*Urszula Zielińska-Borkowska, Katarzyna Wieczorek

Laboratory diagnosis of sepsis – biomarkers

Diagnostyka laboratoryjna sepsy – biomarkery

Department of Anesthesiology and Intensive Care, Medical Center of Postgraduate Education, Warszawa
Head of Departament, a.i.: Małgorzata Malec-Milewska, MD, PhD

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Address/adres:
*Urszula Zielińska-Borkowska
Department of Anesthesiology
and Intensive Care
Medical Center of Postgraduate Education
ul. Czerniakowska 231, 00-416 Warszawa
tel./fax +48 (22) 584-13-42
ula_zielinska@poczta.onet.pl,
oit@szpital-orlowskiego.pl

Summary

Sepsis is a severe, heterogeneous and potentially lethal medical condition. Different clinical manifestation depends on the etiological agent and severity of the infection, but the major role plays patient's immune system. The biomarkers used for diagnosis of sepsis must be characterized by high sensitivity and specificity and meet appropriate criteria. Low index of false positive or false negative results is essential. One of the first biomarkers of the infection, applied in 1921, is erythrocyte sedimentation rate. ESR has also been used in many other medical conditions since that time. Although ESR is still in use, today it plays only minor role in diagnosing infection 178 biomarkers has been detected and described in over 3000 studies in recent years. An important factor in diagnosing infections are test availability and simplicity. Bedside tests would be the most appropriate solution. They would maximally reduce time needed for diagnosis and allow for prompt initiation of proper therapy.

Streszczenie

Sepsa jest ciężkim, niejednorodnym i potencjalnie śmiertelnym stanem chorobowym. Różnorodny obraz kliniczny zależy przede wszystkim od czynnika etiologicznego, masywności zakażenia, a także, a może przede wszystkim od sprawności układu immunologicznego pacjenta. Stosowane w diagnostyce sepsy biomarkery muszą charakteryzować się wysoką czułością i swoistością, a także spełniać odpowiednie kryteria. Muszą także zachować niski odsetek wyników fałszywie dodatnich, jak i ujemnych. Jeden z pierwszych markerów zakażenia zastosowano w 1921 roku. Wskaźnik opadania erytrocytów wykorzystywana jest również w wielu innych schorzeniach. W obecnych czasach jego znaczenie w diagnozowaniu infekcji jest niewielkie. W ostatnich latach w ponad 3000badań wykryto i opisano 178 markerów zakażenia. Ważnym elementem w diagnostyce zakażeń jest dostępność i łatwość wykonania testu. Najbardziej optymalnym rozwiązaniem byłaby możliwość wykonania testu diagnostycznego przy łóżku chorego. Pozwoliłoby to na maksymalne skrócenie czasu diagnostyki i bardzo wczesne wdrożenie postępowania terapeutycznego.

Sepsis is a severe, heterogeneous and potentially lethal disease. Different clinical manifestations depend mainly on the etiological agent, spread of the infection, but most of all, on functioning of the patient's immune system. Cancer, malnutrition, autoimmune diseases, diabetes and organ transplantation are primary risk factors for sepsis. The separate, but very important risk factors, are invasive diagnostic and therapeutic procedures (1, 2). Early diagnosis is the key point for effective treatment. Difficulty in early diagnosis of the infection and evaluation of its severity are very often caused by lack of evident clinical symptoms. Symptoms may be present only at advanced stage of the disease. This applies to patients who have many factors that stimulate immune response. High mortality in severe infections is directly related to delayed introduction of proper treatment. Regardless of the therapeutic option used, the main factor influencing successfulness of the therapy is time from diagnosis to implementation of the treatment. Determining a marker that organism release at early stage of the infection, would enable to introduce proper treatment at the initial stadium. Additionally, early intervention would probably reduce mortality (3).

One of the first biomarkers of the infection, applied in 1921, is erythrocyte sedimentation rate (ESR). ESR has also been used in many other medical conditions since that time. Although ESR is still in use, today it plays only minor role in diagnosing infection (1).

