

GAS6 IN ARDS PATIENTS: DETERMINATION OF PLASMA LEVELS AND INFLUENCE OF PEEP SETTING

Running title: Gas6 in ARDS.

Jean-Luc Diehl MD* (1,2); Nathalie Coolen MD (1); Christophe Faisy MD (1); David Osman MD (3); Gwenaël Prat MD (4); Mustapha Sebbane MD (5); Ania Nieszkowska MD (6); Claude Gervais MD (7); Jean-Christophe M Richard MD (8); Jack Richecoeur MD (9); Laurent Brochard MD (10); Alain Mercat MD (11), Emmanuel Guérot MD (1); Delphine Borgel PhD (12,13)

(1) Service de Réanimation Médicale, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France; (2) INSERM UMR_S765, Faculté de Pharmacie, Université Paris Descartes, Sorbonne Paris Cité, 4 avenue de l'Observatoire, 75006 Paris, France (3) Service de Réanimation Médicale, CHU Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin Bicêtre, France ; (4) Service de Réanimation Médicale, CHU de la Cavale Blanche, boulevard Tanguy Prigent, 29609 Brest, France (5) Anesthésie Réanimation, Hôpital Saint Eloi, 80 Avenue Augustin Fliche, 34295, Montpellier, France (6) Service de Réanimation Polyvalente, CHU de la Pitié Salpêtrière, 47 Boulevard de l'Hôpital, 75013 Paris, France (7) Service de Réanimation Médicale, GHU Caremeau, Place du Professeur Robert Debré, 30029 Nîmes, France (8) Service de Réanimation Médicale, CHU de Rouen, 1 rue de Germont, 76031 Rouen, France (9) Service de Réanimation Polyvalente, Hôpital de Pontoise, 6 Avenue de l'Île-de-France, 95303 Pontoise, France (10) Service de Soins Intensifs, Hôpitaux Universitaires de Genève, CH-1211 Geneve Cedex 14, Suisse (11) Service de Réanimation Médicale et de Médecine Hyperbare, CHU d'Angers, 4 rue Larrey, France (11), Service d'Hématologie, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France (12) Université Paris-Sud, EA4531, F-92296 Châtenay-Malabry Cedex, France

* corresponding author. Tel : 33 1 56093201 ; fax : 33 1 56093202 ; mail : jldiehl@invivo.edu

ABSTRACT

Purpose: Growth arrest-specific protein 6 (Gas6) is a vitamin K-dependent protein expressed by endothelial cells and leukocytes participating in cell survival, migration and proliferation and involved in many pathological situations. The aim of our study was to assess its implication in acute respiratory distress syndrome (ARDS) and its variation according to positive end expiratory pressure (PEEP) setting, considering that different cyclic stresses could alter Gas6 plasma levels.

Methods: Our patients were enrolled in the ExPress study comparing a minimal alveolar distension (“low PEEP”) ventilatory strategy to a maximal alveolar recruitment (“high PEEP”) strategy in ARDS. Plasma Gas 6, IL8 and VEGF levels were measured at day 0 and day 3 by enzyme-linked immunosorbent assay in blood samples prospectively collected during the study for a subset of 52 patients included in 8 centers during year 2005.

Results: We found that Gas6 plasma level was elevated in the whole population at day 0: 106 ng/mL (77-139), (median, IQR), with significant correlations with IL8, the Simplified Acute Physiologic Score II and the Organ Dysfunction and Infection (ODIN) scores. Statistically significant decreases in Gas6 and IL 8 plasma levels were observed between day 0 and day 3 in the “high PEEP” group ($P=0.017$); while there were no differences between day 0 and day 3 in the “low PEEP” group.

Conclusions: Gas6 plasma level is elevated in ARDS patients. The “high PEEP” strategy is associated with a decrease in Gas6 and IL8 plasma levels at day 3, without significant differences in day 28 mortality between the 2 groups.

Trial registration: clinicaltrials.gov Identifier: NCT00188058

INTRODUCTION

Growth arrest-specific protein 6 (Gas6) is a vitamin K-dependant protein sharing 43% of homology with a natural anticoagulant, protein S (1). Gas6 is expressed in various cell types, including endothelial cells, particularly in pro-apoptotic conditions (2). Leukocytes have also been found to release Gas6 (3-4). It is a ligand for 3 tyrosine kinase receptors (Axl, Tyro3 and Mer) whose signaling is implicated primarily in cell survival but also in cell proliferation, adhesion and migration (5).

