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Review Article

Precision Medicine and Enrichment in Sepsis

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Abstract. Sepsis is defined as a dysregulated host response to infection leading to life-threatening organ dysfunction. While this recent iteration of the sepsis definition rightly centralizes organ dysfunction, it does not reflect on the extensive heterogeneity in the host response observed in sepsis patient populations. Heterogeneity in sepsis has hindered the identification of effective therapeutic targets, with current treatment consisting of antimicrobials and supportive care. In order to address the shortcomings in identifying specific therapeutics for sepsis, the focus of various research activities turned towards developing precision medicine approaches. In particular, efforts aimed at stratifying patients into more homogenous subgroups having common dominant pathophysiological features and outcome trajectories, in turn facilitating the delineation of specific therapies. Here, I review current initiatives in prognostic and predictive enrichment strategies in sepsis patient populations, which will be key to identify patients who would benefit, or be harmed, from specific therapeutic interventions.

Keywords: Sepsis, Stratification, Precision, Treatment, Intensive care

1 Introduction

The word "sepsis" was derived from the ancient Greek word "sepo" (σήπω), meaning "I rot". Around 400 BC, Hippocrates described sepsis as the process of hazardous biological decay that could happen in the human body. In the 19th century, with the discovery of microorganisms as causal agents of infection, sepsis was described as a condition associated with severe infections. Since then, the terminology and definitions of sepsis have gone through various iterations. It is a clinical syndrome, not a disease (Vincent et al., 2013), currently defined by consensus as "life-threatening organ dysfunction caused by a dysregulated host response to infection" (Singer et al., 2016). A severe complication of sepsis, that is septic shock, is characterized by vascular hypotension, critical tissue perfusion aberrations and major organ failure, with in-hospital mortality rates reaching as high as 50% (Angus et al., 2013; Hotchkiss et al., 2016). There is no archetypal sepsis patient as it hits individuals indiscriminately across age groups, genders, races, ethnicities or geographical locations. Respiratory or intra-abdominal infections are major determinants of sepsis in elderly and neonatal populations (Rudd et al., 2020). In 2017, sepsis accounted for an estimated 11 million deaths worldwide, equating to age-standardized mortality rates of 148 per 100,000 population (Rudd et al., 2020). The incidence of all-cause sepsis in Malta was estimated at 1349 individuals, responsible for approximately 272 deaths in 2017 (Rudd et al., 2020). While age-standardized incidence and mortality have declined between 1990 and 2017 (Rudd et al., 2020), mainly attributable to advances in antimicrobial therapy and supportive care (Prescott et al., 2018), the incidence of sepsis remained stable and survivors continue to suffer from additional morbidities and poor outcomes (Iwashyna et al., 2010; Shankar-Hari et al., 2016). About 50% of patients who survive sepsis are re-admitted to hospital at least once within the first year, and approximately one-third die (Prescott et al., 2018). The alarming incidence and mortality rates prompted the World Health Organization to adopt a resolution recognizing sepsis as a global health priority (Reinhart et al., 2017). It is expected that sepsis will remain a global problem due to a combination of factors, including a progressively ageing population, surgical interventions, potent immunosuppressive drugs, antimicrobial resistance and emergence of viruses with pandemic potential. Despite remarkable advances in our understanding of the sepsis pathophysiology, par-

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ticularly immunopathological aspects (van der Poll et al., 2017), no specific drug that substantially mitigates poor outcomes has been approved. Numerous clinical trials targeting components of the host response were unsuccessful (Marshall, 2014). Those shortcomings have been ascribed to extensive heterogeneity of the sepsis syndrome, which hinders the identification of patients who would benefit, or be harmed, by specific therapeutic adjuvants, thus motivating current attempts to establish a precision medicine strategy to sepsis diagnosis and treatment. Throughout the last two decades, considerable progress has been made in the stratification of sepsis patients as subgroups by means of host response parameters. Such strategies have been proposed as potentially critical tools to improve on therapies that target specific pathophysiological mechanisms (Marshall, 2014; Stanski et al., 2020). Here, I outline the efforts that have been made to resolve the heterogeneity of sepsis, using either clinical parameters or genomics data, or combinations thereof.

