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Original Article

Surfactant, pulmonary-associated protein D may serve as a biomarker in addition to APACHE II score in predicting mortality rate of ARDS

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Abstract: In the current study, a receiver operating characteristic (ROC) curve was applied to explore predictors for severity and prognosis of acute respiratory distress syndrome (ARDS). Clinical data including mechanical ventilation, clinical manifestation, performance of APACHE II score, and PaO_2/FiO_2 ratio and $AaDO_2$ were obtained within 24-hour of admission to the hospital. Protein levels of surfactant, pulmonary-associated protein A and D (SP-A and -D), Clara cell protein 16 (CC-16), neutrophil elastase (NE) and peptidase inhibitor 3 (PI3) in the patients' serum were determined by ELISA. Mortality of the hospitalized patients was 30.7% and there were significant differences in APACHE II score, PaO_2/FiO_2 ratio and $AaDO_2$, SP-A and -D, CC-16 and NE between survivors and non-survivors. By comparing the areas under the ROC curve of logistic regression model, it was found that APACHE II score and serum SP-D level displayed larger ROC areas with the left shift of the curves. The findings of the current study suggested that, in addition to APACHE II, serum SP-D level could also be used as a biomarker of monitoring mortality rate in ARDS.

Keywords: ROC curve, ARDS, SP-D, PaO_2/FiO_2 ratio, $AaDO_2$

Introduction

ARDS is a rapidly developing, life-threatening medical condition that prevents enough oxygen from getting to the lungs and into the blood. The disease usually develops in people who are already very ill with another disease [1] or who have major injuries [2]. ARDS has been recognized as the most severe form of acute lung injury associated with breakdown of the epithelial and endothelial cells lining the alveoli and lung's blood vessels, however, pathogenesis of ARDS has not been fully elucidated, but the disease has been recognized as the most severe form of acute lung injury associated with breakdown of the epithelial and endothelial cells lining the alveoli and lung's blood vessels [3]. Despite recent therapeutic advances in the field of mechanical ventilation, mortality of ARDS remains relatively stagnant around 40% [4].

Predisposing factors of ARDS are numerous and varied, but mortality of ARDS has not been decreased substantially since the publication of the American-European consensus conference in 1994 [5, 6]. Recently, ROC curve has been used to evaluate risk factors in predicting mortality of severe diseases including ARDS [7-9]. Therefore, using ROC curve analysis, we proposed that diagnostic evaluation would be important in identifying severity of the disease and efficiency of treatment. To accomplish this, 101 cases of ARDS were retrospectively analyzed (excluded patients without ventilation therapy). Specifically, the risk factors of death from the disease were assessed based on changes in areas under the ROC curves. Our results indicated that the serum SP-D level may be considered as a good predictor correlating highly with a mortality rate in the investigated cohort.

Materials and methods

Retrospective study population and inclusion criteria

101 patients aged from 45-72 years old (median, 56 years) were consecutively admit-

ted to intensive care unit (ICU) in our hospital from January 2011 to June 2013. According to the consequence of treatment lasting up to 28 days, survivors and non-survivors with ARDS were classified with an average age of 59.19 and 58.71 years old in each group. Population distribution of male and female patients showed as 47.14% and 52.86% in survivors and 41.94% and 58.06% in non-survivors, respectively. There were no statistical differences in the distribution of age and gender between these two groups (both $P>0.05$). In this retrospective study, clinical data including laboratory tests, use of ventilator and clinical manifestations of the hospitalized patients were collected during the first 24 hours of admission. The study was approved by the Ethics Committee of Friendship Hospital, Beijing in December of 2010 (Approval number: 20101283). A signed written informed consent was obtained from the patients or their relatives. The study was performed in accordance with the Helsinki II declaration.

Inclusion criteria for the study: 1) ARDS as defined by the American-European Consensus Conference [10]; 2) Performance of acute physiology and chronic health evaluation (APACHE) II score was measured [11]; 3) Lung lesions demonstrated by chest radiograph and chest computed tomography scan; 4) Development of extra pulmonary organ dysfunction determined by laboratory tests; 5) No statistical difference in comorbidities including chronic obstructive pulmonary disease, coronary heart disease, hypertension and diabetes between the survivors and non-survivors. Rationale of the including criteria was to exclude patients with HIV infection, cancer patients who had received radiotherapy or chemotherapy, patients with steroid treatment, and patients with immune dysfunction.