Among several functions, previous studies have reported the implication of Gas6 in the inflammatory process, particularly in the physiopathology of severe sepsis (3-4, 6-7). Indeed, Gas6 enhances the interplay of cells implicated in the inflammatory response, endothelial cells, leukocytes and platelets during different conditions of experimental inflammation (4). Models of endotoxemia also suggest an important role of modulation of the immune response (4-5, 8-11).

Given the involvement of Gas6 receptors in experimental sepsis, and the potentially major role of leukocyte apoptosis in the pathophysiology of severe sepsis, previous clinical studies have focused on Gas6 in septic and non-septic critical care patients (3, 6-7). As compared to healthy subjects, levels were higher in critical care patients with one or several failing organs, the highest values being observed in patients with severe sepsis (3). Specifically in severe sepsis patients, a correlation was observed between the number of organ dysfunctions (as reflected by scores such as SOFA and ODIN) reflecting the degree of tissue injury, and Gas6 plasma concentrations (3, 6).

Gas6 can be released by endothelial cells and leukocytes, which are largely implicated in the pathophysiology of acute respiratory distress syndrome (ARDS). Since the pulmonary vascular bed is subjected to cyclic stress in patients with ARDS, and despite the lack of in

vitro or experimental studies evidencing that Gas6 could be modulated by such cyclic stress, we hypothesized that the course of plasma Gas6 levels could differ according to different ventilatory strategies. The aim of this study was to use a multicenter randomized controlled trial ~~therefore~~ to compare the course of plasma Gas6 levels in patients with ARDS from the Expiratory Pressure (ExPress) Study Group (12). Briefly, patients were randomly assigned either to a “low PEEP” strategy (minimal alveolar distension strategy) or to a “high PEEP” strategy (increased alveolar recruitment strategy). The “high PEEP” strategy, avoiding in part consequences of cyclic collapse and excessive hyperinflation, could therefore be associated with less injury and lower plasma Gas6 levels. Additionally, we measured plasma levels of IL-8 and VEGF, as established endothelial and leukocyte markers with important implications in the context of ARDS (13-22).

METHODS

We measured plasma levels of Gas 6, IL-8 and VEGF in a subset of ARDS patients enrolled in the ExPress Study. The 767 enrolled patients were randomized in two groups. In the “low PEEP” strategy group, PEEP and inspiratory plateau pressure were kept as low as possible without falling below oxygenation targets (SpO₂: 88% and/or PaO₂: 55 MmHg). External PEEP was set to maintain total PEEP (the sum of external and intrinsic PEEP) between 5 and 9 cm H₂O. In the “high PEEP” strategy group, PEEP was adjusted based on airway pressure and was kept as high as possible without increasing the maximal inspiratory plateau pressure above 28 to 30 cm H₂O. For both groups, tidal volume (V_T) was set at 6 mL/kg of predicted body weight.

Plasma Gas6, IL-8 and VEGF levels were measured in blood samples prospectively collected during the last year (2005) of the ExPress study at day 0 and day 3 in 8 selected centers. Indeed, the present study concerned only year 2005, as the rationale originated from results

demonstrating the implication of Gas6 in severe sepsis obtained shortly before 2005 (3). Given these conditions, 52 patients were included in the present study: 24 in the “low PEEP” strategy group and 28 in the “high PEEP” strategy group. The study protocol (approval number: 2002/09, 08-Jul-2002) and the corresponding amendment (amendment # 8; 15-Feb-2005) were approved by the ethics committee of the Angers University Hospital (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale). Measurement of plasma Gas6, IL-8 and VEGF levels were therefore determined by enzyme-linked immunosorbent assays (ELISA) as previously described (23-24).

Statistical analysis

Continuous data are expressed as medians and interquartile ranges (IQR) and compared using non parametric tests (Mann-Whitney test, Wilcoxon sign rank test or Kruskal-Wallis test as appropriate). Results are expressed as total number (percentage) for categorical. Comparison of categorical variables was performed by the Chi-squared test. Correlations were assessed with the non parametric Spearman correlation test or with the non parametric test for trend, as appropriate. A *p* value less than 0.05 was considered significant. Analyses were performed using the StatView software (Abacus Concepts, Berkeley, CA).

RESULTS

Plasma Gas6, IL-8 and VEGF levels were measured at day 0 and day 3 in 52 patients from the ExPress study (7 % of the 768 patients). The main clinical characteristics at inclusion, including classification according to the new Berlin definition of ARDS (25), are shown in Table 1. Characteristics at day 3 and relevant physiological and clinical end-points until day 28 are shown in Table 2.

