Interleukin 6 and Lipopolysaccharide Binding Protein - Markers of Inflammation in Acute Appendicitis

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Abstract
The rate of incidence of acute appendicitis is 12% in the case of male patients and 25% in case of women, which represents about 7% of the world population. The appendectomy rate has remained constant (i.e. 10 out of 10,000 patients per year). Appendicitis most often occurs in patients aged between 11-40 years, on the threshold between the third and fourth decades, the average age being 31.3 years. Since the first appendectomy performed by Claudius Amyand (1681/6 – 1740), on December 6th, 1735 to our days, i.e., 270 years later, time has confirmed the efficiency of both the therapy method and the surgical solution. The surgical cure in case of acute appendicitis has proved to be acceptable within the most widely practised techniques in general surgery. The variety of clinical forms has...
reached all age ranges, which in its turn has resulted in a large number of semiotic signs. In the case of acute appendicitis, interdisciplinarity has allowed the transfer of concept and methodology transfer among many areas of expertise, aimed at a better, minute understanding of the inflammatory event itself. Acute appendicitis illustrates inflammation development at digestive level and provides for a diagnostic and paraclinical exploration which continually upgrades. The recent inclusion in the studies of the Lipopolysaccharide binding protein (LBP)-type inflammation markers has laid the foundation of the latter’s documented presence in the case of acute appendicitis-related inflammation. Proof of the correlation between the histopathological, clinical and evolutive forms can be found by identifying and quantifying these inflammation markers. The importance of studying inflammation markers allows us to conduct studies going beyond the prognosis of the various stages in which these markers were identified. The present article shows the results of a 1-year monitoring of the inflammation markers’ values for Interleukin-6 and Lipopolysaccharide binding protein (LBP)-types, both pre-op and 3-days post-op in the case of patients diagnosed with acute appendicitis in the Surgery Clinic IV of the Emergency University Hospital - Bucharest.

The data collected have allowed us to correlate them with the selected parameters, and to draw the conclusions presented in this article.

**Key words:** acute appendicitis, lipopolysaccharide binding protein (LPS-BP, LBP), interleukin-6, pathology, acute phase proteins, correlations, acute inflammation, sepsis

**Introduction**

Within the context of investigating the inflammation specific factors, the dedicated literature also includes data on the specific Lipopolysaccharide binding protein (LPS-BP, LBP) and Interleukines-6 (IL-6).

It is noteworthy that the latest investigation on the digestive inflammatory pathology includes LBP and IL-6 dosing.

Moreover, these investigations can become certainty and prognosis factors of the surgical therapeutic evolution.

**Lipopolysaccharide binding protein (LPS-BP, LBP)** (1)

LBP is a 50-kDa polypeptide mainly synthesized in hepatocytes and released as a 58 - to 60-kDa glycoprotein into the bloodstream after glycosylation (2). Other sources of LBP synthesis have been identified, such as epithelial cells of the skin, the lung, the intestine and human gingival tissues as well as the small muscle cells of the lung arteries, heart muscle cells and renal cells (3,4). Experiments in a model of experimental meningitis suggest a source of LBP within the central nervous system (5).

Human LBP consists of 452 amino acids and has a typical 25-amino-acid signal sequence characterizing secreted proteins (2). The LPS-binding domain has been identified in the N-terminal end of LBP comparable to the structural and functional related bactericidal permeability increasing protein (BPI) (4). Both, the lbp gene and the bpi gene are closely located on chromosome 20 (6).

LBP belongs to the lipid transfer/lipopolysaccharide binding protein (LT/LBP) family, with BPI, the cholesteryl ester transfer protein (CETP) and the phospholipid transfer protein (PLTP) (7).

Lipopolysaccharide (LPS) is a prominent cell wall component of gram-negative bacteria and represents one of the most potent activators of the human innate immune system (8). A high sensitivity of the host for detecting LPS is mandatory in order to mount an early and rapid response against invading gram-negative bacteria (9).

Recently, the existence of a related family of seven human proteins was demonstrated, designated PLUNC proteins, which are expressed in the mouth, nose and upper airways. PLUNC genes are also located on chromosome 20 together with LBP, BPI and PLTP and may participate in host defence (10).

