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Role of biomarkers in sepsis care

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Abstract

Sepsis is one of the leading causes of mortality and morbidity, even with the current availability of extended spectrum antibiotics and advanced medical care. Biomarkers offer a tool in facilitating early diagnosis, in identifying patient populations at high risk of complications, and in monitoring progression of the disease, which are critical assessments for appropriate therapy and improvement in patient outcomes. Several biomarkers are already available for clinical use in sepsis; however, their effectiveness in many instances is limited by the lack of specificity and sensitivity to characterize the presence of an infection and the complexity of the inflammatory and immune processes, and to stratify patients into homogenous groups for specific treatments. Current advances in molecular techniques have provided new tools facilitating the discovery of novel biomarkers, which can vary from metabolites and chemical products present in body fluids to genes and proteins in circulating blood cells. The purpose of this review is to examine the current status of sepsis biomarkers, with special emphasis on emerging markers, which are undergoing validation and may transition into clinical practice for their informative value in diagnosis, prognosis or response to therapy. We will also discuss the new concept of combination biomarkers and biomarker risk models, their existing challenges, and their potential use in the daily management of the sepsis patients.

Keywords

Central venous oxygen saturation; cluster of differentiation-64; C-reactive protein; erythrocyte sedimentation rate; interleukin-6; interleukin-8; interleukin-27; lactate; lipopolysaccharide binding protein; procalcitonin; triggering receptor expressed on myeloid cells-1

Sepsis: Consensus Definition and Management

Sepsis is a systemic response to infection, characterized by hemodynamic, metabolic and inflammatory derangement, which can present in mild cases as systemic inflammatory response syndrome (SIRS) or progress to severe sepsis and septic shock (1). The Sepsis Consensus Conference has defined SIRS by using four clinical and laboratory criteria: heart rate, temperature, respiratory rate, and white blood count. The presence of SIRS with a high clinical suspicion of infection or positive microbial cultures is considered sepsis. Severe

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sepsis is defined as a state of sepsis with concomitant organ dysfunction and hypoperfusion. Patients with sepsis who are hypotensive despite adequate resuscitation are considered to be in septic shock (1, 2). Every year, sepsis affects about 1,000,000 Americans, with a mortality rate estimated between 4% and 10% in pediatric patients and between 28% and 50% in adult patients (3-6). Current management of sepsis relies on the early diagnosis and early implementation of fluid resuscitation and antimicrobial therapy, as well as identification of infection focus and surgical intervention if required. Consequently, in addition to the routine screening of SIRS clinical parameters and identification of the source of infection (7), much effort is being placed on identifying biomarkers that can detect sepsis in an early and reversible phase to decrease the rate of mortality from this disease. In the management of the sepsis patient it is also imperative to closely monitor the progression of the disease and to estimate prognosis as this information may also dictate the individualized clinical intervention (2). Therefore, there has been a need also to discover biomarkers which can aid in the monitoring and staging of sepsis and consequently in the therapeutic management.

Biomarker: Consensus Definition and Characteristics

The National Institutes of Health defines a biomarker as a “characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention” (8). The sensitivity, specificity and predictive value are the most important factors that characterize the ideal biomarker (8). In acute conditions of a disease, which require a prompt clinical intervention, the biomarkers also need to be readily obtainable from body fluids or tissue samples and the test results need to be obtainable in a relative short period.

Biomarkers can be classified based on their clinical application. In the management of sepsis, biomarkers have been used or are currently being developed as diagnostic, prognostic, monitoring, surrogate and stratification biomarkers (Table 1) (9). Diagnostic biomarkers, which can also include screening biomarkers, are used to determine health or disease. Prognostic biomarkers provide information on the likely outcome in an untreated individual and help in establishing the intensity of treatment. Monitoring biomarkers serve to evaluate the disease process and are especially informative of the effectiveness of response to the therapeutic intervention, allowing the clinician to titrate or change the management protocol. A surrogate biomarker is usually an end-point that can be used as an outcome in clinical trials to evaluate safety and effectiveness of therapies instead of measurement of the true outcome of interest. The underlying principle is that changes in the surrogate biomarker follow closely with changes in the outcome of interest. Surrogate biomarkers have the advantage that they may be obtained in a more timely and inexpensive manner than primary end-points such as serious complications and mortality, which require large clinical trials for evaluation (10). Stratification biomarkers are used to identify a group of patients with shared homogenous biological characteristics and help to select the optimal management for the patients and achieve the best possible outcome in terms of disease category. In the context of management of sepsis, which is a complex and heterogeneous pathologic disease, the use of stratification criteria may also help in effectively characterizing risk groups for inclusion into specific treatments or into clinical trials of new interventions (11).

