chen H, Liu L, Xiao P, Xie Y, Geng X, Zhang T, Zhang Y, Lu T, Tan H, Li L, Sun B. Role of Interleukin-17 in Acute Pancreatitis. Front Immunol. 2021;12:674803.
27. Vlachos S, Tsaroucha AK, Konstantoudakis G, Papachristou F, Trypsianis G, Schizas D, et al. Serum Profiles of M30, M65 and Interleukin-17 Compared With C-Reactive Protein in Patients With Mild and Severe Acute Pancreatitis. *J Hepatobiliary Pancreat Sci*.2014; 21(12):911–8.
28. Laha D, Grant R, Mishra P, Nilubol N. The Role of Tumor Necrosis Factor in Manipulating the Immunological Response of Tumor Microenvironment. Front Immunol. 2021;12:656908
29. Hines OJ, Pandol SJ. Management of severe acute pancreatitis. BMJ. 2019;367:l6227.
30. He J, Yu S, Zhang J. Value of serum interleukin-6 and tumor necrosis factor-α in early diagnosis of severe acute pancreatitis.*J ClinHepatol.* 2023;39(7):1657-1664.