In pursuit of precision medicine in the critically ill

Manu Shankar-Hari¹,²,³, Charlotte Summers³*, Kenneth Baillie⁴,⁵*

*Equal contributors

¹Guy's and St Thomas' NHS Foundation Trust, ICU support Offices, 1st Floor, East Wing, St Thomas' Hospital, SE1 7EH, UK;
²Division of Infection, Immunity and Inflammation, Kings College London, SE1 9RT
³Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ
⁴Intensive Care Unit, Royal Infirmary of Edinburgh, Little France, Edinburgh EH16 5SA
⁵Roslin Institute, University of Edinburgh, Easter Bush, Midlothian EH25 9RG

Current address and affiliations of the corresponding author

Dr. Manu Shankar-Hari MSc PhD FRCA FFICM
1st Floor, East Wing, St Thomas' Hospital, Guy’s and St Thomas’ NHS Foundation Trust London, UK, SE17EH; ²Division of Infection, Immunity and Inflammation, Kings College London, SE1 9RT; ³Intensive Care National Audit & Research Centre, Napier House, 24 High Holborn, London WC1V 6AZ, UK
email: manu.shankar-hari@kcl.ac.uk
Tel: +44 20 7188 8769
Fax: +44 20 7188 2284

Sources of support:

Dr Shankar-Hari is supported by the National Institute for Health Research Clinician Scientist Award (CS-2016-16-011). The views expressed in this publication are those of the author(s) and not necessarily those of the NHS, the National Institute for Health Research or the Department of Health.
In pursuit of precision medicine in the critically ill

“For it is not enough to recognize that all our knowledge is, in a greater or less degree, uncertain and vague; it is necessary, at the same time, to learn to act upon the best hypothesis without dogmatically believing it.” From, Philosophy for Laymen, by Bertrand Russell.

Introduction

Critical care medicine is, at present, a specialty of broad syndromes. This reflects the similarity in therapeutic approach required for the final common physiology that follows from many different pathological processes. Since their original definitions and descriptions, sepsis and acute respiratory distress syndrome (ARDS) are the two clinical conditions that have shaped health policy and dominated the research agenda in critical care[1, 2]. It is a truism to state that these are conglomerates of numerous different sub-syndromes; to make this observation is simply to restate the definition of sepsis and ARDS as common patterns arising from numerous different injuries. But it is also clear that, if we take the simple example of organ failure arising from a sterile versus an infectious insult, there is a very high likelihood that patients will respond differently to treatment with antibiotics. Or to take a more ambitious example, if we could diagnose, at presentation, the infectious agent causing sepsis, we could then confidently treat with narrow spectrum antibiotics. In this way, sub-classifications of critical illness are almost certainly directly applicable to clinical practice.

With numerous statistically negative randomized controlled trials reported in both sepsis and ARDS, and strong conceptual arguments that patients presenting with these two conditions are heterogeneous, the idea of providing clinical care based on some patient level characteristic, along similar lines highlighted in cancer
medicine is very appealing. Thus, critical care is contemplating approaches that are considered useful in cancer medicine, and other clinical fields such as respiratory medicine, to inform clinical trials in sepsis and ARDS. However, the challenges with precision medicine in the critically ill could be related to the classic paper by Geoffrey Rose – Sick Individuals and Sick Populations[3]. The key principle is that individual and population approaches to improving health achieve different aims; the individual approach aims to protect susceptible (high-risk) patients, whilst the population approach aims to reduce the group level incidence of or outcome from diseases. In this short perspective, after discussing the rationale for current definitions, we discuss whether the heterogeneity and precision medicine concepts could inform future studies in sepsis and/or ARDS.

