Editorial

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Sepsis biomarkers: past, present and future

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According to the Third International Consensus Definitions for Sepsis and Septic Shock [1], sepsis is currently defined as severe organ dysfunction caused by a dysregulated host response to infection associated with severe injuries to tissues and organs, up to septic shock, multiorgan failure (MOF) and death. Sepsis is a major public healthcare issue, being now considered as the leading cause of mortality from infections, accounting for more than 20 billion US $ hospital costs each year in the US [1].

Recent statistics shows that the actual prevalence of sepsis is as high as 6% in hospitalized adults [2], whilst reliable predictions suggest that its future incidence may nearly double in the next 30 years [3]. The cumulative in-hospital mortality is approximately 15% [2], whereas a much higher death rate is observed in intensive care units (ICUs), typically comprised between 20% and 45% [4]. Although both Gram-positive and -negative bacteria may trigger sepsis, the most frequent of which are *Staphylococcus aureus* (20.5% of cases), *Pseudomonas* species (19.9%), *Escherichia coli* (16.0%), *Klebsiella* species (12.7%), *Enterococcus* (10.9%) and *Staphylococcus epidermidis* (10.8%), sepsis can also be frequently develop in patients infected by *Candida* (17.0%) and other microorganisms [5]. The lungs (36%–42%) are the more frequent infection sites in patients with severe sepsis, followed by genitourinary tract (10%–18%), abdomen (8%–9%) and wounds or soft tissues (7%–9%), whilst no precise source of bacteremia can be identified is as many as 20% of patients [5].

Irrespective of the fact that a timely and accurate diagnosis of sepsis is essential to reverse its otherwise unfavorable clinical course, the diagnostic approach remains challenging. Some scoring systems have been developed in recent years, including the host systemic inflammatory response syndrome (SIRS) criteria, the sequential [sepsis-related] organ failure assessment (SOFA) and the quick SOFA (qSOFA) scores [6]. Nevertheless, all these tools are primarily intended for predicting outcomes, especially death, whilst their early diagnostic efficiency remains unsatisfactory [7]. Even if the Sepsis-3 Task Force reiterates the concept that sepsis shall be identified using clinical criteria for life-threatening organ dysfunction and blood culture [1], both these approaches would only be efficient for delayed diagnosis and thus partially unable to substantially ameliorate an unfavorable prognosis.

For a long time, standard blood culture techniques have represented the only reliable means for diagnosing sepsis, and are still regarded as the gold standard reference methods for detecting and isolating pathogenic organisms from sterile body fluid specimens [8]. Unfortunately, however, blood culture has many drawbacks such as a long turnaround time (TAT) (i.e. 6 h to 5 days are needed for microorganisms to grow to detectable levels, with an additional 24–48 h needed for testing antibiotic susceptibility), low sensitivity, large sample volume, frequent need for repeated testing, risk of false negative test results after initiation of antibiotic therapy (between 30% and 63% of cases) and preanalytical variables (e.g. 2%–4% sample contamination due to non-observance of standard antiseptic procedures during blood drawing) (Figure 1). Finally, albeit that automated approaches have been developed [9], blood culture remains a labor-intensive and time-consuming procedure in many clinical laboratories.

Due to these inherent limitations, a large armamentarium of serum (or plasma) sepsis biomarkers has been commercialized over the past decades. These typically include C-reactive protein (CRP), procalcitonin, presepsin, interleukin 6 (IL-6), lipopolysaccharide-binding protein (LBP), neutrophil CD64 (nCD64), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and the serum soluble urokinase-type plasminogen activator receptor (suPAR), among others. Although none of these biomarkers would thoughtfully fulfil all the ideal features of a sepsis biomarker, as briefly summarized in Table 1, stronger clinical evidence of clinical usefulness has emerged for procalcitonin, presepsin and CRP from many published studies and meta-analyses. Both procalcitonin and presepsin now appear to be the most promising tests, not only for early diagnosis of sepsis, but also for garnering valuable prognostic information and for guiding therapeutic decision-making (i.e. antibiotic stewardship) [7, 10–11]. Nonetheless, their measurement is also plagued by some important drawbacks, such as insufficient standardization [12], the inability to provide information on causative microorganism and suboptimal diagnostic accuracy. Regarding this last aspect, a recent study published by Brodska et al. showed that the diagnostic