**Function of LBP**

LBP is present in normal serum at concentrations of 5 to 10 μg/ml, rising up to 200 μg/ml 24 h after induction of an acute-phase response (11). This rise in LBP levels is caused by transcriptional activation of the LBP gene mediated by interleukin-1 (IL-1) and IL-6 (12). LBP has a concentration-dependent dual role: low concentrations of LBP enhance the LPS-induced activation of mononuclear cells (MNC), whereas the acute-phase rise in LBP concentrations inhibits LPS-induced cellular stimulation (13). LBP binds a variety of LPS (endotoxin) chemotypes from rough to smooth strains of gram-negative bacteria and even lipid A, the lipid moiety of LPS (14,15). LPS molecules, components of the outer membrane of gram-negative bacteria, are important mediators in the pathogenesis of gram-negative sepsis and septic shock (16). Because the lipid A moiety has been shown to be responsible for the biological activity of LPS in most in vivo and in vitro test systems, it has been termed the endotoxin principle of LPS (17).

LPSs activate monocytes and macrophages to secrete inflammatory cytokines (tumor necrosis factor alpha (TNF-α) and IL-1, etc.) and other potent mediators (18) by an intracellular signal amplification pathway. These mediators, in turn, act on additional target cells to produce cardiovascular shock, multisystem organ failure, and septic shock (19,20), one of the major causes of death in intensive care units. Specific cellular responses in organisms are generally mediated by receptors. For endotoxin recognition, a binding protein receptor system has been postulated, involving LBP, the membrane bound and soluble CD14 molecules, members of the family of ‘Toll-like receptors (18,21), and a K+ channel (22, 23). LBP increases the capacity of LPS to induce cytokine release by mononuclear phagocytes (24,25), and neutralization of LBP with rabbit anti-LBP antibodies (Abs) prevents binding...
of LPS to monocytes (25) and protects mice from lethal endotoxemia (26). The important role of LBP in LPS-induced cell activation has been underlined by the observation that blood from mice with a targeted deletion of the LBP gene was hyporesponsive to LPS by at least 1,000-fold (27). In these mice, a transfer of LPS to CD14 was not observed (28). It was shown recently, using reconstituted planar membranes, that LBP intercalates in a directed manner and transmembranously into bilayers composed of an extracellular leaflet with a negative surface charge density. LPS and lipid A were shown to bind to LBP on both sides of the membrane, and binding at the extracellular side led to a conformational change of the protein or a change of its orientation in the membrane (29). Moreover, it has been shown that LBP transfers phospholipids to LPS micelles (30).

Role of LBP in infections

Whereas early PAMP recognition is crucial to mount a sufficient antimicrobial immune response, regulatory mechanisms are also needed to prevent an overreaction followed by development of disease. Regarding its ability to amplify the immune response upon stimulation with bacterial surfaces, high LBP concentrations might be expected to render the host more sensitive and to develop an overwhelming systemic inflammatory response leading to complications of inflammation e.g. septic shock and acute respiratory distress syndrome (ARDS). Early studies using antibodies against LBP in murine models of experimental endotoxemia supported this hypothesis (31,32). LBP knockout mice were protected against septic shock in response to intraperitoneally injected Salmonella LPS.

The absence of LBP led to a reduced LPS responsiveness both in vitro and in vivo (33) whereas the reduced immune response resulted in a higher susceptibility to infections with viable bacteria such as Salmonella typhimurium (33-35). In LBP-wild-type mice S. typhimurium stimulated production of the C-X-C chemokine macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemo-attractant, which was significantly reduced in the LBP-knockout mice resulting in an impaired recruitment of polymorphonuclear leukocytes (PMN) to the peritoneum and subsequently to an increased susceptibility to bacterial infection (1).

Additional confirmation was found in an E. coli peritonitis model in LBP-/– mice which displayed a higher mortality, an earlier bacterial dissemination into the bloodstream and impaired bacterial clearance as compared to wild-type mice (36). Fan et al. investigated the role of LBP in the lung using a model of K. pneumoniae pneumonia in LBP knockout mice (37). They found a significant increase in mortality, an earlier onset of bacteremia, and greater pulmonary bacterial load in the LBP-deficient mice as compared to wild-type mice, presumably caused by a significant reduction of neutrophil recruitment.