178 biomarkers has been detected and described in over 3000 studies in recent years (2). The basic characteristics of each biomarker that is going to be used in clinical practice include high sensitivity and specificity. Additional advantages are low incidence of false positive and negative results.

C-REACTIVE PROTEIN (CRP)

This protein is produced mainly in the liver and adipose tissue and is described as an acute phase protein. Normal serum level in healthy population is not higher than 7-10 mg/l (4). Test sensitivity and specificity are 30-100% (5). CRP level is elevated in infections, autoimmune diseases, massive trauma and cancer diseases. Maximal elevation is observed 1-3 days after damaging insult, depending on the disease. Its level normalizes after 7-10 days of successful treatment (4). Observations in many centers show that CRP is not a good infectious marker in newborn and preterm infants. CRP elevation is delayed in comparison to other acute phase markers (procalcitonin, α-1 antitrypsin, inhibitors and pro-inflammatory cytokines). Therefore it is recommended to use several markers in this age group (4). Determination of CRP level has a greater diagnostic value than white blood count or ESR. However, etiology of the infection can not be determined basing of the CRP serum level (5). Clinical studies by Soetino et al., published in 1989, showed that determination of CRP level in the cerebrospinal fluid might be useful in differentiation between viral and bacterial meningitis. CRP level was estimated in the cerebro-spinal fluid taken from children with bacterial and viral meningitis. C-reactive protein was detected only in patients with bacterial meningitis. It was not present in viral meningitis. Test sensitivity in the purulent infection was 91% comparing to 46% of the microbiological testing (gram stain of the CSF smear) (6). CRP is commonly used as a monitoring tool of the clinical status. It is a diagnostic and prognostic factor in patients with acute pancreatitis, sepsis, meningitis and active inflammatory process. Decrease in CRP level is an indirect indicator of effectiveness of the antibiotic therapy (4).

PROCALCITONIN (PCT)

Procalcitonin has been strongly considered as a marker of inflammation, infection, sepsis and septic shock in the last decade. This tendency is reflected by multifold publications that describe increasing number of multicenter clinical studies and some meta-analysis. Procalcitonin is a peptide composed of 116 aminoacids with molecular weight of 13 kDa. Physiologically it is a precursor of calcitonin and is synthetised in C cells (neuroendocrine cells) in the thyroid gland. In experimental studies procalcitonin was also isolated from adrenal glands, spine, lungs, adipose tissues and gastrointestinal tract. PCT itself does not show any hormonal activity. Its physiological role has not been fully explained. PCT probably takes part in the intercelullar cAMP production, regulates interactions between integrins and influences monocyte chemotaxis. The normal serum level in healthy population is below 0.1 ng/ml. PCT level may be helpful in determining clinical stage of the infection. PCT is eliminated by the renal system (3, 7, 8). Its level increases 2-4 hours after infectious insult to the tissues. The maximal elevation is detected after 6-8 hours. PCT is a relative sensitive and specific factor for the bacterial infection (74-100% and 70-100%, respectively) (5, 9). Studies that compare WBC, CRP and PCT levels indicate that PCT is an early infection marker, which precedes CRP elevation at least for 24 hours. It applies to both – organ and systemic infections (3). Kim et al. estimated role of PCT and CRP as early infection markers in patients with neutropenic fever with suspected infection. PCT and CRP levels were statistically analyzed. Diagrams of ROC and AUC were higher for PCT than CRP. The greater the AUC, the greater test diagnostic power, which has optimal sensitivity and specificity parameters. Authors suggest that procalcitonin is more effective in early diagnosis of the infection in patients with neutropenic fever (10). PCT might be a marker of severe infections, in which etiological factor can not be directly detected and clinical symptoms are unspecific (11). PCT elevation in response to infection occurs prior to increase in CRP, WBC and ESR levels. This plays a crucial role in infections in small children. Though, it should be noticed that elevated PCT levels in newborn maintain for 48 hours after birth (4). Diagnosing infection in children with urinary tract anomalies allows for early initiation of antibiotics and PCT monitoring shortens therapy duration (4, 12). PCT determination is recommended in differentiating between bacterial and viral meningitis. PCT elevation in bacterial meningitis leads to early introduction of the proper antimicrobial therapy. Many studies have not detected PCT in the cerebrospinal fluid (4, 13). These observations are true not only in respect to children. Many studies of the youngest population of patients is concentrated on children with neutropenia, because infection in this group is a negative prognostic factor. Determination of the PCT level enables to differentiate between bacterial and viral infection and initiation of the proper treatment (14). PCT determination is not only limited to differentiation between infectious and noninfectious etiology, but also plays an important role in treatment monitoring and elimination of most serious complications. Nobre et al. analyzed duration of antibiotic therapy in patients with sepsis (15). Patients with abdominal infection were included to the study. Antibiotic was stopped when PCT level decreased for about 90% of the primary value (not until 3 days if PCT was < 1.0 ng/ml or 5 days if PCT level was >1 ng/ml). In the control group antibiotic was given for 9 days (maximal length was 33 days). Therapy duration was reduced by 3.5 days in the study group. Mortality was similar in both groups, but duration of hospitalization was reduced in the study group.

