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Pathogenetic Significance of Biological Markers of Ventilator-Associated Lung Injury in Experimental and Clinical Studies*

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For patients with acute lung injury, positive pressure mechanical ventilation is life saving. However, considerable experimental and clinical data have demonstrated that how clinicians set the tidal volume, positive end-expiratory pressure, and plateau airway pressure influences lung injury severity and patient outcomes including mortality. In order to better identify ventilator-associated lung injury (VALI), clinical investigators have sought to measure blood-borne and airspace biological markers of VALI. At the same time, several laboratory-based studies have focused on biological markers of inflammation and organ injury in experimental models in order to clarify the mechanisms of ventilator-induced lung injury (VILI) and VALI. This review summarizes data on biological markers of VALI and VILI from both clinical and experimental studies with an emphasis on markers identified in patients and in the experimental setting. This analysis suggests that measurement of some of these biological markers may be of value in diagnosing VALI and in understanding its pathogenesis. (CHEST 2006; 130:1906–1914)

Key words: ARDS; critical care; ventilation; ventilator-induced lung injury

Abbreviations: ALI = acute lung injury; CI = confidence interval; IL = interleukin; IL-1Ra = interleukin-1 receptor antagonist; MIP = macrophage inflammatory protein; NO = nitric oxide; PBEF = pre-B-cell colony enhancing factor; PEEP = positive end-expiratory pressure; SP = surfactant protein; sTNFR = soluble tumor necrosis factor receptor 1; sTNFR2 = soluble tumor necrosis factor receptor 2; TNF = tumor necrosis factor; VALI = ventilator-associated lung injury; VILI = ventilator-induced lung injury; vWf:Ag = von Willebrand factor antigen

Although clinicians and researchers have been interested in ventilator-induced lung injury (VILI) for at least 30 years, the reduction in mortality associated with low tidal volume ventilation in pa-

tients with acute lung injury (ALI) and ARDS¹ has directed increasing scientific interest toward the mechanisms of VILI. One of the greatest difficulties in clinical studies has been distinguishing the underlying lung injury responsible for the patient's respiratory failure from the lung injury resulting from the particular settings of the mechanical ventilator. Consequently, investigators have searched for biological markers that will reflect ventilator-attributable lung injury. The term *VILI* generally refers to experimental models in which lung injury is induced directly by an injurious ventilation strategy. Ventilator-associated lung injury (VALI) refers to the additional injury imposed on a previously injured lung by mechanical ventilation in either the clinical setting or in experimental studies. For the purposes of this review, the term *ventilator-attributable injury* encompasses all of these types of injury. Researchers have used a variety of experimental models to deter-

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mine the effects of mechanical ventilation or mechanical strain on the expression of biological markers of inflammation or injury, including whole-animal models of VILI and VALI, *ex vivo* lung preparations with or without perfusion, and isolated alveolar epithelial cells. This review summarizes the published literature on biological markers of VALI with an emphasis on markers that have been consistently identified in both experimental and clinical studies. Although considerable data on biological markers have been generated in other lung injury models and clinical studies of ALI patients, only potential markers of ventilator-attributable injury will be considered in this review.

BIOLOGIC MARKERS OF INFLAMMATION IN CLINICAL STUDIES

The role of the innate immune response and inflammation in the pathogenesis of VILI has been widely studied in recent years. Although some have suggested that inflammation may not be integral to the initiation of VILI, clearly a preponderance of data in this field support a major pathogenetic role for inflammation and lung neutrophil recruitment. The majority of biological markers identified in plasma, serum, pulmonary edema fluid, and BAL fluid in experimental studies are cytokines and chemokines. Although none of these mediators distinguishes ventilator-induced injury from other etiologies of lung injury, the temporal association between changes in levels of these proteins and changes in tidal volume or positive end-expiratory pressure (PEEP) along with inhibitor studies suggests a causative role. Importantly, the precise functional role of each mediator associated with ventilator-attributable lung injury is not completely understood. Table 1 summarizes the potential roles for some of the more widely studied biological markers of VALI; however, our current understanding of the interaction among these mediators during ALI is incomplete. For example, higher levels of potential antiinflammatory mediators have been associated with poorer clinical outcomes (Table 2), a finding that is not surprising considering that many factors simultaneously induce expression of both proinflammatory and antiinflammatory mediators. Therefore, in clinical studies, changes in levels of biological markers have been used primarily in an effort to identify ventilator-attributable injury rather than to study disease pathogenesis. However, ventilator-associated changes in levels of some biological markers have been correlated with patient outcomes, including duration of mechanical ventilation, organ failures, length of hospital stay, and mortality.

