18

Precision Medicine in Critical Illness: Sepsis and Acute Respiratory Distress Syndrome

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Key Point Summary

- Disease heterogeneity in ARDS and sepsis has challenged the conduct of omics studies involving these syndromes, yet precision medicine approaches are sorely needed to improve outcomes.
- Genomics studies of ARDS and sepsis are challenged by small sample sizes and the difficulty in identifying appropriate controls.
- Gene expression studies have identified biologically distinct expression signatures that retroactively identify differential response to routine treatments applied in the ICU. In sepsis, a signature of dysregulated adaptive immune signaling has evidence to stratify patients according to a differential response to systemic steroid therapy. In ARDS, patients with a hyperinflammatory pattern identified in plasma using targeted proteomics responded more favorably to randomized interventions including high positive end-expiratory pressure, volume conservative fluid therapy, and simvastatin therapy.
- Fewer ARDS and sepsis metabolomics and unbiased proteomics studies exist, but as these approaches become more standardized, additional biomarkers may be identified.

Introduction

Sepsis and the acute respiratory distress syndrome (ARDS) are two syndromes causing a sobering proportion of deaths in the intensive care unit (ICU). By one estimate, sepsis was responsible for between one third and one half of inpatient deaths [1], whereas ARDS has been observed to complicate almost one quarter of critical care admissions requiring mechanical ventilation, with a mortality rate exceeding 35% [2]. Despite significant advances in our understanding of the pathologic mechanisms contributing to each of these syndromes, neither sepsis nor ARDS can boast specific pharmacologic therapy with a consistently proven effect. Numerous trials in sepsis

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[3–5] and ARDS [6–8] have failed to establish drug therapy for these deadly syndromes. One major factor that may contribute to this treatment gap is the profound heterogeneity encompassed by patients meeting criteria for each syndrome. A valid concern is that our syndromic definitions [9–11], although useful in identifying patients who share clinical factors and who may benefit from standardized care [12–15], may have almost no utility in predicting a patient's biologic subclassification nor in predicting mortality, expected complications, or response to therapy. Precision medicine options for these syndromes, whereby the correct drug could be targeted to the patients most likely to be helped and least likely to be harmed, are sorely needed.

For complex traits such as asthma or cystic fibrosis, the knowledge of a patient's biologic endotype provides clues about a patient's prognosis, pathophysiology, and expected response to therapy [16–20], yet such a breakthrough has yet to arrive for sepsis or ARDS. In this chapter, we review the contributions of genomic medicine to identifying potential biologic subgroups in sepsis and ARDS, a necessary first step for precision medicine. In addition, we consider specific challenges to pursuing omics approaches in each of these traits, as well as potential opportunities we envision in the near future.

Targeted Proteomics: Laying the Foundation for Precision Medicine of Sepsis and ARDS

Increasingly, we have objective evidence that response to therapy is nonuniform and potentially predictable by factors beyond clinical features. In ARDS, this has been consistently demonstrated among clinical trial populations using an analytic method known as latent class analysis (LCA) to uncover potentially unobserved subpopulations while remaining agnostic to outcomes. In analyses considering clinical variables including vital signs, ventilator data, and laboratory values in addition to exploratory plasma biomarkers representing inflammation, vascular dysfunction, or alveolar injury, a latent class model consistently identified two classes of ARDS trial subjects that differed in their plasma expression of inflammatory biomarkers epitomized by interleukin (IL-) 8 or IL-6, and by their degree of systemic illness, characterized by low blood pressure and low serum bicarbonate [21–24].

Not only were subjects in the "hyperinflammatory" subphenotype group more likely to die, but the LCA group assignment (hyperinflammatory versus non-hyperinflammatory) exhibited significant statistical interaction with randomized treatment effects (Table 18.1) [21, 22, 24]. Thus, when the randomized interventions of higher positive end-expiratory pressure (PEEP), conservative fluid strategy, or simvastatin therapy were analyzed in groups stratified by LCA assignment, each therapy seemed to have a mortality benefit only observed in the hyperinflammatory group, with no signal for improvement in the non-hyperinflamed group [21, 22, 24]. In each trial, there was no evidence for heterogeneity in treatment effect by baseline severity of illness, as defined by the Acute Physiology and Chronic Health Evaluation (APACHE) score, nor by the severity of ARDS, as defined by the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2:FiO_2).

Reproducible biologically defined ARDS subgroups based on plasma protein expression patterns were also reported in a population of sepsis-associated ARDS subjects from a prospective sepsis cohort applying a Ward clustering algorithm [25]. Showing remarkable similarity to the LCA-derived subgroups, the clustering algorithm detected two classes of ARDS, one "reactive" defined by high plasma concentrations of markers of inflammation, coagulation, and endothelial activation compared to the "noninflamed" group, and a significantly higher mortality was observed for the reactive subgroup. Although the overlap in plasma protein signatures between the Calfee "hyperinflamed" and Bos "reactive" subgroups is striking [21–23, 25], both studies sampled fairly similar candidate biomarkers that have previously performed well in human and animal studies of sepsis-associated ARDS [26, 27], so the overlap is less surprising than if the authors

Table 18.1 Apparent heterogeneous response to therapy that may be predictable by biologic testing

Population	Potential classifier	Intervention	Study findings
Pediatric sepsis	Gene expression subtype A vs B	Corticosteroids	Subtype A with higher mortality when treated with steroids Subtype B plus a high predicted mortality displayed a mortality benefit from steroids [117, 118]
Adult sepsis	Plasma IL1RA level	Recombinant IL1RA	High plasma IL1RA subjects with a mortality benefit when randomized to rhIL1RA; low plasma IL1RA no benefit [35]
Adult septic shock	Gene expression subtype SRS1 vs SRS2	Corticosteroids	SRS2 subjects with increased mortality with steroids; SRS1 no effect of steroids [124]
Adult ARDS	Latent class assignment (clinical and plasma protein expression)	High PEEP (positive end-expiratory pressure)	Hyperinflammatory subjects with a mortality benefit when randomized to high PEEP; no benefit in non-hyperinflammatory patients [21]
Adult ARDS	Latent class assignment (clinical and plasma protein expression)	Conservative IV fluid therapy	Hyperinflammatory patients with a mortality benefit when randomized to conservative (dry) fluid strategy; non-hyperinflammatory without benefit [22]
Adult ARDS	Latent class assignment (clinical and plasma protein expression)	Simvastatin therapy	Hyperinflammatory patients with a mortality benefit when randomized to simvastatin; non-hyperinflammatory subjects with no benefit from simvastatin [23]

As evidence for the potential of precision medicine to better target therapy to patients, each study listed describes an apparent statistical interaction between an intervention and study outcome (mortality). Because these were all retrospective studies and many were subgroup analyses, a prospective validation study is needed before altering clinical care
SRS sepsis response signature, *PEEP* positive end-expiratory pressure, *IV* intravenous

had used unbiased discovery or untargeted proteomics approaches.

