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Ch 5. Translational research in ICU: novel approaches for drug development and personalized medicine

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Abstract

The major clinical presentations seen by critical care physicians are sepsis and acute respiratory distress syndrome (ARDS), both of which are heterogenous clinical syndromes rather than specific diagnoses. The current diagnostic criteria provide little insight into the mechanisms underlying these heterogenous syndromes and minimal progress has been made with regard to the development of therapies despite many large randomized controlled trials being undertaken. This review outlines the advances made in improved characterization of critically ill patients, using ARDS as an exemplar, and highlights the need for this improved patient characterization to be coupled with mechanistic science to develop therapies that target specific pathomechanisms.

Keywords:

Acute respiratory distress syndrome; sepsis; phenotype; endotype; biomarker; transcriptome

Critical care syndromes

The major clinical presentations seen by critical care physicians are sepsis and acute respiratory distress syndrome, both of which are heterogenous clinical syndromes rather than specific diagnoses (1,2). More recently, persistent critical illness, or post-ICU syndrome, has been described, which is also heterogenous in nature (3). This review will focus on how precision medicine approaches may be used to improve the development of therapies for critically ill patients, using acute respiratory distress syndrome as an exemplar.

Acute respiratory distress syndrome

Acute respiratory distress syndrome (ARDS) was first described more than 50 years ago as a syndrome characterized by refractory hypoxemia in association with reduced pulmonary compliance in the presence of diffuse alveolar infiltration on a chest radiograph (4). It was not until 1992 when a unified definition of the syndrome was agreed upon (5) (Table 1).

There were felt to be several issues with the American European Consensus Conference (AECC) criteria, including the lack of specification of a time period for the 'acute onset', the absence of accounting for the interplay between positive end expiratory pressure (PEEP) and oxygenation status, the declining use of pulmonary artery catheterization, and the lack of requirement for a risk factor for ARDS, such that in 2011 a new international consensus committee was convened, which produced a revised criteria that attempted to address these questions (6).

ARDS is common, with approximately 190,000 cases and 74,000 deaths per annum in the USA (7). Following introduction of the Berlin definition, an international observational study (LUNG SAFE) was conducted in over 50 countries and showed that >10% of ICU

patients satisfied ARDS criteria (8). Mortality for patients with severe ARDS during this study was 46.1% (95% CI 41.9%-51.4%), which was no improvement on that described 50 years previously. The Extracorporeal Life Support Organization (ELSO) registry report from 2016 showed that even where patients are able to receive extracorporeal membrane oxygenation (ECMO), the mortality for ARDS patients remains at 46% (9).

ARDS can be categorized as being primary (e.g. bacterial or viral pneumonia, direct lung trauma), or secondary in nature (e.g. non-pulmonary trauma, abdominal surgery, non-pulmonary sepsis). Primary and secondary causes are also sometimes referred to as direct or indirect causes. Neither the primary causes of ARDS, nor severity of hypoxemia are independently associated with clinical outcomes. Instead, the factors that are independently associated with mortality tend to be non-modifiable, and include older age, malignancy and non-pulmonary organ failure.

For patients that survive the acute phase of ARDS, there lies ahead a recovery that may be hampered by the effects of long-term critical illness. ARDS survivors often suffer with persistent physical, physiological, and neurocognitive deficits, which prevent recovery to their pre-morbid function. As many as 66% of survivors fail to recover their exercise capacity to pre-morbid levels even 1-2 years after ICU discharge (10,11). There are significant variations in the results of pulmonary function tests following recovery from ARDS, with the consistent identified abnormality across multiple studies being mildly impaired diffusion factor. Cognitive impairment, post-traumatic stress symptoms, anxiety and depression are recognized as common in survivors, and there is a growing need to address the interventions and therapies that might be contributing to the neuropsychological dysfunction (12).

Health-related quality of life (HRQL) scores from ARDS survivors show consistent decrements in both physical and mental health domains. These data are based on outcomes over the first two years after ICU discharge, and the results for ARDS patients are similar to those of the general ICU survivor population (13). The burden of ARDS on families and caregivers (psychological, physical and financial) is often not highlighted during the reporting of epidemiology or outcomes for these patients as there is rarely longer term (>5 year) follow up (10,14). Neuropsychological disorders contribute more to caregiver burden then physical impairment in this population (15). Thirty-one percent of ARDS survivors who were previously employed never returned to work, and 77% reported lost earnings at 5 years (16). ARDS therefore poses a significant burden to society given the loss of productive economic output of the patients and those who care for them.

