Prognostic and Pathogenetic Value of Combining Clinical and Biochemical Indices in Patients With Acute Lung Injury

Lorraine B. Ware, MD, FCCP; Tatsuki Koyama PhD; D. Dean Billheimer, PhD; William Wu, PhD; Gordon R. Bernard, MD, FCCP; B. Taylor Thompson, MD; Roy G. Brower MD; Theodore J. Standiford, MD; Thomas R. Martin, MD; Michael A. Matthay, MD, FCCP; and the NHLBI ARDS Clinical Trials Network*

Background: No single clinical or biologic marker reliably predicts clinical outcomes in acute lung injury (ALI)/ARDS. We hypothesized that a combination of biologic and clinical markers would be superior to either biomarkers or clinical factors alone in predicting ALI/ARDS mortality and would provide insight into the pathogenesis of clinical ALI/ARDS.

Methods: Eight biologic markers that reflect endothelial and epithelial injury, inflammation, and coagulation (von Willebrand factor antigen, surfactant protein D [SP-D]), tumor necrosis factor receptor-1, interleukin [IL]-6, IL-8, intercellular adhesion molecule-1, protein C, plasminogen activator inhibitor-1) were measured in baseline plasma from 549 patients in the ARDSNet trial of low vs high positive end-expiratory pressure. Mortality was modeled with multivariable logistic regression. Predictors were selected using backward elimination. Comparisons between candidate models were based on the receiver operating characteristics (ROC) and tests of integrated discrimination improvement.

Results: Clinical predictors (Acute Physiology And Chronic Health Evaluation III [APACHE III], organ failures, age, underlying cause, alveolar-arterial oxygen gradient, plateau pressure) predicted mortality with an area under the ROC curve (AUC) of 0.82; a combination of eight biomarkers and the clinical predictors had an AUC of 0.85. The best performing biomarkers were the neutrophil chemotactic factor, IL-8, and SP-D, a product of alveolar type 2 cells, supporting the concept that acute inflammation and alveolar epithelial injury are important pathogenetic pathways in human ALI/ARDS.

Conclusions: A combination of biomarkers and clinical predictors is superior to clinical predictors or biomarkers alone for predicting mortality in ALI/ARDS and may be useful for stratifying patients in clinical trials. From a pathogenesis perspective, the degree of acute inflammation and alveolar epithelial injury are highly associated with the outcome of human ALI/ARDS.

CHEST 2010; 137(2):288–296

Abbreviations: ALI = acute lung injury; APACHE III = Acute Physiology and Chronic Health Evaluation III; AUC = area under the receiver operator characteristic curve; ICAM-1 = intercellular adhesion molecule-1; IL = interleukin; NHLBI = National Heart Lung and Blood Institute; PAI-1 = plasminogen activator inhibitor-1; PEEP = positive end-expiratory pressure; ROC = receiver operator characteristic; SP-D = surfactant protein D; TNFR1 = tumor necrosis factor receptor-1; VWF = von Willebrand factor

In patients with acute lung injury (ALI) and ARDS, clinical risk factors such as age, severity of illness scoring, and diagnosis of sepsis have a moderate predictive value for death and other adverse clinical outcomes.^{1,2} Several plasma biologic markers also have predictive value for death, ventilator-free days, and duration of organ failure when considered as single biomarkers in large patient populations.^{3–9} These

biologic markers reflect the complex pathogenesis of ALI/ARDS¹⁰ and include markers of inflammation (interleukin [IL]-6, IL-8, tumor necrosis factor receptor-1 [TNFR1]), lung and systemic endothelial activation and injury (von Willebrand factor [VWF]), lung epithelial injury (surfactant protein D [SP-D], receptor for advanced glycation end products), adhesion molecules (intercellular adhesion molecule-1

[ICAM-1]), and activation of coagulation and inhibition of fibrinolysis (protein C, plasminogen activator inhibitor-1 [PAI-1]). The potential prognostic value of a combination of both biologic markers and clinical risk factors has not been prospectively tested in patients with ALI/ARDS. A risk index that incorporates the prognostic value of both clinical and biologic predictors would be of practical value for clinical use and for enrollment of patients in clinical trials for treatment of ALI/ARDS.