Table 1—Potential General Functional Roles of Biological Markers of VALI*

Potential Proinflammatory	Potential Antiinflammatory	Other
TNF- α	IL-10	Fas-ligand (induces apoptosis)
IL-1 β	IL-1 receptor antagonist	PBEF (function unclear)
IL-8	sTNFR1	SP-A (collectin) \S
IL-6 \dagger	sTNFR2	SP-D (collectin) \S
NO $\dagger\ddagger$	IL-6 \dagger	
	NO $\dagger\ddagger$	

*Precise roles for these mediators in the pathogenesis of VALI have not been established.

\dagger IL-6 and NO have been reported to have immunomodulatory functions that may include potential proinflammatory and anti-inflammatory roles.

\ddagger Measured as nitrite.

\S SP-A and SP-D are part of the collectin family of proteins and may function in part as modulators of the innate immune response.

Clinical Studies: Patients With ALI

Several clinical studies have reported changes in mediators or modulators of inflammation with mechanical ventilation (Tables 2, 3). Ranieri and colleagues² studied 44 patients with ARDS who were enrolled within 8 h of initiation of mechanical ventilation. Patients were randomized to conventional mechanical ventilation with a mean (\pm SD) tidal volume of 11 ± 2 mL/kg and a mean PEEP level of 7 ± 2 cm H₂O, or a lower tidal volume of 8 ± 1 mL/kg and a PEEP level of 15 ± 3 cm H₂O. Plateau airway pressure was limited to < 35 cm H₂O in both groups. These authors² found that from the time of study entry to approximately 36 h, both plasma and BAL levels of interleukin (IL)-1 receptor antagonist (IL-1Ra) and soluble tumor necrosis factor (TNF) receptor 1 [sTNFR1] and soluble TNF receptor 2 (sTNFR2) decreased significantly in the patients receiving mechanical ventilation with lower tidal volumes and higher PEEP levels. In contrast, BAL levels of each of these markers increased in the conventional ventilation group. The mean BAL IL-1Ra in the conventional ventilation group at study entry was 17 μ g/mL and increased to 32 μ g/mL. In the lower tidal volume and higher PEEP group, IL-1Ra at study entry was 19 μ g/mL and decreased to 16 μ g/mL over 36 h. Levels of sTNFR1 and sTNFR2 significantly increased by nearly twofold in the conventional tidal volume group. BAL levels of IL-1 β , IL-6, IL-8, and TNF- α decreased over 36 h in the group with low tidal volume and high PEEP but remained unchanged or increased in the conventional ventilation group. A similar trend was reported for plasma levels of these mediators. In a *post hoc* analysis, the protective ventilation strategy was asso-

Table 2—Biological Markers Found To Decrease With Protective Ventilation Strategies in Experimental and Clinical Studies and Correlated With Outcome in Clinical Studies of VALI

Biological Marker	Experimental Species in Addition to Human	Sample Source	Clinical Outcomes Studied*	References, Clinical	References, Experimental
TNF- α	Mouse, rat, rabbit, sheep	BAL, plasma	Mortality VFD NOF	2, 3	10–23
IL-1 β	Rat, rabbit, sheep	Plasma, BAL, EF	Mortality VFD NOF	2, 3	10, 15, 23–27
IL-6	Rat, rabbit, pig, sheep	Plasma	Mortality VFD OFFD	2–6	10, 15, 19, 22, 26–28
IL-8 and related CXC chemokines†	Rabbit, rat, mouse, sheep	Plasma, BAL, EF	Mortality VFD OFFD	2, 3, 4, 6	10, 12, 15, 20, 23, 29
Fas-ligand	Rat	Plasma	Renal failure	7	7
Nitrite‡	Rat, rabbit	BAL, EF, EBC	Lung injury§	8	37–40
PBEF	Dog, mouse	Lung, BAL, serum	Lung injury§	9	9

