Abstract

Advancements in methods, technology, and our understanding of the pathobiology of lung injury have created the need to update the definition of experimental acute lung injury (ALI). We queried 50 participants with expertise in ALI and acute respiratory distress syndrome using a Delphi method composed of a series of electronic surveys and a virtual workshop. We propose that ALI presents as a “multidimensional entity” characterized by four “domains” that reflect the key pathophysiologic features and underlying biology of human acute respiratory distress syndrome. These domains are 1) histological evidence of tissue injury, 2) alteration of the alveolar–capillary barrier, 3) presence of an inflammatory response, and 4) physiologic dysfunction. For each domain, we present “relevant measurements,” defined as those proposed by at least 30% of respondents. We propose that experimental ALI encompasses a continuum of models ranging from those focusing on gaining specific mechanistic insights to those primarily concerned with preclinical testing of novel therapeutics or interventions. We suggest that mechanistic studies may justifiably focus on a single domain of lung injury, but models must document alterations of at least three of the four domains to qualify as “experimental ALI.” Finally, we propose that a time criterion defining “acute” in ALI remains relevant, but the actual time may vary based on the specific model and the aspect of injury being modeled. The continuum concept of ALI increases the flexibility and applicability of the definition to multiple models while increasing the likelihood of translating preclinical findings to critically ill patients.

Keywords: lung injury; respiratory distress syndrome; pneumonia; hypoxia; extravascular lung water
Overview

The purpose of this report is to update the definition of acute lung injury (ALI) in model systems, revise the relevant measurements that describe the main features of ALI, and reassess the role of time as part of the definition. The ultimate goal is to provide a current framework for defining experimental ALI, which can serve as a standard for the field. The key findings of this workshop are as follows:

- Experimental ALI encompasses a continuum of models ranging from those focusing on gaining specific mechanistic insights to those primarily concerned with preclinical testing of novel therapeutics or interventions.
- We suggest that mechanistic studies may justifiably focus on a single domain of lung injury, but models must document alterations of at least three of the four domains to qualify as "experimental ALI." For preclinical testing of novel therapeutics or interventions, fulfillment of all four domains is recommended.
- Demonstrating alterations in a domain requires at least one measurement identified as "relevant" for that domain.
- We propose that a time criterion defining "acute" in ALI remains relevant, but the actual time may vary based on the specific model and the aspect of injury being modeled.

Introduction

There is significant variability in what researchers consider ALI in an animal or model system. This variability makes it difficult to compare data from different studies and assess rigor and transparency, and it may be a barrier to accurate "bench-to-bedside" translation. Therefore, there is a major need to agree on what constitutes ALI in animals. In 2011, the American Thoracic Society (ATS) published a Workshop Report that used a Delphi approach to identify the main features and measurements defining experimental ALI (1). Since then, advances in imaging, genetic tools, "omics" technologies, and cellular biology have provided new insights into lung injury both in preclinical models and in humans (2–6). These advancements have created a need for a careful reexamination of how ALI is measured and defined, with the goal of facilitating optimal translation of preclinical observations to clinical medicine. The purpose of this update to the 2011 Workshop report is to refine the definition of ALI in model systems, revise the relevant measurements that describe the main features of ALI, and reassess the role of time as part of the definition. The ultimate goal is to provide an updated framework for defining experimental ALI, which can serve as a standard for the field.

Defining the Goal of Experimental ALI Models

A major question regarding experimental models of ALI is what is being modeled. Originally described in 1967 as a case series of 12 patients, acute respiratory distress syndrome (ARDS) was characterized clinically by acute onset of tachypnea, worsening hypoxemia, impaired lung compliance, and widespread alveolar opacities on radiographic imaging (7). Since its original description, the definition of ARDS remains clinical and has evolved to the current 2012 Berlin consensus definition that focuses on "feasibility, reliability, validity and objective evaluation of its performance" (8). The Berlin definition defines the time component of acute onset as "occurring within 7 days of exposure to a recognized predisposing event." It also includes risk factors to consider when ascertaining the origin of edema, categorizes ARDS severity based on physiologic dysfunction or hypoxemia, and provides more explicit criteria for bilateral airspace opacities on radiographic imaging (8). Although animal models are central to studying this clinically defined syndrome, each of the model systems have limitations that need to be considered depending on the goal of the study. For example, small animal models may not replicate all the clinical features of ARDS but are tractable systems that can address mechanisms of disease and provide the framework for rational therapeutic design. Large animal models are less malleable to mechanistic studies but may reproduce clinical features of ARDS and therefore serve as important models for preclinical therapeutic testing (9).