Taken together, LBP is extremely important in bacterial recognition and the control of bacterial growth. The inflammatory response generated by non-replicating surface components such as LPS is significantly decreased in LBP deficient animals. Therefore the potential role of LBP inhibition may be the modification of the immune response after antibiotic lysis of bacteria (1).

Interleukin - 6 (IL-6)

IL-6 type cytokines belong to the long-chain 4α-helix hematopoietic cytokine family. Interleukin-6 is a 212 amino acid cytokine (38); its gene maps to chromosome 7p21 and contains 4 introns and 5 exons. It is observed as a 22- to 28-kDA phosphorylated and variably glycosylated protein (39).

Interleukin-6 targets multiple cell types and induces a broad array of responses. These responses are often simplistically classified as pro- or anti-inflammatory in nature. A key function of IL-6, which was demonstrated in the IL-6 knockout mouse, is mediation of the acute phase response (40,41). The acute phase response occurs when an inflammatory stimulus is severe enough to generate a large number of accompanying systemic changes that reset normal homeostatic mechanisms (42).

Specifically, tissue injury incites a local reaction that includes activation of leukocytes, endothelial cells, and fibroblasts. This activation results in the release of cytokines that induce a systemic response characterized by fever, leukocytosis, and the release of acute phase proteins (APps) (43, 44). There are at least 40 plasma proteins that are classified as such because their concentrations change by at least 25% after an inflammatory stimulus (45). Interestingly, the speed of protein concentration change parallels the magnitude of the inflammatory stimulus (42). Induced proteins include clotting proteins, complement components, antiproteases, and proteins used for transport (45).

Although the acute phase response is classically described as a pro-inflammatory phenomenon, many of the acute-phase reactants have inhibitory effects on the immune system (40,41,46). For example, the APP haptoglobin inhibits the respiratory burst in neutrophils, inhibits lipopolysaccharide (LPS)-induced tumor necrosis factor-α (TNFα) production in monocytes, and inhibits LPS-induced proliferation of lymphocytes (46). C-reactive protein (CRP), another APP, has a variety of important pro-inflammatory functions, such as promoting opsonization, enhancing phagocytosis, activating complement, and stimulating cytokine release and adhesion molecule expression (45). However, CRP also has anti-inflammatory effects, such as inhibiting the neutrophil respiratory burst and degranulation, and thereby limiting tissue injury (47).

Interleukin-6 has a variety of effects on the immune system. It plays an important role in immune cell maturation. It induces immunoglobulin production by B cells and differentiation of T cells (48). Although IL-6 is believed to be essential for antibody production by B cells, it does not affect proliferation of activated B cells (49). Interleukin-6 activates mitogen-stimulated T cells by inducing IL-2 production and IL-2 receptor expression. It acts synergistically with IL-2 in propelling T cell differentiation into cytotoxic lymphocytes (49).

Interleukin-6 activates endothelial cells and induces chemokine production as well as adhesion molecule
expression, leading to the recruitment of leukocytes to sites of inflammation (50). Additional proinflammatory effects of IL-6 include inducing expression of phospholipase A2 (PLA2) (40). In turn, PLA2 actions generate leukotrienes, prostaglandins, and platelet-activating factor (PAF). Platelet activating factor also acts synergistically with IL-6 to prime polymorphonuclear cells (PMNs) (51).

In vitro anti-inflammatory effects of IL-6 include inhibition of TNFα production and IL-1 inhibitor release, as well as induction of tissue inhibitor of matrix metalloproteinase (TIMP) (40,43,48,52,53).

Interleukin-6 also stimulates hemostasis. It stimulates platelet production by megakaryocytes (48,54). In vitro experiments on human platelets have demonstrated morphologic alterations as well as platelet activation as measured by adenosine triphosphate, P-selectin, and dense granule concentrations (54). In vivo experiments in dogs have demonstrated that administration of IL-6 reduces the concentration of thrombin required to activate platelets and enhances their responsiveness to PAF (54).