Pathophysiology

Acute inflammation affecting the alveolar-capillary membrane is the primary finding in ARDS. Increased permeability of the endothelium permits egress of neutrophils and proteinaceous fluid into the airspace resulting in pulmonary edema and a neutrophilic alveolitis. This combination of activated neutrophils and inflammatory exudate damages pneumocytes and inactivates surfactant, causing distal airspace collapse with progressive loss of the available surface area for gas exchange. The inflammatory processes also inhibit hypoxic pulmonary vasoconstriction, which would otherwise regulate the pulmonary vascular tone to prevent shunting of deoxygenated blood into the systemic circulation. The resulting hypoxemia is compounded by impaired pulmonary compliance, which leads to hypercarbic respiratory failure due to accumulation of carbon dioxide, the removal of which can no longer be controlled by increasing minute ventilation.

Efforts to address the deranged arterial blood gas tensions of oxygen and carbon dioxide can be partially mitigated by adjustment of mechanical ventilation settings (positive end-expiratory pressure [(PEEP], mean airway pressure, inspiratory time, minute ventilation) or prone positioning. Increasing airway pressures and exposing the remaining healthy lung tissue to higher energy forces is directly injurious to the lung; This manifests as worsening inflammation, and as baro-(pressure), atelecto-(repeated cycled closure and opening of lung units), volu-(alveolar stretch) trauma. Higher tidal volumes exacerbate pulmonary inflammation and have long been known to be associated with worse survival (17). Paradoxically, patients tend to die from multi-organ failure and not refractory hypoxemia.

Lung biopsies from patients with ARDS show histological changes that are described as 'diffuse alveolar damage', although only approximately half of patients have this finding at post-mortem. Diffuse alveolar damage is characterized by hyaline membrane formation and pulmonary exudates that tend to be rich in neutrophils. ARDS is very heterogeneous, both within a single patient's lung tissue and between different patients with a diagnosis of ARDS, where only some of the above features may be apparent (18).

Human experimental data has been suggested that there is a failure of the lungs to maintain their host defense/immunomodulatory role of trapping activated neutrophils, resulting in breakthrough of these dysregulated immune components into the systemic circulation. Here they cause dysfunction of other organs, most commonly the kidneys, shortly followed by the cardiovascular system and liver (19). This process does not occur in isolation however, as direct and indirect causes of ARDS may also be associated with dysregulated systemic inflammation (sepsis, trauma) or multi-organ dysfunction (major abdominal surgery,

pancreatitis) further complicating attempts to understand the processes that cause our patients to become unwell and often refractory to treatment.

Patients who are successfully supported through the early phases of ARDS may proceed to develop pulmonary fibroproliferation (20). This is associated with slow resolution of pulmonary function, fibroblast proliferation, collagen and fibrin deposition and persistent shunting with associated hypoxemia. There is an increasing recognition that fibroproliferation occurs early in ARDS, with high N-terminal peptide for type III procollagen (N-PCP-III) levels in both the sera and bronchoalveolar lavage fluid (BALF) of ARDS patients within 24 hours, and that this associated with a worse outcome (21).

Treatment for ARDS

There have been many multi-center clinical trials aimed at improving the outcomes for patients with ARDS (Table 2). Of all the interventions studied there have been only two, both supportive therapies, that were shown to improve outcomes:

– Low tidal volume ventilation (6-8 ml/kg Predicted body weight) (17)

– Prone positioning (22)

However, the recently published ROSE trial (22), which also investigated the role of cis-atracurium in ARDS, contradicts the results of the ACCURASYS trial that demonstrated improved survival with early use of neuromuscular blockade (23). Regardless of these conflicting findings, none of the interventions shown to be of potential benefit improved patient outcomes by addressing the underlying disease mechanisms of ARDS. The lack of a well elucidated biological mechanisms to provide suitable targets for the rational development of therapy has been a persistent theme in ARDS. Patient heterogeneity and

limited characterization, using the available diagnostic criteria, have only served to compound the 'noise' in the data when studying this condition. The consequence of this is persistent high mortality rates for those with severe disease (8).

ARDS is not unique in having this repeating pattern of unsuccessful interventions. Randomized controlled trials (RCTs) in other critical illnesses (acute kidney injury, sepsis, cardiogenic shock) have suffered a similar fate (42-45). Each of the promising interventions and therapies in these organ failure syndromes were based on sound physiological reasoning or disease models and expected to deliver improved patient outcomes. The value of these often termed 'negative studies' is that they may have prevented unnecessary, harmful treatments for our patients from being implemented. Examples of this include doctrecogin-alpha, which was found, post-marketing, to be associated with increased mortality (46). Hydroxyethyl starch-based fluids in septic patients, which were found to cause acute kidney injury, also fit into this category (47). The corollary to this is that there may have been a sub-population of ICU patients that would have benefited from these treatments in each of these RCTs, but they have befallen the type 2 error effect of large studies that are unable to apply their interventions to the correct patients.