The form of sepsis biomarkers can vary from metabolites and chemical products present in body fluid to genes and proteins in circulating blood cells. Laboratory tests have focused on biological factors associated with the complex components of the pathophysiology of sepsis, including the oxygenation state of tissue and organs, the oxidative injury by reactive species, the inflammatory and the immune responses, the alteration of the coagulation and

complement cascades, as well as the endothelial dysfunction and the tissue necrosis (12). Because the pathophysiology and the clinical course of sepsis is also influenced by the age of the patients (13), it is also likely that sensitivity and specificity of certain biomarkers are age specific and may show differences when considering neonates, children, adult or elderly patients.

Several biomarkers are currently used in clinical practice, but only a few of them have the characteristics of the ideal laboratory test. Recently, there has been a growing interest in identifying novel biomarkers. The identification of these new candidates has been followed by a thorough operational evaluation to validate its application in the clinical setting. Potential biomarkers must indeed pass several practical tests prior to being applied to clinical practice. Required phases of biomarker development include preclinical exploratory studies, development of the clinical assay, and derivation and validation clinical studies (14). For instance, development of neutrophil gelatinase-associated lipocalin as a biomarker for acute kidney injury is an example of successful transition from candidate biomarker to actual biomarker status (15). However, at this time, only a few novel candidates have achieved actual biomarker status for sepsis, due to failure at the derivation or validation phase.

Markers of acute inflammatory response

One of the most important diagnostic goals for the clinician is the determination of an infective condition and consequently the distinction between SIRS and sepsis, as this dictates therapeutic management decisions with respect to initiation, selection and duration of antibiotics (2). Diagnosis of sepsis may be difficult because the clinical signals and symptoms of the systemic inflammation may overlap with non-infectious critical conditions, such as trauma, burn, pancreatitis, autoimmune diseases and transplant rejection (2).

Commonly utilized biomarkers are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). However, both of them are non-specific markers of inflammation rather than infection, and may be useful in determining the severity and the progression of the disease but not in distinguishing sepsis from SIRS. For example, in burn patients ESR was elevated but not different between infected and non-infected burn patients (16). Another conventional laboratory test for the diagnosis and the monitoring of the progression of the inflammatory process is the measurement CRP levels. CRP is an acute phase reactant, which is synthesized by hepatocytes in response to cytokine stimulation. Normal plasma levels of CRP are usually as <10 mg/L. Plasma levels increase within 4-6 h after initial tissue injury and continue to increase several hundred folds within 24-48 h (17). CRP remains elevated during the acute phase response and returns to normal with resolution of tissue damage. Its plasma concentration has been reported to correlate with the severity of infection (18). Rapid decrease in CRP levels over the first 48 h of therapy has been shown to correlate with an effective response to the initial antimicrobial therapy in septic patients (19). However, its specificity in indicating the presence of an infection has been challenged. For instance, several investigators have reported high levels of CRP among patients with burn injury with or without septic complications (16, 20, 21). CRP has also been shown to have a poor predictor of mortality compared to other biomarkers (22, 23).

Other biomarkers related to the pathophysiology of the inflammatory and immune responses are the pro-inflammatory cytokine interleukin-6 (IL-6) and lipopolysaccharide binding protein (LBP) (24, 25). In the setting of sepsis, clinical studies have shown an elevation of IL-6 in both adult and pediatric populations (26, 27). Serum levels correlate with the severity of septic shock (28) and are higher in non-survivors compared to survivors (29, 30). High serum levels of IL-6 (greater than 1000 pg/ml) have been shown to predict sepsis

related death in adult patients and have been used as stratification tool in sepsis trials investigating the therapeutic efficacy of anti-TNF α antibodies treatment (31, 32). However, IL-6 is also elevated in non-infectious conditions like trauma, surgery and stroke, thus its role as diagnostic tool cannot be completely confirmed (33).

