Review Article

Conventional, molecular methods and biomarkers molecules in detection of septicemia

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Abstract

Sepsis is a leading cause of morbidity and mortality in hospitalized patients worldwide and based on studies, 30–40% of all cases of severe sepsis and septic shock results from the blood stream infections (BSIs). Identifying of the disease, performing laboratory tests, and consequently treatment are factors that required for optimum management of BSIs. In addition, applying precise and immediate identification of the etiologic agent is a prerequisite for specific antibiotic therapy of pathogen and thereby decreasing mortality rates. The diagnosis of sepsis is difficult because clinical signs of sepsis often overlap with other noninfectious cases of systemic inflammation. BSIs are usually diagnosed by performing a series of techniques such as blood cultures, polymerase chain reaction-based methods, and biomarkers of sepsis. Extremely time-consuming even to take up to several days is a major limitation of conventional methods. In addition, yielding false-negative results due to fastidious and slow-growing microorganisms and also in case of antibiotic pretreated samples are other limitations. In comparison, molecular methods are capable of examining a blood sample obtained from suspicious patient with BSI and gave the all required information to prescribing antimicrobial therapy for detected bacterial or fungal infections immediately. Because of an emergency of sepsis, new methods are being developed. In this review, we discussed about the most important sepsis diagnostic methods and numbered the advantage and disadvantage of the methods in detail.

Key Words: Biomarkers, blood-culture, molecular methods, sepsis

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Received: 08.10.2014, **Accepted:** 18.12.2014

INTRODUCTION

The terms sepsis and bacteremia are used commonly in medical microbiology. Sepsis was derived from the ancient Greek for rotten flesh and putrefaction.[1]

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.158027

Bacteremia means the presence of bacteria in the bloodstream.[2] In order to emphasize the need for a diagnosis based on clinical and microbiological criteria, the US Food and Drug Administration guidelines use the term blood stream infection (BSI) instead of bacteremia.[3] Animals mount both local and systemic responses to microbes that traverse epithelial barriers and invade underlying tissues. Fever or hypothermia, leukocytosis or leukopenia, tachypnea, and tachycardia are the cardinal signs of the systemic response often called the systemic inflammatory response syndrome (SIRS). SIRS may have an infectious or a noninfectious etiology. When bacteria enter, the blood called bacteremia.

How to cite this article: Arabestani MR, Rastiany S, Kazemi S, Mousavi SM. Conventional, molecular methods and biomarkers molecules in detection of septicemia. Adv Biomed Res 2015;4:120.

If the infection is suspected or proven, a patient with SIRS is said to have sepsis. When sepsis is associated with dysfunction of organs distant from the site of infection, the patient has severe sepsis. Severe sepsis may be accompanied by hypotension or evidence of hypoperfusion. When hypotension cannot be corrected by infusing fluids, the diagnosis is septic shock. There are several forms of sepsis that always are in relation with bacteremia (or fungemia), but from another point of view, bacteremia and fungemia do not result in sepsis every time. Three usual forms of bacteremia are transient, intermittent, and continuous.[4]

In fact, host's systemic inflammatory response to infections is the reason of sepsis. Although this clinical syndrome lacks specific clinical manifestations, it is considered a major international healthcare problem and is the most important cause of morbidity and mortality in the Intensive Care Unit.[5,6] It is notable that the culture method can prove only 30–40% of all cases of severe sepsis and septic shock; thereby survival rates decrease rapidly in none-detected cases that receiving inadequate antimicrobial therapy within the first 24 h.[7] By applying novel methods that detect BSIs rapidly followed by determination of antibiotic susceptibility, can improve the problems associated with infection control, therapeutic management, and clinical decision-making and finally prevent from over-prescription of antimicrobials and its over-using related problem.[8]

The automatic continues-monitoring usual sterile site culture is the gold standard method for detection of BSI that followed by other routine methods for identification of the organism and also its susceptibilities of antibiotic. But due to rapid answers to guide therapy for sepsis is vital, major limitation of this method is it's time-consuming for final identifying of causal organism and determining of its susceptibilities that ranges from 1 to 5 days or more. Another limitation of this method is because of being difficult or impossibility of culturing fastidious organisms, which this problem may lead to miss possible causing BSI organisms. In addition, antibiotics administration before the blood sampling can also influence the result of culture.