Similarly, in sepsis, it is conceivable that heterogeneity of treatment effect may underlie some of the negative overall findings for such drugs as recombinant human interleukin-1 receptor antagonist (rhIL1RA) [28–30], antitumor necrosis factor [31, 32], or activated protein C [4, 33, 34]. In a subgroup reanalysis of a randomized trial of rhIL1RA for sepsis [29], the mortality benefit of rhIL1RA differed significantly between subjects with high baseline endogenous plasma interleukin-1 receptor antagonist (IL1RA), who seemed to benefit from the drug, and those without elevated plasma IL1RA in whom there was no effect [35]. A separate subgroup reanalysis of the same rhIL1RA trial demonstrated a very strong signal for benefit among subjects with clinically defined macrophage activation syndrome [36]. Although subgroup analyses must be viewed with caution due to underpowering and the risk of unstable effect estimates [37–39], these reports nonetheless highlight the potential for precision application of sepsis therapy if replicated in prospectively defined studies.

What Can Be Learned from Genetic Approaches in Complex, Non-Mendelian Traits?

Neither sepsis nor ARDS is considered a classic monogenic or "Mendelian" trait, whereby the expression of the trait is easily predictable by parsing the inheritance of one genetic locus through several generations. However, multiple lines of evidence support a major interplay between genetic variation and patterned responses to injury and infection. Primary, or inherited, immunodeficiency diseases (PIDD) include over 330 specific disorders caused by at least 320 monogenetic changes [40, 41] and span broad subgroups that include defects in just one aspect of the immune system (e.g., antibody, innate,

T-cell, natural killer cell, neutrophil) to combined immunodeficiencies, autoimmune diseases, or autoinflammatory disorders. Some syndromes present as an inherited susceptibility to one particular type of infection, such as mycobacteria, fungi, or certain bacteria, and thus, elucidation of the respective genetic underpinnings has helped to pinpoint critical host responses to specific pathogens [42–46]. Further, the identification of monogenic conditions causing auto-inflammatory conditions that mimic the clinical features of sepsis while remaining culture-negative [47–49] highlight the primacy of host response in driving shock and organ failure.

Further, there is ample evidence that historic infectious threats likely shaped genetic architecture through natural selection [50, 51]. The single missense variant in the beta-globin gene responsible for sickle cell anemia (rs334) persists at a frequency of 5–10% in genotyped African populations [52, 53], a frequency much higher than expected for such a deleterious mutation. However, because individuals who carry only one copy of rs334 seem to be protected from malarial infection [51, 54], this variant is common in populations where malaria has been, or continues to be, a threat. Similar examples may explain the striking variation in genes encoding cytokines [55], or genes that control activation of the complement syndrome, some of which have also been strongly implicated in inflammatory traits like age-related macular degeneration [56–58].

Accepting that our genes influence response to infection or injury, and that such historic threats have in turn shaped genetic architecture, it remains true that most patients with sepsis do not harbor a single genetic variant that explains their risk for sepsis or sepsis death. Nonetheless, there exists strong evidence that sepsis death exhibits significant heritability. In a classic study merging genealogic records and population health information, biologic parents and their children displayed a much stronger concordance for premature death from infection than did adopted parents and their children, suggesting that genes play a stronger role in response to infection than does environment [59]. The relative risk (RR) of dying prematurely from infection when one parent had also died from infection was almost 6 (95% CI 2.47–13.7), a larger risk than was observed for cancer or even vascular disease [59]. Just as unraveling the monogenic PIDD have suggested precision treatment options that sometimes obviate the need for bone marrow transplantation [47], it may be that better recognition of dysregulated genes contributing to sepsis outcomes suggests novel treatment paradigms for this deadly disease.

Similar data do not exist to support the inherited susceptibility to ARDS, in part because ARDS was only described with the advent of modern ICU care [60], but one might consider the syndrome of acute hypoxia and bilateral lung opacities following a potential insult – ARDS [10] – to be a patterned response to injury or infection. Although to our knowledge no pedigrees exist of ARDS, genetic investigations have suggested novel pathophysiologic processes. Recognizing that we are unlikely to explain a large proportion of the variance in the risk for sepsis or ARDS by one or even a small handful of genes, the dissection of trait-associated pathways may still suggest individuals predisposed to these syndromes via specific mechanisms and, thus, suggest groups who are likely to respond to specific interventions.

Knowledge- and Discovery-Based Genomics Studies

In general terms, there are two major approaches to identify inherited variation that may influence a trait (Fig. 18.1). Knowledge-based approaches, sometimes referred to as candidate gene studies, select specific genes or pathways already hypothesized to contribute to a disease and test for a higher frequency of genetic variants in disease-positive subjects compared to subjects without the disease. Advantages of the candidate approach include the straightforward design, typically as either a case-control or cohort study, low cost, and the fact that next steps after a positive finding are relatively clear. However, precisely because candidate gene studies are predicated on existing knowledge of sepsis or ARDS pathophysiology, these studies have a high risk of failure. For com-

