Profiling the dysregulated immune response in sepsis: overcoming challenges to achieve the goal of precision medicine

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Sepsis is characterised by a dysregulated host immune response to infection. Despite recognition of its significance, immune status monitoring is not implemented in clinical practice due in part to the current absence of direct therapeutic implications. Technological advances in immunological profiling could enhance our understanding of immune dysregulation and facilitate integration into clinical practice. In this Review, we provide an overview of the current state of immune profiling in sepsis, including its use, current challenges, and opportunities for progress. We highlight the important role of immunological biomarkers in facilitating predictive enrichment in current and future treatment scenarios. We propose that multiple immune and non-immune-related parameters, including clinical and microbiological data, be integrated into diagnostic and predictive combitypes, with the aid of machine learning and artificial intelligence techniques. These combitypes could form the basis of workable algorithms to guide clinical decisions that make precision medicine in sepsis a reality and improve patient outcomes.

Introduction

In recent years, the use of evidence-based treatment guidelines, such as those developed as part of the Surviving Sepsis Campaign, has been associated with significant improvements in early-phase sepsis outcomes.[1] Nonetheless, with an estimated 48·9 million incident cases worldwide in 2017, and 11·0 million deaths, sepsis remains a leading cause of morbidity and mortality.[2]

Extensive research has emphasised the role of dysregulated immune responses in sepsis pathophysiology, but only a few immunoadjuvant treatments have shown signals of efficacy and robust positive findings have yet to be produced for clinical endpoints.[3–6] The heterogeneity of immune dysregulation in patients with sepsis explains the many failed attempts to target the dysregulated immune response in clinical trials.[7–9] No individualised immune status assessment is routinely used and no internationally adopted guidelines provide recommendations on preferred biomarkers for sepsis research and clinical practice. In this Review, we emphasise the urgent need to profile the dysregulated immune response in sepsis. Progress in immune profiling would help to make precision medicine a reality in order to improve early-phase outcomes and reduce the incidence of late sepsis complications.[10] We provide an overview of the immunopathophysiology of sepsis and discuss current and innovative strategies to characterise the immune status of patients, including recent advancements in sepsis endotyping, machine learning (ML), and artificial intelligence (AI) approaches. We also examine attempts to implement personalised medicine in sepsis, highlighting associated challenges and proposing solutions. Finally, we draw on the lessons learnt during the COVID-19 pandemic to improve the future treatment of sepsis.

Immunopathophysiology of sepsis

Our understanding of sepsis pathophysiology has evolved substantially during the past century. Currently, sepsis is viewed as a complex and heterogeneous state

Key messages

- A dysregulated immune response with a lack of physiological balance between hyperactivation and hyporesponsiveness is a key component of the pathophysiology of sepsis
- Immune dysregulation in patients with sepsis is heterogeneous and dynamic, requiring personalised treatment strategies
- Successful immunomodulation trials in severe COVID-19 underscore the potential of therapies that target the immune system in the management of sepsis
- Specific immunological biomarkers and high-throughput omics-based techniques provide novel insights into immune dysregulation and allow the identification of sepsis endotypes
- Integration of various types of information, including immunological, clinical, microbiological, and high-throughput omics data in so-called combitypes, with the aid of machine learning and artificial intelligence techniques, could be an important step towards the realisation of precision medicine in sepsis
- The development of internationally adopted guidelines for assessment of the dysregulated host response in sepsis would lead to better standardisation and clinical utility

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characterised by concurrent proinflammatory and anti-inflammatory responses, in which detrimental sequelae can be caused by both persistent hyperinflammation and prolonged hyporesponsiveness of the immune system (figure 1).

The heterogeneity of the immune response can be ascribed to a variety of factors, including age (sepsis typically affects the very old [>70 years] and very young [<1 year]), different infectious causes, sites of infection, host genetics, applied treatments, and rapidly changing illness dynamics. An overview of predisposing factors for sepsis is provided in the appendix (p 1).

In sepsis, the immune response is initially activated through sensing of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors. The activation of pattern recognition receptors induces broad biological responses, including the release of cytokines and other inflammatory mediators,[11] as well as the induction of immune cell death (eg, of macrophages through activation of caspases).[12] Furthermore, activated granulocytes released from the bone marrow produce neutrophil extracellular traps to capture microorganisms, which might aggravate inflammation and tissue injury.[13] The inflammatory response in sepsis is also characterised by neuroendocrine alterations, activation of the complement and coagulation systems, and alterations in lipid mediators—all of which act synergistically to enhance inflammation.[14,15] If systemic or sustained, this