These data show that controlling PCT level is useful in evaluation of antibiotic efficacy (15). Multicenter studies concerning antibiotic efficacy in the ICU patients confirmed that therapy duration had been reduced by 27-37% depending on the clinical diagnosis (16). Monitoring of PCT level probably allows for detection of the infection at early stage, when antibiotic should be introduced regardless of the surgical procedure (17-19). Chromik et al. determined PCT level after colorectal surgery within 3 days after operation (17). Patients were given conventional perioperative antibiotic prophylaxis. PCT elevation > 1.5 ng/ml was an indication for using 3rd generation cephalosporin in the first group of patients. Patients in the second group were given antibiotic after developing clinical symptoms. Early antibiotic introduction in the first group decreased number of severe infectious complications comparing to the 2nd group (18, 20). PCT elevation correlates well with a secondary peritonitis and subsequent multiorgan failure (21). Therefore PCT evaluation leads to early introduction of antibiotic and surgical intervention. PCT elevation after major abdominal surgery is helpful in diagnosing complications in early postoperative period. Elevation of CRP and IL-6 level were not that significant (22). Charles et al. showed that PCT level is significantly higher in cases of sepsis caused by Gram-negative comparing to Gram-positive bacteria (23). PCT levels in developing sepsis are much higher than in patients already presenting symptoms of the infection (24). PCT levels are much higher in sepsis caused by pneumonia than in pneumonia without symptoms of sepsis. In addition, PCT elevation precedes CRP elevation for at least 24 hours (10, 25). All these data show that PCT determination is helpful in diagnosing and monitoring antibiotic therapy in intensive care unit, especially in septic patients (26).

INTERLEUKIN 6

Interleukin 6 belongs to one of the most important and multifunctional cytokine. It is produced mainly by monocytes and macrophages. It may also be produced by fibroblasts, endothelial cells, keratinocytes, chondrocytes, B and T lymphocytes. Its secretion is stimulated by interleukin 1 and lipopolysaccharides (LPS), viruses, interferon (INF) and tumor necrosis factor (TNF). Interleukin 6 stimulates differentiation of B lymphocytes to plasmatic cells, activates T lymphocytes and stimulates hematopoesis. It has also pyrogenic effect and stimulates production of acute phase proteins. Generally, it shows pro-inflammatory characteristics (27). During inflammatory process IL-6 level may be elevated even by several dozen times. Due to its pyrogenic and pro-inflammatory properties it may be an useful marker for diagnosing bacterial infection and sepsis (28). IL-6 elevation precedes CRP elevation. Elevated IL-6 level has been demonstrated in immunological diseases, such as rheumatoid arthritis. Interleukin 6 plays an important role in pathogenesis and development of neoplasms. Though, in patients with cancer, with suspicion of infection, IL-6 may be an unspecific marker (27). IL-6 level is higher in inflammatory response syndrome with sepsis than without sepsis. Correct interpretation of the serum level is difficult. Many biological factors which induce SIRS, apart from cancerogenesis, have influence on its secretion (29).

