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Subphenotypes in critical care: translation into clinical practice

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Summary

Despite the progression of supportive care in intensive care medicine, advancements in disease-modifying therapeutic options have been slow. Many trials conducted in intensive care medicine have failed to identify treatment benefit. One of the reasons implicated is the underlying heterogeneity of critical care syndromes. There are numerous approaches proposed to dividing these populations into more meaningful subgroups (subphenotypes), though some may be more useful than others. Clinical and biomarker-driven subclassification systems have been proposed for acute respiratory distress syndrome, sepsis, acute kidney injury, and pancreatitis. Identifying those systems that are most useful and biologically-meaningful will lead to further understanding of the pathophysiology of critical care syndromes while also allowing us to focus recruitment in future therapeutic trials to predicted responders. This review discusses recently proposed subphenotypes of critical illness syndromes and highlights the issues that will need to be addressed in order to translate them into clinical practice.

Key messages

- A variety of subgroups (subphenotypes) of sepsis, acute kidney injury, and acute respiratory distress syndrome patients have been identified that differ in prevalence and mortality.

- In retrospective analyses, some subphenotypes have shown differential treatment response to randomised interventions which showed no effect in the overall population.

- Mechanistic studies in subphenotypes of critical illness syndromes may allow us to better understand their pathophysiological basis and develop novel targeted therapies.

- In order to translate subphenotypes to the bedside, we will need to develop rapid real-time assays for subphenotype assignment, compare disparate subphenotyping strategies prospectively in heterogeneous cohorts, and freely share data.

Introduction

Most randomised controlled trials (RCTs) of interventions in intensive care medicine have not identified treatment benefit.[1] One potential reason is the heterogeneity of critically-ill populations and the broad defining criteria for associated syndromes.[2] In an attempt to address this problem, population enrichment methods are increasingly being utilised in trials to identify subgroups likely to benefit from treatment, thereby amplifying treatment effect, reducing noise, and reducing required sample sizes.[3] Of particular interest is predictive enrichment, a strategy that aims to identify patients with a higher likelihood of treatment response, often based on biomarkers. A recent sepsis RCT demonstrates the value of a contemporary approach to biomarker-guided predictive enrichment, using clinical measures of coagulopathy to target treatment with thrombomodulin.[4] The use of robust approaches to subdivision based on biomarker panels is an imminent development in critical care, and will radically change the research landscape in the near future.

In recent years, the rise of genomics, transcriptomics, proteomics, and metabolomics coupled with growth in data analytic tools has seen an exponential growth in the identification of novel disease subgroups (subphenotypes) that has led to numerous clinical and biological insights into acute respiratory distress syndrome (ARDS),[5-18] sepsis,[19-37] and acute kidney injury (AKI).[38,39] The advent of these subphenotypes offers the tantalizing prospect of delivering precision-based critical care medicine, as evidenced by data in other fields, such as oncology[40,41] and asthma,[42-44] where similar approaches have been successfully applied. If identified critical care subphenotypes are successfully translated into clinical practice, they could be used to facilitate prospective clinical trials of targeted treatments, allow further understanding of disease classification and pathophysiology, and potentially lead to the clinical use of precision treatments that reduce morbidity and mortality for critical care syndromes.

This review first aims to summarise recent advances in the identification of subphenotypes of critical care syndromes. It then examines in detail correlating and discordant data from different research groups, discusses the implications of identified subphentoypes to future clinical trials and practice, identifies barriers to their translation into clinical practice, and discusses potential solutions to these barriers.

Terminology is particularly difficult in this field. We propose the definitions of phenotype, subphenotype, and endotype presented in Table 1. The imagined application of these definitions is illustrated in Figure 1. This article follows these definitions, and we suggest that future articles follow in the interest of clarity.

THE EXISTING EVIDENCE FOR SUBPHENOTYPES

Acute respiratory distress syndrome (ARDS)

Despite numerous trials of pharmacotherapy, the management of ARDS is limited to supportive therapies. ARDS is clinically defined by the Berlin definition.[45] The heterogeneity contained within this syndromic definition may explain the lack of observed benefit in RCTs testing treatments with strong pre-clinical rationale. In accordance, methods of subdividing ARDS into meaningful subgroups have been attempted and are presented below. Some landmark studies in ARDS subphenotyping are highlighted in Table 2. A comprehensive overview of published ARDS subphenotyping studies can be found in Supplementary material: Table S1. Important upcoming studies that target or aim to identify ARDS subphenotypes are highlighted in Table 3.

Clinical ARDS subphenotypes

The concept of distinct ARDS subphenotypes based on clinical insult is long-standing. "Direct" ARDS results in local lung damage, and is usually caused by pneumonia, aspiration, mechanical ventilation, or contusion. "Indirect" ARDS occurs in the setting of systemic disorders that cause diffuse vascular endothelial damage, such as sepsis, pancreatitis, or cardiopulmonary bypass.[46] Calfee et al. described biomarker differences based on insult pattern in ARDS, showing that whilst endothelial and epithelial injury were ubiquitous, direct ARDS was characterised by a predominance of epithelial injury while indirect ARDS was characterised by a predominance of endothelial injury and inflammation.[7] Indirect and direct ARDS demonstrate divergent radiographic findings, respiratory mechanics, and histopathology, however little evidence supporting differential treatment response has been found.[47]

Alternatively, ARDS has recently been subdivided by clinical imaging as a surrogate marker of lung recruitability.[17] Prior work led to the hypothesis that ARDS localised to the lung bases

("focal ARDS") would respond favourably to low positive end-expiratory pressure (PEEP) while "diffuse ARDS" would respond favourably to high PEEP and recruitment manoeuvres.[48] The Lung Imaging for Ventilator Setting in ARDS (LIVE) study compared a personalised approach to ventilation based on computed tomography (CT) to standard lung-protective ventilation.[17] No difference in 90-day mortality was found for personalised (PEEP and recruitment manoeuvres based on CT morphology) ventilation strategies, though in subgroup analysis of the personalised ventilation limb, patients who were incorrectly classified to focal or diffuse ARDS had increased 90-day mortality. This result demonstrates the inherent subjectivity in using radiographic imaging to subclassify ARDS. More objective methods of describing subphenotypes are needed in order to avoid potential harm incurred by misclassification.

Parsing ARDS by clinical trajectory has also been suggested. A subphenotype of ARDS characterised by rapid improvement (riARDS), who no longer met Berlin criteria or were extubated within one day of study enrolment has been described in ARDS network clinical trials.[18] It is possible that this group may consist of patients who have been misclassified as having ARDS due to limitations of the Berlin definition,[49] though it is also possible that this is a novel clinical or biological subphenotype.

