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*Chest*. Author manuscript; available in PMC 2009 October 20.

Published in final edited form as:
*Chest*. 2006 December ; 130(6): 1906–1914. doi:10.1378/chest.130.6.1906.

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

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## Abstract

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.

### Keywords

ARDS; critical care; ventilation; ventilator-induced lung injury

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 patients with acute lung injury (ALI) and ARDS[1] 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 in 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 determine the effects of mechanical ventilation or mechanical strain on the expression of biological markers of inflammation or

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The authors have no conflicts of interest to disclose.

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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.

### 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 colleagues[2] 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 (± SD) tidal volume of 11 ± 2 mL/kg and a mean PEEP level of 7 ± 2 cm $H_2O$, or a lower tidal volume of 8 ± 1mL/kg and a PEEP level of 15 ± 3 cm $H_2O$. Plateau airway pressure was limited to < 35 cm $H_2O$ in both groups. These authors[2] 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 μg/mL and increased to 32 μg/mL. In the lower tidal volume and higher PEEP group, IL-1Ra at study entry was 19 μg/mL and decreased to 16 μg/mL over 36 h. Levels of sTNFR1 and sTNFR2 significantly increased by nearly twofold in the conventional tidal volume group. BAL levels of IL-1β, 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 associated with more ventilator-free days, fewer organ

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failures, and lower mortality; however, this study[2,3] was not designed to study these outcomes. A follow-up study[43] 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 analysis[4] 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 authors[4] 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 analysis[42] of plasma samples from 565 patients 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 report[44] 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 study[45] 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 reports[64] 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 colleagues[41] 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-1β, and interferon-γ. Therefore, sTNFR1 may be a marker of the alveolar epithelial response to inflammatory mediators in VALI (Table 3).

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In an effort to determine the effect of increasing tidal volume on plasma levels of biological markers, Stuber and colleagues[5] 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 colleagues[8] 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 colleagues[7] 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 study[49] 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 colleagues[9] 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-1β 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 authors[9] 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

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allele was associated with a 7.7-fold higher risk of ALI ($p < 0.001$). Additional transfection studies[9] 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 colleagues[50] 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-1β, 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 colleagues[52] 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.

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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 colleagues[10] 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-1β, 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 the observed changes in airspace cytokines. Similar to the study be Tremblay et al,[10] Veldhuizen and colleagues[54] 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 some[55] 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-2[12] and plasma IL-1β[56] than acid injury alone or noninjurious 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-1β, IL-6, and IL-8 but not TNF-α.[15] In preterm pigs, conventional tidal volume ventilation induced greater increases in leukotriene-$B_4$ 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 colleagues[29] 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 study[59] 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,

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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 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-1β, and TNF-α have been associated with differences in ventilator settings in both clinical and experimental studies. Deceases in plasma levels of IL-6, IL-8, and IL-1β 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 reported[4] 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.

## Acknowledgments

This work was supported by National Institutes of Health grants HL69900 (Dr. Frank) and HL51856 (Dr. Matthay).

## Abbreviations

**ALI**
: acute lung injury

**CI**
: confidence interval

**IL**
: interleukin

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**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

## References

1. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med 2000;342:1301–1308. [PubMed: 10793162]
2. Ranieri VM, Suter PM, Tortorella C, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. JAMA 1999;282:54–61. [PubMed: 10404912]
3. Ranieri VM, Giunta F, Suter PM, et al. Mechanical ventilation as a mediator of multisystem organ failure in acute respiratory distress syndrome. JAMA 2000;284:43–44. [PubMed: 10872010]
4. Parsons PE, Eisner MD, Thompson BT, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med 2005;33:1–6. [PubMed: 15644641]
5. Stuber F, Wrigge H, Schroeder S, et al. Kinetic and reversibility of mechanical ventilation-associated pulmonary and systemic inflammatory response in patients with acute lung injury. Intensive Care Med 2002;28:834–841. [PubMed: 12122519]
6. Lista G, Colnaghi M, Castoldi F, et al. Impact of targeted-volume ventilation on lung inflammatory response in preterm infants with respiratory distress syndrome (RDS). Pediatr Pulmonol 2004;37:510–514. [PubMed: 15114551]

