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REVIEW ARTICLE

The acute respiratory distress syndrome biomarker pipeline: crippling gaps between discovery and clinical utility

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Recent innovations in translational research have ushered an exponential increase in the discovery of novel biomarkers, thereby elevating the hope for deeper insights into "personalized" medicine approaches to disease phenotyping and care. However, a critical gap exists between the fast pace of biomarker discovery and the successful translation to clinical use. This gap underscores the fundamental biomarker conundrum across various acute and chronic disorders: how does a biomarker address a specific unmet need? Additionally, the gap highlights the need to shift the paradigm from a focus on biomarker discovery to greater translational impact and the need for a more streamlined drug approval process. The unmet need for biomarkers in acute respiratory distress syndrome (ARDS) is for reliable and validated biomarkers that minimize heterogeneity and allow for stratification of subject selection for enrollment in clinical trials of tailored therapies. This unmet need is particularly highlighted by the ongoing SARS-CoV-2/COVID-19 pandemic. The unprecedented numbers of COVID-19-induced ARDS cases has strained health care systems across the world and exposed the need for biomarkers that would accelerate drug development and the successful phenotyping of COVID-19-infected patients at risk for development of ARDS and ARDS mortality. Accordingly, this review discusses the current state of ARDS biomarkers in the context of the drug development pipeline and highlight gaps between biomarker discovery and clinical implementation while proposing potential paths forward. We discuss potential ARDS biomarkers by category and by context of use, highlighting progress in the development continuum. We conclude by discussing challenges to successful translation of biomarker candidates to clinical impact and proposing possible novel strategies. (Translational Research 2020; 226:105–115)

INTRODUCTION

Innovations in laboratory biochemistries, molecular biology, and "omics" medicine have ushered in an era with the potential to unravel the Gordian knot of identifying validated molecular markers of disease.[1,2] The emergence of precision medicine and high throughput precision technologies elevated aspirations for defining novel biomarkers that would accelerate improved treatment of diverse adverse health conditions by facilitating the identification of responders to promising novel or repurposed therapeutic strategies.[3,4] A cursory

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Submitted for Publication May 10, 2020; received submitted June 17, 2020; accepted for publication June 23, 2020.

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1931-5244/$ - see front matter

https://doi.org/10.1016/j.trsl.2020.06.010

review of the medical literature[5-7] over the past 3 decades revealed the emergence of an increasing number of biomarker candidates. However, the exponential rate of initial discovery has now completely outpaced the ability of the biomedical community to successfully develop and validate the clinical utility of prospective biomarkers.[7,8] In fact, only ~0.1% of potentially clinically relevant biomarkers described in the literature have progressed to utility as a meaningful and routinely utilized clinical readout.[9] The reasons for this massive disconnect are multifactorial including the stark reality that the majority of biomarkers identified are by investigators in government-funded university laboratories that are ill-resourced to complete the biomarker development and validation continuum.[5] This realization led the U.S. Congress under the 21st Century Cures Act of 2016, to encourage the U.S. Food and Drug Administration (FDA) to create the biomarker qualification program as part of the drug development toolkit, an effort to guide researchers and accelerate the development of promising biomarkers.[10-13]

Prior reviews of biomarkers in acute respiratory distress syndrome (ARDS), a serious critical care illness in dire need of validated and clinically useful biomarkers, have largely served as diligent but descriptive approaches outlining new technologies or summarizing the pathobiology of current biomarkers.[14-20] In contrast, this current review is highly divergent from prior reports and seeks to discuss the current state of ARDS biomarkers in the context of the drug development pipeline and to highlight the gaps between discovery and clinical implementation while proposing potential paths forward. Our intent is to shift the paradigm from a focus on biomarker discovery that is currently relegated to demonstrating a correlation between a specific biomarker and either the development of ARDS or ARDS severity, to a focus on the clinical utility and implementation of the biomarker within well-defined contexts of use including subject stratification in clinical trials.[4,5] The need for such a translational focus is particularly highlighted by the ongoing SARS-CoV-2/COVID-19 pandemic. COVID-19-induced ARDS has strained health care systems across the world and exposed the need for biomarkers that would accelerate disease phenotyping and drug development.