Though clinical classifications of ARDS allow us to conceptually map disease characteristics and rationalise currently-available supportive therapies in select subgroups, they lack a clear link to the biological mechanisms underlying the development of ARDS.

Biomarker-driven ARDS subphenotypes

Data-driven classification approaches based on biological data are yielding insights into potential ARDS mechanisms and subphenotypes. These approaches may lead to targeted treatments with loftier therapeutic goals than are possible with clinical classification systems. Unsupervised clustering analyses of large datasets of ARDS using high-dimensional biological

variables may identify subgroups that reveal underlying biological mechanisms and identify treatable traits.

The most recognised subphenotypes of ARDS are those described by Calfee et al., who identified two distinct groups using latent class analysis (LCA) of clinical and biomarker data[6] from the ARDSnet trials of lower tidal volume ventilation (ARMA)[50] and of high vs. low PEEP (ALVEOLI).[51] Latent class analysis is a type of structural equation modelling that identifies unrecognised subgroups in categorical and/or continuous data. The "hyperinflammatory" class was characterised by a higher level of circulating plasma markers of inflammation (interleukin-6 [IL-6], interleukin-8 [IL-8], soluble tumour necrosis factor receptor-1 [sTNFR-1], and plasminogen activator-inhibitor [PAI-1]), a higher use of vasopressors, a higher degree of metabolic acidosis, and greater prevalence of sepsis. The "hypoinflammatory" cohort had higher serum bicarbonate, higher protein C, higher systolic blood pressure, higher platelet count, and lower mortality.[6] These findings were confirmed in retrospective analysis of the "Fluids and Catheters Treatment Trial" (FACTT) cohort.[52] The hyperinflammatory and hypoinflammatory groups also demonstrated differential treatment response to high vs. low PEEP ventilation strategies, and fluid-liberal vs. fluid-conservative resuscitation strategies.[6,8] Of note, an erratum has been published for the Famous et al.[8] study of the FACTT cohort, correcting an exchange of subphenotype terminology that reversed their differential responses.[53] Regardless, the conclusion that these subphenotypes respond differently to fluids is unchanged.

These subphenotypes were also verified in post-hoc analysis of two clinical trials of statins in ARDS.[9,10] In analysis of the "Hydroxymethylglutaryl-CoA reductase inhibition in Acute lung injury to Reduce Pulmonary dysfunction" (HARP2) cohort,[54] the hyperinflammatory subgroup had increased 28-day survival when randomised to simvastatin.[9] Although subphenotypes were again identified in the ARDSnet "Statins for Acutely Injured Lungs from Sepsis" (SAILS) cohort,[55] a differential survival benefit with rosuvastatin was not identified.[10] Most recently, a 3-variable model that can be used to prospectively classify ARDS subphenotype has been

developed through analysis of five clinical trials. A model using IL-8, bicarbonate, and protein C performed best achieving an area under the receiver operating characteristic curve (AUROC) of 0·94, however different 3-variable models using other biomarkers (soluble TNF receptor 1 and IL-6) also performed well.[11] The prevalence and mortality of the hypo- and hyperinflammatory subphenotypes are closely comparable across five RCTs.[6,8-11] Furthermore, latent class analysis revealed similar predictive biomarker panels for these subphenotypes across all analysed RCTs.

In separate work, Kitsios et al. used LCA to retrospectively identify two subphenotypes that closely corresponded to hyper- and hypoinflammatory ARDS in a prospectively-enrolled convenience sample of patients with respiratory failure.[12] The finding the hyper- and hypoinflammatory subphenotypes also exist in a population with respiratory failure not meeting ARDS criteria is especially intriguing, illustrating the limitations of clinical classification systems.[12] Similar results were recently shown by another group in a retrospective LCA of 203 patients in the FACTT cohort and 49 prospectively-enrolled ARDS patients.[13] As compared to phenotype B, phenotype A showed higher plasma levels of angiopoietin-2, IL-8, interleukin-1 receptor antagonist (IL-1RA), and IL-6 as well as higher 28-day mortality.[13]

Disparate subphenotypes in patients with ARDS, termed “uninflamed” and “reactive”, have also been identified.[14] These groups were identified in an observational cohort using cluster analysis of biomarker data only. This work utilised a simplified panel of biomarkers to classify the subphenotypes, consisting of IL-6, interferon gamma (IFNγ), angiopoietin-1/2, and PAI-1.[14] Retrospective cohort analysis later demonstrated that the uninflamed subphenotype responded preferentially to therapy with low dose macrolides as compared to the reactive group (though treatment was not randomised).[15] The same investigators, using whole blood transcriptomics and canonical pathway analysis, found notable differences in gene expression. The reactive subphenotype was associated with upregulation of genes that map to oxidative phosphorylation and cholesterol synthesis pathways. The uninflamed subphenotype was associated with

upregulation of the MAP2K4 and RAF1 dependent mitogen-activated protein kinase (MAPK) pathways, which are involved in cell proliferation, differentiation, motility and survival.[16] While this data comes from a large prospective observational study,[14] at present the uninflamed and reactive subphenotypes have been demonstrated in one cohort only and were derived using a limited set of 20 biomarkers.

It is tempting to equate the reactive subphenotype from Bos et al.[14] to the hyperinflammatory subphenotype from Calfee et al.[6] due to presumed underlying inflammatory state. In fact, the hyperinflammatory and reactive subphenotypes share characteristics such as increased circulating levels of IL-8 and PAI-1, as well as decreased serum bicarbonate.[6,8,14] Study of these similarities may lead to novel insights into ARDS biology.

Sepsis

In sepsis, limitations of clinical definitions have again been implicated in trials that showed no treatment benefit.[56] Newly outlined Sepsis-3 definitions[57] improve clarity through differentiating sepsis from simple infection and shock from hypotension, but do little to reduce heterogeneity. Some landmark studies in sepsis subphenotyping are highlighted in Table 2. A comprehensive overview of published sepsis subphenotyping studies can be found in Supplementary material: Table S2. Important upcoming studies in sepsis subphenotyping are highlighted in Table 3.

Clinical sepsis subphenotypes

Several investigators have sought to subdivide sepsis using readily-available clinical data. Zhang et al. developed sepsis subgroups using latent profile analysis (LPA), a technique similar to latent class analysis that identifies subgroups using only continuous variables.[19] They identified four sepsis subphenotypes: profile 1 (baseline group, low mortality), profile 2 (respiratory dysfunction), profile 3 (multiple organ dysfunction, highest mortality), and profile 4

(neurological dysfunction).[19] Profile 3 seemed to respond favourably to intravenous fluids in terms of mortality, while profile 4 responded poorly.