Combining clinical and biologic markers for diagnosis and prediction of clinical outcomes has been of major value in many clinical disorders, including solid tumors, hematopoietic malignancies, and rheumatologic conditions. The largest studies integrating clinical and biologic predictors have been done in patients at risk for and with existing cardiovascular disease.¹¹ For example, current consensus recommendations for the diagnosis of acute myocardial infarction include clinical findings, such as electrocardiographic changes, and circulating levels of cardiac troponins.¹² Trevelyan and colleagues¹³ reported that adding cardiac troponin measurements to standard World Health Organization criteria for myocardial infarction classified an additional 26.1% of patients as having myocardial infarction as compared with World Health Organization criteria alone, and produced an overall diagnostic alteration in 11.5%. Combining clinical and biochemical markers has also been used in predicting risk for cardiovascular disease. In a recent study, the addition of circulating biomarkers to clinical risk factors improved risk prediction.¹⁴

Manuscript received June 25, 2009; revision accepted September 6, 2009.

Affiliations: From the Division of Allergy, Pulmonary, and Critical Care Medicine, Department of Medicine (Drs Ware and Bernard), and the Department of Biostatistics (Drs Koyama and Wu), Vanderbilt University School of Medicine, Nashville, TN; the Department of Agricultural and Biosystems Engineering, and Arizona Statistics Consulting Laboratory (Dr Billheimer), University of Arizona, Tucson, AZ; Pulmonary and Critical Care Medicine (Dr Thompson), Massachusetts General Hospital, Boston, MA; Pulmonary and Critical Care Medicine (Dr Brower), Johns Hopkins Hospital, Baltimore, MD; Pulmonary and Critical Care Medicine (Dr Standiford), University of Michigan, Ann Arbor, MI; Pulmonary and Critical Care Medicine (Dr Martin), University of Washington and Medical Service, VA Puget Sound Healthcare System, Seattle, WA; and the Cardiovascular Research Institute and Departments of Medicine and Anesthesia (Dr Matthay), University of California, San Francisco, CA.

*A complete list of study participants is located in the Appendix.

Funding/Support: This study was supported by the National Institutes of Health [Grants HL81332, HR46059, HL74005, HL73996, HL74024, HL73994].

Correspondence to: Lorraine B. Ware, MD, FCCP, Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University, T1218 MCN, 1161 21st Ave S, Nashville, TN 37232-2650; e-mail: lorraine.ware@vanderbilt.edu

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DOI: 10.1378/chest.09-1484

The primary aim of this study was to determine whether combining clinical risk factors with biologic markers in plasma would enhance the prediction of adverse outcomes in patients with ALI/ARDS who were enrolled in the National Heart, Lung, and Blood Institute (NHLBI) ARDS Clinical Trials Network clinical trial of two different levels of positive end-expiratory pressure (PEEP).¹⁵ We hypothesized that the combination of biologic and clinical risk factors would provide a superior prognostic index for mortality in patients with ALI/ARDS as compared with either biologic or clinical risk factors alone. Furthermore, we reasoned that a biologic index that is combined with clinical risk factors might provide new insight into the pathogenesis of human acute lung injury. Portions of this work have been published in abstract form.¹⁶

MATERIALS AND METHODS

Patients

Patients were eligible for inclusion if they participated in the NHLBI ARDS Clinical Trials Network multicenter randomized controlled trial of two different PEEP titration strategies (the ALVEOLI study).¹⁵ There were 549 patients enrolled in the clinical trial. Of these patients, 528 were included in the current study, based solely on the availability of baseline plasma samples. The study protocol was approved by the institutional review board at each hospital. Informed consent was obtained from all patients or their surrogates.

Clinical Trial Procedures

Inclusion and exclusion criteria and the study protocol for the parent study have been described.¹⁵ Patients were randomized to one of two PEEP titration strategies within 36 h of meeting inclusion criteria. Plasma samples for the current study were collected on day 0 (prior to randomization) and stored at -80°C until analyzed.