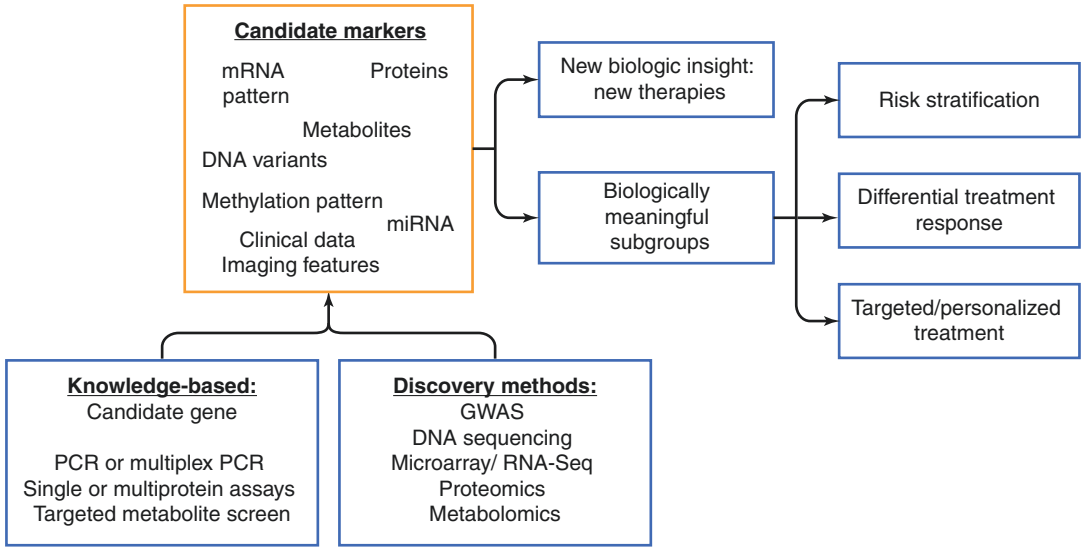

Fig. 18.1 Both knowledge-based and discovery-based methods can identify new candidate markers that span all aspects of biology and clinical features. Novel analytic techniques can then combine clinical, genetic, transcriptomic, metabolomic, and proteomic data to achieve the goals of precision medicine: identifying new therapies via refined mechanistic insight and unpacking clinical heterogeneity into biologically meaningful subgroups. PCR polymerase chain reaction, GWAS genome-wide association study, RNA-Seq RNA sequencing, mRNA messenger RNA

plex traits like sepsis or ARDS, the expected effect size of any given genetic variant is modest, with an odds ratio less than 1.4, and consequently most studies are statistically underpowered to detect an effect even if one exists. Further, even if the selected candidate gene does play a central role in ARDS pathophysiology, researchers still need to genotype the causal part of the gene responsible for the trait or, leveraging linkage disequilibrium [61], a variant in linkage with the causal variant. We are only beginning to understand the complexities of genetic regulation beyond the traditional paradigm of *cis* regulation, whereby local DNA sequence dictates local messenger RNA (mRNA) sequence, which in turn explains protein sequence. With the application of next-generation sequencing techniques to better understand DNA-protein binding, noncoding RNA regulatory elements, epigenetic changes that may silence or activate gene expression, and the impact of three-dimensional chromatin organization [62–66], it is now apparent that early genotyping strategies may have been too simplistic. Thus, the failure to detect associations does not exonerate a gene from playing a significant role. Finally, the candidate gene approach now seems highly inefficient in the era of next generation sequencing approaches.

Genome-wide association studies (GWAS) assay single nucleotide polymorphisms (SNPs) at over 500,000 loci across the genome using nanofabricated arrays of oligonucleotide probes specific for individual SNPs. Then, using knowledge of linkage disequilibrium between SNPs based on large-scale genotyping of multiple populations [52, 67, 68], investigators can impute genotypes at loci that were not genotyped, allowing dense characterization of genetic variation for less than 100 US dollars per sample. However, though the array-based GWAS does characterize DNA variation "across the genome" and is considered a discovery approach, it is not truly bias-free, as the arrays are built using oligonucleotide probes for known SNPs and imputation steps rely upon preexisting knowledge of LD relationships between SNPs. The technology that yields data closest to truly bias-free genome sequences is next-generation sequencing (NGS), in which massively parallel sequencing of fragmented input DNA occurs. NGS is the preferred method to discover new variants or private variants that occur in only one family, or only one individual, as well as to study rare variants [69].

Factors beyond the inherited genomic DNA sequence influence the expression of genes. Profiling of messenger RNA (mRNA) or, more broadly, entire transcriptomes (i.e., transcriptomics) has enabled major advances in the understanding of cancer and complex traits like asthma [16, 70, 71]. Gene expression studies also can be thought of as following either knowledge-based or discovery-based paths. To understand the mRNA abundance or expression pattern of a specific transcript, one could use traditional polymerase chain reaction (PCR) methods using an oligonucleotide probe or probes complementary to the sequence(s) of interest. When roughly 20–30 thousand probes are arrayed onto a single nanofabricated platform to assess global gene expression, we term this a whole genome microarray, which is a discovery method, albeit based on probes and, thus, not bias-free. For broader unbiased characterization of transcriptomes, investigators can use RNA-sequencing (RNA-Seq), a NGS approach that sequences a complementary DNA (cDNA) library prepared from input RNA. Gene expression studies have unique challenges compared to genomics in that the former are cell type and context specific and highly dynamic. Beyond mRNA, multiple noncoding RNA species have been identified and demonstrated to influence transcription, translation, message stability, splicing, enhancing/silencing, epigenetic regulation, and even molecular scaffolding [72]. Bias-free sequencing has elucidated the breadth of the noncoding RNA landscape, and the study of noncoding RNA in critical illness remains in its infancy. Epigenetic changes, which describe inherited but modifiable DNA-protein interactions, such as histone modifications or DNA methylation patterns, also modify gene expression and can be assessed in targeted, high-throughput, or bias-free applications.

Unique Challenges to Achieving Precision Medicine in Critical Care

Although the promise of omics techniques to contribute to precision medicine options is undeniable, specific challenges complicate the application of these methods to sepsis and ARDS. Both syndromes are complex genetic traits, requiring both an extreme environmental insult like infection or exposure to a ventilator and host susceptibility. This gene-by-environment interaction can be complex to study and poses unique barriers to identifying omics signals even when present.

First, the genomic signature of sepsis or ARDS will never occur in isolation. Sepsis by definition is a systemic disease, and multiple organ dysfunction is often central to its diagnosis. Dissecting out the signature of sepsis from that of secondary kidney, lung, brain, or liver injury requires unique analytic tools as well as potentially arbitrary decisions classifying changes as sepsis related or not. With genomic material from the infecting microbe potentially circulating in blood, sequencing techniques might amplify bacterial, viral, parasitic, or fungal genomes rather than the patient's cells. ARDS is also frequently complicated by coincident non-lung organ failure and frequently occurs on the background of sepsis, such that identifying the specific ARDS signature may be difficult. Further, liver and kidney dysfunction can alter the clearance of proteins and metabolites. When metabolic or proteomic changes seem to distinguish ARDS cases from non-cases, these could represent an important feature of the causal pathway in sepsis or may simply reflect end-organ dysfunction with impaired clearance.