In another clinical classification, Bhavani et al.[20] identified sepsis subphenotypes using group-based trajectory modelling of repeated temperature measurements. Four subtypes were identified: hyperthermic, slow resolvers (10·2% mortality); hyperthermic, fast resolvers (3% mortality); normothermic (4·5% mortality); and hypothermic (9·0% mortality). The hypothermic group were older, while the hyperthermic, fast resolvers had higher C-reactive protein (CRP) and faster erythrocyte sedimentation rate (ESR).[20]

Recently, investigators employed k-means clustering to develop sepsis subphenotypes from clinical data at emergency department presentation.[21] A composite database comprising 47,712 patients was used to identify four subphenotypes that differed in prevalence, mortality, and clinical characteristics. The α subphenotype (33% prevalence, 2% mortality) had fewer abnormal laboratory values and less organ dysfunction; the β subphenotype (27% prevalence, 5% mortality) were older, had more chronic illness, and more renal dysfunction; the γ subphenotype (27% prevalence, 15% mortality) had more inflammation, lower albumin, and higher temperature; and the δ subphenotype (13% prevalence, 32% mortality) had higher lactate, higher transaminases, and more hypotension.[21] Further analyses suggest that subphenotypic heterogeneity of recruited patients could explain previous equivocal results in sepsis trials, though it should be noted that this study relied heavily on multiple imputation and the results should be cautiously interpreted.

In another recent study, LCA has been performed on a database of 36,390 patients to define subphenotypes based on multi-morbidity state.[22] Identified groups were: the “cardiopulmonary” (6·1% prevalence) and “cardiac” subphenotypes (26·4% prevalence), consisting of older patients with cardiopulmonary conditions; the “young” subphenotype (23·5% prevalence) consisting of young, healthy patients; the “hepatic/addiction” subphenotype (9·8% prevalence)

consisting of middle-aged patients with high rates of depression, substance abuse, and liver failure; the “complicated diabetics” subphenotype (9·4% prevalence); and the “uncomplicated diabetics” subphenotype (24·8% prevalence).[22] The highest mortality groups were the “hepatic/addiction” subphenotype, followed by the “cardiac” subphenotype, then the “cardiopulmonary” and “complicated diabetics” subphenotypes. This study is the first to apply LCA to multi-morbidity and provides robust evidence for differing clinical outcomes based on multi-morbidity cluster.

As in ARDS, subphenotypes derived using clinical data provide limited mechanistic insight. Biomarker-driven approaches to subphenotyping and unbiased statistical analyses could provide a better understanding of sepsis biology than is afforded by a clinical classification system alone.

Biomarker-driven sepsis subphenotypes

Secondary analyses of sepsis RCTs have yielded insight into biomarker-defined subphenotypes. Shakoory et al. defined a group of patients with hepatobiliary dysfunction and disseminated intravascular coagulation and re-analysed data from an RCT of interleukin-1 receptor antagonist (IL-1RA) to demonstrate that this group likely benefited from trial drug.[23] In a separate trial also focusing on the interleukin-1 pathway, Meyer et al. performed retrospective subgroup analysis on a trial of recombinant human IL-1RA in sepsis, showing that patients with a baseline high level of endogenous IL-1RA benefited from study drug.[24] This notably counterintuitive result highlights our limited understanding of the pathophysiology of sepsis. Due to the complexity of the syndrome, it is likely that a single biomarker like IL-1RA is inadequate to precisely identify subgroups. To that end, researchers have employed biomarker panels to classify sepsis into subphenotypes.

Subphenotypes of sepsis defined by biomarker panels were first described in paediatric sepsis by Wong et al.[25] Genome-wide expression of whole blood-derived RNA was employed in a prospective cohort of 98 children with septic shock.[25] Data was then subjected to unsupervised hierarchical clustering to identify three subphenotypes (A, B, C). Patients in subclass A were younger, had higher illness severity, higher degrees of organ failure, and higher mortality. Further, subclass A differed from subclasses B and C in that genes associated with adaptive immunity, glucocorticoid receptor signalling, and zinc biology were repressed.[25] Subsequently, investigators described a 100-gene signature model to distinguish subphenotypes.[26,27] This model was developed into a multiplex messenger RNA quantification platform that was prospectively tested in another cohort.[30] Mosaics representing expression patterns of the 100 subphenotype-defining genes for each patient were compared to reference mosaics and group was assigned by least difference. In this cohort no patients met criteria for subclass C. Children from subclass A had worsened mortality when prescribed corticosteroids, though allocation was non-randomised.[30] This work on paediatric sepsis subphenotypes has been translated into a protein biomarker-based classification and regression tree (CART) model for mortality risk that has been employed in children and adults.[28,29]

Alternative biomarker-derived sepsis subphenotypes have been described in adults. In a series of studies, investigators identified distinct transcriptomic subphenotypes by cluster analysis of peripheral blood leucocyte genome-wide transcription profiles in a prospective cohort of 265 adult patients with sepsis secondary to community-acquired pneumonia.[33] These findings were validated in a second independent cohort[34] and tested for differential treatment response in secondary analysis of a randomised controlled trial.[35] The first subphenotype (sepsis response signature 1 [SRS1]), had gene-expression patterns indicative of a relatively immunosuppressed pattern, suggesting endotoxin tolerance, T-cell exhaustion, and human leukocyte antigen (HLA) class II downregulation. Mortality was higher in SRS1 subphenotype

compared to sepsis response signature 2 (SRS2).[33,34] Further, in secondary analysis of an RCT, using a simplified model consisting of seven genes, investigators again identified the two subphenotypes, and corticosteroid therapy was associated with increased mortality in the SRS2 subphenotype.[35] This evidence suggests not only a clinical application but also a model that may be more feasible at the bedside.

Research from a Dutch group used machine learning and cluster analysis of whole blood genome-wide expression profiles to identify four sepsis subphenotypes, termed Mars1-4.[36] These subphenotypes were derived in a prospective cohort and subsequently validated in an adult and paediatric retrospective cohort. The Mars1 subphenotype was associated with poor prognosis and downregulation of genes associated with the innate and adaptive immune system. Mars2, which had intermediate mortality risk, was associated with increased expression of genes involved in pattern recognition (recognition of pattern-associated and damage-associated molecular patterns [PAMPs and DAMPs]), cytokine, cell growth, and mobility pathways (e.g. nuclear factor kappa B [NF- κB], IL-6, and inducible nitric oxide synthase). The Mars3 subphenotype was associated with upregulation of adaptive immune function, upregulation of T-cell function, and lower risk of mortality. Mars4, similarly to Mars2, was associated with intermediate mortality and increased expression of genes involved in pattern recognition and cytokine pathways, though different specific pathways were implicated (e.g. interferon signalling and retinoic acid-inducible gene-I-like receptors [RIG1] signalling).