**Rationale for ARDS and sepsis definitions with predictive validity**

The latest ARDS[2] and sepsis definitions[1] and the corresponding clinical criteria[4-6] were derived to identify patient populations with predictive validity, by combining the consensus conference discussions with empirical evaluation of clinical data; resulting in valid and reliable critical illness syndromic definitions. ARDS is defined as acute onset hypoxic respiratory failure despite a positive end expiratory pressure of 5 or greater, with non-cardiogenic pulmonary edema evidenced by bilateral chest opacities. With this definition, stages of mild, moderate, and severe ARDS based on severity of hypoxemia, were associated with significantly higher mortality and increased median duration of mechanical ventilation in survivors[2]. Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, with organ dysfunction defined as an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more[4]. Septic shock was defined as a subset of sepsis in which profound circulatory, cellular, and
metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Patients with septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2mmol/L in the absence of hypovolemia[5]. With this definition, sepsis and septic shock were associated with significantly higher mortality, compared to uncomplicated infection[1]. The predictive validity for mortality categories could potentially inform clinical care and trial design. For example, worsening ARDS severity has been aligned with treatment options, with severe ARDS aligned with need for neuromuscular blockade, prone position and extracorporeal support[6].

Heterogeneity

Heterogeneity is the inter-individual variation in susceptibility to either the illness or the outcome from illness or both. When risk factors for the illness or outcome are reported, the unstated (and likely incorrect) assumption is that these risk factors confer similar risk to individuals in a population, which is often overlooked during design, conduct and interpretation of studies [7]. Variation in the risk of illness or outcome is generated either as a random phenomenon, or due to measurable differences in biological characteristics in patients [8, 9], resulting from genetic and/or environmental influences. It is challenging to discern the relative contribution of each of these influences on outcomes in a critically ill ARDS or sepsis patient. For contextualization to ARDS and sepsis patients, heterogeneity could be categorized in patient, illness biology and treatment response level differences.

Patient level heterogeneity

Patient level heterogeneity contemplates broadly two questions – (a) why did this patient get this disease at this time? [3] and (b) why did this patient have a different
outcome, compared to another patient who appeared similar in many aspects of illness? The answer to these questions rely on understanding the determinants of susceptibility amongst individuals to the illness (risk of illness) and the illness related outcomes (risk of outcome). For example, as sepsis is infection related organ dysfunction, the risk factors for sepsis would include risk factors for infection and risk factors for developing organ dysfunction in the context of infection. The risk factors for developing organ dysfunction in the context of infection is poorly understood. The risk factors for infection include age with an inverted parabolic distribution with highest risk at extremes of age, male sex, ethnicity with black race and Asians with a higher risk and presence of one or comorbidity[10]. Genetic predisposition to infectious diseases is strongly heritable[11, 12], presumably due to the strong selective pressure exerted by pathogens on our ancestors. Identifying – and understanding – the genetic factors underlying predisposition may lead directly to tractable therapeutic targets in the host[13]. Several strong associations with susceptibility to infections have been discovered (e.g. HIV, WNV, TB, Malaria, Flu, meningococcus)[14-16], but these tend to be highly pathogen-specific. Whether there is general genetic susceptibility to sepsis, or even more broadly to a deleterious response to sterile injury, remains an open question. However, several lines of evidence suggest that responses to critical illness are likely to exhibit heritable variation in human populations. Firstly, the consensus in critical care medicine, supported by many years of clinical and animal model research, is that organ failure seen during critical illness is complex, driven by immune-mediated injury and alterations in bioenergetics [17, 18], alongside individual predisposition discussed as patient heterogeneity. Immune phenotypes tend to be strongly heritable, and numerous genetic associations for both
autoimmune and infectious diseases have been discovered. Importantly, many of these disease associations are pleiotropic[19]. Hence, variation in the host responses to severe systemic injury is likely to be in part genetically-determined. Finally, and most directly, the results of the GAINS and GenoSept studies have discovered some genetic associations with outcome in sepsis [20], which will be important candidates for further biological investigation.