Diagnostic features	Blood culture	Serum biomarkers	Molecular biology
Etiological diagnosis	Yes	No	Yes
Standardization	Acceptable	Limited	Limited
Rapidity	Limited	High	Limited
Accuracy	Partial	Partial	Partial
Predictiveness	No	Yes	No
Antibiotic resistance	Yes	No	No
Preanalytical issues	Yes	Limited	Yes

Figure 1: Diagnostic features of past, present and future sepsis biomarkers.

Table 1: Ideal features of sepsis biomarkers.

- Being present at symptoms onset (or even earlier), to allow for an early diagnosis
- Being highly sensitive and specific for infections, to allow for an accurate differential diagnosis between infectious and non-infectious diseases
- Being capable of identifying the causative microorganism
- Being informative on the clinical course
- Providing valuable information on the prognosis
- Guiding therapeutic decisions (e.g. antibiotic stewardship)

accuracy of some of the currently available biomarkers remains limited (i.e. <80%), and that presepsin even failed to outstrip more conventional sepsis biomarkers such as procalcitonin and CRP for both diagnosing sepsis and prognosticating death in critically ill patients [13].

Nucleic acid amplification is one of the most promising perspectives in sepsis diagnostics. The current techniques are essentially based on rapid amplification of DNA or RNA of pathogen origin, up to obtaining detectable levels which can then be assayed using mass spectrometry (MS), high-resolution melting or sequencing. Several commercial methods have been commercialized and cleared by many regulatory agencies worldwide, as is thoughtfully reviewed elsewhere [8]. Albeit recent evidence suggests that the diagnostic performance of these assays is comprised between 70% and 90%, the ability to accurately detect pathogens is still limited by some preanalytical issues, the need for effective lysis across a broad range of bacteria, interference from host DNA or other inhibitory substances, off-target interactions and amplification bias [8]. Moreover, antimicrobial stewardship may remain virtually unchanged even after molecular biology detection, so that clinical efficiency and cost-effectiveness of these techniques remain largely untested [14]. Hence, further refinements of molecular assays would be needed to overcome the current limitations in their diagnostic performance.

Early diagnosis of sepsis remains crucial for the managed care of patients with severe infections, who need timely treatment much earlier than after any signs and symptoms of organ failure have appeared. Prompt pathogen identification would also be highly effective for limiting inappropriate antibiotics usage, thus lowering the risk of antimicrobial resistance, which is one of the biggest threats to global health according to the World Health Organization (WHO). As previous work suggested, neither the past, present or future tests would fulfil all the features of an ideal sepsis biomarker and thus being considered the panacea that clinicians are expecting (Table 1). On the other hand, the integration of these existing technologies would probably be effective for counterweighing individual drawbacks but will exponentially increase the healthcare expenditure. Future cost-effectiveness studies aimed at comparing and validating their diagnostic usage, alone or in combination, will thus be necessary.

The most reliable strategy for early diagnosis of sepsis appears to be that suggested by Schuetz et al. in this issue of the journal [15], relying on diagnostic algorithms integrating the pretest probability of infection, clinical features and results of *in vitro* diagnostic testing (e.g. procalcitonin). This approach, entailing close collaboration and cooperation between laboratory professionals and clinicians, reflects the current value of the

so-called "clinical laboratory stewardship" [16], which must be seen as an essential step forward for improving the appropriateness of laboratory test ordering and the accuracy of interpretation of test results. Nonetheless, additional practical issues should be addressed, such as the current lack of standardization of procalcitonin immunoassays, as is also highlighted in another interesting article published by Chambliss et al. in this issue of the journal [17].

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