Elsewhere, Sweeney et al. used a novel clustering algorithm[58] to derive subphenotypes in sepsis based on whole blood genome-wide expression profile data retrieved retrospectively from a composite of multiple small studies in adult and paediatric populations.[37] Investigators identified three subphenotypic clusters,[37] termed the Inflammopathic (innate immune activation, higher mortality), Adaptive (adaptive immune activation, lower mortality), and Coagulopathic (gene expression suggestive of platelet degranulation, coagulation dysfunction; higher mortality;

older) groups. Of note, 16% of patients in the discovery cohort were not clustered to a subphenotype. Through analysis of clinical data, investigators suggested that the Adaptive subphenotype are a less sick group, while the Inflammopathic and Coagulopathic subphenotypes split the more severe sepsis cohort into younger and older groups, respectively. This result should be interpreted with caution, however, as only 36% of patients had age and severity data available.

There are some unexpected data points around sepsis subphenotypes that require future examination. Counterintuitive results with regards to responsiveness to recombinant IL-1RA are discussed earlier.[24] Also counterintuitively, retrospective analysis of the "Vasopressin vs. Norepinephrine as Initial Therapy in Septic Shock" (VANISH) trial cohort[59] revealed increased mortality in SRS2 patients randomised to receive corticosteroids (OR 7·9, 95% CI 1·6-39·9) but found no treatment effect for SRS1.[35] While hypotheses are presented to explain these findings in the primary sources, these unexpected results highlight our limited understanding of sepsis biology.

There are overlaps and conflicts between existing biomarker-driven approaches to sepsis classification. Of note, both paediatric subclass A and adult subphenotype SRS1 have gene-expression patterns suggestive of relative immunosuppression but appear to exhibit disparate responses to corticosteroids. When given corticosteroids, children in subclass A had increased mortality.[30] A similar effect was not observed in SRS1 adults. In fact, it was the relatively immunocompetent SRS2 that exhibited increased mortality in response to steroids.[35] In other comparisons, investigators noted that the Mars3 subphenotype was correlated with the SRS2 subphenotype, with both groups demonstrating heightened expression of genes involved in adaptive immunity.[36] Similarly, Sweeney et al. observed that their Inflammopathic subphenotype most closely corresponded to SRS1 and paediatric subclass B, while the Adaptive subphenotype corresponded to SRS2.[37] Whilst the proposed similarities are

encouraging, it is noteworthy that there were also substantial discordances between subphenotype allocations in all comparisons. The interactions between existing subphenotypes raise several fundamental questions about sepsis pathophysiology, and further study herein may glean insights into its underlying mechanisms.

Acute kidney injury (AKI) subphenotypes

Acute kidney injury is another heterogeneous critical care syndrome. The current definitions[60-61] do not provide information on the biology of AKI. AKI "stages" do not accurately represent renal pathophysiology that could potentially present pharmacological targets.[62] One landmark study in AKI subphenotyping is highlighted in Table 2. A complete overview of published AKI subphenotyping studies can be found in Supplementary material: Table S3.

In 2016, Bhatraju et al. identified subphenotypes of AKI based on creatinine trajectory.[38] In secondary analysis of two prospective trials, patients with AKI were classified as "resolving" or "non-resolving" based on creatinine trajectory in the first 72 hours. Non-resolving patients had 68% higher mortality (RR 1·68, 95% CI 1·15-2·44), even after adjustment for AKI severity stage.[38] The same research group then identified AKI subphenotypes using clinical data and serum biomarkers in retrospective analysis of two clinical trials using LCA.[39] Compared to AKI subphenotype 1 (AKI-SP1), AKI subphenotype 2 (AKI-SP2) was characterised by poorer renal function, higher vasopressor use, and higher concurrence of sepsis with ARDS.[39] AKI-SP2 also showed more endothelial activation, lower bicarbonate, higher IL-6, and higher IL-8.[39] Most interestingly, in post-hoc analysis of the "Vasopressin and Septic Shock Trial" (VASST),[63] AKI-SP1 showed improved mortality with vasopressin as opposed to noradrenaline (27% vs. 46%, p = 0·02), though no benefit was observed for AKI-SP2 (45% vs. 49%, p = 0·99).[39]

Similarly to research in ARDS, some distinguishing biomarkers for AKI subphenotypes are IL-6, IL-8, and bicarbonate. This raises the question of parallels between critical illness syndromes and may suggest shared mechanisms that transcend syndromic definitions.

Acute pancreatitis subphenotypes

Neyton et al. recently used unsupervised clustering of proteomic, transcriptomic, and metabolomic data to describe four subphenotypes of acute pancreatitis dubbed "hypermetabolic", "hepatopancreaticobiliary", "catabolic", and "innate immune".[64] The hypermetabolic subphenotype exhibited increased markers of glutathione synthesis, gastrointestinal metabolism of dopamine, the tricarboxylic acid cycle, and sphingolipid biosynthesis (e.g. γ-glutamyl transferase 2 [GGT2], citrulline, and serine palmitoyltransferase subunit B [SPTSSB]); the hepatopancreaticobiliary subphenotype was associated with bilirubin glucuronidation and bile transporters; the catabolic subphenotype was associated with proteolysis and apoptosis; and the innate immune subphenotype was associated with complement regulation and immune cell adherence.[64] Pancreatitis is a "late entry" to the field of critical care subphenotyping and while these results are interesting, they are based on small sample sizes and have not yet been subject to peer review. Whether or not these groupings are robust and clinically meaningful remains unanswered. Proposed pancreatitis subphenotypes are summarised in Supplementary material: Table S4.

TRANSLATION OF SUBPHENOTYPES INTO CLINICAL PRACTICE

In critical care, we need to resolve whether we should be treating syndromes, subphenotypes, or some combination of the two. How to do this is not entirely clear and will undoubtedly require a larger body of evidence and discussion than is currently available. To this end, there has been a rapid recent growth in critical care subphenotyping studies with a number currently recruiting worldwide. Some important ongoing and upcoming studies of subphenotypes in critical care are highlighted in Table 3.[65-70]

As we move towards the era of precision medicine in critical care, numerous barriers will need to be overcome to translate our current knowledge into clinical practice. We present an overview of these barriers and potential solutions in Table 4.

Limited understanding of pathophysiology

We have a superficial knowledge of the pathophysiological mechanisms of critical illnesses, hindering their study. We can identify differences between subphenotypes in terms of biomarkers using unbiased analytical approaches, but these differences are difficult to interpret without insights into how the biomarkers interact and are regulated. This knowledge gap is apparent in the counterintuitive results in sepsis involving IL-1RA responsiveness[24] or in the divergent response to corticosteroids between paediatric subclass A[30] and SRS1.[35] We know very little about the mechanisms of critical care illnesses in general, let alone those of the proposed subtypes. While the biomarkers used to identify subphenotypes in humans may be useful to guide future study, there is currently no evidence to confirm that they are mediators rather than simply biomarkers.

