

Biomarkers and sepsis endotypes. New evidence

Biomarcadores y endotipos en la sepsis. Nuevas evidencias

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Abstract

Background: Sepsis is an important cause of disease and death worldwide, and it has a complex and heterogeneous disease process. It is now recognized that biological markers improve sepsis classification and may facilitate the identification of different subclasses or endotypes of patients. **Objective:** To analyze the current concepts in translational biology that help to understand the heterogeneity in the response of the patient with sepsis. **Conclusions:** There are a number of biomarkers that could be useful for constructing endo-compound types that predict uncomplicated results in sepsis. These endotypes could help identify patients who deserve early antimicrobial onset or discontinuation.

Keywords: Sepsis. Biology. Endotypes. Biomarkers.

Resumen

Antecedentes: La sepsis es una causa importante de enfermedad y muerte en todo el mundo, y es un proceso complejo y heterogéneo. Ahora se reconoce que los marcadores biológicos mejoran la clasificación de la sepsis y pueden facilitar la identificación de distintas subclases o endotipos de pacientes. **Objetivo:** Analizar los conceptos actuales en biología traslacional que ayudan a entender la heterogeneidad en la respuesta del paciente con sepsis. **Conclusiones:** Hay una serie de biomarcadores que podrían ser útiles para construir endotipos compuestos que predicen resultados no complicados en la sepsis. Estos endotipos podrían ayudar a identificar a los pacientes que merecen el inicio temprano de antimicrobianos o la interrupción de estos.

Palabras clave: Sepsis. Biología. Endotipos. Biomarcadores.

Introduction

Sepsis is an important cause of disease and death worldwide, and is a complex and heterogeneous process associated with or caused by a variety of microorganisms and sources of infection. Currently, there

is a promising shift from outcome prediction to an understanding of the diversity in host response to sepsis based on pathobiology. Now, it has been recognized that biological markers improve sepsis classification and may facilitate different patient subclasses or endotypes identification.

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The purpose of this work is to review current concepts of translational biology that help understand the response heterogeneity of patients with sepsis.

Sepsis profiles

One of the first studies to use hierarchical clustering to study sepsis subgroups within the intensive care unit (ICU) population was carried out in a cohort of 98 ICU-admitted children diagnosed with septic shock¹.

Using genome expression patterns, three subclasses were identified: endotype A (29%), endotype B (46%) and endotype C (26%). These three classes significantly differed in clinical phenotypes, including ICU mortality (highest in endotype A, with 36%, vs. 11% and 12% for endotypes B and C, respectively), disease severity, and degree of organ failure (both higher in endotype A), and age (younger in endotype A).

A similar methodology has recently been applied to a critically ill adult population. Among 265 patients diagnosed with sepsis due to community-acquired pneumonia, as part of the Genomic Advances in Sepsis (GAINs) trial, transcriptomic profiles defined two sepsis-response signals: SRS1 (41%) and SRS2 (59%)². In comparison with SRS2 patients, patients in the SRS1 group had a higher 14 day-mortality (22% vs. 10%). Assignment to SRS1 was associated with relative immunosuppression, endotoxin tolerance, T-cell depletion, class II human leukocyte antigen (HLA) downregulation, and metabolic disorders (change from oxidative phosphorylation to glycolysis). Out of more than 3,000 differentially-expressed genes, seven reliably predicted SRS group membership. In future studies, patients prospectively assigned to SRS1 may benefit from therapies that stimulate their immune system and prevent nosocomial infection. This analytical approach was subsequently used by the same researchers to study gene expression patterns in 117 patients with fecal peritonitis³. Again, two distinct groups were identified, SRS1_FP (46%) and SRS2_FP (54%), and patients in the SRS1_FP group also had higher 14-day mortality (19% vs. 4%). The findings were strongly correlated with the SRS groups identified in the CAP study², showing, again, increased endotoxin tolerance and T-cell activation, but also cell death, apoptosis, and necrosis. From the more than 1,000 differentially-expressed genes, a simpler set of six genes was derived, which predicted SRS group membership. It should be noted that when the gene expression patterns that distinguished the SRS groups

were tested in the above-described pediatric cohort¹, the same increase was not observed (i.e., SRS1_FP³ and endotype A¹).

