Clinica Chimica Acta 460 (2016) 203–210

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Review

Diagnosing sepsis – The role of laboratory medicine

Shu-Ling Fan, Nancy S. Miller, John Lee, Daniel G. Remick *

Department of Pathology and Laboratory Medicine, Boston University School of Medicine/Boston Medical Center, United States

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 29 June 2016
Accepted 2 July 2016
Available online 4 July 2016

Keywords:
Biomarkers
Sepsis
Cytokines
Procalcitonin
Lactate
IL-6
D-dimer
Bloodstream infections
Bacteremia
Rapid diagnostics

ABSTRACT

Sepsis is the host response to microbial pathogens resulting in significant morbidity and mortality. An accurate and timely diagnosis of sepsis allows prompt and appropriate treatment. This review discusses laboratory testing for sepsis because differentiating systemic inflammation from infection is challenging. Procalcitonin (PCT) is currently an FDA approved test to aid in the diagnosis of sepsis but with questionable efficacy. However, studies support the use of PCT for antibiotic de-escalation. Serial lactate measurements have been recommended for monitoring treatment efficacy as part of sepsis bundles. The 2016 sepsis consensus definitions include lactate concentrations >2 mmol/L (>18 mg/dL) as part of the definition of septic shock. Also included in the 2016 definitions are measuring bilirubin and creatinine to determine progression of organ failure indicating worse prognosis. Hematologic parameters, including a simple white blood cell count and differential, are frequently part of the initial sepsis diagnostic protocols. Several new biomarkers have been proposed to diagnose sepsis or to predict mortality, but they currently lack sufficient sensitivity and specificity to be considered as stand-alone testing. If sepsis is suspected, new technologies and microbiologic assays allow rapid and specific identification of pathogens. In 2016 there is no single laboratory test that accurately diagnoses sepsis.

Contents

* Corresponding author at: 670 Albany Street, Room 407, Boston, MA 02118, United States.
E-mail address: remickd@bu.edu (D.G. Remick).

http://dx.doi.org/10.1016/j.cca.2016.07.002
0009-8981/

1. Introduction

Sepsis is a significant public health problem across the world, with >31 million cases annually and a 17% mortality [1]. Sepsis is a systemic host response to microbial pathogens that results in significant morbidity and mortality. The concept of the Systemic Inflammatory Response Syndrome (SIRS) was proposed in 1992 [2] to help identify critically ill patients and the original criteria are listed in Table 1. Sepsis and SIRS can closely mimic one another and present a diagnostic challenge. A 2016 report, defined as Sepsis-3, detailed the Third International Consensus Definitions for Sepsis and Septic Shock [3]. These definitions are listed in Table 2. The lay definition of sepsis by this group is succinct and easy to communicate to patients: "Sepsis is a life-threatening condition that arises when the body's response to an infection injures its own tissues and organs". With these updated definitions it is appropriate to review the role of the clinical laboratory in the diagnosis of sepsis.

A biomarker with high sensitivity, specificity, speed and accuracy would be revolutionary for differentiating sepsis from noninfectious SIRS, given the limitations and time required for microbial verification of pathogens. Furthermore, 40% of the sepsis patients remain culture negative [4]. It is important to differentiate culture negative sepsis patients from those with noninfectious SIRS, as these disease conditions require different therapeutic regimens. The Surviving Sepsis Campaign recommends that antibiotics should be administered within 1 h of the onset of septic shock [5,6]. Every hour of delay in antibiotic administration has been shown to increase the mortality of septic shock by 7.6% [7]. Conversely, noninfectious SIRS patients misdiagnosed as sepsis may be inappropriately treated with broad spectrum antibiotics, which delays treatment of the underlying systemic inflammation and contributes to the emergence of antibiotic resistance [8]. Biomarkers may also improve the prediction of mortality, especially in the early phase of sepsis when levels of certain pro-inflammatory cytokines and proteins are elevated.

2. Clinical chemistry

2.1. FDA-approved tests

2.1.1. Procalcitonin (PCT)

PCT, the precursor of the hormone calcitonin, is elevated in patients with invasive bacterial infections. It is produced by many tissues, not just cells at the local site of infection, and is part of the systemic response in severe sepsis. PCT is thought to have pro-inflammatory effects similar to CRP. The FDA has approved a commercially available PCT assay [9] for the assessment of risk for developing severe sepsis in critically ill patients upon their first day of admission to intensive care units. It should be noted that the 2016 sepsis-3 definitions no longer include the category of severe sepsis [3].

2.1.1.1. PCT may accurately differentiate sepsis from SIRS. Recently, Wacker et al. [10] performed a meta-analysis including 30 studies with a total of 3244 patients and found that PCT can differentiate effectively between true sepsis and SIRS of noninfectious origin. Bivariate analysis yielded a mean sensitivity of 77% and specificity of 79%. The receiver operating characteristic curve area under the curve (AUC) was 0.85 (95% CI 0.81–0.88) with similar results for medical, surgical, and pediatric patients.

Anand et al. [11] concluded in a prospective study that PCT can accurately differentiate culture-negative (AUC = 0.89) and culture-positive (AUC = 0.96) sepsis from noninfectious SIRS and thereby contribute to early diagnosis and effective management of these conditions. In the culture-negative group, the best cutoff point for PCT was at 1.43 ng/mL (92% sensitivity; 83% negative predictive value).

2.1.1.2. PCT to guide antibiotic de-escalation. Randomized trials have been conducted to examine whether PCT levels may be used in algorithms to stop antibiotic therapy. A meta-analysis of 14 studies done in the United States included 4467 patients [12]. This analysis included patients from primary care, the emergency department and those in intensive care settings. There was a consistent reduction in both the use of antibiotics as well as the number of days antibiotics were given. Importantly, the trials did not show any difference in mortality when using the PCT algorithms, showing that early termination of antibiotic therapy was safe.

2.1.1.3. Limitations. Although PCT is closely associated with inflammation, it may not be completely specific for infection [13]. Evidence has shown that it may be elevated in a number of disorders in the absence of infection, especially following trauma [14]. Therefore, using a single concentration value for the diagnosis or prognosis of sepsis is not practical. Normal serum values are below 0.05 ng/mL, and a value of 2.0 ng/mL suggests a significantly increased risk of sepsis and/or septic shock. Values <0.5 ng/mL represent a low risk while values of 0.5–2.0 ng/mL suggest an intermediate likelihood of sepsis and/or septic shock. The meta-analysis done by Wacker et al. [10] only indicated a modest diagnostic performance with a sensitivity of 77% and a specificity of 79%. PCT is not particularly useful in making the final diagnosis in patients with values in the intermediate range. PCT should always be interpreted carefully in the context of medical history and other clinical information as recommended in the Surviving Sepsis Campaign [6].

2.2. FDA-approved analytes not specifically approved for sepsis

2.2.1. Lactate

Sepsis may progress rapidly to septic shock that is often associated with micro- and macro-circulatory dysfunction, arterial hypotension, and decreased delivery of oxygen and nutrients into peripheral tissues.

Table 1
Systemic Inflammatory Response Syndrome (SIRS) criteria patients are diagnosed with SIRS if they meet two of the four criteria [5].

