ARTICLE

Extracellular Matrix Turnover, Angiogenesis and Endothelial Function in Acute Lung Injury: Relation to Pulmonary Dysfunction and Outcome

Naglaa K Idriss[1], Sherif Sayed[2] and Hayam G Sayyed[3]

[1]Department of Medical Biochemistry, [2]Department of Anaesthesiology and [3]Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

Corresponding author: Dr. Hayam G Sayyed Email: hayam_said3@yahoo.com

Published: 01 September 2012
Ibnosina J Med BS 2012,4(5):170-182
Received: 09 March 2012
Accepted: 09 July 2012
This article is available from: http://www.ijmbs.org

Abstract

Background: Acute lung injury (ALI) is a syndrome with a diagnostic criteria based on hypoxemia and a classical radiological appearance, with acute respiratory distress syndrome at the severe end of the disease. Occurrence of rupture of the basement membranes and interstitial matrix remodeling during ALI. Matrix metalloproteinases (MMPs) participate in tissue remodeling related with pathological conditions such as acute lung injury. We hypothesized the interrelationships between extracellular matrix (ECM) turnover as MMP-9 and indicator of angiogenesis such as angiopoietin-2 (Ang-2) as well as plasma von Willebrand factor (vWF) and their correlation with arterial partial pressure of oxygen (PaO2), oxygen saturation (SaO2) and mortality in ALI/ARDS. **Methods**: Eighty eight mechanically ventilated patients (68 male, mean (SD) age 61 (10) years) were compared to 40 healthy controls (36 male, mean (SD) age 57 (10)). All biomarkers were measured by Enzyme linked immunosorbent assay (ELISA). Oxygenation, body temperature, leucocytes, and platelet counts were noted. **Results:** Plasma levels of all biomarkers were significantly different, among ALI/ARDS subjects ($p<0.001$). They were inversely related to PaO2 and SaO2 and positively related to mortality. In addition, increased MMP-9, Ang-2 and vWF levels were interrelated on the first day of admission. **Conclusions**: The observed diversity in plasma levels of MMP-9, Ang-2 and vWF in ALI/ARDS patients revealed the activity and severity of the disease, shedding more light onto the pathogenesis and/or presentation of ARDS.

Introduction

Acute lung injury (ALI) syndrome and acute respiratory distress syndrome (ARDS) are defined by radiographic and physiological changes that characterize patients with acute lung failure (36). American-European Consensus Conference (AECC), defines ALI as "a clinical syndrome of severe dyspnea of rapid onset, hypoxemia, and diffuse pulmonary infiltrates leading to respiratory failure, a ratio of arterial

oxygen tension to fraction of inspired oxygen (PaO2/FiO2) of < 300, in the absence of cardiac failure" (4). Whereas acute respiratory distress syndrome (ARDS) is a sequence of the ALI, characterized by acute onset of tachypnea and hypoxemia, bilateral pulmonary infiltrates on chest radiography and reduced lung compliance, PaO2/FiO2 of < 200 mmHg, in completely healthy young individuals (13).

The pathogenesis of ARDS remains unknown. The cause of disease transition from the acute inflammatory stage to the fibrotic stage is undefined, and there is fibrosis and immune impairment from the initial stage of ARDS (21). Pro-inflammatory mediators, such as tumour necrosis factor-α, interleukin-6, nitric oxide and prostaglandin E2, play a crucial role in the progression of the disease (21,26). This inflammatory response resulting in an increase in alveolar-capillary permeability leads to alveolar edema and leukocyte infiltration, impaired surfactant function with alveolar collapse, and results in a severe impairment of gas exchange (21).

Matrix metalloproteinases (MMPs) represent a family of structurally related zinc-dependent enzymes, which are capable of debasing all extracellular matrix components of the basement membrane (20,23,25). MMPs are secreted in an inactive form called proMMP. Subsequent activation of proMMP occurs in response to a variety of stimuli such as growth factors, cytokines and chemokines, and endogenous inflammatory mediators (34). Based on their structure and function, they are classified as collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs (49). MMP-9 is a constituent of gelatinase subgroup (11). MMP-9 is created by several lung cell types. These include macrophages, Clara cells, alveolar type II cells, smooth muscle cells, fibroblasts and bronchial epithelial cells. Many studies have implicated the role of MMP-9 in the injurious process of ALI (2), while others have shown the protective role of MMP-9 in different models of ALI (6,27,37,47).

Angiopoietins (Angs) are growth factors that enhance angiogenesis. Four members of Angs have been identified including Ang-1, Ang-2, Ang-3 and Ang-4, which act by binding to the endothelium-specific receptor tyrosine kinase called Ties (38,48). Ang-2 acts as a "trigger" of vascular remodeling as it "destabilizes" the vessel to be sensitive to other angiogenic growth factors (24,38). Raised plasma angiopitein-2 level is present in ARDS patients (10,17).

Additionally, von Willebrand factor (vWF) is a plasma glycoprotein that mediates platelet adhesion to an injured endothelial surface and carries clotting factor VIII in the circulation to protect it from renal clearance (44,45). It is synthesized by endothelial cells and megakaryocytes (8). It is documented to be a circulating marker of endothelial damage/dysfunction (39).

The aims of this study were (1) to determine the interrelationship between plasma MMP-9, Ang-2 and vWF and their correlation with arterial partial pressure of oxygen and oxygen saturation, and (2) to study the predictive value of MMP-9, Ang-2 and vWF for ICU mortality.

Patients and Methods

Settings and Protocols

This study was carried out in the intensive care unit (ICU) of Assiut University Hospital, Egypt, over an 18-month period. The protocol was approved by the local ethics committee and written informed consent was obtained from all patients.

A total of 88 consecutive mechanically ventilated patients were included in the present study. Ages ranged from 40 to 65 years. Of the 88 patients, 42 met the American-Europe Consensus Definition for ALI: PaO2/FIO2 < 300 mmHg regardless of the level of positive end expiratory pressure (PEEP), bilateral pulmonary infiltrated visible on an anterior/posterior chest radiograph with no clinical evidence of left-sided heart failure. The remaining patients (46) were suffering from ARDS with PaO2/FIO2 < 200 mmHg. In addition, study indices were compared with 40 healthy control subjects who were either members of the hospital staff or patient relatives. Each patient and control was subjected to full clinical assessment including (a) demographic information (b) clinical conditions associated with development of ARDS, (c) routine laboratory measurements, including kidney function tests, blood sugar level, plain X-ray, lab panel and arterial blood gases. Patients with the following criteria were excluded: cancer, concurrent systemic infection, inflammatory bowel disease, hepatic impairment, renal failure, connective tissue disease, or those on hormone replacement therapy. Healthy controls were healthy by virtue of careful clinical history and examination, with normal basic blood tests and ECG.