Better understanding of human genetic variation and the use of metabolomics are key aspects for separating groups of patients with similar responses, and the use of these techniques might help stratify patient risk. Scicluna et al.⁴ report the results of a matrix-based transcriptomics study using blood samples collected at ICU admission from 526 sepsis patients at two ICUs in the Netherlands (discovery and first validation cohort), and in 265 patients with pneumonia-associated sepsis from 29 ICUs in the United Kingdom (second validation cohort), with the purpose to define different functional and homogeneous subtypes, i.e., endotypes. Blood transcriptomes of these endotypes had a different host response, which ranged from immunosuppression to hyperinflammation. They found that there could be four endotypes, which are the groups into which they divide them according to their genetics-dominated immune response. Each group reacts in a certain way, and predicting the possibility of death at each group is possible. Group 1 (called MARS 1) has the highest mortality and a decreased response of the recognition pattern and cytokine signaling, while the MARS 3 group has the best prognosis (Fig. 1). High levels of circulating pro-inflammatory mediators are known to be predictive of increased mortality⁵, but poor patient evolution is also associated with higher concentrations of anti-inflammatory cytokines⁶. This observation might reflect a suppression of immune activity (commonly known as immunoparalysis) in patients with the Mars 1 endotype, which would contribute to the development of opportunistic infections that increase sepsis severity.

In a group of 306 patients admitted to the ICU in the Netherlands, as part of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project, four molecular endotypes (Mars 1-4) were identified⁴. Mortality at 28 days differed between subgroups and was higher in the Mars 1 group, with 39%, in comparison with 22% in Mars 2, 23% in Mars 3 and 33% in Mars 4. The Mars 1 poor-prognosis endotype had a decrease in the expression of genes involved in innate and adaptive immune functions (Toll-like receptor, nuclear factor kappa B [NF- κ B] signaling, antigen presentation and T-cell receptor signaling) and increased expression of specific cellular metabolic pathways (heme biosynthesis), which are processes that are analogous to immune depletion. Mars 2 and 4 endotypes had an

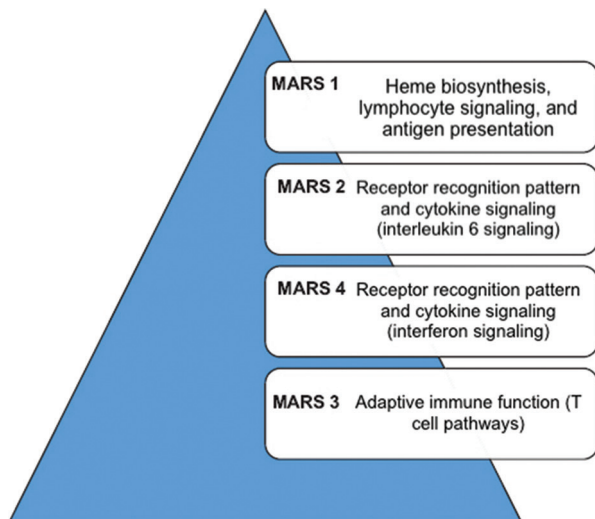


Figure 1. Sepsis endotypes. Group 1 (called MARS 1) has the highest mortality.

upregulation of recognition patterns and cytokine pathways (interleukin [IL] 6, NF- κ B, interferon signaling, inducible nitric oxide synthase), which represents a hyperinflammatory state. Finally, Mars 3 was a low-risk endotype with increased expression of adaptive immune pathways (T-helper cells, natural killer cells, IL-4 signaling, B-cell development), which was highly correlated with SRS2 low-risk endotype². An expression ratio of two genes was obtained to allow each endotype classification at ICU admission.