It is increasingly apparent that critical illness is a collection of poorly characterized, heterogeneous clinical syndromes rather than distinct diseases. Our current, routine biochemical tests and physiological measurements are still unable to differentiate the nuances between different subtypes. Stratification is required in order to determine which groups of patients might benefit from a therapeutic intervention and those who are likely to come to harm, or we will be doomed to repeat the failures of the past 50 years. This problem is not unique to critical care patients, but given there are no definitive, diagnostic biomarkers for

many critical illnesses (ARDS, sepsis), the diagnostic uncertainty in these patients compounds the potential errors. In light of the many discarded treatments, there is an urgent need for a tailored approach. Characterization of patients based on accurate disease subtypes, whilst taking into account their genomic, physiological and biological response to acute illness is the goal. This individualization of care is often referred to as precision medicine. The expectation is the ability to seamlessly incorporate all of this multi-modal information to targeted therapy to change the disease trajectory.

Stratification attempts in ARDS

Methods used to attempt stratification of ARDS can broadly be classified into:

- Biomarkers
- Genomics
- Physiology

Biomarkers in ARDS

The FDA define biomarkers as: *"A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how a patient feels, functions, or survives."* BEST (Biomarkers, EndpointS, and other Tools) Resource. FDA-NIH Biomarker Working Group 2016.

In ARDS, efforts have focused on diagnostic biomarkers and prognostic biomarkers. Diagnostic biomarkers determine the absence or presence of a disease or disease subtype (e.g. sweat chloride in cystic fibrosis), whereas prognostic biomarkers indicate the likelihood

of a future clinical event in an identified population (e.g. prostate specific antigen and likelihood of prostate cancer progression). Predictive biomarkers are used to identify individuals that might respond differently to a given treatment or environmental exposure (e.g. thiopurine methyltransferase (TPMT) genotype or activity and risk of toxicity from azathioprine). An identified biomarker may not attribute a mechanism to the disease in question, hence positive associations and correlations must be interpreted with caution.

In ARDS diagnostic and prognostic biomarkers have been sought by measuring cytokines and chemokines in serum and in bronchoalveolar lavage fluid (BALF). The search for the ARDS equivalent of a high sensitivity troponin following myocardial injury remains elusive. The mediators that have been measured in ARDS have for the most part been present in the blood for pragmatic reasons in the critically unwell. The measured proteins can be classified as pulmonary-derived, vascular-derived, or cytokines.

Pulmonary:

- Soluble receptor for end glycosylation products (sRAGE) is highly expressed in lung epithelium, especially alveolar type 1 cells. sRAGE plasma levels in patients with severe ARDS have been shown to correlate with mortality in patients ventilated with high tidal volumes. High concentrations of plasma sRAGE is not specific to ARDS, as other pulmonary and non-pulmonary diseases are associated with elevated levels. (48)
- Surfactant protein D (SP-D) is one of four surfactant proteins. Raised concentrations correlate with ARDS mortality and tend to be higher in ARDS secondary to a direct etiology. (48)

- Raised concentrations of Krebs von den Lungen-6 (KL-6, now officially named Mucin-1) is associated with mortality in ARDS. It is large glycoprotein expressed on type II alveolar cells and is associated with lung inflammation. (48)

Vascular markers tend to relate to endothelial function or coagulation and include angiopoietin-2 (Ang-2), von Willebrand factor (vWF) and plasminogen activator inhibitor-1 (PAI-1). Raised Ang-2 plasma concentrations, in both ARDS and at-risk patients, are predictive of mortality. Elevated concentrations also correlate with the development of ARDS in trauma patients (49,50). vWF levels correlate with mortality in ARDS, although circulating concentrations are reduced in direct ARDS, where the primary injury is to the pulmonary epithelium.

The cytokines intereleukin (IL)-2, IL-4, IL-6, IL-8, IL-1b, tumor necrosis factor (TNF)-α have all been associated with ARDS and mortality. Their overlap with sepsis and other inflammatory states (trauma, burns) makes them less useful as single predictors in ARDS. A recent meta-analysis indicated that IL-8 was the most strongly associated with the diagnosis of ARDS, while IL-2 and IL-4 were strongly associated with mortality (51).

Do combinations work better?

Ware *et al.* developed a predictive model that used a combination of physiological features (APACHE III, age, number of organ failures, alveolar-arterial oxygen difference, age) with eight biomarkers (SP-D, vWF, IL-6, IL-8 TNF receptor (TNFR)-1, plasminogen activator inhibitor (PAI)-1, intercellular adhesion molecule (ICAM)-1 and protein C) in 528 patients to predict mortality in ARDS (52). They developed models in both sepsis and trauma-associated ARDS, using non-ARDS patients as controls. The strongest predictors common to

both the trauma and sepsis-associated ARDS were the combinations of APACHE-III, IL-8 and SP-D (AUC =0.834). This approach was validated by Zhao *et al.* using the same predictors in 1,538 patients; the model performed well (AUC = 0.74), but not quite as well as previously (AUC = 0.85) (53). The same group has also used a similar strategy for the diagnosis of ARDS, using 100 patients to develop a five biomarker panel (SP-D, RAGE, IL-8, CC-16 and IL-6) for diagnosing ARDS in sepsis (AUC = 0.75) (54).