*In all cases, lower levels of these mediators were associated with better clinical outcomes. EF = pulmonary edema fluid; Lung = tissue messenger RNA or protein expression; EBC = exhaled breath condensate; VFD = ventilator-free days (number of days of the first 28 study days in which a patient was not receiving mechanical ventilation); NOF = number of failing organs (total cumulative number of failing organs by predefined clinical criteria); OFFD = organ failure-free days (number of days of the first 28 study days in which a patient had no organ failures).

†CXC chemokines that bind CXCR2, including CXCL1 and CXCL2/3.

‡As a marker of endothelial or inducible NO synthase activity.

§Physiologic and radiographic abnormalities.

ciated with more ventilator-free days, fewer organ failures, and lower mortality; however, this study^{2,3} was not designed to study these outcomes. A follow-up study⁴³ of BAL neutrophils in samples collected from patients in this study demonstrated increased neutrophil activation in the samples from patients receiving mechanical ventilation with the conventional strategy.

The ARDS Network low tidal volume study,¹ which studied the effect of lower tidal volume,

plateau pressure-limited ventilation compared with more conventional tidal volumes in 861 patients, has provided considerable data on several biological markers of VALI. In an analysis⁴ of samples from patients in this clinical trial, higher baseline plasma levels of IL-6, IL-8, and IL-10 were each associated with an increased risk of death in all patients independent of the ventilation protocol to which they were randomized. In the low tidal volume group, plasma levels of IL-6 decreased by 26% and IL-8 levels decreased by 12%, but levels of IL-10 did not change by the third study day. In 703 of the 861 patients enrolled in the study, IL-6 was measured on both day 0 and day 3. The odds ratio for mortality for plasma IL-6 level on day 3 of the study was 3.7 (95% confidence interval [CI], 2.7 to 5.7) per 10-fold increase in plasma IL-6 level. It is noteworthy that IL-6 and IL-8 levels did vary with clinical risk factor for ARDS and that factors such as infection could increase levels of these mediators. However, the authors⁴ calculated that the proportion of treatment effect in the low tidal volume group captured by day 3 IL-6 level was 30% (95% CI, 8 to 88%), suggesting that IL-6 may be a surrogate marker for mortality reduction attributable to low tidal volume ventilation. From these clinical studies, it appears that low tidal volume ventilation may lead to a more rapid attenuation of the inflammatory response as measured by changes in plasma and BAL cytokines. Another analysis⁴² of plasma samples from 565 pa-

Table 3—Biological Markers Found To Increase With Protective Ventilation in Clinical Studies but Not Confirmed or Reported in Experimental Studies*

Biological Marker	Sample Source	Clinical Outcomes Studied	References
IL-1Ra	BAL	Mortality VFD NOF Oxygenation	2, 3, 5
sTNFR1	Plasma, BAL	Mortality VFD OFFD	2, 3, 41
sTNFR2	Plasma, BAL	Mortality VFD OFFD	2, 3†
SP-D	Plasma	Mortality VFD OFFD	42

*See Table 2 for expansion of abbreviations.

†Not confirmed in Ye et al.⁹

tients in the ARDS Clinical Trials Network low tidal volume study found that higher plasma surfactant protein (SP)-D levels at baseline were associated with an increased risk of death and that the low tidal volume strategy was further associated with a significant decrease in SP-D by study day 3. Because SP-D is specific to alveolar epithelial cells, this result may indicate reduced alveolar epithelial cell injury or decreased alveolar epithelial permeability to protein in the patients treated with the low tidal volume ventilation strategy. Plasma SP-A levels did not correlate with outcome or with ventilation strategy in that study; however, a previous report⁴⁴ suggested that increased plasma SP-A levels are associated with fewer ventilator-free days and higher mortality in ARDS and ALI patients independent of tidal volume.