In the most recent and comprehensive attempt to identify sepsis subtypes, the results of 14 transcriptomic datasets from 700 patients revealed three robust host response groups across the sepsis spectrum⁷. These groups were called: 1) inflammopathic (increased innate and reduced adaptive immune signal marked by IL-1 receptor increased expression, pattern recognition receptor activity, and complement activation); 2) adaptive (reduced immune and high adaptive pathways signaling with lower mortality, marked by interferon signaling); and 3) coagulopathic (coagulation and complement system irregularities, including platelet degranulation and glycosaminoglycan binding). As in previous analyses, the three groups differed in 30-day mortality, with the highest rate being observed in the inflammopathic group, with 30%, in comparison with 8% in the adaptive groups and 25% in the coagulopathic groups. A simplified 33-gene classifier was obtained to facilitate group assignment. Assignment to the high-mortality inflammopathy group corresponded to SRS1, and the low-mortality adaptive group to SRS2². Early transcriptional changes may have also

identified patients at risk of sepsis-associated complications at ICU admission. A study from our research group that included 57 patients with sepsis, found that differential expression of key mediators of neutrophil initial response to infection identified patients with acute respiratory distress syndrome (ARDS) ($n = 29$), in comparison with those with sepsis who had no ARDS ($n = 28$); a finding that could not be attributed to neutrophil count⁸.

So far, genome-wide expression sepsis studies have used entire populations of leukocytes. However, there are different gene expression patterns between subsets of granulocytes and lymphocytes that represent the specialized function of each one of these immune cells⁹. Given that the transcriptome profile depends on inflammatory cell type, it is possible for the gene expression patterns that distinguish the subclasses to reflect different populations of leukocytes rather than gene expression differences within cells. These findings also require validation in large cohorts encompassing different countries, since ethnicity variation is a strong determinant of gene expression¹⁰. However, these studies provide evidence of different categories of host response to sepsis, and potential novel therapeutic targets based on molecular pathways that distinguish patient endotypes.

Proteins

A more established and more feasible method for biological sub-classification of patients with sepsis is plasma protein quantification, and a large number of studies have classified sepsis using this approach¹¹⁻¹³. One important method that might offer a novel way to obtain sepsis subclasses is to combine molecular and protein biomarkers in order to predict the outcome in patients with septic shock. This approach was used to stratify the risk of pediatric septic shock with a previously-validated risk score that consists of five plasma protein biomarkers (PERSEVERE decision tree)¹³ and their combination with four housekeeping genes mortality assessments¹⁴. An improvement in the performance of the risk score that estimates the risk of mortality at 28 days was observed (PERSEVERE-XP, the area under the curve increases from 0.78 to 0.91). Plasma biomarkers were associated with dysfunctional inflammation and cell damage, while genes were related to tumor protein 53 (TP53, p53), a transcriptional factor that acts as a tumor suppressor, thus preventing the generation and persistence of cells with genomic damage¹⁴.