In order to translate critical care subphenotypes into endotypes, studies will need to be undertaken that employ discovery approaches followed by the establishment of causality in model systems. Our proposed approach to this problem first involves collecting cell isolates of

interest (e.g. peripheral leucocytes, alveolar macrophages, glomerular cells) from subphenotyped patients and comparing whole-genome expression profiles to identify differentially-expressed genes that may represent candidate mechanistic pathways. Then studies that employ protein quantification and characterisation methods should follow in order to link this transcriptomic data to proteomic differences between subphenotypes. The use of selective inhibitors to some of these identified proteins *in vitro* and *in vivo* may then establish causality. Such an approach would require the concomitant development of *in vitro* and animal subphenotype models, none of which currently exist.

An example hypothesis comes from Bos et al.,[16] who note that the reactive subphenotype of ARDS exhibits upregulation of genes associated with neutrophil activation, oxidative phosphorylation, and mitochondrial dysfunction as compared to the uninflamed subphenotype. One could hypothesise that alveolar damage in this subphenotype is dependent on neutrophil serine proteases, such as neutrophil elastase, and test this hypothesis using fluorescence resonance energy transfer (FRET) and a specific inhibitor such as sivelestat.[16]

An alternative approach to identifying candidate mechanisms is evidenced by Jones et al., who recently used an application of Mendelian randomisation to suggest from an observational study a causal role for soluble receptor for advanced glycation end products (sRAGE) in ARDS.[71] This follows from previous work that similarly suggested a causative role for angiopoietin-2.[72] Though such an approach requires genomic data and still requires further replication, it could be co-opted for subphenotype studies in which causal inference methods are used to identify those genes that may be linked to mechanism. This information could then be used to inform *in vitro* or *in vivo* studies of selective inhibitors.

While such early studies are exciting, it is likely that mechanistic differences between critical care endotypes will be more complex than single targets and mediators.

Comparison of subphenotypes

Within syndromes, it remains unclear how much identified subphenotypes overlap with each other due to differences between the patient populations studied, clinical characteristics and biomarkers used, and methods of analysis. Some studies that have identified critical care subphenotypes use small discovery cohorts and have yet to be replicated. Furthermore, the various cluster analysis methods that have been used to identify critical care subphenotypes tend to generate different results depending on the variables chosen for analysis and the method of clustering.[73] A number of different clustering methods have been used to identify critical care subphenotypes. It is therefore possible that some described subphenotypes are spurious findings.

Disparities between identified subphenotypes are evidenced in sepsis. Of particular note is the conceptual analogy between paediatric subclass A and adult subphenotype SRS1. Both subphenotypes have characteristics suggesting relative immunosuppression, but they appear to exhibit disparate responses to treatment with steroids.[30,35] In an attempt to address this inconsistency, Wong et al. conducted analysis showing a weak positive correlation between paediatric subphenotype and analogous SRS subphenotype, though demonstrating an interaction between age and group assignment.[31]

There are also notable disparities within ARDS subphenotyping systems. In retrospective analysis of the HARP2[54] study, a treatment interaction was found, with the hyperinflammatory group exhibiting improved survival with simvastatin.[9] Conversely, in analysis of the SAILS[55] study, no treatment effect was found for rosuvastatin.[10] This discrepancy may be related to differential drug levels, differential ARDS aetiology in the two trials (sepsis-related versus all-cause), differential ARDS severity, or differential hydrophilicity of the two statins employed. Nonetheless, these data illustrate our limited knowledge of critical care subphenotypes and demonstrate that trial recruitment methods may directly influence the

results of retrospective biomarker analyses. Hence, it is important in future that the methods used to define subphenotypes are reported in detail to facilitate comparisons and analysis.

Stability of subphenotypes

A major remaining question is whether or not subphenotypes are stable over time, in response to treatment, and across multiple sampling sites and methods. It is possible that different subphenotypes represent different temporal stages in the evolution of the syndrome in question. Since some subphenotypic definitions of ARDS are based on inflammatory biomarkers, it is conceivable that changes in inflammation in response to disease course could affect the reliability of subphenotype allocation. Recently, latent transition analysis (LTA), a clustering approach used to determine movement between subgroups over time, demonstrated stability of the hypo- and hyperinflammatory groups retrospectively at day 0 and day 3 in the ARMA[50] and ALVEOLI[51] clinical cohorts.[74] Most patients assigned to a class at day 0 remained assigned to the same class at day 3 (>94%).[74] Stability will be required for recruitment to future clinical trials targeting a specific ARDS subphenotype. However, stability of ARDS subphenotypes past study day 3 and/or in response to specific treatments remains unknown.

Even more unclear are the temporal stability of sepsis subphenotypes and the stability of sepsis subphenotypes across age demographics. Wong et al. demonstrated that subphenotypes derived in children may not be clinically useful in older adults, as evidenced by the weak correlation between paediatric subphenotype and SRS.[31] These findings suggest that sepsis subphenotype may be age-dependent and response to corticosteroids may change accordingly, implying complex changes in the immune landscape with host age. Age will undoubtedly need to be accounted for in future assessment of the host immune transcriptome in sepsis and other syndromes, and further study of its role in subphenotype determination may provide insights into the underlying pathophysiology of these syndromes.

Within adult populations temporal instability is evident. In the study of faecal peritonitis by Burnham et al., 46% of patients that had serial samples moved between SRS group over time, changing gene expression profiles.[34] This result indicates that SRS group may be an indicator of current host immune state rather than representing endotypes of sepsis.

The potential influence of time on sepsis subphenotypes is demonstrated in a recent study of myeloid-derived suppressor cells (MDSCs) in persistent organ dysfunction after sepsis.[75] MDSCs are a heterogeneous group of immature myeloid cells that have been implicated in sepsis pathobiology.[76] Expansion and infiltration of MDSCs after sepsis is thought to induce persistent organ dysfunction through host immunosuppression and inhibition of lymphocyte proliferation. Surprisingly, Hollen et al. recently demonstrated that MDSC function evolves over time, with MDSCs 4 days after sepsis actually being stimulatory toward T cells. This result suggests that future precision medicine approaches in sepsis will need to consider temporal instability in immune states and may suggest that sepsis subphenotypes that are defined by immune function are in fact different points on a temporal continuum.

There is a further possibility that biomarker and transcriptomic signatures will vary with cell or tissue type sampled, limiting the generalisability of many current subphenotyping strategies. In ARDS, we do not know how accurately subphenotypes defined by blood biomarker panels reflect what is happening in the lung. In sepsis, since contemporary transcriptome analysis strategies use heterogeneous cell populations (whole blood or peripheral blood leukocytes), they could be subject to instability with disease course. If mRNAs that are used to quantify differential gene expression are expressed specifically or preferentially by one or more leukocyte subtypes, changes in differential cell count that occur with disease progression may affect stability of subphenotype allocation.