Criteria	Metric	Comment
Temperature	>100.4 °F (>38.0 °C) or <96.8 °F (<36.0 °C)	Either hyperthermia or hypothermia is a SIRS criteria
Heart rate	>90 beats per minute	Only tachycardia
Respiratory rate	>20 breaths per minute	If the patient is mechanically ventilated, $PaCO_2$ <32 mm Hg
White blood count	>12,000/mm^3 or <4000/mm^3 or >10% immature forms	Any one of these parameters is sufficient for this category

Table 2
Sepsis definitions as defined by the Third International Consensus Task Force [3]. The severe sepsis category was removed.

Diagnosis	Definition
Sepsis	Life threatening organ dysfunction caused by a dysregulated host response to infection.
Septic Shock	A subset of sepsis with profound circulatory, cellular, and metabolic abnormalities associated with increased mortality.

Lactate levels have been a useful marker for organ dysfunction and may also serve as an endpoint for resuscitation in patients with sepsis and septic shock as part of the sepsis bundles [6,15]. In the 2016 Sepsis-3 definitions lactate levels were included in defining patients with septic shock [3], described in more detail below

2.2.1.1. Prognostic value of lactate measurement. The diagnostic and prognostic value of lactate in septic patients have been well-documented in the setting of an emergency department, intensive care unit or in the trauma patient. High lactate is strongly associated with poor outcome and high mortality. In a study in patients admitted with an infection ($n = 1278$), lactate levels could correctly stratify the patients' mortality into three categories (Table 3, [16]). Those with the highest levels of lactate had the highest mortality. Howell et al., who studied essentially the same patient population, recruited patients admitted from the emergency department with a clinically suspected infection [17], and Mikkelsen et al. included patients with severe sepsis. Both studies confirmed that elevated lactate levels were associated with mortality, independent of shock [18]. Similar observations were also demonstrated in other studies [19,20]. Indeed, the 2013 Surviving Sepsis Campaign international guidelines lists a lactate level >2 mmol/L as one of the criteria defining severe sepsis and a lactate level >4 as defining septic shock [5]. However, the criteria have become stricter in the recently published Sepsis-3 definitions [3]. Patients with septic shock can be identified with a lactate level >2 mmol/L after adequate fluid resuscitation and requiring a vasopressor to maintain a mean blood pressure of 65 mm Hg or greater. The criteria was further verified by a systematic review of 44 studies reporting septic shock outcomes (total of 166,479 patients).

Serial lactate measurements may be useful in monitoring treatment effectiveness to various therapeutic interventions, and therefore, is recommended in the sepsis bundle for septic shock, especially when the initial level is high [5]. Monitoring the clearance of lactate through serial measurements has been demonstrated to be a useful predictor of morbidity and mortality. Patients with a decrease in an initially elevated lactate level within 24 h have significantly better outcomes than patients whose lactate remains elevated [15]. In a study of 90 severe septic patients, <10% lactate clearance (measured upon admission and 6 h after) could predict a higher organ dysfunction rate and mortality [3].

2.2.1.2. Limitations. Although lactate is currently the most commonly used analyte measured to follow the patient's response to treatment, there are limitations to using increased lactate levels as a diagnostic biomarker. Elevated lactate levels can be seen in a wide variety of conditions, such as cardiac arrest, trauma, seizure or excessive muscle activity. Elevated levels of lactate are not considered specific for either the diagnosis of sepsis, or predicting mortality, unless thoughtfully coupled with the overall clinical picture. In addition, lactate may not be as sensitive as previously believed. A normal lactate level is often interpreted as indicating a good prognosis in sepsis, but studies suggest that this may be a false assurance. For example, in a study by Dugas et al., 45% of patients in vasopressor-dependent septic shock did not have lactate levels >2.4 mmol/L initially, but their mortality remained high [21]. The reasons why some patients have elevated lactate levels compared to others is not well understood.

Table 3
Lactate levels as predictor of mortality. Lactate levels in emergency department correlate with survival [16]. Lactate measurements have also been included in the 2016 consensus definitions of sepsis and septic shock [3].

Concentration mmol/L	28 day mortality (95% confidence interval)
0–2.4	4.9% (3.5–6.3%)
2.5–3.9	9.0% (5.6%–12.4%)
>4.0	28.4% (21–36%)

2.2.2. C reactive protein (CRP)

CRP is an acute phase reactant synthesized in the liver in response to infection or inflammation and is frequently measured to monitor response to therapy in patients with chronic inflammatory conditions such as rheumatoid arthritis. Serum concentrations can increase up to 1000-fold during acute inflammatory events, which increases its value as a biomarker of infection and inflammation compared to other acute phase reactants. Because of wide availability, good reproducibility, and low cost, CRP concentrations have been investigated as an attractive biomarker to diagnose sepsis

2.2.2.1. CRP as a diagnostic and prognostic marker. Ugarte et al. [22] measured CRP concentrations in patients with ($n = 111$) and without ($n = 79$) infection. The median was significantly higher in infected patients (12.1 vs. 5.6 mg/dL), with an optimal discrimination value of 7.9 mg/dL. However, 33% of the noninfected patients had a CRP >7.9 mg/dL on admission, making it difficult to discriminate patients with and without infection based on CRP measurement.

Similar observations were made by Reny et al. [23] and Povoa et al. [24]. The Reny study also identified that the change in CRP concentrations between admission and day 4 was the best predictor for recovery [23]. Povoa et al. found that CRP values correlated well with the severity of the infection. For a cut-off of 8.7 mg/dL, the sensitivity and specificity of CRP for infection diagnosis were 93.4 and 86.1%, respectively. When combined with a temperature >38.2 °C, the specificity increased to 100%. Subsequent studies by this group further validated a CRP cutoff level of 8.7 mg/dL and concluded that this value had an 88% risk of infection [25].

Lobo et al. [26] observed that CRP concentrations at ICU admission ($n = 303$) were associated with organ dysfunction, ICU length of stay, and mortality. A CRP concentration >10 mg/dL was associated with proven infection in 73% of patients as compared to 31% when the CRP was <1 mg/dL. In patients with CRP concentrations >10 mg/dL, decreasing concentrations in the first 48 h was associated with a mortality of 15%, whereas mortality reached 61% for patients in whom the CRP concentration increased. A study by Castelli et al. [27] provided similar results.

2.2.2.2. Serial CRP measurements confirming the adequacy of antibiotic therapy. Confirming that serial measurements of CRP concentrations are more important than a single admission value, Povoa et al. observed no significant differences between CRP in survivors and non-survivors until day 2 of antibiotic therapy in a multicenter, 891 patient study [24]. A similar observation was made by Schmit and Vincent [28], where CRP concentrations decreased faster during the first 48 h if the antibiotic therapy was adequate. An increase in CRP concentration above 2.2 mg/dL over the 48-hour period was predictive of inadequate antibiotic therapy with a sensitivity of 77% and a specificity of 67% [25].

Povoa et al. [25] suggested the importance of daily measurement of CRP concentrations in the assessment of appropriate antibiotic therapy in bacteremia and found it was necessary to wait until day 4 to observe a relationship with outcome. This observation was also suggested in the meta-analysis performed by Zhang and Ni [29].