Animal models of ALI have played a fundamental role in the development of key effective interventions for the treatment of ARDS. For example, the concept that overexpansion of alveolar spaces was due to...
excessive tidal volumes or inspiratory pressures was initially derived from animal studies and eventually led to low tidal volume ventilation (10–15). Similarly, animal studies of the impact of gravity on pulmonary perfusion during lung injury provided the initial rationale for prone ventilation in ARDS (16). However, challenges exist in the direct application of criteria used to define ARDS in humans, and one must first determine the goal of the study to identify the optimal model of experimental ALI (Figure 1). For instance, rodent models of ALI rarely use hypoxemia as a defining criterion despite it being the primary criterion of physiologic dysfunction in humans. This is partly because of the technical challenges of obtaining an arterial blood gas in small animals, which is more feasible in large animal models. In contrast, rodent models may serve the goal of investigating genetic variants and predisposing conditions more readily than large animal models (Figure 1). Although patients with ARDS can be supported with invasive or noninvasive mechanical ventilation, mechanical ventilation is possible in rodent models of experimental ALI for only short periods of time and is not a practical approach to study the resolution phase of injury in either small or large animal models. Despite these limitations, animal models remain essential to advance the understanding of the biology of ALI, and working toward uniformity on what constitutes ALI in a preclinical experimental model may accelerate progress related to specific therapeutics in ARDS.

**Methods**

**Selection of Participants**

Recommendations for workshop panelists were solicited from participants of the 2011 workshop report as well as from the Assembly Chair and Planning Committee Chair of the ATS Assemblies that cosponsored the workshop (Allergy, Immunology, and Inflammation; Critical Care; Environmental, Occupational, and Population Health; Pediatrics; Pulmonary Circulation; Pulmonary Infections and Tuberculosis; Respiratory, Cell, and Molecular Biology; and Respiratory Structure and Function). A list of 50 participants was finalized to include those working in the fields of experimental ALI and human models of ARDS as well as to have representation from diverse geographical regions, genders, and seniority levels (Table E1 in the data supplement). For each domain, a lead was identified to collate and coordinate survey responses from each round and report back to the committee chairs. Potential conflicts of interest were disclosed and managed in accordance with the policies and procedures of the ATS.

**Delphi Approach**

Similar to the 2011 workshop, a Delphi method was used to solicit measurements of experimental ALI (Figure 2). In Round 1 (Table E2), the 50 participants were asked to complete an electronic survey, wherein they stated all the measurements that they thought would be helpful to assess 1) histological evidence of tissue injury, 2) alteration of the alveolar–capillary barrier, 3) presence of an inflammatory response, and 4) evidence of physiologic dysfunction (1). The “acute” component of ALI was defined as “24 hours from the initial injurious intervention.” We convened the current workshop to update the ALI definition by answering the following questions: 1) Should a time criterion continue to be included in the definition of experimental ALI, and, if so, what should that time be? 2) What is the minimum number of domains that should show alterations to determine that ALI has occurred? 3) What are the measurements that determine that one of these domains has been altered? To address these questions, we queried a broad and diverse range of international experts in the field of ALI and ARDS, using a Delphi approach.
Virtual Discussion
The original proposal included an in-person meeting, but because of the coronavirus disease (COVID-19) pandemic, this was replaced by virtual approaches. First, a video containing a presentation of the results was prerecorded by the domain leads and shared with the participants. Certain questions that were perceived by the domain leads to be important for the appropriate interpretation of the document were also included in this presentation (Table E5). Subsequently, two 90-minute virtual sessions in which the domain leads presented the results were held in the same week. Participants were encouraged to give their opinions about the questions in a live, online setting. All participants were asked to attend at least one of these sessions.

Post-Meeting Survey
The participants were subsequently emailed a post-meeting survey composed of the same questions that were discussed at the virtual meeting (Table E6). A free-text box was also provided for participants to provide feedback on any aspects of the project, including the methodology and/or the results. These results were tabulated by the domain leads and have been incorporated into the manuscript to demonstrate the extent of agreement on each of the questions.

Results
The concept of four domains reflecting the key pathophysiologic features and underlying biology of experimental ALI were retained from the 2011 workshop report (Table 1). Additionally, the time criterion defining “acute” was retained based on responses from the participants. Key results for each domain and the time criterion are presented below.