Blood gas analysis and ventilator settings

Blood samples were collected by arterial puncture and used for gas analysis. A ratio of PaO_2 (partial pressure of oxygen in arterial blood) and FiO_2 (fraction of inspired oxygen) was determined typically as the hyperemia criterion to characterize ARDS. $AaDO_2$ (alveolar-arterial oxygen tension difference) is calculated as an index to assess mismatch of ventilation and perfusion of the lungs.

Positive end-expiratory pressure (PEEP) was applied for some patients within 24 hours of admission following the previously published protocol [10]. Specifically, PEEP was adjusted from 4-15 mH_2O according to the results of blood gas analysis.

Determination of potential diagnostic markers in serum

Five ml blood were collected and serum was stored at -80°C until being used for quantification of SP-A and -D, CC-16, NE and PI3 by ELISA following the manufacture's instruction (BD Biosciences, NJ US). Briefly, 100 µl of the substrate solution was added to 100 µl of the serum sample in microtiter plates in duplicate tests and then incubated for 30 min at room temperature. The reaction was stopped by adding 50 µl of 4 M sulfuric acid, and the OD values were read in a microtiter autoreader at 450 nm.

ROC curve analysis

A ROC curve was created by plotting the true positive rate against the false positive rate at various threshold settings, which was expressed as a graph of sensitivity (y-axis) versus 1-specificity (x-axis) with Prism. Maximizing sensitivity and specificity corresponded to some large y and to a small x value on the curve, respectively. The closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test [12].

In this study, the optimal cutoff point for the predictors of death from ARDS was selected on the ROC curve moving from the vicinity of the upper left corner over toward the upper right corner. We randomly picked one from the dead cases and one from the survival patients and do the test on both. The patient with the more abnormal test result should be the one from non-survivors. The area under the curve was the percentage of randomly drawn pairs for which this was true. Multivariable logistic regression model was established as a direct probability model that was developed to predict a binary response based on more predictor variables.

Statistical analyses

A CI was applied to observe a percentage of population distribution of the patients. Median

Table 1. Comparison of clinical data of ARDS patients during the 1st day after admission (excluded patients without ventilation therapy)

Variables	Survivors (n=70)	Non survivors (n=31)	*P*
APACHE II score	19 (16, 23)	28 (25, 35)	0.000
PaO_2/FiO_2	236 (164, 268)	162 (102, 188)	0.000
A-aDO_2	103 (58, 174)	152 (115, 238)	0.005
SP-D (pg/ml)	447 (267, 663)	994 (818, 1085)	0.000
SP-A (pg/ml)	24 (21, 29)	30 (27, 33)	0.001
CC-16 (pg/ml)	162 (128, 208)	228 (169, 258)	0.000
NE (ng/ml)	16 (10, 24)	15 (10, 18)	0.124
PI3 (pg/ml)	1650 (959, 2777)	1442 (829, 2809)	0.845
NE/PI3	8 (5, 15)	9 (4, 14)	0.656

Table 2. Changes in areas under the ROC curves in the studying population (excluded patients without ventilation therapy)

Variables	Area under ROC curve (95% CI)	*P**	*P***
PI3	0.512 (0.386, 0.638)	0.8465	<0.001
HNE	0.597 (0.482, 0.712)	0.100	<0.001
A-aDO_2	0.676 (0.567, 0.785)	0.002	<0.001
SP-A	0.715 (0.608, 0.822)	<0.001	<0.001
PaO_2/FiO_2	0.740 (0.642, 0.838)	<0.001	<0.001
CC-16	0.760 (0.656, 0.865)	<0.001	<0.001
SP-D	0.909 (0.839, 0.979)	<0.001	0.019
APACHE II	0.948 (0.908, 0.987)	<0.001	> 0.05
Logistic Model I	0.978 (0.953, 1.000)	<0.001	> 0.05
Logistic Model II	0.978 (0.956, 1.000)		

*, **compared to 0.5 (a diagonal line from 0.0 to 1.1 on the ROC curve) and Logistic model II.

was calculated in some of the results from the investigated cohort. The Spearman rank correlation between two variables was performed using Statistical Package for the Social Science (SPSS, version 13.0). Comparisons between groups were implemented by student's unpaired *t* test. The Chi-square test (χ^2) was conducted to analyze the significance of parameters within groups. A *P* value of <0.05 was considered significant.