**Similarity within sepsis and ARDS biological heterogeneity**

Acute immune changes in sepsis, studied using whole blood transcriptomics, identifies a complex set of pro and anti-inflammatory abnormalities in the innate and adaptive immune systems and alteration in genes highlighting mitochondrial dysfunction [21]. Ability of antigen presenting cells such as dendritic cells, monocytes-macrophage system, follicular dendritic cells are impaired and there is accelerated depletion of B and T lymphocytes. When the same biology is studied by looking for unsupervised clustering algorithms, between 2 and 4 different subphenotypes within sepsis have been observed [18, 22-25]. At clinical characteristics level, site of infection, numbers of organ dysfunction, type of organ dysfunction and combination of organ dysfunctions also influence outcome from sepsis and add to this heterogeneity [26]. In ARDS, the structural and functional disruption of the alveolar endothelial and epithelial barrier, result from generation of inflammasome and signalosome complexes, by leukocyte sensing of danger signals [27, 28]. The endothelial abnormalities and inflammatory responses observed in ARDS are also seen in sepsis and trauma [23, 29]. Furthermore, ARDS patients could also be grouped into hyperinflammatory and non-reactive ARDS subphenotypes based on biomarkers and/or clinical variables [30-34] (Table-1).
Heterogeneity in treatment responses

For any treatment, the essential drivers arguing for a precision medicine approach, either in clinical setting or trial setting, include the between patient differences in treatment responses, patient-level interaction with treatment due to individual heterogeneity and the variation in treatment response determined by stage of illness due to variability in the lag time between onset of illness to treatment [35]. A simple example for the impact of time on treatment effect is the relationship between time to antibiotic treatment and outcome. In a cohort study, Seymour et al, highlighted that in patients who had the 3-hour Surviving Sepsis Campaign Bundle (blood culture, antibiotic therapy and measure lactate) completed within a 12-hour period, every extra hour taken to complete the bundle is associated with a significant increase in mortality [36]. Similarly, treatment effect of drugs has been shown to vary with illness severity, using activated protein C trials in sepsis and effect of PEEP in ARDS [37] as examples. A related concept in this context is heterogeneity in treatment response, which is a crude omnibus test for differences in responses to treatment and illness related outcomes arising from all factors contributing to heterogeneity and stochasticity of risk. This has been illustrated using simulation of sepsis and ARDS RCTs [38] and by using completed RCT data from intravenous immunoglobulin trials in sepsis [39]. As the risk of death changes in a trial population, the differences in mortality between the intervention arm and the usual arm also changes. This could potentially highlight a risk of outcome specific sub-group within ARDS and sepsis who are likely to benefit the most from the intervention.

Stratified medicine and enrichment

Stratified medicine refers to identifying groups of patients based on either characteristics of disease, or likely treatment response at a population levels.
Enrichment markers are biomarkers that help identify either treatment responders and/or patients with higher risk of certain outcomes[40]. Thus, in the context of sepsis and ARDS, stratified medicine (enrichment) of clinical trial populations is a potentially viable strategy, as differential biological mechanisms and the technical ability to prospectively identify patient subsets exist. For example, in children with septic shock, using a 100-gene profile and serum protein biomarkers, it is possible to identify two patient subsets, with different outcomes, and differential responses to corticosteroid treatment [41]. Similarly, in adults with septic shock, corticosteroid responders can be identified using a three-biomarker panel [42]. Similarly, ARDS subsets, that respond differently to ventilator and fluid management, have been identified using data from completed ARDS trials [32, 33].

In 2007, Trusheim et al proposed three necessary conditions [40] required for effective stratified medicine for a disease: (a) differential biological mechanisms, (b) multiple treatment options, and (c) a clinical biomarker that links patient subsets to treatment responses. Sadly, in critical care medicine, we are a long way from meeting the second criterion: multiple treatment options.

However, as efforts have progressed to achieve these goals, both in critical care medicine and beyond, it has become clear that a necessary first step is the identification of a pattern, or subgroup, within heterogeneous patient populations. In itself, this is purely an exercise of academic interest, but where a common biological mechanism can be found, there is a reasonable chance that some current or future therapy might have a different effect in patients belonging to a given subgroup - this essentially is the definition of a disease endotype [43]. The process of identifying endotypes is conceptually identical to the approach taken by our medical forebears: a syndrome becomes a disease when the underlying mechanism is thought to be
known. Given the interplay of multiple mechanisms, two or more endotypes are likely in ARDS and sepsis.