LBP is a polypeptide mainly synthesized in the liver and released into the bloodstream after glycosylation. LBP has a dual role, enhancing the lipopolysaccharide (LPS) signaling at low levels while at high levels it inhibits LPS signaling (25). Serum LBP levels increase several fold in sepsis (34). Studies of LBP in sepsis have shown varying results in different age populations. In critically ill neonates and children, LBP was a better marker of sepsis than IL-6 and PCT (35). In the adult population, both IL-6 and LBP appeared to be superior to PCT as diagnostic markers of sepsis when tested in a cohort of 106 adults with community acquired infections and sepsis (36). On the other hand, in a recent prospective study, LBP only moderately discriminated sepsis from SIRS (AUC 0.66) in a cohort of surgical ICU patients and was inferior to IL-6 (AUC 0.76) and PCT (AUC 0.86) (37).

Among molecules related to the organ damage pathophysiology, serum soluble endothelial markers such as fms-like tyrosine kinase 1, E-selectin, intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and plasminogen activator inhibitor 1 have been measured in adult patients and found to be associated with sepsis severity, organ dysfunction, and mortality (38, 39). However, these data await confirmation in larger clinical studies.

Potential marker of infection: Procalcitonin

Procalcitonin (PCT) is a 116 amino-acid peptide precursor of the hormone calcitonin, which has been proposed as a reliable diagnostic and prognostic marker of sepsis, better differentiating inflammatory responses from bacterial infections. In healthy individuals, PCT is secreted only in neuroendocrine cells of the thyroid (40); however, during an infection, PCT is released up to a thousand fold increase from nearly all tissues and cell types in the host in response to cytokines and bacterial products (41). In experimental studies in hamsters, PCT levels were increased after *in vivo* treatment with TNF. However, when PCT was administered to the animals, it did not increase levels of IL-1 or TNF, suggesting that this hormone precursor is a secondary mediator of the inflammatory septic response (42). On the contrary, production of PCT is attenuated by viral-induced interferon- γ (23, 40, 41), further supporting its sensitivity and specificity for bacterial infections, rather than viral infections.

PCT has many favorable characteristics. In a study in healthy volunteers subjected to endotoxin administration, serum levels of PCT increased by 3 h with peak values between 6-24 h. Although progressively declining, serum levels remained above normal up to 7-14 days (43, 44).

PCT levels are significantly high in patients with bacteremia and moderately elevated in patients with fungemia (45). On the contrary, levels fall rapidly when infection gets controlled and correlate with down regulation of its gene in several tissues (46). Continuous veno-venous hemofiltration does not significantly influence PCT plasma concentrations or kinetics in patients with sepsis and PCT can be used for diagnostic purposes in this patient population (47). Circulating levels of PCT demonstrate a superior diagnostic accuracy when compared to other established biomarkers and indicators of sepsis (48, 49), and are unaffected by the administration of anti-inflammatory therapy, such as glucocorticoids, when compared to CRP (50). The sensitivity and specificity to discriminate infection from the inflammatory response has been also reported in pediatric patients with SIRS and sepsis (51, 52). In liver transplanted children, PCT levels were useful to differentiate SIRS

associated with graft rejection from sepsis (53). In children with cardiopulmonary bypass, PCT was the most reliable diagnostic marker of probable/definite sepsis compared to CRP and immature-to-total neutrophil ratio with an area under the curve (AUC) of 0.84 (95% confidence interval, 0.75-0.92) for PCT (54). In pediatric patients with community acquired pneumonia, PCT level can help in differentiating viral and bacterial pneumonia better than CRP, white cell count and IL-6 levels (55).

The prognostic value of PCT has also been evaluated. In a retrospective study of postoperative critically ill adult patients, PCT levels were measured on first post-operative day and were found to predict mortality using a cut off point of 1.44 ng/mL (AUC 0.871, sensitivity 80.8%, specificity 80.4%) (23). PCT levels have been also shown to be a more sensitive marker in predicting late mortality at 30 days with an AUC of 0.73 when compared to an AUC of 0.64 for CRP levels (56).