PENTRAXIN 3 (PTX3)

Pentraxins belong to proteins of the first line unspecific immune response system. They are early markers of many infectious and inflammatory diseases. Pentraxins are produced by many cell types, such as macrophages, dendritic and endothelial cells. Pentraxins can be divided into short and long groups basing on their protein structure. Signals for long pentraxin production, such as PTX3, is elevation of TNFα and inerleukin-1βconcentrations. PTX3 is also stimulated by lipopolysaccharide (LPS) which is a component of cell wall of Gram-negative bacteria (30, 31). PTX3 shows antibody-like functions: blockade of pathogens, activation and regulation of complement system, pathogen opsonization. It also regulates inflammatory process. Serum PTX concentration is below 2 ng/ml. PTX3 elevation is observed in inflammatory diseases, which include:

- autoimmune diseases: SLE – systemic lupus erythematosus, systemic vasculitis, rheumatoid arthritis,
- angina pectoris, arterial hypertension, myocardial ischemia (in the first day of symptoms) (31),
- infection with *Aspergillus fumigatus*, tuberculosis, leptospirosis, dengue fever and meningitis.

PTX3 elevation was also observed in women with pathologic course of pregnancy. In patients with acute lung injury/acute respiratory distress syndrome high PTX3 concentrations in alveoli are related to the infection. PTX3 concentration correlates with degree of lung injury and severity of the disease course. Persistently high PTX concentrations in the first five days correlate with mortality. Moreover, elevated PTX3 concentrations in septic patients correlate with symptoms, severity of clinical manifestation measured by SAPS (Simplified Acute Physiology Score) and SOFA (Sequential Organ Failure Assessment) scales and number of insufficient organs (32).

CD64 RECEPTORS

Receptor expression on neutrophiles – as a marker of their activity in infection – was evaluated in the clinical practice in inflammatory states in order to differentiate between excessive inflammatory response and sepsis (1, 33). To this group belong CD43, CD50, CD62L, CD64 and MAC-1. The best known is CD64 (1). Expression of CD64 is induced in neutrophiles stimulated by cytokines, such as interleukin 12, interferon γ and GSCF (granulocyte colony-stimulating factor) (34). CD64 shows small expression on neutrophiles of healthy people (35). In clinical studies elevated CD64 expression was observed in bacterial infections, probably in response to components of bacterial

cell wall, such as lipopolysaccharide (35). Neutrophil receptor expression may be analyzed by flow cytometry with monoclonal antibodies. This is a qualitative but also quantitative evaluation of mature and immature neutrophiles, which have different number of surface receptors (33). CD64 may be used for differentiation between SIRS and sepsis, especially in patients with fever of unknown origin (35). Most of clinical studies of CD64 was conducted in neonatal intensive care units. Sensitivity is 88-96%, specificity 77-97% (1). CD64 expression remains stable for about 36 hours in heparinized blood sample in room temperature, which makes it appropriate for laboratory testing (34). CD64 is not a universal marker. Increased CD64 level is observed in patients who had been treated with interferon gamma or GSCF. Therefore increased CD64 expression may be present in this group of patients even by lack of infection (36).

SERUM AMYLOID A

Serum amyloid A (SAA) is a relatively sensitive inflammatory marker. It belongs to acute phase proteins. It is an apolipoprotein. Its production in the liver is influenced by proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and according to Misse et al. also IL-22. SAA function is connected with HDL metabolism. It also causes monocyte and macrophage migration to vessels, in particular to atherosclerotic plaque. SAA induces leukocyte and platelets adhesion, inhibiting their aggregation in the same time (37). Potentially it may be a biomarker of sepsis. SAA level may be increased even by 1000 times in the first 24 hours after infectious insult. Its level normalizes after 3-7 days of successful treatment. SAA elevation is not equal in every kind of inflammatory state. The largest variation in SAA levels is observed in patients after organ transplantation, in cases of bacterial or fungal infection and in diabetic patients. However, there is a large group of inflammatory states, autoimmune diseases and neoplasms, in which amyloid level is increased (38).