2 Precision Medicine

The term precision medicine refers to the concept that fundamentally moves diagnosis, prognosis and treatment strategies away from the "one-size-fits-all" mindset, that is taking into consideration individual patient characteristics (Collins et al., 2015; Wong, 2017). This personalized approach was conceptualized in the field of oncology, which has advanced greatly in comparison to sepsis. Technological innovations in genomics, transcriptomics, proteomics, epigenomics and immune profiling have enabled the identification of tumour molecular markers that can be targeted with tailored therapeutic agents. For example, the Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT) studies (Tsimberidou et al., 2012; Tsimberidou et al., 2014). Targeted molecular therapies that include immune checkpoint blockade inhibitors and anti-programmed cell death (PD)-1 have anti-tumour activity against numerous types, including melanoma, lung, breast and bladder cancers, however only 10%–30% of patients benefit from these immunotherapeutic agents (Larkin et al., 2015). Hence, development of molecular biomarkers to identify those patients who would benefit from tailored treatment strategies are key to establishing precision medicine approaches (National Academies Press (US), 2016). In recognition of important advances in cancer treatment facilitated by making use of precision medicine methods, the federal government of the United States launched the "Precision Medicine Initiative" in 2015 (White House, 2015). The Malta National Cancer Plan, launched by the Ministry for Health in 2021, also recognized the importance of developing precision medicine to aid in cancer treatment strategies (Ministry of Health, 2021). Against this backdrop, the natural next step would be the application of precision medicine approaches to other heterogeneous diseases and syndromes, such as sepsis. To this end, a currently ongoing proof-of-concept "Personalized Immunotherapy in Sepsis: a Multicentre and Multinational, Double-blind, Double-dummy Randomized Clinical Trial" (IMMUNOSEP; ClinicalTrials.gov identifier: NCT04990232), coordinated by the Hellenic Institute for the Study of Sepsis, seeks to provide benchmark evidence for the application of precision medicine principles in the field of critical illness due to sepsis. The concept of "enrichment" is a key principle of precision medicine (Wong, 2017), which refers to reducing population level heterogeneity. It is subcategorized as either prognostic or predictive enrichment of patient populations (figure 1). Prognostic enrichment describes selection of a subgroup of patients who are more likely to meet relevant outcomes or clinical endpoints, for example mortality (Prescott et al., 2016; Stanski et al., 2020). An example of prognostic enrichment is the successful "Effect of Prone Positioning on Mortality in Patients With Severe and Persistent Acute Respiratory Distress Syndrome (ARDS)" trial (PROSEVA; ClinicalTrials.gov Identifier: NCT00527813) (Guérin et al., 2013). The PROSEVA trial of proning therapy enrolled only those patients with an arterial to inspired oxygen ratio (PaO_2/FiO_2) less than 150 mmHg, hence enriching the trial for patients with moderate-to-severe ARDS. The trial showed that prolonged prone-positioning significantly increased survival in patients with severe ARDS (Guérin et al., 2013). Predictive enrichment refers to the selection of a subgroup of patients who are more likely to respond favorably to a given treatment targeting a specific biological mechanism relative to unselected patients (Prescott et al., 2016; Stanski et al., 2020). A typical example of predictive enrichment is shown in the successful use of trastuzumab, a recombinant monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2), in patients with HER2-positive breast cancer in the Herceptin Adjuvant (HERA) trial (Piccart-Gebhart et al., 2005). In the context of sepsis, predictive enrichment is challenging due to relatively limited knowledge of the dominant pathobiological mechanisms driving sepsis. In general, there is consensus among researchers and clinicians that to successfully establish precision medicine in sepsis necessitates simultaneous prognostic and predictive enrichment (Shankar-Hari et al., 2019; Stanski et al., 2020). In order to move towards this goal, it is crucial to gain a deeper understanding of the pathobiological mechanisms underlying the sepsis syndrome, which will not be achieved by considering the typical translational research model using inappropriate animal models and patient se-

lection criteria (Cavaillon et al., 2020), but by developing interdisciplinary strategies to disentangle drivers of heterogeneity in sepsis, and in turn utilizing the information to stratify patients into robust treatable subgroups.