In a separate study⁴⁵ using samples from 559 patients in the ARDS Network low tidal volume study, an increased plasma level of von Willebrand factor antigen (vWf:Ag), which is released from platelets and endothelial cells, was associated with increased mortality in patients with sepsis and ARDS. Previous reports⁶⁴ have found an association between vWf:Ag levels in plasma and lung injury severity. However, there was no difference in plasma vWf:Ag levels between patients ventilated with the low tidal volume (6 mL/kg) or the more conventional tidal volume (12 mL/kg).⁴⁵

Parsons and colleagues⁴¹ reported that elevated plasma levels of sTNFR1 and sTNFR2 were associated with higher mortality in 377 patients with ARDS or ALI in the ARDS Network study. The odds ratios for mortality were 5.76 (95% CI, 2.63 to 12.6) for every 10-fold increase in plasma sTNFR1 and 2.58 (95% CI, 1.05 to 6.31) for every 10-fold increase in plasma sTNFR2.⁴¹ In agreement with the previous study by Ranieri and colleagues,² plasma sTNFR1 significantly decreased from enrollment to day 3 only in patients receiving mechanical ventilation with the low tidal volume strategy ($p = 0.037$). In companion *in vitro* studies,⁴¹ alveolar epithelial-like cells (A549 cells) released sTNFR1 but not sTNFR2 in response to stimulation with TNF- α , IL-1 β , and interferon- γ . Therefore, sTNFR1 may be a marker of the alveolar epithelial response to inflammatory mediators in VALI (Table 3).

In an effort to determine the effect of increasing tidal volume on plasma levels of biological markers, Stuber and colleagues⁵ measured plasma IL-8, TNF- α , IL-1Ra, IL-10, and IL-6 in 12 patients with ALI before, during, and after changing tidal volume from 6 to 12 mL/kg for 1 h. Higher tidal volume ventilation induced a transient increase in plasma levels of each of these mediators. Levels returned to baseline with tidal volume reduction. Interestingly,

BAL levels of each of the cytokines increased throughout the study in the subset of patients who underwent BAL. These data, although collected in a small number of patients, implicate higher tidal volume ventilation as a contributor to increased plasma concentrations of these mediators in lung injury patients.⁵

Nitrite has been used as a marker for nitric oxide (NO) synthase activity and NO production in clinical and experimental studies. Increased production of reactive oxygen species and NO-derived reactive nitrogen species have been implicated in the development and progression of ALI.⁴⁶ Most of the injurious effects of NO have been attributed to the formation of peroxynitrite, which is formed from the reaction of NO with superoxide.⁴⁷ Peroxynitrite contributes to nitrosylation of proteins and other biological molecules, potentially altering their function. Pulmonary edema fluid from patients with ARDS contains higher levels of nitrosylated proteins than edema fluid from patients with hydrostatic pulmonary edema.⁴⁸ Gessner and colleagues⁸ found that exhaled breath condensate nitrite levels were strongly correlated with tidal volume in 28 ARDS patients ($r = 0.79$, $p = 0.001$). They further reported that the ratio of exhaled breath condensate nitrite to tidal volume correlated with lung injury severity as measured by Murray Lung Injury Severity Score ($r = 0.84$, $p = 0.0001$).⁸ Therefore, the increase in nitrite for a given tidal volume was greater with more severe lung injury. These data support the hypothesis that more injured lungs are more susceptible to VALI and that nitrite levels increase with VALI.

Clinical and Experimental Studies in Parallel

Two reports have examined the roles of specific mediators in VALI in combined experimental and clinical studies. Imai and colleagues⁷ found that rabbits with acid-induced lung injury exposed to high tidal volume ventilation (12 mL/kg) had more severe pulmonary edema and increased epithelial apoptosis in the kidney and small intestine. Plasma from rabbits ventilated with high tidal volume induced apoptosis in renal tubule epithelial cells in culture. Apoptosis in the cultured cells was significantly decreased by a Fas ligand blocking antibody. In the clinical portion of the study, plasma levels of Fas ligand were found to correlate with higher plasma creatinine levels in 11 patients with ARDS.⁷ A previous study⁴⁹ reported increased Fas ligand levels in pulmonary edema fluid from patients with ARDS. These data support the hypothesis that injurious ventilation leads to an increased circulating level of

Fas ligand, which induces apoptosis in distal organs and potentially contributes to multiple organ failure.