The identification of a disease endotype has immediate clinical relevance, since it is likely that patients with a given endotype will respond differently to some therapies when compared with patients having other patterns of disease. Once an endotype is convincingly discovered, considerable investment of academic and commercial resource is applied to identifying both treatments and viable biomarkers with which to make the diagnosis [44]. It is therefore important to consider carefully what criteria must be met for this enterprise to proceed. Differential response to therapy is probably too high a bar in critical care medicine, because in many cases the fundamental problem is that we lack specific diseases and therapies for those diseases. Thus, a major aspiration of this field is that, by better understanding the underlying biology, we may be able to create or repurpose drug treatments to modulate the host response to injury. At present, however, efforts to achieve this goal have failed. Aside from heterogeneity, this is not necessarily because of lack of understanding of molecular mechanisms involved, but due to the complex interplay of many mechanisms contributing to the final outcome, and targeting one particular mechanism may not yield the desired treatment benefit. Progress in identifying distinct disease processes in critical illness should not be held back by this limitation. We therefore propose the following, permissive, criteria for concluding that a valid and reliable endotypes exist in critically ill populations. Subgroups should be:

1. Consistency
2. Biologically plausible
3. Clinically plausible
4. Feasibility of implementation in clinical care and/or trials
Consistency can be measured using standard criteria for generalizability to other populations of similar patients. Where, as is often the case, expensive new technologies have been used to observe patterns in a group of patients, consistency must necessarily be determined within the original population, using a bootstrapping approach or similar. Plausibility is a vague and subjective concept but we contend that most investigators know it when they see it. Biological plausibility can – in some cases – be determined by statistical tests applied to the biological signature that defines membership of a subgroup. Such signatures may depend on systematic collections of known biology, for example for pathway enrichments, or genome-wide methods[45], such as co-expression module enrichment [46]. If the number of tests performed is faithfully reported, these approaches can provide convincing evidence that a given grouping is biologically real. Clinical plausibility is an extension of this concept. Biological plausibility has obvious limitations: there are many real subgroupings of any population of patients. Hence if there is not a predictable mechanism by which a given subgrouping could turn out to have a differential treatment effect, or at least a differential effect on prognosis, then the risk of failure is expected to be high. Finally, feasibility represents a compromise between the truth of detection (validity) and reliability of measurements to identify subgroups. For example, a cytokine profile for identifying corticosteroid responders in septic shock [42] could be considered to have greater feasibility compared to 100-gene expression panel [41], but with different reliability and validity. Importantly, it is possible, but not necessary, for clinical outcome to be different between endotypes: patients with different diagnoses can have identical statistical probabilities of a given outcome. A focus on outcome runs the risk of detecting severity markers, rather than distinct biological processes.
**Precision medicine**

Fundamentally, precision medicine represents a scenario where detecting one or more biological abnormalities in patients help pair them to treatment(s), based on the individuals’ favorable treatment response – adverse effect profile. Most precision medicine advances have been in oncology, although consistent success is limited. For example, super-responders to Everolimus treatment considered as exemplar for precision oncology [47] have not been consistently replicated. Furthermore, the cancer free survival amongst patients with relapse and/or refractory tumors is not impressive and super-responders to targeted chemotherapy may be a much smaller cancer population than previously considered [48]. For example, a recently published phase II multicenter RCT enrolled 741 adult patients with any kind of metastatic solid tumour refractory to standard of care. From this population, 40% of patients had at least one molecular alteration that matched with one of the 10 treatment regimens and 195 patients were randomized to receive experimental treatment specific for pathway mutations or standard of care. There were no differences in efficacy or adverse event end points between the intervention and control arms [49]. These lessons from precision oncology approaches must be seriously considered [50], when testing precision medicine in critically ill patients with heterogeneous syndromes such as sepsis or ARDS.

**Conclusions**

ARDS and sepsis often occur in older patients with comorbidity resulting in critical illness. Heritable characteristics and environmental factors influence the incidence and outcomes from ARDS and sepsis. We have begun to group ARDS and sepsis patients with similar biological characteristics and consider stratified or precision medicine as the solution for overcoming statistically negative clinical trials. Key
biological and clinical plausibility challenges need to be addressed to achieve major breakthroughs in future trials and the clinical care of sepsis and ARDS patients.
References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM et al: The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016, 315(8):801-810.

2. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS: Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012, 307(23):2526-2533.