True bacterial or fungal sepsis is believed to exist in more than 50% of the blood cultures (BCs) that are reported to be negative.[9] According to the International Sepsis Committee recommendation, clinical judgment rather than culture results should be base of decisions regarding antibiotic administration, treatment alterations or its stop. Therefore, regarding above statements and also considerations such as being too late, incomplete, and potentially misleading for coming results, it is thought that BC is not an ideal gold standard method.[10] Because of limitation in BCs method for identifying sepsis, other methods like nucleic acid technology (NAT) is considered, which able to detect and identify the pathogenic microorganisms including bacteria and fungi in blood with rapid, more sensitive and specific manner. Molecular methods can primarily be divided into two categories: (1) Methods that applying after cultivation steps and using of positive BCs or single colonies, and (2) assays that can be applied directly to drawn blood or other primarily sterile specimens like cerebrospinal fluid (CSF).[11] In addition, there have been interests for development of new tests for detecting nonspecific and specific biomarkers of sepsis.[12]

PREVALENCE AND SIGNIFICANT IN SEPSIS SEPTIC SHOCK

Prevalence of bacteremia and sepsis is increasing worldwide, and nowadays, they are the most serious health problems that third sepsis-related deaths are as a result of them.[13] Depending on the analysis, rates of mortality resulted from sepsis in hospitalized patients range from 18% to 35%; to the degree that investigations about the epidemiology of sepsis demonstrated that currently sepsis is the third most common cause of mortality after coronary heart disease and myocardial infarction with incidence of 12.4%, and of 11% for severe sepsis with septic shock.[14] Besides, a multicenter study in Germany showed that the third most common cause of death in that country was becoming sepsis.[14]

Three studies showed that the proportion of infections due to Gram-negative bacteria was more than that of infections due to Gram-positive bacteria in the total number of sepsis cases; these studies reported Gram-negative caused infections ranged from 30% to 80% while Gram-positive caused infections ranged from 6% to 24%, and the remainder was other pathogenic organisms.[15] Nevertheless, the sepsis caused by Gram-positive bacteria has increased. In the early 1990s, it accounted for more than 50% of all cases of septicemia that *Staphylococcus aureus* and *Staphylococcus epidermidis* were responsible for more than half of the cases.[16] Using catheters and other invasive equipment, as well as chemotherapy and immunosuppression in patients with organ transplants or inflammatory diseases are possibly caused of increasing rates of septicemia. The old people are especially common group that involved with sepsis.[17]

DETECTION OF MICROORGANISMS BY CONVENTIONAL METHOD

Blood-cultures

For the diagnosis of bacteremia, BCs are considered as the "gold standard" method and possibly detection of causal organisms including bacteria and fungi in blood is one of the most important practices of the clinical microbiology laboratory.[18] In other words, for discovering the infectious etiology of a patient's illness, BCs are one of the diagnostic choices. Other advantages of this method are providing the causal organism for further antimicrobial susceptibility testing and optimization of the antimicrobial therapy of patients.[19,20]

Identity of organism

Identifying of the causal organism by BC is often a very useful sign for the fact that the results may or may not represent contamination.[21] According to Weinstein *et al.'s* study, it is suggested that almost when certain organisms including *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli* and other *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Candida albicans* isolated from a BC, it can be considered that they are association with true bacteremia or fungemia.[19] Furthermore, certain organisms including *Clostridium perfringens*, *Bacillus* species other than *Bacillus anthracis*, coagulase-negative *Staphylococci* (CoNS), *Micrococcus* species, *Propionibacterium acnes*, *Viridans* group *Streptococci*, *Enterococci*, and *Corynebacterium* species are considered as a contamination in a significant proportion of cases when isolated from a BC.[19,22] Nevertheless, *E. coli* can be considered both infectious or contamination. Between mentioned contaminant organisms, the CoNS represent important frequent cases which are the most common BC contaminants typically representing 70–80% of all contaminated BCs.[23,24] Nevertheless, recently, investigations have demonstrated that these organisms are an increasing source of true bacteremia in patients with prosthetic devices and central venous catheters.[19,25]