Histological Evidence of Tissue Injury
Most of the histological features of ALI proposed by the panel (Table 2) represent individual aspects of tissue injury, but one of them deserves specific mention: a “validated histologic score,” ranked number 2 overall and recommended by 63% of respondents. However, there is no validated score at the present time with demonstrated intra- and interobserver reproducibility. This situation has not changed from 2011, when such a score did not yet exist. In the prior report, an example of a potential score was proposed, but many readers understood it as a “recommended score” rather than a “proposal for a score.” Therefore, we purposely do not include any scores in the present update. Instead, the issue of histological scoring and injury quantification was further discussed at the virtual meeting, and in the post-meeting survey, panelists were asked, “how should histologic injury be quantified” (Table E6, Q.2)? The majority (57%) of respondents answered that a blinded assessment of lung injury features in several nonoverlapping fields with a clear methodologic description of each measure was preferred. Other respondents (36%) stated that the field should develop a validated lung injury score to be used in histology, followed by a smaller group of
respondents stating that no quantification is necessary (5%). Automated assessment using image analysis software was suggested by one respondent (2%).

In addition to a validated histologic score, other features ranked “most relevant” to the domain by the highest number of panelists (number 1 and number 3, respectively) were “filling of the alveolar space with proteinaceous alveolar fluid and debris” (82% of respondents) and “evidence of alveolar epithelial injury” (57% of respondents). Each of these reflect consequences of destruction of the alveolar–capillary barrier and flooding of the alveoli with protein-rich fluid. Interestingly, “neutrophilic infiltration” and “interstitial edema” were both ranked lower but remained above the 30% cutoff required for a “recommended measurement.” We speculate that this may reflect the perception that these two assessments are less specific for ALI; for example, “interstitial edema” can also be seen in cardiogenic pulmonary edema. Finally, the other two measurements recommended by 30% or more of the respondents and therefore considered “most relevant” were “diffuse alveolar damage pattern” (43%) and “hyaline membranes or presence of fibrin derivatives in the airspaces” (41%).

**Alteration of the Alveolar–Capillary Barrier**

Disruption of the alveolar–capillary barrier is a central feature of ARDS, leading to flooding of the airspace with protein-rich fluid. Loss of barrier integrity differentiates pulmonary edema caused by ARDS and edema from cardiogenic causes. Experimental measures of alveolar–capillary barrier dysfunction reflect the major pathophysiologic changes that result from barrier loss. The feature considered “most relevant” by the highest number of panelists was the direct measurement of high concentrations of albumin or, alternatively, IgM or another large-molecular-weight plasma protein, which nearly 90% of the panel considered to be in the top five “most relevant” measures of alveolar–capillary barrier dysfunction (Table 3). Other direct measures of leakage of plasma components into the airspace or interstitium were also considered “most relevant” and included elevated BAL total protein, Evan’s blue dye–labeled albumin and protein accumulation in lung homogenate, rate of accumulation of a tagged marker in the airspace, and transport of a large-molecular-weight substance. Although Evan’s blue dye is
**Table 1. Main Features of Experimental ALI**

**Main Features**

| Rapid onset (with a defined period of time, specific to the model utilized) plus* | Histological evidence of tissue injury |
| --- | --- |
| Alteration of the alveolar–capillary barrier | Presence of an inflammatory response |
| Evidence of physiological dysfunction |

*Definition of abbreviation: ALI = acute lung injury. To state ALI has occurred, at least one accepted “relevant” measurement under at least three out of four domains should be reported.

Commonly used as a tracer because it binds tightly to albumin and other proteins, thus reflecting transit of these proteins into the airspace, unbound Evan’s blue is only 0.98 kD and acts as a small molecule. When using this method, caution should be taken to ensure that free Evan’s blue is not present. Endothelial-specific permeability can be assessed by the measurement of the filtration coefficient, but this measure is technically challenging and is not available in all laboratories. Another feature considered “most relevant” to the domain by ≥30% of the panelists was assessment of pulmonary edema accumulation as measured by lung wet weight to dry lung weight or body weight ratio. Currently, the ability to quantify edema readily and easily is a unique feature of animal models. Although extravascular lung water can be measured with thermodilution techniques (18), these methods have limited clinical applicability and are not as accurate as gravimetric methods (19).