The clinical definition of the highly heterogeneous ARDS includes acute arterial hypoxemia and a ratio of partial pressure of arterial oxygen [PaO_2] to fraction of inspired oxygen [FiO_2] that is less than 300, bilateral pulmonary opacities, and the exclusion of cardiac failure or other reversible primary causes.[21] Since lung biopsies are not routinely obtained in ARDS, this clinical definition aims to identify patients with noncardiogenic pulmonary edema, a process characterized by increased protein permeability of the alveolar-capillary membrane.[22,23] Diagnostic uncertainty in ARDS further exacerbates disease heterogeneity and is a potential source of bias in conducting clinical trials.[23] There is a compelling unmet medical need to identify clinical and/or disease-specific biochemical parameters that risk-stratify patients for both accurate prognostication and clinical trial purposes. Stratification of ARDS patients with reliable biomarkers that are predictive of mortality would optimize participant selection for clinical trial enrollment by focusing on those subjects most likely to benefit from novel clinical interventions.[24,25] More than 45 promising candidate biomarkers in ARDS have been described in the medical literature, however, to date no biomarker has been successfully developed as an accepted point of care surrogate marker of disease.[14,15] The heterogeneity of the ARDS phenotype, the variability of candidate biomarkers, and the focus on biomarker discovery without consideration of biomarker development, are all serious contributors to the abysmal record for ARDS biomarker development and the poor performance record of ARDS clinical trials.

Biomarkers, objectively measured as characteristic of clinical, pathologic, or physiologic processes, can be bioanalytical (proteins, metabolites, DNA genetic variants, RNA types), histologic, or radiographic.[12,26] The ideal bioanalytical biomarker is easily measured in blood or in other bodily fluids, has an excellent analytical sensitivity, high statistical sensitivity and specificity, varies rapidly in response to impactful therapies, aids in subject stratification, and exhibits biologic plausibility.[26] Although traditional clinical or laboratory variables such as blood pressure readings, PaO_2, hemoglobin A_{1C}, and glomerular filtration rates are examples of "biomarkers," in the context of translational research, the term often refers to a marker used to provide insight into a "personalized medicine" approach to phenotyping and care.[27]

In this review, we have attempted to summarize the current state of ARDS biomarkers based upon FDA-proposed categories with assessment of the advancement of each ARDS biomarker in the drug development continuum. Finally, we have sought to identify potential challenges to the successful translation of candidates through the pipeline of biomarker development. Of note, the biomarkers covered in this review are not an exhaustive list of possible ARDS biomarkers.

ARDS BIOMARKERS BY CATEGORY

The pathogenesis of ARDS includes a combination of endothelial injury, epithelial injury, an intense inflammatory cascade, dysregulated coagulation, fibrosis, and

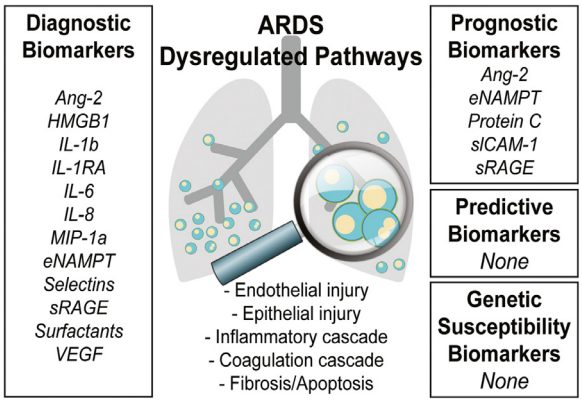

Fig 1. ARDS biomarkers by category.

The majority of candidate ARDS biomarkers involve dysregulation of the following pathways: endothelial injury, epithelial injury, inflammatory cascade, coagulation cascade, or fibrosis and apoptosis. Studies have mostly assessed the diagnostic and prognostic performance of these candidate biomarkers. Ang-2, angiopoietin 2; HMGB1, high mobility group box nuclear protein 1; IL-1β, interleukin 1 beta; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IL-8, interleukin 8; MIP-1α, macrophage inflammatory protein-1α; eNAMPT, extracellular nicotinamide phosphoribosyltransferase; sRAGE, soluble receptor for advanced glycation end products; VEGF, vascular endothelial growth factor.