PCT has also been studied as a monitoring biomarker for antibiotic stewardship. A recent meta-analysis of randomized controlled trials has suggested that implementation of a PCT-based algorithm may reduce antibiotic exposure in adult septic patients without compromising clinical outcomes (57). PCT also performs with high sensitivity and specificity as a prognostic and stratifying biomarker. In a pediatric prospective observational study, PCT was superior to CRP in distinguishing severity of illness when patients were classified into six groups (negative, SIRS, localized infection, sepsis, severe sepsis, and septic shock) with an AUC of 0.91 for PCT versus 0.75 for CRP (58). In addition to absolute serum concentrations, dynamic changes of PCT may have predictive value during the hospital stay. A recent study in septic shock reported that a decrease of PCT level by 25% over a 5-day period was a useful indicator of survival in patients who exhibited PCT concentrations greater than 10 ng/ml at day 1 of enrollment (59). Because of significant heterogeneity among studies and selection criteria, however, other meta-analyses have not confirmed the superior diagnostic performance of PCT versus other sepsis biomarkers (60, 61), although still suggesting that PCT may be a helpful diagnostic tool. Therefore, although elevated levels of PCT appear indicative of sepsis, this laboratory test has to be carefully interpreted in the context of the clinical situation and used in conjunction with other laboratory findings for a correct diagnosis.

Markers of impaired metabolism: central venous oxygen saturation (ScvO₂) and lactate

Oxygen delivery, oxygen consumption, and tissue acidosis are the main variables that are considered for determining adequate tissue perfusion and oxygenation. The most commonly used parameters are the mixed venous O₂ saturation (SvO₂), the central venous O₂ saturation (ScvO₂) and serum lactate, which provide information about the degree of oxygenation and anaerobic metabolism and are used as diagnostic, prognostic and monitoring biomarkers.

ScvO₂ has been used to detect imbalance between oxygen delivery and consumption. A low ScvO₂ indicates low oxygen delivery to tissues, an increased extraction at the cellular level, or a combination of the two mechanisms. Optimization of ScvO₂ is considered one of the main resuscitation targets of the early-goal-directed therapy along with optimization of central venous and mean arterial pressure and urine output as recommended by the Surviving Sepsis Campaign 2012 guidelines (2). A recent multicenter study has, in fact, reported that inability to achieve ScvO₂ >70% within the first 6 h of early goal-directed therapy is associated with significantly increased mortality in patients with sepsis (62). A meta-analysis including 5 studies with 11,530 patients found that patients reaching values of

ScvO₂ >70% were twice more likely to survive compared to patients not reaching this endpoint (63).

Serum lactate levels have been used as a biomarker for tissue hypoxia and anaerobic metabolism. Energy production at the cellular level depends on the availability of glucose and oxygen. Glucose is converted into pyruvate via the glycolytic pathway, which yields a small amount of ATP, the driver of all energy linked metabolic processes in the cell. Pyruvate in turn is taken up by mitochondria to enter the Krebs cycle to produce more ATP via the electron transport chain (a process referred to as aerobic metabolism). However, in the absence of oxygen, pyruvate remains in the cytoplasm and is converted into lactate yielding a small amount of ATP in the process. With the ongoing tissue hypoxia during the state of shock, other mechanisms may further contribute to hyperlactatemia. Such mechanisms include the excessive or prolonged catecholamine release, which may cause elevated lactate levels through stimulation of glycolysis, and hepatic failure, which may cause decrease of lactic acid clearance (12). Several clinical studies have demonstrated a correlation between serum lactate and outcome, as both the duration and degree of hyperlactatemia are important predictors of morbidity and mortality. In a large retrospective study, admission lactate levels >2 mmol/L was a significant independent risk factor for mortality in patients admitted to intensive care units. In the same study, the odds ratio increased from 1.94 to 10.89 dependent on the level of hyperlactatemia compared to patients with admission lactate of <2 mmol/L (64). In addition, sustained hyperlactatemia has been shown to be predictive of in-hospital mortality (65). On the contrary, early lactate clearance was associated with improved outcomes in patients with severe sepsis and septic shock (66). In critically ill patients, including patients with sepsis, who had hyperlactatemia on admission, early-guided therapy was shown to significantly reduce hospital mortality when 20% reduction of lactate levels was used as a target of the resuscitation protocol (67). The utility of serial blood lactate monitoring and its value as predictive marker of in-hospital mortality have been further confirmed by a recent systematic review (68). It also appears that lactate screening may be a valuable risk-stratification tool even in normotensive, hemodynamically stable patients. In a study of 1,278 patients with infection, lactate levels less than 2.5 mmol/L were associated with a 4.9% mortality rate compared to patients with lactate levels ≥ 4 mmol/L who had an in-hospital mortality of 28.4% (69). Recently, data from the derivation cohort of the Vasopressin Septic Shock Trial and further validation in a single-center septic shock cohort has suggested that even minimal increase in arterial lactate concentration within the normal range (1.4-2.3 mmol/L) may predict 28-day mortality (sensitivity of 86%, specificity of 27%). Furthermore, the data suggested that a low cut off value (lactate level <1.4 mmol/L) might be used as a therapy guide for vasopressin infusion (70).