Clinical Data

Clinical data were recorded as part of the parent study at baseline and on days 1 to 4, 7, 14, 21, and 28. Acute Physiology And Chronic Health Evaluation III (APACHE III) scores were calculated at enrollment.¹⁷ The primary clinical risk factor for ALI/ARDS was determined prospectively prior to randomization by the clinical coordinator and physician investigator at each center. Patients were followed for 60 days or until discharge home with unassisted breathing. The primary outcome in the present study was mortality before discharge home with unassisted breathing. Secondary outcomes included the number of ventilator-free days and nonpulmonary organ failure-free days over a 28-day period after enrollment.¹⁵

Clinical Predictors

Clinical predictors were based on published studies as well as analysis of patients enrolled in the NHLBI ARDS Network low tidal volume study and the NHLBI ARDS Network lisofylline

study^{18,19} using a forward stepwise selection scheme to identify predictors of mortality from the 27 baseline variables recorded for 473 patients who received the strategy of mechanical ventilation involving lower tidal volumes as described in the ALVEOLI study.¹⁵ The clinical predictors for the current study included age, the underlying cause of ALI/ARDS (ie, sepsis, trauma, aspiration of gastric contents, and so forth), APACHE III score,¹⁷ plateau pressure, number of organ failures, and alveolar-arterial difference in the partial pressure of oxygen measured at enrollment prior to randomization. Because the APACHE III score is cumbersome to calculate at the bedside and is infrequently used by clinicians, predictive models were built both with and without the inclusion of the APACHE III score.

Biomarker Measurements

A panel of eight biomarkers was measured in duplicate in plasma samples that were collected at the time of enrollment prior to randomization to one of two ventilator strategies. The eight biomarkers were chosen based on prior demonstration of their association with adverse outcomes, including mortality,³⁻⁸ in patient groups derived from the first ARDS Network low tidal volume study.¹⁸ All biomarkers were measured using commercially available singleplex ELISA kits using standards provided by the manufacturers: SP-D (Yamasa Corporation; Tokyo, Japan); VWF (Diagnostica Stago; Parsippany, NJ); IL-6, IL-8, TNFR1, and sICAM1 (all from R&D Systems; Minneapolis, MN), PAI-1 (American Diagnostica; Stamford, CT), and protein C (Helena Laboratories; Beaumont, TX).

Statistical Analysis

Baseline demographic and clinical variables for all patients were assessed using the Wilcoxon rank sum test for continuous variables and the Fisher exact test for categorical variables. All values for biomarkers were transformed using the $\log_{10}$ function to achieve approximate normality. Biomarker values below the detection limit were imputed at half the lower limit of detection for each biomarker.

The strength of the marginal relationship, measured by Spearman rank correlation coefficient, to the response variable was used to eliminate from further consideration variables that showed only a very weak relationship to mortality. Nonlinear predictor effects were allowed by using restricted cubic splines on the variables that showed relatively high correlations. The clinical predictor model and biomarker-only model were fitted using all available respective variables. The full model, which combines all of the clinical and biomarker variables, was also fitted and used as the standard of comparison to assess the performance of the reduced model.

Candidate models were constructed through a backward elimination procedure. From the full model with 14 predictors (13 for the model without Apache III), variables were dropped one at a time, and a *P* value comparing the new reduced model to the full model was computed. The predictor that gave rise to the largest *P* value was eliminated from further consideration. Then from the model with 12 predictors, the same step was repeated to determine the next predictor to be dropped. This backward elimination process was repeated until only one variable remained. The predictors were then ranked from most significant (the last one to remain in the model) to least significant (the first one eliminated). The consecutive models were compared based on their predictive performance, as judged by the area under the receiver operator characteristic (ROC) curves. The reason for using area under ROC curves (AUC) is that our ultimate goal was to establish a predictive model judged by its

performance based on AUC. We took into consideration our preferences and previous knowledge of clinical markers and biomarkers, and thus not all decisions were based solely on statistical significance. Comparisons of the models based on AUC were conducted using the integrated discrimination improvement proposed by Pencina et al.²⁰ The ROC curves for the full model, clinical predictor model, biomarker-only model, and the reduced model were presented graphically, and the 95% CI for each AUC was computed using a bootstrap method. Calibration of the final model was assessed graphically by plotting the predicted probability of death on the abscissa and the estimate of the actual probability of death computed via locally weighted scatterplot smoothing on the ordinate. Finally, the odds ratio for each predictor was computed from the final model and presented with a 95% CI. For continuous variables, odds ratios represent comparisons between the values of lower and upper quartiles.

RESULTS

Patient Characteristics and Baseline Clinical and Biomarker Variables

Patient characteristics are shown in Table 1. The overall mortality was 27.3% and did not differ significantly by ventilator group. In univariable analysis, patients who died had higher APACHE III scores, more organ failures at enrollment, a higher alveolar to arterial oxygen gradient, and higher plateau pressures, and were more likely to have nonpulmonary sepsis as the underlying cause of ALI/ARDS. Patients who died also had significantly fewer organ failure-free and ventilator-free days. A comparison of baseline biomarker levels between patients who lived and died is shown in Table 2. The plasma concentrations of all eight biomarkers differed significantly between survivors and nonsurvivors.