Calfee *et al.* showed that measurements of single proteins performed adequately as predictive and diagnostic biomarkers. By comparing the relative concentrations of different cytokines in patients with direct (pneumonia, aspiration, thoracic trauma) and indirect (sepsis, trauma) ARDS, they found concentrations of SP-D and sRAGE to be significantly higher in direct ARDS, and angiopoietin-2 (Ang-2) to be significantly higher in indirect ARDS. These results were validated from samples collected and analyzed *post-hoc* during a multi-center study of patients with ARDS (n = 853) (55). Their findings were consistent with direct ARDS being associated with pulmonary epithelial injury, whilst non-direct ARDS was associated with endothelial inflammation. Unlike other attempts to find predictive signals in ARDS, this approach described differential biological processes, albeit crudely, compared with a purely statistically-driven, model optimization approach.

Genomics of ARDS

There have been six transcriptomic studies (all using microarrays) in ARDS and two genome wide association studies (GWAS). These have, for the most part, been small in size. The largest transcriptomic study to date was by Sweeney *et al* who investigated whole blood gene expression in 148 patients with ARDS and 268 controls, finding a set of 30 differentially regulated genes, which enriched for expression in metamyelocytes and granulocytes. The

authors attributed this signal to inflammatory and non-pulmonary processes. A seven-gene subset of the thirty performed poorly and had a low generalizability for diagnosis of ARDS. There were no new proposed mechanisms from these results. (56)

Kangelaris *et al.* investigated differential gene expression between patients with ARDS and sepsis controls (n = 57), creating two models, one of which was adjusted for age, sex, batch, type of ARDS (direct / indirect) and neutrophil counts. Fifteen genes were differentially expressed, of which four were consistently upregulated. qPCR was undertaken to confirm the higher expression of these genes. One of these genes was CD24, the granulocyte receptor for platelet P-selectin, which is involved in platelet-neutrophil interactions and was later identified in a genome wide association study (GWAS) by Bime *et al*. (see below). The other three genes were lipcocalin-2 (also known as NGAL), bactericidal permeability-increasing protein (BPI) and neutrophil collagenase (MMP-8) all of which are strongly associated with neutrophils. (57)

Howrylak *et al*. examined whole blood gene expression in 13 patients with ARDS alongside sepsis controls, finding eight differentially expressed genes, the strongest of which was the ferritin heavy chain. The role of raised ferritin was presumed to be a sign of oxidative stress in patients with ARDS, however their results did not suggest any further mechanism due to small numbers (58). Chen *et al.* used the same data collected by Howrylak and submitted to the gene expression omnibus (GEO) repository. Using different informatics methods, they found twenty differentially expressed genes (12 upregulated, 8 downregulated). Following enrichment, they focused occludin (OCLN) and HLA-DQB1. OCLN is a membrane protein involved in tight junction assembly which may be influenced by TNF-a and IL-18 signaling HLA-DQB1 is a major histocompatibility complex class II protein (59).

Dolinay *et al* also undertook whole blood gene expression on 88 patients with sepsis and ARDS. The focus of their study was on validating the role of inflammasomes and IL-18 in ARDS using a mouse model. Results from the expression data confirmed the high expression of inflammasome related genes (caspase-1 and ASC) in ARDS compared with sepsis. Kangelaris *et al.* attempted to replicate these findings but found that relative expression of IL-18 to be reduced (57,60).

Juss *et al.* compared the transcript profiles of purified blood neutrophils in from patients with ARDS to those of healthy volunteers (n = 12) and found 1,319 differentially expressed genes. This reduced to 216 differentially expressed genes when the healthy volunteer neutrophils were treated with granulocyte-macrophage-colony stimulating factor (GM-CSF) *ex vivo*. There was an interesting overlap between the upregulated, differentially expressed genes from these neutrophils and the results from leucocyte gene expression of burns patients. (61)

Two GWAS have been published in patients with ARDS. Christie *et al.* identified 159 enriched single nucleotide polymorphism (SNPs) in 812 patients following major trauma (600 discovery, 212 validation) (62). One locus, PPFIA1, was significant following expression quantitative trait loci (eQTL) analysis of a B-lymphoblastoid cell line. This result was of nominal statistical significance and no polymorphism had genome wide significance. PPFIA1 encodes liprin-a which is involved in cell adhesion and cell-matrix interactions.

A second GWAS undertaken in 232 African-American patients with ARDS identified an intragenic SNP in SELPLG as being associated with increased susceptibility This gene encodes P-selectin glycoprotein ligand-1 (PSGL1) which was subsequently validated in

murine models of ventilator-induced and lipopolysaccharide-induced lung injury (63). This protein had previously been identified by Kangaleris *et al.*, which offered some validity to this finding (57). Although both GWAS highlighted some potential insights into ARDS, they require considerably more work before the results might be translated into a diagnostic tool or potential target for new treatments.