3 Patient Stratification in Sepsis

Recent efforts to develop precision medicine strategies for sepsis have leveraged on the concepts of unsupervised clustering and machine learning to stratify sepsis patients as subgroups using several demographic, clinical and/or molecular parameters. Here, the terminology tends to be inconsistent. For clarity, patient subgroups identified by using routine clinical measurements, not necessarily reflecting a potential underlying biological mechanism, are defined as "subphenotypes", whereas "endotypes" indicate biological subtypes defined by distinct pathophysiological mechanisms. Several attempts have been made in recent years to split sepsis patient populations into subgroups, using clinical parameters and/or molecular measurements as data inputs in machine learning approaches, as well as the more traditional unsupervised clustering techniques, for example k-means or latent class analysis (DeMerle et al., 2021; Reddy et al., 2020). These initial attempts were pioneered by Hector Wong and colleagues in pediatric sepsis (Wong, 2022), who have provided benchmark evidence that prognostic and predictive enrichment strategies can unlock precision medicine in sepsis and septic shock.

3.1 Prognostic enrichment

A classic example of prognostic enrichment in sepsis is the Pediatric Sepsis Biomarker Risk Model (PERSEVERE) (Wong et al., 2012). On the basis of a discovery approach utilizing genome-wide gene expression profiling of blood leukocytes purified from pediatric septic shock patients, Wong and colleagues derived a candidate panel of 12 serum protein biomarkers for patient stratification and outcome prediction. Protein biomarkers were measured in serum samples from a cohort of 220 unselected children presenting with septic shock. Serum samples were obtained during the first 24 hours of admission to the intensive care unit (ICU). Applying Classification and Regression Tree (CART) analysis on both serum biomarkers and routinely available clinical variables a decision tree was built to predict 28-day all-cause mortality. This approach also reduced the dimensionality of serum biomarkers to a panel of 5 proteins, namely C–C chemokine ligand 3 (CCL3), Interleukin (IL)–8, granzyme B, heat shock protein (HSP) 70 kDa member 1B and matrix metallopeptidase (MMP)–8 (Wong et al., 2012). In the derivation cohort, sensitivity, specificity, negative and positive predictive values equated to 91% (95% confidence interval (CI): 70–98), 86% (95% CI: 80–90), 43% (95% CI: 29–58), and 99% (95% CI: 95–100), respectively (Wong et al., 2012). In the test (validation) cohort, sensitivity and specificity equated to 89% (95% CI: 64–98) and 64% (95% CI: 55–73), respectively. This model has been prospectively validated in other cohorts (Wong et al., 2014b), including adult septic shock patients (Wong et al., 2014a). The model progressed through additional reiterations that include thrombocyte counts to the original candidate biomarker panel (PERSEVERE-II) (Wong et al., 2016), as well as having leukocyte gene expression data included in the model (PERSEVERE-XP) (Wong, 2017). These studies provide compelling examples of the clinical utility of decision-making models built on combinations of clinical and molecular data. Another example of a combinatorial strategy in prognostic enrichment is a study that combined demographic (patient age), clinical (hematocrit, serum lactate measurements) and circulating metabolite levels, namely 4-cis-decenoylcarnitine, 2-methylbutyroylcarnitine, butyroylcarnitine and hexanoylcarnitine, obtained from patients on hospitalization for the development of a model to predict survival from sepsis (Langley et al., 2013). A support vector machine (SVM) was utilized to develop a weighted prediction model of sepsis survival, which resulted in a receiver-operator-characteristic (ROC) area-under-the-curve (AUC) of 0.819 and 0.74 in the training and validation cohort, respectively. In a community approach, a group of researchers assembled various patient cohorts with genome-wide gene expression data from whole blood leukocytes and clinical parameters, including Acute Physiology and Chronic Health Evaluation (APACHE) and Sequential Organ Failure Assessment (SOFA) scores, to the aim of identifying a gene set that is able to predict mortality due to sepsis (Sweeney et al., 2018b). The group trained four models using 12 cohorts as discovery set encompassing 485 survivors and 157 non-survivors. Model performance was tested in 9 heterogeneous validation cohorts that included 419 survivors and 52 non-survivors. Using ROC-AUCs as metrics of model performance, a joint model that included gene expression profiles and clinical indices of severity performed better than gene expression indices in isolation; albeit with extensive variability in ROC AUCs ranging from 0.537 to 1.0 in the discovery cohorts (Sweeney et al., 2018b). Other investigators combined host genetics, systemic metabolite levels and cytokine measurements in patients, to the goal of identifying a mortality predictor with pathophysiological implications (Wang et al., 2017). By also testing their findings in a mouse model, Wang and colleagues uncovered a role for the methionine salvage pathway in the pathophysiology of sepsis. High plasma concentrations