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7. Imai Y, Parodo J, Kajikawa O, et al. Injurious mechanical ventilation and end-organ epithelial cell apoptosis and organ dysfunction in an experimental model of acute respiratory distress syndrome. JAMA 2003;289:2104–2112. [PubMed: 12709468]
8. Gessner C, Hammerschmidt S, Kuhn H, et al. Exhaled breath condensate nitrite and its relation to tidal volume in acute lung injury. Chest 2003;124:1046–1052. [PubMed: 12970036]
9. Ye SQ, Simon BA, Maloney JP, et al. Pre-B-cell colony enhancing factor as a potential novel biomarker in acute lung injury. Am J Respir Crit Care Med 2005;171:361–370. [PubMed: 15579727]
10. Tremblay L, Valenza F, Ribeiro SP, et al. Injurious ventilatory strategies increase cytokines and c-fos m-RNA expression in an isolated rat lung model. J Clin Invest 1997;99:944–952. [PubMed: 9062352]
11. Cheng KC, Zhang H, Lin CY, et al. Ventilation with negative airway pressure induces a cytokine response in isolated mouse lung. Anesth Analg 2002;94:1577–1582. [PubMed: 12032030]
12. Chiumello D, Pristine G, Slutsky AS. Mechanical ventilation affects local and systemic cytokines in an animal model of acute respiratory distress syndrome. Am J Respir Crit Care Med 1999;160:109–116. [PubMed: 10390387]
13. Imai Y, Kawano T, Iwamoto S, et al. Intratracheal anti-tumor necrosis factor-α antibody attenuates ventilator-induced lung injury in rabbits. J Appl Physiol 1999;87:510–515. [PubMed: 10444606]
14. Takata M, Abe J, Tanaka H, et al. Intraalveolar expression of tumor necrosis factor-α gene during conventional and high-frequency ventilation. Am J Respir Crit Care Med 1997;156:272–279. [PubMed: 9230760]
15. Naik AS, Kallapur SG, Bachurski CJ, et al. Effects of ventilation with different positive end-expiratory pressures on cytokine expression in the preterm lamb lung. Am J Respir Crit Care Med 2001;164:494–498. [PubMed: 11500356]
16. Haitsma JJ, Uhlig S, Goggel R, et al. Ventilator-induced lung injury leads to loss of alveolar and systemic compartmentalization of tumor necrosis factor-α. Intensive Care Med 2000;26:1515–1522. [PubMed: 11126266]
17. Murphy DB, Cregg N, Tremblay L, et al. Adverse ventilatory strategy causes pulmonary-to-systemic translocation of endotoxin. Am J Respir Crit Care Med 2000;162:27–33. [PubMed: 10903215]
18. Kornecki A, Tsuchida S, Kumar Ondiveeran H, et al. Lung development and susceptibility to ventilator-induced lung injury. Am J Respir Crit Care Med 2005;171:743–752. [PubMed: 15640366]
19. Rich PB, Douillet CD, Hurd H, et al. Effect of ventilatory rate on airway cytokine levels and lung injury. J Surg Res 2003;113:139–145. [PubMed: 12943823]
20. Wilson MR, Choudhury S, Goddard ME, et al. High tidal volume upregulates intrapulmonary cytokines in an in vivo mouse model of ventilator-induced lung injury. J Appl Physiol 2003;95:1385–1393. [PubMed: 12807894]
21. Guery BP, Welsh DA, Viget NB, et al. Ventilation-induced lung injury is associated with an increase in gut permeability. Shock 2003;19:559–563. [PubMed: 12785012]
22. Stamme C, Brasch F, von Bethmann A, et al. Effect of surfactant on ventilation-induced mediator release in isolated perfused mouse lungs. Pulm Pharmacol Ther 2002;15:455–461. [PubMed: 12406668]
23. Ribeiro SP, Rhee K, Tremblay L, et al. Heat stress attenuates ventilator-induced lung dysfunction in an *ex vivo* rat lung model. Am J Respir Crit Care Med 2001;163:1451–1456. [PubMed: 11371417]
24. Narimanbekov IO, Rozycki HJ. Effect of IL-1 blockade on inflammatory manifestations of acute ventilator-induced lung injury in a rabbit model. Exp Lung Res 1995;21:239–254. [PubMed: 7774527]
25. Caruso P, Meireles SI, Reis LF, et al. Low tidal volume ventilation induces proinflammatory and profibrogenic response in lungs of rats. Intensive Care Med 2003;29:1808–1811. [PubMed: 12904859]
26. Vreugdenhil HA, Haitsma JJ, Jansen KJ, et al. Ventilator-induced heat shock protein 70 and cytokine mRNA expression in a model of lipopolysaccharide-induced lung inflammation. Intensive Care Med 2003;29:915–922. [PubMed: 12734649]
27. Allen GB, Suratt BT, Rinaldi L, et al. Choosing the frequency of deep inflation in mice: balancing recruitment against ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol. 2006(Epub PMID:16698851)

