# Chapter 6

## Plasma protein levels are markers of pulmonary vascular permeability and degree of lung injury in critically ill patients with or at risk for acute lung injury/acute respiratory distress syndrome

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*Critical Care Medicine*
*2011;39:89-97*

## ABSTRACT

**Introduction** To evaluate the diagnostic value of plasma protein levels for pulmonary vascular permeability and acute respiratory distress syndrome (ARDS). During acute lung injury (ALI) and ARDS, increased vascular permeability induces protein-rich fluid extravasation. We hypothesized that plasma protein levels predict increased vascular permeability and ARDS.

**Design, Setting, and Patients** A prospective, observational study, including 83 consecutive, mechanically ventilated patients with or at risk for ALI/ARDS, of whom 18 had sepsis. Patients with increased pulmonary capillary wedge pressures (PCWP) or central venous pressures (CVP), were excluded.

**Interventions** Patients were subjected to PCWP/CVP-guided fluid loading with saline or colloid fluids.

**Measurements and Main Results** We measured plasma albumin and transferrin levels, and determined the $^{67}$Gallium-transferrin pulmonary leak index (PLI), the American European Consensus Conference (AECC) criteria and the lung injury score (LIS). Measurements were performed before and after fluid loading, to evaluate effects of fluid loading. Plasma albumin and transferrin levels were about 30% lower in ARDS than in non-ALI ($P<0.01$) and ALI patients ($P<0.05$). Protein levels inversely related to the PLI (standardized regression coefficient [src] -0.28, $P<0.001$ for albumin; src -0.30, $P=0.003$ for transferrin) and the LIS (src -0.19, $P=0.01$ for albumin), independently of presence of sepsis, severity of disease and fluid loading. Albumin and transferrin levels had 77-96% sensitivity and negative predictive value for elevated pulmonary vascular permeability and ARDS (AECC criteria and LIS). Addition of hypoalbuminemia (<17.5 g/L) and hypotransferrinemia (<0.98 g/L) as criterion to the AECC criteria or the LIS increased their predictive values for elevated pulmonary vascular permeability.

**Conclusion** In critically ill patients, decreased plasma albumin and transferrin levels parallel increased pulmonary vascular permeability irrespective of underlying disease and fluid status. While normal levels help to exclude ARDS, hypoalbuminemia and hypotransferrinemia increase the diagnostic accuracy of the AECC criteria and LIS for elevated pulmonary vascular permeability.

## INTRODUCTION

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) frequently complicate critical illness in the intensive care unit (ICU). Forming a continuum, the syndromes are characterized by inflammation of the alveolar-capillary barrier, vascular hyperpermeability and protein-rich pulmonary edema, potentially decreasing plasma protein levels [1]. A low colloid osmotic pressure and positive fluid balance may further aggravate pulmonary edema and contribute to a poor outcome [2].

ALI/ARDS is defined according to the American European consensus conference (AECC) criteria [3] or the lung injury score (LIS) [4] to characterize permeability edema of the lungs, but these definitions can be criticized [5,6]. The $^{67}$Gallium (Ga) transferrin pulmonary leak index (PLI), obtainable at the bedside and directly measuring pulmonary vascular permeability, may further help to discriminate between permeability and hydrostatic pulmonary edema, and to characterize and grade the former [7-10]. However, the method is laborious and prompts for surrogate markers of increased pulmonary vascular permeability and injury, including low plasma protein levels [11-13], high edema fluid to plasma protein ratios [14], elevated urinary albumin excretion rate [15], high plasma angiopoietin-2 levels [16], positive fluid balance [2,17] and high extravascular lung water to blood volume ratios measured by transpulmonary thermodilution [18,19]. However, the diagnostic values of the latter surrogate indices differed among studies, because of different a priori chances and patient populations, often including hydrostatic pulmonary edema [2,11-13,17-19]. Indeed, the diagnostic value of protein levels can be overestimated when, in a population of patients with pulmonary edema, cardiogenic edema is included, since plasma protein levels may be elevated in the latter [12,20-22]. Otherwise, diagnostics can be hampered when technical demands are high and clinical applicability is low [14-16,18,19].

In search for simple biomarkers of ARDS, we evaluated the predictive values of plasma albumin, transferrin and total protein levels, together with fluid balance for pulmonary vascular permeability and lung injury. The study was performed in septic and non-septic critically ill patients, with or at risk for ALI/ARDS, in whom hypervolemia was excluded. Pulmonary vascular permeability was studied by the $^{67}$Ga PLI, while lung injury was characterized according to the AECC criteria and the LIS. We hypothesized that low plasma protein levels relate to increased pulmonary vascular permeability and lung injury, and thereby predict ARDS, regardless of underlying disease and fluid status.

## METHODS AND PATIENTS

This was a prospective, observational study, involving 83 consecutive, mechanically ventilated patients with or at risk for ALI/ARDS (18 septic and 65 non-septic patients), considered a representative critical care patient population. The study was approved by the local ethical committee, and written informed consent was given by patients or relatives. Patients were taking part in randomized controlled trials on the effects of saline, gelatin 4%, hydroxyethyl starch 6%, or albumin 5%, results of which have been published elsewhere [23,24]. In the current study, patients receiving albumin 5% were excluded from analysis.

**Patient population**

Patients were included in the study within 12 hours after meeting criteria for sepsis or 3 hours after major surgery, severe trauma or gastro-intestinal bleeding. We excluded patients with hypervolemia, arbitrarily defined as a systolic pressure ≥110 mmHg and increased filling pressures (a pulmonary capillary wedge pressure (PCWP) >13 mmHg in the presence of a pulmonary artery catheter (n=26), or a central venous pressure (CVP) >12 mmHg when positive end-expiratory pressure (PEEP) ≤15 $cmH_2O$ and >16 mmHg when PEEP >15 $cmH_2O$ in the presence of a central venous catheter (n=57)). Other exclusion criteria were age ≥78 years, pregnancy, known anaphylactic reaction to colloid fluids and life expectancy <24 hours. Sepsis was diagnosed if two or more of the following criteria were met: abnormal body temperature (<36 °C or >38 °C), tachycardia (>90/min), tachypnea (>20/min or a partial pressure of arterial carbon dioxide ($P_aCO_2$) <32 mmHg (4.3 kPa), abnormal white blood cell counts (<4 or >12x$10^9$/L) and a microbiologically proven or clinical source of infection.
According to the AECC criteria, ALI or ARDS were diagnosed in the presence of the following criteria: a) acute onset, b) a partial pressure of arterial oxygen ($P_aO_2$)/fraction of inspired oxygen ($FiO_2$) ratio between 200 mmHg (26.6 kPa) and 300 mmHg (39.9 kPa), or <200 mmHg (26.6 kPa), respectively, c) bilateral infiltrates on the anteroposterior chest radiograph, d) a PCWP <18 mmHg or clinical absence of left atrial hypertension (3). To study the influence of underlying risk factors, ARDS was further categorized into primary ARDS (due to pulmonary risk factors like pneumonia and sepsis from pulmonary origin) and secondary ARDS (due to systemic risk factors like sepsis from non-pulmonary origin, surgery and trauma). In addition, all patients were characterized as being at risk for or having direct lung injury (due to pneumonia or sepsis from pulmonary origin) or indirect lung injury (due to surgery, trauma, or sepsis from extrapulmonary origin). The LIS was calculated after scoring a) the number of alveolar consolidations on the anteroposterior chest radiograph, b) hypoxemia, c) pulmonary compliance, and d) PEEP. ALI or ARDS were characterized by a LIS between 1 and 2.5 or a LIS ≥2.5, respectively [4].