In another set of experiments, Ye and colleagues⁹ induced lung injury in dogs with saline solution lavage and then ventilated the animals with a low tidal volume zero PEEP strategy for 6 h. Mice given intratracheal endotoxin were also ventilated with a high tidal volume of 17 mL/kg for 2 h. Lung tissue samples from the two animal models were analyzed for gene expression using a gene chip microarray system. In both models, one gene demonstrating a significant increase in expression in the injured lungs was pre-B-cell colony enhancing factor (PBEF). This gene had not previously been associated with lung pathology. In addition, human umbilical vein endothelial cells in culture exposed to cyclic strain of 18% surface area change were found to have significantly increased expression of PBEF. The increase in PBEF in these cells was augmented by adding IL-1 β to the culture medium. BAL and serum levels of PBEF were found to be significantly higher in patients with ALI as compared to patients without lung injury. The authors⁹ went on to identify two single-nucleotide polymorphisms in the PBEF gene in a population of patients with ALI and sepsis, severe sepsis, and healthy subjects. One allele was associated with a 7.7-fold higher risk of ALI ($p < 0.001$). Additional transfection studies⁹ with the two alleles combined with a reporter construct in cultured human umbilical vein endothelial cells demonstrated a 1.8-fold decrease in transcription rate in response to mechanical stretch for the allele associated with a lower risk for ALI. This series of experiments supports the hypothesis that both polymorphisms in the PBEF gene and PBEF levels are associated with an increased risk of ALI and VALI.

Clinical Studies in Patients Without ALI

Other clinical data have demonstrated that high tidal volume ventilation in the absence of preexisting lung injury does not affect plasma cytokine and chemokine levels in patients. Wrigge and colleagues⁵⁰ reported that in 62 patients undergoing major thoracic or abdominal surgery randomized to ventilation with either 12 mL/kg or 6 mL/kg tidal volume and similar PEEP levels, ventilation strategy had no effect on plasma levels of TNF- α , IL-1 β , IL-8, IL-6, or IL-10 after 3 h. In an earlier report,⁵¹ the same group found that among 39 patients who were American Society of Anesthesiologists physical status I or II ventilation with either 15 mL/kg tidal volume without PEEP, 6 mL/kg without PEEP, or 6 mL/kg with PEEP had no effect on plasma levels of TNF- α , IL-1Ra, IL-6, or IL-12. In contrast, Tsangaris and colleagues⁵² reported that among patients

without lung injury who were placed on mechanical ventilation for airway protection, BAL levels of platelet-activating factor increased over 1 week of ventilation. Although platelet-activating factor has been shown to be a mediator of pulmonary edema,⁵³ no data regarding pulmonary edema or lung injury were reported in the study by Tsangaris et al.⁵²

EXPERIMENTAL STUDIES OF VENTILATOR-ATTRIBUTABLE INJURY

In an effort to understand and extend data from clinical trials, many investigators have attempted to model ventilator-attributable injury under more controlled experimental conditions. One of the most commonly used models is the VILI model in which normal lungs are injured with overtly harmful high or low lung volumes. Others have attempted to model the effects of more physiologic ventilator settings on previously injured lungs. This type of model, termed VALI, may more directly mimic the clinical setting; however, just as in clinical studies, these models are potentially confounded by the variability and unpredictability of the underlying lung injury. Other models such as *ex vivo* lung preparations have also been widely reported in the literature. In this model system, lungs are removed from an animal and exposed to injurious ventilation. While some have used isolated and perfused lungs, others have ventilated lungs without perfusion. In addition, isolated alveolar epithelial cells or alveolar epithelial-like cell lines have been used to determine the effects of mechanical strain on markers of inflammation or injury.

