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IL-6 AS A DIAGNOSTIC MARKER FOR THE INFLAMMATORY PROCESS OF ACUTE APPENDICITIS

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Abstract

The action of interleukin 6 (IL-6) on lymphoid and non-lymphoid cells is a modulatory mechanism 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. Cytokines bind to specific membrane receptors on cells. IL-1 and TNF-α have as primary inducers the production of IL-6 and IL-8 and both occur systematically in acute appendicitis. 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 the acute appendicitis and precedes the growth of the C-reactive protein by 24-36 hours. IL-8 is the secondary mediator of TNF-α-induced neutrophil activation.

Key words: interleukin, pro-inflammatory, neutrophils, acute appendicitis

INTRODUCTION

Reginald Fitz of Boston uses the term appendicitis and recommended surgical treatment of the disease. Chester McBurney described characteristic migratory pain as well as the surgical approach area located along an imaginary oblique line from the anterior superior iliac spine to the umbilical scar. Since 1940 the use of antibiotics, computed tomography, ultrasound and laparoscopic examination has resulted in decreased morbidity and mortality and increased diagnostic accuracy. Laparoscopic appendectomy was first reported by gynaecologist Kurt Semm in 1982, but it has gained wide-spread acceptance only in recent years.

The vermiform appendix is a rudimentary part of the large intestine. The average length is 8-9 cm, the outer diameter is 5-8 mm and the one of

the inner canal of 1-3 mm, a canal that continues with the cecum through the appendicular ostium that is provided with a fold of mucous membrane - Gerlach's valve. Its insertion site is located at the junction of the three taeniae coli, 2-3 cm distal to the ileocecal valve. The wall structure is identical to that of the cecum. In young people approximately 200 lymphatic follicles are present in the submucosa that is also called the "abdominal tonsil" (Zviedre et al., 2016). Blood supply is provided by the appendicular artery, derived from the ileocolic artery, the final major branch of the superior mesenteric artery. The appendicular artery is situated in the mesentery of the appendix. The veins accompany the artery and drain the blood into the superior mesenteric vein. The lymphatic drainage is done to the colic and ileocolic ganglia, but depending on the position of the appendix the lymph can be drained to the duodenum, gallbladder or ovarian ganglia. A very interesting element is that of the position of the appendix; while its base and the cecum are located in the right iliac fossa, its tip may be directed either upward or downward. The most frequent positions are the retrocaecal-retrocolic (75%) and the subcaecal-pelvic ones (20%) (Widdison and Karanja, 1993) while the meso-celiac, subhepatic, herniated ones are also described.

According to the colocalization 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 (Halang et al., 2002). Excessive trypsin (Halang et al., 2002) disrupts the protease-antiprotease balance by consumption of specific (PSTI) and nonspecific trypsin inhibitors (α1-antitrypsin and α2-macroglobulin). This will activate other zymogens (chymotrypsinogen, proelastase, phospholipase) as well as various protease systems (complement, kinin, coagulation and fibrinolytic factors) triggering a strong inflammatory reaction (Rivera-Chavez et al., 2003). 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 (Sack et al., 2006). Cellular degradation will result 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.

Sack et al., 2006 in the study on the role of inflammatory blood markers when diagnosing acute appendicitis in children revealed that secondary transport in the systemic circulation of many substances produced during the inflammatory reaction will lead to complications: cardiac circulatory failure, acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation, acute renal failure, multiple organ

dysfunction (MODS). All these pathophysiological data enhance our understanding of the interest given in the dosage of biological markers (CPR, interleukins, PMN) (Sack et al., 2006).

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 (Wang et al., 1996).

MATERIAL AND METHOD

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

In this regard, a group of 90 patients diagnosed with acute appendicitis was created. All patients were hospitalized during 2019 in the surgical units of the County Emergency Clinical Hospital Oradea.