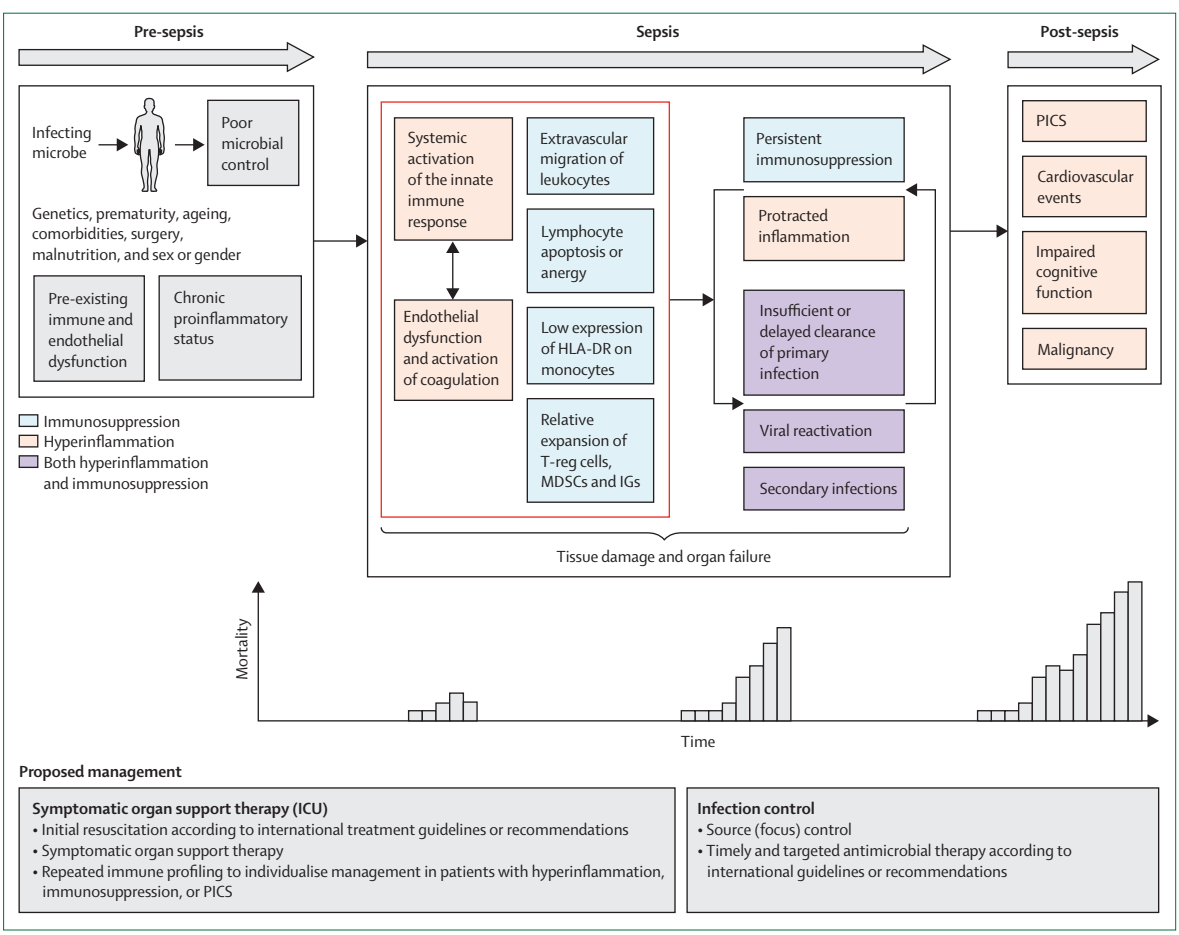

Figure 1: **Sequence of immune dysregulation in sepsis**
Many patients with sepsis have predisposing factors, such as ageing or comorbidities, that are known to contribute to immune dysregulation (appendix p 1), thereby impairing their ability to mount an effective response against an infecting microbe. An impaired initial response typically translates into poor microbial control, leading to excessive activation of the innate immune response and acute-on-chronic endothelial dysfunction or activation of coagulation. This hyperinflammatory signature is paralleled by the emergence of an immunosuppressive phenotype, exemplified by uncontrolled migration of leukocytes to the extravascular space, increased apoptosis and impaired function of lymphocytes, defective expression of molecules required for antigen presentation such as HLA-DR, as well as relative expansion of suppressor cells such as T-reg cells, MDSCs, and IGs. This immunosuppressive phenotype frequently leads to sepsis-associated immunosuppression, which renders the host unable to clear the primary infection, provokes reactivation of dormant viruses, and renders the host vulnerable to secondary infections, culminating in a vicious pathogenic cycle. These events are compounded by the induction of tissue injury by microbial and immunological factors, leading to organ dysfunction. Many sepsis survivors face long-term clinical consequences, including so-called PICS, frequently accompanied by cardiovascular and neurological complications, and cancer, which represent chronic immunological sequelae. ICU=intensive care unit. IGs=immature granulocytes. MDSCs=myeloid-derived suppressor cells. PICS=persistent inflammation, immunosuppression, and catabolism syndrome. T-reg cells=regulatory T cells.

inflammatory cascade potently activates the endothelium, which loses its homoeostatic functions, becomes pro-coagulant and leaky, and contributes substantially to the development of shock, organ dysfunction, and ultimately death in a considerable proportion of patients.[16] An important mechanism leading to imbalanced coagulation and endothelial dysfunction in septic shock is depletion of activated protein C, which has important anticoagulant and anti-inflammatory effects, and can promote fibrinolysis and inhibit thrombin generation.[17] However, in the PROWESS-SHOCK trial, treatment with recombinant human activated protein C did not reduce 28-day mortality in adults with septic shock.[18]

In a subset of patients, an uncontrolled hyperinflammatory response might occur, resulting in hyperferritinaemic sepsis, macrophage activation-like syndrome (MALS), or in the most severe form, haemophagocytic lymphohistiocytosis. All these conditions are associated with high mortality and typically occur early in the disease course (figure 1).[19,20]

However, in some patients with sepsis, concurrent immunoregulatory compensatory mechanisms might be predominant (figure 1).[21,22] For example, early findings revealed that circulating monocytes of patients with sepsis display impaired proinflammatory cytokine secretion in response to PAMP exposure.[23,24] Similarly, innate immune cells (eg, dendritic cells, natural killer cells, and neutrophils) also display a reduced capacity to produce mediators essential for effective pathogen clearance.[25–27] Instead, these cells secrete increased amounts of anti-inflammatory or immunoregulatory molecules, such as interleukin (IL)-10, that dampen antimicrobial effector mechanisms.[28] These innate immune defects are explained by one or both of the following: (1) metabolic and epigenetic reprogramming triggered by the initial inflammatory or hyperinflammatory insult;[29] and (2) altered leukocyte differentiation or generation in the bone marrow.[30] Sepsis-induced immunosuppression is also characterised by major defects in adaptive immune function.[31] For instance, T-cell counts are reduced due to apoptosis, while surviving T cells frequently display an exhausted phenotype. This phenotype is characterised by and might be due to increased expression of immune checkpoint molecules, as well as impaired production of immunostimulatory cytokines such as interferon (IFN)-γ. Finally, a relative expansion of regulatory T (T-reg) cells has repeatedly been reported.[32–35] In most cases, hyporesponsiveness of the immune system in sepsis presents in the post-acute phase, although its molecular hallmarks are apparent very early after the onset of sepsis. This immunocompromised state can have serious consequences, such as reactivation of latent viral infections—a phenomenon that can negatively affect recovery and overall outcome.[36,37] Furthermore, sepsis-induced immune alterations render the host highly vulnerable to secondary infections, often with opportunistic, difficult-to-treat pathogens.[38]

Sepsis-induced immune alterations might persist, at a low grade, for years after hospital discharge. There is compelling evidence that a significant proportion survivors do not fully recover after sepsis, but have dismal long-term functional, cognitive, and physical derangements.[10,39] In these individuals, evidence for the presence of persistent low-grade inflammation, immune suppression, and lean tissue wasting has been reported.[40] Patients with so-called persistent inflammation, immunosuppression, and catabolism syndrome (PICS; figure 1) are now recognised as having a specific syndrome of chronic critical illness associated with poor long-term outcomes.[41]

Taken together, the evidence suggests that the pathophysiology of sepsis involves a major imbalance in the immune response to infection. Depending on the host, pathogen, and sepsis phase, this response might take the form of a dominant hyperinflammatory or immunosuppressive phenotype. Both are linked to poor prognosis, but might necessitate contrasting immunoadjuvant therapeutic approaches (figure 2). Due to the absence of clinical signs indicative of a patient's immune status, immune monitoring is crucial in identifying an appropriate treatment and therapeutic window.

Biomarkers to profile immune status in sepsis

An overview of the significance and suggested use of currently used biomarkers to profile immune status in sepsis is provided in the appendix (pp 3–7). Technologies

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See **Online** for appendix

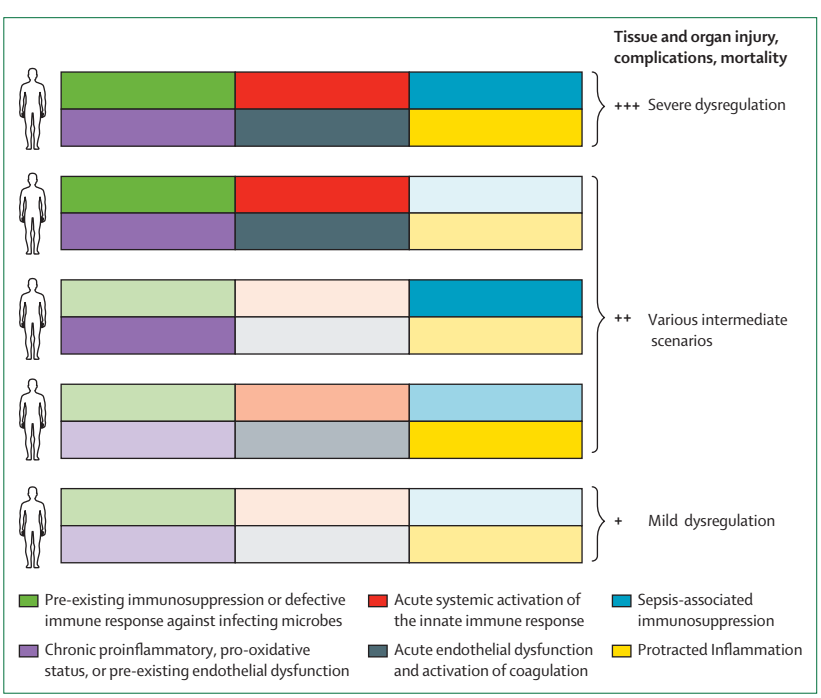

Figure 2: **Potential immunological phenotypes in sepsis**
Patients with sepsis can present with different features of immunological dysfunction and related endothelial and coagulation disturbances. These multisystem derangements can coexist, present with different magnitudes over the disease course, and are linked to varying degrees of organ failure. Accurate longitudinal profiling of the type and dimension of these features can guide appropriate interventions, not only with immunomodulatory drugs, but also with therapeutics targeting specific elements of the endothelial or coagulation derangements.