2.2.3. Cytokines

Cytokines are regulators produced by the host immune system in response to an infection or injury which have a role in the complex pathophysiology of sepsis. Interleukin-6 (IL-6), IL-8 and IL-10 have been the most widely studied cytokines to diagnose sepsis, evaluate the level of the inflammatory response and help determine the prognosis for the patient. IL-6 is a prototype of proinflammatory cytokine, IL-8 is a major chemokine, and IL-10 represents an important anti-inflammatory cytokine

Cytokines may be useful for monitoring inflammatory responses. Cytokine levels in septic patients have been investigated and provide a quantitative assessment of the severity of sepsis, which may relate to

outcome. IL-6 levels are increased in patients with infectious complications and have been used to differentiate SIRS from sepsis [30]. Studies have shown that IL-6 and IL-10 levels are correlated with the mortality rate in septic patients [31]. IL-8 has been used to predict the severity of sepsis in pediatric patients, although the utility of IL-8 has not been confirmed in adults [32,33]. None of the cytokine markers has been proven to be more sensitive or specific than PCT or CRP [34]. Nevertheless, the determination of cytokines may be valuable in monitoring the intensity of the inflammatory response although elevated levels are also present in SIRS of noninfectious origin. There are currently no studies demonstrating that the treatment of sepsis based on these markers influences the treatment strategy or improves the clinical outcome.

2.2.4. D-dimer

Sepsis is associated with defects in hemostasis and the development of disseminated intravascular coagulation. D-dimer is a product of fibrin degradation after fibrinolysis. As early as 1990, D-dimer was shown to predict the presence of bacteremia in septic patients and was correlated with sepsis severity [35]. The marked elevation of D-dimer in patients with sepsis was confirmed by the PROWESS study [36].

2.2.5. Proadrenomedullin (ProADM)

ProADM is a potent vasodilator that belongs to the calcitonin peptide superfamily with PCT. It is upregulated in inflammatory and infectious conditions, and expressed in many clinical conditions including sepsis, respiratory infections and pneumonia, as well as also heart failure and myocardial infarction [37,38]. ProADM has been used as a prognostic marker, either alone or in risk stratification with other hormonal propeptides in patients with sepsis and severe pneumonia [39]. Importantly, ProADM has been shown to improve clinical pneumonia risk scores, and in a pilot intervention study, tended to decrease the length of stay without increased risk for readmissions by improving physicians' admission and early discharge decisions [40].

2.2.6. Myocardial biomarkers

Myocardial biomarkers, such as troponin, natriuretic peptides and myoglobin, have also been investigated since myocardial dysfunction is a frequent complication in sepsis patients. Sepsis associated myocardial dysfunction was first described decades ago, and it has only been recognized recently due to the extensive use of echocardiography in the ICUs.

The Albumin Italian Outcome Sepsis (ALBIOS) was a multicenter, randomized trial that enrolled 1818 patients with severe sepsis or septic shock in 100 ICUs [41]. Despite the controversial conclusion on albumin replacement as a therapeutic approach [42], they found a high prevalence of elevated levels for N-terminus pro-basic natriuretic peptide (NT-pro-BNP) and high-sensitive cardiac troponin T (hs-cTnT) (97.4% and 84.5%, respectively). They also found that early changes (from day 1 to day 2 after enrollment) of both markers were independently associated with mortality in patients with septic shock. Notably, these changes had a greater prognostic value than lactate or lactate clearance. Furthermore, NT-pro-BNP was a better predictive marker than hs-cTnT for mortality in the ICU and at 90 days [41]. Similar results previously have been found in smaller studies [43,44].

Myoglobin is a sensitive yet non-specific marker for myocardial injury. Yao et al. studied the correlation of myoglobin, along with CRP and PCT, in 70 septic patients [45]. The data indicated that myoglobin was increased gradually within 24 h of admission, and the degree of increase correlated with the severity of sepsis ($p < 0.05$). Moreover, they identified a cutoff value of 922.4 μg/L of myoglobin in predicting the 28-day mortality using a receiver operator curve (ROC), area under the curve (AUC) (AUC = 0.824, 95% CI 0.728–0.920, $p < 0.05$). The Kaplan-Meier survival curve showed that the patients with a myoglobin level above the cutoff had decreased 28-day survival compared to the patients had a lower myoglobin level (26.3% vs. 76%, $p < 0.05$), although the correlation of myoglobin level and Sequential Organ Failure Assessment (SOFA) score was poor ($r = 0.641$). The authors concluded that high myoglobin could predict more severe sepsis with a poorer prognosis.

2.2.6.1. Multi-marker approach to sepsis diagnosis. Even with close monitoring during the course of a patient's hospital stay, no single marker accurately reflects the rapid immunological changes of sepsis. This is demonstrated by the important lesson from the PASS study of sepsis patients showing that PCT, when used as a single marker, failed to provide useful information [46]. Consequently, some studies have proposed applying a multi-marker approach for improved risk assessment.

Kelly et al. from the CDC Prevention Epicenters Program studied the performance of 9 biomarkers, including the cutoffs and sampling time, in 69 SICU patients with suspected sepsis [47]. With optimal cutoff values, the combination of baseline alpha-2 macroglobulin and 72-hour PCT offered a 75% negative predictive value (95% CI 54–96%), and differentiated bacterial sepsis from SIRS among SICU patients with suspected sepsis.

A multicenter study performed by Kellum et al. [31], which included 1886 patients hospitalized with community-acquired pneumonia, revealed a strong association between elevated levels of several plasma cytokines and 90-day mortality. The worst outcomes were observed in the subset with increased levels of both pro-inflammatory and anti-inflammatory cytokines (IL-6 and IL-10, respectively). Shapiro et al. analyzed samples from 10 emergency departments ($n = 1000$) to predict the development of sepsis within 72 h [48]. The investigators, using multivariate logistic regression, narrowed over 150 different biomarkers down to a panel of 3 markers that best predicted the development of sepsis: IL-1 receptor antagonist (IL-1ra), protein C and neutrophil gelatinase associated lipocalin (NGAL). The Area Under the Curve (AUC) of the Receiver Operator Characteristic Curve for accuracy to predict severe sepsis, septic shock and death are 0.80, 0.77 and 0.79, respectively. A similar bioscore, utilizing the results of three more traditional biomarkers (PCT, CD64 and sTREM-1) has also been proposed [15].

The best panel of biomarkers for the diagnosis of sepsis or prediction of developing septic shock is likely to include both pro-inflammatory and anti-inflammatory markers. Andaluz-Ojeda et al. measured almost 20 different cytokines concurrently using an automated multiplexed immunoassay approach in approximately 30 patients with severe sepsis [49]. They found that levels of IL-6, IL-8, and MCP-1 (pro-inflammatory markers), as well as IL-10 (anti-inflammatory marker) were all higher in patients who died (mortality rate was 59%). The combined score was more predictive than any one cytokine, even when the hazard ratio was adjusted for the APACHE score. This multi-marker approach may be more likely to succeed in predicting the onset of severe sepsis in future studies.