apoptosis in response to diverse stimuli[14] (Fig 1). This acute dysregulation of various biochemical and cellular pathways allows for the generation of numerous potential biomarkers associated with risk or severity of ARDS.[14,15] However, to address the specific unmet medical needs in ARDS, biomarkers must provide specific contexts such as early diagnosis, pathobiologic disease classification, or guide successful development of novel therapies (Fig 1). For example, a diagnostic biomarker could lead to the early diagnosis of ARDS, promote improved patient selection for clinical trial enrollment and provide useful and reliable implications for clinical care. A predictive biomarker in the disease-causal pathway could enhance our understanding of the pathophysiology and, by extension, identify likely novel therapeutic targets and strategies. A prognostic biomarker would optimally stratify ARDS patients by likelihood of treatment response, again enriching future clinical trials for detection of a treatment effect. Whereas previous efforts have focused on discovery by demonstrating an association between a specific biomarker and ARDS risk and mortality, Table 1 presents a summary of ARDS biomarkers classified by general biomarker categories and potential examples of the corresponding context for biomarker use as recommended by the FDA.[11]

Diagnostic biomarkers in ARDS. In the context of ARDS, an ideal diagnostic biomarker is a surrogate that identifies the early stages of the syndrome, minimizes heterogeneity, reflects the natural history, and is a potential target for a clinical trial. Currently, the most promising diagnostic biomarkers do correlate with ARDS susceptibility, however, they do not meet the full criteria for surrogacy. Specific examples include:

Table 1. Biomarker categories and examples of corresponding context of use

Biomarker category	Context of use example
Diagnostic biomarkers	• Select subjects for clinical trial enrollment
Predictive biomarkers	• Identify subjects on the basis of effect of a specific intervention or exposure
Prognostic biomarkers	• Stratify subjects by likelihood of a relevant clinical outcome (ex. mortality or response to treatment) • Enrich clinical trial subject selection by refining inclusion and exclusion criteria
Biomarkers for susceptibility or risk	• Indicate the potential for developing a disease or sensitivity to an exposure
Biomarkers of treatment responses and pharmacodynamics	• Assess differential dose-response effects based upon biology • Assess the efficacy of specific therapy in subgroups
Biomarkers of safety	• Assess the presence or extent of toxicity related to an intervention or exposure

Receptor for advanced glycation end products (RAGE). RAGE, a transmembrane pattern recognition receptor of the immunoglobulin superfamily is abundantly expressed in the lung and primarily located on the basal surface of alveolar type 1 epithelial cells (AT1).[28,29] The soluble form of RAGE (sRAGE) comprising the extracellular domain is produced through cleavage by matrix metalloproteinases. Soluble RAGE is a marker of AT1 cell injury and a key mediator of alveolar inflammation[30-32] and sRAGE expression is enhanced in the early stage of ARDS.[33] Plasma and bronchoalveolar lavage (BAL) fluid levels of sRAGE are elevated during ARDS and correlate with disease severity by lung CT. Although a very promising diagnostic biomarker, sRAGE measurements have not progressed to clinical utility and validation.

Angiopoeitin-2 (Ang-2). Ang-2 is an endothelial-derived protein that increases the junction instability of the endothelial junction thereby enhancing vascular

leak.[34] As a potential diagnostic biomarker, higher plasma levels of Ang-2 were associated with development of ARDS in a cohort of patients admitted to the ICU.[35] Similarly, among surgical ICU patients, plasma levels of Ang-2 were higher in ARDS patients compared to those without ARDS.[36] Ang-2 remains a very promising diagnostic biomarker but without validation of clinical utility in ARDS.

Vascular endothelial growth factor (VEGF). VEGF belongs to the platelet-derived growth factor superfamily which play central roles in the regulation of angiogenesis and lymphangiogenesis.[37] VEGF is released from various alveolar type 2 epithelial (AT2) cells, neutrophils, alveolar macrophages, and activated T cells and depending on the degree of epithelial or endothelial damage, VEGF expression in ARDS varies.[14] Studies suggesting a correlation of plasma or BAL VEGF levels with the diagnosis of ARDS have not been consistently replicated.[14]

Surfactant proteins. Because of the vital role in maintaining the integrity of the alveolar-capillary interface, the surfactant-associated proteins (SP-A, SP-B, SP-C, and SP-D) were considered early on as natural candidate diagnostic biomarkers in ARDS.[38,39] However, initial reports correlating high plasma levels of SP-A and SP-B or reduced BAL levels of SP-D with a diagnosis of ARDS have not been confirmed.[35,40]

Selectins. These membrane-associated glycoproteins mediate the adhesion of leukocytes and platelets to the vascular endothelium.[41] Plasma levels of P-selectin and E-selectin are elevated in patients with acute lung injury compared to those with sepsis without lung injury.[42,43] However, these initial reports have not been robustly replicated.