Laboratory methods

Patients admitted to the intensive care unit (ICU) of Assiut University Hospital, were screened daily. Patients (or their families) provided informed consent before they were enrolled. Initial arterial blood gases and hemodynamic data were obtained from each participant (10 cc fasting blood samples obtained by venipuncture). On admission within 24 hours, and after one week, 10 cc of venous blood was again obtained from the antecubital vein into citrated tubes then centrifuged at 3,000 rpm (1,000 *g*) for 20 min at 4°C. All aliquots were stored at –70°C to allow batch analysis of

biomarkers. Plasma biomarkers were measured by enzyme-linked immunosorbent assays (ELISA) using commercial reagents with strict adherence to the manufacturers' guidelines.

Statistical analysis

Following application of the Shapiro-Wilkes test to determine normal distribution, non-categorical data distributed normally are expressed as mean (standard deviation) and data distributed non-normally are expressed as median (interquartile range). Mann-Whitney test or ANOVA test was used for comparison between groups. Demographic and severity score data were analyzed by two factor analysis of variance. Correlations were required by Spearman's rank method. A probability value of less than 0.05 was considered statistically significant. The sample size estimate was modeled in Graph pad prism 4.

Results

Laboratory data

The routine laboratory data of study subjects were summarized in Table 1. ALI/ARDS patients were grouped into categories by survivor or non-survivor. All laboratory data showed no significant difference between ALI/ARDS patients and healthy controls except blood sugar, blood urea and serum creatinine were significantly higher in non-survivor ALI/ARDS patients compared to healthy controls and survivor ALI/ARDS patients.

Arterial blood gas analysis

Arterial blood gas analysis were summarized in Table 2. All indices showed no significant difference between ALI/ARDS patients and healthy controls except PaO2 and SaO2 were significantly lower in both survivor and non-survivor ALI/ARDS patients compared to healthy controls

Table 1. Routine laboratory data in patients with acute lung injury/acute respiratory distress syndrome (ALI/ARDS) compared to healthy controls. Values are mean (SD) or mean (interquartile range).

Indices	Healthy controls (n = 40)	ALI/ARDS Patients (n = 88)	
		Survivor (n = 59)	Non-survivor (n = 29)
Blood sugar (mg/dl)	4.6 ± 0.5	4.5 ± 0.4	6.6 ± 0.3***###
Blood urea (mmol/L)	0.0 ± 0.2	4.1 ± 0.3	7.3 ± 1.1***###
Serum creatinine (μmol/L)	77.8 ± 2.4	70.9 ± 5.2	156.0 ± 28.8***###
WBCs/cmm	9.22 ± 0.52	9.25 ± 0.74	9.54 ± 0.83
RBCs/cmm	4.70 ± 0.29	4.54 ± 0.22	4.63 ±0.64
Hb (gm/dl)	11.30 ± 0.78	11.08 ± 0.29	11.16 ± 0.40
HCT %	38.30 30.10 - 39.20	36.30 25.10 - 49.80	37.20 26.20 – 45.50
MCV (fL)	88.20 73.20 - 99.20	89.20 78.80 - 81.00	88.60 75.60 – 88.40
MCH (pg)	28.60 26.10 - 38.90	29.90 20.30 - 38.90	28.90 24.20 – 38.10
MCHC (g/L)	28.70 24.20 - 32.40	29.10 23.70 - 34.30	29.00 24.00 – 33.10
Platelet/cmm	292 235 - 640	268 135 – 631	280 200 – 620

P value by ANOVA, ***: $p < 0.001$ (Tukey's test) compared to healthy controls, ###: $p < 0.001$ compared to survivor ALI/ARDS. Hb: hemoglobin; HCT: hematocrit value; RBCs: red blood cells; MCV: mean corpuscular volume; WBCs: white blood cells; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

Table 2. Arterial blood gas analysis in acute lung injury/acute respiratory distress syndrome (ALI/ARDS) patients on admission (1st day of admission) compared to healthy controls. Values are either median (interquartile range) or mean (Standard Deviation).

Indices	Healthy controls (n = 40)	ALI/ARDS Patients (n = 88)	
		Survivor (n = 59)	Non-survivor (n = 29)
pH	7.4 7.1–7.6	7.4 7.1 – 7.5	7.4 7.1 – 7.4
$PaCO_2$ (mmHg)	34.9 25.9– 48.2	36.9 27.5– 57.1	37.2 25.9 – 57.3
PaO_2 (mmHg)	92.4 58.2– 21.2	68.4*** 49.3–89.5	65.5*** 46–84
HCO_3 (mmol/L)	25.7(1.0)	27.3(1.0)	26.8(1.0)
Base Excess (mmol/L)	4.3 (0.9)	4.5 (1.2)	4.3(1.0)
SaO_2%	98.0 79.0 – 99.2	92.8*** 79.2 – 97.0	90.5*** 79.0 – 95.5

P value by ANOVA, ***p < 0.001 (Tukey's test) compared to healthy controls
$PaCO_2$: partial pressure of carbon dioxide in the arterial blood; PaO_2: partial pressure of oxygen in arterial blood; HCO_3: bicarbonate ion; SaO_2: oxygen saturation.

(p < 0.001).

Plasma levels of MMP-9, Ang-2 and vWF on the day of admission

Plasma levels of MMP-9, Ang-2 and vWF on the day of admission were summarized in Table 3. Plasma level of MMP-9, Ang-2 and vWF were significantly higher in ALI/ARDS patients compared to healthy controls (582.00 ± 87.36 vs. 90.23 ± 17.94, p < 0.001, 297.30 ± 52.51 vs. 12.48 ± 3.26, p < 0.001, 367.40 ± 64.39 vs. 124.50 ±38.69, p < 0.001, respectively).