2.2.7. Analytes to evaluate sequential (sepsis-related) organ failure (SOFA)

Septic patients may develop organ failure directly related to the septic process, including declining function of the pulmonary, coagulation, hepatic, cardiovascular, central nervous system and renal systems. These changes may be quantified by calculating the SOFA score [3]. Clinical laboratory tests are essential in determining pulmonary function (arterial blood gases), hepatic function (bilirubin) and renal function (creatinine). The status of the coagulation system is determined by measuring the number of platelets.

2.2.8. Experimental analytes under investigation

2.2.8.1. Emerging sepsis biomarkers. Several new biomarkers have been proposed recently ranging from cytokines to small cellular proteins. These markers offer the potential to improve the diagnosis and treatment of sepsis. Unfortunately, even these newer biomarkers have failed to provide the necessary specificity to allow a prompt, sensitive and specific diagnosis of sepsis. Table 4 provides a list of recent biomarkers, in addition to some of the classic biomarkers such as CRP and PCT.

Table 4
Diagnostic biomarkers for sepsis. The area under the curve for the receiver operator characteristic is listed. The data were from human studies [67,85].

Biomarker	Area under the curve, receiver operator characteristic
C-reactive protein (CRP)	–
Procalcitonin (PCT)	0.89
Interleukin 6 (IL-6)	0.86
Soluble urokinase plasminogen activator receptor (suPAR)	0.62–0.79
Pro-adrenomedullin	0.72
Presepsin	0.74–0.82
Lipopolysaccharide binding protein	0.73
Soluble Triggering Receptor Expressed on Myeloid Cells (sTREM)	0.87

Weber et al. have demonstrated, first in a mouse model and later in a human cohort, that IL-3 is the key mediator that induces downstream cytokine expression in sepsis. IL-3 levels during the first 24 h after the onset of sepsis predicted death in patients. High IL-3 levels are associated with poor prognosis and high mortality rate, even after adjusting for prognostic indicators [50].

O'Callaghan et al. isolated monocytes from patients with severe sepsis ($n = 16$), healthy volunteers ($n = 15$), and critically ill patients with noninfectious SIRS ($n = 8$). The basal and lipopolysaccharide-induced tumor necrosis factor (TNF) levels were measured. TNF-α–converting enzyme (TACE) is a trans-membrane protease enzyme that cleaves membrane-bound TNF to produce soluble TNF. Patients with sepsis had substantially elevated levels of basal TACE activity that were refractory to lipopolysaccharide stimulation. In patients with SIRS, monocyte basal TACE and its induction by lipopolysaccharide appeared similar to healthy controls [51].

Read et al. identified peptidoglycan (PGN) recognition protein 1 (PGLYRP1) as a ligand for TREM-1, a known proinflammatory receptor expressed on monocytes/macrophages and neutrophils. When complexed with PGN, PGLYRP1 is able to activate TREM-1 and enhance cytokine production in human neutrophils and macrophages [52].

Motal et al. studied the level of vaspin in sepsis patients. Vaspin, a visceral adipose tissue-derived serpin, was first identified as an insulin-sensitizing adipose tissue hormone, and its anti-inflammatory function has recently been demonstrated. Plasma vaspin concentrations were measured from patients with severe sepsis ($n = 57$) and critically ill patients as control group ($n = 48$) on the day of diagnosis. Vaspin concentrations were significantly higher in septic patients compared to the control group (0.3 ng/mL vs. 0.1 ng/mL, respectively; $P < 0.001$). The investigators also demonstrated a weak positive correlation between the concentration of vaspin and CRP ($r = 0.31$, $P = 0.002$). Although there seems to be some relationship between vaspin and inflammation, its role in human sepsis needs to be evaluated further [53].

MicroRNAs (miRNAs) are a group of small (20–24 nucleotides) RNA molecules that do not encode for proteins, but regulate gene expression that mediates physiological and pathophysiological processes. miRNAs have also been detected in the blood and might serve as biomarkers. In addition to their stability, circulating miRNAs do not undergo post-processing modifications and have a less complex chemical structure. Thus, circulating miRNAs might be superior to other classes of serum protein based biomarkers [54]. In the last years, miRNAs have been suggested as biomarkers in the context of sepsis [54,55]. However, there are striking inter-study variances of miRNA-regulation patterns in the different cohorts of patients with sepsis, which are most likely due to a lack in standardization of sample collection, data normalization, and analysis [54,55]. If these problems can be solved, miRNAs offer attractive options as "next generation" biomarkers in sepsis. Additionally, some studies proposed monitoring oxidative stress in septic patients [56]. However, this process of tissue ischemia leading to multi-organ failure is not specific to sepsis, it is also seen in SIRS.

3. Other laboratory testing used for clinical evaluation of sepsis

3.1. Hematology

Hematologic parameters are one of the four SIRS criteria (Table 1), including a white blood cell count. Certainly neutrophils, as a major component of the innate immune system, are important in the pathogenesis of sepsis. The SIRS criteria were published over 20 years ago [2] and additional hematology measurements may be useful for the diagnosis of sepsis. One approach is measuring a change in neutrophil antigen expression as a marker for sepsis. As mentioned, platelet measurement is one parameter of the SOFA score.

3.1.1. Neutrophil antigen expression

Many neutrophil antigens have been evaluated in the sepsis setting and some groups have studied soluble markers such as CD14 [57] and soluble CD16 (which is cleaved from the neutrophil cell surface after apoptosis). However, the most frequently studied antigen is CD64, a high affinity Fc receptor for immunoglobulin G (IgG) that is expressed on neutrophils during an infectious or inflammatory state. CD64 is one of the most frequently studied antigens because it is a good laboratory marker because its expression increases in a graded manner [58]. In addition, neutrophil CD64 expression is negligible to minimal under normal conditions which makes detection of a change more obvious [58], unlike other neutrophil antigens [59]. Unfortunately only a few studies are available comparing neutrophil CD64 expression to other antigens [60].

CD64 expression has been evaluated in infections, bacterial and non-bacterial, as well as non-infectious inflammatory states. In a bacterial infection, an increase in the number or the density of CD64 antigens as well as an increase in the percentage of neutrophils showing increased CD64 expression, has been reported [61]. In contrast to a viral infection, only an increase in the percentage of neutrophils showing CD64 expression is typically observed. CD64 expression is also increased in patients who have non-infectious inflammatory systemic conditions such as sickle cell crisis [62] as well as localized inflammatory conditions such as the synovial fluid of rheumatoid arthritis patients [63].

When comparing sepsis with SIRS, the percentage of CD64 positive neutrophils is highest in the patients who had sepsis, followed by SIRS, hospitalized patients who did not have sepsis or SIRS, and finally normal controls [64]. In terms of CD64 expression density, the patients with sepsis had the highest density, however, the density of CD64 expression did not differ significantly between the SIRS patients, hospitalized patients, and controls [64]. One group has advocated the utility of neutrophil CD64 expression over other traditional hematologic markers in predicting clinically determined sepsis or infection [65]. These findings have also been extended to the neonatal population, a population in which the diagnosis of sepsis is more difficult and associated with greater morbidity and mortality [66].