Second is the problem of identifying a suitable control population and appropriately designing the study. In many diseases, large convenience cohorts of healthy adults can be used as controls, and large-scale genomic resources such as the UK Biobank can be very powerful to detect a genetic signal [73]. For ICU diseases, however, the use of healthy controls may be problematic, precisely because of the gene-by-environment interaction that requires a severe environmental insult to manifest sepsis or ARDS. A person's genome may contain multiple risk variants for ARDS, but if she never develops sepsis, exposure to a ventilator, or another ARDS precipitant, she may never exhibit lung flooding. The presence of such a subject in the control group would attenu-

ate any signal for ARDS risk, even if multiple ARDS cases carry the same variant. Similar issues arise when designing a sepsis study. In a case-control design, should controls be a group of patients who never had pneumonia or, instead, a group infected with pneumonia and thus at risk to develop sepsis, but who remained relatively well? For this reason, many critical care investigators choose a cohort design, which alleviates the concern for selection bias, but comes at increased cost and lower efficiency. Design issues may complicate the analytic phase as well. A frequent criticism of a potential new prognostic marker for sepsis or ARDS is that it merely reflects severity of illness, and investigators are asked to confirm that associations remain independent of illness severity characterized by simplified acute physiology or APACHE scores [74–76]. While appropriate adjusting for potential confounders has face validity for any analysis, there is a counterargument that the very processes driving acute physiologic derangement and captured by such scores may be in the causal path influencing sepsis or ARDS risk and outcome. Observing that an association persists across multiple levels of illness severity or predicted mortality can sometimes mitigate this concern [77].

Third, timing of biospecimen sampling is both critically important and yet challenging to enact. For both sepsis and ARDS, genomic, proteomic, and metabolomic signatures change substantially within hours to days, and the timeframe to collect samples is highly compressed. Whereas genomic DNA samples should be stable over time and could be collected after the acute event, RNA, protein, or metabolite profiling often requires specific collection strategies and is only relevant if collected during the illness itself. At the same time, critically ill patients are frequently unable to consent for themselves, suffer from anemia [78], and have multiple competing clinical needs that may limit the ability to conduct observational research in the early hours of ICU admission. Furthermore, it is challenging to define "time zero" for sepsis; is it when the patient presents to the emergency room, the first low blood pressure, or the first fever? The importance of time-course analysis in sepsis was highlighted by gene expression work by Sweeney et al. [79], in which a clear sepsis gene expression signature emerged in early sepsis, but was later swamped by recovery signals. For ARDS, does the clock start when exogenous oxygen exceeds 4 liters per minute, when the chest radiograph is first abnormal, or when the patient is intubated and meeting all consensus criteria [10, 80]? For each omics study, these issues should be carefully considered and protocolized to ensure the highest possible scientific rigor.

Finally, there is the issue of which tissue warrants profiling. Peripheral blood – easily obtained by a blood draw and either left whole or segmented into constituent blood cells – is convenient, widely available, and relevant, as a potential snapshot of circulating host response. Buffy coat gene expression signatures reproducibly separate sepsis cases from controls [81, 82], and circulating inflammatory cells may be the critical actor in sepsis pathology. However, peripheral blood has numerous limitations. Genes that are expressed exclusively by endothelium, epithelium, stromal tissue, or tissue specific to the infected organ will not be captured by whole blood or leukocyte gene expression profiling. Though there may be strong interest to evaluate vascular mRNA, a vessel biopsy will remain highly unlikely and circulating endothelial cells are difficult to collect and may be fundamentally distinct compared to intact vasculature [83]. For ARDS, there is general consensus that lung tissue would provide the maximal utility for gene, protein, and metabolite expression. However, patients with ARDS are rarely subjected to lung biopsy due to their tenuous stability and the risk of the procedure [84]. Easily obtained peripheral blood is not a consistent surrogate for omics states in the lung. In ARDS, peripheral blood gene expression may be swamped by sepsis severity rather than lung injury per se [85]. Even when limiting analysis to only the mononuclear cell fraction and carefully timing blood draws to coincide with bronchoalveolar lavage (BAL), the signature of alveolar macrophages in ARDS is markedly distinct from synchronous peripheral blood monocytes [86]. Despite these formidable

challenges, there are numerous examples of incremental and occasionally transformational progress toward precision medicine that speak to tremendous potential of omics approaches in sepsis and ARDS (Table 18.1).

Sepsis Genetics: Hints at Heterogeneous Treatment Effects

Although the promise of genetics to contribute to precision medicine options for sepsis remains strong, progress to date has been relatively modest. Candidate gene association studies have yielded a number of variants that reliably associate with increased susceptibility to specific infections and occasionally with a higher frequency of hypotension or death [87–89]. As candidate gene studies extend from our preexisting paradigm of sepsis, most of the interrogated genes have been those influencing host response, immune regulation, or vascular regulation. Genome-wide studies of sepsis outcome – a highly heritable trait [59] – have also been published and suggest novel pathways that merit consideration.