Correlation and linear regression of plasma biomarkers with PaO2 and SaO2

Correlation and linear regression of plasma levels of MMP-9, Ang-2 and vWF with PaO2 (A) and SaO2% (B) in ALI/ARDS patients on the first day of admission were summarized in Figures 1-3. Plasma levels of MMP-9 were negatively correlated with PaO2 and SaO2% in ALI/ARDS patients (– 0.75, p < 0.0001 and r = – 0.81, p < 0.0001). Also, plasma level of Ang-2 was negatively correlated with PaO2 and SaO2% in ALI/ARDS patients (r = – 0.68, p < 0.0001 and r = – 0.63, p < 0.0001). Plasma levels of vWF were negatively related with PaO2 and SaO2% in ALI/ARDS patients (r = – 0.76, p < 0.0001 and r = – 0.69, p < 0.001).

Association of plasma levels of MMP-9, Ang-2 and vWF with outcomes

Comparisons of plasma levels of MMP-9 (A), Ang-2 (B) and vWF (C) on first day and day seven between survivors and non-survivor patients with ALI/ARDS were shown in Figure 4. At the first day, plasma levels of MMP-9, Ang-2 and vWF were significantly higher in non-survivors than in survivors (635.8 ± 73.39 vs. 528.3 ± 64.37, p < 0.001, 345.2 ± 20.45 vs. 256.6 ± 18.87, p < 0.01 and 407.8 ± 55.44 vs. 327.1 ± 45.56, p < 0.001, respectively). At day seven, plasma levels of MMP-9, Ang-2 were significantly decreased in survivors (391.3 ± 69.57, p < 0.001, 163.1 ± 47.54, p < 0.01 and 259 ± 29.41, p < 0.05) while, in non-survivors this remained high (611.1 ± 76.48, 279.9 ± 60.66 and 395.6 ± 49.57, respectively).

The interrelation between MMP-9, Ang-2 and vWF levels on the day of admission

MMP-9 levels were positively related to Ang-2 and vWF

Table 3. Plasma levels of all biomarkers in all Cohort study on admission (1st day of admission).

Mean (SD)	Healthy Controls (n=40)	ALI/ARDS Patients (n=88)	p value
MMP-9 (ng/ml)	[a]90.2 (17.9)	582.0 (87.4)	$p < 0.001$
Ang-2 (ng/mL)	[a]12.5 (3.3)	297.3 (52.5)	$p < 0.001$
vWF (U/mL)	[a]124.5 (38.7)	367.4 (64.4)	$p < 0.001$

Data presented as mean (SD). MMP-9: matrix metalloproteinase-9; Ang-2: angiopiotein-2; vWF: von Willebrand factor. p value by Mann-Witney test. ALI/ARDS patients were significantly different compared to healthy controls. [a]: $p < 0.001$.

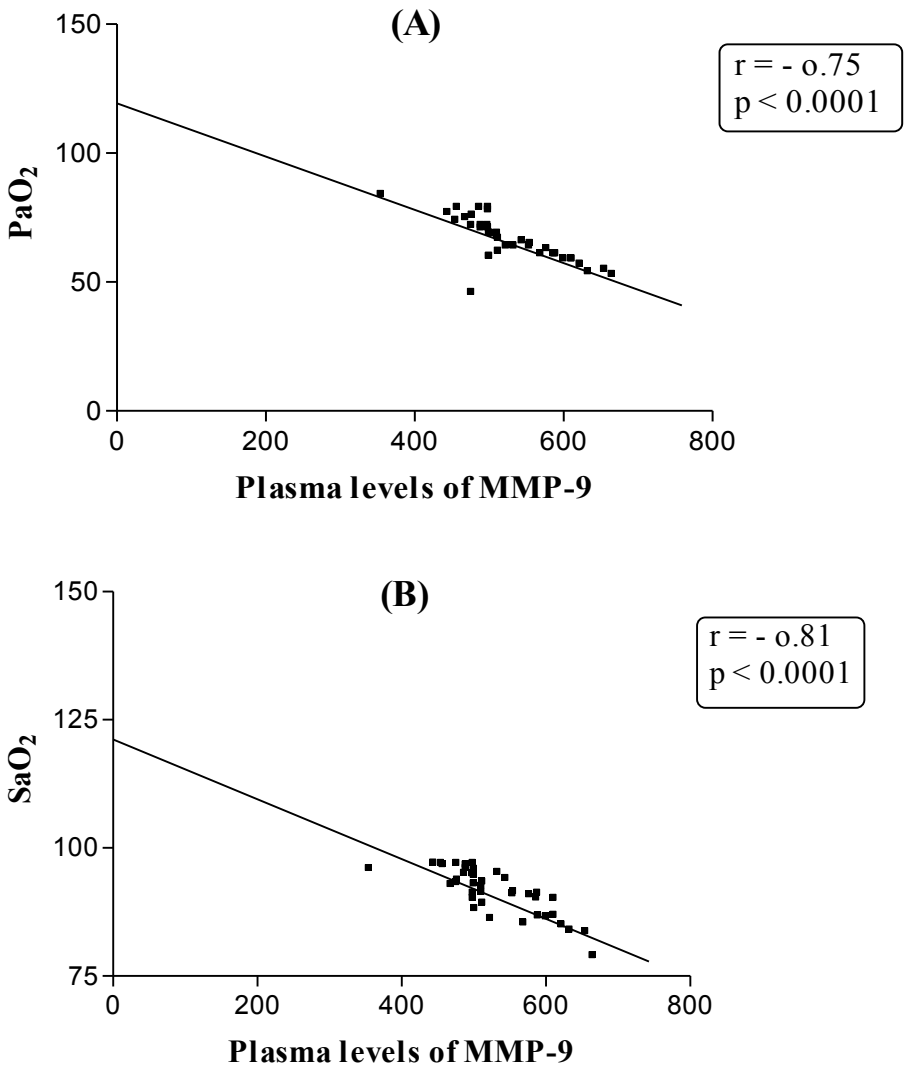

Figure 1. Correlation and linear regression of plasma level of matrix metalloproteinase-9 (MMP-9) with partial pressure of oxygen in arterial blood (PaO2) (A) and oxygen saturation (SaO2%) (B) in ALI/ARDS patients.