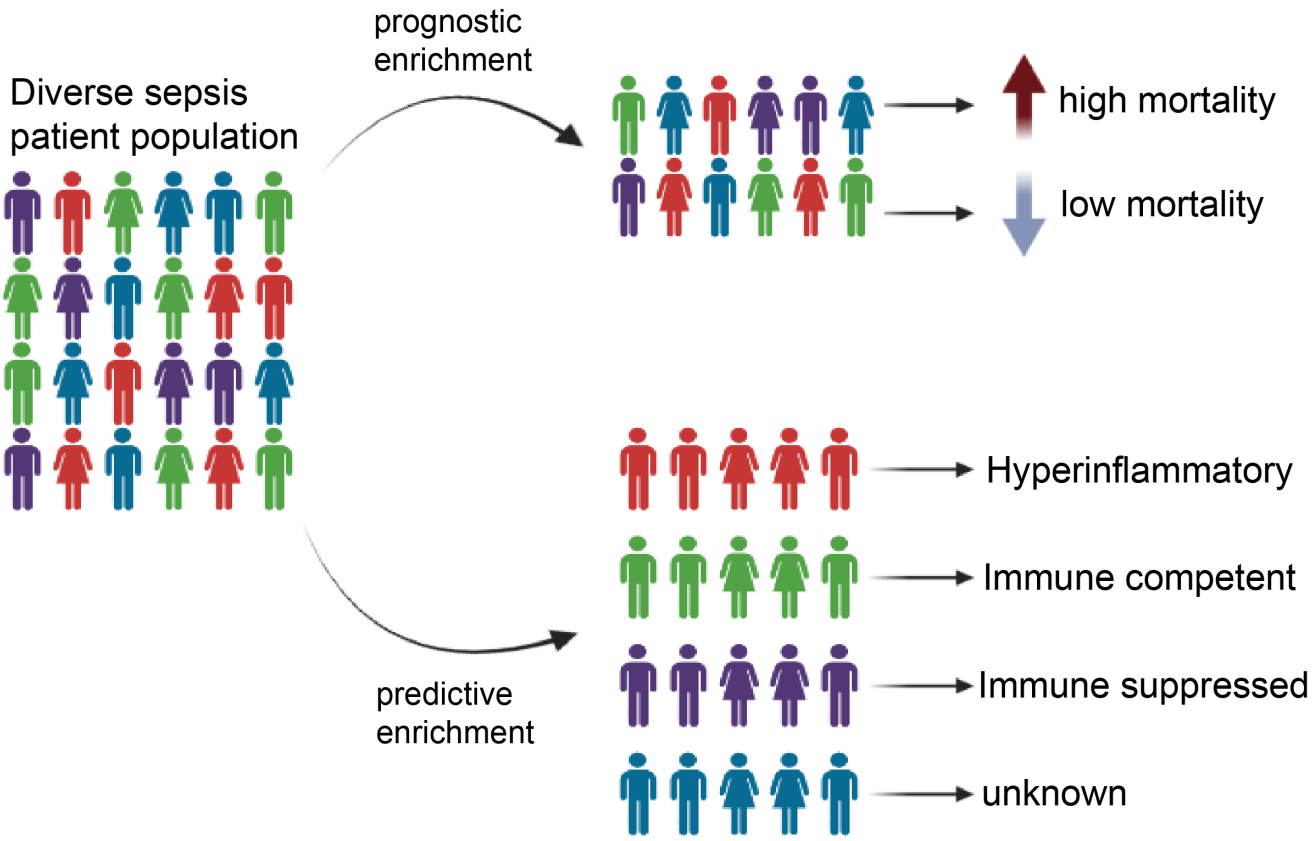

Figure 1: Illustration of prognostic and predictive enrichment of sepsis patient populations. A diverse population of critically ill patients with sepsis is analyzed in two ways: (1) prognostic enrichment to identify a subphenotype of patients at risk of adverse clinical endpoints or outcome, for example mortality, and (2) predictive enrichment to cluster patients as endotypes reflecting dominant pathobiologies amenable to specific therapeutic interventions, for example immune compromised patients treated with immune activating therapies. Both approaches to enrichment are not intended to run in isolation, but rather in combination to identify risk subgroups with underlying biological mechanisms.

of methylthioadenosine were associated with mortality in patients with sepsis and was significantly correlated with pro-inflammatory cytokine concentrations. By combining plasm levels of methylthioadenosine and other variables the investigators showed their combinatorial method could predict death due to sepsis with 80% accuracy (Wang et al., 2017). Researchers have also used readily available clinical and routine laboratory test results for prognostic enrichment. In a retrospective analysis, Seymour and colleagues identified four subphenotypes (designated α, β, ɣor δ) by utilizing data obtained from 16,552 unique patients who met Sepsis-3.0 criteria (Singer et al., 2016), within 6 hours of presentation at the emergency department in twelve Pennsylvania, USA, hospitals utilizing 29 variables in a k-means consensus clustering approach (Seymour et al., 2019). With a prevalence of 33%, the αsubphenotype was the most common and included patients with the lowest organ dysfunction and 2% mortality rate. The βsubphenotype (prevalence = 27%) had older patients with more chronic illness and renal dysfunction, as well as 5% mortality rate. The ɣsubphenotype (prevalence = 27%) included patients exhibiting hyperinflammatory patterns, higher