In one of the first published GWAS for sepsis survival, investigators from the Genetics of Sepsis and Septic Shock in Europe (GenOSept) consortium used a discovery population of approximately 1000 subjects with community-acquired pneumonia and replicated findings in an additional 1000 individuals from clinical trials or an ongoing pneumonia cohort. The GenOSept authors reported a fairly convincing LD peak on chromosome 5 in the *FER* gene encoding Fps/Fes-related tyrosine kinase that associated with lower risk of death (meta-analysis odds ratio 0.56, 95% confidence interval 0.41–0.66) with a *p*-value robust to multiple comparison testing ($p = 5.6 \times 10^{-8}$) [90]. Interestingly, the association with death was attenuated by expanding the population to include septic subjects with abdominal infections, suggesting that the protective association may be relevant only to pulmonary sepsis. The *FER* gene encodes a non-receptor protein tyrosine kinase implicated in actin cytoskeleton regulation as well as chemotaxis and leukocyte migration – areas already of interest in sepsis pathophysiology [91–93] – thus, it is an attractive sepsis candidate gene. Mortality was 25% for homozygous carriers of the dominant allele compared to 10% in homozygous recessive carriers [90], suggesting that genotype might act as a prognostic enrichment tool to help select a high-risk population [11]. However, as is often the case with genomic findings, replication of this variant has been inconsistent, and it was not associated with mortality in a smaller population of septic subjects enrolled in clinical trials in Germany [94]. A second GWAS found that a rare missense variant in gene *VSP13A* was associated with very high risk for mortality, and this *VSP13A* SNP was associated with higher sequential organ failure assessment score in a separate pneumonia study, providing possible replication. *VSP13A* encodes for vacuolar protein sorting 13 homolog A and has been implicated in autophagy, another pathway relevant to sepsis [95].

Examples of genetic studies contributing to precision therapy in sepsis are indirect, but a few do exist. Meyer et al. identified a synonymous coding SNP in the gene *IL1RN* encoding interleukin-1 receptor antagonist (IL1RA) that associated with reduced ARDS risk in both trauma and sepsis populations, as well as with reduced sepsis mortality in the VASST septic shock trial [96–98]. The SNP seemed to be functional, associating with increased plasma IL1RA among patients at risk for ARDS, lower plasma interleukin-1 beta (IL1β) during septic shock, and as a site of allelic imbalance with more efficient *IL1RN* gene expression following endotoxin challenge. As these data suggested that more efficient plasma IL1RA generation might be protective in sepsis, Meyer and colleagues used plasma from a completed clinical trial of recombinant human IL1RA (rhIL1RA) for sepsis to phenotype sepsis patients for pre-randomization plasma IL1RA and IL1β expression. They detected a differential effect of rhIL1RA on mortality based on plasma IL1RA expression, such that rhIL1RA seemed to reduce mortality among "IL1RA-high" subjects [adjusted risk difference (ARD) −12%, 95% CI −23% to −1%], $p = 0.044$, but not among "IL1RA-low" subjects (ARD +7%, 95% CI −4% to +17%), resulting in a statistically significant

Table 18.2 Genetic associations with sepsis or ARDS with potential impact on therapeutic response

Gene *(Official gene ID)*	Population	Outcome associated with gene variant	Potential therapy with pharmacogenetic response
Interleukin-1 receptor antagonist (*IL1RN*)	Adult sepsis trial populations Adult trauma cohort	Reduced sepsis death; reduced ARDS risk [97, 98]	Recombinant human interleukin-1 receptor antagonist (rhIL1RA)
Leucyl/cystinyl aminopeptidase or vasopressinase (*LNPEP*)	Adults with septic shock	Higher sepsis mortality [132]	Vasopressin
Protein C *(PROC)*	Adults with sepsis	Higher sepsis mortality, higher organ failure score [133, 134]	Drotrecogin alpha (activated protein C)
Pre-elafin *(PI3)*	Adult at risk for ARDS	ARDS [135, 136]	Human neutrophil elastase inhibitors
Angiotensin-converting enzyme *(ACE)*	Adults with ARDS compared to at-risk or healthy controls	ARDS risk ARDS mortality [137, 138]	ACE inhibitors, angiotensin receptor blockers, or ACE2 analogs
Surfactant protein B *(SFPTB)*	Adults with ARDS compared to at-risk or healthy controls; children with pneumonia	ARDS risk [139], mechanical ventilation risk [140]	Exogenous surfactant
Angiopoietin-2 *(ANGPT2)*	Adult trauma at-risk ARDS Adult sepsis	ARDS; plasma Angiopoietin-2 level [103, 114]	Anti-angiopoietin-2 agent; TIE2 agonist

In each case, a genetic association has been reported in at least one population, and drugs exist to target the gene's pathway

interaction term [35]. As a subgroup analysis, the observation of lower mortality was insufficient evidence to change practice [77, 99] yet it demonstrates that individualized sepsis treatment based on plasma biomarker expression is possible. Given numerous potential genetic associations with sepsis in pathways associated with drug targets (Table 18.2), testing for heterogeneous treatment effect by genotype or plasma protein expression as a routine addition to interventional trials is an approach that, if adequately powered, could be promising to identify precision targets.

ARDS Genetics and the Search for Causal Intermediates

Numerous candidate gene studies have been undertaken in ARDS populations to elucidate key factors associated with either risk of ARDS or ARDS mortality [100, 101]. Among the best replicated loci, genes contributing to inflammatory response (*IL6*, *IL10*, *IL1RN*, *PI3*, *MBL2*, *NFKB1*, *TLR1*), vascular regulation (*ACE*, *VEGFA*, *MYLK*, *ANGPT2*, *SERPINE1*), oxidant stress (*NFE2L2*, *HMOX1*), and lung epithelial function (*SFTPB*) are overrepresented. Discovery approaches have also been published [102] and have contributed new candidate genes such as *PPFIA1*, which encodes for liprin alpha 1, a gene expressed in lung and numerous tissues that plays a role in regulating focal adhesions and cell-matrix interactions. As previously mentioned, medium-throughput candidate gene DNA array studies identified risk variants in ARDS that may have potential therapeutic implications, as for *IL1RN*, the gene encoding IL1RA, or *ANGPT2*, the angiopoietin-2 gene that contributes to vascular permeability [98, 103].

Given results of GWAS over the past decade, it is usually unreasonable to expect that common variants will be associated with complex diseases with effect sizes large enough to be statistically significant in studies involving fewer than 2000 cases. In complex traits where numerous relatively

frequent SNPs are hypothesized to alter risk with modest effect sizes (odds ratio 1.1–1.5) [104], a well-powered study should include many thousands of cases and non-cases, and no such ARDS population yet exists. Nonetheless, an approach by which genetics may help advance a precision medicine platform is by integrating genetic association results with those of other omics studies to prioritize candidate biomarkers. For example, plasma markers could be prioritized based on genetic results to identify those with the most direct relationships with ARDS risk or mortality. To borrow from a cardiology example, genetics provided strong inferential evidence that plasma concentration of low-density lipoprotein cholesterol (LDL) was the major risk factor for coronary artery disease (CAD) risk and mortality. Plasma LDL is strongly genetically regulated and variants that influence plasma LDL strongly associate with CAD in a consonant fashion; genetic variants that lower LDL associate with reduced lifetime CAD risk and those that elevate LDL associate with high CAD and mortality [105–107]. Thus, drugs targeting LDL are a mainstay of CAD prevention and treatment, and we term LDL a "causal" marker for CAD. Causal markers are lacking for ARDS, but if a causal marker were identified, it could speed drug development via the design of high-throughput screens to identify compounds that alter the marker.