Physiology/Clinical variable-based subtypes of ARDS

Attempts to score, stratify or predict outcomes in patients with ARDS based on clinical variables have not been shown outperform existing scoring systems (APACHE-III, SAPS, SOFA). Even the Berlin definition is a relatively poor predictor of outcomes (64,65). Villar *et al.* described four phenotypes based on a combination of PaO2/FiO2 ratio <150 mmHg (20 kPa) and PEEP >10 cmH2O (66). This differs from the Berlin definition which stratifies patients at PaO2/FiO2 ratios of 300 mmHg (40 kPa), 200 mmHg (26.7 kPa) and 100 mmHg (13.3 kPa) whilst receiving PEEP >5 cmH2O. This method was more predictive of mortality if applied at 24 hours after ICU admission. Groups with more severe respiratory failure and higher PEEP requirements had a significantly increased mortality, but they also had worse APACHE scores and higher incidence of multi-organ failure. The authors acknowledged this in their discussion, but did not incorporate these covariates into their analysis or make adjustments for them. Bos *et al.* (2016) used this scoring system for patients with ARDS in the Netherlands and found the phenotypic groups to align with 30-day mortality. (67)

From a patient perspective, an important finding by Wang *et al.* was that although acute physiological derangement might predict short term outcomes (ICU / 30-day mortality), these factors fail to predict 1-year mortality (68). In their multivariate model, the strongest predictors of 1-year survival were age, co-morbidities and discharge destination. They found

patients admitted to ICU had a 24% hospital mortality, reflecting the improvements in supportive care, but 41% 1-year mortality.

Each of the methods described above (biomarkers, genomics and physiology) have similar problems of low predictive validity and poor choice of control populations/comparator groups. Low predictive validity arises where the study findings cannot be externally validated. A recurring issue with control populations is that septic patients have been used as the comparator in many of these studies, with minimal appreciation of the inherent heterogeneity within sepsis itself. Treating the septic control patients as a 'statistically static target' seems flawed. These patients were not equivalent to controls from an *in vitro* experiment or animal model. Adjustment for clinical covariates, or stratification by clinical variables will not mitigate these differences if they do not reflect the underlying biology. Patients with different biological processes can arrive at the same physiological endpoints e.g. PaO2/FiO2 ratio < 100 mmHg / vasoplegic shock / high SOFA score as any practicing ICU physician could easily demonstrate.

Bos *et al.* have recently been able to demonstrate ARDS endotypes within a heterogeneous sepsis population (69). In this study, patients with sepsis from the MARS cohort, with and without ARDS, were characterized into hyper-reactive and hypo-reactive subtypes based on their cytokine profiles. Differential gene expression, from whole blood transcriptome microarray analysis between the patients in these two subtypes, revealed a number of processes that might be at work to differentiate uninflamed and reactive ARDS. However, this study failed to acknowledge the degree of overlap between the sepsis and ARDS patients, which was apparent in their k-means figure. Here the k-means-based medioids of the first two principal components of the differentially expressed genes were plotted against

each other to differentiate uninflamed ARDS / reactive ARDS / sepsis (as a single entity) from healthy controls. The medioid derived from the sepsis patients was based on the entire sepsis transcriptome, which showed more variation than the ARDS subsets derived from this data.

Successful endotyping approaches in other diseases

Diseases that might have previously been well characterized by their symptoms, natural history, clinical signs and investigations are now being recognized as collections of heterogeneous variants with different underlying pathophysiology. Examples include asthma, chronic obstructive pulmonary disease (COPD) and breast cancer. The opportunity to better describe these syndromes has emerged due to complimentary improvements in scientific (genomics, high sensitivity assays) and computational (high dimensional data analysis, neural networks) methods all of which are increasingly accessible.

Detailed biological data can now be combined with symptomatology and treatment information into models that identify patient sub-clusters that might not otherwise have been apparent. The characteristics that these specific subgroups share are better enriched for mechanisms than grouping patients based on observable features alone (disease phenotypes). This might account for the failures of genetic studies to reveal new disease insights in asthma or COPD outside of rare subsets (e.g. alpha-1 antitrypsin deficiency). Phenotypes that describe specific pathobiological mechanisms are referred to as ‘endotypes’. As an illustrative example, sickle cell disease could be considered an endotype of anemia. Hinks *et al.* have shown that whilst clinically-based phenotypes fail to stratify patients, mechanistically plausible endotypes of patients with asthma can be identified (70).

Breast cancer

Molecular features (hormone receptors, HER2 status) have long been known to be associated with outcomes in breast cancer. Dawood *et al* developed a composite multi-variable model of immunohistochemical features, which they were able to validate in a large cohort of 1,957 patients. They found five distinct tumor phenotypes of which 'luminal A' had a worse prognosis than the other four (71). The advantage of this approach was that it could be carried out on preserved histological samples, without the need for gene expression profiling using microarrays. The same group also found that ductal carcinoma in situ (DCIS) displayed the same five phenotypes found in invasive breast cancer but at different relative frequencies in a sample of 2,897 patients (72).