Results

Clinical characteristics of ARDS patients

A total of 101 patients with ARDS were recruited for this retrospective study. Patients' demographic and clinical data were collected during the first 24 hours of hospitalization. All of the enrolled ARDS patients' information was collected until being discharged from hospital or deceased within 28 days of hospitalization. As shown in **Table 1**, 31 patients died from the disease with a mortality rate of 30.7%. Furthermore, 63.4% patients had lung lesions (≥ two regions) and 50% patients had non-pulmonary organ damage (≥ one organ), respectively.

Comparison of clinical data between survivors and non-survivors

Results of mechanical ventilation and clinical manifestations of ARDS patients were compared in survivors and non-survivors. In statistical analysis, a population distribution of the hospitalized patients received mechanical ventilation was shown as 72.9% in survivors and 86.1% in non survivors. Furthermore, the non-survivors stayed in hospital longer than the survivors, but it was not statistically different. Incidence of lung lesions (≥ two separate lesions) was 60.4% in the survivors and 72.2% in the non-survivors, respectively, which was not significantly different (*P*>0.05). In contrast, 42.7% of the survivors, while 69.4% of non-survivors, had extra pulmonary organ dysfunction (≥ one organ failure) and there was significant difference between the two groups (*P*<0.05).

Comparison of ARDS mortality predictors between survivors and non-survivors

Risk factors for predicting ARDS mortality were assessed within 24 hours of admission to the hospital and the results were expresses by median values (Interquartile Range, IQR) as shown in **Table 1**. The APACHE II score were 19 (16, 23) and 28 (25, 35) in survivors and non-survivors, respectively, and the difference was statistically significant (*P*=0.000). Blood gas analysis from the dead cases displayed a significant decrease associated with an obvious increase in the PaO_2/FiO_2 ratio and A-aDO_2 as compared to those in the survival patients (both *P*<0.005). Serum levels of SP-A, SP-D, and CC-16 were significantly increased in the non-survival group compared to those in the

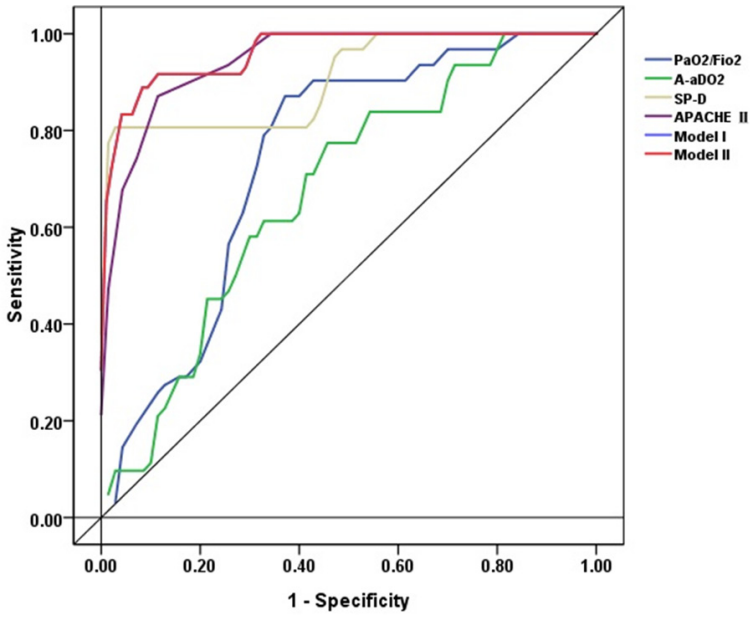

Figure 1. The ROC curves graph for diagnostic tests. Areas under the ROC curves for SP-D (A), PaO_2/FiO_2 (B), A-aDO_2 (C) and APACHE II score (D) were assessed in ARDS patients, respectively. Model I and II were established with multivariable logistic regression data that excluded and included the APACHE II score.

survival group (P<0.05); while NE was slightly but significantly decreased in the non-survival group than that in the survival group (P<0.05). Level of PI3 and ratio of NE/PI3 were not significantly different (P=0.845 or 0.656).