All patients were endotracheally intubated and mechanically ventilated (Evita 3, Dräger, Lübeck, Germany), with a volume-controlled regimen in non-septic and a pressure-controlled regimen in septic patients. Patients with ALI/ARDS were on pressure-controlled ventilation aiming at a tidal volume of 6-8 mL/kg, with an inspiratory:expiratory ratio of 1:2. Respiratory rate, $O_2$-air mixture and PEEP were adjusted aiming at normocapnia and a $P_aO_2$ of >60 mmHg (8.0 kPa). Patients were otherwise treated by attending intensive care physicians, who were not involved in the study.

**Protocol**

At baseline, clinical and demographic data were recorded, together with ventilatory variables. Arterial blood samples were taken to assess $P_aO_2$ and $P_aCO_2$ (Rapidlab 865, Bayer Diagnostics, Tarrytown, New York, USA). Immediately after inclusion, the $^{67}$Ga PLI was measured as previously described (7-9). Autologous erythrocytes were labeled with $^{99m}$Technetium ($^{99m}$Tc, 11MBq, physical half-life 6h; Mallinckrodt Diagnostica, Petten, The Netherlands). Transferrin was labeled in vivo following intravenous injection of $^{67}$Ga-citrate (4.5 MBq, physical half-life 78 hours; Mallinckrodt Diagnostica). Patients were in the supine position and two scintillation detection probes (Eurorad C.T.T., Strasbourg, France) were positioned over the apices of the right and left lung. From the time of $^{67}$Ga-citrate injection, radioactivity was detected during 30 minutes. The counts were corrected for background radioactivity, physical half-life, spill-over, and expressed as counts per minute (CPM) per lung field. Blood samples (2 mL aliquots) were taken every 4 minutes for 30 minutes; samples were weighed, radioactivity was determined with a single well counter (LKB Wallac 1480 Wizard, Perkin Elmer, Life Science, Zaventem, Belgium), and corrected for background radioactivity, physical half-life, and spill-over. Results were expressed as CPM/gram. For each blood sample, a time-matched CPM over each lung was taken. A radioactivity ratio was calculated ($^{67}$Ga lung/$^{99m}$Tc lung)/($^{67}$Ga blood/$^{99m}$Tc blood) and plotted against time. The PLI was calculated from the slope of the increase of radioactivity ratio divided by the intercept, to correct for physical factors in radioactivity detection. The values for both lung fields were averaged. The PLI represents the transport rate of $^{67}$Ga-transferrin from the intra- to the extravascular space of the lungs, and is a measure of pulmonary vascular permeability. Reproducibility for measurement is 14% [8]. The upper limit of normal for PLI is $14.7 \times 10^{-3}$/min; a PLI $\geq 14.7 \times 10^{-3}$/min was considered to indicate ALI, while PLI $\geq 30.0 \times 10^{-3}$/min was chosen as cut-off value for ARDS, since PLI is typically raised twofold or more in ARDS [7,8]. Concomitantly with the measurement of PLI, venous blood samples were taken to measure albumin (bromocresol purple colorometry), transferrin (immunonephelometry) and total protein (biuret colorometry) concentrations. Albumin concentrations of 35-52 g/L, transferrin concentrations of 2.0-3.6 g/L, and total protein concentrations of 60-80 g/L were considered normal. Albumin and transferrin have similar molecular weight of about 65-75 kDa.

Patients were randomized to receive either saline 0.9% (n=28), gelatin 4% (Gelofusine®, 40 g/L, B.Braun Melsungen AG, Melsungen, Germany, in 154/120 mM NaCl, n=27) or hydroxyethyl starch 6% (HES, Hemohes®, MW 200.000, substitution 0.45-0.55, B. Braun Melsungen AG, in saline, n=28). Fluids were given in a time span of 90 minutes until predefined increases in PCWP/CVP were reached [23,24]. Per 10 minutes a maximum of 200 mL was given, while total loading volumes did not exceed 1800 mL. Directly after fluid loading, previously mentioned measurements were repeated. The fluid balance was recorded during the 90 minutes study interval from inclusion in the study until completion of fluid loading. Patients were followed until death or discharge from the ICU.

**Statistical analysis**

For statistical analysis, PLI, LIS and transferrin values were logarithmically transformed to obtain normal distributions (Kolmogorov-Smirnov test $P>0.05$). Data are presented as mean and standard deviation (SD) or median and interquartile range, where appropriate. Pearsons correlation coefficient and kappa statistics were used to evaluate agreement on measurements of the PLI, AECC diagnosis and LIS before and after fluid loading. For comparison of study variables between groups a Student's t-test or one-way analysis of variance with Tukey post-hoc analysis was performed. To control for possible confounding in the relation between pulmonary vascular permeability and plasma protein concentrations, we performed generalized estimating equations (GEE), in which PLI, AECC diagnosis or LIS were taken as dependent variable, albumin, transferrin, total protein levels, fluid balance, and APACHE II score as covariate, while sepsis and type of lung injury (direct versus indirect lung injury), or type of resuscitation fluid were taken as factor. Hence, repeated measurements in the same patient were taken into account. The standardized regression coefficient (src) was calculated. To ease clinical interpretation of these relations, partial correlations controlled for presence of sepsis and APACHE II score are presented. Coefficients of determination ($r^2$) are reported. For each surrogate marker, receiver operating curves (ROC) were calculated. After plotting sensitivity to 1-specificity, cut-off values with the best combination of sensitivity and specificity (the maximal sum of both values) were taken. To evaluate the additive value of plasma protein levels in predicting increased pulmonary vascular permeability, predictive values of AECC and LIS alone for a PLI$\geq 30.0 \times 10^{-3}$ were determined. Subsequently, hypoalbuminemia and hypotransferrinemia were added as criterion to the AECC criteria and the LIS, and combinations were evaluated for predictive values (Fisher's exact test). Likelihood ratios for positive results were calculated as sensitivity / (1-specificity). A P-value $<0.05$ was considered statistically significant. All statistics were performed using SPSS 15.0 (SPSS Inc., Chicago, USA).