Modeling of Mortality Using Clinical and Biomarker Variables: Models With APACHE III

Mortality was modeled using clinical predictors alone, biomarker predictors alone, and using clinical and biomarker predictors together. ROC curves were used to assess model performance. As shown in Figure 1, the AUC for the clinical predictors alone was 0.815 (95% CI, 0.790-0.866). The AUC for the biomarkers alone was 0.756 (95% CI, 0.733-0.821). A model that contained all of the clinical and biomarker predictors (full model) had an AUC of 0.850 (95% CI, 0.813-0.883). Modeling of mortality using a backward elimination strategy yielded a reduced model that contained APACHE III score, age, SP-D, and IL-8, with an AUC of 0.834 (95% CI, 0.789-0.862). The reduced model that combined clinical variables with these two biomarker variables performed significantly better than either the clinical predictors or the biomarker variables alone (*P* < .001).

Table 1—Baseline Patient Characteristics by Survival

	No.	Alive (n = 384)	Dead (n = 144)	Overall (N = 528)	P Value
Age	528	47 (37-61)	58 (47-73)	50 (39-65)	< .001
Sex: male	528	46%	42%	45%	.37
Race	528				.18
White		76%	73%	75%	
Black		14%	12%	14%	
Other		10%	15%	11%	
Cause of ARDS	528				.03
Pneumonia		39%	42%	40%	
Nonpulmonary sepsis		20%	27%	22%	
Aspiration		16%	14%	15%	
Multiple transfusions		5%	3%	5%	
Trauma		10%	3%	8%	
Other		10%	11%	10%	
Treatment type: low PEEP	528	51%	47%	50%	.38
Nonpulmonary organ failure-free days	525 (382/143)	23 (16-26)	0 (0-0)	19 (4-25)	< .001
Number of nonpulmonary organ failures	528				< .001
0		45%	26%	40%	
1		43%	36%	39%	
2		11%	26%	16%	
3		3%	10%	5%	
4		1%	1%	1%	
Ventilator-free days	528	22 (16-25)	0 (0-0)	18 (0-24)	< .001
Alveolar-arterial O ₂ difference, mm Hg	506 (368/137)	260 (191-381)	331 (245-506)	287 (208-428)	< .001
APACHE III score	522 (379/143)	83 (67-104)	114 (94-135)	92 (71-114)	< .001
Plateau airway pressure, cm H ₂ O	417 (306/111)	26 (21-31)	28 (23-32)	26 (22-31)	.002

Data given as median (interquartile range) or percent of patients; No. is the number of nonmissing values. Wilcoxon tests (continuous variables) and Fisher exact tests (categorical variables) were used to compare between survivors and nonsurvivors. APACHE = Acute Physiology and Chronic Health Evaluation; PEEP = positive end-expiratory pressure.

Modeling of Mortality Using Clinical and Biomarker Variables: Models Without APACHE III

Mortality was modeled without the baseline APACHE III score using clinical predictors alone, biomarker predictors alone, and clinical and biomarker predictors together. As shown in Figure 2, the AUC for the clinical predictors alone was 0.757 (95% CI, 0.731-0.822). The AUC for the biomarkers alone was 0.756 (95% CI, 0.733-0.821). A model that contained all of the clinical and biomarker predictors (full model) had an AUC of 0.823 (95% CI, 0.813-0.883).

Modeling of mortality using a backward elimination strategy yielded a reduced model that contained age, number of organ failures, alveolar-arterial oxygen difference, SP-D, IL-8, PAI-1, and TNFR1, with an AUC of 0.811 (95% CI, 0.789-0.862). The reduced model that combined both clinical and biomarker variables performed significantly better than either the clinical predictors or the biomarker variables alone ($P < .001$). The calibration curve for the reduced model without APACHE III is shown in Figure 3. The model performed well across the range of predicted