ROC curves analysis of ARDS mortality predictors

Patients' demographic and clinical data correlating with risk factors of death for ARDS patients were compared according to the change in the areas under the ROC curves and the results were shown in **Table 2**. Multivariable logistic regression models were developed to identify independent predictors of mortality and other adverse outcome of the hospitalized patients. The logistic model I and II were established with the predictors with or without APACHE II score in each individual model. Our data exhibited model I (95% CI, 0.953 to 1.000) and II (95% CI, 0.956 to 1.000) with accuracy to be over 93% of the positive correct. The ROC curve areas for diagnostic tests were quantified as APACHE II score > SP-D > CC-16 > PaO_2/FiO_2 > SP-A > A-aDO_2 > NE > PI3 in the order of the size. The APACHE II score (95% CI, 0.908 to 0.987) and the serum SP-D level (95% CI, 0.839 to 0.979) showed a good relationship as tested by the logistic models. All tests but not the PI3 test displayed significant differences in the areas as compared to 0.5 and the model II (All P<0.05 or 0.001).

In analysis of the ROC curve graph, the true positive rate (sensitivity) was plotted in function of the false positive rate (specificity) for different cut-off points. The logistic regression models were used as positive controls to indicate a high risk of mortality associated with the hospitalized patients. To observe major change in the variables in highly correlation with a mortality rate, ROC plots for the four predictors of APACHE II score, SP-D, PaO_2/FiO_2, A-aDO_2 were illustrated and the results are shown in **Figure 1**. Similar to the curves of the logistic models, the ROC curves of the four diagnostic tests started in the same way from the lower left corner, went straight up to the upper left corner with different altitudes, and then to the upper right corner on the ROC curves. The curves of APACHE II score and serum SP-D corresponded to a leftward movement in the ROC curves and displayed larger areas under the curves as compared to other curves.

Discussion

While there is no specific treatment for ARDS yet, goal of ventilation therapy is simply to support breathing and allow the patient's lungs to heal [13, 14]. Thus, diagnostic evaluation of death risk factors applied for the hospitalized patients would be necessary in predicting disease severity and providing various alternative treatments for repairing lung injuries. In this content, the current study retrospectively analyzed mortality and predicting factors of ARDS. It was found that 30.7% of the enrolled patients died from ARDS during 28 days of hospital stay, which was lower than mortality rate reported in other studies [15, 16]. 63.4% and 50% of the patients presented with lung lesions or non-pulmonary organ dysfunction, respectively.

Mortality of ARDS varies widely depending on factors such as disease severity, application of

mechanical ventilation and presence of other medical conditions [17, 18]. In the current study, in order to eliminate inappropriate statistical analysis of the death-related factors, demographic data of mechanical ventilation and clinical manifestations of the patients were compared by quantifiable statistics between survivors and non-survivors. While the rate of failing to use mechanical ventilation was lower in non-survivors, incidence of extra pulmonary organ dysfunction was significantly higher in non-survivors than that in survivors, suggesting mechanical ventilation may be not associated with occurrence of extra-pulmonary organ dysfunction.

Risk factors for death were screened from the clinical data with the APACHE II scores obtained from ICU surveillance and patients' records. Incidence proportion of the non-survivors was significantly increased with the score reaching to a median value of 10 over the survivors, demonstrating that high APACHE II scores were related to the disease severity with high mortality of the patients [10]. Since ARDS is a disease of the microscopic air sacs of the lungs that leads to decreased exchange of oxygen and carbon dioxide [1], blood gas analysis was performed and found that PaO_2/FiO_2 ratio was dramatically decreased while A-aDO_2 was significantly increased in the group of non-survivors compared to the group of survivals. These findings indicated not only a failure of the lung to transport oxygen into the blood, but also severe hypoxemia relative to the underlying disease was progressing.