4. Microbiology

Time to appropriate antimicrobial therapy is an independent predictor of death from sepsis [67] and current clinical guidelines require timely empiric or directed antimicrobial therapy [5,68]. However, the time and labor-intensive nature of traditional culture-based testing marginalizes the microbiology laboratory during the acute stage of sepsis recognition and management [69]. Also, 30% to 50% of blood cultures can be negative in patients with a clinical diagnosis of sepsis [67] or in suspected cases of bacteremia or candidemia [70]. Therefore, new diagnostics have focused on culture-limited or culture-independent technologies. Rapid, meaningful pathogen detection for sepsis diagnosis implies on-site, time-saving test logistics.

4.1. Sequence-based methods: molecular hybridization probe detection after enrichment by blood culture

A current incarnation of this approach, Peptide Nucleic Acid Fluorescent In Situ *Hybridization* (PNA FISH) (bioMérieux), detects pathogen ribosomal RNA (rRNA) using labeled DNA mimic-molecules and FISH that is performed on smears made from "positive" blood cultures. Assays with 25 min-to-result allow the use of simultaneous critical action value reporting for Gram stain and PNA FISH identification. PNA-FISH kits perform acceptably [69] and provide the accuracy of molecular testing in a familiar manual format. Optimal PNA-FISH implementation benefits from active antimicrobial stewardship [71].

4.2. Multiplex real-time molecular assays in sample-to-answer format

The past decade saw wide commercialization of user-friendly, sample-to-answer platforms that obviate the need for expertise while minimizing hands-on-time and risk of amplicon contamination by combining multiple steps in one reaction vessel. Commercial sequence-based assays are performed on aliquots from positive blood cultures with appropriate Gram-stain findings. A highly-multiplexed approach is very practical for sepsis diagnostics because approximately 90% of bloodstream infections are caused by the same 20–25 pathogens and simultaneous inquiry is cost- and time-effective and useful for polymicrobial infections [72]. One FDA-approved highly-multiplexed platform is the FilmArray (Biofire Dx/bioMérieux) Blood Culture Identification Panel. It tests for 24 bacterial and yeast pathogens plus 3 antibiotic resistance markers. Other options are the FDA-cleared Verigene (Nanosphere) BC-GP and BC-GN assays that use Gold/Ag nanoparticle probes and micro-array for detection of bacterial pathogens and several resistance markers. Both platforms allow random access testing – an asset for STAT sepsis diagnostics. Overall concordance with traditional phenotypic methods is reported to be very good to excellent (≥95% sensitivity/specificity) for adult blood cultures [72] and similar for pediatric cultures [73]. Faster pathogen identification can facilitate reduced time to susceptibility profiling with potential for better antimicrobial stewardship, clinical outcomes, and decreased hospital costs [74].

4.3. Database-dependent "fingerprint" methods

Matrix Assisted Laser Desorption-Time Of Flight Mass Spectrometry (MALDI-TOF MS) is now adapted for clinical microbiology by exploiting 'soft ionization' of bacterial or fungal isolates to preserve components key for detection and analysis. The result of MALDI-TOF MS is a species-specific spectral "fingerprint" that is compared to a database of organisms based on rRNA DNA sequences. Using agar plate culture growth, MALDI-TOF MS takes minutes compared to hours or days for biochemical identification methods and costs are considerably less per isolate [75]. In the U.S. two FDA-cleared MALDI-TOF MS systems are the Vitek-MS (bioMérieux) and the BioTyper (Bruker Daltronics). MALDI-TOF MS performance is equivalent or superior to phenotypic identification methods, recently reviewed by Clark et al. [76]. Alternative workflow efficiencies have been tried including testing pellets from signal-positive blood culture broth instead of from agar subcultures – sometimes in conjunction with similar "off-label" susceptibility testing plus real-time antimicrobial stewardship, with mixed but promising results [77].

4.4. Direct pathogen detection without culture amplification, but without sample-to-answer format

Several approaches to culture-independent direct pathogen detection form the basis for the next-generation of diagnostics. These use broad range or universal PCR primers adapted for pathogen detection in small volumes of whole blood, sometimes followed by species-specific primers and sequencing [78]. Studies using culture independent technologies in the context of sepsis are detailed in two 2014 reviews [67,79]. High-throughput DNA sequencing may become more accessible to clinical microbiology [79] especially for detection of non-cultivable or complex polymicrobial infections that defy the technical resolution of PCR and Sanger-based sequencing. The Iridica platform combines PCR and electrospray ionization mass spectrometry to respectively amplify and detect microbial pathogens directly from patient samples without prior culture.

4.5. Direct sample-to-answer pathogen detection from uncultured blood samples

An ideal sepsis diagnostic would use molecular analytics in a sample-to-answer format with the ability to rapidly test blood samples directly without prior culture, and be universally suitable for point-of-impact use [69]. In 2014 this was partly achieved by the bench top T2Dx instrument with its inaugural T2Candida assay (T2 Biosystems). T2 relies on changes in a sample's T2 magnetic resonance (T2MR) signal caused by hybridization of PCR-amplified pathogen DNA to capture probe-decorated nanoparticles. T2Candida detects 5 *Candida* species of yeast in 1 mL of uncultured whole blood in about 3 h with a claimed limit of detection as low as 1 colony forming unit/ml, with good agreement with simulated blood cultures [80].

4.6. The future of sepsis diagnostics – point-of-impact, next-gen phenotypics, genomics and proteomics for pathogen identification

The increasing interest in very rapid, point-of-impact diagnostics for infectious diseases and the global need for in-field devices for low resource settings [81] have yielded a plethora of prototype miniaturized devices that feature clever chemistries, microfluidics, and minimal power requirements. Another technology uses minimal culture times and molecular padlock probes to detect bacterial ribosomal RNA and detection of antibiotic-resistant bacteria [82]. Even with the rapid diagnosis of an infection, host genes affect the prevalence and severity of infectious diseases. With advancing knowledge in the human genome, studies have now focused on understanding the immune response in sepsis. New methodologies, such as DNA and RNA microchips, have aided complex investigations to answer questions including whether gene expression patterns differ with infectious and non-infectious etiologies. Boldrick et al. demonstrated the immune response gene expression is stereotypical with infection but varied with different infectious agents [83]. Prucha et al., using expression profiling, showed the exclusiveness of the immune response in systemic inflammation of infection [35]. A recent study looking at multiple datasets identified 11 genes that accurately differentiated sepsis from sterile inflammation in patients [84]. Despite these promising findings, studies of genetic polymorphism of the innate immune system and cytokines have not produced reproducible results that may be readily translated into clinical practice. The major issue is that genotype does not always predict phenotype. Therefore, efforts have been directed to the study of proteomics with the objective of identifying new biomarkers that can aid in the diagnosis, monitoring, or predicting progression and outcome of sepsis. With the complexity of etiology, this approach may lead to treatment solutions of personalized medicine in septic patients [35].

Acknowledgements

This work was supported in part by NIH grants R21AI112887, R01GM97320 and T32GM86308 (DGR).