Determinations were made according to the following criteria:

- preparing the patient – fasting (in a fasting state);
- collected specimen - venous blood;
- collecting tube – vacutainer with sodium citrate 0.105 M (ratio sodium citrate/blood=1/9);
- collected quantity
- as much as the vacuum allows; in order to prevent the partial coagulation of the sample, the proper mixing of the blood and anticoagulant was ensured by movements of tube inversion (5-6 gentle inversions);
- causes of rejection of the evidence
- vacutainer is not full (at least 90%);
- hemolysed or coagulated sample, the sample collected in a tube other than the one with citrate;
- processing required after collection - the sample was centrifuged 15 minutes at 2,500g;
- sample stability - the sample is stable for 8 hours at room temperature;
- the separated plasma is stable for 3 weeks at -20ºC; > 1 year at -70ºC. Prior to analysis, frozen samples were thawed rapidly in 3-5 min at 37°C. Defrosting at lower temperatures can cause cryoprecipitate.

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 (Adkinson et al., 2008; Zvedre et al., 2016). 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 (Synevo, 2010). 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) (Richard et al., 2007; Robert et al., 2008). 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 (285). Like IL-1, IL-6 is secreted by macrophages, being also synthesized by T and B lymphocytes, fibroblasts and endothelial cells, keratinocytes, synoviocytes, chondrocytes, epithelial cells (Adkinson et al., 2008; Richard et al., 2007; Robert et al., 2008). Thus, IL-6 is produced in response to bacterial and viral infections, inflammation or trauma, rapidly reaching detectable plasma levels unlike many other cytokines (Robert et al., 2008). Cytokine IL-6 is considered the major mediator for the hepatic production of acute-phase reactants: fibrinogen, serum amyloid A, haptoglobin, C-reactive protein (Sack et al., 2006). Following exposure to IL-6, the liver decreases the albumin and transferrin synthesis, initiating processes of hepatocyte regeneration instead (Synevo Laboratory, 2014). IL-6 cytokine stimulates humoral and cellular immune responses by acting on both B and T lymphocytes. IL-6 plays a role in the differentiation and growth of the B cells and stimulates their production of immunoglobulins (Laborator Synevo, 2014). It also promotes T cell activation, growth and differentiation. It is involved in the pathogenesis of multiple myeloma, being used as a prognostic factor of the disease. IL-6 stimulates haematopoiesis (acts synergistically with IL-3), induces ACTH secretion and other pituitary hormones (prolactin, growth hormone, luteinising hormone) (Boucher, 2000). 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 and stimulates the synthesis of IL-1RA (Adkinson et al., 2008).

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 (Laboratory Corporation of America, 2014).

Preparing the patient - fasting (in a fasting state) or postprandial (www.labcorp.com 2014)

Collected specimen - venous blood (www.labcorp.com 2014)

Collecting tube - vacutainer with no anticoagulants, with / without separator gel (www.labcorp.com 2014)

Collected quantity - minimum 0.5 mL serum (Synevo Laboratory, 2014).

Causes of sample rejection - intensely hemolyzed, jaundiced, lipemic or bacterially contaminated samples; samples that did not arrive frozen at the laboratory (www.labcorp.com 2014).

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 (www.labcorp.com 2014).

Sample stability – the serum is stable for one month at -20°C; do not defrost/refreeze (Laborator Synevo, 2014).

Method - the immunochemical method with chemiluminescence detection (Laborator Synevo, 2014) **(CLIA)**

Reference values - <3.8 pg / mL (Synevo Laboratory, 2014).