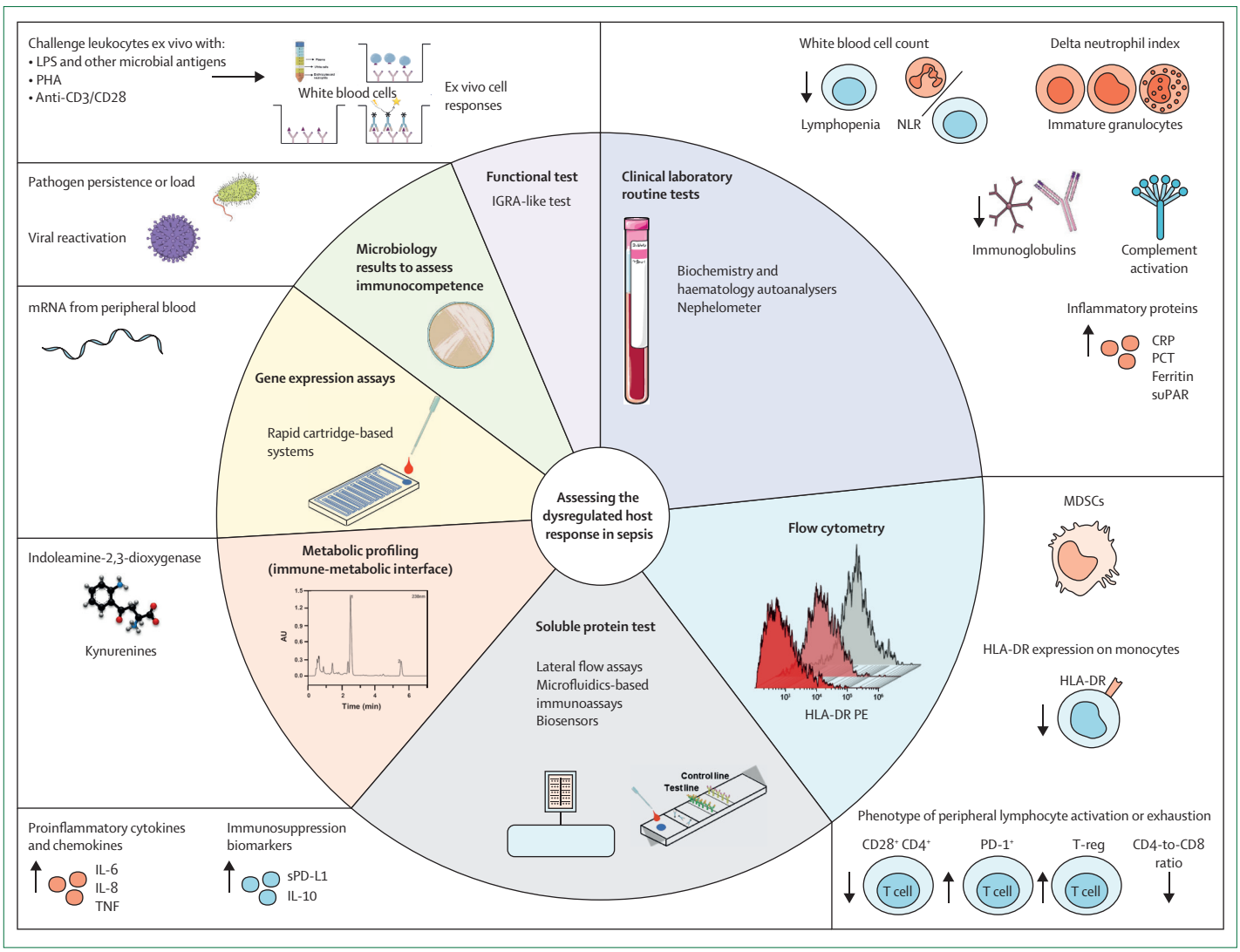

Figure 3: **Technologies to profile immune status in sepsis**
The presented technologies are either already available in most hospital settings or moving towards broad-scale clinical application. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3·0 unported license. CRP=C-reactive protein. IGRA=interferon-γ release assay. IL=interleukin. LPS=lipopolysaccharide. MDSCs=myeloid-derived suppressor cells. NLR=neutrophil-to-lymphocyte ratio. PCT=procalcitonin. PD-1=programmed cell death 1. PE=phycoerythrin. PHA=phytohaemagglutinin. sPD-L1=soluble programmed cell death 1 ligand 1. suPAR=soluble urokinase plasminogen activator receptor. T-reg=regulatory T cell. TLR=toll-like receptor. TNF=tumour necrosis factor.

to profile immune status in sepsis that are already available in most hospitals and moving towards broad-scale clinical application are displayed in figure 3.

Routine clinical laboratory markers

Only a few immune biomarkers are available for routine use by clinicians in the management of sepsis. Acute-phase inflammatory proteins, such as C-reactive protein (CRP), and more specific markers of bacterial infection, including procalcitonin, are used as diagnostic markers of infection and can aid in monitoring the response to antibiotics[42]—in particular, in paediatric patients.[43,44] These markers might also have prognostic value.[45] Similarly, a decreased lymphocyte count was identified as a marker of poor prognosis in sepsis and pneumonia.[46,47] An increased immature granulocyte fraction (assessed through measurement of the delta neutrophil index by some blood-cell analysers) has been proposed as a marker for early diagnosis and prognostication in sepsis.[48–51] The latter also applies to alterations in the neutrophil-to-lymphocyte ratio.[52] Finally, routinely measured ferritin is typically used to identify possible MALS.[53]

Soluble markers

An early elevation in inflammatory cytokines (eg, IL-6 and IL-8) might be indicative of a hyperinflammatory phenotype.[54–58] Conversely, increased concentrations of

IL-10 and transforming growth factor-β are associated with immunosuppression and might be related to reduced expression of HLA-DR on monocytes (as discussed later).[59,60] Activation of the complement system in sepsis leads to generation of complement C5a. This anaphylatoxin has been proposed as a therapeutic target, given that complement C5a inhibition enhanced immune function in preclinical studies.[61–63] Furthermore, complement activation leads to consumption of factors such as complement C3, and a relationship between complement C3 depletion and the expansion of T-reg cells was identified in patients with sepsis.[64] Finally, plasma concentrations of the soluble fraction of the immune-inhibitory receptor programmed cell death 1 ligand 1 (PD-L1) are elevated in sepsis.[65,66] Although several of these markers (eg, some cytokines) are already routinely measured in some hospitals, they could be broadly implemented on clinical analysers, pending further evidence of clinical utility, and developed into point-of-care tests to facilitate their use in daily clinical practice.