Markers of innate immune response: Cluster of differentiation-64 (CD64) and triggering receptor expressed on myeloid cells-1 (TREM-1) as emerging markers of infection

CD64 is a high affinity immunoglobulin Fc gamma receptor I and is expressed constitutively on monocytes, but only to a very low extent on resting polymorphonuclear cells (PMNs). In conditions of infections, however, CD64 expression on PMNs increases remarkably and indicates an attempt by the innate immune response to increase phagocytosis. In this regard, CD64 has several desirable biomarker characteristics and can be used for differentiating bacterial infection from other inflammatory disorders. Its expression is low on resting neutrophils and is increased significantly after activation within a few hours (71). For example, CD64 expression has been shown to increase rapidly in polymorphonuclear cells from healthy individuals exposed to lipoteichoic acid (72) or lipopolysaccharide (73),

reaching a plateau within 20 min to 2 h and persisting for 48 h (72, 73). CD64 expression returns to its basal level in a few days after the stimulus disappears (71). Moreover, CD64 is relatively stable after blood collection and its expression is easily measured by flow cytometry (71). Upregulation of CD64 has been demonstrated in PMNs of patients with severe bacterial infections (73). Interestingly, overexpression of CD64 in blood monocytes and neutrophils in septic patients appears to be associated with leukocyte dysfunction when compared to CD64-negative cells (74). In a recent meta-analysis of 13 studies including pediatric and adult patients, CD64 expression on PMNs appeared to be a useful diagnostic cell-based parameter of bacterial infections with a pooled sensitivity and specificity of 79% (95% CI: 70-86%) and 91% (95% CI: 85-95%), respectively (75). When conducted in neonates and children, a prospective observational study also reported that CD64 expression had higher diagnostic accuracy for infection than PCT and CRP (76). In a clinical study with adult patients, a CD64 index of 1.19 was predictive of negative blood culture results, while an index of >1.19 was predictive of an ultimate clinical and/or culture diagnosis of infection with a sensitivity and specificity of 94.6% and 88.7%, respectively. CD64 indices also seemed to change in response to antibiotic therapy (77).

TREM-1 is a trans membrane glycoprotein expressed on the surface of neutrophils, mature monocytes, and macrophages, which has been demonstrated to be involved in the signaling of the inflammatory response during infection (78, 79). A soluble counterpart of TREM-1 (sTREM-1) has been detected in biological fluids of patients and animals suffering from a variety of infections and diseases and often correlates with disease severity (79). Experimental studies have also suggested that TREM-1 is an important pathogenetic factor of organ dysfunction and inhibition of the TREM-1 pathway affords beneficial effects in *Streptococcus pyogenes*-induced sepsis (80) and hemorrhagic shock models (81). In a prospective multi-biomarker clinical study of patients admitted to intensive care units with suspected infection, plasma sTREM-1 levels had the highest discriminative value to differentiate SIRS, sepsis, severe sepsis and septic shock, with an AUC of 0.97, followed by PCT (AUC 0.85) and CRP (AUC 0.77) (82). A recent small prospective study (n=63) of patients older than 18 years with severe sepsis and septic shock found significantly higher plasma sTREM-1 levels in non-survivors compared to survivors at the time of admission and before early goal directed therapy (514.1 pg/ml vs 182.4pg/ml, p=0.001) (83). In addition, the plasma sTREM-1 levels remained significantly higher until death in non-survivors compared to survivors and performed better at predicting mortality than PCT and CRP (83). However, data are not yet consistent among clinical studies. In a similar study, Gibot and colleagues reported that sTREM-1 levels were significantly lower at admission in non-surviving patients compared to surviving patients. Nevertheless, even with lower levels at admission, sTREM-1 concentrations remained stable or even increased in non-survivors while decreasing in survivors (84). Studies in patients after cardiac surgery and cardiac arrest without infection have suggested that sTREM-1 and PCT are not specific for infection and can also increase markedly in acute inflammation without infection in a similar degree as patients with sepsis (85). Furthermore, a recent meta-analysis including eleven studies showed that plasma sTREM-1, when used individually, was not sufficient in differentiating sepsis from SIRS (86).