Interpretation of results

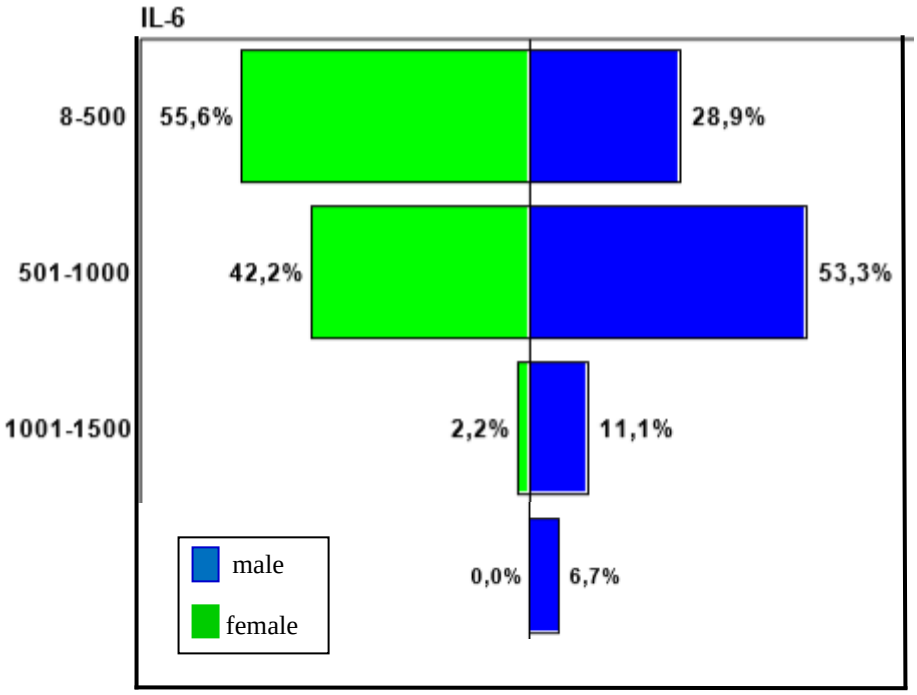
Increased levels of the marker are found in:

- rheumatoid arthritis (www.labcorp.com 2014; Fionula et al., 2008).
- multiple myeloma: prognostic factor (Greer et al., 2004)
- autoimmune diseases – lymphomas (www.labcorp.com 2014)
- sepsis – AIDS (www.labcorp.com 2014)
- alcoholic liver disease (www.labcorp.com 2014)
- viral infections (www.labcorp.com 2014)
- transplant rejection (www.labcorp.com 2014)
- severe preeclampsia.

RESULTS AND DISCUSSION

Bioclinical examinations were recorded in 90 patients (33.5%), 45 women and 45 men, representing 30.2% of the female group and 37.5% of the male group.

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 (Atkinson et al., 1998). These markers are a priority in the current medical research due to their role in human pathology in general and to the fact that these bioactive compounds may become therapeutic targets. The ideal marker should be an objective indicator and non-obverver-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.



Graph 1. Distribution of cases by gender and IL-6

There are significant differences between the two genders, male and female, in terms of IL-6 ($p < 0.001$). Thus, the number of female patients with normal values of IL-6 is almost two times higher than the number of male patients (55.6% versus 28.9%) and the average value of acute appendicitis is within normal limits (425.0 pg /mL).

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. The experimental evidence showed that IL-6 overexpression induces severe forms of acute appendicitis and its counteraction with monoclonal antibodies has protective effects. In acute appendicitis the increased values correlated with the complicated and lethal forms of the disease. Recently it has been reported that IL-6 is the best prognostic marker of respiratory failure. 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 appendicitis.

CONCLUSIONS

The inflammatory markers appear in the serum later than the previous ones, the acute-phase proteins, C-reactive protein and ESR, but they present the advantage of monitoring the clinical evolution, offering complementary opportunities.

These biochemical parameters could help identify the "ideal marker", which is why they should be evaluated prospectively, on large samples of patients that include significant percentages of severe cases.

In addition to the diagnostic role, these new markers may become therapeutic targets, their manipulation allowing the prevention or alleviation of sever acute appendicitis.

The hopes for the future are related to the markers of appendix injury and those of inflammation, in particular cytokines, provided that a dosing methodology that will make them useful in emergency cases is developed.

Genetic manipulation is a new promise in identifying the markers of severe acute appendicitis.

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