Proinflammatory cytokines. The intense inflammatory cascade characteristic of ARDS is associated with increased plasma and alveolar levels of a number of inflammatory cytokines such as IL-1β and TNF-α that are both secreted by activated macrophages in the early inflammatory phase of ARDS and drive the release of other proinflammatory chemokines including monocyte chemotactic protein-1, macrophage inflammatory protein-1α, IL-6, and IL-8.[44] These proinflammatory chemokines propagate the inflammatory cascade by further damage of the endothelial-alveolar barrier, recruitment of inflammatory cells into the airspaces, and impairment of fluid transport.[44] Naturally, these proinflammatory cytokines have been extensively studied as possible diagnostic candidate biomarkers in ARDS.[14,15] However, to date, no individual proinflammatory cytokine has been clinically validated as a robust diagnostic biomarker.

Anti-inflammatory cytokines. The innate immune system responds to the acute inflammatory cascade with specific (IL-1 receptor antagonist or IL-1RA) and nonspecific anti-inflammatory systems (IL-10).[44] IL-1RA[45] but not IL-10[46] has emerged as a promising diagnostic ARDS biomarker but has yet to be validated.

Cytozymes. Cytozymes are a class of proteins that retain an intracellular function as an enzyme but are highly proinflammatory when secreted into the extracellular space or circulation. High mobility group box nuclear protein 1 (HMGB1) dually serves an intracellular function as a DNA nuclear binding protein and is an inflammatory cytokine when secreted by monocytes and macrophages.[47] Plasma and alveolar levels of HMGB1 increase early after severe trauma and correlate with development of ARDS.[47] However, critical significant associations between HMGB1 levels at ICU admission and clinical outcomes in critically ill patients has yet to be demonstrated.[48] Macrophage migration inhibitory factor (MIF) is an intracellular tautomerase that is a potent inflammatory mediator when secreted. MIF is postulated to play a crucial pathological role in the development of alveolar inflammation in ARDS. MIF, and its close structural D-dopachrome homolog (MIF-2 or DDT), are potential diagnostic biomarkers in sepsis, trauma and ARDS that require further clinical validation.[49,50] Similarly, the nicotinamide phosphoribosyltransferase known as NAMPT (also known as pre-B-cell colony-enhancing factor or visfatin) regulates intracellular NAD metabolism, whereas extracellular secreted eNAMPT is an innate immunity regulator which binds Toll-like receptor 4.[51] eNAMPT levels are increased in ARDS with trending toward ARDS severity, but requires robust clinical validation as a diagnostic biomarker.[52-54]

Plasminogen activator inhibitor (PAI-I). Plasminogen activator (PA) and PAI-I regulate fibrinolysis through the conversion of plasminogen to plasmin.[55] During lung injury, alveolar epithelial cells and activated macrophages overexpress PAI-1, thus contributing to decreased alveolar fibrinolytic activity.[55] Initial reports suggesting PAI-1 as a diagnostic biomarker were not confirmed in a large prospective cohort.[56]

Predictive biomarkers in ARDS. Many promising therapies for ARDS have failed in phase 3 studies due to phenotypic heterogeneity, a challenge potentially addressed by validated predictive biomarkers that identify individuals more likely to respond to a treatment type. Predictive biomarkers that reside in the causal pathway of the disease are obvious potential therapeutic targets. By identifying patients in whom a larger treatment effect can be obtained, biomarkers may significantly impact clinical trial design and sample size considerations. The best example of a causal pathway biomarker is low-density lipoprotein which has been implicated in the development of atherosclerotic cardiovascular disease.[57] Casual

inference of low-density lipoprotein and atherosclerotic cardiovascular disease is supported by evidence from genetic risk studies on inherited disorders of metabolism, prospective epidemiologic studies, Mendelian randomization studies, and randomized controlled trials.[57] In ARDS, no single biomarker has been shown to reliably predict clinical outcomes.

Prognostic biomarkers in ARDS. Prognostic biomarkers provide information addressing the overall disease outcome and are potentially useful in stratifying patients for enrollment in clinical trials. In ARDS, enrichment of study subjects most likely to have a poor outcome can aid the design of clinical trials and enhance the ability to detect beneficial effects from potential therapies.