The success of these approaches in oncology has led to development of platform trial design. Platform trials evaluate multiple treatments in a heterogeneous population and assume that treatment effects might also be heterogeneous. Treatment groups can change over time as data from the study is evaluated, and may even be dropped if there is evidence of harm or futility. The advantage of this approach is that it enriches the treatment groups for patients that might be more likely to benefit from a particular treatment or intervention (response-adaptive randomization). Lower numbers are required in each group, and unsuccessful interventions can be discarded earlier, which is more economical for sponsors whilst reducing the number of patients being exposed, unnecessarily, to adverse events. These studies are dependent on specialized Bayesian statistical methods whereby new information collected by the trial is used to inform allocation of patients to intervention arms.

The I-SPY2 trial is an example of a platform trial in breast cancer, where patients are stratified using biomarkers (estrogen receptor, progesterone receptor, HER2 status,

microarray results) and are randomized to receive either paclitaxel with one of three new drugs, or paclitaxel and trastuzumab with one of three new drugs as neoadjuvants prior to surgery (73). I-SPY2 has led to six new investigational treatments being advanced to phase 3 trials with each drug matched to biomarker signatures where they were most efficacious (74).

Similar approaches are now being pursued in intensive care medicine and infectious diseases. The PREPARE research network is an EU-funded platform collaboration designed to offer a rapid clinical research-based response to new or re-emergent epidemics (75). The MERMARIDS-ARI study is a PREPARE-funded, observational platform study of acute respiratory infections in 2000 adults that finished recruiting in April 2019.

Sepsis

Two recent studies have used whole blood RNA sequencing to identify endotypes in patients with sepsis, one from the Molecular Diagnosis and Risk Stratification of Sepsis (MARS Consortium) and the other from the Genomic Advances in Sepsis (GAinS) study (76-78). The MARS consortium used consensus clustering to find the best clustering method, and random forests to select the best genes that classified each endotype. Using the 140 gene-based classifier they found four groups that they labelled Mars 1-4. 306 patients were used in the discovery cohort, and their findings were validated externally using another cohort from the Netherlands (n = 206) and results from the GAinS study (n = 265, in this paper).

The Mars-1 endotype was found to have the worst 28-day survival, and was the most consistent across the validation cohorts in terms of mortality. Using combinations of the identified genes in the 140-gene classifier and gene expression ratios, they attributed two top performing genes to each endotype. In Mars-1 these were bisphosphoglycerate mutase

(BPGM) and transporter 2, ATP binding cassette subfamily B member (TAP2). BPGM is a 2,3-diphosphoglycerate, which modulates oxygen affinity to hemoglobin. TAP2 is a member of the superfamily of ATP-binding cassette transporters involved in antigen presentation. Enrichment of gene signatures differentially expressed in Mars-1 identified downregulated innate and adaptive cell functions, whilst increased expression of heme biosynthesis and aberrant functioning of metabolic pathways. The authors suggested that given the metabolic dysfunction previously described in sepsis, this endotype could represent a failure of the immunometabolic circuits leading to immunoparalysis and poor survival.

The GAinS study recruited patients admitted to ICU with sepsis due to either community acquired pneumonia or feculent peritonitis. Using blood transcriptomics from 265 patients they were able to identified a sepsis response signature (SRS) associated with higher mortality, and a T-cell exhaustion, immuno-incompetent phenotype; this was conducted using hierarchical clustering on the 10% most variable gene probes, followed by fitting of each cluster to mortality using sparse generalized linear models. 41% of the patients were categorized as SRS-1. Validation was in a separate 106 patient cohort. Enrichment and pathway analysis of the 3,080 differentially expressed genes showed functional differences related to T-cell activation, apoptosis, phagocyte movement, endotoxin tolerance and hypoxic response. A seven gene subset was identified as being predictive of SRS-1 and then successfully used to validate endotype assignment in the validation cohort of 106 patients. Similar outcomes in terms of organ failure and mortality were observed in the validation SRS-1 group. The authors proceeded to investigate genomic-level modulation of sepsis by using their gene expression results as a quantitative trait for cis- and trans-eQTL mapping.

Although these methods enriched for known immune-related pathways and genes (PI3K signaling, antigen presentation, mitochondrial dysfunction), they were unable to reproduce an association with the intronic FER variant that was described in their previously published GWAS of septic patients (79). Of note is the finding that the Mars-3 and SRS-2 endotypes, both low risk groups, correlated well with each other. Both were characterized by heightened expression of genes predominantly involved in adaptive immune functions, adding a degree of external validity to both of these studies.