Table 2—Baseline Biomarker Values by Survival

Biomarker	No.	Alive (n = 384)	Dead (n = 144)	Overall (N = 528)	P Value
IL-6, pg/mL	521 (381/140)	209 (92-610)	322 (103-1,670)	244 (94-742)	.004
IL-8, pg/mL	528	35 (13-74)	64 (13-255)	40 (13-98)	< .001
TNFR, pg/mL	528	3,668 (2,478-6,872)	6,914 (3,291-12,917)	4,283 (2,604-8,484)	< .001
PROTC, % control	528	82 (49-127)	68 (36-102)	78 (45-121)	.011
PAI-1, ng/mL	524 (381/143)	54 (29-110)	111 (34-233)	60 (30-143)	< .001
ICAM, ng/mL	528	854 (581-1,280)	1,072 (774-1,536)	924 (605-1,384)	< .001
SP-D, ng/mL	510 (372/138)	92 (49-192)	124 (55-317)	101 (50-217)	.010
VWF, % control	528	370 (223-553)	477 (292-732)	394 (247-624)	< .001

Data given as median (interquartile range) or percent of patients; No. is the number of nonmissing values. When fewer than 528 patients were included in the analysis, the numbers in parentheses show the numbers in the alive and dead groups that were compared for each analyte. Wilcoxon tests (continuous variables) and Fisher exact tests (categorical variables) were used to compare between survivors and nonsurvivors. ICAM = intercellular adhesion molecule; IL = interleukin; TNFR = tumor necrosis factor receptor; PROTC = protein C; PAI = plasminogen activator inhibitor; SP-D = surfactant protein D; VWF = von Willebrand factor.

FIGURE 1. Receiver operator characteristic curves for predictive models of mortality that include the Acute Physiology And Chronic Health Evaluation (APACHE) III score in 528 patients with acute lung injury (ALI)/ARDS. The full model includes all six clinical predictors and all eight biomarker variables. The reduced model includes APACHE III score, age, surfactant protein D (SP-D), and interleukin (IL)-8. AUC = area under the receiver operator curve.

mortalities. The predicted probability of mortality associated with changes in each variable in the final reduced model without APACHE III is shown in Figures 4 and 5.

FIGURE 2. Receiver operator characteristic curves for predictive models of mortality that include the APACHE III score in 528 patients with ALI/ARDS. The full model includes all six clinical predictors and all eight biomarker variables. The reduced model includes age, number of organ failures, alveolar-arterial oxygen difference, SP-D, IL-8, plasminogen activator receptor (PAI)-1, and tumor necrosis factor receptor (TNFR) 1. See Figure 1 legend for expansion of other abbreviations.

This study provides strong evidence that a combination of biologic and clinical risk factors provides a superior prognostic index for mortality in patients with early ALI/ARDS, as compared with either biologic or clinical risk factors alone. In addition, we found that two plasma biomarkers, IL-8 and SP-D, had major prognostic value when combined with clinical risk factors, suggesting the importance of acute inflammation and alveolar epithelial injury in the pathogenesis and recovery from human ALI.

Prediction of clinical outcomes in patients with ALI/ARDS has several potential applications. In addition to establishing prognosis in individual patients for clinical purposes, a prognostic index that combines clinical and biologic risk factors could be used for risk assessment for enrollment in clinical trials.² Standard clinical definitions of ALI and ARDS²¹ select a heterogeneous population of patients with a wide spectrum of severity of illness. As demonstrated in studies of recombinant activated protein C in severe sepsis, the risk-to-benefit ratio of a therapy may be different depending on the severity of illness.²² For this reason, stratification or inclusion based on a combined clinical and biologic risk index might be used to select a more homogeneous population. This approach is supported by our recent finding in a reanalysis of the NHLBI ARDS Network low tidal volume trial, that the beneficial effect of low tidal volume on mortality was evident only in patients with more severe lung epithelial injury as measured by higher plasma receptor for advanced glycation end products levels at enrollment.⁹

Prediction of clinical outcomes using plasma levels of biologic markers also provides important information about the pathogenesis of clinical ALI/ARDS. Across all models tested, the two best performing biomarkers were IL-8 and SP-D. IL-8 is a potent neutrophil chemoattractant that plays an important role in neutrophil chemoattraction in animal models of ALI.²³⁻²⁶ High levels of IL-8 have been reported in the pulmonary edema fluid²⁷ and BAL fluid of patients at risk for and with ALI/ARDS,^{28,29} and serum levels have been associated with severity of illness. In the ARDS Network low tidal volume study, baseline levels of IL-8 in the plasma were highly associated with mortality and were reduced by the protective ventilatory strategy.³ Taken together with these prior studies, the strong association between plasma IL-8 concentrations and mortality in the current study supports the important role of this chemokine in the pathogenesis of clinical ALI/ARDS. Plasma levels of SP-D were also associated with mortality in all models tested. SP-D is a protein that is almost exclusively produced by alveolar epithelial type 2 cells. The presence