Phenotypic cellular markers

HLA-DR is part of the MHC-II expressed by antigen-presenting cells at the interface between innate and adaptive immunity. Its expression is generally measured by flow cytometry, although mRNA expression has been used as well.[67,68] Several studies have shown an association between decreased monocyte HLA-DR expression (mHLA-DR) and functional alterations of monocytes, such as reduced proinflammatory cytokine release and diminished antigen presentation capacity.[69,70] Most importantly, low mHLA-DR expression was shown to be independently associated with an increased risk of secondary infections and death in critically ill patients.[71] Recent work identified clusters of patients with septic shock who exhibited specific mHLA-DR trajectories related to unfavourable outcomes.[72,73] A wide consensus in the scientific community, therefore, claims that low mHLA-DR expression constitutes a surrogate marker of sepsis-induced immunosuppression.[22,74]

More complex cellular phenotyping by flow cytometry can provide detailed information on cellular activation or exhaustion, maturation, and function in patients with sepsis. With respect to neutrophils, low surface expression of CD16 and CD10 is indicative of low-density immature granulocytes, and increased abundance of these immunosuppressive myeloid-derived suppressor cells[75–77] is associated with an increased risk of death and secondary infections in sepsis.[75,78] T-cell exhaustion is defined by three inter-related features: (1) impaired effector function; (2) sustained increase in cell-surface expression of inhibitory immune checkpoint molecules such as programmed cell death 1 (PD-1) on T cells or its ligand PD-L1 on antigen-presenting cells; and (3) a distinct transcriptional state that impairs development of functional T-cell memory. Increased expression of PD-1 and PD-L1 is associated with progression of infection to sepsis,[79] risk of nosocomial infections,[80] and a more severe disease state.[81] In older patients with sepsis, especially those who do not survive, a relative abundance of the immunocompetent $CD28^+$ subset of $CD4^+$ T cells was found to be decreased, whereas that of the immunosuppressive $PD\text{-}1^+$ T cells and $CD4^+CD25^+Foxp3^+$ T-reg cells was increased.[82] An increased proportion of T-reg cells has been repeatedly observed in sepsis,[32–35] and is related to a decreased lymphoproliferative response.[32] The CD4-to-CD8 cell ratio is another potential marker of immune functionality. For example, among older patients with sepsis, the mean CD4-to-CD8 T-cell ratio is significantly lower in non-survivors than in survivors.[83,84] Finally, an increasing number of studies has shown the feasibility of using dedicated flow cytometry panels in multicentric and longitudinal clinical studies. This analytical capacity illustrates its potential for clinical application.[79,85,86]

Functional tests

Functional testing is an attractive method to identify immune deficiencies in sepsis because it directly assesses the ex vivo capacity of a cell population to respond to an immune challenge by measuring its cytokine production, oxidative burst capacity, proliferation, or activation status. Low tumour necrosis factor (TNF) production by ex vivo-stimulated whole blood was used as an inclusion criterion to apply immunostimulatory therapy in paediatric patients with sepsis.[87] Although commercial easy-to-use products are emerging,[88] routinely available assays are yet to be developed in a standardised format, similar to the assay for IFN-γ release in response to tuberculosis antigens.

Monitoring of viral reactivation

There is growing evidence of reactivation of latent viruses in patients with sepsis.[36,89,90] Compared with other critically ill patients (eg, those with burns or trauma), patients with sepsis show elevated herpes virus titres (mainly determined as DNAaemia via PCR assays available in most hospitals)[91,92] that correlate with transcriptomic[37] or humoral[37] features of immuno-suppression, and are associated with disease severity and mortality.[91–93] Although viral reactivation might be pathogenic and contribute to disease severity,[37,94,95] viral titres are a potentially useful metric to gauge immuno-competence.

Broad profiling of immune status in sepsis

The emergence of high-throughput omics technologies combined with advanced data analysis is helping to delineate a broader picture of the immune status in sepsis. This approach is referred to as sepsis systems immunology.[96]

Transcriptomics

Whole-blood (ie, leukocyte) gene expression profiling has contributed extensively to the derivation of transcriptomic panels that aid rapid diagnosis of sepsis and identification of different sepsis subclasses, also known as endotypes—eg, specific patterns of gene expression that are related to the host response in sepsis.[97–108] Endotypes that are characterised by reduced expression of genes involved in lymphocyte signalling and antigen presentation pathways are indicative of impaired innate or adaptive immune responses, and identify patients with poor prognosis. These include the so-called sepsis response signature 1[101] and the Mars1 endotype in adults,[100] as well as the A endotype in children.[109] The findings suggest that impaired immunity is a common signature associated with severity in sepsis. The identification of a cluster of patients with sepsis who were characterised by an overall activation of adaptive immunity and improved survival supports this notion.[98] Furthermore, endotypes have been shown to evolve during hospitalisation in parallel with disease severity, and the persistence of proinflammatory or coagulopathic endotypes predicts worse outcomes.[110] Achieving the overarching goal of stratification of patients with sepsis into endotypes of clinical utility will require an international collaboration to establish a consensus sepsis endotype model, including variability stemming from geographically diverse patient populations. In addition, although blood transcriptomics have been key to improved understanding of the host response in sepsis, most studies have focused on samples obtained at intensive care unit (ICU) admission (ie, in acute sepsis). Recent work showed that blood transcriptional patterns of patients with surgical sepsis who developed chronic critical illness were partly distinct at both day 1 and day 14 post-sepsis from those of survivors who rapidly recovered, in support of the PICS endotype.[111] Further work is needed to clarify whether survivors of sepsis exhibit consistent transcriptomic alterations in connection with these endotypes.[40]

A major evolution in transcriptomic analysis is the development of single-cell RNA sequencing (scRNA-seq) technologies, which permits the analysis of transcriptomic profiles at the level of single cells. So far, a few small studies limited to peripheral blood mononuclear cells have used scRNA-seq in the context of sepsis.[112–116] Despite their limitations, these studies have uncovered potentially important cellular features—eg, identification of a novel $CD14^{+}$ monocyte subset (termed MS1) that was expanded in patients with bacterial sepsis.[115] Furthermore, among survivors who develop chronic critical illness, scRNA-seq analysis (in combination with cellular indexing of transcriptomes and epitopes by sequencing [CITE-seq]) revealed lymphocytes characterised by gene expression profiles attuned to simultaneous immunosuppressive and low-grade proinflammatory states.[114] Another study reported that myeloid-derived suppressor cells maintain a transcriptomic profile reflective of an immunosuppressive state in patients with late sepsis,[116] whereas scRNA-seq of circulating haematopoietic stem and progenitor cells revealed altered granulopoiesis in patients with sepsis and poor outcome.[117] These studies show that scRNA-seq is a powerful tool that has potential in resolving diversity in patients' immune status. Large population studies that profile millions of single cells, including the challenging granulocytes, are needed to confirm, refine, and operationalise scRNA-seq.