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28. Merz U, Klosterhalfen B, Hausler M, et al. Partial liquid ventilation reduces release of leukotriene $B_4$ and interleukin-6 in bronchoalveolar lavage in surfactant-depleted newborn pigs. Pediatr Res 2002;51:183–189. [PubMed: 11809912]
29. Altemeier WA, Matute-Bello G, Frevert CW, et al. Mechanical ventilation with moderate tidal volumes synergistically increases lung cytokine response to systemic endotoxin. Am J Physiol Lung Cell Mol Physiol 2004;287:L533–L542. [PubMed: 15145786]
30. Strand M, Ikegami M, Jobe AH. Effects of high $PCO_2$ on ventilated preterm lamb lungs. Pediatr Res 2003;53:468–472. [PubMed: 12595596]
31. Rai S, Engelberts D, Laffey JG, et al. Therapeutic hypercapnia is not protective in the in vivo surfactant-depleted rabbit lung. Pediatr Res 2004;55:42–49. [PubMed: 14561781]
32. Belperio JA, Keane MP, Burdick MD, et al. Critical role for CXCR2 and CXCR2 ligands during the pathogenesis of ventilator-induced lung injury. J Clin Invest 2002;110:1703–1716. [PubMed: 12464676]
33. Sinclair SE, Altemeier WA, Matute-Bello G, et al. Augmented lung injury due to interaction between hyperoxia and mechanical ventilation. Crit Care Med 2004;32:2496–2501. [PubMed: 15599157]
34. Mourgeon E, Isowa N, Keshavjee S, et al. Mechanical stretch stimulates macrophage inflammatory protein-2 secretion from fetal rat lung cells. Am J Physiol Lung Cell Mol Physiol 2000;279:L699–706. [PubMed: 11000130]
35. Quinn DA, Moufarrej RK, Volokhov A, et al. Interactions of lung stretch, hyperoxia, and MIP-2 production in ventilator-induced lung injury. J Appl Physiol 2002;93:517–525. [PubMed: 12133859]
36. Whitehead TC, Zhang H, Mullen B, et al. Effect of mechanical ventilation on cytokine response to intratracheal lipopolysaccharide. Anesthesiology 2004;101:52–58. [PubMed: 15220771]
37. Frank JA, Pittet JF, Lee H, et al. High tidal volume ventilation induces NOS2 and impairs cAMP-dependent air space fluid clearance. Am J Physiol Lung Cell Mol Physiol 2003;284:L791–798. [PubMed: 12562562]
38. Broccard AF, Feihl F, Vannay C, et al. Effects of L-NAME and inhaled nitric oxide on ventilator-induced lung injury in isolated, perfused rabbit lungs. Crit Care Med 2004;32:1872–1878. [PubMed: 15343015]
39. Hammerschmidt S, Schiller J, Kuhn H, et al. Influence of tidal volume on pulmonary NO release, tissue lipid peroxidation and surfactant phospholipids. Biochim Biophys Acta 2003;1639:17–26. [PubMed: 12943964]
40. Choi WI, Quinn DA, Park KM, et al. Systemic microvascular leak in an *in vivo* rat model of ventilator-induced lung injury. Am J Respir Crit Care Med 2003;167:1627–1632. [PubMed: 12663326]
41. Parsons PE, Matthay MA, Ware LB, et al. Elevated plasma levels of soluble TNF receptors are associated with morbidity and mortality in patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 2004;288:L426–L431. [PubMed: 15516488]
42. Eisner MD, Parsons P, Matthay MA, et al. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax 2003;58:983–988. [PubMed: 14586055]
43. Zhang H, Downey GP, Suter PM, et al. Conventional mechanical ventilation is associated with bronchoalveolar lavage-induced activation of polymorphonuclear leukocytes: a possible mechanism to explain the systemic consequences of ventilator-induced lung injury in patients with ARDS. Anesthesiology 2002;97:1426–1433. [PubMed: 12459668]
44. Cheng IW, Ware LB, Greene KE, et al. Prognostic value of surfactant proteins A and D in patients with acute lung injury. Crit Care Med 2003;31:20–27. [PubMed: 12544988]
45. Ware LB, Eisner MD, Thompson BT, et al. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. Am J Respir Crit Care Med 2004;170:766–772. [PubMed: 15201135]
46. Haddad, I.; Pitt, B.; Matalon, S. Nitric oxide and lung injury. In: Fishman, AP., editor. Pulmonary diseases and disorders. New York, NY: McGraw-Hill; 1996. p. 337-346.
47. McAndrew J, Patel RP, Jo H, et al. The interplay of nitric oxide and peroxynitrite with signal transduction pathways: implications for disease. Semin Perinatol 1997;21:351–366. [PubMed: 9352609]