Seymour *et al.* have recently described four sepsis phenotypes (a, b, g, d) derived from a combination of pooled observational studies and randomized controlled trials (RCTs; PROWESS, ProCESS, ACCESS) of patients with sepsis (80). The PROWESS, ProCESS and ACCESS trials were all RCTs undertaken in septic patients where activated protein-C, goal directed fluid therapy and eritoran respectively were investigated. Clinical variables were combined with 27 protein biomarkers and the optimum number of phenotypes was derived using a combination of consensus k-means clustering, and methods called OPTICS (ordering points to identify clustering structure). Genomic data were not included in this analysis. Latent class analysis (LCA) was used as an independent, confirmatory method to determine the optimal phenotype number, based on Bayesian information criterion and posterior probabilities. The mean values for standardized variables in each group were consistent when comparing the different phenotypes, whether they had been derived using the consensus k-means or LCA methods.

The a and d phenotypes were well separated for short-term mortality outcomes. The authors suggested that the d phenotype, which was associated with poor outcomes, cardiovascular and liver dysfunction, aligned with the SRS-1 and Mars-2 endotypes from the GAinS and

MARS sepsis studies. The a phenotype, which was associated with better short-term outcomes aligned with the SRS-2 and MARS-2 endotypes. Simulations were constructed to determine the outcomes of patients that might have been randomized to the PROWESS and ACCESS trials. They conducted this analysis by enriching baseline characteristic of patients for a given endotype, before running a simulation of the trial. They compared the expected difference in mortality with the same simulation, only where the baseline characteristics had not been enriched for a particular phenotype. The results of these simulations suggested that patients with the d phenotype, would have suffered harm from eritoran and goal-directed therapy, whilst the a phenotype would have had better outcomes with goal-directed fluid management. Given that the endotypes were derived from clinical variables and biomarkers, and did not require analysis of gene expression, they might be amenable to use in future platform studies.

Successful Endotyping Approaches in ARDS

Calfee *et al.* demonstrated that by using latent class analysis (LCA) on the physiological, biochemical and cytokine data from the ARDSnet ARMA and ALVEOLI trials, two endotypes, which they declared as hyperinflammatory and hypoinflammatory, could be identified (81). Latent class analysis is a structural equation model-based method of using latent (hidden) structure to explain outcomes. It has been widely used in the social and psychological sciences, where it has been used to predict social behaviors, voting trends, and psychopathology, usually from survey data. The hyperinflammatory group, who constituted one third of the enrolled patients, was characterized by higher IL-6, soluble TNF receptor-1 (sTNFR-1), plasminogen activator inhibitor-1 (PAI-1) concentrations, and lower bicarbonate and platelet concentrations. Importantly, the degree of respiratory failure did not differ

between the groups, which was an important finding since this had been the established method of categorizing ARDS.

Stratified treatment responses in each arm of the ALVEOLI study (high PEEP and low PEEP strategies) were identified in patients from each latent class. Patients in the hyperinflammatory group had improved mortality if they were randomized to the high PEEP arm of the study. There were no benefits of a high PEEP strategy to patients in the hypoinflammatory group. Follow up studies have applied the same methods to the FACTT and HARP-2 RCTs, and have demonstrated the existence of the same two latent classes in the patients enrolled into both of these trials. Furthermore, following *post hoc* stratification of patients into each treatment arm, they found the primary interventions to have significantly benefited the patients assigned to the hyperinflammatory class (conservative fluid strategy and simvastatin). This methodology of using LCA to assign patients in an unsupervised manner, defining classes that do not align with many of the pre-existing biases relating to ARDS (degree of respiratory failure, primary diagnosis), followed by external validation in multiple studies, sets this work apart from all others to date. (82,83)

Although LCA lends some mechanistic insights into ARDS, it is not a complete model. The same group were unable to replicate their findings in a *post hoc* analysis of the SAILS study (rosuvastatin in ARDS). Although they successfully identified two latent classes that were consistent with their previous findings, there was no benefit of rosuvastatin to patients classed as hyperinflammatory. The authors attributed this to the relative lipophilicity of different statins. In addition, one might consider a two-class model as identifying only a single subtype (hyperinflammatory), whilst assigning the rest to an alternative group. Although the two class LCA model performed best in each of the studies it was applied to, as

determined by Bayesian information criterion, this method did not capture any of the heterogeneity in or explain any features of the larger, hypoinflammatory group. (84)