RAGE. Monitoring of sRAGE levels have been used to identify the subgroup of ARDS patients more likely to respond to alveolar recruitment maneuvers.[58] Unfortunately, however, initial reports of an association between plasma levels of sRAGE and 28-day or 90-day mortality in patients with ARDS[58,59] have not been consistently replicated.[60]

Angiopoeitin-2 (Ang-2). Elevated levels of Ang-2 have been associated with increased risk of mortality among patients with infection-related ARDS.[61,62] Higher levels of Ang-2, measured as part of a panel of 6 biomarkers was associated with increased risk of mortality.[63,64]

Soluble intercellular adhesion molecule-1 (sICAM-1). sICAM-1 is an inducible glycoprotein that is expressed on the endothelial cell surface.[65] Levels of sICAM-1 are upregulated during inflammation in response to stimulation by interferon-γ or IL-1.[66] In multiple studies, elevated baseline levels of sICAM have been associated with increased mortality from ARDS.[67-69]

Protein C. Protein C is synthesized in the liver as an inactive form and transformed on cell surface to its active form by the thrombomodulin-thrombin complex.[70,71] Activated protein C is an important endogenous regulator of coagulation and fibrinolysis with anti-inflammatory properties that can improve endothelial permeability,[72] and exert antiapoptotic effects.[73] Activated protein C also inactivates PAI-1 thus promoting fibrinolysis.[74] Low plasma levels of protein C have been associated with higher ARDS mortality.[75]

Proinflammatory cytokines. High plasma levels of IL-1β, TNF-α, IL-6, IL-1, and IL-18 have been associated with increased mortality from ARDS.[15,44] However, none of these proinflammatory biomarkers have sufficient specificity to serve as a stand-alone prognostic biomarker. Higher plasma levels of IL-6, IL-8, IL-1RA, measured as part of a panel of 6 biomarkers were associated with increased risk of mortality.[63] High eNAMPT levels at the time of admission to the intensive care unit correlate with disease severity and may predict mortality in patients with sepsis and ARDS.[76,77] Unfortunately, no single biomarker has been shown to reliably provide information about the patient's overall disease outcome and thus stratify patients for enrollment in clinical trials. However, recent efforts to combine biomarkers demonstrate that prognostic ability can be greatly enhanced.[24,25,63]

Biomarkers of ARDS genetic susceptibility. Despite the challenges in studying ARDS phenotypes, genomic/genetic methodologies have generated novel insights into the pathogenesis of ARDS[78] and elucidated previously unknown mechanistic pathways in the pathogenesis of ARDS.[79,80] For example, the association between *NAMPT* and development of ARDS and ventilator-induced lung injury (VILI)-driven pathobiology was discovered utilizing high throughput functional genomic approaches.[52] Extensive microarray-based lung gene expression profiling in canine, murine, and human acute lung injury models identified increased expression of *NAMPT*[52] whose genetic variants are now confirmed to be associated with ARDS susceptibility and severity.[52,53,81] Single nucleotide polymorphisms (SNP)-driven ARDS approaches, including genome-wide association studies for ARDS susceptibility, are historically limited by low statistical power and the daunting heterogeneity of the ARDS phenotype. However, these strategies have identified a S1PR3[82,83] and the polypeptide-interacting protein alpha-1 (*PPFIA1*) as risk factors for developing acute lung injury including after major trauma.[84] Recently, an association between variants in the selectin P ligand gene (*SELPLG*) and the development of ARDS in African-American patients with sepsis[85] was described with the potential as a viable ARDS biomarker. Despite herculean efforts, no genotype-driven biomarker of genetic susceptibility to ARDS has reached the level of clinical utility. Mendelian randomization analysis with genetic variation as an instrumental variable linked to plasma levels of a biomarker can infer causal inference under certain assumptions.[78,86] Plasma levels of Ang-2, sRAGE, and S1P3 have been identified as potential casual biomarkers in sepsis-associated ARDS using these techniques.[87,88]