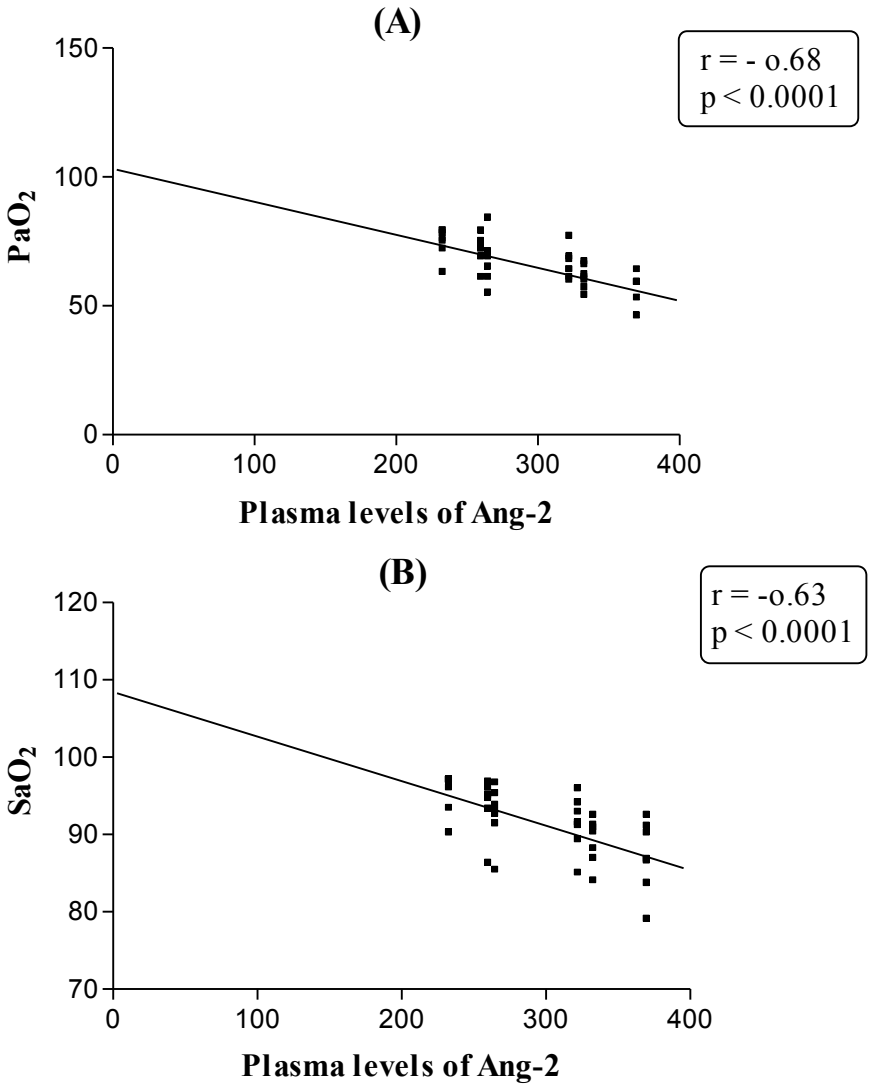

Figure 2. Correlation and linear regression of plasma level of angiopoitein-2 (Ang-2) with partial pressure of oxygen in arterial blood (PaO2) (A) and oxygen saturation (SaO2%) (B) in ALI/ARDS patients.

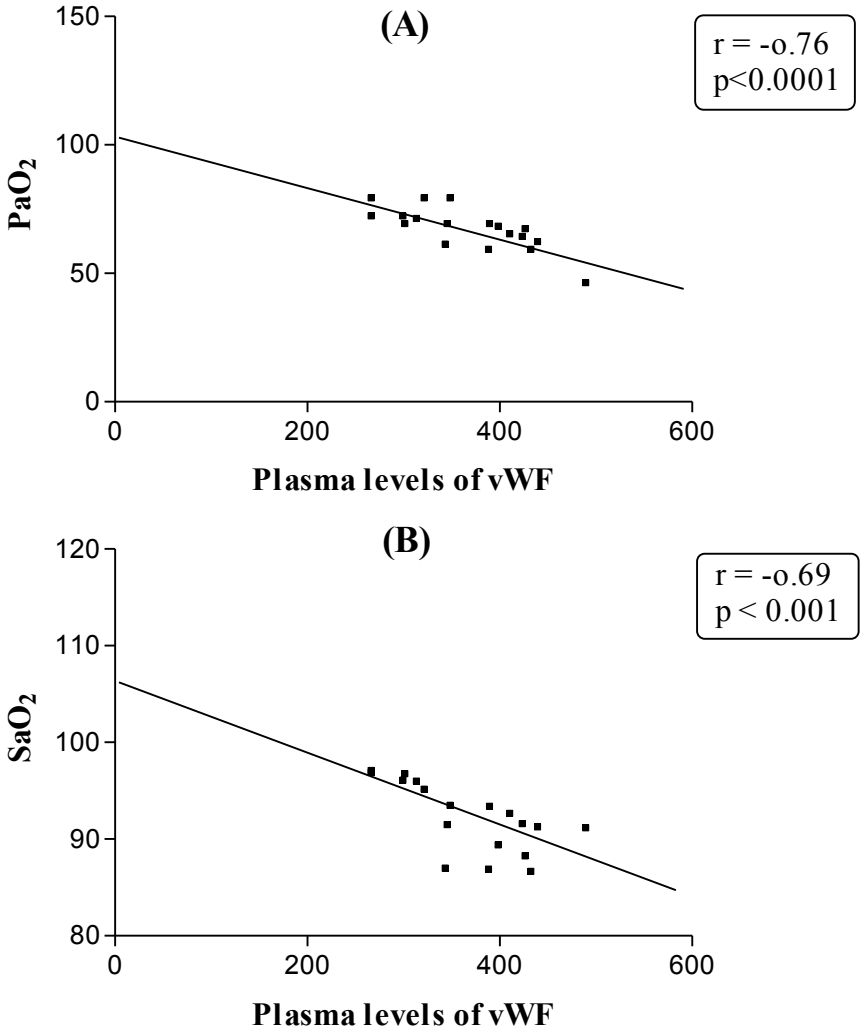

Figure 3. Correlation and linear regression of plasma level of von Willwbrand factor (vWF) with partial pressure of oxygen in arterial blood (PaO2) (A) and oxygen saturation (SaO2%) (B) in ALI/ARDS patients.