## RESULTS

### Patient population

Patient characteristics are presented in Table 1. Eighteen patients (22%) had sepsis, while 65 patients (78%) were included after surgery, trauma or gastro-intestinal bleeding. The frequencies of a PLI $\geq 30x10^{-3}$/min and ARDS according to the AECC criteria and the LIS are stated in Table 2. Severity of disease was higher in septic than in non-septic patients, as indicated by a higher APACHE II score, a higher mortality (Table 1), lower plasma albumin and transferrin levels and a higher ARDS incidence (Table 2).

**Table 1 - Patient characteristics**

| | Total (n=83) | Sepsis (n=18) | Non-sepsis (n=65) |
|---|---|---|---|
| Age (year) | 60 ± 12 | 60 ± 12 | 61 ± 12 |
| Sex (male/female) | 65/18 (79/21) | 14/4 (78/22) | 51/14 (78/22) |
| BMI (kg/m$^2$) | 25.7 ± 3.7 | 24.4 ± 3.6 | 26.1 ± 3.7 |
| Source of sepsis | | | |
| Blood/catheter | | 1 | |
| Abdominal tract | | 3 | |
| Respiratory tract | | 9 | |
| Urogenital tract | | 1 | |
| Other | | 4 | |
| Microbiology | | | |
| Gram-positive bacteria | | 6 | |
| Gram-negative bacteria | | 7 | |
| Fungi | | 4 | |
| No micro-organisms cultured | | 3 | |
| Type of surgery/trauma | | | |
| Major thoracic surgery | | | 30 |
| Major abdominal surgery | | | 28 |
| Orthopedic surgery | | | 3 |
| Trauma | | | 3 |
| Gastrointestinal bleeding | | | 1 |
| APACHE II score | 10 ± 5 | 14 ± 5 | 9 ± 4 |
| Vasoactive treatment (yes/no) | 56/27 (67/33) | 16/2 (89/11) | 25/40 (39/61) |
| Duration of mechanical ventilation (hr) | 144 ± 305 | 304 ± 279 | 100 ± 298 |
| ICU mortality | 11 (13) | 7 (39) | 4 (6) |

Patient characteristics at inclusion. Mean ± SD or total number (percentage) where appropriate. APACHE = acute physiology and chronic health evaluation; BMI = body mass index; ICU = intensive care unit.

**Table 2 - Study variables in septic and non-septic patients before and after fluid loading.**

| | Before fluid loading | | | After fluid loading | | |
|---|---|---|---|---|---|---|
| | Total (n=83) | Sepsis (n=18) | Non-sepsis (n=65) | Total (n=83) | Sepsis (n=18) | Non-sepsis (n=65) |
| PLI (x$10^{-3}$/min) | 22.0 (13.8-50.3) | 54.8 (42.1-142.1) | 18.5 (12.5-27.5)** | 23.8 (14.3-47.3) | 63.8 (40.0-130.4) | 19.3 (13.0-33.4)** |
| PLI<14.7 | 27 (33) | 0 (0) | 27 (43) | 20 (24) | 0 (0) | 20 (31) |
| 14.7≥PLI<30 | 27 (33) | 10 (56) | 25 (40) | 29 (35) | 2 (11) | 27 (42) |
| PLI≥30 | 27 (33) | 8 (44) | 11 (17) | 33 (40) | 16 (89) | 17 (27) |
| AECC criteria | | | | | | |
| No ALI/ARDS | 48 (58) | 2 (11) | 46 (71) | 54 (65) | 2 (11) | 52 (80) |
| ALI | 21 (25) | 7 (39) | 14 (21) | 15 (18) | 7 (39) | 8 (12) |
| ARDS | 14 (17) | 9 (50) | 5 (8) | 14 (17) | 9 (50) | 5 (8) |
| LIS | 1.25 (0.75-1.75) | 2.38 (1.69-2.81) | 1.00 (0.75-1.38)** | 1.25 (0.75-1.75) | 2.25 (1.69-3.00) | 1.00 (0.75-1.50)** |
| LIS<1 | 31 (37) | 1 (6) | 30 (46) | 23 (28) | 0 (0) | 23 (35) |
| 1≤LIS<2.5 | 39 (47) | 8 (44) | 31 (48) | 47 (57) | 10 (56) | 37 (57) |
| LIS≥2.5 | 13 (16) | 9 (50) | 4 (6) | 13 (16) | 8 (44) | 5 (8) |
| $PaO_2/FiO_2$ ratio | 291 ± 111 | 205 ± 49 | 315 ± 112** | 304 ± 115 | 193 ± 60 | 335 ± 108** |
| Albumin (g/L) | 19 ± 5 | 13 ± 3 | 21 ± 4** | 17 ± 5 | 11 ± 3 | 18 ± 4** |
| Transferrin (g/L) | 1.10 (0.89-1.35) | 0.71 (0.53-0.86) | 1.20 (0.99-1.40)** | 0.92 (0.76-1.30) | 0.64 (0.530.73) | 1.00 (0.88-1.30)** |
| Total protein (g/L) | 44 ± 7 | 46 ± 5 | 44 ± 7 | 40 ± 7 | 41 ± 6 | 39 ± 8 |
| Infusion (mL) | 1547 ± 368 | 1503 ± 307 | 1592 ± 343 | | | |
| Urine flow (mL) | 454 ± 368 | 169 ± 144 | 535 ± 373** | | | |
| Fluid balance (mL) | 1139 ± 457 | 1333 ± 300 | 1085 ± 480* | | | |

Study variables measured before and after fluid loading. Before fluid loading, PLI measurement was missing in 2 patients (n=81), after fluid loading PLI measurement was missing in 1 patient (n=82). Mean ± standard deviation, median (interquartile range) or total number (percentage) where appropriate. * P<0.05, ** P<0.01 compared to sepsis group calculated by Student's t-test. AECC = American European consensus conference; ALI = acute lung injury; ARDS = acute respiratory distress syndrome; $FiO_2$ = fraction of inspired oxygen; LIS = lung injury score; $PaO_2$ = partial pressure of arterial oxygen; PLI = pulmonary leak index.