Combining biomarkers to improve diagnostic, predictive, and prognostic value. Thus far, no individual ARDS biomarker candidate has demonstrated acceptable statistical sensitivity and specificity to serve as an ideal predictive, diagnostic, or prognostic biomarker. Recently, researchers have combined biological markers to improve the sensitivity and sensitivity. For example, a panel of 7 biomarkers (sRAGE, procollagen peptide III, brain natriuretic peptide, Ang-2, IL-10, TNF-α, and IL-8) were recently found to exhibit a high diagnostic accuracy for differentiating acute lung injury among trauma patients from controls as reflected by an area under the receiver operating characteristic curve (AUC – 0.86).[89] Similarly, a combination of

biomarkers of epithelial injury (CC16, SP-D, sRAGE) and inflammation (IL-6, IL-8) was demonstrated to exhibit reasonable diagnostic value for ARDS in patients with severe sepsis (AUC – 0.75).[90] Recently, a combination of 6 biomarkers (Ang-2, MIF, IL-8, IL-1RA, IL-6, and eNAMPT) showed promising prognostic value.[63] A subphenotype of ARDS patients with high plasma levels of these cytokines exhibited significantly higher mortality when compared to those with lower levels.[63] Additional attempts to combine clinical and biological markers to enhance the diagnostic accuracy or stratify ARDS patients by prognosis have met with less success.[24,25,91]

ARDS BIOMARKERS IN THE DEVELOPMENT CONTINUUM

The biomarker development pipeline can be divided into the following phases: biomarker discovery, analytical validation, validation for clinical utility, regulatory qualification, and approval (Fig 2). Biomarker discovery is the initial preclinical description of an association with a disease process. Analytical validation involves the assessment of the performance of the assay in specific samples. How reliably does the test measure the analytes of interest in the patient specimen? Clinical validation assesses how robustly and reliably is the test result correlated with the clinical phenotype or outcome of interest. Regulatory qualification and approval is required prior to clinical implementation of a biomarker. Many candidate biomarkers in ARDS, such as TNF-α, IL-6, and IL-8, are based on preclinical investigations of dysregulated cellular pathways characteristic of acute lung injury[14,15,44] and have proven to share an element of analytical validity.[44] Ideally, novel biomarker assays would typically undergo optimization and confirmation of analytical validity.[92] Unfortunately, without standardization of platform techniques and robust external analytical validation, it is difficult to rule out analytical flaws and laboratory errors in many studies claiming an association between blood biomarker candidates and ARDS. Over the past 60 years, immunoassays used for qualitative and quantitative detection of analytes have evolved from uniplex/conventional enzyme-linked immunosorbent assay formats that rely on colorimetric enzymatic substrates for detection to multiplex enzyme-linked immunosorbent assay systems that adopt chemiluminescent/fluorescent reporter systems.[92] Contemporary multiplex immunoassay systems include platforms such as Luminex, Cytometric Bead Arrays, and Bio-PlexPro that employ a suspension format or platforms that rely on a planar format such as the Mesoscale Discovery Technology Platform (MSD) and the Q-Plex array (Quansys Biosciences).[92] While these multiplex platforms present a theoretical benefit in terms of comprehensive profiling in complex phenotypes such as ARDS, challenges such as cross-reactivity of capture and/or detection antibodies may compromise readout viability.[93,94] Fig. 3