A few examples of leveraging genetics to infer causal ARDS intermediates are worth highlighting. Recognizing that platelets contribute to both microvascular and immune system activation during ARDS and that the lung is a major site of platelet biogenesis [108–110], Wei and colleagues focused on genes shown to strongly associate with platelet counts in healthy subjects and verified that variants in the gene *LRRC16A* also associate with platelet count in a critically ill population. Further, the same platelet-associated variant is also associated with ARDS risk, and a small but significant portion of the ARDS risk was mediated through platelet count, implicating thrombocytopenia as a causal intermediate for ARDS risk [111]. The same group then identified an independent locus in the *LRRC16A* gene associated with both a falling platelet trajectory in the ICU and ARDS mortality [112] and statistically demonstrated that declining platelet count mediated the association between *LRRC16A* and death. These examples of genetic mediation analysis are one demonstration of using genetic data to adapt causal inference methodology for the identification of causal disease intermediates. By mathematically disassembling an association between an explanatory variable (gene variant) and outcome (ARDS) into direct (gene-ARDS) and indirect (gene-platelet and platelet-ARDS) effects, one can infer the relative proportion of effect for the candidate mediator. The concept of using drugs to target platelet abundance or platelet trajectory to modify ARDS risk or mortality may seem unfamiliar, yet there was strong rationale for the LIPS-A study that tested whether aspirin reduced ARDS risk and found that it reduced ARDS risk though with a smaller effect size than anticipated [113]. Future work in this area may be fruitful.

A complementary approach termed Mendelian randomization (MR) leverages the association between genetic variants and intermediates such as plasma biomarkers to infer a biomarker's causality. Each individual's genetic variants are independently assigned by random assortment of parental alleles, according to the law of independent assortment, and alleles are distributed independently of any potential confounder. MR leverages this independence and applies the instrumental variable method to reduce a potential predictor variable to the portion that is least confounded, least susceptible to measurement error, and least vulnerable to reverse causation. Reilly and colleagues used MR to infer a potential causal role for plasma angiopoietin-2 (ANG2) and ARDS risk following sepsis; ANG2 was selected as a marker based on a genetic association between the angiopoietin-2 gene (*ANGPT2*) and ARDS they had previously identified [114]. Further, via mediation analysis, they found that plasma ANG2 mediated a substantial proportion (>34%) of the association between *ANGPT2* variants and ARDS risk [114], whereas no direct effect between *ANGPT2* and ARDS was observed. Together, these data highlight the potential for drugs that block ANG2 signaling to

improve ARDS outcomes and the promise of applying a similar study design to prioritize interventions in future trials.

Sepsis Gene Expression Studies: Ready for Clinical Launch?

Gene expression studies are close to yielding findings that can be clinically translated into prognostic and predictive biomarkers in sepsis. Much of the early work demonstrating the power of whole blood, or peripheral leukocyte, gene expression signatures to discriminate biologically meaningful sepsis subgroups originated with Hector Wong's work in pediatric populations. Using unsupervised hierarchical clustering, his group consistently identified three patterns of gene expression among pediatric patients with septic shock [82, 115, 116]: "subclass A" patients, characterized by repression of adaptive immunity genes and glucocorticoid receptor signaling, who exhibited higher severity of illness, fewer ICU-free days, and higher mortality.

Given that glucocorticoids are frequently administered to patients with septic shock, the same group then asked whether the effect of glucocorticoids on sepsis mortality was associated with baseline gene expression patterns. They reported a potential interaction ($p = 0.089$) with steroids increasing mortality in subclass A patients with an odds ratio of 4.1 (95% CI: 1.4–12.0), but not in subclass B patients [117]. Further, by using a plasma biomarker risk stratification tool as a prognostic marker that reliably identified high risk for mortality [11], along with the glucocorticoid-response gene expression subclassification signature, Wong and colleagues established preliminary proof that the gene expression signature could be used to identify subjects who respond favorably to glucocorticoids among those with high predicted mortality [118].

This work set the stage for a precision clinical trial of corticosteroids leveraging the PERSEVERE pediatric biomarker risk model [119], which is based on plasma expression of five biomarkers (C-C chemokine ligand 3, interleukin 8, heat shock protein 70 kDa 1B, granzyme B, and matrix metallopeptidase 8), to identify high-risk subjects. Subsequently, a 100-gene mRNA classifier was used to identify sepsis subclass and limit enrollment to subclass B patients. By focusing on high-risk individuals, the prognostic approach seeks to improve trial efficiency, whereas the predictive approach, in theory, will limit the potential for the intervention to harm patients predicted to do worse with corticosteroid treatment. The Stress Hydrocortisone in Pediatric Septic Shock (SHIPSS) is a phase III randomized double-blind placebo-controlled trial to test whether steroids are beneficial in refractory septic shock; the trial will use plasma and gene expression classifiers to determine whether the above approach is ready for clinical use.