Measurements that less than 30% of panelists considered “most relevant” included markers of alveolar epithelial injury (e.g., BAL or plasma RAGE [receptor for advanced glycation end products] or SP-D [surfactant protein-D]), surfactant function, and evidence of large defects in the alveolar–capillary barrier (e.g., red blood cells in the airspace and transfer of very large-molecular-weight proteins across the barrier). Although these measures were not considered “most relevant” to the domain by ≥30% of the panelists, they may be highly relevant to specific studies of alveolar epithelial injury, surfactant dysregulation, and hemorrhage into the airspace. In a follow-up questionnaire, we asked the panel if, in aggregate, measures of alveolar epithelial injury should be added as a measure that would fulfill this domain for purposes of defining ALI (Table E6, Q.3). The majority of respondents (52%) were of the opinion that epithelial injury alone should not be used to fulfill this domain. It was noted that although epithelial injury is a prominent feature of both ARDS and experimental ALI, there can be some degree of epithelial injury without significant breakdown of the alveolar–capillary barrier (20–22). Thus, epithelial injury alone is insufficient as a measure of alveolar–capillary barrier dysfunction. Overall, measures in this domain reflect the barrier breakdown and extravascular accumulation of lung fluid and protein that are key features of human ARDS.

**Presence of an Inflammatory Response**

Nearly all respondents (96%) chose “increase in chemokine and cytokine expression in the BAL or lung tissue” as a relevant measurement indicating the presence of an inflammatory response (Table 4). Soluble mediators stated by respondents included chemokines such as IL-8 (CXCL8) or its murine homolog chemokines KC (CXCL1) and MIP-2 (macrophage inflammatory protein-1 or CXCL2), MCP-1 (monocyte chemotactic protein-1 or CCL2), and MCP-3 (CCL7) as well as cytokines such as IL-6, TNF-α (tumor necrosis factor–α), IL-1β, IL-18, sTNFRI (soluble TNF receptor 1), and IL-10. The majority of respondents (55%) recommended measuring these inflammatory mediators as examples indicating the presence of an inflammatory response, and there was broad agreement that this list of mediators is neither comprehensive nor specific to lung injury per se (Table E6, Q.5). When asked in what compartment (e.g., blood, lung tissue, airspace compartment) should endothelial cell adhesion molecule expression or mediator release be measured, the majority of respondents (52%) stated that the compartment should not be specified, whereas the rest of the respondents were split among the blood, lung tissue, and airspace compartments (Table E6, Q.4).

Measurements that less than 30% of panelists considered “most relevant” included transcriptomic signatures that mirror those found in clinical ARDS; soluble damage associated molecular patterns such as sICAM-1 (soluble intercellular adhesion molecule), sVCAM-1 (soluble vascular cell adhesion molecule), Ang-2 (angiopoietin-2), and vWF (von Willebrand Factor) are relevant measurements of endothelial injury. The majority of respondents (55%) recommended providing measurements of one or more of these mediators (e.g., sICAM-1, sVCAM-1, Ang-2, vWF), recognizing that the list is neither exhaustive nor specific to lung injury per se (Table E6, Q.5). When asked in what compartment (e.g., blood, lung tissue, airspace compartment) should endothelial cell adhesion molecule expression or mediator release be measured, the majority of respondents (52%) stated that the compartment should not be specified, whereas the rest of the respondents were split among the blood, lung tissue, and airspace compartments.
as extracellular ATP, extracellular DNA, and HMGB1 (high mobility group box 1); proteases such as matrix metalloproteinases and elastase; changes in acute response genes; inflammasome activation; mitochondrial dysfunction; and neutrophil extracellular traps (Table 4). The relevant measurements of this domain reflect a broader recognition of the changing composition of the inflammatory cellular profile depending on the experimental animal model and the phase of injury being studied. In addition, the inflammatory response domain now includes endothelial cell adhesion molecule expression or endothelial mediator release as a relevant measurement.