3. Rose G: Sick individuals and sick populations. Int J Epidemiol 2001, 30(3):427-432.

4. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, Rubenfeld G, Kahn JM, Shankar-Hari M, Singer M et al: Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016, 315(8):762-774.

5. Shankar-Hari M, Phillips GS, Levy ML, Seymour CW, Liu VX, Deutschman CS, Angus DC, Rubenfeld GD, Singer M, Sepsis Definitions Task F: Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016, 315(8):775-787.

6. Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, Brochard L, Brower R, Esteban A, Gattinoni L et al: The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. Intensive Care Med 2012, 38(10):1573-1582.

7. Aalen OO, Valberg M, Grotmol T, Tretli S: Understanding variation in disease risk: the elusive concept of frailty. Int J Epidemiol 2015, 44(4):1408-1421.

8. Smith GD: Epidemiology, epigenetics and the 'Gloomy Prospect': embracing randomness in population health research and practice. Int J Epidemiol 2011, 40(3):537-562.

9. Pearson H: Epidemiology: Study of a lifetime. Nature 2011, 471(7336):20-24.

10. Mayr FB, Yende S, Angus DC: Epidemiology of severe sepsis. Virulence 2014, 5(1):4-11.

11. Petersen L, Sorensen TI, Andersen PK: A shared frailty model for case-cohort samples: parent and offspring relations in an adoption study. Stat Med 2010, 29(7-8):924-931.
12. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW: Genetic and environmental influences on premature death in adult adoptees. *N Engl J Med* 1988, 318(12):727-732.

13. Baillie JK: Translational genomics. Targeting the host immune response to fight infection. *Science* 2014, 344(6186):807-808.

14. Hill AV: Evolution, revolution and heresy in the genetics of infectious disease susceptibility. *Philos Trans R Soc Lond B Biol Sci* 2012, 367(1590):840-849.

15. Everitt AR, Clare S, Pertel T, John SP, Wash RS, Smith SE, Chin CR, Feeley EM, Sims JS, Adams DJ et al: IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* 2012, 484(7395):519-523.

16. Glass WG, McDermott DH, Lim JK, Lekhong S, Yu SF, Frank WA, Pape J, Cheshier RC, Murphy PM: CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J Exp Med* 2006, 203(1):35-40.

17. Singer M: The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. *Virulence* 2014, 5(1):66-72.

18. van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG: The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol* 2017, 17(7):407-420.

19. Sivakumaran S, Agakov F, Theodoratou E, Prendergast James G, Zgaga L, Manolio T, Rudan I, McKeigue P, Wilson James F, Campbell H: Abundant Pleiotropy in Human Complex Diseases and Traits. *Am J Hum Genet* 2011, 89(5):607-618.

20. Rautanen A, Mills TC, Gordon AC, Hutton P, Steffens M, Nuamah R, Chiche JD, Parks T, Chapman SJ, Davenport EE et al: Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study. *The Lancet Respiratory medicine* 2015, 3(1):53-60.

21. Sweeney TE, Shidham A, Wong HR, Khatri P: A comprehensive time-course-based multicohort analysis of sepsis and sterile inflammation reveals a robust diagnostic gene set. *Sci Transl Med* 2015, 7(287):287ra271.

22. Burnham KL, Davenport EE, Radhakrishnan J, Humburg P, Gordon AC, Hutton P, Svoren-Jabalera E, Garrard C, Hill AVS, Hinds CJ et al: Shared and Distinct Aspects of the Sepsis Transcriptomic Response to Fecal Peritonitis and Pneumonia. *Am J Respir Crit Care Med* 2017, 196(3):328-339.
23. Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AV et al: Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *The Lancet Respiratory medicine* 2016, 4(4):259-271.

24. Rautanen A, Mills TC, Gordon AC, Hutton P, Steffens M, Nuamah R, Chiche J-D, Parks T, Chapman SJ, Davenport EE et al: Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study. *The Lancet Respiratory Medicine* 2015, 3(1):53-60.

25. Scicluna BP, van Vught LA, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, Nurnberg P, Schultz MJ, Horn J, Cremer OL et al: Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *The Lancet Respiratory Medicine* 2017.