Proteomics

Liquid chromatography-based separation methods coupled with mass spectrometry have contributed to the identification of up to 3000 proteins involved in sepsis pathology—for instance, in the inflammatory response, induction of oxidative stress, and mitochondrial dysfunction.[118] Other simpler tests to profile immunological proteins are the bead-based multiplex assays, which have been used extensively for simultaneous quantification of cytokines, chemokines, and neutrophil degranulation markers in sepsis.[119,120] A relatively new technology, proximity extension assay, combines a targeted immunoassay with PCR to provide multiplex quantification of hundreds of proteins in minimal volumes of any biological fluid.[121] This technology has been used to identify new biomarkers in sepsis and COVID-19.[122,123]

Metabolomics

Metabolic pathways are crucial in regulating immune responses.[124] An integrated metabolomics and proteomics analysis of septic patients' plasma revealed a dysregulation of amino acid metabolism related to inflammation and immunity.[125] For example, tryptophan degradation and kynurenine generation via the indoleamine-2,3-dioxygenase pathway is a driver of sepsis-associated immunosuppression, and is associated with increased T-cell apoptosis, decreased T-cell proliferation, and generation of T-reg cells.[126] Furthermore, metabolomic profiling of neutrophils of patients with sepsis revealed that inhibition of glycolysis contributed to the immunosuppressed cellular phenotype.[127] In addition, leukocytes from patients with sepsis exhibit generalised metabolic defects at the level of both glycolysis and oxidative metabolism, which are restored in recovered patients and when exposed to ex vivo treatment with IFN-γ or IL-7.[29,128] Finally, recent work has shown that metabolomics might have value in identifying subphenotypes of sepsis-induced acute respiratory distress syndrome.[129]

Cytomics

The limitations of traditional flow cytometry, related to resolution and number of fluorescence channels, do not

apply to two novel cytomics technologies: mass cytometry by time of flight (CyTOF)[130] and spectral cytometry.[131] Both technologies enable simultaneous analysis of more than 40 markers. In sepsis, CyTOF identified shifts in B-cell subpopulations and novel subsets of myeloid cells.[132] Furthermore, a barcoding method for standardised immunophenotyping to derive immune trajectories in critically ill patients has recently been reported.[133]

Emerging tools to rapidly profile immune status in sepsis

Although omics-based technologies can help to identify immune profiles relevant to sepsis diagnosis and prognosis, their application in clinical practice remains difficult given their complexity, labour intensity, and lack of standardisation. Emerging technologies have the potential to solve this problem. For example, new reverse transcription amplification technologies allow profiling of gene expression signatures within minutes.[134,135] Such methods have already shown value for rapid diagnosis of sepsis.[134,136] Furthermore, an automated multiplex quantitative PCR of several targeted genes on whole-blood samples (results within 60 min) identified patients with sepsis with low mHLA-DR expression. The same technology was recently used to calculate a transcriptomic score that identified patients at risk of ICU-acquired infections.[137] New microfluidics-based platforms are able to quantify immunological and endothelial proteins in a multiplex format in less than 2 h.[138] Finally, biosensors are emerging to rapidly profile host response proteins, cell-surface markers, and even functional parameters such as neutrophil motility.[139,140]

Machine learning and artificial intelligence

An intuitive step towards establishing precision medicine in sepsis is the integration of various types of information, including immunological, clinical, microbiological, and high-throughput omics data in meaningful combinations—so-called combitypes. ML and AI algorithms have the potential to facilitate implementation of combinatorial strategies by assimilating large datasets to learn key patterns that could deliver timely and accurate information on the status of a patient and guide treatment decisions.[141]

Examples of AI approaches that have been used in sepsis research include a natural language processor-enabled AI algorithm that was trained on clinicians' free-text and electronic medical records for the early detection and diagnosis of sepsis.[142] Additionally, unsupervised k-means clustering (a type of ML) was used to derive clinical subphenotypes of sepsis (α, β, γ, and δ),[8] and another AI method with training on a 29-gene signature was able to discriminate acute bacterial and viral infections.[143] Although most studies have focused on different data modalities in isolation, combinatorial strategies using ML-based and AI-based methods deserve more attention, given evidence for their efficacy. For example, a ML algorithm was able to classify patients with all-cause sepsis as blood transcriptomic endotypes (Mars1–4), which when combined with Acute Physiology and Chronic Health Evaluation (APACHE) IV scores improved on mortality prediction compared with APACHE IV scores in isolation.[144] A retrospective analysis of the PROWESS study (testing recombinant human activated protein C) reported that patient assignment to α, β, γ, and δ subphenotypes in the context of the treatment effect was significantly modified by integrating clinical microbiological data in a host–pathogen model versus host-only model.[145] Recently, a ML approach that analysed blood transcriptional profiles and metagenomic data identified a multiomic signature that accurately distinguished sepsis from non-infectious critical illness.[146] Ultimately, combitypes need to be integrated into workable algorithms or decision trees that can be used effectively by clinicians. One example is the paediatric PERSEVERE model, which combines soluble markers of immune and endothelial responses, platelet counts, and mRNA expression to generate a prediction model and serve as a prognostic enrichment tool.[147–150]

Another emerging and futuristic data-driven approach is the creation of virtual replicas of a patient's physiology. This concept, known as digital twinning, can facilitate an even more refined personalised treatment plan based on a patient's unique physiological characteristics.[151–153] In theory, an AI clinician[154] could use data from a patient's digital twin to identify the most effective treatment strategies, considering their individual response to different medications and interventions, but the approach needs to be prospectively assessed. Panel 1 shows examples of emerging applications of immunological profiling in sepsis.[65,98,104,134,146,149,155–165]

Towards precision medicine in sepsis

Precision medicine is a new concept that embraces individual patient characteristics in clinical decision making, thus abandoning a one-size-fits-all approach.[166,167] This personalised approach was first conceptualised in oncology and has become standard of care for the treatment of many cancers.[168] For precision medicine to succeed, population-scale heterogeneity needs to be reduced by applying an enrichment strategy.[167] The two main approaches, which are not mutually exclusive, are prognostic and predictive enrichment. In prognostic enrichment, a subgroup of patients who are more likely to meet clinically defined endpoints or outcomes (eg, mortality) is selected from a larger, heterogeneous patient population.[169,170] In predictive enrichment, a subgroup of patients who are more likely to respond to a specific biologically driven therapeutic intervention is selected from a diverse

Panel 1: **Applications of immunological profiling in sepsis**

Predicting risk of sepsis

- A profoundly altered peripheral adaptive immune compartment after critical injury is a potential biomarker to identify individuals at a high risk of developing sepsis (measured by flow cytometry in peripheral blood mononuclear cells).[155]

Identifying pre-sepsis or facilitating early diagnosis of sepsis

- Host biomarker signatures might be able to identify postoperative infection or sepsis up to 3 days in advance of clinical recognition.[156]
- Distinct immune signatures precede the onset of severe sepsis and lethality, providing a method for early triage of patients with sepsis.[104]

Combining strategies to improve diagnosis and severity stratification

- Combining host response and microbial signatures improves sepsis diagnosis.[146]
- Combining transcriptomic, lipidomic, and targeted proteomics facilitates early detection of neonatal sepsis.[157]
- Combining gene expression in leukocytes and inflammatory mediators improves infection diagnosis (SeptiCyte LAB plus C-reactive protein).[158]
- Combining gene expression and protein quantification improves severity stratification (PERSEVERE-XP).[149]