Much is left to be understood about the stability of biomarkers in critical illness, and consequently, subphenotype stability. It is possible that fluctuations in group allocation may limit the applicability of subphenotypes in recruitment to future clinical trials, though these

fluctuations may also be productively harnessed to other means. Perhaps in future, changes in subphenotype allocation could be employed to monitor syndrome progression or to monitor response to treatment.

Multi-morbidity

The problem of multi-morbidity in critical care patient will present a challenge to precision medicine.[77] Many trials in which subphenotypes have been identified exclude patients with considerable co-morbidity. It is possible that, through studying subphenotypes in retrospective analyses of RCTs, we are eliminating the complexity introduced by chronic disease and multi-morbidity, potentially limiting applicability of subphenotypes at the bedside. This issue is most pronounced in those subphenotypes that are evidenced from retrospective analyses of highly-selected clinical trial cohorts. It is also possible that biomarker-based subphenotypes are representative of differing multi-morbidity states. In a recent study, multi-morbidity subphenotypes of sepsis were recognised and assigned by LCA.[22] This approach could provide interesting insights if compared to biomarker-based classification approaches.

Diminishing returns with increasing subdivision

Deciding on the utility of further subdividing syndromes will be another issue. As we incorporate more data, there is the potential to subdivide syndromes into a large number of subphenotypes. While this increased resolution represents a closer approximation of truly individualised medicine, healthcare economics and clinical utility may necessitate a stopping point. As an extreme example, at some level every patient represents an individual subphenotype, and unique treatments for individual patients are unlikely to be feasible. Some generalisability will be required, at least in the foreseeable future of medicine, and the optimal way to subdivide syndromic conditions could depend on the treatment in question and biological plausibility.

Difficulties with speed of subphenotype assignment

For subphenotypes to become clinically-viable, real-time diagnostic assays must be available. At present, sepsis typing is limited by the time required to perform transcriptomic analysis. In paediatric sepsis, Wong et al. initially attempted to address this issue by rationalising their microarray data into an assay for 100 genes that can classify subphenotype in 8-12 hours[30] and then further simplifying to a decision tree involving four genes that is likely more clinically feasible.[32]

In ARDS, there has been significant interest in the hypo- and hyperinflammatory subphenotypes being seen across five RCTs, and their apparent interaction with treatment effect to simvastatin, PEEP, and fluid management strategies.[6,8,9] However, the latent class analysis models used in these cohorts require multiple predictor variables, making them impractical for clinical use. A simplified parsimonious model that can be used to prospectively identify ARDS subphenotype has recently been reported.[11] In order to bring prospective ARDS classification to fruition in the clinical setting, this data will have to be combined with real-time testing for associated biomarkers. Unfortunately, at present no such commercially-available test exists, though candidate point-of-care assays are in development and may bring subphenotypes to the bedside.

The need for large prospective studies

In future, we need to determine which subphenotypes are reproducible, which are spurious, which overlap, and establish their stability across patient demographics (i.e. are they the same in children and adults?). In order to answer these questions, the most compelling dataset will involve prospective validation of multiple subphenotyping strategies in large, heterogeneous patient cohorts. A single such study would allow comparisons between subphenotyping strategies and would help to delineate their overlap, interactions, and clinical applicability. Reproducing these results in other cohorts would then help to determine

subphenotype stability. Our ongoing study, "Clinical Evaluation of a Point of Care Assay to Identify Phenotypes in the Acute Respiratory Distress Syndrome" (PHIND)[65] is one such initiative that aims to do this. In addition, the "Reanimation Low Immune Status Markers" (REALISM) project aims to immunophenotypically characterise a large cohort of intensive care patients,[69] and the subsequent IMPACCT (Immune Profiling of ICU Patients to Address Chronic Critical Illness and Ensure Healthy Ageing) study aims to prospectively allocate subphenotypes of sepsis identified in REALISM and allocate them at the bedside.

Global co-operation

Prospective validation of subphenotyping strategies will require sharing of datasets as well as discriminant algorithms between investigators. International databanks could be used to identify generalisable subphenotypes. The ideal solution would be a decentralised open-access databank akin to tumour registries currently used in oncology. However, there are potential issues with data sharing, including the issues of international transferability and differences in patient privacy law. Decentralisation of health records and greater levels of cyber-security are needed before this can be fully realised.

A further issue impeding global collaboration is the reluctance of some investigators to openly share subphenotype-defining algorithms. While understandable, such competition is counterproductive. A useful approach to overcoming this barrier is the establishment of collaborative organisations for critical care subphenotyping, in which many investigators contribute to all publications (akin to the ARDSnet group), thereby facilitating recognition. Such efforts are underway as of the time of preparation of this article.

Conclusions and future directions

In this article, we have outlined recent advances in the identification of subphenotypes and their implications to the future of critical care. Numerous interesting data points have arisen

from this discussion, highlighting gaps in our knowledge and the aforementioned barriers to translating subphenotypes into clinical practice. Undoubtedly, in order to bring the promise of precision medicine to fruition, a large body of research and international co-operation will be needed.

We propose an approach for precision medicine in critical care in Figure 2. Establishing the existence of subphenotypes is only the first part of the puzzle. Currently, opportunities are arising to streamline current subphenotyping strategies, compare them, and prospectively validate them in real-time using parsimonious assays and algorithms. Then, in order to progress from subphenotypes to endotypes, and from endotypes to clinically-valuable treatable traits, we will need to undertake basic science studies that establish mechanistic differences between subphenotypes and develop treatments targeted to plausible mechanisms of disease pathology. This will require the development of new *in vitro* and *in vivo* models. We will then need to test targeted interventions prospectively, thereby attributing clinical value to subphenotypes and endotypes.

Pursuing subphenotypes may lead to the development of beneficial new treatments, provide insights into pathophysiology, and provide opportunities to identify commonalities across syndromes, leading us to redefine critical illness by biological similarity rather than clinical symptomatology. Since critical illness syndromes are often multi-system insults, there is a possibility that subphenotypes may transcend current disease definitions and describe multi-system inflammatory states, changing how we understand critical illness. There is no doubt that this is an exciting time, and we can expect a strong focus on subphenotypes in critical care research in the coming years.

Search strategy and selection criteria

References for this review were identified through searches of PubMed (MEDLINE) for articles published before January 1, 2020 by use of the terms “critical care”, “intensive care”, "ARDS", "AKI", "pancreatitis", "sepsis", “phenotype”, “sub-phenotype”, and “endotype”. Primary research and reviews resulting from this search and relevant references cited in those articles were included. The initial search was conducted in May 2019. The search was updated in November 2019 and in January 2020.