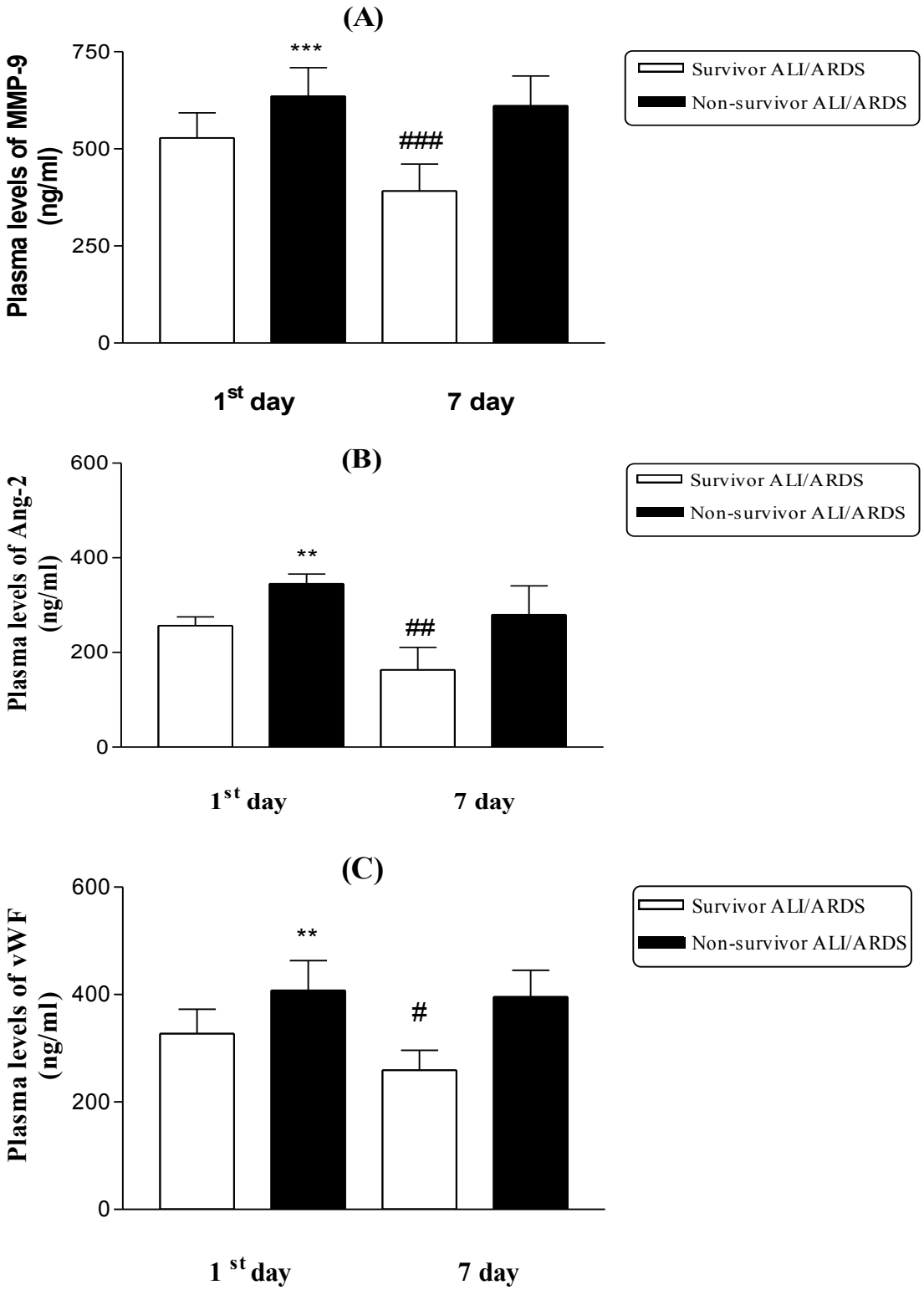

Figure 4. Comparison of plasma levels of MMP-9 (A), Ang-2 (B) and vWF (C) at the first day and day 7 between survivors and non-survivors patients with ALI/ARDS. p value by ANOVA test followed by Tukey test. At the first day plasma levels of MMP-9, Ang-2 and vWB factor were significantly higher in non-survivor than survivor ALI/ARDS patients. On day 7 plasma levels significantly decreased in survivors while, they did not change in non-survivors. ***: $p < 0.001$, $p < 0.01$ compared to survivor ALI/ARDS patients, ###: $p < 0.001$, $p < 0.01$ compared to the level at the first day.