Similar efforts have been undertaken in adult sepsis, with the results of several large, highly cited adult sepsis trials highlighted in Table 18.3. In every case, authors were able to identify a subset of patients at increased risk of death, though the precise method of detecting classes and the number of clusters varied. The UK Genomic Advances in Sepsis (GAiNS) group performed whole blood gene expression profiling by microarray on a discovery cohort of 265 subjects with severe pneumonia, with replication in a second pneumonia population of 106 [120]. Using unsupervised hierarchical cluster analysis of the most variable 10% of transcript probes, they identified two dominant clusters which they termed "sepsis response signatures 1 and 2" (SRS1, SRS2). The SRS1 subtype had a 27% mortality at 28 days, while SRS2 had 17% mortality. Although SRS1 subjects were more likely to require vasoactive medications and had higher Sequential Organ Failure Assessment (SOFA) scores at baseline, there was no statistically significant difference in baseline APACHE II score, need for mechanical ventilation, or use of renal replacement therapy. Combinations of clinical covariates performed poorly at predicting SRS membership with misclassification rates of 20–40%. Thus, SRS grouping seemed to add prognostic value beyond typical clinical scoring systems [120]. Investigators were able to reduce their classifier to seven transcripts that predicted SRS classification in both discovery and validation cohorts. In pathway analysis annotating the genes

Table 18.3 Sepsis gene expression cohort studies

Author, *population*	Population	Sepsis source	*N* Dis[a]	*N* Rep[b]	RNA source	Statistical approach	Subphenotype identified	Gene set, *highlighted genes*
Scicluna, *MARS* [121]	Adults with sepsis	CAP	306	216/265	Whole blood RNA, first 24 hours	Unsupervised clustering	4 MARS clusters	140 gene classifier *BPGM*, *TAP2*
Davenport *UK GAiNS* [120]	Adults with sepsis	CAP	265	106	Peripheral blood leukocyte RNA, first 5 days	Unsupervised hierarchical clustering	2 clusters, SRS1 and SRS2	7-gene classifier *DYRK2*, *TDRD9*, *CCNB1IP1*, *ZAP70*, *ARL14EP*, *MDC1*, *ADGRE3*
Burnham, *UK GAiNS* [141]	Adults with sepsis	CAP	73	53	Peripheral blood leukocyte RNA, first 5 days	Unsupervised hierarchical clustering	2 clusters, SRS1 high mortality	
Burnham, *UK GAiNS* [141]	Adults with sepsis	Fecal peritonitis	64	53	Peripheral blood leukocyte RNA, first 5 days	Unsupervised hierarchical clustering	2 clusters SRS1 and SRS2; very similar to CAP profile	
Wong [116]	Children <age 11 in ICU	Diverse bacterial and virus	98	82	Whole blood RNA, 24 hours	Clustering via computer-based image analysis of gene expression mosaics	3 clusters; type "A" with increased mortality	Dysregulated adaptive immunity, repressed GC receptor signaling
Sweeney; *meta-analysis* [123]	14 datasets, diverse ages, <48 h	Bacterial (diverse)	700	600 (9 datasets)	Whole blood or leukocyte RNA	COMMUNAL multiple clustering	3 clusters: adaptive, coagulopathic, inflammopathic	500-gene classifier inflammopathic-IL-1 receptor, PRP activity, complement activity Adaptive: adaptive immunity, interferon signaling Coagulopathic: platelet degranulation, coagulation cascade, GAG binding

Study populations are named by their published acronyms
CAP community-acquired pneumonia, *IL-1* interleukin 1, *PRP* pattern recognition receptor, *GAG* glycosaminoglycan
[a]Discovery population
[b]Replication population

that were most differentially expressed between SRS1 and SRS2 groups, the high-mortality SRS1 group did not exhibit increased expression of cytokine or inflammatory genes, but, rather, exhibited dysregulation of genes related to T-cell activation, cell death, apoptosis, necrosis, cytotoxicity, and phagocyte movement, in addition to upregulation of genes that characterize endotoxin tolerance [121, 122], suggesting a defective adaptive immune signature characterized SRS1 subjects.

In consonant fashion, a group from the Netherlands created the observational Molecular Diagnosis and Risk Stratification of Sepsis (MARS) cohort and applied slightly different clustering methodology to identify four clusters of sepsis subjects [121]. A 140-gene classifier reliably identified cluster membership when applied to two additional adult sepsis populations, and it identified three of four clusters in a pediatric sepsis population. Although the particular genes that best discriminated the high-risk cohort varied by study, this may be due to high correlation among many dysregulated genes, rather than a failure to replicate. Indeed, a meta-analysis by Sweeney et al. [123] that included mortality data from 14 diverse datasets of bacterial infection, including both adult and child cohorts, identified three clusters (a high-mortality "inflammopathic" cluster, a low-mortality "adaptive" cluster, and an additional "coagulopathic" cluster characterized by abnormal coagulation profiles) that could be distinguished with an 11-gene signature. They assessed overlap of their signature-based clusters with the high-mortality clusters identified in the MARS and Wong et al. pediatric cohorts, and they found substantial overlap in patients identified by each cohort's high-mortality/high-inflammation endotype.

Thus, while the specific genes selected for gene expression signatures vary, across numerous populations, a high-mortality subset of septic patients with dysregulated adaptive immunity can be identified, and "high-risk" gene expression status enhances mortality prediction over the APACHE score [121]. As mentioned above, the PERSEVERE trial is using these methods for targeted enrollment into a personalized trial of corticosteroid therapy for high-risk patients, demonstrating the potential such signatures have for personalized medicine trials. Adults with sepsis may also exhibit heterogeneous response to glucocorticoids, and one study has suggested that response may be predicted by whole blood gene expression patterns. Using a parsimonious classifier based on the expression of seven transcripts that distinguished the high-mortality, adaptive immune dysregulated SRS1 group [120], Antcliffe et al. retrospectively assigned SRS classification to subjects with septic shock in the VANISH trial [124, 125]. Although there was no mortality benefit observed for corticosteroids in the overall trial, a statistical interaction was detected between steroid allocation and SRS grouping, such that SRS2 subjects exhibited a higher risk of death from sepsis when randomized to steroids [124]. As SRS2 is classically the low-mortality group of sepsis characterized by higher levels of adaptive immune signaling, it may be that corticosteroids disrupt an otherwise favorable host response to infection in some patients.

Gene Expression Studies in ARDS

Four groups have published whole blood gene expression studies in ARDS to date (including a pediatric cohort of acute respiratory failure), and an additional two publicly available datasets from the GLUE grant of trauma also include ARDS phenotyping. Each cohort is individually small (13–67 cases) and heterogeneous in terms of timing of sampling. In each case, a unique set of ARDS-associated genes has been identified, and the ability to rigorously replicate the genes identified in other cohorts has been modest [126]. Sweeney et al. performed a meta-analysis study of all six publicly available datasets to attempt to find a common gene expression signature across the disparate cohorts, but in contrast to the clear signal found with these methods in septic cohorts [79, 127], no expression signature could robustly distinguish ARDS cases from controls [85]. Limiting the ARDS cohorts to more homogenous groups (e.g., only adults with sepsis, excluding those with trauma) did not enhance the classifier

signal. Interestingly, the top ARDS-associated genes in the meta-analysis are related to sepsis according to Gene Ontology classifiers, suggesting a potential overwhelming signal from sepsis and severity of illness may have obscured any lung-specific ARDS signals.