Diagnosis of viral infection is usually based on immunological assays of antigen and antibody from class IgG and IgM. IFN-γ-inducible protein (IP-10) is a pro-inflammatory chemokin. It causes chemotaxis of macrophages and activated lymphocytes. Clinical relevance of IP-10 evaluation was demonstrated in clinical studies of infections with rhinovirus, respiratory syncythial virus (RSV), hepatitis type B and C viruses and influenza virus H5N1. IP-10 level does not identify an etiological factor, but may play a role in differentiating between bacterial and viral infections (5).

Mannan and anti-mannan in blood of patients with symptoms of sepsis may be potential biomarkers in invasive candidiasis and aspergillosis. Determination of both biomarkers significantly increases sensitivity and specificity in identifying invasive fungal infection. Additional advantages are short testing time and using blood sample, which is easy to obtain.

Toll-like receptors are membrane receptors from a group of pattern recognition receptors which activate innate and acquired immunity. These receptors are divided into five groups and have affinity to various pathogens. They are present on cell membranes and in cytoplasm of various cell types, which make them responsible for a great part of immune response. As ligands for many pathogens they activate immune response. They induce and simultaneously activate many cytokines. TLR2 has great affinity mainly to bacteria. It may bind to ligands of many microorganisms: peptidoglycan and lipoteichoic acid of Gram-positive bacteria and lipoaranbinomannan of mycobacteria, fungal zymosan, glycolipids of *Treponema maltophilium* and many others. Probably it also binds to LPS of microorganisms other than Gram-negative bacteria, such as *Porphyromonas gingivalis*, *Leptpspira* sp. or *Helicobacter pylori*. It also activates immune response against those pathogens (39, 40). Increased TLR2 expression was observed not only in bacterial infections, but also in influenza type A and B viruses, cytomegelovirus (CMV), RSV, measles and varicella zoster virus (5).

TLR2 is probably the main receptor of toll-like receptors. TLR2, TLR3 and TLR4 take part in stimulation of protein kinases in cells infected with Gram-positive pathogens (39). TLR5 is the only Toll-like receptor that binds to flagellin. Flagellin is a structural protein of motor apparatus of Gram-positive and negative microorganisms. It activates pulmonary epithelial cells to production of pro-inflammatory cytokines (40, 41). TLR5 enhances immunity by activation of monocytes and T limfocytes. There are also toll-like receptors with affinity to unknown microorganisms or to unknown ligand. TLR11 has affinity to uropathogenic *E. coli* strains, but the ligand remains unknown. Detailed studies of mechanisms, how toll-like receptors bind to ligands of microorganisms, will probably allow for early diagnosis of sepsis. Furthermore, complete understanding of mechanisms mentioned above will lead to introduction of therapy that influences disease course and modulates immune response (5, 39, 40).

There is a large group of biomarkers that indicate infection indirectly. They determine insufficiency of the organ that is damaged by sepsis. These include coagulation factors, which may be damaged in sepsis or troponin that is released in cardiovascular insufficiency. Biomarkers used in diagnosis of sepsis are presented in table 1 (2).

CONCLUSIONS

Diagnosis of sepsis at early stage, when clinical symptoms are not evident, is extremely difficult in many cases. Identification of etiologic factor may be even impossible. Combination of some biomarkers determined periodically may significantly help in confirming etiology and severity of the disease. It will also allow for differentiation between fungal, bacterial and viral

Table 1. Biomarkers used in diagnosis of sepsis (2).