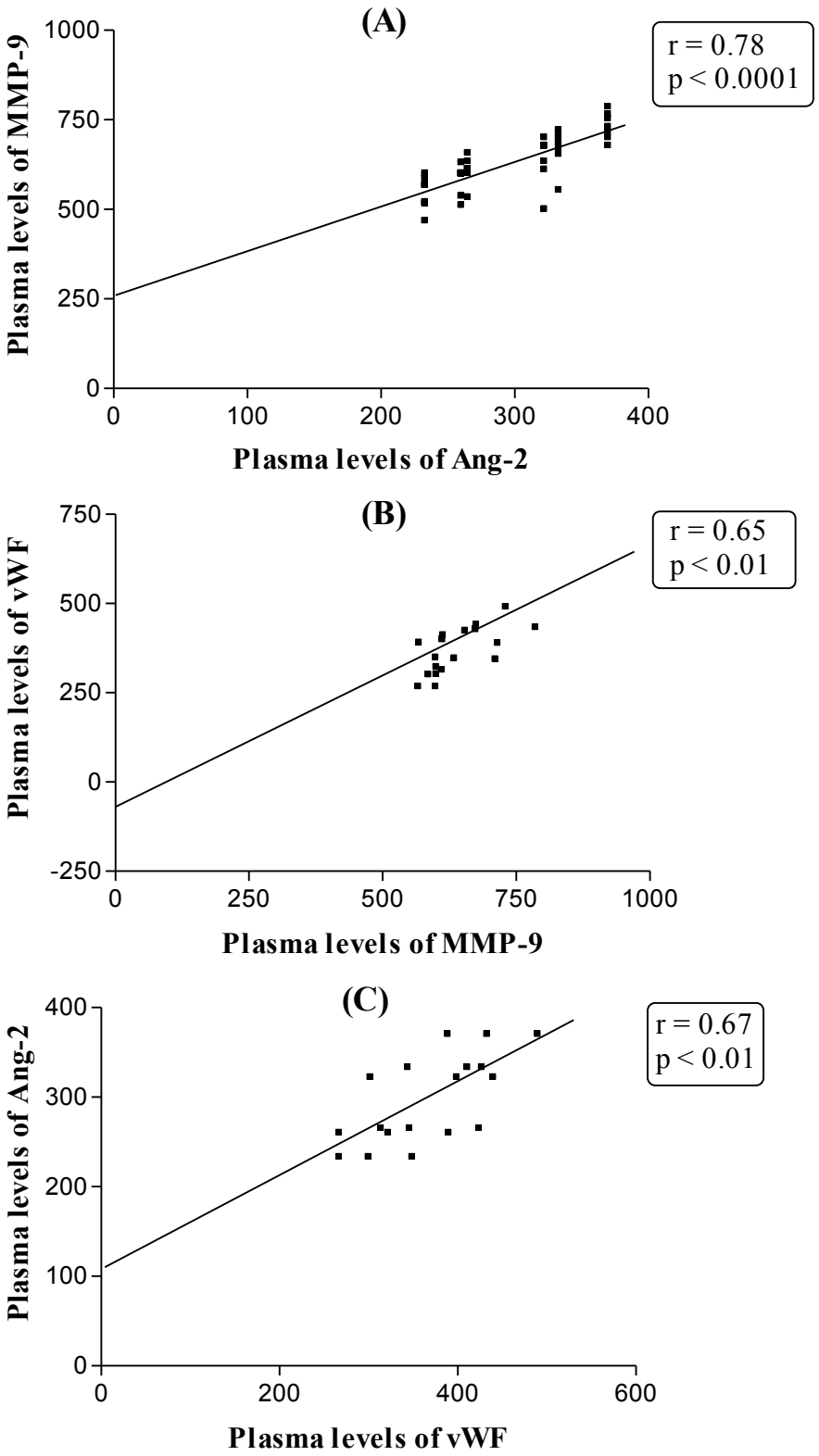

Figure 5. The interrelationship between MMP-9, Ang-2 and vWF plasma levels in ALI/ARDS patients at the first day of admission.

levels ($r = 0.78$ and $p < 0.0001$, $r = 0.65$ and $p < 0.01$). Also, Ang-2 levels were positively related to vWF levels ($r = 0.67$ and $p < 0.01$), Figure 5.

Discussion

Protein rich alveolar edema, reduced lung compliance, and acute severe hypoxemia are associated with ALI/ARDS (35). Understanding the mechanisms that contribute to ALI/ARDS may ultimately lead to the development of effective therapeutic strategies.

We reported higher circulating levels of ECM turnover, angiogenesis and endothelial dysfunction markers (MMP-9, Ang-2 and vWF, respectively) in patients with ALI/ARDS and their relation to pulmonary dysfunction and outcomes of the patients. Additionally, we found an interrelationship between plasma levels of MMP-9, Ang-2 and vWF on the day of admission.

MMP-9 plays a vital role in lung injury by remodeling the extracellular matrix, increasing cell migration, enhancing angiogenesis, enhancing inflammatory cell migration by modulating proinflammatory cytokines and chemokines and contributing to lung tissue remodeling (22).

Our results revealed that MMP-9 plasma levels are elevated in ALI/ARDS patients compared to healthy controls, and show an inverse relation to PaO2 and SaO2 and a positive relation to patient mortality.

Studies in various models of lung injury showed that MMP-9 is implicated in the pathogenesis of lung injury (1, 9). Michele, et al. (29), demonstrated up-regulation of MMP-9 activity in ALI patients in comparison to controls. Furthermore, Guillermo, et al. (20), reported that MMP-9 is related to the severity of lung injury. Moreover, inhibition of MMP-9 in lung injury has a protective role (1) and improved the pathological changes of lung tissue (12, 19). However, this result is contrary to the results of O'Kane, et al. (30), who reported that up-regulation of MMP-9 activity by salbutamol, beta agonist, in vitro and in vivo in patients with ARDS was associated with reduced extravascular lung water, and they concluded that MMP-9 has a valuable role in decreasing pulmonary edema in ARDS. Ang-2 has been implicated in neovascularization, a mitogen for vascular endothelial cells, and an activator of certain protease cascades (e.g. matrix metalloproteinases and plasminogen-activated pathways), all of which are proteases that have been related to the pathophysiology of ALI/ARDS (22, 24, 38).

The present study showed that Ang-2 plasma levels are elevated in ALI/ARDS patients compared to healthy controls, and have an inverse relation with PaO2 and SaO2, and a positive relation with mortality.

The present study is in broad agreement with several studies that suggested that Ang-2 is a mediator in the mechanism of pulmonary vascular permeability and injury and its correlation with severity and mortality of lung injury (5, 10, 14, 17, 31, 43).

Fiedler, et al. (15), reported that Ang-2 plays a central role in the pathogenesis of the pulmonary inflammation and permeability of ALI/ARDS by sensitizing the pulmonary endothelium to proinflammatory cytokines. Recent studies documented that Ang-2 enhanced the hyperpermeability response of cultured human pulmonary microvascular endothelial cells by disrupting the adherence junctions (5, 43).

In addition, vWF is a marker of pulmonary endothelial injury and is associated with an increased risk of death in ALI (7). The present study showed that vWF plasma levels are elevated in ALI/ARDS patients compared to healthy controls, and show an inverse relation with PaO2 and SaO2 and positive relation with patient mortality.

This result is concomitant with several studies signifying that baseline levels of vWF in patients with ALI are associated with worse clinical outcomes (28, 32, 33). Moreover, Flori, et al. (16), and Stapleton, et al. (39), found that plasma vWF levels in ALI were associated with an increased risk of death and prolonged mechanical ventilation.

Our result is contrary to the result of Covarrubias, et al. (8), who found no association between plasma vWF levels and acute lung injury occurring within 72 h following lung transplantation. Moreover, Bajaj, et al. (3), and Ware, et al. (46), demonstrated failure of vWF to expect the sequence of ARDS in at-risk patients. In our study, increased MMP-9, Ang-2 and vWF levels were interrelated on the day of admission. This is concomitant with the finding of previous studies (18, 40). The relation between Ang-2 and vWF claimed to be their secretion together from the endothelial Weibel-Palade body (41).

In conclusion, our study revealed a gradation in all biomarkers was inversely related to PaO2 and SaO2 and directly related to mortality in ALI/ARDS subjects. Moreover, we found positive correlations between their levels which might reflect a common pathway of their alteration. This may reflect the relative roles of these molecules in the pathophysiology of ALI/ARDS.