core temperatures, more pulmonary dysfunction and 13% mortality rate. With a prevalence of 13%, the δsubphenotype was the most severe with patients presenting higher serum lactate levels, more hypotension and a mortality of 32%. When investigators considered all cohorts and trials, both 28-day and 1-year mortality rates were significantly highest among patients classified as δsubphenotype, relative to the other 3 subphenotypes (Seymour et al., 2019). Notably, Monte-Carlo simulations showed that the proportion of randomized control trials (RCTs) reporting benefit, harm, or no effect changed substantially by taking into account varying frequencies of the four subphenotypes in the study population, suggesting that α, β, ɣor δsubphenotypes may aid in better understanding the heterogeneity of treatment effects (Seymour et al., 2019). A Monte-Carlo simulation is a computational method that is used to understand the risk of a particular outcome given the presence of random variables that introduce uncertainty (Harrison, 2010). In practice, the model generates numerous results by assigning multiple values to an uncertain variable, for example treatment benefit or harm, which are subsequently averaged to obtain an estimate. Other investigators examined core temperature trajectories of sepsis patients, delineating four subphenotypes with distinct mortality risks, termed "hyperthermic, slow resolvers", "hyperthermic, fast resolvers", "normothermic" and "hypothermic" patient subphenotypes (Bhavani et al., 2019). The "hypothermic" subphenotype had the highest mortality risk concomitant with the lowest levels of inflammatory markers. Although the subphenotypes delineated by clinical parameters alone do not reflect dominant biological mechanisms, they may provide a more practical and feasible approach to risk stratification of patients with sepsis since routinely acquired clinical variables do not require advanced molecular techniques or sophisticated data analysis strategies.