Timing of blood-culture collection

Most of the authors believe that high temperature is most important indicator and using 2–3 BC with interval of 20–30 min is the best policy. However, it is regarded to be less imperative in some clinical situations (e.g. in case of there be no need for immediate antimicrobial therapy), and therefore probably, the BC collecting time is less affected over time. In comparison, one study showed that when comparing simultaneously collected BC sets with those collected at different times over a 24-h period, there is no difference in the ability to detect bacteremia.[26]

Number of positive blood culture bottles within a blood culture set

The number of positive BC sets is one of proven methodology that can help to differentiate contaminated BC from true infection. If there are true bacteremia, multiple BC sets will usually grow the same organism and in fact as indicated in the College of American Pathologists Q-Probes study, this theory has been demonstrated one of the most commonly considered sign for discriminating true bacteremia from contamination.[19,24]

Volume of blood cultures

Although improvements in producing BC media as well as continuous monitoring blood culture systems (CMBCS) enabled bacteria are detected rapidly and more often, there is still common consensus regarding volume of collected blood that it is the most significant for detecting bacteremia due to the fact that the most bacteremia cases in adults have a low density of microorganisms (often 10 CFU/ml).[27]

There is less available information about pediatric BCs. It is assumed that BSI in young children has a larger amount (often 100 CFU/ml) than bacteremia in adults.[28] It has been recommended former that in order to performing BCs, blood volumes of 1–2, 2–3, 3–5, and 10–20 ml need to be collected in cases of neonates, infants (age 1–24 months), older children, and adolescents, respectively.[29]

Considerations for current blood-culture systems

The introduction of automated CMBCS was one of the great advances in medical microbiology. Nowadays, there are three different commercially available systems. These systems are the BACTEC 9000 series (Becton-Dickinson, Sparks, MD, USA), the BacT/Alert 3D system (bio-Merieux, Durham, NC, USA) and VersaTREK (TREK Diagnostic System, Cleveland, OH, USA). The function of the first two systems is based on colorimetric or fluorimetric detection of increased CO_2 levels as a result of microorganism growth; and the function of third system is based on measuring the change in gas pressure (production of CO_2) in the headspace of the BC bottle result from microbial growth. These systems measure gas pressure in a certain interval depend on the type of the system ranging from 10 to 24 min. Captured data by three mentioned systems are analyzed using computer algorithms. When present appropriate criteria are denoting significant CO_2 increase in BC bottles, then the system signaled as positive and identified for removal and subculture.[30]

It has been demonstrated that in order to detect the most commonly encountered bacterial and fungal

organisms, usually 4–5 days of incubation would be sufficient.[31] The BC incubation time is often recommended that extend for patients with presumed endocarditis. In the case of clinical suspicion of bacteremia caused by fastidious organisms including *Brucella* species, nutritionally variant *Streptococci*, *Francisella* species and the HACEK group organisms, it was indicated for prolonged time of incubation of broth-based BC bottles as well.

AEROBIC VERSUS ANAEROBIC BLOOD-CULTURE COLLECTION

Anaerobes are capable to cause infection at almost all body sites such a BSI and, therefore, early identification and performing susceptibility antimicrobial test are significant for appropriate treatment of the patients with these microorganisms.[32,33] Based on reported studies, until the 1990s, the rates of anaerobic bacteremia were up to 20% of all BSIs and then decline during the 1990s.[34]

Considering the increase anaerobic bacteremia in patients with underlying and immunosuppressed diseases like malignancies etc., anaerobic bacteremia has re-emerged as a significant clinical problem.[35]

PREVENTION FROM CONTAMINATION

Contamination of BC leads to false-positive results and declines the specificity of the BC. By definition, specificity is directly related to the rate of false-positive results. In order to improve specificity and better performance of BC test, the contamination should be reduced. In trying to reduce BC contamination, several factors have been investigated including source of culture (catheter vs. percutaneous), the use of dedicated phlebotomy teams, and the use of commercial BC collection kits.[36]

Skin preparation

It is believed that the patient's skin is the most common source of percutaneous BCs contamination. Disinfection of the skin before blood collection cannot completely prevent the contamination of BCs from skin flora. Studies have shown that as many as 20% of skin-related bacteria survive disinfection, as culturing antiseptic skin samples harvested with a sterile surgical technique were positive.[37,38]