References

1. Adrian D, Thomas SH, Dorota P, Jolanta S, Justyna F, David HJ, Grzegorz S. Effects of MMP-9 inhibition by doxycycline on proteome of lungs in high tidal volume mechanical ventilation-induced acute lung injury. Proteome Sci 2010;8:3. doi:10.1186/1477-5956-8-3.
2. Albaiceta GM, Gutierrez-Fernandez A, Parra D, Astudillo A, Garcia-Prieto E, Taboada F, Fueyo A. Lack of matrix metalloproteinasec9 worsens ventilator-induced lung injury. Am J Physiol Lung Cell Mol Physiol 2008;294:L535-43.
3. Bajaj MS, Tricomi SM. Plasma levels of the three endothelial-specific proteins von Willebrand factor, tissue factor pathway inhibitor, and thrombomodulin do not predict the development of acute respiratory distress syndrome. Intensive Care Med 1999;25:1259-66.
4. Bhadade RR, de Souza RA, Harde MJ, Khot A. Clinical characteristics and outcomes of patients with acute lung injury and ARDS. J Postgrad Med 2011;57(4):286-90.
5. Bhandari V, Choo-Wing R, Harijith A, Sun H, Syed MA, Homer RJ, Elias JA. Increased hyperoxia-induced lung injury in nitric oxide synthase 2 null mice is mediated via angiopoietin 2. Am J Respir Cell Mol Biol 2012 May;46(5):668-76.
6. Cabrera S, Gaxiola M, Arreola JL, Ramirez R, Jara P, D'Armiento J, Richards T, Selman M, Pardo A. Overexpression of MMP9 in macrophages attenuates pulmonary fibrosis induced by bleomycin. Int J Biochem Cell Biol 2007;39:2324-38.
7. Collard HR, Calfee CS, Wolters PJ, Song JW, Hong SB, Brady S, Ishizaka A, Jones KD, King TE, Matthay MA, Kim DS. Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2010;299(1):L3-7.
8. Covarrubias M, Ware LB, Kawut SM, De Andrade J, Milstone A, Weinacker A, Orens J, Lama V, Wille K, Bellamy S, Shah C, Demissie E, Christie JD. Plasma intercellular adhesion molecule-1 and von Willebrand factor in primary graft dysfunction after lung transplantation. Am J Transplant 2007;7(11):2573-8.
9. Davey A, McAuley DF, O'Kane CM. Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair. Eur Respir J 2011;38(4):959-70.
10. Davis JS, Yeo TW, Piera KA, Woodberry T, Celermajer DS, Stephens DP, Anstey NM. Angiopoietin-2 is increased in sepsis and inversely associated with nitric oxide-dependent microvascular reactivity. Crit Care 2010;14:3:89.
11. Deepali PS, Preeti MC, Hemlata RP, Savita SM, Sadhana RJ. Matrix Metalloproteinase-1 and -9 in human placenta during spontaneous vaginal delivery and caesarean sectioning in preterm pregnancy. PLoS One 2012;7(1):e29855. doi: 10.1371/journal.pone.0029855.
12. Doroszko A, Hurst TS, Polewicz D, Sawicka J, Fert-Bober J, Johnson DH, Sawicki G. Effects of MMP-9 inhibition by doxycycline on proteome of lungs in high tidal volume mechanical ventilation-induced acute lung injury. Proteome Sci 2010;29(8):3. doi:10.1186/1477-5956-8-3.
13. Dushianthan A, Grocott MP, Postle AD, Cusack R. Acute respiratory distress syndrome and acute lung injury. Postgrad Med J 2011;87(1031):612-22. doi:10.1136/pgmj. 2011.118398.
14. Ebihara I, Hirayama K, Nagai M, Kakita T, Sakai K, Tajima R, Sato C, Kurosawa H, Togashi A, Okada A, Usui J, Yamagata K, Kobayashi M. Angiopoietin balance in septic shock patients with acute lung injury: effect of direct hemoperfusion with polymyxin B-immobilized fiber. Ther Apher Dial 2011;15(4):349-54. doi:10.1111/j.1744-9987.2011.00963.x.
15. Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzenrath M, Rosseau S, Suttorp N, Sobke A, Herrmann M, Preissner KT, Vajkoczy P, Augustin HG. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 2006;12(2):235-9.
16. Flori HR, Ware LB, Milet M, Matthay MA. Early elevation of plasma von Willebrand factor antigen in pediatric acute lung injury is associated with an increased risk of death and prolonged mechanical ventilation. Pediatr Crit Care Med 2007;8(2):96-101.Gallagher DC, Parikh SM, Balonov K, Miller A, Gautam S, Talmor D, Sukhatme VP. Circulating angiopoietin 2 correlates with mortality in a surgical population with acute lung injury/adult respiratory distress syndrome. Shock 2008;29:656-61.
17. Ganter MT, Cohen MJ, Brohi K, Chesebro BB, Staudenmayer KL, Rahn P, Christiaans SC, Bir ND,