Furthermore, IL-6 induces tissue factor expression in monocytes; in turn, the binding of tissue factor to factor VIIa eventually leads to thrombin and fibrin generation (55). Interleukin-6 plays an important role in the neuroendocrine system. Interleukin-6 binds to the hypothalamus and induces fever (56).

Interleukin-6 is a "robust" stimulant of the hypothalamic-pituitary-adrenal axis, both centrally and at the adrenal gland. It stimulates the release of corticotrophin releasing factor from the central nervous system (CNS), adrenocorticotropic hormone (ACTH) release from the pituitary gland, and cortisol release from the adrenal gland (57-59) Conversely, cortisol inhibits IL-6 production (59).

Interleukin-6 also stimulates vasopressin and growth hormone secretion by the pituitary gland but inhibits thyroid-stimulating hormone secretion (59). Several IL-6 family members, including IL-6, IL-11, ciliary neurotropic factor (CNTF), and oncostatin M (OSM), also mediate the "immuno-neuroendocrine interface" (60).

Interleukin-6 induces hyperglycemia by releasing glucose from hepatic glycogen stores; increased serum concentrations of IL-6 are associated with insulin resistance (61). Conversely, hyperglycemia increases IL-6 serum concentrations by augmenting IL-6 production by monocytes (61). In vitro experiments suggest that insulin resistance in a variety of disease states, including infection, may be mediated by IL-6 (62). Interleukin-6 has been shown to inhibit insulin signaling in hepatocytes; this in turn may be mediated by induction of the suppressor of cytokine signaling-3 (SOCS-3) proteins (62).

Interleukin-6 is also expressed and secreted by osteoblasts and osteoclasts upon stimulation with parathyroid hormone, Vitamin D, or IL-8; it also activates these cells (48,53,63, 64). Finally, IL-6 stimulates multilineage blast cell colony formation in haematopoietic stem cells, G0 to G1 cell-cycle progression, differentiation of neural cells, and proliferation of keratinocytes (48,49,59). Knowledge of this myriad of actions is essential to understanding the multiple roles that IL-6 plays in surgery, trauma, and critical illness.

Serum IL-6 concentrations increase in surgical patients, in proportion to the magnitude of the surgical stress (48,65, 66) Similarly, IL-6 concentrations increase in sepsis, in proportion to the severity of illness and correlate with adverse outcome (67,68).

Materials and Methods

The present research intends to highlight in their dynamic the markers chosen as representative for acute appendicitis prognosis, i.e., LPS-BP, IL-6, both in preoperative diagnosis settlement and during the immediate postoperative evolution, that is 72 hrs after surgery.

Therefore, we proceeded to identifying the study sample, namely ER patients, diagnosed with acute appendicitis, in absence of any associated pathology, who underwent acute appendicitis specific surgery and in whose case the following elements were considered:

A. Subjective data;
B. Objective data;
C. Paraclinical data.

These patients had been diagnosed with acute appendicitis, in accordance with the investigation methodology.

The patients who, admitted to hospital in accordance with the inclusion criteria, had refused surgery in the B moment of the study, for last minute personal reasons (one patient could not afford the C test of the inflammation markers) were excluded from the lot. Equally excluded were the patients diagnosed with acute appendicitis in whom, during surgery, genitally caused haemoperitoneum, perforated ulcer with biliar peritonitis, Meckel diverticulitis (1 case), cecal tumor and appendicular tumor, simultaneity with Crohn’s disease (1 case), posttraumatic spleen rupture, pneumonia, myocardial infarctus, and retroperitoneal haematoma were found.

The study lasted for one year and was aimed at highlighting in their dynamic the inflammation markers during the pre- and post operatory stages, 72 hrs. after surgery.

The research criteria consisted in making up three distinct groups:

Group A – Preoperative

Comprises general data on the patient, subjective symptoms, status upon admission. The objective examination consisted in filing the local exam results, and was focused on: abdomen aspect, lower right quadrant pain, pain on sudden abdomen decompression – the Bloomberg sign – and the rectal exam.

Among the paraclinical investigations we can mention imagistic and routine ones.

The first sample of inflammation markers was drawn from these patients upon admission, including the Lipopolysaccharide binding protein and Interleukine-6.