Probably, povidone-iodine (an iodophor) is the most common studied and traditionally utilized antisepsis for blood culturing. Another commonly antiseptics utilized for skin preparation for BC is alcohol-based products. In the studies by Calfee and Farr that performed a randomized comparative trial of four skin antiseptics (10% povidone-iodine, 70% isopropyl alcohol, tincture of iodine, or povidone-iodine plus 70% ethyl alcohol) for 12,692 percutaneous BCs showed that the antiseptics that contained alcohol may have greater effectiveness.[23]

Among the multiple factors that can affect the antiseptics to be effective for preventing from contamination, the time required for the antiseptic to have maximal effect is a significant consideration. For instance, the contact time required for maximum effect of povidone-iodine and tincture of iodine preparations is 1.5–2 min and 30 s, respectively. Thus, having knowledge of the minimum contact time for chosen skin antiseptic is important.[22]

IDENTIFICATION OF PATHOGENS BY STANDARD AND ADVANCED CULTURE-BASED METHODS

By performing gram staining after determining of positive BC signal, individuals can select appropriate media to allow growth of single colonies for further processing. Currently, identification of the pathogens in blood as well as their antibiotic susceptibility testing is usually performed using microdilution broth identification with automated continuously-monitoring systems, such as Vitek 2 (bioMe'rieux), Phoenix (Becton Dickinson, New York, USA), Micro Scan Walk Away (Siemens Medical Solutions, Erlangen, Germany), or semi-automated systems Micronaut (Merlin Diagnostica, Bornheim-hersel, Germany). Depending on the organism, the continuously monitoring expert-based systems have decreased turnaround time to a mean of 6.75 h for complete identification of bacteria, or approximately 18 h for fungi (Vitek 2 data) including antibiotic susceptibility reporting.

Staphylococci and *E. coli* are the most common causes of BSIs and are identified after a mean of 6–8 h; on the other hand, for full identification of the nonfermenters such as *Pseudomonas* and *Acinetobacter* spp. need more than 10 h by the microdilution systems. The molecular technologies (NATs) can detect pathogens faster in comparison to the standard automated culture-based identification.

IDENTIFICATION BY MICROBIAL NUCLEIC-ACID TECHNOLOGY

Detection and identification of pathogenic microorganisms including bacteria and fungi in blood by NATs can primarily be divided into two categories: (1) Methods that applying after cultivation steps and using positive BCs or single colonies, and (2) assays that can be applied directly to drawn blood or other primarily sterile specimens, like CSF. The

NATs that directly applied lead to a reduction of the time to get a final result, in contrast, NATs applied after cultivation have only minor turnaround time. There are three procedures for applying NAT assays in infectious disease diagnosis: (1) Pathogen-specific methods, (2) multiplex assays covering several different pathogens typical for a certain infection type, and (3) using universal broad-range assays involving conserved target sequences, such as the eubacterial 16S or 23S rDNA/RNA, and the pan fungal 8S or 18S rDNA/RNA.[11,39]

Pathogen identification from positive blood cultures by nucleic-acid technology

In order to detect most bacteria and fungi, the target of primers located in a conserved region in the 16S or 23S rRNA/rDNA gene for bacteria, and 8S or 18S rRNA/rDNA gene for fungi, required.[40] Since these rDNA are frequently present in multiple copies thus allowing for more sensitive detection through the multiple target sites. Several analysis approaches including restriction fragment length polymorphism profile analysis, single-strand conformation polymorphism,[41] and 16S rDNA-based universal polymerase chain reaction (PCR) with sequencing[42] has been successfully performed for direct identification of unselected bacteria or fungi from positive BCs.

Direct identification of pathogens from blood by nucleic-acid technology

Direct detection method for identification of pathogens in blood without former cultivation was performed the first time for rapid detection of *Salmonella typhi* to diagnose typhoid fever.[43] Since then, several pathogen-specific PCR assays have been described such as *Mycobacteria*,[44] or different *Salmonella*.[45-47]

Several studies have shown that real-time multiplex PCR assays using TaqMan, molecular beacon or the Smart Cycler Technology have high sensitivity for direct detection of *S. aureus*, methicillin-resistant *S. aureus*, and *Enterococci* as well as highly pathogenic bacteria directly from blood within 1.5 h.[48] Rapid PCR-based identification of Gram-negative sepsis was performed experimentally for *E. coli*.[49]

Other more advanced assay is the multiplex real-time PCR-based assay with commercial name of "SeptiFast" (Roche Diagnostics, Mannheim, Germany) that capable to identify simultaneously 25 clinically important bacteria and fungi (covering about 90% of the causal microorganisms for nosocomial bacteremia) at the species level by melting peak differences directly from whole blood in <6 h.