26. Shankar-Hari M, Harrison DA, Rowan KM: Differences in Impact of Definitional Elements on Mortality Precludes International Comparisons of Sepsis Epidemiology-A Cohort Study Illustrating the Need for Standardized Reporting. *Crit Care Med* 2016, 44(12):2223-2230.

27. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH, Fredenburgh L, Massaro AF, Rogers A, Gazourian L, Nakahira K et al: Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am J Respir Crit Care Med* 2012, 185(11):1225-1234.

28. Matthay MA, Ware LB, Zimmerman GA: The acute respiratory distress syndrome. *J Clin Invest* 2012, 122(8):2731-2740.

29. Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, Hayden DL, Hennessy L, Moore EE, Minei JP et al: A genomic storm in critically injured humans. *J Exp Med* 2011, 208(13):2581-2590.

30. Bos LD, Schouten LR, van Vught LA, Wiewel MA, Ong DSY, Cremer O, Artigas A, Martin-Loeches I, Hoogendijk AJ, van der Poll T et al: Identification and validation of distinct biological phenotypes in patients with acute respiratory distress syndrome by cluster analysis. *Thorax* 2017.
31. Calfee CS, Janz DR, Bernard GR, May AK, Kangelaris KN, Matthay MA, Ware LB: Distinct molecular phenotypes of direct vs indirect ARDS in single-center and multicenter studies. *Chest* 2015, 147(6):1539-1548.

32. Famous KR, Delucchi K, Ware LB, Kangelaris KN, Liu KD, Thompson BT, Calfee CS, Network A: Acute Respiratory Distress Syndrome Subphenotypes Respond Differently to Randomized Fluid Management Strategy. *Am J Respir Crit Care Med* 2017, 195(3):331-338.

33. Calfee CS, Delucchi K, Parsons PE, Thompson BT, Ware LB, Matthay MA, Network NA: Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *The Lancet Respiratory medicine* 2014, 2(8):611-620.

34. Calfee C, Matthay M: Clinical immunology: Culprits with evolutionary ties. *Nature* 2010, 464(7285):41-42.

35. Senn S: Mastering variation: variance components and personalised medicine. *Stat Med* 2016, 35(7):966-977.

36. Seymour CW, Gesten F, Prescott HC, Friedrich ME, Iwashyna TJ, Phillips GS, Lemeshow S, Osborn T, Terry KM, Levy MM: Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. *N Engl J Med* 2017.

37. Shankar-Hari M, Rubenfeld GD: The use of enrichment to reduce statistically indeterminate or negative trials in critical care. *Anaesthesia* 2017, 72(5):560-565.

38. Iwashyna TJ, Burke JF, Sussman JB, Prescott HC, Hayward RA, Angus DC: Implications of Heterogeneity of Treatment Effect for Reporting and Analysis of Randomized Trials in Critical Care. *Am J Respir Crit Care Med* 2015, 192(9):1045-1051.

39. Welton NJ, Soares MO, Palmer S, Ades AE, Harrison D, Shankar-Hari M, Rowan KM: Accounting for Heterogeneity in Relative Treatment Effects for Use in Cost-Effectiveness Models and Value-of-Information Analyses. *Med Decis Making* 2015, 35(5):608-621.

40. Trusheim MR, Berndt ER, Douglas FL: Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers. *Nat Rev Drug Discov* 2007, 6(4):287-293.
41. Wong HR, Atkinson SJ, Cvijanovich NZ, Anas N, Allen GL, Thomas NJ, Bigham MT, Weiss SL, Fitzgerald JC, Checchia PA et al: Combining Prognostic and Predictive Enrichment Strategies to Identify Children With Septic Shock Responsive to Corticosteroids. Crit Care Med 2016, 44(10):e1000-1003.

42. Bentzer P, Fjell C, Walley KR, Boyd J, Russell JA: Plasma cytokine levels predict response to corticosteroids in septic shock. Intensive Care Med 2016, 42(12):1970-1979.

43. Russell CD, Baillie J: Treatable traits and therapeutic targets. Current Opinion in Systems Biology 2017.

44. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, Lemanske RF, Jr., Wardlaw AJ, Wenzel SE, Greenberger PA: Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011, 127(2):355-360.