Biomarker combination approach

Since no clinical study has provided conclusive evidence of an ideal biomarker with sufficient sensitivity and specificity to be utilized in the management of septic patients, recently a biomarker combination approach has been proposed. In a prospective study in patients suspected of having community-acquired infections, Kofoed and colleagues used a panel of six biomarkers including sTREM-1, CRP, PCT, soluble urokinase-type plasminogen activator, macrophage migration inhibitory factor and neutrophil count. The

diagnostic accuracy for detecting bacterial versus non-bacterial causes of inflammation was significantly greater than that of the single markers (87). In another prospective study, which included both inception and validation cohorts, Gibot and colleagues (88) developed a bioscore using three biomarkers, CD64 index, PCT and sTREM-1 levels. When compared to the single biomarker, the bioscore demonstrated a higher performance in diagnosing sepsis in the critically ill patients. The probability of sepsis paralleled the increase of bioscore with rates of infection of 3.8% for a bioscore of 0 (all three markers below their respective thresholds) to 100% for a bioscore of 3 (all three markers above threshold) with an AUC of 0.97 (95% CI, 0.95–0.99) (88).

Discovery of novel candidates with the ‘omic’ approach

The rapid development of high-throughput technologies in the field of genomics, proteomics and metabolomics has recently allowed identifying candidate biomarkers using a large-scale approach (89). The cellular and molecular heterogeneity of sepsis and the large number of genes potentially involved in its pathogenesis emphasize the importance of studying gene expression changes on a global scale. The underlying concept is based on the hypothesis that active genes and their respective products, and other organic chemicals made by the cell are unique identifiers that may represent the ‘molecular signature’ of a specific disease. However, application of these technologies is not easily translatable into clinical routine analysis, as it requires laboratory-based assays, which are expensive and time-consuming. For this reason, an important step in the identification of novel genomic, proteomic or metabolic candidates has been the development of approaches, which can be easily implemented in clinical laboratories (89).

The identification of interleukin-27 (IL-27) as a potential biomarker illustrates the usefulness of genomics and its application in clinical medicine. In a recent large multi-center study led by our intensive care unit, genome-wide expression analysis between pediatric patients with SIRS and pediatric patients with sepsis identified 221 gene probes, which were differentially regulated between the two groups. At cross validation, the *Ebstein-Barr-virus-induced gene 3 (EBI3)* was found to have the highest predictive strength for bacterial infection of all predictor genes. Since the *EBI3* gene transcribes for the EBI3 protein subunit of IL-27, an important immune regulatory cytokine, the utility of serum levels of IL-27 protein to predict bacterial infection was further tested in healthy control children and in a large cohort of critically ill patients. Using a cutoff point of 5.0 ng/ml, IL-27 was found to have a specificity and positive predictive value of >90% for identifying bacterial infection in these patients (90).

Another example of biomarker discovery through genome-wide analysis of gene expression is the identification of IL-8 as a stratification biomarker in pediatric septic shock (91). With microarray analysis of whole-blood-derived RNA from samples obtained within 24 h of admission of patients with septic shock, the *IL8* gene was identified as one of 34 genes highly expressed in nonsurviving patients. Serum levels of the IL-8 protein greater than 220 pg/ml had 75% sensitivity and specificity in predicting mortality at 28 days and a negative predictive value of 95%. A validation study further confirmed the predictive value of IL-8 for mortality (91). However, a prospective study in adult patients did not confirm the ability of IL-8 to serve as a stratification biomarker in sepsis (92). Nevertheless, a combination approach of serum IL-8 levels with other cytokines, such as IL-6 and IL-10, was associated with a worse outcome in adult patients with sepsis (93).