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48. Zhu S, Ware LB, Geiser T, et al. Increased levels of nitrate and surfactant protein a nitration in the pulmonary edema fluid of patients with acute lung injury. Am J Respir Crit Care Med 2001;163:166–172. [PubMed: 11208643]
49. Albertine KH, Soulier MF, Wang Z, et al. Fas and fas ligand are up-regulated in pulmonary edema fluid and lung tissue of patients with acute lung injury and the acute respiratory distress syndrome. Am J Pathol 2002;161:1783–1796. [PubMed: 12414525]
50. Wrigge H, Uhlig U, Zinserling J, et al. The effects of different ventilatory settings on pulmonary and systemic inflammatory responses during major surgery. Anesth Analg 2004;98:775–781. [PubMed: 14980936]
51. Wrigge H, Zinserling J, Stuber F, et al. Effects of mechanical ventilation on release of cytokines into systemic circulation in patients with normal pulmonary function. Anesthesiology 2000;93:1413–1417. [PubMed: 11149435]
52. Tsangaris I, Lekka ME, Kitsiouli E, et al. Bronchoalveolar lavage alterations during prolonged ventilation of patients without acute lung injury. Eur Respir J 2003;21:495–501. [PubMed: 12662008]
53. Goggel R, Winoto-Morbach S, Vielhaber G, et al. PAF-mediated pulmonary edema: a new role for acid sphingomyelinase and ceramide. Nat Med 2004;10:155–160. [PubMed: 14704790]
54. Veldhuizen RA, Slutsky AS, Joseph M, et al. Effects of mechanical ventilation of isolated mouse lungs on surfactant and inflammatory cytokines. Eur Respir J 2001;17:488–494. [PubMed: 11405530]
55. Ricard JD, Dreyfuss D, Saumon G. Production of inflammatory cytokines in ventilator-induced lung injury: a reappraisal. Am J Respir Crit Care Med 2001;163:1176–1180. [PubMed: 11316656]
56. Frank JA, Matthay MA. Science review: mechanisms of ventilator-induced injury. Crit Care 2003;7:233–241. [PubMed: 12793874]
57. Imai Y, Nakagawa S, Ito Y, et al. Comparison of lung protection strategies using conventional and high-frequency oscillatory ventilation. J Appl Physiol 2001;91:1836–1844. [PubMed: 11568170]
58. Steinberg JM, Schiller HJ, Halter JM, et al. Alveolar instability causes early ventilator-induced lung injury independent of neutrophils. Am J Respir Crit Care Med 2004;169:57–63. [PubMed: 14695106]
59. Frank JA, Gutierrez JA, Jones KD, et al. Low tidal volume reduces epithelial and endothelial injury in acid-injured rat lungs. Am J Respir Crit Care Med 2002;165:242–249. [PubMed: 11790662]
60. Behnia R, Molteni A, Waters CM, et al. Early markers of ventilator-induced lung injury in rats. Ann Clin Lab Sci 1996;26:437–450. [PubMed: 8879362]
61. Yoshikawa S, Miyahara T, Reynolds SD, et al. Clara cell secretory protein and phospholipase $A_2$ activity modulate acute ventilator-induced lung injury in mice. J Appl Physiol 2005;98:1264–1271. [PubMed: 15608088]
62. Mutschler DK, Larsson AO, Basu S, et al. Effects of mechanical ventilation on platelet microparticles in bronchoalveolar lavage fluid. Thromb Res 2002;108:215–220. [PubMed: 12617984]
63. Frank JA, McAuley DF, Gutierrez JA, et al. Differential effects of sustained inflation recruitment maneuvers on alveolar epithelial and lung endothelial injury. Crit Care Med 2005;33:181–188. [PubMed: 15644667]
64. Ware LB, Conner ER, Matthay MA. von Willabrand factor antigen is an independent marker of poor outcome in patients with acute lung injury. Crit Care Med 2001;29:2325–2331. [PubMed: 11801836]

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**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-1β | IL-1 receptor antagonist | PBEF (function unclear) |
| IL-8 | sTNFR1 | SP-A (collectin)§ |
| IL-6† | sTNFR2 | SP-D (collectin)§ |
| NO†‡ | IL-6† | |
| | NO†‡ | |

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

†IL-6 and NO have been reported to have immunomodulatory functions that may include potential proinflammatory and antinflammatory roles.

‡Measured as nitrite.

§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.

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**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<br>VFD<br>NOF | 2, 3 | 10–23 |
| IL-1β | Rat, rabbit, sheep | Plasma, BAL, EF | Mortality<br>VFD<br>NOF | 2, 3 | 10, 15, 23–27 |
| IL-6 | Rat, rabbit, pig, sheep | Plasma | Mortality<br>VFD<br>OFFD | 2–6 | 10, 15, 19, 22, 26–28 |
| IL-8 and related CXC chemokines† | Rabbit, rat, mouse, sheep | Plasma, BAL, EF | Mortality<br>VFD<br>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.

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**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]

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**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.[8]