FIGURE 3. Calibration curve for the reduced model that includes age, number of organ failures, alveolar-arterial oxygen difference, SP-D, IL-8, PAI-1, and TNFR1. The gray line represents perfect calibration. Where the observed proportion of death (black line) is above the gray line, there are more deaths than predicted by the model. The model performs well across the spectrum of predicted probabilities of mortality. See Figure 1 and 2 legends for expansion of abbreviations.

of SP-D in the plasma in patients with ALI/ARDS is thought to reflect injury and increased permeability of the alveolar epithelial barrier.⁵ The association between circulating SP-D levels and mortality in the current study suggests that injury to the alveolar epithelial barrier has major prognostic implications in clinical ALI/ARDS, a finding that has also been reported in studies of alveolar epithelial function³⁰ and studies of markers of type 1 epithelial cell injury in clinical ALI/ARDS.⁹

We modeled mortality with and without the APACHE III score. The APACHE III score uses seven chronic health status items, 78 disease groups, and 17 physiologic variables to assess disease severity and predict outcomes in critically ill patients.¹⁷ The performance of the APACHE III scoring system has declined over time³¹ leading to modifications³² and

recent publication of the APACHE IV scoring system.³³ In addition to changes in performance over time, the APACHE III scoring system was designed to predict mortality in populations rather than individuals. Furthermore, it is challenging for bedside clinicians to use, because of the large number of variables that must be entered into the calculation. Although training and information technology can facilitate accurate APACHE scoring,³³ we questioned whether a predictive model for mortality in ALI/ARDS that included APACHE III would ultimately have clinical usefulness. For this reason we compared models with and without the APACHE III score. The reduced model developed without the APACHE III score contains age, number of organ failures, the alveolar-arterial oxygen difference, SP-D, IL-8, PAI-1, and TNFR1, and had good performance, with an AUC of 0.811.

This study has some limitations. Although the clinical data and plasma samples were collected prospectively, this analysis was done retrospectively. Because of limited sample volume, the analysis was limited to eight biomarkers that have been previously associated with mortality in ALI/ARDS. It is possible that other biomarkers might have better performance. Indeed, some of the biomarkers assayed had a fair degree of collinearity; the need for orthogonal biomarkers in multimarker predictors has been emphasized.^{34,35} Because subjects were enrolled in a large multicenter clinical trial, they represent a selected group of patients with clinical characteristics that may differ from less selected clinical populations in daily practice and the findings may not be generalizable to a broader patient population. Also, the additional predictive value of the plasma biomarkers added to the clinical predictors alone is modest (AUC of 0.850 vs 0.815); thus, further work will be needed to test the value of these biomarkers over clinical predictors alone. In addition, it should be noted that the biomarkers were all measured by immunoassay and as such provide information only about antigenic levels and not biologic activity. Finally, although the clinical

FIGURE 4. Predicted probability of death for each clinical variable in the reduced model without APACHE III when all other variables are fixed at their median values. Median values are: age, 50 years; number of organ failures at enrollment, 0; arterial-alveolar oxygen difference, 286 mm Hg. Dotted lines in each plot represent the 95% CI. See Figure 1 legend for expansion of abbreviation.

FIGURE 5. Predicted probability of death for each biomarker variable in the reduced model without APACHE III when all other variables are fixed at their median values. Median values are: IL-8, 40.4 pg/mL; SPD, 99.0 ng/mL; PAI, 61.3 ng/mL; TNFR1, 4,283 pg/mL. Dotted lines in each plot represent the 95% CI. See Figure 1 and 2 legends for expansion of abbreviations.

predictors and the biomarkers have been studied previously in the first ARDS Network study of a low tidal volume protective ventilator strategy, the current study represents the first assessment of a multi-marker panel in a single cohort and the risk of overfitting should be considered. For this reason, this model should be validated prospectively.

In summary, a combination of biomarkers and clinical predictors is superior to either clinical predictors or biomarkers alone for predicting mortality in ALI/ARDS. The two best performing biomarkers are markers of lung epithelial barrier injury (SP-D) and inflammation and neutrophil chemotaxis (IL-8), confirming the importance of these pathways in the pathogenesis of clinical ALI/ARDS. A combined clinical and biomarker index of predicted

mortality should be useful for stratifying patients in clinical trials.