Evidence of Physiological Dysfunction

Physiological dysfunction, defined here not only as impaired alveolar–capillary gas exchange, lung mechanics, or alveolar fluid clearance but also as systemic manifestations of ALI and notably altered appearance of lung tissue on radiographic imaging, is the cardinal feature of clinical ARDS. The 2012 Berlin definition of ARDS is primarily based on impaired oxygenation and evidence of bilateral opacities on chest imaging (8). As such, different measures of gas exchange, including partial pressure of arterial oxygen, ratio of partial pressure of arterial oxygen to fractional inspired oxygen, oxygen saturation, alveolar–arterial oxygen gradient, partial pressure of carbon dioxide, diffusing capacity of carbon monoxide, or ventilation–perfusion mismatch, were commonly named by all respondents (Table 5). Of these, arterial blood gas measurements of oxygenation were ranked by ≥30% of the panelists as being “most relevant” to the domain. It was, however, noted by several respondents that although arterial blood gases are probably the “ideal” measurement, blood gas analyses in small rodent models require specific instrumentation (e.g., small animal blood gas analyzer) and experience in blood sampling. Furthermore, arterial blood sampling for blood gas analysis is commonly a terminal procedure in mice unless an indwelling arterial catheter has been placed; even in the latter case, the number of possible blood samplings remains limited by the small murine blood volume. As such, noninvasive assessment of oxygenation by pulse oximetry may provide a reasonable alternative (albeit signal quality deteriorates commonly in hemodynamic shock) and was accordingly also considered by nearly half the respondents as one of the top four “most relevant” measures of physiological dysfunction. Second to changes in gas exchange, impaired lung mechanics were considered “most relevant” by the highest number of panelists. Of these, changes in lung or respiratory system compliance were considered the “most relevant,” followed by changes in dead space, airway and/or tissue resistance, total or inspiratory lung capacity, or atelectasis. Several respondents highlighted the forced oscillation technique as a widely accepted, accurate measure of lung mechanics in small rodents. Third, a

Table 2. Measurements of Histological Evidence of Tissue Injury

| Domain Recommendations                                                                 | n (%) |
|----------------------------------------------------------------------------------------|-------|
| Filling of the alveolar space with proteinaceous alveolar fluid and debris*             | 40 (82)|
| A validated histologic injury score*                                                   | 31 (63)|
| Evidence of alveolar epithelial injury (cell death, epithelial denudation, or ATII proliferation)* | 28 (57)|
| Neutrophil infiltration of the alveolar space*                                         | 26 (53)|
| Thickening of alveolar septae and/or interstitial edema*                               | 25 (51)|
| Diffuse alveolar damage pattern*                                                       | 21 (43)|
| Hyaline membranes or presence of fibrin or derivates in the airspaces*                 | 20 (41)|
| Evidence of intraalveolar hemorrhage or extravasated red cells                         | 14 (29)|
| Evidence of capillary and/or endothelial cell death                                     | 13 (27)|
| Neutrophil infiltration of alveolar septae or interstitium                              | 11 (23)|
| Perivascular inflammation, including intravascular accumulation of neutrophils        | 8 (16 )|
| Perivascular edema or cuffing                                                         | 3 (6 )|
| Hepatization                                                                           | 2 (4 )|
| Loss of tight junctions                                                                 | 2 (4 )|
| Presence of microthrombi                                                                | 1 (2 )|

Definition of abbreviation: ATII = Type II alveolar epithelial cell.
*Features or measurements that were considered as being “most relevant” to the domain by 30% or more of the respondents.

Table 3. Measurements of Alteration of the Alveolar–Capillary Barrier

| Domain Recommendations                                                                 | n (%) |
|----------------------------------------------------------------------------------------|-------|
| Elevated BAL albumin, IgM, or other large circulating protein*                          | 44 (90)|
| Increased lung wet-to-dry weight ratio, lung wet weight to body weight ratio, or extravascular lung water* | 38 (78)|
| Elevated BAL total protein*                                                             | 30 (61)|
| Evan’s blue dye accumulation in lung homogenate*                                      | 24 (49)|
| Pulmonary vascular permeability index and/or filtration coefficient*                    | 21 (43)|
| Rate of accumulation of tagged marker (fluorescent probe, I-131 albumin, etc.) in the airspace* | 20 (41)|
| Transport of large-molecular-weight substance (~70 kDa or larger, e.g., dextran)*       | 18 (37)|
| Accumulation of airspace-injected tracers into the circulation                         | 9 (18 )|
| Circulating markers of epithelial and/or airway injury (e.g., RAGE, SP-D, KL-6)        | 9 (18 )|
| Increased markers of ATI or ATII injury in the airspace                                 | 9 (18 )|
| Hemorrhage and/or RBCs in airspace                                                     | 8 (16 )|
| Elevated BAL RAGE                                                                      | 7 (14 )|
| Transport of a very large (~300 kDa) tracer across barrier                             | 7 (14 )|
| Surfactant function                                                                    | 1 (2 )|

Definition of abbreviations: ATI = Type I alveolar epithelial cells; KL-6 = Kreb von den Lungen-6; RAGE = receptor for advanced glycation end products; RBCs = red blood cells; SP-D = surfactant protein-D.
*Features or measurements that were considered as being “most relevant” to the domain by 30% or more of the respondents.
change in alveolar fluid clearance was considered by more than half of the respondents as one of the top four “most relevant” features of physiological dysfunction, although it was pointed out that quantification of alveolar fluid clearance, particularly in rodent models, is performed in only a few specialized laboratories. Measures of impaired breathing such as increased respiratory rate, visualized difficulties in breathing, or increased minute ventilation were also considered as one of the top four “most relevant” measures. Notably, the applicability of this criterion will depend on the model of ALI and its time course (i.e., impaired breathing will be typically observed after longer time periods in spontaneously breathing animals).