3.2 Predictive enrichment

In contrast to prognostic enrichment, predictive enrichment does not utilize demographic, clinical parameters, indices of severity, and/or outcome to stratify patients into subgroups. Predictive enrichment seeks to stratify patients into biologically meaningful subgroups or "endotypes", based on unbiased computational approaches that leverage on molecular patterns representing underlying biological mechanisms. The ultimate goal of this approach is to define biomarkers that inform the attending physician on a key pathobiological feature that is potentially amenable to therapeutic intervention, that is a treatable trait (Russell et al., 2017; Scicluna et al., 2019). Genome-wide whole blood leukocyte gene expression studies are the most common strategies in predictive enrichment. So far, four clustering methods have been used in different clinical contexts to classify patients with sepsis based on whole blood leukocyte gene expression patterns, including pediatric septic shock, adult sepsis secondary to pneumonia and all-cause adult sepsis, outlined below. Hector Wong and colleagues were the first to report subgrouping of pediatric septic shock cases by means of leukocyte gene expression profiling (Wong et al., 2009; Wong et al., 2011). Patients were initially classified as either subclass (endotype) A, B or C on the basis of k-means clustering of 6,934 genes. Using a leave-one-out cross-validation strategy, a 100-gene set was derived having the highest predictive value for the delineation of the three endotypes. Evaluation of the association between endotype assignment and clinical parameters revealed patients classified as endotype A were younger, more severely sick and had higher mortality rates relative to patients classified as endotypes B or C (Wong et al., 2009). Biological pathway analysis revealed endotype A was characterized by reduced expression of genes involved in adaptive (lymphocyte) immunity and glucocorticoid receptor signaling. Notably, and in line with pathway analysis, treatment of patients assigned to endotype A with corticosteroids was associated with higher risk of mortality (Wong et al., 2015). Thus, this predictive enrichment strategy demonstrates detrimental effects of corticosteroid treatment in a proportion of septic shock patients, which lends further weight to the controversy surrounding corticosteroids being prescribed without