Contributors

KR prepared the first draft and subsequent revisions of the manuscript. PS, CO'K, ACG, CSC, and DFM contributed to the writing of the manuscript, reviewed and edited the manuscript, and approved this final version of the manuscript.

Declaration of Interest

KR has no conflict of interest to declare.

PS has no conflict of interest to declare.

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Table 1: suggested definitions of “phenotype”, “subphenotype”, “endotype”, and “treatable trait” in future critical care literature. These definitions draw from similar literature in asthma, as described by Lötvall et al.[42]

Phenotype Clinical features of a group of patients who share a common syndrome/condition. *e.g.* the Berlin definition of ARDS
Subphenotype A group of patients sharing a phenotype that has a different shared risk factor, trait, diagnostic feature, expression marker, mortality risk, or outcome in response to treatment as compared to other subphenotypes. *e.g.* hypoinflammatory versus hyperinflammatory ARDS, or SRS1 versus SRS2
Endotype A subgroup of patients that shares a biological mechanism of disease and anticipated response to treatment, which may be indicated by shared mortality risk, clinical course, or treatment responsiveness. As we know little about the mechanisms of critical illness, true endotypes do not yet exist in critical care. *e.g.* allergic asthma versus aspirin-sensitive asthma versus late-onset hypereosinophilic asthma[42]
Treatable trait A subgroup characteristic that can be successfully targeted by an intervention. *e.g.* the BRAF V600E mutation (substitution of glutamic acid for valine at position 600 in the BRAF gene) of melanoma being targeted by vemurafenib[41]

Table 2: Select landmark studies identifying subphenotypes in ARDS, sepsis, and acute kidney injury. This table highlights the significance of each study and compares subphenotype prevalence, mortality, and differential treatment effect. Biomarker-driven studies were chosen for this table based on novelty, number of citations, relative contribution to the field, and demonstration of differential treatment effect. Comprehensive analyses of subphenotyping studies in ARDS, sepsis, AKI, and pancreatitis are available in Supplementary material: Tables S1-4.

Syndrome	Study	Significance	Subphenotypes (prevalence %)	Mortality (%)	Differential treatment response
ARDS	Calfee et al. (2014)[6]	First to identify hyper- and hypoinflammatory subphenotypes in ARDS	hypoinflammatory (67-74%); hyperinflammatory (26-33%)	hypoinflammatory (19-23%); hyperinflammatory (44-51%)	Differential response to high and low PEEP ventilation strategies for hypoinflammatory and hyperinflammatory ARDS
ARDS	Calfee et al. (2018)[9]	First to demonstrate differential response to pharmacologic treatment in ARDS	hypoinflammatory (65%); hyperinflammatory (35%)	hypoinflammatory (22%); hyperinflammatory (46%)	Higher 28-say and 90-day survival with simvastatin in hyperinflammatory ARDS
ARDS	Bos et al. (2017)[14]	First to identify uninflamed and reactive subphenotypes in ARDS	uninflamed (48%); reactive (52%)	uninflamed (21·6-22%); reactive (37·7-39·1%)	Not tested
Sepsis	Wong et al. (2009)[25]	First to identify subphenotypes in (paediatric) sepsis	subclass A (29%); subclass B (46%); subclass C (26%)	subclass A (36%); subclass B (11%); subclass C (12%)	Not tested
Sepsis	Wong et al. (2015)[30]	First to demonstrate differential response to corticosteroids in sepsis	subclass A (34-48%); subclass B (52-66%)	subclass A (17-21%); subclass B (5-10%)	Increased mortality in subclass A when prescribed corticosteroids
Sepsis	Davenport et al. (2016)[33]	First to identify subphenotypes of adult sepsis	SRS1 (35-41%); SRS2 (59-65%)	SRS1 (22-59%); SRS2 (10-29%)	Not tested
Sepsis	Antcliffe et al. (2019)[35]	First to demonstrate differential response to corticosteroids in adult sepsis	SRS1 (47%); SRS2 (53%)	SRS1 (33-37%); SRS2 (8-42%)	SRS2 mortality increased with hydrocortisone
Sepsis	Scicluna et al. (2017)[36]	First to describe Mars subphenotypes	Mars1 (13-29%); Mars2 (34-44%); Mars3 (23-37%); Mars4 (6-13%)	Mars1 (28·6-43·3%); Mars2 (16·2-26·7%); Mars3 (7·2-28·2%); Mars4 (5·3-32·5%)	Not tested
AKI	Bhatraju et al. (2019)[39]	First to describe biomarker-derived subphenotypes in AKI	AKI-SP1 (58-63%); AKI-SP2 (37-42%)	AKI-SP1 (6-24%); AKI-SP2 (25-43%)	Mortality decreased with vasopressin as opposed to noradrenaline in AKI-SP1

Table 3: Ongoing and upcoming studies in critical care subphenotyping. Many of these studies aim to prospectively validate or define subphenotypes. Of note, the PHIND study will prospectively study a rapid assay for subphenotype allocation that may be clinically viable.[65] The ProCoCo study is the only study that will target treatment to subphenotype, though the subphenotypes used are investigator-defined based on hypothesised response to corticosteroids.[66] Upcoming and ongoing studies were identified for this table from a search of clinical trial registries and included based on novelty.
*The IMPACCT study is currently not referenced online, though it is funded and in the recruitment phase.

Syndrome	Study	Design	Novelty	Recruitment countries and period
ARDS	Clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome (PHIND)[65]	Multicentre prospective cohort (480 patients)	Prospectively validating hyper- and hypoinflammatory subphenotypes and allocating them at the bedside	UK and Ireland; currently recruiting
ARDS	ProCollagen-3 driven Corticosteroids for persistent acute respiratory distress syndrome (ProCoCo)[66]	Multicentre randomised controlled trial (356 patients)	Targeting corticosteroid administration to subphenotype (procollagen III-high) in a randomly-allocated parallel arm study	France; currently recruiting
ARDS	Linking Endotypes and Outcomes in Paediatric Acute Respiratory Distress Syndrome (LEOPARDS)[67]	Multicentre prospective cohort (500 patients)	Identifying subphenotypes in paediatric ARDS	USA; not yet recruiting
ARDS	Identifying PARDS {paediatric ARDS) endotypes[68]	Single-centre prospective case-control (60 patients)	Correlating nasal and bronchial epithelial gene expression to serum biomarkers and determining their efficacy in subphenotype identification	USA; currently recruiting
Sepsis	The REAnimation Low Immune Status Markers (REALISM) project[69] and IMmune Profiling of ICU pAtients to address Chronic Critical illness and ensure healThy ageing (IMPACCT)*	Initial single-centre prospective cohort of 160 patients with sepsis (REALISM) followed by multi-centre prospective cohort (IMPACCT)	Two stage process, clarifying optimal markers to identify immunosuppressed subphenotypes in sepsis and then prospectively validating and allocating them at the bedside	UK, France and Sweden; not yet recruiting
Sepsis	Stress Hydrocortisone In Paediatric Septic Shock (SHIPSS)[70]	Multicentre randomised controlled trial (1032 patients)	Examining differential response of paediatric subphenotypes A and B to steroids in a randomly-allocated fashion (exploratory outcome only)	USA and Canada; currently recruiting

Table 4: An overview of potential barriers to clinical implementation of subphenotypes in critical care and their possible solutions.