Adding immune signatures to clinical scores to improve outcome prediction

- A 29-mRNA host response whole-blood signature added to the quick Sepsis Related Organ Failure Assessment (qSOFA) improves mortality prediction.[159]

Improving differential diagnosis of sepsis of bacterial or viral origin using rapid transcriptomic tests in whole blood

- The host response bacterial or viral (HR-B/V) host gene expression test rapidly and accurately discriminates bacterial and viral infection (better than procalcitonin).[160]
- The TriVerity test uses two algorithms (IMX-BVN and IMX-SEV) to produce three separate scores that determine the likelihood of bacterial infection, viral infection, and requirement for organ supportive therapy.[134,161]

Using leukocyte transcriptomics to improve diagnosis of fungal infections

- Transcriptional analysis of circulating leukocytes differentiates candidaemia from viral and bacterial infection.[162]

Understanding interactions between the immune response and the coagulation system

- Prolonged prothrombin time is associated with stronger anomalies in pathways implicated in the pathogenesis of sepsis, suggesting that activation of coagulation affects other host response mechanisms.[163]
- Advanced computational techniques used on transcriptomic datasets identify inflammopathic, adaptive, and coagulopathic clusters.[98]

Profiling endotypes to predict response to steroids

- In a secondary analysis of the VANISH randomised trial, the immunocompetent SRS2 endotype was associated with significantly higher mortality when treated with corticosteroids compared with placebo.[164]

Understanding implications of sepsis-associated immunosuppression for antibiotic stewardship

- The upcoming RISC-sepsis trial will provide insights into the impact of sepsis-associated immunosuppression on a biomarker-guided antibiotic duration intervention. The primary outcome measures are monocyte HLA-DR; neutrophil CD88; programmed cell death 1 on monocytes, neutrophils, and T lymphocytes; and the percentage of regulatory T cells.[165]

Understanding long-term consequences of sepsis

- In a study that profiled long-term host immune response trajectories,[65] persistent elevation of inflammation and immunosuppression biomarkers occurred in two-thirds of patients who survived hospitalisation for sepsis and was associated with worse long-term outcomes.

population.[169,170] In contrast to oncology, enrichment strategies in sepsis, particularly predictive enrichment, are challenging because of the highly dynamic nature of sepsis and incomplete knowledge of the pathophysiological mechanisms of a given sepsis phenotype. There is consensus among researchers and clinicians that successful application of precision medicine in sepsis requires a balanced application of both prognostic and predictive enrichment strategies.[170,171] Although limited, we present an overview of clinical studies evaluating adjunctive immunotherapy in sepsis using precision medicine approaches. Unlike the numerous failed trials of immunomodulatory therapies in unselected patients with sepsis, these studies have produced some promising results.

Anti-inflammatory treatments

Methylprednisolone was investigated in a multicentre, double-blind randomised controlled trial (RCT) performed in patients with sepsis due to severe community-acquired pneumonia and pronounced inflammation (CRP >150 mg/L at admission).[172] The methylprednisolone treatment group exhibited significantly less late treatment failure and a reduced mortality trend. These results are in line with those of a recent multicentre prospective cohort study, in which glucocorticoids significantly reduced 30-day mortality in the subgroup of patients with septic shock or requiring mechanical ventilation and with a CRP of more than 150 mg/L.[173]

The effect of the anti-TNF monoclonal antibody afelimomab on survival was studied in the RAMSES

RCT, which was conducted in patients with sepsis who had elevated serum IL-6 concentrations (>1000 pg/mL).[174] Although high IL-6 concentration identified a sepsis subgroup with higher mortality, the study was terminated early because the modest mortality reduction in the afelimomab-treated group was unlikely to achieve statistical significance, even with more enrolled patients. A few years later, in the larger MONARCS RCT, which used the same IL-6-based stratification, treatment with afelimomab showed a covariate-adjusted 5·8% reduction in the risk of death.[175] Furthermore, afelimomab significantly reduced circulating TNF and IL-6 concentrations, and rapidly improved organ failure scores compared with placebo.[175]

Anakinra is a recombinant, engineered variant of the IL-1 receptor antagonist, blocking activity of both IL-1α and IL-1β. Two previous phase 3 RCTs showed no efficacy of anakinra treatment in an unstratified patient population with severe sepsis.[176,177] A reanalysis of the first trial showed a mortality benefit of anakinra in patients with an initial plasma IL-1 receptor antagonist concentration above an empirical threshold of 2071 pg/mL.[178] Moreover, a subgroup analysis of the second trial reported an absolute 30% reduction of 28-day mortality in anakinra-treated patients with MALS (identified by hepatobiliary dysfunction and disseminated intravascular coagulation) who were treated with anakinra.[179] The PROVIDE pilot study stratified patients with septic shock into three endotypes based on circulating ferritin concentrations and mHLA-DR.[180] Patients with septic shock and MALS (ferritin >4420 ng/mL) or immunosuppression (mHLA-DR expression <5000 mAb per cell) were randomly assigned to receive placebo (n=21) or personalised immunotherapy (n=15). Anakinra treatment in patients with MALS was associated with improved Sequential Organ Failure Assessment scores, absolute lymphocyte counts, and international normalised ratio during the first week, but no improvement in survival by day 28.[180]

Immunostimulatory therapies

The use of IFN-γ to restore immune function in sepsis was first reported in 1997, in a case series of nine patients with sepsis with low mHLA-DR expression.[23] IFN-γ restored mHLA-DR expression, enhanced TNF secretion by ex vivo monocytes stimulated with lipopolysaccharide, and resulted in clearance of sepsis in eight patients. Several case reports and series showing similar results in patients with sepsis with signs of overt immunosuppression were published in the following decades,[181–183] the most recent example being the use of IFN-γ in five critically ill patients with COVID-19 who had impaired cellular immunity.[184] So far, the aforementioned PROVIDE pilot study [180] on personalised immunotherapy has been the only RCT to evaluate the effects of IFN-γ therapy in sepsis-associated immunosuppression. However, only two patients with mHLA-DR expression <5000 mAb per cell were included in this study, so no conclusions can be drawn.

An RCT with a small sample size (n=38) showed promising results for granulocyte-macrophage colony-stimulating factor (GM-CSF) in restoring mHLA-DR in patients with persisting sepsis-associated immunosuppression (mHLA-DR <8000 mAb per cell).[185] GM-CSF treatment was also associated with reduced duration of mechanical ventilation, although the study was not powered for this clinical endpoint.[185] A double-blind RCT in patients who were admitted to the ICU with mHLA-DR expression <10 000 mAb per cell after surgery showed that treatment with GM-CSF was safe and effective in restoring mHLA-DR, and reduced the duration of infection.[186] Recently, the GRID study failed to show a beneficial effect of GM-CSF in terms of the prevention of ICU-acquired infections. However, the study was underpowered due to premature termination of the trial.[187]

IL-7 is a non-redundant potent cytokine involved in T-cell development, survival, and proliferation. As such, recombinant human IL-7 has primarily been developed to treat lymphopenia-associated disorders,[188–190] but is currently under investigation in sepsis and COVID-19. Importantly, all published clinical reports of recombinant human IL-7 use (phase 2 RCT and clinical cases) were guided by an absolute lymphocyte count of 900 cells per µL or lower.[191–195] Preliminary results indicated that recombinant human IL-7 enhances lymphocyte counts in patients with sepsis in the absence of severe side-effects.[191–195]