consideration of the underlying immune status of the patient. Moreover, this study was the first report on the potential for transcriptomic endotypes as treatable traits. Investigators from the United Kingdom enrolled adult patients with sepsis secondary to community-acquired pneumonia in the Genomic Advances in Sepsis (GAinS) study, and analyzed leukocyte gene expression data by unsupervised hierarchical clustering of the top 10% most variable genes (n=2619 genes). In doing so, two endotypes or sepsis response signatures (SRS) 1 and 2 were identified in a discovery cohort of 256 patients (Davenport et al., 2016). Patients assigned to SRS1 (prevalence = 41%) were more severely ill and at higher risk of mortality as compared to SRS2 patients (prevalence = 59%). Biological pathway analysis revealed SRS1 was characterized by genes involved in endotoxin tolerance, T cell exhaustion and reduced expression of genes linked to the major histocompatibility complex class II (Davenport et al., 2016). In a follow-up study that included patients diagnosed with sepsis due to fecal peritonitis, SRS 1 and 2 signatures were validated (Burnham et al., 2017). A seven gene signature was derived for the classification of patients as either SRS1 or 2, namely DYRK2, CCNB1IP1, TDRD9, ZAP70, ARL14EP, MDC1, and ADGRE3 (Davenport et al., 2016). Moreover, the same research group performed a post hoc analysis of a double-blind, randomized clinical trial in septic shock, that is the Vasopressin vs. Norepinephrine as Initial Therapy in Septic Shock (VANISH; Clinicaltrials.gov identifier: ISRCTN 20769191) trial. The group reported no association with vasopressor choice, however, corticosteroid treatment prescribed to patients classified as the relatively low-risk, immunocompetent endotype SRS2 was associated with increased mortality and an adjusted odds ratio of 7.9 (95% CI: 1.6–39.9) (Antcliffe et al., 2019). Therefore, the proposed interaction between steroid treatment and the relatively less-severe SRS2 endotype implies opposing effects of the same therapeutic intervention across and also within distinct patient endotypes (Scicluna et al., 2019). Replication of these findings is certainly needed, particularly because a recent re-analysis of the VANISH trial showed that steroid treatment was associated with increased mortality in adult septic shock patients classified as (pediatric) endotype A, which was shown to have similarities with immune compromised SRS1, not immune competent SRS2 (Wong et al., 2021). In the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) study, other investigators analyzed whole blood transcriptomic data (n=5000 genes) obtained from adult patients with all-cause sepsis on ICU admission, that is sepsis due to different infectious etiologies, identifying four molecular endotypes termed MARS1 to MARS4 (Scicluna et al., 2017). The MARS1 endotype was associated with poor prognosis, having high total SOFA scores, higher prevalence of septic shock (44%), and mortality rates reaching 39% after 28-day patient follow-up. The MARS3 endotype was relatively less severe, with patients having lower SOFA scores and septic shock presentation (17%), with 28-day mortality rate equating to 23% (Scicluna et al., 2017). By combining APACHE IV scores and molecular endotype assignment in an analysis of the net reclassification improvement showed this clinicomolecular model significantly improved risk prediction relative to only considering clinical risk prediction. Biological pathway analysis revealed the poor prognosis MARS1 endotype was associated with reduced innate and adaptive immune functions attuned to an immunosuppressed state, whereas gene expression profiles of the low-risk MARS3 endotype were consistent with elevated capacities for adaptive immune reactions, particularly increased lymphocyte functions (Scicluna et al., 2017). The capacity to identify immunosuppressed patients is especially appealing since these patients will not benefit from steroid treatment, but more likely to respond favorably to therapeutic interventions aimed at restoring immune function, for example Interferon-γ treatment, which has been shown to reverse immune paralysis in a small cohort of sepsis patients (Cheng et al., 2016). The MARS investigators proceeded to derive a 140-gene classifier that enabled the validation of the MARS endotypes in two additional cohorts, including the previously described GAinS cohort (Davenport et al., 2016). To facilitate translation to the clinic, MARS investigators refined their gene expression classifier to a panel of eight genes (BPGM, TAP2, GADD45A, PCGF5, AHNAK, PDCD10, IFIT5 and GLTSCR2). Comparing MARS and SRS endotype membership demonstrated significant overlap between the low-risk endotypes MARS3 and SRS2 (Davenport et al., 2016; Scicluna et al., 2017). Moreover, MARS investigators also tested their endotype classification strategy in the aforementioned pediatric septic shock cohort (Wong et al., 2009). The relatively low-risk MARS3 endotype, characterized by gene expression patterns attuned to heightened adaptive immune responses, was not reliably delineated in the pediatric sepsis cases. The investigators argued that the selection of septic shock patients in the pediatric cohort, as well as an under-developed adaptive immune system in children may explain the lack of MARS3 assignments (Scicluna et al., 2017). The complicated relationship between patient age and endotype classification is notable, which was demonstrated in a study that sought to classify adult sepsis patients to pediatric endotypes (Wong et al., 2017). These observations suggest that a unifying model across patient ages may not be feasible, but dis-

tinct classification strategies for pediatric and adult patients may be necessary. Pooling gene expression data from 14 bacterial sepsis cohorts (n=700), including pediatric and adult patients admitted to hospitals in seven countries, Sweeney and colleagues used two clustering algorithms, that is k-means clustering and Partitioning Around Medoids (PAM), identifying three transcriptomic endotypes (Sweeney et al., 2018b). The three endotypes were termed "inflammopathic", "adaptive", and "coagulopathic". Considering results of both discovery and validation sets, the "adaptive" endotype was associated with a lower clinical severity and lower mortality rate. In contrast, the "coagulopathic" endotype was associated with older age, higher mortality and coagulation dysfunction (Sweeney et al., 2018a). Similarities between classification strategies was also reported, specifically the "inflammopathic" endotype overlapped SRS1 and pediatric endotype B. The "adaptive" endotype corresponded to the SRS2 endotype. On the basis of a 33-gene classifier to assign each endotype ("inflammopathic", "adaptive", or "coagulopathic"), 97 patients with coronavirus infectious disease (COVID) 2019 (Sweeney, Timothy E. et al., 2021), the disease caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). COVID-19 patients were assigned to "inflammopathic" (29%), "adaptive" (44%), or "coagulopathic" (27%) endotypes, with similar proportions to the previous study in bacterial sepsis (Sweeney et al., 2018a). Notably, patients assigned to the "adaptive" endotype were less severe and no deaths, whereas "coagulopathic" and "inflammopathic" endotypes were more severe, having mortality rates equating to 42% and 18%, respectively.