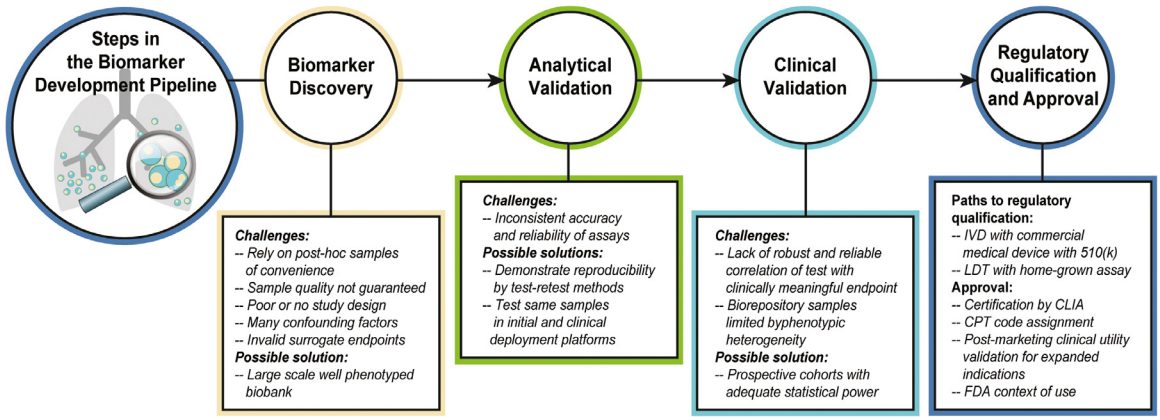

Fig 2. Steps in the biomarker development pipeline. There are 4 phases in the biomarker development pipeline. Biomarker discovery is the initial preclinical description of an association with a disease process. Analytical validation involves the assessment of the performance of the assay in specific samples. How reliably does the test measure the analytes of interest in the patient specimen? Clinical validation assesses how robustly and reliably is the test result correlated with the clinical phenotype or outcome of interest. Regulatory qualification and approval is required prior to clinical implementation of a biomarker. The FDA regulates initial biomarker qualification in the USA. IVD, in vitro diagnostic products are classified by the FDA to determine the appropriate premarket process. The FDA does not enforce premarket review of LDT – laboratory developed test – in vitro diagnostic test designed, manufactured and used within a single laboratory. CLIA, Clinical Laboratory Improvement Amendments, regulate laboratory testing and require clinical laboratories to be certified by the Center for Medicare and Medicaid Services (CMS) before they can accept human samples for diagnostic testing. CPT, Common Procedural Terminology.

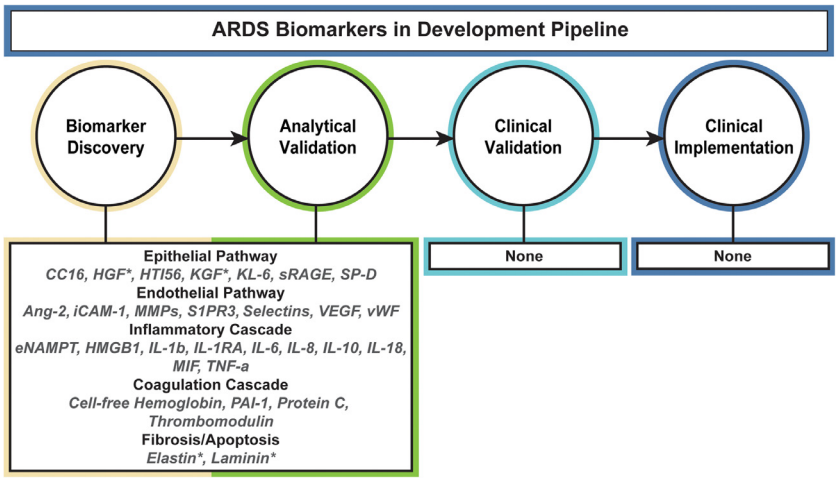

Fig 3. ARDS biomarkers in the development continuum. The majority of the current candidate biomarkers have laboratory developed or commercially available assays that have undergone some form of analytical validation. No biomarker has cleared the threshold for robust and reliable clinical validation. Biomarkers are listed by type of dysregulated pathway. Ang-2, angiopoietin 2; CC16, Clara cell secretary protein; HGF, hepatocyte growth factor; HTI_{56}, human alveolar type I cell protein; KL-6, Krebs von den Lungen-6; HMGB1, high mobility group box nuclear protein 1; iCAM-1, intracellular adhesion molecule 1; IL-1β, interleukin 1 beta; IL-1RA, interleukin 1 receptor antagonist; IL-6, interleukin 6; IL-8, interleukin 8; KGF*, keratinocyte growth factor; MMP, matrix metalloproteinase; MIF, macrophage migration inhibitory factor; MIP-1α, macrophage inflammatory protein-1α; eNAMPT, extracellular nicotinamide phosphoribosyltransferase; PAI-1, plasminogen activator inhibitor-1; S1PR3, sphingosine-1phosphate receptor 3; SP-D, surfactant protein D; sRAGE, soluble receptor for advanced glycation end products; TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor. Elastin*, Laminin*. *No evidence of analytical validation.

summarizes the state of ARDS biomarkers in the development continuum and a majority of the current candidates have commercially available assays that have undergone a degree of analytical validation. However, details regarding the quality of samples and reproducibility of the platform are not always reported.[92]

Validation for clinical utility should distinguish between at-risk ICU controls and ARDS patients and between ARDS survivors and nonsurvivors. Ideally, validation for clinical utility should include an assessment of performance against clinically meaningful outcomes in multiple prospective cohort studies. For ARDS, mortality is the universal endpoint, however, the context of use determines the endpoint chosen. As noted above, the historical focus in the ARDS biomarker research community has been on discovery. No ARDS biomarker candidate has cleared regulatory qualification and approval.