Pittet JF. Angiopoietin-2, marker and mediator of endothelial activation with prognostic significance early after trauma? Ann Surg 2008;247:320-6.
18. Ge S, Gong WH, Zhang CX, Zhang L, Han PH, Zhang SQ, Feng JB, Zhou DC. Protective role of MMP-9 inhibitor batimastat in acute lung injury after cardiopulmonary bypass. Zhonghua Wai Ke Za Zhi 2010;48(1):57-61.
19. Guillermo MA, Ana G, Emilio G, Xose SP, Diego P, Aurora A, Cristina Campestre, Sandra C, Adrian G, Antonio F, Francisco T, Carlos L. Absence or Inhibition of Matrix Metalloproteinase–8 Decreases Ventilator-Induced Lung Injury. Am J Respir Cell Mol Biol 2010;43(5):555-63.
20. Hayes M, Curley G, Laffey JG. Mesenchymal stem cells - a promising therapy for Acute Respiratory Distress Syndrome. F1000 Med Rep 2012;4:2.
21. Heikki L, Anna H, Urpo L, Kristina B. Matrix metalloproteinase-9 deficiency worsens lung injury in a model of bronchopulmonary dysplasia. Am J Respir Cell Mol Biol 2009;41(1):59-68.
22. Hua G, Jingjing Z, Ting L, Weiyun S. Rapamycin prevents endothelial cell migration by inhibiting the endothelial-to-mesenchymal transition and matrix metalloproteinase-2 and -9: An in vitro study. Mol Vis 2011;17:3406-14.
23. Iribarren C, Phelps BH, Darbinian JA, McCluskey ER, Quesenberry CP, Hytopoulos E, Vogelman JH, Orentreich N. Circulating angiopoietins-1 and -2, angiopoietin receptor Tie-2 and vascular endothelial growth factor-A as biomarkers of acute myocardial infarction: a prospective nested case-control study. BMC Cardiovasc Disord 2011;11:31.
24. Jae-Won J, Jung-Kil L, Soo-Han K. Activation of matrix metalloproteinases-9 after photothrombotic spinal cord injury model in rats. J Korean Neurosurg Soc 2011;50(4):288-92. doi:10.3340/jkns.2011.50.4.288.
25. Li Y, Chung-Hsin Y, Ming-Ling Y, Yu-Hsiang K. Luteolin Suppresses Inflammatory Mediator Expression by Blocking the Akt/NFκB Pathway in Acute Lung Injury Induced by Lipopolysaccharide in Mice. Evid Based Complement Alternat Med 2012;383608. doi:10.1155/2012/383608.
26. Lukkarinen H, Hogmalm A, Lappalainen U, Bry K. Matrix metalloproteinasec9 deficiency worsens lung injury in a model of bronchopulmonary dysplasia. Am J Respir Cell Mol Biol 2009;41:59-68.
27. Magda C, Sandra B, Anil S, Michael AM, Gwynne C. Biological markers of lung injury before and after the institution of positive pressure ventilation in patients with acute lung injury. Crit Care 2006;10(5): R126.
28. Michele YFK, Amit G, Yao L, Margaret W, Blalock JE, Clancy JP. Matrix metalloproteinase activity in pediatric acute lung injury. Int J Med Sci 2009;6(1): 9-17.
29. O'Kane CM, McKeown SW, Perkins GD, Bassford CR, Gao F, Thickett DR, McAuley DF. Salbutamol up-regulates matrix metalloproteinase-9 in the alveolar space in the acute respiratory distress syndrome. Crit Care Med 2009;37(7):2242-9.
30. Ong T, McClintock DE, Kallet RH, Ware LB, Matthay MA, Liu KD. Ratio of angiopoietin-2 to angiopoietin-1 as a predictor of mortality in acute lung injury patients. Crit Care Med 2010;38(9):1845-51.
31. Parsons PE, Matthay MA, Ware LB, Eisner MD. Elevated plasma levels of soluble TNF receptors are associated with morbidity and mortality in patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 2005a;288:L426-31. doi: 10.1152/ajplung.00302.2004.
32. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, Wheeler AP. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Crit Care Med 2005b;33:1-6. doi: 10.1097/01.CCM.0000149854.61192.DC.
33. Pirrone F, Pastore C, Mazzola S, Albertini M. In vivo study of the behaviour of matrix metalloproteinases (MMP-2, MMP-9) in mechanical, hypoxic and septic-induced acute lung injury. Vet Res Commun 2009;33(1):S121-S4. doi:10.1007/s11259-009-9255-y.
34. Price LC, McAuley DF, Marino PS, Finney SJ, Griffiths MJ, Wort SJ. Pathophysiology of Pulmonary Hypertension in Acute Lung Injury. Am J Physiol Lung Cell Mol Physiol 2012;(Epub ahead of print).
35. Proudfoot AG, Hind M, Griffiths MJ. Biomarkers of acute lung injury: worth their salt? BMC Med 2011;12(9):132. doi:10.1186/1741-7015-9-132.
36. Renckens R, Roelofs JJ, Florquin S, de Vos AF, Lijnen HR, van't Veer C, van der Poll T. Matrix metalloproteinase-9 deficiency impairs host defense against abdominal sepsis. J Immunol

2006;176:3735-41.
37. Sazan R, Marie HR, Aysegul I, Katharina L, Ludwig W, Alexandra K. Circulating angiopoietin-2 and soluble Tie-2 in type 2 diabetes mellitus: a cross-sectional study. Cardiovasc Diabetol 2011;10:55. doi:10.1186/1475-2840-10-55.
38. Stapleton RD, Dixon AE, Parsons PE, Ware LB, Suratt BT. The association between BMI and plasma cytokine levels in patients with acute lung injury. Chest 2010;138(3):568-77.
39. Van der Heijden M, van Nieuw Amerongen GP, van Hinsbergh VW, Groeneveld AB. Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients. Thorax 2008;63:903-9.
40. Van der Heijden M, Pickkers P, van Nieuw Amerongen GP, van Hinsbergh VW, Bouw MP,van der Hoeven JG, Groeneveld AB. Circulating angiopoietin-2 levels in the course of septic shock: relation with fluid balance, pulmonary dysfunction and mortality Intensive Care Med 2009;35:1567-74.
41. Van der Heijden M, van Nieuw Amerongen GP, van Hinsbergh VW, Groeneveld AB. The interaction of soluble Tie2 with angiopoietins and pulmonary vascular permeability in septic and nonseptic critically ill patients. Shock 2010;33:263-8.
42. Van der Heijden M, van Nieuw Amerongen GP, van Bezu J, Marinus AP, Johan AB, van Hinsbergh VWM. Opposing effects of the angiopoietins on the thrombin-induced permeability of human pulmonary microvascular endothelial cells. PLoS One 2011;6(8):e23448. doi:10.1371/journal.pone.0023448.
43. Wang JW, Eikenboom J. Von Willebrand disease and Weibel-palade bodies. Hamostaseologie 2010;30(3):150-5.
44. Wang JW, Groeneveld DJ, Cosemans G, Dirven RJ, Valentijn KM, Voorberg J, Reitsma PH, Eikenboom J. Biogenesis of Weibel-Palade bodies in von Willebrand disease variants with impaired von Willebrand factor intrachain or interchain disulfide bond formation. Haematologica 2011;29.
45. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. Am J Respir Crit Care Med 2004;1(170):766-72.
46. Yoon HK, Cho HY, Kleeberger SR. Protective role of matrix metalloproteinasec9 in ozone-induced airway inflammation. Environ Health Perspect 2007;115:1557-63.
47. Zijing L, Yu L, Yuhui S, Xiuping H. Expression of Ets-1, Ang-2 and maspin in ovarian cancer and their role in tumor angiogenesis. J Exp Clin Cancer Res 2011;30(1):31. doi:10.1186/1756-9966-30-31.
48. Zitka O, Kukacka J, Krizkova S, Huska D, Adam V, Masarik M, Prusa R, Kizek R. Matrix metalloproteinases. Curr Med Chem 2010;17(31):3751-68.