Similarly, PLI, LIS, APACHE II score and mortality were higher in patients with ARDS (AECC criteria), than in patients with ALI or non-ALI (Supplementary Table 1), while the PLI was higher in patients with a LIS >2.5 than in patients with a LIS <2.5 (P=0.003). Before fluid loading, the PLI and the frequencies of a PLI ≥30x10$^{-3}$/min and LIS ≥2.5 were higher in patients with primary ARDS than in patients with secondary ARDS, while plasma proteins tended to be lower in the former (Supplementary Table 2). Within the sepsis group, no difference in study variables could be detected between patients with direct lung injury and patients with indirect lung injury (Supplementary Table 3). The PLI before fluid loading correlated with that after loading ($r^2$=0.71, P<0.001). The kappa coefficient for agreement before and after fluid loading was 0.72 (P<0.001) for AECC criteria and 0.72 (P<0.001) for LIS. Plasma albumin and transferrin levels strongly correlated at both time points ($r^2$=0.66, P<0.001 before and $r^2$=0.64, P<0.001 after fluid loading).

**Proteins, permeability and AECC/LIS**

Before fluid loading, levels of albumin and transferrin were lower in patients with a PLI ≥30x10$^{-3}$/min, than in patients with a PLI between 14.7 and 30x10$^{-3}$/min (P<0.001 for both albumin and transferrin levels) and those with a PLI <14.7x10$^{-3}$/min (P<0.001 for both albumin and transferrin levels) (Supplementary Figure 1). Similarly, protein levels were lower in patients with ARDS than in patients with non-ALI and ALI according to AECC criteria and LIS (Figures 1 and 2 for data before fluid resuscitation). These differences persisted after fluid loading (data not shown), even though at slightly decreased protein concentrations (Table 2). The fluid balance was more positive in patients with a PLI ≥30x10$^{-3}$/min than in patients with a PLI <14.7x10$^{-3}$/min (Figure 3).



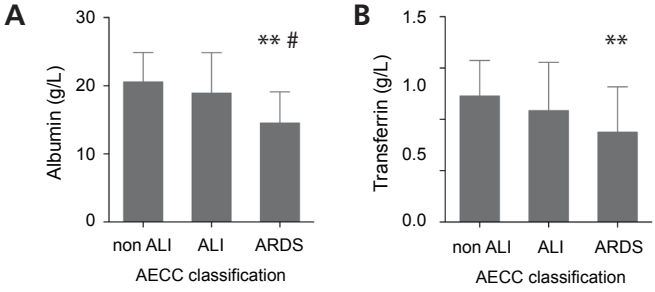


**Figure 1 – Concentrations of albumin (A) and transferrin (B) in the different AECC subcategories before fluid loading.** Error bars represent +/- standard deviation (A) or interquartile range (B). ** = P<0.01 compared to non-ALI, # = P<0.05 compared to ALI calculated by one-way analysis of variance with Tukey post-hoc analysis. ALI = acute lung injury; ARDS = acute respiratory distress syndrome; AECC = American European consensus conference.

In GEE, the relation of PLI and LIS to plasma proteins and fluid balance was adjusted for presence of sepsis, type of lung injury and severity of disease (APACHE II score). The PLI inversely related

to plasma albumin (src -0.28, P=0.002), transferrin (src -0.30, P=0.003) and total protein levels (src -0.16, P=0.003), and positively to fluid balance (src 0.26, P=0.04). LIS inversely related to albumin (src -0.19, P=0.01) and total protein levels (src -0.08, P=0.04). No influence of the type of resuscitation fluid could be detected. Partial correlation adjusted for presence of sepsis and APACHE II score revealed that PLI correlated with albumin ($r^2$=0.08, P=0.02 before and $r^2$=0.23, P<0.001 after fluid loading) and transferrin ($r^2$=0.15, P<0.001 before and $r^2$=0.18, P<0.001 after fluid loading) (see also Figure 4). Albumin further correlated with PEEP ($r^2$=0.14, P=0.001 before and $r^2$=0.10, P=0.005 after fluid loading) and compliance ($r^2$=0.10, P=0.005 before and $r^2$=0.12, P=0.002 after fluid loading). Likewise, transferrin correlated with PEEP ($r^2$=0.10, P=0.005 before and $r^2$=0.07, P=0.01 after fluid loading) and compliance ($r^2$=0.09, P=0.008 before and $r^2$=0.12, P=0.002 after fluid loading).

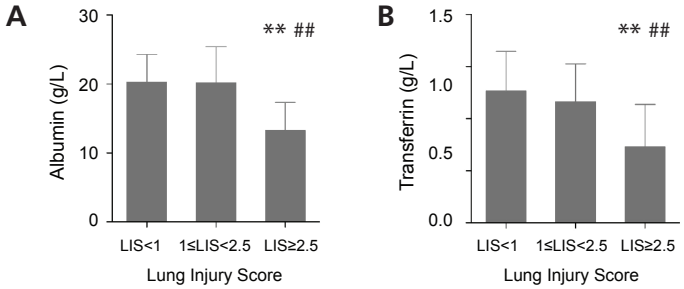


**Figure 2 – Concentrations of albumin (A) and transferrin (B) in the different LIS subcategories before fluid loading.** LIS<1 represents non-ALI patients, 1≤LIS<2.5 ALI patients, and LIS≥2.5 ARDS patients. Error bars represent +/- standard deviation (A) or interquartile range (B). ** = P<0.01 compared to LIS<1, ## = P<0.01 compared to 1≤LIS<2.5 calculated by one-way analysis of variance with Tukey post-hoc analysis. ALI = acute lung injury; ARDS = acute respiratory distress syndrome; LIS = lung injury score.