Immune checkpoint inhibitors targeting PD-1, PD-L1, and CTLA-4, among others, are revolutionising cancer treatments. A double-blind, phase 1b RCT evaluated the anti-PD-1 treatment nivolumab in adult patients with sepsis stratified by absolute lymphocyte count (≤1100 cells per µL). The therapy was safe, without signs of a cytokine storm, and was associated with the restoration of mHLA-DR.[196] Similarly, anti-PD-L1 therapy was well tolerated in a small group of patients with sepsis with absolute lymphocyte counts of 1100 or fewer cells per µL, with no evidence of drug-induced hypercytokinaemia.[197] Furthermore, at higher doses, anti-PD-L1 treatment increased mHLA-DR expression, an effect that persisted beyond 28 days. In two case reports, nivolumab combined with IFN-γ conferred a clinical benefit in patients who were immunosuppressed in the ICU with invasive bacterial and fungal infections. This result was based on the monitoring of PD-1 expression on T cells and mHLA-DR.[198,199] In the appendix (p 2), we discuss additional biomarker-guided immunoadjuvant treatments using extracorporeal therapy and immunoglobulin supplementation.

Lessons learnt from COVID-19

COVID-19, in its most severe form, fulfils the diagnostic criteria for sepsis; however, it is far less heterogeneous

than classic sepsis due to pathogen cause and focus. The treatment strategies tested in COVID-19 have resurrected the notion that anti-inflammatory therapies could improve survival in sepsis,[200–202] especially when applied to selected patients. For instance, in the SAVE-MORE trial, enrolment was guided by a biomarker (suPAR) that acts as a danger-associated molecular pattern.[203] In that enriched patient population, treatment with anakinra improved survival.[203] Furthermore, both dexamethasone and the IL-6 receptor antagonist tocilizumab were found to be most effective in patients with high CRP concentrations,[204,205] and there was evidence of harm from tocilizumab in patients with low CRP.[205] The results obtained with IL-6 receptor antagonists and Janus kinase inhibitors have renewed interest in these therapies for biomarker-guided treatment of patients with sepsis without COVID-19 who have acute lung injury and hypercytokinaemia.[206,207] The fact that the general COVID-19 patient population is relatively homogeneous is a possible reason why the above therapies were frequently effective in unselected COVID-19 patient populations. This observation might underscore the promise of targeted application of immunomodulatory therapies in classic sepsis, following characterisation of immune status. Nonetheless, an important consideration for future intervention studies involving anti-inflammatory drugs in bacterial sepsis is the potential impact on the resolution of ongoing infections and the risk of developing secondary infections. In this respect, a low risk of protracted and secondary infections might also contribute to the beneficial effects of anti-inflammatory therapies in severe COVID-19.[200,201] For example, patients with COVID-19 have less pronounced immune suppression (measured by mHLA-DR expression) compared with patients with bacterial sepsis.[208] Additionally, peak viral shedding typically occurs early in the course of COVID-19,[209] whereas anti-inflammatory agents were shown to improve outcomes in hospitalised patients who progressed to severe disease.[200,202,210] Unlike influenza, in which high-dose corticosteroids increase mortality[211] and bacterial superinfections are common and contribute to poor clinical outcomes, bacterial co-infection on admission is rare in COVID-19.[212–214] As a result, the potential negative effect on pathogen clearance from the use of anti-inflammatory drugs in studies of severe and critical COVID-19 might have been minimal. These considerations underscore the potential value of the combitypes in future studies investigating anti-inflammatory agents in bacterial sepsis. Using such an approach, patients could be characterised according to their immunological endotype, enriched by clinical and microbiological features. The pandemic has also highlighted the importance of sex-based differences in the dysregulated immune response to infection, as men with COVID-19 are at a higher risk of worse outcomes,[215] which underscores the need to consider sex as an important factor in sepsis-immunology research.

Challenges and opportunities for progress

In this Review, we describe the status of profiling of the dysregulated host response in sepsis and consider future clinical perspectives. Although targeted immunotherapies have the potential to transform care for patients with sepsis, current challenges include the following: (1) poor performance of routinely available biomarkers; (2) limited access in routine clinical care to more specific immune-related biomarkers; (3) uncertainty about when and how often immune biomarkers should be measured; (4) absence of direct therapeutic implications; and (5) failure of single biomarkers to profile the full complexity of the dysregulated host response. In the paragraphs below, we discuss these challenges and identify steps that could be taken to create opportunities for progress in the care of patients with sepsis.

First, routinely used biomarkers—including CRP, procalcitonin, and lymphocyte counts—do not reflect the functionality of the immune system. These markers have no specificity and are insufficient to guide individual treatments or to monitor effects of immunotherapy. Nevertheless, they have shown value in enrichment strategies in preliminary RCTs that have evaluated immunoadjuvant therapies in sepsis and COVID-19. Increased use of these markers in clinical practice could expand clinicians' empirical knowledge of the dysregulated immune responses in patients with sepsis and spark new ideas about how to use these (and related) biomarkers to improve patient outcomes. Additionally, increased use in clinical practice would increase the possibility of conducting large-scale sepsis studies based on retrospective data, considering at least some form of immunological profiling.

Second, this Review also highlights the potential of more specific markers (eg, mHLA-DR, viral reactivation, multicytokine panels, or functional tests) to distinguish patients with overriding hyperinflammation from those with persisting immunosuppression. These markers, primarily available in specialised and large centres, are increasingly used to guide decisions about individual patient treatments and as enrichment strategies in clinical trials. For example, detection of immunosuppression can facilitate more rapid identification of secondary infections and viral reactivation through increased preparedness for specific nosocomial or opportunistic pathogens. This knowledge of immunosuppression should further motivate clinicians to combine immunological markers with therapeutic drug monitoring, because it is crucial to ensure that appropriate antibiotic administration is provided for patients whose immune responses fail to efficiently resolve infections. With ongoing technical improvements and the development of point-of-care devices, we

anticipate that such immune monitoring markers will become broadly available and integrated into routine care within the next decade.

Third, establishing the optimal timing of immune monitoring is challenging because sepsis is highly dynamic. Consequently, immune monitoring should start on the first day of ICU admission to identify patients who might benefit most from early interventions (eg, targeting hyperinflammation). In addition, to distinguish between homoeostatic physiological responses and pathogenic immunosuppression, markers related to sepsis-induced immunoregulatory mechanisms should not be interpreted on the basis of a single measurement obtained at ICU admission. Instead, repeated measurements over time or assessment of levels after a few days of ICU admission could reveal persisting immunosuppression and help to identify patients who fail to recover or return to immune homoeostasis.