45. Khatri P, Sirota M, Butte AJ: Ten years of pathway analysis: current approaches and outstanding challenges. PLoS Comput Biol 2012, 8(2):e1002375.

46. Forrest AR, Kawaji H, Rehli M, Baillie JK, de Hoon MJ, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, Itoh M et al: A promoter-level mammalian expression atlas. Nature 2014, 507(7493):462-470.

47. Iyer G, Hanrahan AJ, Milowsky MI, Al-Ahmadie H, Scott SN, Janakiraman M, Pirun M, Sander C, Socci ND, Ostrovnaya I et al: Genome Sequencing Identifies a Basis for Everolimus Sensitivity. Science 2012, 338(6104):221.

48. Prasad V: Perspective: The precision-oncology illusion. Nature 2016, 537(7619):S63-S63.

49. Le Tourneau C, Delord JP, Goncalves A, Gavoille C, Dubot C, Isambert N, Campone M, Tredan O, Massiani MA, Mauborgne C et al: Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol 2015, 16(13):1324-1334.

50. Stewart DJ, Kurzrock R: Fool’s gold, lost treasures, and the randomized clinical trial. BMC Cancer 2013, 13(1):193.
**Table-1: Recent studies describing Acute respiratory distress syndrome and sepsis subphenotypes**

Abbreviations: IL = interleukins; sTNFR1 = soluble tumor necrosis factor receptor; IFN-γ = interferon gamma; ANG1/2 = Angiotensin; PAI-1 = plasminogen activator inhibitor-1; DYRK2 = dual specificity tyrosine phosphorylation regulated kinase 2; CCNB1|P1 = cyclin B1 interacting protein 1; TDRD9 = tudor domain containing 9; ZAP70 = zeta chain of T-cell receptor associated protein kinase 70; ARL14EP = ADP ribosylation factor like GTPase 14 effector protein; MDC1 = mediator of DNA damage checkpoint 1; ADGRE3 = adhesion G protein-coupled receptor E3; BPGM = bisphosphoglycerate mutase; TAP2 = ATP binding cassette subfamily B member transporter 2; GADD45A = growth arrest and DNA damage inducible alpha; PCGF5 = polycomb group ring finger 5; AHNAK = AHNAK nucleoprotein; PDCD10 = programmed cell death 10; NOP53 = ribosome biogenesis factor

| Reference     | Subphenotypes                              | Comment                                                                 |
|---------------|--------------------------------------------|-------------------------------------------------------------------------|
| Calfee C et al[33] [ARDS] | Hyper-inflammatory phenotype versus Phenotype-1 | Latent class analyses based grouping based on clinical and biomarker data. The discriminant markers between phenotypes were IL-6; sTNFR1; vasopressor use; IL-8; bicarbonate |
| Famous et al[32] [ARDS] | Hyper-inflammatory phenotype versus Phenotype-1 | Latent class analyses based grouping based on clinical and biomarker data. The discriminant markers between phenotypes were IL-8; sTNFR1; vasopressor use; bicarbonate; minute ventilation |
| Bos et al[30] [ARDS] | Reactive phenotype versus Uninflamed phenotype | Agglomerative hierarchical cluster analyses based only on biomarker data. The discriminant markers between phenotypes were IL-6; IFN-γ; ANG1/2; PAI-1 |
|           | Sepsis response signature-1 Versus Sepsis response signature-2 | Agglomerative hierarchical clustering based on Ward’s method using panleukocyte transcriptome using microarray. The discriminant markers between two phenotypes were seven genes - DYRK2, CCNB1IP1, TDRD9, ZAP70, ARL14EP, MDC1, and ADGRE3 |
|-----------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Davenport EE et al[23] [Sepsis] | Four molecular endotypes named as Mars1 to Mars4 in sepsis | Agglomerative hierarchical clustering based on Ward’s method using panleukocyte transcriptome using microarray. The study shows that 140-gene expression signature reliably stratified patients with sepsis to the four endotypes. The study also reports biomarkers for each endotype to facilitate clinical use: Mars1 = BPGM and TAP2; Mars2 = GADD45A and PCGF5; Mars3 = AHNAK and PDCD10; Mars4 = IFIT5, NOP53 |