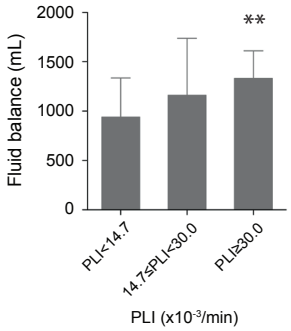


**Figure 3 – Fluid balance in the different PLI subcategories.** PLI<14.7x$10^{-3}$/min represents non-ALI patients, 14.7x$10^{-3}$/min≤PLI<30x$10^{-3}$/min ALI patients, and PLI≥30x$10^{-3}$/min ARDS patients. Error bars represent +/- standard deviation. ** = P<0.01 compared to PLI<14.7x$10^{-3}$/min calculated by one-way analysis of variance with Tukey post-hoc analysis. ALI = acute lung injury; ARDS = acute respiratory distress syndrome; PLI = pulmonary leak index.

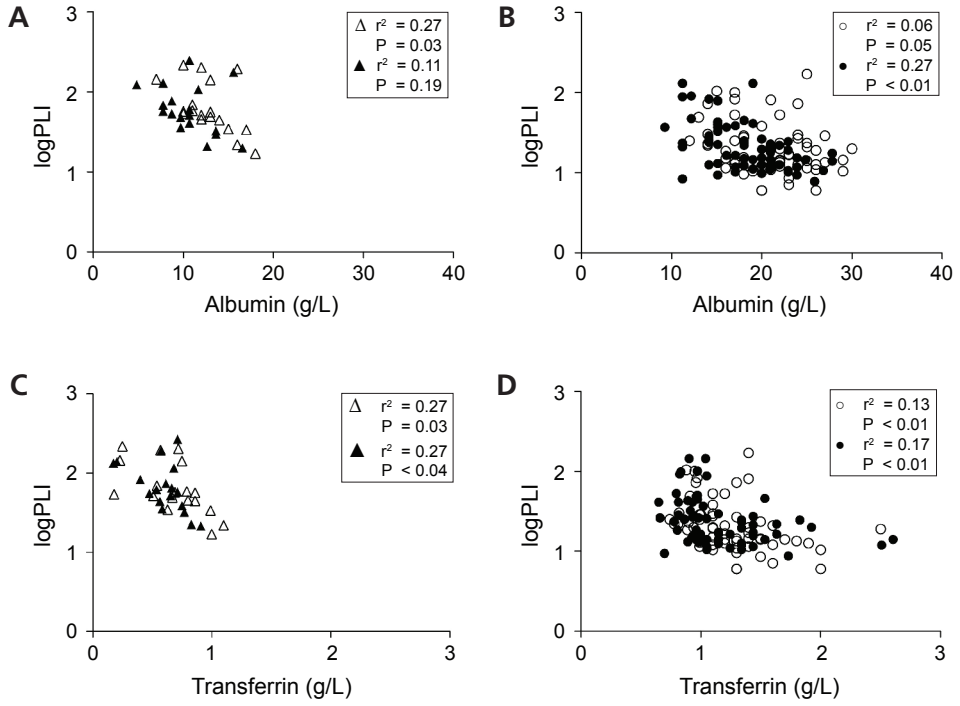


**Figure 4 – Concentrations of albumin (A,B) and transferrin (C,D) plotted against logarithmically transformed PLI for septic patients (A,C) and non-septic patients (B,D).** Open symbols represent measurements before fluid loading, closed symbols represent measurements after fluid loading. Coefficients of determination ($r^2$) represent partial correlation coefficients adjusted for APACHE II score. PLI = pulmonary leak index.



### Predictive values

Figure 5 shows the ROC of albumin and transferrin for PLI $\geq 30 \times 10^{-3}$/min and for ARDS according to the AECC criteria and the LIS before fluid loading. Albumin and transferrin levels had a high sensitivity, but moderate specificity for predicting a PLI $\geq 30 \times 10^{-3}$/min and ARDS according to the AECC criteria and the LIS (Table 3). After fluid loading, optimal cut-off levels were slightly lower than before, as a result of hemodilution. Since the cut-off values at the best combination of sensitivity and specificity were far below normal protein concentrations, predictive values for a cut-off value closer to the reference range (24.5 g/L for albumin, 1.55 g/L for transferrin) were calculated (Table 4). It is shown that normal albumin and transferrin values virtually exclude ARDS in this population. Albumin or transferrin levels further contributed to AECC criteria and the LIS in predicting an elevated PLI; adding hypoalbuminemia (<17.5 g/L), hypotransferrinemia (<0.98 g/L) or both as criterion to the AECC criteria and the LIS resulted in higher predictive values for elevated vascular permeability than the AECC criteria and LIS alone (Table 5).

**Table 3 – Predictive values for acute respiratory distress syndrome**

| | | Before fluid loading | | | | After fluid loading | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | AUC of ROC | Best cut-off | Sens/Spec | PPV/NPV | AUC of ROC | Best cut-off | Sens/Spec | PPV/NPV |
| PLI | Albumin (g/L) | 0.85 (P<0.001) | 17.5 | 85/75 | 60/92 | 0.89 (P<0.001) | 16.5 | 87/78 | 73/90 |
| (≥30x10$^{-3}$/min) | Transferrin (g/L) | 0.85 (P<0.001) | 0.98 | 81/75 | 59/90 | 0.86 (P<0.001) | 0.93 | 87/71 | 67/89 |
| | Total protein (g/L) | 0.57(P=0.31) | - | - | - | 0.72 (P=0.001) | 40.5 | 76/67 | 61/81 |
| | Fluid balance (mL) | 0.69 (P<0.001) | 1205 | 65/62 | 43/80 | | | | |
| AECC (ARDS) | Albumin (g/L) | 0.80 (P=0.001) | 17.5 | 79/64 | 31/94 | 0.84 (P=0.001) | 15.5 | 86/64 | 33/96 |
| | Transferrin (g/L) | 0.78 (P=0.001) | 1.05 | 93/65 | 35/98 | 0.81 (P=0.002) | 0.83 | 86/78 | 44/96 |
| | Total protein (g/L) | 0.45 (P=0.57) | - | - | - | 0.52 (P=0.82) | - | - | - |
| | Fluid balance (mL) | 0.54 (P=0.61) | - | - | - | | | | |
| LIS (≥2.5) | Albumin (g/L) | 0.86 (P<0.001) | 17.5 | 85/64 | 31/96 | 0.80 (P=0.001) | 15.5 | 85/64 | 31/96 |
| | Transferrin (g/L) | 0.82 (P<0.001) | 1.05 | 85/63 | 30/96 | 0.78 (P=0.002) | 0.83 | 77/77 | 39/95 |
| | Total protein (g/L) | 0.52 (P=0.78) | - | - | - | 0.49 (P=0.899) | - | - | - |
| | Fluid balance (mL) | 0.57 (P=0.46) | - | - | - | | | | |

Predictive values of albumin, transferrin, total protein and fluid balance for ARDS according to the different diagnostic definitions. Cut-off values are chosen with the best combination of sensitivity and specificity. Sensitivity, specificity, positive and negative predictive values in %. AECC = American European consensus conference; AUC = area under the curve; LIS = lung injury score; NPV = negative predictive value; PLI = pulmonary leak index; PPV = positive predictive value; ROC = receiver operating curve; Sens = sensitivity; Spec = specificity.