4 Future perspectives

The application of prognostic and/or predictive enrichment strategies to sepsis patients have the potential to provide much-needed precision to diagnosis, treatment and clinical trial design. Much work is needed for the field to progress to the same extent as in oncology. An important step towards the ultimate goal of predictive and prognostic enrichment, that is risk stratification of sepsis patients into subphenotypes or endotypes for use in the clinic, will require international collaborative efforts to establish a consensus sepsis endotype model. The importance of a consensus model is exemplified by work in the field of colorectal cancer. By combining high-dimensional gene expression studies from six independent research groups, all with their own subtype classification strategies, a consensus four-subtype model was developed that standardized colorectal cancer subclassification for further studies (Guinney et al., 2015; Linnekamp et al., 2018). While the observed similarities between the earlier-mentioned methods for sepsis patient stratification as transcriptomic endotypes are reassuring, we do not know whether overlapping endotypes describe the same subgroup of patients. It is essential to investigate the similarities and differences between patient endotypes, particularly across different geographical populations. To date, most studies were limited to people of Northern European ancestry, which certainly restricts our ability to identify sources of inter-individual variation in the host response, and consequently generalizability of patient endotypes. Thus, including more diverse patient populations in stratification studies will go a long way to developing veritable consensus sepsis endotypes. Until now, the vast majority of genomics studies for the purpose of patient stratification utilized whole blood leukocyte transcriptomes obtained on ICU admission. While whole blood is extremely relevant biological specimen to the clinic owing to its accessibility, it does complicate the interpretation of transcriptomic endotypes. Whether the leukocyte gene expression patterns observed in sepsis patients on ICU admission reflect dominant pathobiological mechanisms, especially those that ensue at the primary anatomical site of infection is unknown. Establishing a connection between organ-specific pathophysiology in sepsis and transcriptomic endotype membership cannot be overstated. It is crucial to better understand the relationship between transcriptomic endotypes, particularly those that emerge from consensus endotype efforts, and organ-specific biology in sepsis. In addition, it is envisaged that future studies will be designed in a longitudinal manner, that is obtaining specimens at various time points of a patient's ICU stay. The host response to infection is a highly dynamic and temporally coordinated process, exemplified by the time-dependent patterns observed in the human endotoxemia model (Perlee et al., 2018; Scicluna et al., 2020). This is particularly pertinent to sepsis endotypes studies since it was observed that approximately 30% of children with septic shock switch endotype membership during the first 72 hours after ICU admission (Wong et al., 2018).

5 Concluding Remarks

Technological advances have heralded important discoveries in sepsis pathophysiology, particularly in the immunology of sepsis (van der Poll et al., 2021). Despite an improved understanding of the immunopathology of sepsis, translation to effective treatments remains problematic. It is evident that unraveling the complexities that underlie the heterogeneity in sepsis is a challenging task, requiring more than reductionist approaches alone. Embracing the concepts of integrative biology, that is bringing together investigators of diverse specialties, for example anatomy, physiology, biochemistry, pathology, mo-

lecular biology, genetics, genomics and mathematics to address the problem of sepsis not only in a multidisciplinary manner but also transdisciplinary. The current method of choice is utilizing high-dimensional "omics" data and data science for immune-profiling in a "multi-omics" approach, permitting analysis of multiple molecular strata that include DNA, RNA, proteins and metabolites from the same sample. While useful in building platforms for the derivation of new hypotheses, it will be critical to build these models not only using systemic molecular profiles, but also at the tissue-site of infection (Cavaillon et al., 2020). More attention should be given to designing longitudinal "multi-omics" studies, including samples not only obtained during a patient's ICU stay, but also after hospital discharge. This approach will allow for a more holistic integrative model of the septic response, patient trajectories and the long-term consequences. In this way, host response biomarkers will be derived reflecting dominant pathobiological mechanisms during the acute and/or convalescent phases, which despite its challenges is envisaged to progress to realizing the promise of precision medicine approaches in sepsis.

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