Limitation of nucleic acid testing

Subjects including the range of detection, antimicrobial susceptibility, turnaround time, throughput, technical complexity, time, and effort requirement, as well as overall costs should be included in order to assessment of NAT-based technologies for BSI diagnosis. Other more important subjects that should be considered are the fact regarding differentiation viable from nonviable microorganisms or in other word microbial DNA aemia (DNA from live microorganisms versus DNA from dead microorganisms or free circulating DNA versus phagocytised DNA in immunocompetent host cells).

Interpretation of obtained results of new NATs associated with BCs for diagnosis of BSI is challenging when a positive result for bacterial or fungal nucleic acids is compared with a negative result for culture.[50] The reason for this contrary may be association with detection of pathogens as a result of a higher sensitivity of NAT for certain slow-growing or fastidious organisms, or associated with circulating nucleic acids or nonproliferating, nonviable or degraded pathogens, which may be lack of clinical importance.

BIOMARKERS OF SEPSIS

A biomarker is the biological compound that can be used to evaluate the condition of the individual that is, assess normal or pathologic process, or even evaluate response of the body to therapeutic intervention.[51] Because of limitation in BCs method for identifying sepsis, there have been interests for development of new tests that are sensitive and rapid for detecting nonspecific biomarkers of sepsis.[12] Many efforts have been performed with aiming for determining and identification of biomarkers that are helpful in the differential diagnosis of sepsis and other infectious diseases.

Optimal management of patients with sepsis present multiple challenges because of the variable manifestations of condition and the clinical and biochemical similarities to other, noninfective, systemic inflammatory states (e.g., trauma and blood transfusion reaction); thereby diagnosis of sepsis with high sensitivity and specificity is difficult now. Identifying sepsis precisely at an early stage has significant advantages including quickly administration of appropriate treatment such as antibiotic therapy, applying radiological/surgical drainage if necessary, also avoid unnecessary interventions, leads to reduction of antibiotic over administration; thereby decreasing in emerging bacterial resistance, *Clostridium difficile* infection as

well as the various other complications associated with antibiotics taking such as renal or hepatic dysfunction. Biomarkers offer more diagnostic tools to optimize and facilitate the clinical diagnosis and, in addition, improve definitions of the SIRS and sepsis.[52]

C-reactive protein

C-reactive protein (CRP) is one of general acute-phase plasma proteins, which belongs to the pentraxin (PTX) family of acute-phase reactants. Synthesizing of this protein is mainly by hepatocytes. As the inflammation and/or infection occur, CRP response mediated by cytokine stimulation triggers and thus its plasma concentration considerably increases.[53] The reason of considering and frequently using CRP as a biomarker of the significant inflammation or infectious disease including sepsis is fact that the levels of CRP in comparison to the other acute phase reactants, rise considerably during acute inflammation; therefore according to reports, the CRP evaluation test is used especially in pediatrics,[54] and more recently, as a biomarker of the inflammation that accompanies atherosclerosis and cardiovascular disease.[55] As whole, CRP cannot differentiate sepsis precisely but can be helpful.

It has been found that the CRP test can differentiate patients with pneumonia from those with endotracheal infections[56] as well as bacterial from viral infections[57] and is helpful in the diagnosis of appendicitis.[58] It has been shown that failure of CRP levels declining even with antimicrobial therapy are associated with increased morbidity and mortality and may be indicating of an inappropriate choice for antimicrobial therapy.

Prohormone of calcitonin

Regulation of the prohormone of calcitonin (PCT) induction during sepsis and infection is different from that of hormonal activities of the mature hormone.[59] The transcription of the CALC-I gene related to PCT is limited to neuroendocrine cells in the thyroid gland and the lung. Although the serum PCT levels are low, typically <0.1 ng/mL in healthy individuals (i.e., in noninfectious conditions),[60] during infections particularly systemic infections such as sepsis, the gene expression of the CALC-I gene upregulated and PCT is constitutively released from almost all tissues and cell types in the body.