Other studies have also used latent class analysis in the attempt to describe ARDS endotypes; Reilly *et al.* retrospectively studied 1,245 major trauma patients with an injury severity score (ISS) >15, of which 394 developed ARDS (189 derivation, 205 validation) (85). They used LCA to determine three classes, which were principally defined by the time from admission of developing ARDS. The model was simplified to two groups, using 48-hours as the threshold for defining early or late onset ARDS. The early group were defined as being more likely to have had thoracic injury, lower blood pressure and have received a blood transfusion. This group had higher Ang-2 and sRAGE levels, but only the Ang-2 level was significantly higher after multiple comparisons correction. Mortality was similar in both groups. On validation, the thoracic injury and blood pressure features remained consistent, but the requirement for blood transfusion was not present in the early onset validation group. Details about model fit, misclassification rates and receiver operating curves were not included in the published manuscript. This study successfully identified a subset of ARDS due to hemorrhagic shock that had features consistent with the published literature on ARDS and trauma (raised Ang-2). However, their model was based on incorporating a large number of variables in a relatively small sample size, and so the frequency of clinical features and events will have been relatively low. This is relevant because variations in data quality and recording during initial acute trauma care compared with late, in-hospital care might influence this modeling. Similar to the other studies listed, there was very little exploration of the second identified class.

The key limitation of all the attempts at classification of ARDS detailed above, is that very few of them have been coupled with mechanistic studies to validate their findings and allow the elucidation of targets for therapeutic development. If we are to make substantial progress in treating critical illness of all types, we need to integrate discovery biology with improved patient characterization, so we can move towards administering the right drug to the right patient at the right time.

Table 1

	Timing	Oxygenation	Chest radiograph	Pulmonary artery wedge pressure
Acute lung injury (ALI) criteria	Acute onset	PaO_2/FiO_2 <300 mmHg, regardless of positive end expiratory pressure (PEEP)	Bilateral infiltrates see on a frontal chest radiograph	<18 mmHg or no clinical evidence of left atrial hypertension
Acute respiratory distress syndrome (ARDS) criteria	Acute onset	PaO_2/FiO_2 <200 mmHg, regardless of positive end expiratory pressure (PEEP)	Bilateral infiltrates see on a frontal chest radiograph	<18 mmHg or no clinical evidence of left atrial hypertension

Table 1: American European Consensus Conference (AECC) Criteria.

Adapted from (5)

Table 2

Timing		Within one week of a known clinical insult or new/worsening respiratory symptoms
Chest imaging		Bilateral opacities not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema		Respiratory failure not fully explained by cardiac failure or fluid overload. Need objective assessment (e.g., echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation	Mild	200 mmHg $<PaO_2/F_IO_2<$300 mmHg with PEEP or CPAP >5 cm H_2O
	Moderate	100 mmHg $<PaO_2/F_IO_2<$200 mmHg with PEEP or CPAP >5 cm H_2O
	Severe	$PaO_2/F_IO_2<$100 mmHg with PEEP or CPAP >5 cm H_2O

Table 2: The Berlin definition of acute respiratory distress syndrome.

Adapted from (6)

Study	Published	n	Intervention	Primary Outcome
ARDSnet:ARMA (17)	2000	861	Low tidal volume ventilation	Improved hospital mortality
ARDSnet:KARMA (25)	2000	234	Ketoconazole	No difference in 28-day mortality
ARDSnet:LARMA(26)	2002	235	Lisofylline	No difference in 28-day mortality
ARDSnet:ALVEOLI (27)	2004	549	High PEEP strategy	No difference in hospital mortality
Taylor *et al* (28)	2004	385	Nitric Oxide	No difference in VFD at 28 days
ARDSnet:LaSRS (29)	2006	180	Methylprednisolone	No difference in 60-day mortality
ARDSnet:FACTT (30)	2006	1000	Liberal or conservative fluid strategy	No difference in 60-day mortality
ACURASYS (24)	2010	340	Cis-atracurium	Improved 90-day mortality
ARDSnet:Omega (31)	2011	272	Omega-3 fatty acid	Stopped early for futility
ARDSnet:ALTA (32)	2011	282	Albuterol (inhaled)	No difference in VFD at day 28
BALTI-2 (33)	2012	162	Salbutamol (IV)	Increased mortality, stopped early
ARDSnet:EDEN (34)	2012	1000	Trophic vs Full enteral nutrition	No difference in VFD at day 28
HARP-2 (35)	2014	540	Simvastatin	No difference in VFD at day 28
OSCAR (36)	2013	795	HFOV	No difference in 30-day mortality
OSCILLATE (37)	2013	548	HFOV	Increased mortality, stopped early
PROSEVA (22)	2013	466	Prone positioning	Improved 28-day mortality
ARDSnet:SAILS (38)	2014	745	Rosuvastatin	Stopped early for futility
LIPS-A(39)	2016	390	Aspirin	No difference in the incidence of ARDS
KARE (40)	2017	60	Keratinocyte growth factor	No difference in oxygen index at day 7
EOLIA (41)	2018	247	ECMO	No difference in 60-day mortality
ROSE (23)	2019	1006	Cis-atracurium	Stopped early for futility

Table 3: Randomized controlled trials in ARDS

VFD - ventilator free days. PEEP - positive end expiratory pressure. HFOV - high frequency oscillatory ventilation. ECMO - extracorporeal membrane oxygenation

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