There are several critical differences between the commonly used experimental models and clinical VALI. The most obvious differences are in time frame and lung size, as most studies are done over 1 to 8 h in mice, rats, or rabbits. In clinical studies of ventilator lung injury, data are collected on a daily or longer basis. The acute events occurring within the first few hours of the initiation of mechanical ventilation have not been fully studied clinically, and therefore our ability to generalize much shorter duration experimental studies is uncertain. Inherent differences in the structural makeup of the lung and chest wall as well as the effects of gravity on the edematous lung are potentially important in the pathogenesis of VALI and differ between humans and small animals. The wide variety of models used in experimental studies also complicates interpretation of the literature.

There is no ideal model of clinical VALI. Each model has inherent strengths and limitations. The data that are similar across several models as well as

in clinical studies (Table 2) are perhaps the most helpful in unraveling the mechanisms of clinical VALI.

Considerable experimental data have demonstrated a variety of biological markers of inflammation and cellular injury following injurious ventilation. As already discussed, several of these biological markers have also been reported in clinical studies of VALI (Table 2). Among the most studied biological markers in VILI and VALI are proinflammatory cytokines and chemokines.

Cytokines and Chemokines in Experimental Studies

Isolated Lung Models: Tremblay and colleagues¹⁰ ventilated isolated nonperfused rat lungs for 2 h and found that high tidal volume, zero PEEP ventilation induced a much greater increase in airspace TNF- α , IL-1 β , IL-6, IL-10, macrophage inflammatory protein (MIP)-2, and interferon- γ than lower tidal volume and high PEEP ventilation with similar peak inspiratory pressure or low tidal volume and zero PEEP ventilation. When rats were pretreated with endotoxin 2 h before removal of the lungs and the ventilation procedure, levels of each of these mediators were significantly higher, with the exception of TNF- α in the high tidal volume group.¹⁰ Ventilation of isolated mouse lungs with negative end-expiratory pressure has also been associated with increased BAL levels of TNF- α ,¹¹ affirming the hypothesis that excessively low lung volume ventilation and collapse of alveolar units may account for some of the observed changes in airspace cytokines. Similar to the study by Tremblay et al,¹⁰ Veldhuizen and colleagues⁵⁴ found that in isolated mouse lungs, ventilation with a tidal volume of 20 mL/kg without PEEP resulted in significant increases in BAL levels of TNF- α and IL-6. Although some⁵⁵ have criticized the nonperfused, isolated lung model as unreliable, changes in levels of many of the mediators identified in this model have been found to be markers of VALI in other experimental models. It should also be noted that the usefulness of a biological marker is as much related to its ability to identify a condition as its role in the pathogenesis of a disease. Therefore, the role of these cytokines in the initiation of VILI may be separate from their usefulness as markers of the condition.

In Vivo Models of VALI: There are several reports of mechanical ventilation-attributable increases in cytokines and chemokines in whole-animal models. Following acid aspiration-induced lung injury in rats, higher tidal volumes are associated with greater increases in plasma and BAL TNF- α and MIP-2¹² and plasma IL-1 β ⁵⁶ than acid injury alone or nonin-

jurious ventilation. Others^{13,14,57,58} have reported similar results in surfactant-depleted rats, rabbits, and pigs ventilated with high or low tidal volume or high-frequency oscillatory ventilation. Antibody blockade of TNF- α decreased histopathologic lung injury in surfactant-depleted rats,¹³ while blockade of IL-1 with IL-1Ra attenuated lung injury after 8 h of ventilation in surfactant-depleted rabbits.²⁴ These data suggest a pathogenetic role for TNF- α and IL-1 in VALI. In preterm lambs, higher lung volume ventilation with high PEEP resulted in greater increases in IL-1 β , IL-6, and IL-8 but not TNF- α .¹⁵ In preterm pigs, conventional tidal volume ventilation induced greater increases in leukotriene-B₄ and IL-6 than high-frequency oscillatory ventilation or partial liquid ventilation; however, BAL levels of TNF- α were not different among the groups.²⁸ Altemeier and colleagues²⁹ pretreated rabbits with endotoxin before mechanical ventilation with a tidal volume of 15 mL/kg without PEEP for 8 h and found that endotoxin combined with mechanical ventilation resulted in significant increases in BAL levels of TNF- α , IL-8, growth-related oncogene- α , and MIP-1. Endotoxin or mechanical ventilation alone did not increase these cytokines.²⁹ Others^{16,17} have also reported that endotoxin augments BAL and plasma levels of TNF- α following injurious ventilation.