**Table 4 – Predictive values for acute respiratory distress syndrome at higher cut-off values**

| | | Before fluid loading | | After fluid loading | |
|---|---|---|---|---|---|
| | | Sens/Spec | PPV/NPV | Sens/Spec | PPV/NPV |
| PLI | Albumin (g/L) | 96/24 | 38/93 | 100/10 | 43/100 |
| | Transferrin (g/L) | 100/6 | 37/100 | 100/14 | 44/100 |
| AECC (ARDS) | Albumin (g/L) | 93/20 | 19/93 | 100/7 | 18/100 |
| | Transferrin (g/L) | 93/13 | 18/90 | 100/10 | 19/100 |
| LIS (≥2.5) | Albumin (g/L) | 100/21 | 20/100 | 100/6 | 17/100 |
| | Transferrin (g/L) | 92/13 | 17/90 | 100/10 | 17/100 |

Predictive values of albumin and transferrin for ARDS according to the different diagnostic definitions at cut-off values of 24.5g/L for albumin and 1.55g/L for transferrin. Sensitivity, specificity, positive and negative predictive values in %. AECC = American European consensus conference; LIS = lung injury score; NPV = negative predictive value; PLI = pulmonary leak index; PPV = positive predictive value; Sens = sensitivity; Spec = specificity.

**Table 5 – Predictive values of AECC criteria and the LIS with and without plasma protein levels for an elevated PLI**

| | Sensitivity | Specificity | P-value (Fisher's exact test) | PPV/NPV | Likelihood Ratio for PLI>30.0x10$^{-3}$/min |
|---|---|---|---|---|---|
| AECC | 72 (49/68) | 62 (8/13) | 0.02 | 48/82 | 1.9 |
| AECC + Alb (<17.5g/L) | 73 (51/70) | 73 (8/11) | 0.005 | 57/85 | 2.7 |
| AECC + Tf (<0.98g/L) | 71 (50/70) | 78 (7/9) | 0.006 | 61/85 | 3.2 |
| AECC + Alb (<17.5g/L) + Tf (<0.98g/L) | 71 (50/70) | 78 (7/9) | 0.006 | 61/85 | 3.2 |
| LIS | 74 (50/68) | 69 (9/13) | 0.005 | 54/84 | 2.4 |
| LIS + Alb (<17.5g/L) | 74 (52/70) | 82 (9/11) | 0.001 | 67/86 | 4.1 |
| LIS + Tf (<0.98g/L) | 74 (52/70) | 100 (9/9) | <0.001 | 100/89 | ∞ |
| LIS + Alb (<17.5g/L) + Tf (<0.98g/L) | 74 (52/70) | 100 (9/9) | <0.001 | 100/89 | ∞ |

Predictive values of AECC criteria and the LIS with or without plasma albumin and transferrin concentrations for a PLI>30.0x10$^{-3}$/min before fluid loading. Sensitivity, specificity, positive and negative predictive values in %. AECC = American European Consensus Conference; Alb = albumin; LIS = lung injury score; NPV = negative predictive value; PLI = pulmonary leak index; PPV = positive predictive value; Tf = transferrin.

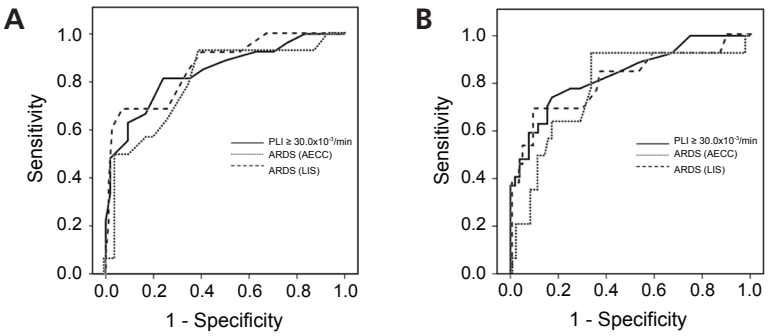


**Figure 5 – Predictive values of albumin and transferrin for ALI/ARDS.** ROCs of albumin (A) and transferrin (B) for a PLI≥30.0x10$^{-3}$/min (solid, AUC=0.85, P<0.001 for albumin, and AUC=0.85, P<0.001 for transferrin), for ARDS according to the AECC criteria (square dots, AUC=0.79, P=0.001 for albumin, and AUC=0.78, P=0.001 for transferrin), and for ARDS according to the LIS (dashed, AUC=0.86, P<0.001 for albumin, and AUC=0.82, P<0.001 for transferrin) before fluid loading. AUC = area under the curve; ARDS = acute respiratory distress syndrome; PLI = pulmonary leak index; ROC = receiver operating curve.

## DISCUSSION

We found a direct relation between decreased plasma albumin and transferrin levels and increased pulmonary vascular permeability and lung injury in critically ill, non-hypervolemic patients, independent of underlying disease and fluid status. Protein levels had a high sensitivity and negative predictive value for increased pulmonary vascular permeability and ARDS, and were of additive diagnostic value to the AECC criteria and the LIS.