APPENDIX

Participants in the National Heart, Lung, and Blood Institute (NHLBI) ARDS Clinical Trials Network were as follows: Investigators (principal investigators are marked with an asterisk): Cleveland Clinic Foundation — H. P. Wiedemann,* A. C. Arroliga, C. J. Fisher, Jr, J. J. Komara, Jr, P. Periz-Trepichio; Denver Health Medical Center — P. E. Parsons; Denver Veterans Affairs Medical Center — C. Welsh; Duke University Medical Center — W. J. Fulkerson, Jr,* N. MacIntyre, L. Mallatratt, M. Sebastian, J. Davies, E. Van Dyne, J. Govert; Johns Hopkins Bayview Medical Center — J. Sevransky, S. Murray; Johns Hopkins Hospital — R. G. Brower, D. Thompson, H.E. Fessler, S. Murray; LDS Hospital — A. H. Morris,* T. Clemmer, R. Davis, J. Orme, Jr, L. Weaver,

C. Grissom, F. Thomas, M. Gleich (deceased); McKay-Dee Hospital — C. Lawton, J. D'Hulst; MetroHealth Medical Center of Cleveland — J. R. Peerless, C. Smith; San Francisco General Hospital Medical Center — R. Kallet, J. M. Luce; Thomas Jefferson University Hospital — J. Gottlieb, P. Park, A. Girod, L. Yannarell; University of California, San Francisco — M. A. Matthay,* M. D. Eisner, J. Luce, B. Daniel, T. J. Nuckton; University of Colorado Health Sciences Center — E. Abraham,* F. Piedalue, R. Jagusch, P. Miller, R. McIntyre, K. E. Greene; University of Maryland — H. J. Silverman,* C. Shanholtz, W. Corral; University of Michigan — G. B. Toews,* D. Arnoldi, R. H. Bartlett, R. Dechert, C. Watts; University of Pennsylvania — P. N. Lanken,* J. D. Christie, B. Finkel, B. D. Fuchs, C. W. Hanson, III, P. M. Reilly, M. B. Shapiro; University of Utah Hospital — R. Barton, M. Mone; University of Washington/Harborview Medical Center — L. D. Hudson,* G. Carter, C. L. Cooper, A. Hiemstra, R. V. Maier, K. P. Steinberg, Margaret Neff, Patricia Berry-Bell; Utah Valley Regional Medical Center — T. Hill, P. Thaut; Vanderbilt University — A. P. Wheeler,* G. Bernard,* B. Christman, S. Bozeman, T. Swope, L. B. Ware; Clinical Coordinating Center, Massachusetts General Hospital, Harvard Medical School — D. A. Schoenfeld,* B. T. Thompson, M. Ancukiewicz, D. Hayden, MA, F. Molay, N. Ringwood, C. Oldmixon, A. Korpak, R. Morse; NHLBI Staff — D. B. Gail, A. Harabin,* P. Lew, M. Wacławski,* Steering Committee — G. R. Bernard (chair); Data and Safety Monitoring Board — R. G. Spragg (chair), J. Boyett, J. Kelley, K. Leeper, M. Gray Secundy, A. S. Slutsky, B. Turnbull; Protocol Review Committee — J. G. N. Garcia (chair), S. S. Emerson, S. K. Pingleton, M.D. Shasby, W. J. Sibbald.

ACKNOWLEDGMENTS

Author contributions: Dr Ware: contributed to designing the study, supervising all measurements made, analyzing the results, and writing the manuscript.

Dr Koyama: contributed to biostatistical analysis and editing the manuscript.

Dr Billheimer: contributed to biostatistical analysis and editing the manuscript.

Dr Wu: contributed to biostatistical analysis.

Dr Bernard: contributed to designing the study, analyzing the results, and editing the manuscript.

Dr Thompson: contributed to designing the study, analyzing the results, and edited the manuscript.

Dr Brower: contributed to designing the study, analyzing the results, and editing the manuscript.

Dr Standiford: contributed to designing the study, analyzing the results, and editing the manuscript.

Dr Martin: contributed to designing the study, analyzing the results, and editing the manuscript.

Dr Matthay: contributed to designing the study, analyzing the results, and editing the manuscript.

Financial/nonfinancial disclosures: The authors have reported to CHEST that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

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