Measurements that less than 30% of panelists considered “most relevant” included the quantity of pathogen (in pneumonia, sepsis, or peritonitis models), systemic illness and/or systemic organ dysfunction, weight loss, systemic hemodynamics (for monitoring vital signs), or temperature. Notably, the low ranking for some of these features (e.g., pathogen quantity) may be attributable to individual models used, as numerous animal models of ALI, such as ventilator-associated lung injury, bleomycin, oleic acid, or acid aspiration, are sterile in nature and do not involve the administration of infectious pathogens. Another measurement that less than 30% of panelists considered “most relevant” was the appearance of lung tissue on radiographic imaging. As this measurement constitutes one of the cardinal features of ARDS in humans, we asked respondents why lung imaging may be less relevant in animal models than in humans (Table E6, Q6). Most respondents (84%) conceded that imaging is an important tool to document ALI in large animals. Yet in small animals, in which the bulk of ALI research is performed, imaging is considered as a potentially powerful and evolving technology that is, however, not widely used or available. It is noteworthy that relevant measurements of physiological dysfunction received much broader support as compared with the 2011 definition, which only listed hypoxemia and an increased alveolar–arterial oxygen difference as “very relevant” features. In contrast, in addition to impaired gas exchange, impaired lung mechanics, alveolar fluid clearance, and signs of increased respiratory effort (e.g., elevated respiratory rate, difficulty breathing, and minute ventilation) were now also considered as “most relevant” features by at least 30% of respondents.

### Recommended Definition of Experimental ALI

The 2011 workshop report recommended that alterations in at least three of the four domains should be present to determine that ALI has occurred in an experimental model (Table 1). In this revised report, we propose that experimental ALI encompasses a continuum of models ranging from those focusing on gaining specific mechanistic insights to models that are largely concerned with preclinical testing of therapeutics or promising interventions (Figure 3). This new framework acknowledges that mechanistic studies may justifiably focus on one or two domains of lung injury (i.e., “potential lung injury”; Figure 3). However, to fully qualify as “experimental ALI” (i.e., “demonstrated lung injury”; Figure 3), a model should demonstrate alterations in at least three of the four domains, reflecting the multidimensional aspects of human ARDS (Table 1). We propose that demonstrating alterations in a domain requires at least one measurement identified as “relevant” for that domain (Tables 2–5). “Relevant measurements” are those that were ranked among the “top 4–5 most relevant measurements” by at least 30% of the respondents (Tables 2–5). Tables 2–5 also list measurements that did not reach the 30% cutoff. These measurements may be included or reported in support of changes in a domain, but at least one of the “relevant” measurements should also be reported to fulfill that domain. As the goal of experimental modeling advances from studying basic mechanisms to preclinical drug testing and the need to ensure translation to clinical studies increases, we suggest demonstrating alterations of all four domains using at least one “relevant measurement” per domain (Figure 3).

### Time Criterion as a Part of the Definition of Experimental ALI

Nearly three-quarters of respondents considered that the definition of experimental ALI should include a time criterion to define “acute.” However, the time range for this criterion was broad. Among those who responded “yes,” nearly one-third proposed the range to be within 24 hours from the onset of exposure to the stimulus. The next most common answer was 72 hours (14%). Approximately 10% of
specific mechanisms to studies primarily concerned with preclinical testing of novel therapeutics or promising interventions (Figure 3). Accordingly, the stringency of the requirements to identify lung injury in an experimental model should also follow a continuum that matches study goals. This new framework acknowledges that mechanistic studies may justifiably focus on a single aspect or domain of lung injury, but in those cases, they would suffice as “potential lung injury.” However, for a model to qualify as “experimental ALI,” we recommend demonstrating alterations in at least three of the four domains of lung injury (Table 1). For preclinical testing of novel therapeutics or interventions, fulfillment of all four domains is recommended. Thus, although the definition of ALI remains binary, the fulfillment of domains represents a continuum based on the purpose of the model, with a greater number of domains fulfilled increasing the confidence of demonstrating ALI in the model. Additionally, our revised definition decreases the number of “relevant measurements” required to fulfill an ALI domain to only one, thus increasing flexibility for the field. This “continuum” concept seeks to improve bench-to-bedside translation without imposing excessive burden to researchers doing highly mechanistic, focused studies. This concept also emphasizes the need for experimental ALI to reflect the multidimensional aspects of human ARDS.