Due to the fact that levels of PCT increase within 4–12 h upon occurring inflammation or induction, and its circulating levels decline daily when the infection is controlled using antibiotic therapy or by the host immune system, it is considered a more approving kinetic profile than CRP and cytokines.[61]

Lipopolysaccharide-binding protein

Similar to CRP and PCT, lipopolysaccharide-binding protein (LBP) is also an acute-phase protein[62] stimulated by interleukin 6 (IL-6) and IL-1.[63] Several organs including the liver, lungs, kidneys, heart, and the intestine are involved in the production of LBP.[62,64] The base levels of LBP in serum are low (1–15 g/ml) however, they increase greatly during infection.[65] Limitations of LBP as a biomarker are the fact that it cannot differentiate between recent and old infection and also Gram-positive bacteremia. Function of LBP includes binding the lipopolysaccharide (LPS) of Gram-negative bacteria and forms a complex that in turn binds to CD14 and to toll-like receptors and thereby triggering a signal transduction and finally leads to release of IL-1 and induction of macrophages and neutrophils for effectively neutralizing the LPS.[66] Studies have proved that binding of LBP to killed bacteria is noticeably higher than the binding to living bacteria.[67]

Recently, it has been introduced that the soluble form of CD14 can be used as a biomarker of sepsis because it has been demonstrated that its levels be equivalent to PCT.[68]

Inflammatory cytokines

The three cytokines tumor necrosis factors (TNF), IL-1b, and IL-6 mediate the initial response of the innate immune system to injury or infection and considered pro-inflammatory cytokines. The release of TNF-α, IL-1b, IL-8, and IL-6 in response to infectious pathogens and host injury may result in SIRS, therefore, could be considered potentially helpful biomarkers of sepsis. Studies have shown that in the case of endotoxin related Gram-negative sepsis, TNF and IL-1b levels are both elevated. IL-6 has been particularly received the most attention. It can be measured more reliably in plasma than the other two cytokines (TNF and IL-1b), having a long half-life[16] and also other potential clinical uses such as diagnosis and management of autoimmune rheumatic disorders. According to many studies, elevated levels of IL-6 in septic patients are associated with an increased mortality.[69]

The chemotactic cytokines called chemokines are another group of pro-inflammatory cytokines which have been introduced as biomarkers of sepsis. There are two major types of chemokines based on function: Homing chemokines and inflammatory chemokines.[70] The latter type leads to attraction of polymorphonuclears (PMNs) and monocytes to inflammatory sites and enhance diapedesis (migration) of them through the blood vessel wall. Thus, many inflammatory chemokines are considered as potential

biomarkers of sepsis. Chemokine IL-8 for diagnosis of sepsis[71] and monocyte chemoattractant protein-1 for prediction of sepsis mortality[72] are examples of used biomarkers.

Pro-adrenomedullin and pro-vasopressin

Vasopressin or arginine vasopressin (AVP) and adrenomedullin (ADM) are the two described biomarkers related to regulation of the vasotonus. Serum levels of AVP have been shown to rise during the early phase of septic shock and to decrease during the later phases of septic shock.[73,74] Pro-ADM was showed to be useful compared with other biomarkers (PCT, CRP, IL-6)[75] because it was an excellent prognostic biomarker for the severity and outcome of sepsis, and superior to other biomarkers such as CRP and PCT. It has been demonstrated in a recent study of 99 patients with septic shock that serum concentrations of both pro-AVP and pro-ADM were considerably higher in nonsurvivors compared with survivors, and concluded that both biomarkers seemed to be good predictors of 28-day mortality after septic shock.[76]