The specific feature of the study consisted in drawing the inflammation markers every 72 hours, as they were considered prognosis elements. All admitted and operated patients had given their written consent as to being supplementally investigated.
The prelevation technique closely followed the protocol. The patients were divided into several groups, differentiated by the pre-op therapeutic attitudes.

**Group B – Intraoperative**

The second data prelevation way was the intraoperative one; during this stage the following data were filed:

1. The macroscopic aspect of the appendix, i.e. catharal, flegmonous, gangrenous, with/without perforation, appendicular abscess, mesenteric adenitis, or other occurrences.
2. Visual inspection of the last 20 cm of the ileum, to identify the possible existence of the Meckel diverticulum.
3. Prelevation of cultures from the peritoneal secretion, antibiogram of the operative incision.

The excision piece was directed to a histopathology exam.

**Group C – Postoperative**

The third research module was dedicated to the postoperative stage, during which numerous parameters were under observation.

72 hrs postoperative, the laboratory investigations were repeated, using the same prelevation technique as the preoperative one; the focus was the same, i.e., on the Lipopolysaccharide binding protein and Interleukine-6.

**Data analysis**

The total number of patients included in the study was 47, admitted in the 4th Surgery Clinic of the Emergency University Hospital of Bucharest.

According to the histopathological exam results, the excision pieces were divided into: catarrhal, flegmonous and gangrenous, with or without perforation.

The histopathological form matches statistical data, the catharal forms prevailing (53%), followed by the flegmonous (34%) and gangrenous (12.7%) ones.

According to the anatomopathological form, the patients were divided into 25 catarrhal, 16 flegmonous, 6 gangrenous (Graphic 1).

In the case of catarrhal inflammation of the appendix, the Interleukin-6 value is hard to detect preoperatively, in 31% of the cases being under the detection limit of the lab (i.e., <2 pg/ml). In 69% of the cases, Interleukin-6 can be detected by the lab, its value exceeding 2 pg/ml, but still below the normal limits (< 9.7 pg/ml) in 100% of the cases, and with a maximum value of 5.7 pg/ml. Postoperatively, 72 hrs after surgery, the Interleukin-6 value decreases in 100% of the cases; in 84.4% it decreases under the detection limit of the laboratory, whereas in 15.6% of the cases it stays within a detectable value limit. The smallest decrease between the pre- and postoperative value was 0.7 pg/ml, recorded in a single case (Graphic 2).

In the case of flegmonous inflammation, the Interleukin-6 value is easy to detect preoperatively, being in 100% of the cases above the detection limit of the laboratory (i.e., >2 pg/ml). In 93.7% of the cases, the Interleukin-6 value stays between the normal limits (< 9.7 pg/ml), but ranges at the upper limit of the physiological value. Only in 6.25% of the cases did the Interleukin-6 value exceed the normal threshold, with a maximum value of 10.5 pg/ml. Postoperatively, 72 hrs after surgery, the Interleukin-6 value decreases in
100% of the cases, ranging at the detection limit of the laboratory in 100% of the cases and having a minimum value of 2.8 pg/ml. No case was registered in which the Interleukin-6 value decreased below the detection limit of the laboratory. The most significant decrease between the pre- and postoperative value was 6.6 pg/ml, whereas the smallest decrease was 2.8 pg/ml (Graphic 4).

In the case of flegmonous inflammation, the Lipopolysaccharide binding protein value is visibly higher than in the case of catarrhal forms, being present in 100% of the cases, but far below the pathological limit (> 15 μg/ml), with minimum values of 3.2 μg/ml and maximum ones of 8.3 μg/ml. Postoperatively, 72 hrs after surgery, the Lipopolysaccharide binding protein value increases in 100% of the cases, approximately 5 times, getting closer to the pathological limit in 25% of the cases, discretely exceeding it in 18.75% of the cases and ranging between the normal limits in 56.25% of the cases. The highest registered postoperative value was 15.9 μg/ml. The greatest decrease between the pre- and postoperative value was 11.6 μg/ml, while the smallest was 0.5 μg/ml (Graphic 5).