No	Biomarkers	Biomarker name	Results
1	aPTT	activated partial thromboplastin time	high NPV
2	CD11b	cell receptor	high values in children with sepsis
3	CD25	cell receptor	differentiation between sepsis and SIRS
4	CD64	cell receptor	low sensitivity and specificity in differentiation between viral and bacterial infection
5	C3, C4, C5a	complement system	differentiation between sepsis and SIRS
6	EA complex	elastase-1-antitripsin	elevation earlier than CRP in sepsis
7	ELAM-1	E-selectin	increase in trauma in patients with sepsis vs patients without sepsis
8	Endocan	endothelial protein	differentiation between sepsis and SIRS
9	E-selectin	–	differentiation between sepsis and SIRS
10	FDP	fibrinogen degradation products	high NPV
11	Gas6	stimulates cell proliferation	high values in acute septic patients vs patients with organ failure without sepsis
12	G-CSF	granulocyte colony stimulating factor	differentiation between sepsis and SIRS
13	Gelsolin	actin binding protein	high values in patients with sepsis vs patients without sepsis
14	IL-1	receptor IL-1 antagonist	early diagnosis of sepsis in neonates
15	IL-8	interleukin 8	high values in septic patients with neutropenia vs neutropenic patients without sepsis
16	IL-10	interleukin 10	high values in patients with septic shock vs patients with cardiogenic shock
17	IL-12	interleukin 12	diagnosis of sepsis in paediatric patients
18	IL-18	interleukin 18	differentiation between Gram-positive and Gram-negative bacteria
19	IP-10	chemokin	early diagnosis of sepsis in neonates
20	laminin	synthetized in epithelial cells	differentiation between bacterial and fungal sepsis
21	LBP	lipopolysaccharide	differentiation between Gram-positive and Gram-negative infections
22	MCP-1	chemokin	differentiation between sepsis and SIRS in paediatric patients with neutropenia
23	NO	nitrous oxide	high values in patients with sepsis and cardiogenic shock
24	osteopontin	protein in bone tissue matrix	differentiation between sepsis and SIRS
25	PAI 1	plasminogen activator inhibitor 1	high values in septic patients with DIC vs patients without
26	pentraxin	protein	differentiation between sepsis and SIRS
27	peptidoglikan	cell wall component	high values in patients with infection in postoperative period
28	pFN	fibronectin	differentiation between sepsis and SIRS
29	PLA2-II	A2 phospholipase	differentiation between bacterial and non-bacterial infection
30	lysozyme	enzymatic activity	differentiation between sepsis and organ transplant rejection in patients after transplantation
31	ST2	interleukin 1 inhibitor	high values in septic patients
32	surfactant proteins (A, B, C, D)	proteins	early ARDS diagnosis in sepsis
33	TREM-1	glycoprotein from immunoglobulin superfamily	differentiation between sepsis and SIRS in pneumonia
34	troponin	regulatory protein	cardiac insufficiency diagnosis in sepsis

NPV – negative predictive value

etiology with higher likelihood (fig. 1). Biomarkers used in diagnosis of sepsis must be highly sensitive and specific and meet appropriate criteria. They also must be characterized by low number of false positive and negative results.

Test availability and simplicity are important factors in diagnosing infection. The most appropriate would be bed-side tests. They would maximally reduce time needed for diagnosis and allow for prompt initiation of proper therapy.

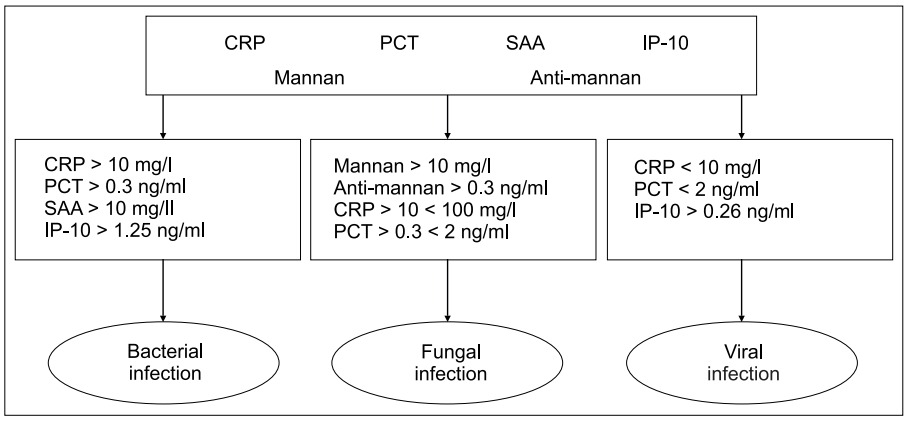

Fig. 1. Biomarkers used in diagnosis of sepsis (5).
CRP – C reactive protein, PCT – prokalcitonin, SAA – serum amyloid A, IP-10 – IFN-γ inducible protein-10

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