Pentraxin

Pentraxins belong to the superfamily of proteins involved in acute immunological responses which act as pattern recognition receptors. Pentraxin 3 (PTX3) has similar structures to CRP, which may be produced by various cells primarily by inflammatory cells including leukocytes and endothelial cells rather than the liver. The studies have shown that alike CRP, increased levels of PTX3 are associated with the severity of sepsis.[77] Function of PTX3 includes binding to specific patterns of fungi, bacteria, and viruses and stimulating phagocytosis as a result of binding to complement component C1q.[78] Persisting high levels of circulating PTX3 over the 1st day from the onset of sepsis in patients with severe sepsis and septic shock has been reported to associate with mortality; in addition, correlation of PTX3 with the severity of sepsis and with sepsis-associated coagulation/fibrinolysis dysfunction has been shown. It has also proven that PTX3 is predictive for patients with positive BCs.[79] Maximum PTX3 levels between days 1 and 4 in bacteremic patients were reported to be noticeably higher in nonsurvivors compared with in survivors;[80] also, presenting high PTX3 levels in hematologic patients with febrile neutropenia after chemotherapy have been predicted to be septic shock and bacteremia at the onset of febrile neutropenia.[81]

Macrophage migration inhibitory factor

The level of macrophage migration inhibitory factor (MIF) as a regulator of innate immunity[82] is raised in septic shock. MIF has been demonstrated to distinguish among survivors and nonsurvivors[83] but failed to discriminate infectious from noninfectious causes of inflammation.[84]

New biomarkers

Triggering receptor expressed on myeloid cells-1

Triggering receptor expressed on myeloid cells-1 (TREM-1) is one of the recently discovered biomarkers which belong to the immunoglobulin superfamily of receptors. It has been shown that bacterial or fungal infections can lead to inducing expression of TREM-1. Similar to CD64, TREM-1 is up-regulated when bacteria expose to PMNs.[85] However, the studies regarding clinical use of the soluble form of TREM-1 as a biomarker are under investigations.[86] It has been shown that sensitivity and specificity of used TREM-1 as a diagnostic biomarker to be more than CRP and PCT.[87] Furthermore, Urine sTREM-1 has been reported to be more sensitive compared to other tests e.g. evaluation of serum CRP or PCT, and routine white blood cell counts for the primary diagnosis of sepsis, dynamic evaluations of severity and prognosis.

CD163

New biomarker CD163 is a transmembrane molecule until now only revealed on the membrane of mononuclear phagocytes. CD163 has been found to regulate the expression of anti-inflammatory molecules such as IL-10 and hemeoxigenase-1.[88] Based on some studies, it has been shown that blood levels of the serum sCD163 have prognostic importance for several inflammatory diseases and can be used as a biomarker of inflammatory diseases in clinical applications for dynamic evaluation of sepsis prognosis.[89]

MicroRNAs

Other agents that received the most attention as biomarkers are microRNAs (miRNAs) a type of endogenous noncoding small RNAs with approximately 22 nucleotides in length.[90] They have been shown to play significant roles by inhibiting the expressions of messenger RNAs.[91] According to investigational observation, a considerable number of miRNAs present outside of cells within various body fluids. Because of stability characteristics of the cell-free miRNAs in body fluids below unpleasant conditions including boiling, low or high pH and multiple freeze-thaw cycles,[92] Circulating miRNAs have been recently identified as promising biomarkers for sepsis.

Based on several finding regarding miRNAs, it is suggested that miR-150 might be in related to some of the immune system dysfunctions in sepsis patients, and it presents a new potential pathogenetic mechanism of sepsis.

CONCLUSION

Direct detection and identification of pathogens in clinical samples specifically in blood specimens of septicemia patients is a promising idea for early appropriate treatment. Using blood samples for detection of pathogens, such as bacteria or fungi directly by molecular methods is very challenging since there are some inhibitors and the load of bacteria or fungi is very low. In spite of the improvement of quality and reproducibility of molecular methods in diagnostic BSIs, there are some limitations for using this method in direct and also expensive and technically demanding technologies. At present, by introducing a few biomarkers in diagnostic of septicemia (e.g. CRP, PCT, LBP, inflammatory cytokines, presepsin, PTX, presented new biomarkers such as: TREM-1, CD163, and especially miRNA involved in BSIs) it seems that they could use for primarily screening or even that for diagnostic in practice. By the way, using the combination of BC technique, molecular methods and biomarkers in diagnostic of septicemia would be better than the application of one method since each of this method has some advantages and disadvantages in real.

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Source of Support: Nil, **Conflict of Interest:** None declared.