In the case of gangrenous inflammation, the Interleukin-6 value is easy to detect preoperatively, being in 100% of the cases above the detection limit of the laboratory (i.e., >2 pg/ml). In 100% of the cases, the Interleukin-6 value exceeds the normal value (9.7 pg/ml), with a maximum value of 18.9 pg/ml. Postoperatively, 72 hrs after surgery, the Interleukin-6 value decreases in 100% of the cases, has a minimum value of 5.6 pg/ml and stays at the detection limit of the laboratory. No case was registered in which the Interleukin-6 value decreased below the detection limit of the laboratory. The greatest decrease between the pre- and postoperative value was 11.3 pg/ml, while the smallest was 5.9 pg/ml (Graphic 6).

In the case of gangrenous inflammation, Lipopolysaccharide binding protein value is significantly higher than in the case of the flegmonous forms, being present in 100% of the cases much above the pathological limit (> 15 μg/ml), with minimum values of 20.9 μg/ml and maximum ones of 38.1 μg/ml. Postoperatively, 72 hrs after surgery, the Lipopolysaccharide binding protein value decreases in 100% of the cases, approximately 3-4 times, gets closer to the pathological limit in 33% of the cases, discretely exceeds it in 16% of them and ranges within the normal limits in 50.7% of the cases. The highest registered postoperative value was 17.5 μg/ml. The greatest decrease between the pre- and postoperative value was 25.7 μg/ml, while the smallest was 11.7 μg/ml (Graphic 7).

**Discussion**

The element of accuracy in the assessment of the inflammation degree in acute gangrenous and flegmonous appendicitis belongs to the Lipopolysaccharide binding protein. The LBP value is significantly higher in all cases of acute gangrenous appendicitis. Recovery of the normal values is somewhat spectacular only in the case of LBP. IL-6 has slight improvement as compared to LBP recovery to normal values speed.

In the case of catarrhal and flegmonous appendicitis,
without peritoneal reaction, there is an intersection of the IL-6 and LBP curves, in the sense that the lower the former, the higher the latter. This "X" is valid only in the acute catharal and flegmonous appendicitis. The difference between the two histopathological forms lies in the quasi-normal value of the inflammation markers, and the attitude is the same both in the acute catharal appendicitis and the flegmonous one.

We were unable to catch the moment of dramatic increase of the shift from flegmonous to gangrenous appendicitis, but in the gangrenous forms, significantly increased LBP in agreement with IL-6 was noticed.

There were borderline cases considered gangrenous, with normal LBP, in which the histopathological result indicated flegmonous aspect, although during surgery the laceration rather suggested the gangrenous aspect.

LBP represents the most accurate inflammation marker which underscores and correlates with histopathology results (the encoding element of the inflammation, both macroscopically and mainly microscopically).

LBP increase was not influenced by postoperative antibiotherapy in the catharal and flegmonous cases with initially low LBP, the latter continuing to increase up to the upper normal limit during the first 72 hrs postoperative.

Conclusions

The ways of objectifying the prognosis factors in acute appendicitis are currently based on the following findings:

1. The research on Interleukin – 6 and Lipopolysaccharide binding protein and their dosage in acute appendicitis represents a stage in the synchronization of clinical data with morphopathological ones.

2. The inclusion in the paper of the Lipopolysaccharide binding protein as an acute phase protein is a breakthrough in the Romanian surgical pathology.

3. The present paper offers important data on the research of the correlations between the clinical, paraclinical and histopathological elements of the diagnosis, which allows us to identify new prognosis data as well as to better assess the evolution stages of the inflammation pathological process in appendicitis.

4. The research on these acute inflammation markers, especially the Lipopolysaccharide binding protein, offers new opportunities to further enlarge knowledge of physiopathology in surgical major interest areas (e.g. MSOF).

5. The aim of the study is not to draw some specific therapeutic conclusions; nevertheless, we are certain that – in certain situations – it can provide the practicing with the adoption of a more nuanced attitude insofar as antibiotic therapy efficiency monitoring is concerned.

Author contribution

For the work for Dr. Brănescu that will be published in no. 2 of the Romanian Surgery Journal (Chirurgia) the contribution of the first author (Mihai Cristian Brânescu), the one of the corresponding author (the second author) (Dragoș Şerban) and the last author (Costel Ţălovescu) is equal.

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