References

[1] C. Fleischmann, A. Scherag, N.K. Adhikari, C.S. Hartog, T. Tsaganos, P. Schlattmann, et al., Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations, Am. J. Respir. Crit. Care Med. 193 (2016) 259–272.

[2] R.C. Bone, R.A. Balk, F.B. Cerra, R.P. Dellinger, A.M. Fein, W.A. Knaus, et al., Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The accp/sccm consensus conference committee. American college of chest physicians/society of critical care medicine, Chest 101 (1992) 1644–1655.
[3] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, et al., The third international consensus definitions for sepsis and septic shock (sepsis-3), JAMA 315 (2016) 801–810.
[4] J.L. Vincent, Y. Sakr, C.L. Sprung, V.M. Ranieri, K. Reinhart, H. Gerlach, et al., Sepsis in european intensive care units: results of the soap study, Crit. Care Med. 34 (2006) 344–353.
[5] R.P. Dellinger, M.M. Levy, A. Rhodes, D. Annane, H. Gerlach, S.M. Opal, et al., Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012, Crit. Care Med. 41 (2013) 580–637.
[6] M.M. Levy, A. Rhodes, G.S. Phillips, S.R. Townsend, C.A. Schorr, R. Beale, et al., Surviving sepsis campaign: association between performance metrics and outcomes in a 7.5-year study, Crit. Care Med. 43 (2015) 3–12.
[7] A. Kumar, D. Roberts, K.E. Wood, B. Light, J.E. Parrillo, S. Sharma, et al., Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock, Crit. Care Med. 34 (2006) 1589–1596.
[8] R.P. Wenzel, M.B. Edmond, Managing antibiotic resistance, N. Engl. J. Med. 343 (2000) 1961–1963.
[9] USA B, Press Release fda Approved pct for Sepsis, http://www.marketwired.com/press-release/brahms-receives-fda-clearance-to-market-automated-procalcitonin-pct-test-841588.htm (Accessed May 26, 2015 2015).
[10] C. Wacker, A. Prkno, F.M. Brunkhorst, P. Schlattmann, Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis, Lancet Infect. Dis. 13 (2013) 426–435.
[11] D. Anand, S. Das, S. Bhargava, L.M. Srivastava, A. Garg, N. Tyagi, et al., Procalcitonin as a rapid diagnostic biomarker to differentiate between culture-negative bacterial sepsis and systemic inflammatory response syndrome: a prospective, observational, cohort study, J. Crit. Care 30 (2015) (218 e7-12).
[12] P. Schuetz, V. Chiappa, M. Briel, J.L. Greenwald, Procalcitonin algorithms for antibiotic therapy decisions: a systematic review of randomized controlled trials and recommendations for clinical algorithms, Arch. Intern. Med. 171 (2011) 1322–1331.
[13] P. Sridharan, R.S. Chamberlain, The efficacy of procalcitonin as a biomarker in the management of sepsis: slaying dragons or tilting at windmills? Surg. Infect. 14 (2013) 489–511.
[14] J.D. Faix, Biomarkers of sepsis, Crit. Rev. Clin. Lab. Sci. 50 (2013) 23–36.
[15] L.W. Andersen, J. Mackenhauer, J.C. Roberts, K.M. Berg, M.N. Cocchi, M.W. Donnino, Etiology and therapeutic approach to elevated lactate levels, Mayo Clin. Proc. 88 (2013) 1127–1140.
[16] N.I. Shapiro, M.D. Howell, D. Talmor, L.A. Nathanson, A. Lisbon, R.E. Wolfe, J.W. Weiss, Serum lactate as a predictor of mortality in emergency department patients with infection, Ann. Emerg. Med. 45 (2005) 524–528.
[17] M.D. Howell, M. Donnino, P. Clardy, D. Talmor, N.I. Shapiro, Occult hypoperfusion and mortality in patients with suspected infection, Intensive Care Med. 33 (2007) 1892–1899.
[18] M.E. Mikkelsen, A.N. Miltiades, D.F. Gaieski, M. Goyal, B.D. Fuchs, C.V. Shah, et al., Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock, Crit. Care Med. 37 (2009) 1670–1677.
[19] D. Junhasavasdikul, P. Theerawit, A. Ingsathit, S. Kiatboonsri, Lactate and combined parameters for triaging sepsis patients into intensive care facilities, J. Crit. Care 33 (2016) 71–77.
[20] T.G. Shin, I.J. Jo, S.Y. Hwang, K. Jeon, G.Y. Suh, E. Choe, et al., Comprehensive interpretation of central venous oxygen saturation and blood lactate levels during resuscitation of patients with severe sepsis and septic shock in the emergency department, Shock 45 (2016) 4–9.
[21] A.F. Dugas, J. Mackenhauer, J.D. Salciccioli, M.N. Cocchi, S. Gautam, M.W. Donnino, Prevalence and characteristics of nonlactate and lactate expressors in septic shock, J. Crit. Care 27 (2012) 344–350.
[22] H. Ugarte, E. Silva, D. Mercan, A. De Mendonca, J.L. Vincent, Procalcitonin used as a marker of infection in the intensive care unit, Crit. Care Med. 27 (1999) 498–504.
[23] J.L. Reny, A. Vuagnat, C. Ract, M.O. Benoit, M. Safar, J.Y. Fagon, Diagnosis and follow-up of infections in intensive care patients: value of c-reactive protein compared with other clinical and biological variables, Crit. Care Med. 30 (2002) 529–535.
[24] P. Povoa, L. Coelho, E. Almeida, A. Fernandes, R. Mealha, P. Moreira, H. Sabino, C-reactive protein as a marker of infection in critically ill patients, Clin. Microbiol. Infect. 11 (2005) 101–108.
[25] P. Povoa, L. Coelho, E. Almeida, A. Fernandes, R. Mealha, P. Moreira, H. Sabino, Early identification of intensive care unit-acquired infections with daily monitoring of c-reactive protein: a prospective observational study, Crit. Care 10 (2006) R63.
[26] S.M. Lobo, F.R. Lobo, D.P. Bota, F. Lopes-Ferreira, H.M. Soliman, C. Melot, J.L. Vincent, C-reactive protein levels correlate with mortality and organ failure in critically ill patients, Chest 123 (2003) 2043–2049.
[27] G.P. Castelli, C. Pognani, M. Cita, A. Stuani, L. Sgarbi, R. Paladini, Procalcitonin, c-reactive protein, white blood cells and sofa score in icu: diagnosis and monitoring of sepsis, Minerva Anestesiol. 72 (2006) 69–80.
[28] X. Schmit, J.L. Vincent, The time course of blood c-reactive protein concentrations in relation to the response to initial antimicrobial therapy in patients with sepsis, Infection 36 (2008) 213–219.
[29] Z. Zhang, H. Ni, C-reactive protein as a predictor of mortality in critically ill patients: a meta-analysis and systematic review, Anaesth. Intensive Care 39 (2011) 854–861.