Metabolomics Studies in Sepsis and ARDS

Metabolomics is the study of all small molecules in an organism or tissue, including peptides, lipids, nucleic acids, and carbohydrates. Metabolite levels can change rapidly in response to cellular perturbations, such as the switch to anaerobic metabolism in exercise or sepsis that leads to lactate production. Thus, metabolites are particularly promising targets for personalized medicine because they reflect dynamic changes in the host. In contrast to well-established profiling methods for gene expression or SNP assessment, metabolomic profiling methods are rapidly evolving. The number of detected and quantified human metabolites included in the human metabolome database (HMDB) has increased from 8000 in 2010 to 18,000 in 2019, and the presumed number of actual metabolites is likely >100,000 [128]. This growth in the number of identifiable metabolites, and the parallel growth of metabolomic analytic and statistical methods, makes it difficult to compare studies across years and cohorts.

Metabolic changes in sepsis are widely recognized, as lactate, the end product of anaerobic metabolism, is the most widely used sepsis biomarker. Lactate measurement is a Centers for Medicare & Medicaid Services quality measure for patients with sepsis. Elevated lactate (>4 mmol/L) defines septic shock in the most recent sepsis guidelines, and serial lactate measurement and clearance can be used to assess adequacy of resuscitation [9, 129]. In addition to lactate, numerous metabolites are measured in basic chemistry (e.g., serum bilirubin, creatinine) that are followed as part of standard ICU care and comprise critical elements of our ICU scoring systems [74, 76].

Broad profiling of the plasma metabolome in sepsis has shown promise for biomarker identification. Langley et al. performed nontargeted profiling of plasma in 63 sepsis survivors and 31 non-survivors and identified widespread metabolic abnormalities between the two groups, involving pathways such as fatty acid transport, β-oxidation, gluconeogenesis, and the citric acid cycle. This group identified a biomarker panel of five metabolites, along with age and hematocrit, that outperformed lactate and APACHE score in mortality prediction in several studies [130]. Interestingly, when Rogers et al. examined the same populations with different metabolomics analytic strategies, a separate predictive biomarker panel was identified, likely a result of the high correlation among many metabolites [131] and emphasizing the lack of consistency among different metabolomics analysis strategies.

ARDS metabolomics studies are summarized in Table 18.4. These studies vary widely in terms of fluid studied (plasma, free edema, exhaled breath condensate, bronchoalveolar lavage), control population, and metabolic profiling techniques. All are fairly small, involving fewer than 50 ARDS patients in any individual study. Although an ideal control population might have respiratory failure and a condition that mimics the hypoxia of ARDS, such as hydrostatic pulmonary edema or pneumonia, in practice, most studies used convenience or non-critically ill controls. Not surprisingly, the ARDS-associated metabolites in such disparate populations are far from conclusive in terms of either pathway or individual metabolite. Further, given the extent of heterogeneity in sample type and control population, these data are not amenable to meta-analysis. Larger ARDS metabolomics studies are needed, with a focus on careful phenotyping and consistent sample preparation methods.

Table 18.4 Published metabolomic studies in ARDS

Author, year	Sample type	*N* Cases/ control	Control population	*N* Metabolites profiled	Major findings	Ref
ARDS vs control						
Schubert, 1998	Exhaled breath	19/18	Surgical ICU patients	9	Decreased isoprene	[142]
Stringer, 2011	Plasma	13/8	Healthy	40	4 metabolites: total glutathione, adenosine, phosphatidylserine, sphingomyelin	[143]
Rai, 2013	Mini-BAL	21/9	Ventilated ICU	>100	11 metabolites including arginine, glycine, aspartic acid, glutamate, muscle breakdown	[144]
Evans, 2014	BAL	18/8	Healthy	>500 untargeted	37 metabolites; amino acid metabolism, glycolysis/ gluconeogenesis, fatty acid synthesis, phospholipids, purine metabolism	[145]
Bos, 2014	Exhaled breath	23/30 19/29	Ventilated ICU (some with CHF or pneumonia)	>500	Octane, acetaldehyde, 3-methylheptane; improved ability to discriminate ARDS	[146]
Singh, 2014	Serum	26/19	Ventilated ICU	>100	Many differ, focus on lipids	[147]
Rogers, 2017	Undilute edema fluid	16/13	Hydrostatic edema	>700	2 subsets of ARDS, higher mortality	[148]
Izquierdo-Garcia, 2018	Serum	12/18 13/13	H1N1 without ARDS	>100	7 metabolites: 90% discrimination of ARDS; correlates w SOFA	[149]
Within ARDS variability						
Viswan, 2017	Mini-BAL	36 ARDS 23 severe vs 12 non-severe		29 metabolites	6 metabolites ➔ ~80% classification	[150]
Maille, 2018	Plasma	30 ARDS 22 survivors vs 8 fatality		359 lipids	90 lipids, >90% increased	[151]

For each study the biofluid profiled (sample type), the number of ARDS cases and controls (*N* cases/controls), characteristics of the control population, the number (*N*) of metabolites profiled, and major findings are shown
Ref reference, *Mini-BAL* bronchoalveolar lavage using small volume of saline, *BAL* bronchoalveolar lavage, *H1N1* a strain of influenza known to be virulent and an ARDS precipitant

Conclusion

Despite unique challenges in sepsis and ARDS, the past 10 years have shown substantial advances in the prospect of precision medicine for these deadly diseases. Large-scale gene expression and targeted proteomics plasma studies are identifying biologically distinct patterns of expression that at least retroactively identify a differential response to routine treatments applied in the ICU. Once metabolomics and proteomics approaches become more standardized, investigators may identify additional biomarkers for use in clinical trials that serve either as enrollment criteria to enrich for high-risk subgroups or for potential predictive enrichment to select a population for whom an intervention is more likely to have a positive effect. Prospective randomized trials based on biologic classification will be a reality in the near future, and the era of critical illness precision medicine might thus begin.

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