It is important to note that the extensive data from the genome-wide expression analysis offers a powerful tool for the development of multi-biomarker panels, which can be used for stratification strategy. For example, in our pediatric intensive care unit, we have derived and

tested a biomarker-based risk stratification model, PERSEVERE (PEdiatRiC SEpsis biomarkEr Risk modEl), which appears to reliably predict mortality in children with septic shock. Using twelve candidate genes from the genomic data, which were identified for their differential regulation between survivors and non-survivors, ideal biomarkers were further tested on two important characteristics of plausibility and feasibility. Specifically, the expressed product of the gene was selected according to its likelihood to have a biological and mechanistic role in the context of the infection and/or the pathological inflammatory and immune dysregulatory responses; and according to its easy access in a biological fluid. The derived decision tree included five final serum biomarkers, C-C chemokine ligand 3, neutrophil elastase 2, heat shock protein 70, IL-8 and lipocalin 2. In a combined derivation and study cohort, the decision tree showed sensitivity for mortality of 93%, specificity of 74%, positive predictive value of 32%, and negative predictive value of 99% (94). Therefore, it appears that, once the model will be further validated, PERSEVERE has the potential to serve as stratification tool for sepsis management.

Metabolomic profiling may also represent a valid strategy for the development of multi-biomarker panels and predictive models, which can be used for diagnosis and prognosis of sepsis. For example, using proton nuclear magnetic resonance spectroscopy, specific metabolic changes were found in pediatric patients with sepsis when compared with healthy control children (95). The predictive models consisted of metabolites mainly associated with the energy deregulation, the inflammatory response and the organ failure of sepsis. These constructed metabolomics models showed an excellent separation between survivors and non-survivors and a very good distinction between the observed and expected outcomes, and were also able to identify age-specific responses to sepsis (95). Further validations studies will determine whether these metabolite spectra may be reproducible in large cohorts of patients and may represent reliable chemical fingerprints of the pathophysiology and dysmetabolism of sepsis.

Conclusion

Many advances have been made in the identification of novel biomarkers for sepsis care. However, substantial discovery still remains to be made in this field. Novel high-throughput methodologies hold the promise to identify molecular signatures of the disease. However, extensive clinical validation of these novel biomarkers remains one of the most significant challenges. Nevertheless, in the process of searching for reliable markers, valuable insights may derive into the pathophysiologic mechanisms of sepsis that could possibly lead to novel treatment strategies.

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Table 1
Description of clinical goals of selected biomarkers of sepsis

Use	Clinical Goal	Biomarker used or evaluated in clinical studies and reference number
Diagnostic	Used to reliably and readily determine the presence or absence of the disease.	Cluster of differentiation-64 (75, 76), Interleukin-27 (90) Lactate (11) Lipopolysaccharide binding protein (35, 36) Procalcitonin (11, 21, 47, 48, 49, 51, 52, 53, 54) Triggering receptor expressed on myeloid cells-1 (82)
Prognostic	Used in estimating the likely outcome, without considering treatment, to establish the intensity of treatment.	C-reactive protein (22) Interleukin-6 (22, 23, 29, 30) Interleukin-8 (91) Lactate (68, 69, 70, 64, 65) Procalcitonin (46,57) ScvO ₂ (62, 63) Triggering receptor expressed on myeloid cells-1 (83)
Monitoring	Used in assessing the effectiveness and adverse effects of a treatment.	Cluster of differentiation-64 (77) C-reactive protein (19) Lactate (67) Procalcitonin (22, 56, 59) ScvO ₂ (62)
Surrogate	Used as a short-term outcome in predicting a long-term primary clinical outcome for a quick assessment of the safety and efficacy of a therapy.	Lactate (67)
Stratification	Used to classify groups of patients in terms of stage, severity or outcome of the disease.	Lactate (11, 69, 70) Interleukin-6 (31, 32) Procalcitonin (11, 58) Triggering receptor expressed on myeloid cells-1 (82)