[30] B. Du, J. Pan, D. Chen, Y. Li, Serum procalcitonin and interleukin-6 levels may help to differentiate systemic inflammatory response of infectious and non-infectious origin, Chin. Med. J. 116 (2003) 538–542.
[31] J.A. Kellum, L. Kong, M.P. Fink, L.A. Weissfeld, D.M. Yealy, M.R. Pinsky, et al., Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the genetic and inflammatory markers of sepsis (genims) study, Arch. Intern. Med. 167 (2007) 1655–1663.
[32] H.R. Wong, N. Cvijanovich, D.S. Wheeler, M.T. Bigham, M. Monaco, K. Odoms, et al., Interleukin-8 as a stratification tool for interventional trials involving pediatric septic shock, Am. J. Respir. Crit. Care Med. 178 (2008) 276–282.
[33] C.S. Calfee, B.T. Thompson, P.E. Parsons, L.B. Ware, M.A. Matthay, H.R. Wong, Plasma interleukin-8 is not an effective risk stratification tool for adults with vasopressor-dependent septic shock, Crit. Care Med. 38 (2010) 1436–1441.
[34] S.D. Carrigan, G. Scott, M. Tabrizian, Toward resolving the challenges of sepsis diagnosis, Clin. Chem. 50 (2004) 1301–1314.
[35] M. Prucha, G. Bellingan, R. Zazula, Sepsis biomarkers, Clin. Chim. Acta 440C (2014) 97–103.
[36] G.T. Kinasewitz, S.B. Yan, B. Basson, P. Comp, J.A. Russell, A. Cariou, et al., Universal changes in biomarkers of coagulation and inflammation occur in patients with severe sepsis, regardless of causative micro-organism [isrctn74215569], Crit. Care 8 (2004) R82–R90.
[37] P. Schuetz, R.J. Marlowe, B. Mueller, The prognostic blood biomarker proadrenomedullin for outcome prediction in patients with chronic obstructive pulmonary disease (copd): a qualitative clinical review, Clin. Chem. Lab. Med. 53 (2015) 521–539.
[38] E. Grolimund, A. Kutz, R.J. Marlowe, A. Vogeli, M. Alan, M. Christ-Crain, et al., Long-term prognosis in copd exacerbation: role of biomarkers, clinical variables and exacerbation type, COPD (2014).
[39] P. Schuetz, D. Aujesky, C. Muller, B. Muller, Biomarker-guided personalised emergency medicine for all - hope for another hype? Swiss Med. Wkly. 145 (2015) w14079.
[40] L. Debiane, R.Y. Hachem, I. Al Wohoush, W. Shomali, R.R. Bahu, Y. Jiang, et al., The utility of proadrenomedullin and procalcitonin in comparison to c-reactive protein as predictors of sepsis and bloodstream infections in critically ill patients with cancer*, Crit. Care Med. 42 (2014) 2500–2507.
[41] S. Masson, P. Caironi, C. Fanizza, S. Carrer, A. Caricato, P. Fassini, et al., Sequential n-terminal pro-b-type natriuretic peptide and high-sensitivity cardiac troponin measurements during albumin replacement in patients with severe sepsis or septic shock, Crit. Care Med. 44 (2016) 707–716.
[42] P. Caironi, G. Tognoni, S. Masson, R. Fumagalli, A. Pesenti, M. Romero, et al., Albumin replacement in patients with severe sepsis or septic shock, N. Engl. J. Med. 370 (2014) 1412–1421.
[43] C. Tettamanti, T. Hervet, S. Grabherr, C. Palmiere, Elevation of nt-probnp and cardiac troponins in sepsis-related deaths: a forensic perspective, Int. J. Legal Med. (2016).
[44] H. Cheng, W.Z. Fan, S.C. Wang, Z.H. Liu, H.L. Zang, L.Z. Wang, et al., N-terminal pro-brain natriuretic peptide and cardiac troponin i for the prognostic utility in elderly patients with severe sepsis or septic shock in intensive care unit: a retrospective study, J. Crit. Care 30 (2015) (654 e9–14).
[45] L. Yao, Z. Liu, J. Zhu, B. Li, C. Chai, Y. Tian, Higher serum level of myoglobin could predict more severity and poor outcome for patients with sepsis, Am. J. Emerg. Med. (2016).
[46] J.U. Jensen, L. Hein, B. Lundgren, M.H. Bestle, T.T. Mohr, M.H. Andersen, et al., Procalcitonin-guided interventions against infections to increase early appropriate antibiotics and improve survival in the intensive care unit: a randomized trial, Crit. Care Med. 39 (2011) 2048–2058.
[47] B.J. Kelly, E. Lautenbach, I. Nachamkin, S.E. Coffin, J.S. Gerber, B.D. Fuchs, et al., Combined biomarkers discriminate a low likelihood of bacterial infection among surgical intensive care unit patients with suspected sepsis, Diagn. Microbiol. Infect. Dis. 85 (2016) 109–115.
[48] N.I. Shapiro, S. Trzeciak, J.E. Hollander, R. Birkhahn, R. Otero, T.M. Osborn, et al., A prospective, multicenter derivation of a biomarker panel to assess risk of organ dysfunction, shock, and death in emergency department patients with suspected sepsis, Crit. Care Med. 37 (2009) 96–104.
[49] D. Andaluz-Ojeda, F. Bobillo, V. Iglesias, R. Almansa, L. Rico, F. Gandia, et al., A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis, Cytokine 57 (2012) 332–336.
[50] G.F. Weber, B.G. Chousterman, S. He, A.M. Fenn, M. Nairz, A. Anzai, et al., Interleukin-3 amplifies acute inflammation and is a potential therapeutic target in sepsis, Science 347 (2015) 1260–1265.
[51] D.J. O'Callaghan, K.P. O'Dea, A.J. Scott, M. Takata, A.C. Gordon, Monocyte tumor necrosis factor-alpha-converting enzyme catalytic activity and substrate shedding in sepsis and noninfectious systemic inflammation, Crit. Care Med. 43 (2015) 1375–1385.
[52] C.B. Read, J.L. Kuijper, S.A. Hjorth, M.D. Heipel, X. Tang, A.J. Fleetwood, et al., Cutting edge: identification of neutrophil pglyrp1 as a ligand for trem-1, J. Immunol. 194 (2015) 1417–1421.
[53] M.C. Motal, D.A. Klaus, D. Lebherz-Eichinger, B. Tudor, T. Hamp, M. Wiegele, et al., Increased plasma vaspin concentration in patients with sepsis: an exploratory examination, Biochem. Med. 25 (2015) 90–96.
[54] F. Benz, S. Roy, C. Trautwein, C. Roderburg, T. Luedde, Circulating micrornas as biomarkers for sepsis, Int. J. Mol. Sci. 17 (2016).
[55] R. Dumache, A.F. Rogobete, O.H. Bedreag, M. Sarandan, A.C. Cradigati, M. Papurica, et al., Use of mirnas as biomarkers in sepsis, Anal. Cell. Pathol. (Amst.) 2015 (2015) 186716.
[56] D. Bar-Or, M.M. Carrick, C.W. Mains, L.T. Rael, D. Slone, E.N. Brody, Sepsis, oxidative stress, and hypoxia: are there clues to better treatment? Redox Rep. 20 (2015) 193–197.
[57] S. Masson, P. Caironi, C. Fanizza, R. Thomae, R. Bernasconi, A. Noto, et al., Circulating presepsin (soluble cd14 subtype) as a marker of host response in patients with