Taking PLI and LIS as continuous parameters of pulmonary vascular permeability and lung injury, respectively, we observed that decreased plasma protein levels parallel the severity of ALI/ARDS. This may reflect cause-effect relationships, rather than diminished production in the course of severe disease only, following an increase in transcapillary protein flux [25,26]. Decreased protein levels themselves may contribute to fluid extravasation by lowering colloid osmotic pressure [27,28] and increasing transendothelial permeability [29]. Although low transferrin levels decrease oxidative stress and mortality in animal models [30,31], previous human studies suggest that low plasma levels of albumin [12,13], transferrin [12,32,33] and total protein [11] predict development and mortality of ARDS in a variety of populations. Using the PLI method to differentiate permeability from hydrostatic pulmonary edema in a small series of critically ill patients having either type of edema or a combined form, we already demonstrated that circulating protein levels were of help in the differentiation [12]. In addition to previous studies, the current study strongly suggests that increased permeability underlies relations between low plasma protein levels and lung

injury, relatively independent of the type of lung injury, underlying disease, and fluid status. The high sensitivity and negative predictive value found in this study indicate that normal protein levels virtually exclude ARDS. Adding plasma protein concentrations as an extra criterion to the AECC criteria and the LIS resulted in higher predictive values for elevated pulmonary vascular permeability (Table 5). These results indicate that augmenting clinical definitions like the AECC criteria and the LIS with the supposed indicators of increased permeability, i.e. hypoalbuminemia and/or hypotransferrinemia, further increases diagnostic accuracy. In fact, the AECC criteria and the LIS correlated moderately with the PLI (Table 5), indicating that clinical definitions of ARDS poorly predict elevated pulmonary vascular permeability. These findings are in line with previous studies, which demonstrated that clinical definitions inadequately reflect pathological changes [5] or pathophysiological characteristics [6]. Despite the differences in ARDS frequency between diagnostic methods (Table 2), albumin and transferrin levels had a comparable sensitivity and negative predictive value for pulmonary vascular permeability and lung injury. The low specificity of albumin and transferrin levels for permeability and lung injury in our study may result from factors other than endothelial permeability determining plasma protein levels in critically ill patients [34]. As opposed to ARDS versus non-ARDS, small differences in albumin and transferrin levels between ALI and non-ALI patients (Figures 2&3 and Supplementary Figure 1) precluded separating these conditions. The predictive values of plasma albumin and transferrin alone in this study resemble those of the edema fluid to plasma protein ratios reported before, but these can only be obtained in the presence of florid edema and with help of deep tracheal suctioning or bronchoalveolar lavage [14,21]. Moreover, protein contents of edema fluid may change during reabsorption, independently of permeability [20]. No predictive values of total protein levels were found in this study, in spite of those of albumin and transferrin levels. This may be explained by albumin and transferrin levels constituting highly varying fractions of total proteins (from 18 to 63% for albumin in this study) and higher permeability for albumin and transferrin than for high molecular weight proteins and immunoglobulins [34]. The strong correlation between albumin and transferrin indicates that these proteins are almost interchangeable in this population, rendering albumin measurement a feasible substitute for the more arduous transferrin assay.

The PLI positively related to the fluid balance, irrespective of underlying disease and fluid status. Although predictive values for ARDS were low, this relation confirms the previously suggested marker role of fluid balance in pulmonary vascular permeability [2,17,35]. We only included patients without hypervolemia to exclude patients with hydrostatic pulmonary edema and to allow for fluid loading. Indeed, hydrostatic or cardiogenic edema is characterized by transudation of protein-low fluid and increased plasma albumin levels, thereby potentially increasing the value of plasma proteins in discriminating hydrostatic from permeability edema [12,20-22].

This study contains several limitations. First, the influence of ventilator settings on pulmonary vascular permeability was not addressed. However, tidal volumes exceeded 10 mL/kg in only six patients, showing minimal risk for volutrauma in this population. Second, lung water content was not evaluated in this study. Nonetheless, protein permeability does not necessarily imply pulmonary edema, for instance if hydrostatic driving pressure is low [19]. Third, overall severity of disease and mortality in the present study population were low, while causes for ARDS were diverse. These are relevant factors, but we primarily targeted for a population homogenous with respect to fluid status, to exclude fluid status as a confounder. In addition, the current population is considered representative for our overall critical care patient population, broadening the applicability of the results.

In conclusion, decreased plasma albumin and transferrin levels parallel increased pulmonary vascular permeability and lung injury in critically ill patients, irrespective of underlying disease and fluid status. Since normal levels have a high sensitivity and negative predictive value and help to exclude ARDS, hypoalbuminemia and hypotransferrinemia increase the diagnostic accuracy of the AECC criteria and LIS for elevated pulmonary vascular permeability. A larger number of patients is required to validate the best cut off values, which may help future refining of the diagnosis of ALI/ARDS.

## ACKNOWLEDGEMENTS

We thank J. Verheij for help in collecting the data, and M.W. Heymans for review of the statistical analysis. This work was supported by B.Braun, Melsungen, Germany. There are no conflicts of interest to declare. JA was supported by The Netherlands Heart Foundation (The Hague, grant 2003T3201). MvdH was supported by the European Society of Intensive Care Medicine (ECCRN 2007 Levi Montalcini Biomedical Sciences Award). GPvNA was supported by The Netherlands Heart Foundation (The Hague, grant 2003T032).

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# Chapter 6
# -
# Supplementary Information

**SupplementaryTable 1 – Patient characteristics and study variables in the different AECC categories**

| | Before fluid loading | | | After fluid loading | | |
|---|---|---|---|---|---|---|
| | **Non-ALI (n=48)** | **ALI (n=21)** | **ARDS (n=14)** | **Non-ALI (n=54)** | **ALI (n=15)** | **ARDS (n=14)** |
| PLI (x$10^{-3}$/min) | 20.0 (13.5-30.0) | 16.5 (12.3-55.5) | 44.5 (21.8-64.0)† | 20.0 (13.8-36.8) | 23.5 (14.5-126.0) | 45.5 (32.0-65.9)* |
| PLI ≥30x$10^{-3}$/min | 11 (23) | 8 (38) | 8 (57)* | 15 (28) | 7 (47) | 11 (79)* |
| LIS | 0.75 (0.56-1.00) | 1.75 (1.25-2.38) | 2.50 (2.00-3.00)** | 1.00 (0.69-1.25) | 1.50 (1.25-2.25) | 2.88 (2.25-3.06)** |
| LIS ≥2.5 | 0 (0) | 5 (24) | 8 (57)** | 0 (0) | 3 (20) | 10 (71)** |
| $PaO_2/FiO_2$ ratio | 350 ± 104 | 250 ± 27 | 153 ± 36** | 359 ± 97 | 256 ± 27 | 144 ± 38** |
| Sepsis | 2 (4) | 7 (33) | 9 (64)** | | | |
| APACHE II | 9 ± 4 | 10 ± 4 | 12 ± 5* | | | |
| Mortality | 2 (4) | 5 (24) | 4 (29)* | | | |

Patients characteristics and study variables for patients with non-ALI, ALI or ARDS according to the American European Consensus Conference criteria. Mean±SD, median (interquartile range), or number (percentage), where appropriate. † P<0.08, * P<0.05,** P<0.01, calculated by one-way analysis of variance with Tukey post-hoc analysis or Chi-square test, where appropriate. ALI = acute lung injury; APACHE = acute physiology and chronic health evaluation; ARDS = acute respiratory distress syndrome; $FiO_2$ = fraction of inspired oxygen; LIS = lung injury score; $PaO_2$ = partial pressure of arterial oxygen; PLI = pulmonary leak index.