Barrier	Solution(s)
Limited understanding of critical illness pathophysiology	Targeting mechanistic studies at biomarkers identified by unbiased, "bottom-up" approaches to syndrome classification (e.g. latent class analysis) Transcriptomic analysis of subphenotypes, followed by studies identifying candidate protein mediators, followed by causal studies in model systems Development of novel animal models or appropriation of animal models (e.g. identify existing model with transcriptomal changes similar to an identified human subphenotype)
Unclear overlap and correlation between existing subphenotypes	Validation of similar subphenotypes in large prospective cohorts (e.g. Mars and hypo-/hyperinflammatory ARDS in one cohort)
Stability of subphenotypes	Repeated prospective cohort studies validating subphenotypes that differ in disease stage and severity Repeated subphenotype assignment at multiple time points in prospective cohorts Comparisons of subphenotype-defining biomarker panels across varying tissue types (e.g. blood vs. lungs in ARDS)
Multi-morbidity	Validation in large prospective cohorts with few exclusion criteria
Diminishing returns with increasing subdivision	Focusing on subphenotypes with strong biological rationale and plausible heterogeneity of treatment effect
Difficulties with speed of subphenotype assignment	Development and validation of parsimonious subphenotype assignment algorithms Development and validation of point-of-care biomarker assays
Poor global co-operation	Decentralisation of patient data away from hospitals and towards collaborative databanks International consortiums on critical care subphenotyping

Figure 1: An imagined application of the suggested definitions for “phenotypes”, “subphenotypes”, “endotypes”, and “treatable traits”. Note that not all subphenotypes are necessarily endotypes. This figure also details methods by which subphenotypes, endotypes, and treatable traits might be identified. Subphenotypes defined by biomarkers have been repeatedly identified by techniques such as latent class analysis (LCA) and cluster analysis. Identified candidate markers should then be investigated to identify mechanistic differences between subphenotypes. If these mechanistic differences are proven, the subphenotype becomes an endotype. If a biologically-plausible treatment can be successfully targeted to an endotypic mechanism, it then becomes a treatable trait.

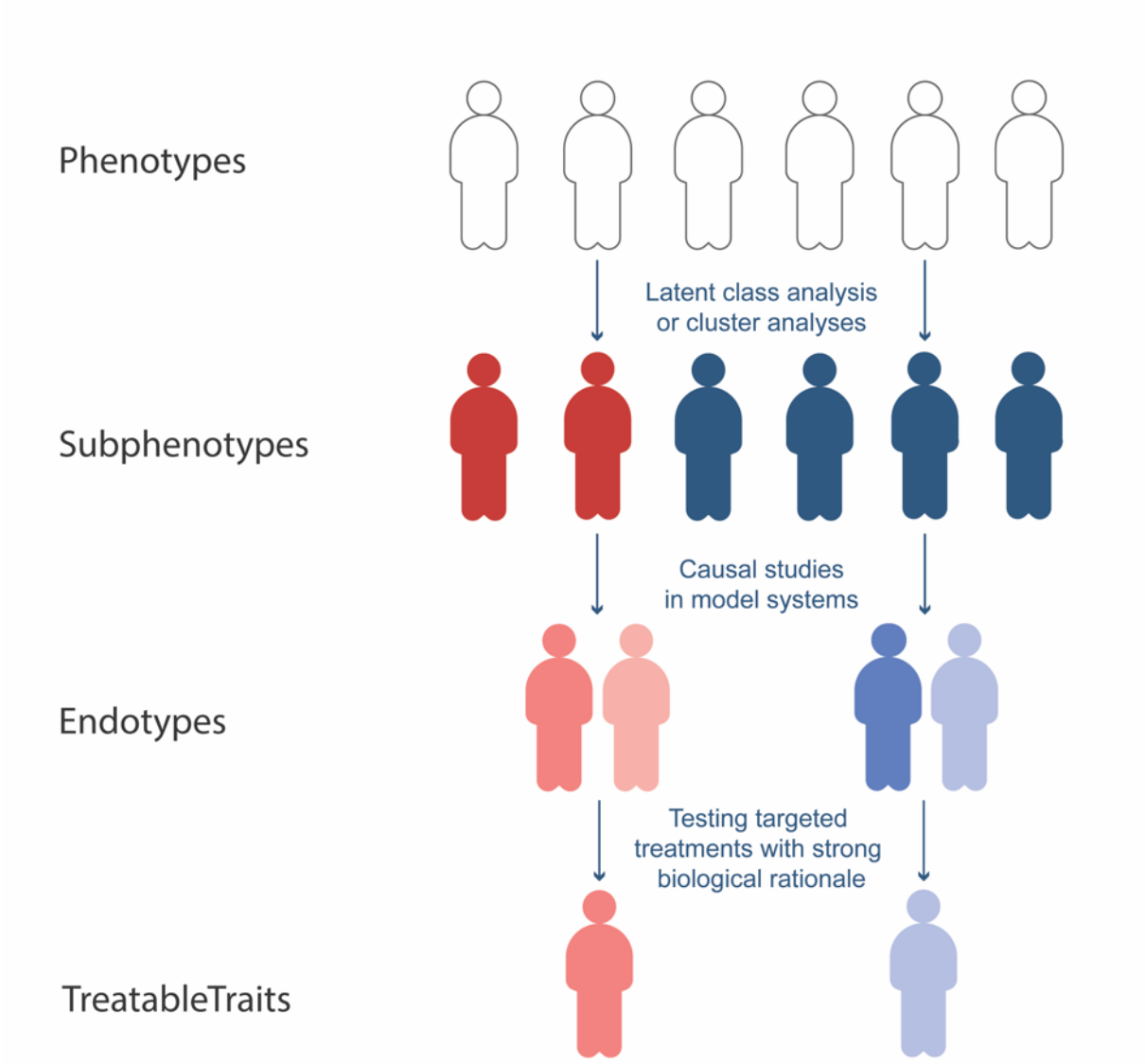

Figure 2: An approach to precision medicine in critical care. We propose patients are rapidly screened for multiple subphenotype assignments at ICU admission and are directed to endotype-specific therapies for each. Many more subphenotype assignments and treatment options than those pictured will likely be available.