We were also able to build on feedback from the workshop participants that the original time criterion of 24 hours for defining “acute” was too restrictive. We continue to recommend a time criterion in the definition of experimental ALI, but we now recommend against a fixed time cutoff required to define “acute.” Instead, we acknowledge that the time cutoff may vary depending on the specific injury-causing agent, the model, doses studied, and any use of adjunctive therapies (e.g., antibiotics). We foresee our revision facilitating additional rigor in the field while allowing for newer, experimental models to evolve, which would more accurately represent ARDS in humans (17).

The four “domains” of experimental ALI emerged iteratively during the Delphi process of the 2011 workshop (1), and, therefore, we decided to keep these domains as a conceptual framework for our project. In the current workshop, we did not ask for reference values for any measurement, realizing that these are model specific and may differ between laboratories depending on techniques used. Additionally, we deliberately chose not to specifically discuss any individual lung injury models. There were several reasons for this. First, there are many excellent review papers on this topic (26–29). Second, we wanted to provide a flexible conceptual framework that could be applied to different lung injury models. Third, we wanted to encourage innovation in ALI model development (9). We reasoned that providing a list of models, or even examples, might be limiting for investigators wishing to develop new ways of modeling ALI. We hope our approach will increase the flexibility and applicability of the proposed definition for ALI.

“Histological evidence of tissue injury” has been considered as the most relevant defining feature of experimental ALI (1). However, the definition of ARDS is entirely clinical and does not include pathology (8). It has been repeatedly demonstrated that histological patterns other than diffuse alveolar damage can be seen in patients who otherwise meet the current Berlin definition of ARDS (30–32). Therefore, in the experimental setting, it may be important to avoid defining a single histological pattern as “diagnostic” or “required” to establish that ALI is present in an animal and instead focus on specific pathological features that are relevant for the hypothesis being tested in the study. For example, a study investigating whether deleting a particular gene impairs neutrophil recruitment may find it useful to focus on different assessments of the histological distribution of neutrophils in the lungs. In contrast, a study investigating a potentially novel therapeutic may want to
emphasize the coexistence of several different histological measurements of lung injury (Table 2). Thus, an important difference in the present recommendations compared with the 2011 workshop is that the specific measurements of histological injury should be tailored to the specific scientific question being asked in each study. Members of the panel emphasized that histologic features of lung injury should be assessed in a rigorous manner that minimizes the potential for technical bias. We refer the reader to literature discussing important technical considerations (33).

Throughout the Delphi process, there was much discussion of the best way to assess histologic lung injury. A “validated histologic score” ranked highly among the list of features for assessing tissue injury despite the lack of a validated score that serves as a standard. This highlights the urgent need for an unbiased measurement schema in experimental ALI. Resolving this issue is beyond the scope of the current workshop, and we hope that the lung injury field works to develop a validated histologic score that can be broadly applied by ALI investigators. We acknowledge that histological scores may not be equally suitable for all ALI models, and some models may require specific histological criteria (34, 35). There is ongoing work in digital imaging and analysis to develop unbiased, automated computer-based scoring systems for lung injury, either involving computerized pixel-counting algorithms or the use of deep neural networks. Even if successful, these approaches require sophisticated computational and imaging-capture resources, which may be beyond the reach of most investigators. Simpler semiquantitative scores relying mostly on a microscope and basic image-analysis software are sufficient for most purposes.

There is broader recognition that the cellular composition of the inflammatory response may not be exclusively neutrophilic and instead differs in terms of the model system used and the phase of injury. This is exemplified by the inclusion of “increases in inflammatory monocyte and macrophage (and/or lymphocyte) subpopulations in BAL or lung tissue” as a relevant feature, likely reflecting the importance of these infiltrating cell types in models such as bacterial- or viral-induced lung injury as well as in sterile injury (36–40). The inclusion of “endothelial cell adhesion or activation markers” as a relevant feature of the inflammation domain is also new from the original workshop report and reflects a wider understanding of the activated endothelium as an important component of the inflammatory response (41, 42). However, the compartment in which endothelial cell adhesion or activation markers should be measured was not specified, as there was a recommendation that the report should provide general “good practice” guidelines for the field without being overly restrictive. In addition, we acknowledge that the presence of inflammatory features is not specific to ALI and may reflect the underlying insult that led to ALI rather than ALI itself (e.g., in pneumonia or sepsis).