Fourth, a major reason why immune-related biomarkers are used infrequently is the lack of high-grade evidence of the efficacy of biomarker-guided immunoadjuvant treatments in sepsis. As a result, there are no internationally defined guidelines for biomarker use in patients with sepsis. However, several small or retrospective studies in sepsis and COVID-19 suggest that a biomarker-driven approach is feasible and associated with beneficial outcomes. COVID-19 studies have shown that anti-inflammatory therapies can improve outcomes in subgroups of patients with hyperinflammation. However, there are significant disparities between severe COVID-19 and bacterial

***Panel 2*: Recommendations for research**

Pathophysiology

Improve understanding of the immune response during sepsis using systems immunology:

- Local versus systemic responses: identify shared or specific immunological alterations leading to the failure of specific organs in sepsis, and identify direct or indirect markers of the dysregulated immune response in different body compartments
- Pathogen-specific responses: develop tests to assess specific immunological responses against different types of infecting microbe, to assess the likelihood that a patient can clear the infection (immunobiogram)
- Delineate subpopulation-specific responses, such as differences due to age (eg, children or older patients), sex or gender, or immune status (eg, immunocompromised patients)
- Compare with and learn from immune responses in non-infectious conditions
- Describe evolution over time, including long-term consequences

Basic immune monitoring

Current standardised tests profile mostly non-specific markers of systemic inflammation, which are also elevated in other non-infectious conditions. Make better use of already available or basic markers:

- Use repeated measurements of these markers because features of immune dysfunction change over the course of sepsis
- Define international guidelines for the list of optimal immunological markers to be followed in patients and the measurement kinetics required
- Develop a list of markers that should be provided systematically in observational or clinical studies focused on the immune response in sepsis (minimal reported information)
- To achieve this goal, develop and support a large collaborative effort to standardise measurements of cellular and soluble biomarkers of the dysregulated immune response in sepsis to allow comparisons between studies, going beyond enumeration to also address functionality
- Use microbiology data more extensively because the presence of certain pathogens is associated with impaired immunity, and the persistence of positive results or a high microbial burden in blood or other samples serves as an indirect marker of impaired immunity in sepsis

New (combinations of) markers

Studies of the effects of endotypes derived from omics analysis combined with routinely assessed immunological biomarkers on outcome and response to treatment are warranted. In an effort to standardise omics technologies to transfer them from research laboratories to routine laboratories, we recommend the following:

- Identify consensual panels
- Perform cross-validation
- Transfer panels to simpler and standardisable assays or technologies
- Combine these markers with other parameters (microbiological data or clinical and demographic data) to generate combitypes
- Provide clinical validation of newly developed panels or combitypes
- Use validated panels or combitypes to create simple workable algorithms that can guide clinical decisions or patient enrichment in trials

Precision medicine

Provide the rationale for a precision medicine approach in sepsis by performing biomarker-guided randomised clinical trials with a high likelihood of a positive outcome:

- Define the appropriate design, target population, endpoint, treatment, marker or panel, combitype, and measurement techniques
- Show the safety of this approach (monitoring of side-effects)
- Show efficacy on clinically relevant endpoints

sepsis regarding the risk of impaired pathogen clearance when using anti-inflammatory agents. Therefore, future stratification strategies in patients with sepsis without COVID-19 should be based on simultaneous evidence of a hyperinflammatory phenotype and assessment of clinical and microbiological features. The clinical and microbiological features would be indicative of the presence of infections that are or are not amenable to effective antimicrobial treatment. In other words, anti-inflammatory treatments should be withheld when the causative pathogen is known to cause complicated infections. In this context, the recently published CAP-COD trial provides valuable insights, as it showed that low-dose hydrocortisone improved survival in patients with severe community-acquired pneumonia that was predominantly caused by *Streptococcus pneumoniae,* an easy-to-treat pathogen.[3] Recent findings further support the important role of microbial cause in treatment responses and the derivation of subphenotypes of sepsis.[9,145] The ongoing IMMUNOSEP (NCT04990232) and IGNORANT (NCT05843786) trials will provide more information about the efficacy of biomarker-guided immunoadjuvant treatment.

Search strategy and selection criteria

Literature for this Review was gathered through PubMed searches using a combination of MeSH terms and free-text keywords, including the terms "sepsis", "septic shock", or "covid-19" in conjunction with at least one of the following terms: "pathophysiology", "risk factors", "immune response", "biomarker", "biomarkers", "precision medicine", "phenotypes", "endotypes", "immune monitoring", "immune status", "immune dysregulation", "immunity", "immunological", "artificial intelligence", "immunoadjuvant", and "anti-inflammatory agents". Filters were applied to include human studies, articles with full text available in English, and specific article types (clinical studies, reviews, and meta-analyses) published from Jan 1, 2000, to Aug 31, 2023. Preprint publications were excluded. The choice of topic-specific search terms and selection of articles for consideration and citation were determined by the authors responsible for each section to allow wide scrutiny of the literature. In a first step, selected articles in each section were approved for inclusion by the authors responsible for the section, but final inclusion required approval by all authors. Inclusion criteria for articles consisted of selection of leading articles with relevance to the aims of this Review and their contribution to a comprehensive understanding of the topic and a balanced view of the field. The reference lists of identified articles were also reviewed to ensure a thorough search, and relevant articles from those lists were included if deemed to be appropriate. Any potential conflicts of interest were managed (ie, all authors approved the final list) to maintain objectivity in our review of the literature.

Fifth, considering the complexity and diversity of mechanisms involved in immunological dysregulation in sepsis, combinations of multiple immune and non-immune-related parameters such as clinical information and microbiology data are key in realising the potential of precision medicine. The use of such combitypes will be facilitated by the rapidly evolving development of high-throughput omics-based techniques and advanced point-of-care tests that measure multiple (panels of) biomarkers simultaneously and are subjected to ML or AI analytical scrutiny. In the final operationalisation step, the most precise combitypes would be integrated into simple workable algorithms to guide complex clinical decisions. Considering the challenges outlined in this Review, panel 2 delineates our specific recommendations for future research.

Conclusion

Accurate profiling of the dysregulated host response will be an essential step in the optimisation of care for patients with sepsis. Several candidate biomarkers have shown potential for patient enrichment in trials investigating immunomodulatory therapies. Nevertheless, additional approaches to assess immune status that are combinatorial in nature need to be developed or refined for clinical operationalisation. A multifaceted research effort could pave the way for improved disease conceptualisation, sepsis diagnosis, disease prediction, patient enrichment, and therapeutic decision making. Ultimately, refined clinical trials that confirm the clinical benefits of biomarker-guided therapeutic strategies will be needed to achieve the widely desired goal of personalised medicine in sepsis.

Contributors

Each author co-wrote at least one section of this paper as part of a panel composed of three or more authors who were selected based on their expertise (biochemistry, immunology, critical care, respiratory medicine, or infectious diseases). The panels (and their corresponding sections) were as follows: SC, FV, SBF, IR, MSW, GL, TS, BPS, MAW, AT, JFB-M, SC, MO, MAW, and IR (Immunopathophysiology of sepsis); FV, JCS, AC, MSW, GL, TS, BPS, MG, AG-S, EJG-B, MAW, and GM (Biomarkers to profile immune status in sepsis); MK, JFB-M, BPS, IM-L, RAM, TS, MAW, MB, MS-H, and J-MC (Broad profiling of immune status in sepsis); FV, MK, JCS, GL, BPS, MG, MO, AG-S, EJG-B, GM, and FMB (Towards precision medicine in sepsis); FV, SC, MK, and JFB-M (Lessons learnt from COVID-19). All authors also served as internal reviewers for other sections. JFB-M, RAM, and FV designed and created the figures. SC, MK, JFB-M, JCS, and FV wrote the abstract, Key messages, Introduction, Conclusion, and the section on Challenges and opportunities for progress. SC, MK, JFB-M, JCS, and FV compiled all sections, and provided final editing of the manuscript. MK and SC managed the reference list, and SC and FV coordinated work on the paper. All authors participated in literature searches and critical analysis of published data. All authors read and approved the final manuscript. All authors are members of the European Group on Immunology of Sepsis.

Declaration of interests

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