Serum protein levels of SP-A, SP-D and CC-16 were significantly increased in the non-survivors compared to that in survivors. In contrast, serum NE and ratio of NE/PI3 were dramatically decreased in non-survivors compared to that in survivors. Consistent with previous reports that these proteins involve in injury and repair of the alveolar wall [19, 20] and lung inflammatory response [21, 22], findings of the current study suggested that these proteins play a crucial role in the progression of the disease.

Mortality differential for predictor variables was examined by comparing areas under the ROC curve that may measure discrimination, that is, the ability of the test to correctly classify the variables with or without a death risk [23, 24]. Multivariable logistic regression models were established by regression analysis. While ROC spaces increased in most of the tests, areas for APACHE II score (95% CI, 0.908-0.987) and serum SP-D (95% CI, 0.839-0.979) were significantly enlarged with the curves moving far away from the diagonal line as compared to others, indicating the two variables highly correlate with mortality of the patients by reaching to 82%-98% of the highest accuracy in the cohort. APACHE II score is designed to measure the severity of a disease with an integer score from 0 to 71 of several measurements [11]. Consistent with previously published results by other investigators [25], the result of quantitative ROC analysis in the current study fully demonstrated that higher APACE II score is corresponding to more severe condition of the illness and a higher risk of death in the study.

SP-D is a collagenous, carbohydrate-binding glycoprotein synthesized and secreted into the air spaces of the lung by the respiratory epithelium [26, 27]. Though lung is the major site of SP-D expression, it is likely that the protein has more generalized roles in host defense and the acute response to infection and tissue injury [27]. It has been found that a high SP-D plasma level might positively correlate with lung injury in ARDS patients [28]. ROC analysis of the current study revealed a strong association of serum SP-D level and severity of lung injury as well as high mortality of the disease, suggesting that serum SP-D level may be considered as a biomarker in predicting mortality rate of ARDS.

Taken together, this study generated ROC curve analysis and documented a highly sensitive and specific association of the optimal cut-off points of the SP-D serum level with ARDS patients' mortality, which could be a prognostic factor for ARDS in addition to APACHE II score.

Disclosure of conflict of interest

None.

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References

[1] Ware LB and Matthay MA. The acute respiratory distress syndrome. N Engl J Med 2000; 342: 1334-1349.