severe sepsis or septic shock: data from the multicenter, randomized albios trial, Intensive Care Med. 41 (2015) 12–20.
[58] K.C. Petroni, L. Shen, P.M. Guyre, Modulation of human polymorphonuclear leukocyte igg fc receptors and fc receptor-mediated functions by ifn-gamma and glucocorticoids, J. Immunol. 140 (1988) 3467–3472.
[59] A.C. Muller Kobold, J.G. Zijlstra, H.R. Koene, M. de Haas, C.G. Kallenberg, J.W. Tervaert, Levels of soluble fc gammariii correlate with disease severity in sepsis, Clin. Exp. Immunol. 114 (1998) 220–227.
[60] M. Groselj-Grenc, A. Ihan, M. Derganc, Neutrophil and monocyte cd64 and cd163 expression in critically ill neonates and children with sepsis: comparison of fluorescence intensities and calculated indexes, Mediat. Inflamm. 2008 (2008) 202646.
[61] L. Leino, K. Sorvajarvi, J. Katajisto, M. Laine, E.M. Lilius, T.T. Pelliniemi, et al., Febrile infection changes the expression of igg fc receptors and complement receptors in human neutrophils in vivo, Clin. Exp. Immunol. 107 (1997) 37–43.
[62] E. Fadlon, S. Vordermeier, T.C. Pearson, A.R. Mire-Sluis, D.C. Dumonde, J. Phillips, et al., Blood polymorphonuclear leukocytes from the majority of sickle cell patients in the crisis phase of the disease show enhanced adhesion to vascular endothelium and increased expression of cd64, Blood 91 (1998) 266–274.
[63] J.A. Quayle, F. Watson, R.C. Bucknall, S.W. Edwards, Neutrophils from the synovial fluid of patients with rheumatoid arthritis express the high affinity immunoglobulin g receptor, fc gamma ri (cd64): role of immune complexes and cytokines in induction of receptor expression, Immunology 91 (1997) 266–273.
[64] S.S. Qureshi, S.M. Lewis, V.A. Gant, D. Treacher, B.H. Davis, K.A. Brown, Increased distribution and expression of cd64 on blood polymorphonuclear cells from patients with the systemic inflammatory response syndrome (sirs), Clin. Exp. Immunol. 125 (2001) 258–265.
[65] B.H. Davis, N.C. Bigelow, Comparison of neutrophil cd64 expression, manual myeloid immaturity counts, and automated hematology analyzer flags as indicators of infection or sepsis, Lab. Hematol. 11 (2005) 137–147.
[66] P.C. Ng, K. Li, R.P. Wong, K.M. Chui, E. Wong, T.F. Fok, Neutrophil cd64 expression: a sensitive diagnostic marker for late-onset nosocomial infection in very low birthweight infants, Pediatr. Res. 51 (2002) 296–303.
[67] F. Bloos, K. Reinhart, Rapid diagnosis of sepsis, Virulence 5 (2014) 154–160.
[68] C.A. Schorr, S. Zanotti, R.P. Dellinger, Severe sepsis and septic shock: management and performance improvement, Virulence 5 (2014) 190–199.
[69] N. Mancini, S. Carletti, N. Ghidoli, P. Cichero, R. Burioni, M. Clementi, The era of molecular and other non-culture-based methods in diagnosis of sepsis, Clin. Microbiol. Rev. 23 (2010) 235–251.
[70] A. Bacconi, G.S. Richmond, M.A. Baroldi, T.G. Laffler, L.B. Blyn, H.E. Carolan, et al., Improved sensitivity for molecular detection of bacterial and candida infections in blood, J. Clin. Microbiol. 52 (2014) 3164–3174.
[71] C. Holtzman, D. Whitney, T. Barlam, N.S. Miller, Assessment of impact of peptide nucleic acid fluorescence in situ hybridization for rapid identification of coagulase-negative staphylococci in the absence of antimicrobial stewardship intervention, J. Clin. Microbiol. 49 (2011) 1581–1582.
[72] A. Hemmert, N. Garrone, Rapid methods for pathogen detection in blood stream infections, MLO Med. Lab. Obs. 45 (2013) 38–39.
[73] X. Zheng, W. Polanco, D. Carter, S. Shulman, Rapid identification of pathogens from pediatric blood cultures by use of the filmarray blood culture identification panel, J. Clin. Microbiol. 52 (2014) 4368–4371.
[74] E. Wenzler, J.R. Wong, D.A. Goff, C.A. Jankowski, K.A. Bauer, Controversies in antimicrobial stewardship: focus on new rapid diagnostic technologies and antimicrobials, Antibiotics 5 (2016).
[75] C. Wojewoda, Education Committee of the Academy of Clinical Laboratory P, Scientists, Pathology consultation on matrix-assisted laser desorption ionization-time of flight mass spectrometry for microbiology, Am. J. Clin. Pathol. 140 (2013) 143–148.
[76] A.E. Clark, E.J. Kaleta, A. Arora, D.M. Wolk, Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology, Clin. Microbiol. Rev. 26 (2013) 547–603.
[77] K.K. Perez, R.J. Olsen, W.L. Musick, P.L. Cernoch, J.R. Davis, G.A. Land, et al., Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs, Arch. Pathol. Lab. Med. 137 (2013) 1247–1254.
[78] S.A. Barghouthi, A universal method for the identification of bacteria based on general pcr primers, Indian J. Microbiol. 51 (2011) 430–444.
[79] O. Liesenfeld, L. Lehman, K.P. Hunfeld, G. Kost, Molecular diagnosis of sepsis: new aspects and recent developments, Eur. J. Microbiol. Immunol. 4 (2014) 1–25.
[80] N.D. Beyda, M.J. Alam, K.W. Garey, Comparison of the t2dx instrument with t2candida assay and automated blood culture in the detection of candida species using seeded blood samples, Diagn. Microbiol. Infect. Dis. 77 (2013) 324–326.
[81] A.N. Abou Tayoun, P.R. Burchard, I. Malik, A. Scherer, G.J. Tsongalis, Democratizing molecular diagnostics for the developing world, Am. J. Clin. Pathol. 141 (2014) 17–24.
[82] A. Mezger, E. Gullberg, J. Goransson, A. Zorzet, D. Herthnek, E. Tano, et al., A general method for rapid determination of antibiotic susceptibility and species in bacterial infections, J. Clin. Microbiol. 53 (2015) 425–432.
[83] J.C. Boldrick, A.A. Alizadeh, M. Diehn, S. Dudoit, C.L. Liu, C.E. Belcher, et al., Stereotyped and specific gene expression programs in human innate immune responses to bacteria, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 972–977.
[84] T.E. Sweeney, A. Shidham, H.R. Wong, P. Khatri, A comprehensive time-course-based multicohort analysis of sepsis and sterile inflammation reveals a robust diagnostic gene set, Sci. Transl. Med. 7 (2015) 287ra71.
[85] C. Henriquez-Camacho, J. Losa, Biomarkers for sepsis, BioMed Res. Int. 2014 (2014) 547818.