CHALLENGES TO SUCCESSFUL TRANSLATION OF ARDS BIOMARKERS

As noted above, general biomarker development, including specific utility in ARDS, remains a limited priority and pales in comparison to efforts in government-funded university laboratories where novel biomarker discovery is the focus and centers on demonstrating disease associations[5] but do not address issues of clinical validation. The focus of these laboratories is entirely understandable given the lack of capacity and resources to undertake the stringent biomarker profiling required to attract investment in clinical trials.[5] Unidentified flaws at the time of biomarker discovery can hinder the subsequent progression along the development pipeline. Such flaws include, but are not limited to, the poor quality of biospecimens (frequent freeze-thawing, etc.), insufficient sample numbers (leading to inadequate statistical power), and incomplete phenotypic clinical data linked to the assayed samples.[5] Another challenge is the variability in test accuracy and reproducibility of analytical platforms, resulting in inconsistent performance of various measurement assays and inability to replicate the original claims. Publication bias in favor of positive results is another challenge to proper clinical validation. There are currently no standards, best practices, or guidelines to guide investigators in the ARDS biomarker research and development. A final concern is the relative absence of multidisciplinary coordinated efforts in the ARDS research community to address the unmet need for ARDS biomarkers which requires integrative and collaborative approaches.

THE WAY FORWARD

In order to address the major barriers of moving forward beyond association and toward causation, mechanism, and predicting response, researchers need to veer from working in "silos" and instead move to the forging of new collaborative, integrative approaches to biomarker development.[5] This is important because specific failures of biomarkers begin at the discovery and analytical validation phases. The need for broadly accepted standards to inform every module and decision point of biomarker development cannot be overemphasized.[5] In terms of clinical validation, beyond strategies such as increasing statistical power, deeper phenotyping to minimize heterogeneity, and more robust replication, there is a need for innovative study designs such as cell based screening of candidate biomarkers,[95] single patient (N-of-1) designs based on biomarker profiles,[96] adaptive signature designs[97] and the use of mediation analysis[98] or Mendelian randomization.[99] Another emerging approach is to leverage the ready availability of rich and expansive datasets and advances in multi-omic technologies and computational platforms to identify novel biomarkers.[100,101] Machine learning unsupervised algorithms capitalize on the vast amount of human genetic information in large populations, comparing transcriptomic, proteomic, and metabolomics profiling of patients with disease vs healthy individuals to identify novel biomarkers.[102] Strategies to improve the rates of ARDS biomarker validation should include new collaborative research networks that include all stakeholder communities (researchers, funding agencies, and pharmaceutical companies) mobilizing resources and diverse expertise. For example, the national biomarker development alliance has proposed standards-based, end-to-end systems approach to facilitate the seamless flow of meritorious biomarker candidates within and across the modules of the discovery and development pipeline.[5] The ongoing SARS-CoV-2/COVID-19 pandemic presents a unique opportunity given the dramatic increase in number of COVID-19-associated ARDS cases worldwide. Therefore, a national federal-funded ARDS biomarker consortium in partnership with industry would appear to serve as an excellent starting point.

CONCLUSIONS

A critical gap exists between the fast pace of biomarker discovery in ARDS and successful translation to clinical use. This gap underscores the fundamental biomarker conundrum across various acute and chronic disorders: how does a biomarker address a specific unmet need? In addition, this gap highlights the need to shift the paradigm from a focus on biomarker discovery to greater translational impact. In ARDS, the unmet need is for reliable validated ARDS biomarkers that minimize heterogeneity and allow for stratification of subject selection for enrollment in clinical trials, tailored therapies for specific endotypes as suggested by biomarkers, and a more streamlined drug approval process. This will require multilateral collaboration and, while challenging, has never before been as within reach as it is today.

ACKNOWLEDGMENTS

Conflicts of Interest: All authors declare no relevant conflict of interest.

Funding: This work was supported by K08 HL141623 (C.B.); P01 HL126609 (J.G.N.G.); R41 HL147769 (J.G.N.G.); R42 HL145930 (J.G.N.G.).

All authors have read the journal's authorship agreement and the manuscript has been reviewed by and approved by all named authors. The authors did not have any editorial support for preparation of this manuscript.

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