[2] de Haro C, Martin-Loeches I, Torrents E and Artigas A. Acute respiratory distress syndrome: prevention and early recognition. Ann Intensive Care 2013; 3: 11.
[3] Dushianthan A, Grocott MP, Postle AD and Cusack R. Acute respiratory distress syndrome and acute lung injury. Postgrad Med J 2011; 87: 612-622.
[4] Brun-Buisson C, Minelli C, Bertolini G, Brazzi L, Pimentel J, Lewandowski K, Bion J, Romand JA, Villar J, Thorsteinsson A, Damas P, Armaganidis A, Lemaire F and Group AS. Epidemiology and outcome of acute lung injury in European intensive care units. Results from the ALIVE study. Intensive Care Med 2004; 30: 51-61.
[5] Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A and Spragg R. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med 1994; 149: 818-824.
[6] Phua J, Badia JR, Adhikari NK, Friedrich JO, Fowler RA, Singh JM, Scales DC, Stather DR, Li A, Jones A, Gattas DJ, Hallett D, Tomlinson G, Stewart TE and Ferguson ND. Has mortality from acute respiratory distress syndrome decreased over time?: A systematic review. Am J Respir Crit Care Med 2009; 179: 220-227.
[7] Bos LD, Weda H, Wang Y, Knobel HH, Nijsen TM, Vink TJ, Zwinderman AH, Sterk PJ and Schultz MJ. Exhaled breath metabolomics as a noninvasive diagnostic tool for acute respiratory distress syndrome. Eur Respir J 2014; 44: 188-197.
[8] Zhu B, Zheng F, Liu N, Zhu MH, Xie J, Ye JR, Zhang J, Jiang DQ, Yang C and Jiang Y. Diagnostic value of surfactant protein-a in severe acute pancreatitis-induced acute respiratory distress syndrome. Med Sci Monit 2014; 20: 1728-1734.
[9] Bauman ZM, Gassner MY, Coughlin MA, Mahan M and Watras J. Lung Injury Prediction Score Is Useful in Predicting Acute Respiratory Distress Syndrome and Mortality in Surgical Critical Care Patients. Crit Care Res Pract 2015; 2015: 157408.
[10] Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L and Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307: 2526-2533.
[11] Knaus WA, Draper EA, Wagner DP and Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985; 13: 818-829.
[12] Zweig MH and Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; 39: 561-577.
[13] Putensen C, Theuerkauf N, Zinserling J, Wrigge H and Pelosi P. Meta-analysis: ventilation strategies and outcomes of the acute respiratory distress syndrome and acute lung injury. Ann Intern Med 2009; 151: 566-576.
[14] Needham DM, Yang T, Dinglas VD, Mendez-Tellez PA, Shanholtz C, Sevransky JE, Brower RG, Pronovost PJ and Colantuoni E. Timing of low tidal volume ventilation and intensive care unit mortality in acute respiratory distress syndrome. A prospective cohort study. Am J Respir Crit Care Med 2015; 191: 177-185.
[15] Zambon M and Vincent JL. Mortality rates for patients with acute lung injury/ARDS have decreased over time. Chest 2008; 133: 1120-1127.
[16] Mancebo J, Fernandez R, Blanch L, Rialp G, Gordo F, Ferrer M, Rodriguez F, Garro P, Ricart P, Vallverdu I, Gich I, Castano J, Saura P, Dominguez G, Bonet A and Albert RK. A multicenter trial of prolonged prone ventilation in severe acute respiratory distress syndrome. Am J Respir Crit Care Med 2006; 173: 1233-1239.
[17] Needham DM, Colantuoni E, Mendez-Tellez PA, Dinglas VD, Sevransky JE, Dennison Himmelfarb CR, Desai SV, Shanholtz C, Brower RG and Pronovost PJ. Lung protective mechanical ventilation and two year survival in patients with acute lung injury: prospective cohort study. BMJ 2012; 344: e2124.
[18] Kregenow DA, Rubenfeld GD, Hudson LD and Swenson ER. Hypercapnic acidosis and mortality in acute lung injury. Crit Care Med 2006; 34: 1-7.
[19] Crouch E and Wright JR. Surfactant proteins a and d and pulmonary host defense. Annu Rev Physiol 2001; 63: 521-554.
[20] Hermans C and Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. Am J Respir Crit Care Med 1999; 159: 646-678.
[21] Belaaouaj A, Kim KS and Shapiro SD. Degradation of outer membrane protein A in Escherichia coli killing by neutrophil elastase. Science 2000; 289: 1185-1188.
[22] Tejera P, Wang Z, Zhai R, Su L, Sheu CC, Taylor DM, Chen F, Gong MN, Thompson BT and Christiani DC. Genetic polymorphisms of peptidase inhibitor 3 (elafin) are associated with acute respiratory distress syndrome. Am J Respir Cell Mol Biol 2009; 41: 696-704.
[23] Hanley JA and McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143: 29-36.
[24] Knaus WA, Zimmerman JE, Wagner DP, Draper EA and Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. Crit Care Med 1981; 9: 591-597.

[25] Wu WH, Niu YY, Zhang CR, Xiao LB, Ye HS, Pan DM and Zeng M. Combined APACH II score and arterial blood lactate clearance rate to predict the prognosis of ARDS patients. Asian Pac J Trop Med 2012; 5: 656-660.

[26] Crouch EC. Collectins and pulmonary host defense. Am J Respir Cell Mol Biol 1998; 19: 177-201.

[27] Crouch EC. Surfactant protein-D and pulmonary host defense. Respir Res 2000; 1: 93-108.

[28] Ware LB and Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 2001; 163: 1376-1383.