Metabolomics

Metabolomics is a method under development for deciphering sepsis heterogeneity. It refers to global evaluation of small metabolites in any biological sample, which represents gene expression, enzyme activity and the physiological landscape¹⁵. More than 5,000 metabolites can be detected in cells, tissues or biofluids (blood components, urine) using magnetic resonance (MR) spectrometry or mass spectrometry, with the latter being more sensitive and able to detect low-abundant metabolites¹⁶. ¹H MR spectroscopy was used to analyze and compare serum samples from adults with septic shock and from controls at the ICU¹⁷. Sixty metabolites were recognized, out of which 31 were able to distinguish ICU-patients with septic shock, with a composite pattern of biomarkers that could differentiate between these groups of patients being proposed. The metabolites involved in energy metabolism included glucose, 3-hydroxybutyrate, O-acetylcarnitine, succinate, creatine, creatine phosphate, and a decreased level of branched-chain amino acids and arginine. These results suggest that, in early sepsis, metabolites involved in energy metabolism have a role in sepsis pathophysiology. Alteration of those metabolites involved in energy metabolism has also been recognized as key for distinguishing sepsis survivors from non-survivors using mass spectroscopy to characterize lower-plasma concentration metabolites. In a sub-study of the Albumin Italian Outcome Result Sepsis (ALBIOS) trial, which enrolled 1,818 patients with severe sepsis or septic shock, plasma samples from days 1 and 7 of 20 patients were analyzed, 45% of whom died on day 28¹⁸. Another large cohort, in which mass spectrometry was used to study metabolite biomarkers in 60 ICU survivors and 30 ICU non-survivors found that, out of 187 tested metabolites, 57 were associated with mortality at 28 days¹⁹, and 31 of them were replicated in the CAPSOD validation cohort²⁰. These metabolites included various lipid products, carbohydrates, amino acids and nucleotides. Higher values of tyrosine and phenylalanine catabolism products and lower values of lipid metabolites were associated with mortality. The largest study to investigate metabolites using mass spectrometry in patients with sepsis included 406 subjects, out of whom 268 were included in a discovery cohort (42 patients with systemic inflammatory response syndrome, 67 with community-acquired pneumonia, 60 with intra-abdominal infection, 73 with urinary tract infection, and 26 with bloodstream

infection)²¹. The analysis also indicated a large heterogeneity in metabolite patterns, depending on the source of anatomical infection and, therefore, a single-metabolite model for predicting an unfavorable outcome for each type of infection was proposed.

Biomarkers of microcirculatory damage

Adrenomedullin is a peptide produced by various tissues during physiological and infectious stress, with different functions. Levels of the mid-regional pro-adrenomedullin (MR-proADM) fragment are correlated with the degree of organ failure in sepsis. Elke et al.²² demonstrated that low concentrations of MR-proADM identify patients with sepsis with a very low risk of death during ICU stay. MR-proADM has a negative predictive value (NPV) of 94% for ruling out mortality at 28 days and of 96% at 10 days²².

Immune biomarkers

Human leukocyte antigen DR (HLA-DR) represents the capacity of monocytes for antigen presentation and connection with auxiliary T cells, which allows adaptive immune system activation. HLA-DR in monocytes is considered a surrogate marker of sepsis-induced immunosuppression. Survivors show a significant increase in the percentage of HLA-DR-positive monocytes during the course of sepsis²³. Using flow cytometry in a group of patients with severe sepsis, Wu et al.²⁴ showed that a 4.8% increase on day 3 of ICU stay yielded a 98% NPV for ruling out mortality at 28 days, while a 9% increase on day 7 yielded a NPV of 97.2%.

Biomarkers related to coagulation and neutrophil protease

Neutrophil proteases are involved in the pathogenesis of the coagulation abnormalities observed in sepsis and mediate endothelial damage. We have identified that neutrophil protease activity (matrix metalloproteinase 8 and lipocalin-2-LCN2/NGAL) is associated with the degree of organ failure and mortality in sepsis²⁵. Evidence of low or decreased neutrophil protease values during hospitalization is also an additional candidate signal for identifying uncomplicated patients. Neutrophil proteases mediate proteolysis and, using mass spectrometry, Bauzá-Martínez et al.²⁶ demonstrated that septic shock survivors are characterized by an absence of increased proteolysis throughout the disease evolution (evidenced by peptide abundance).

Conclusions

There is a series of promising biomarkers involved in proteolytic, vascular, immune and coagulation anomalies that could be useful for constructing composite endotypes to predict uncomplicated outcomes in sepsis. These endotypes could help identify patients requiring antimicrobials early initiation or discontinuation. Further efforts are needed in order to identify optimal biomarker combinations for the best composite endotype obtain through repeated evaluations over the course of the disease. New metabolomics technologies, flow cytometry, immunoassays and messenger RNA quantification technologies will facilitate accurate identification of these endotypes.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments have been performed on humans or animals for this research.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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