Cell-Specific Markers of Injury or Impaired Function

Relatively few experimental studies have examined cell type-specific markers of injury or dysfunction in the context of VALI (Table 3). In one study⁵⁹ of VALI in rats, acid aspiration was followed by ventilation with either 12 mL/kg tidal volume and high or low PEEP, 6 mL/kg and high PEEP, or 3 mL/kg tidal volume and high PEEP. In the absence of acid injury, mechanical ventilation alone did not induce a measurable lung injury. Following acid injury, 4 h of mechanical ventilation with a tidal volume of 12 mL/kg resulted in more severe lung injury as measured by the severity of pulmonary edema and histopathologic and ultrastructural markers of cell injury. Higher tidal volume ventilation was also associated with higher airspace and plasma levels of RTI40, a rat type I pneumocyte-specific integral membrane protein.⁵⁹ Furthermore, a reduction of tidal volume from 6 to 3 mL/kg resulted in a further significant reduction in plasma RTI40. Therefore, higher tidal volume ventilation resulted in greater type I epithelial cell injury and higher airspace and plasma levels of RTI40. Similarly, lung expression of SP-C messenger RNA, a type II cell specific protein, was lower following high volume

Table 4—Cell-Specific Markers of Injury Identified in Experimental Studies of VALI but Not Confirmed or Reported in Clinical Studies

Biological Marker	Experimental Species	Cellular Source	Sample Source	Change in Marker with Injurious Ventilation	References
Angiotensin-converting enzyme activity	Rat	Endothelium	Lung	Decrease	60
Clara-cell secretory protein	Mouse	Clara cell	Plasma	Increase	61
Platelet microparticles	Pig	Platelets	BAL	Increase	62
RTI40	Rat	Type I pneumocytes	Plasma, BAL	Increase	38, 63
SP-C	Rat	Type II pneumocytes	BAL, lung	Decrease	38
vWf:Ag	Rat	Endothelium platelets	Plasma	Increase*	38

*No difference with ventilation strategy observed in clinical study.⁵

ventilation, although SP-C levels were not directly measured in the airspace fluid. To date, similar alveolar epithelial cell-specific markers of injury have not been tested in clinical studies. Table 4 summarizes the reported changes in cell-specific biological markers of injury in experimental VALI and VILI.

CLINICAL IMPLICATIONS

Measurement of biological markers in experimental and clinical studies has provided insight into the pathogenesis of VALI. Both clinical and experimental data have characterized VALI as a condition of ongoing lung inflammation resulting from overdistention of the lung and potentially from excessively low lung volume. Although experimental studies have not been entirely concordant with clinical studies, there are several remarkable similarities. Temporal changes in plasma levels of IL-6, IL-8, IL-1 β , and TNF- α have been associated with differences in ventilator settings in both clinical and experimental studies. Decreases in plasma levels of IL-6, IL-8, and IL-1 β in clinical studies are in part attributable to lung protective ventilation and are associated with better clinical outcomes. In particular, decreasing plasma levels of IL-6 have been reported⁴ to be a marker for clinical benefit from protective ventilation. Although the precise role of each mediator in the pathogenesis of VALI is not fully understood, measurement of these biological markers may identify patients in whom VALI is more likely and protective ventilation strategies most useful. Currently there have been no prospective clinical studies to validate the sensitivity or specificity of any of these biological markers in patients at risk for VALI. Specific markers of alveolar epithelial cell injury have been found to correlate well with physiologic, histologic, and ultrastructural indexes of experimental ventilator-attributable lung injury, and more studies of analogous biological markers in patients are needed. Clinical data have demonstrated

that tidal volume reduction improves mortality in ALI and ARDS patients; however, whether a truly safe ventilation strategy exists is uncertain. Although currently our ability to recognize ongoing VALI in patients is limited, experimental studies have indicated that measuring biological markers may be a valuable tool for identifying patients at risk, as well as for determining prognosis and understanding pathogenesis.

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