**Supplementary Table 2 - Patient characteristics and study variables in patients with primary and secondary acute respiratory distress syndrome**

| | Before fluid loading | | After fluid loading | |
|---|---|---|---|---|
| | Primary ARDS (n=5) | **Secundary ARDS (n=9)** | **Primary ARDS (n=5)** | **Secundary ARDS (n=9)** |
| PLI ($x10^{-3}$/min) | 58.5 (48.0-136.8) | 27.0 (17.4-42.9)* | 58.5 (44.0-75.0) | 39.0 (27.3-67.3) |
| PLI≥30$x10^{-3}$/min | 5 (100) | 3 (38)* | 5 (100) | 6 (67) |
| LIS | 3.00 (2.25-3.25) | 2.00 (1.75-2.75) | 3.00 (2.50-3.25) | 2.75 (2.13-3.00) |
| LIS≥2.5 | 4 (80) | 4 (44) | 4 (80) | 6 (67) |
| $PaO_2/FiO_2$ ratio | 153 ± 31 | 152 ± 41 | 144 ± 50 | 144 ± 33 |
| Albumin (g/L) | 12 (11-13) | 17 (12-18)† | 10 (9-11) | 14 (9-17) |
| Transferrin (g/L) | 0.72 (0.53-0.83) | 0.99 (0.77-1.00)† | 0.57 (0.44-0.74) | 0.76 (0.61-0.96) |
| Total protein (g/L) | 46 (45-50) | 42 (38-52) | 37 (36-42) | 39 (31-45) |
| Fluid balance | 1661 (1033-1740) | 865 (795-1560) | | |

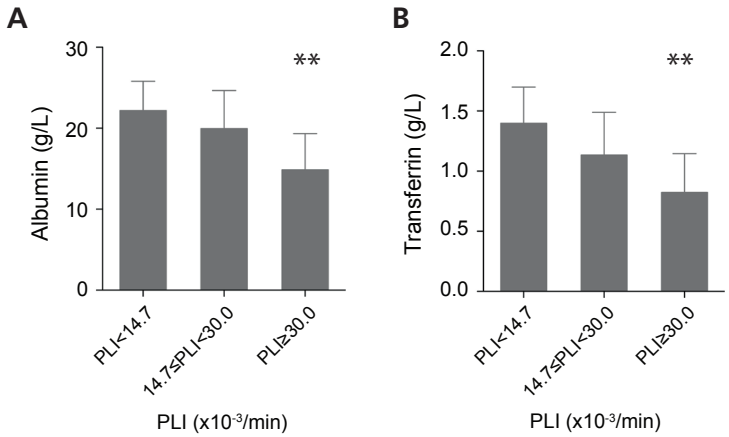
Study variables in patients with ARDS according to the AECC criteria divided into primary (pneumonia, sepsis with pulmonary origin) and secondary (sepsis, trauma, surgery) ARDS. Mean±SD, median (interquartile range) or number (percentage), where appropriate. † P<0.07, * P<0.05, calculated by Mann-Whitney-U test or Chi-square test, where appropriate. ARDS = acute respiratory distress syndrome; $FiO_2$ = fraction of inspired oxygen; LIS = lung injury score; $PaO_2$ = partial pressure of arterial oxygen; PLI = pulmonary leak index.

**Supplementary Table 3 - Study variables in septic patients with direct versus indirect lung injury**

| | Before fluid loading | | After fluid loading | |
|---|---|---|---|---|
| | **Direct lung injury (n=9)** | **Indirect lung injury (n=9)** | **Direct lung injury (n=9)** | **Indirect lung injury (n=9)** |
| PLI ($x10^{-3}$/min) | 57.5 (46.8-132.3) | 53.5 (27.8-142.8) | 61.5 (44.0-148.3) | 69.5 (28.5-134.8) |
| LIS | 2.50 (1.75-3.00) | 2.25 (1.25-2.63) | 2.75 (1.88-3.13) | 2.00 (1.38-2.88) |
| $PaO_2/FiO_2$ ratio | 186 ± 46 | 225 ± 45 | 183 ± 60 | 202 ± 61 |
| APACHE II | 15 ± 6 | 12 ± 5 | | |
| Albumin (g/L) | 13 ± 2 | 13 ± 4 | 11 ± 3 | 11 ± 4 |
| Transferrin (g/L) | 0.67 (0.56-0.76) | 0.80 (0.24-1.00) | 0.58 (0.51-0.72) | 0.67 (0.41-0.81) |
| Total protein (g/L) | 46 ± 3 | 46 ± 7 | 40 ± 6 | 41 ± 7 |
| Fluid Balance (mL) | 1450 ± 288 | 1217 ± 279 | | |

Comparison of study variables in septic patients with direct (sepsis from pulmonary origin) versus indirect (sepsis from extrapulmonary origin) lung injury. Mean±SD or median (interquartile range), where appropriate. No statistical difference between the groups (direct versus indirect lung injury) could be detected, as calculated with Student's t-test or Whitney-U test, where appropriate. APACHE = Acute Physiology And Chronic Health Evaluation; $FiO_2$ = fraction of inspired oxygen; LIS = lung injury score; $PaO_2$ = partial pressure of arterial oxygen; PLI = pulmonary leak index.

## SUPPLEMENTARY FIGURE




**Supplementary Figure 1 – Concentrations of albumin (A) and transferrin (B) in the different PLI subcategories before fluid loading.** PLI<14.7x10-3/min represents non-ALI patients, 14.7x10-3/min≤PLI<30x10-3/min ALI patients, and PLI≥30x10-3/min ARDS patients. Error bars represent +/- standard deviation (A) or interquartile range (B). ** = P<0.01 compared to PLI<14.7x10-3/min, ## = P<0.01 compared to 14.7x10-3/min≤PLI<30x10-3/min calculated by one-way analysis of variance with Tukey post-hoc analysis.. ALI = acute lung injury; ARDS = acute respiratory distress syndrome; PLI = pulmonary leak index.