Measurement of alterations in alveolar–capillary barrier permeability can be divided into two main groups: measures of increased extravascular lung water and measures of increased permeability of the alveolar–capillary barrier. These two main aspects have not changed substantially since the 2011 workshop report. Here, measurement of endogenous molecules or tagged tracers in the airspace or measurement of lung wet-to-dry weight ratios remain among the top features that fulfill this domain. One interesting new addition is the suggestion, made by many panelists, of measuring markers of lung epithelial injury (such as RAGE). Although none of these markers reached the >30% threshold and panelists did not feel that a measure of epithelial injury alone fulfills this domain, their inclusion reflects the growing appreciation of their potential to provide insight into the development of ALI and ARDS (43, 44). As our understanding of epithelial biology and specific subpopulations of lung epithelial cells continues to grow, future recommendations for modeling alveolar–capillary barrier dysfunction may become more nuanced.

Although many of the features from the 2011 workshop were still ranked as “most relevant” in our current report, two additional physiological features are now considered to demonstrate physiological dysfunction satisfactorily. First, impaired lung function, specifically reduced respiratory compliance or increased elastance, was considered one of the “most relevant” features to the domain by at least 30% of the respondents. A sizeable proportion (about one-fourth) of the respondents who suggested lung function measurements recommended the use of the forced oscillation technique rather than body plethysmography, in line with the reported
higher sensitivity and specificity of the former technique in small rodent models of airway disease (45–47). Second, impaired alveolar fluid clearance is now included as a relevant parameter of physiological dysfunction, although it was noted that the measurement of alveolar fluid clearance by single or double indicator dilution techniques (48) is challenging in mice and rats and, as such, is not widely available. Overall, physiological measurements remain technically demanding, yet they provide the unique advantage that their absolute values can commonly be compared across studies, although it was noted that histological evidence of injury should always be demonstrated to document “lung injury.” However, we deliberately created the framework to be as flexible as possible, and we feel that flexibility in this regard is more important than a firm insistence on specific measures needed to fulfill ALI criteria. Interpreting the relevance of a particular ALI model will be up to the reader.

We also acknowledge that the domain concept has limitations. For example, not all measurements fit clearly within only one domain. Lung imaging, such as by computed tomography, was suggested as a measurement in all four domains. Because most respondents included it in the physiology domain, after extensive discussion, we elected to include imaging in that domain, but we recognize that this choice was arbitrary. There were additional controversies regarding lung imaging in experimental ALI. Although lung imaging did not reach the 30% cutoff required to be considered a “relevant measurement,” some panelists pointed out that it is of very high value in large animal models. We speculate that the failure of lung imaging to make the cutoff may be due to its lower applicability to small animal models, in which the bulk of ALI research is presently performed. We suspect that the relevance of lung imaging will increase and perhaps become an ALI domain of its own as the availability and resolution of imaging techniques such as micro-computed tomography, small animal magnetic resonance imaging, and other methodologies increases (49).

Conclusions
In summary, this Workshop Report revises and updates the previous 2011 report by emphasizing that models of experimental ALI span a continuum ranging from those used in highly focused mechanistic studies on one end of the spectrum to those used in preclinical testing of novel therapeutics or promising interventions on the other end. Accordingly, the highly mechanistic studies concerned with only one domain of lung injury may focus on multiple measurements of that single domain. However, for a model to qualify as “experimental ALI,” it must demonstrate evidence of alterations in at least one relevant measure from at least three of the four ALI domains and thus reflect the multidimensional aspect of human ARDS. For preclinical testing of novel therapeutics or interventions, fulfillment of all four domains is recommended. This continuum framework increases the flexibility and applicability of the definition, simultaneously inviting innovation and model development while increasing rigor for preclinical studies, and hopefully increases the translational potential. In addition, this revised workshop retains a time criterion as a part of the definition of experimental ALI but emphasizes that the specific time required to define “acute” is model dependent. Although the four-domain framework has proven useful for defining experimental ALI, this framework will need to be reevaluated in future workshops as new technologies become available and concepts within the field evolve.
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