As expected, there was a statistically significant difference between PEEP levels at day 3: 7 cmH₂O (5-8) vs 15 cmH₂O (10-17), while there were no differences in PEEP levels at day 0, nor in V_T levels at days 0 and at day 3.

The main biological results at day 0 and day 3 are shown in Table 3. Gas6 plasma level was very high in patients at the onset of ARDS: 106 ng/mL (77 - 139), with no statistically significant difference between the “low PEEP” group and the “high PEEP” group: 121 ng/mL (80 - 143) vs 93 ng/mL (73 - 123) respectively, $P = 0.17$ (Figure 1). Considering all 52 patients, there was a trend ($P = 0.15$) to lower Gas6 values in the less severe ARDS according to the Berlin classification (25): mild ARDS: 92 ng/mL (80 - 96), moderate ARDS: 115 ng/mL (82-143) and severe ARDS: 127 ng/mL (67-144).

Gas6 correlated positively with the severity of disease, as assessed by the Simplified Acute Physiologic Score II ($\rho = 0.43$, $P = 0.002$) and the Organ Dysfunction and Infection (ODIN) score ($\rho = 0.40$, $P = 0.007$), which indicates the number of organ dysfunction (varying between 0 and 7, including clinically evident infection as an organ dysfunction). There was no correlation between Gas6 level at day 0 and PEEP and V_T levels. There was no difference between Gas6 levels at day 0 between septic ($n = 33$) and non-septic patients ($n = 19$).

A statistical difference between plasma Gas6 levels at day 0 and day 3 was found in the “high PEEP” group ($P = 0.017$) but not in “low PEEP” group ($P=0.83$).

We found elevated plasma levels of IL-8 at day 0, with no statistical difference between the 2 groups. There was a statistically significant correlation between plasma levels of Gas6 and of IL-8 at day 0 ($\rho = 0.40$, $P = 0.006$), but not at day 3. A statistical difference between plasma IL-8 levels at day 0 and day 3 was found in the “high PEEP” group ($P = 0.02$) but not in “low PEEP” group ($P=0.63$).

We found elevated plasma levels of VEGF at day 0, with no statistical difference between the 2 groups. There was no statistically significant correlation between plasma levels of VEGF

and Gas6 either at day 0 or at day 3. Similarly, there was no statistically significant correlation between plasma levels of VEGF and of IL-8 either at day 0 or at day 3. Finally, there were no statistically differences between VEGF levels at day 0 and at day 3, either in the “high PEEP” or in the “low PEEP” groups.

There were no differences between IL-8 and VEGF levels at day 0 between septic and non-septic patients.

DISCUSSION

As expected, according to a previous study among more heterogeneous critically ill patients, Gas6 plasma level was very high in patients at the onset of ARDS: 106 ng/mL (77 - 139) as compared to reference values observed in spontaneously breathing healthy subjects: 54 ng/mL (49-68) (3). We found also elevated plasma levels of IL-8 and VEGF at the onset of ARDS, confirming previous series (13-15, 17,19-21). A positive correlation was found between Gas6 and IL-8 plasma levels. A significant decrease in Gas6 and IL-8 plasma levels was observed in the “high PEEP” group, but not in the “low PEEP” group.

This is the first report of elevated Gas6 plasma levels in a homogeneous group of ARDS patients. Obviously, over-expression of Gas6 was very likely in relation with disease severity (as assessed by correlation with SAPS II and ODIN scores) and sepsis (33 patients in the series). Since it was previously proposed that Gas6 originates from endothelial cells and leukocytes (3-4), increase in Gas6 production could also in part be related to pulmonary neutrophil infiltration and to diffuse pulmonary endothelial damage which are key features of ARDS (13, 26). Moreover, despite the lack of surrounding experimental studies, it was tempting to assume that mechanical ventilation, by applying cyclic stress over pulmonary endothelial cells and by exacerbating leukocytes’ stimulation, could *per se* be a contributing factor to the high Gas6 plasma levels observed at day 0 and day 3.

As previously described in patients with severe sepsis (3,6), Gas6 correlated positively with the severity of disease, as assessed by the Simplified Acute Physiologic Score II and the ODIN score. The correlation values were in the same range that previous published data from our group and from others adding external validity to our results (3,6). However, they are just indicators of some degrees of association between the severity of the disease, as assessed by the scores, and the Gas6 plasma levels; any causal relationship between these parameters remaining to be investigated. We found no correlation between Gas6 level at day 0 and PEEP and V_T levels, parameters which obviously influence cyclic stress exerted on pulmonary vasculature. However, one can observe that V_T at day 0 was set at a level close to 6 mL/kg IBW, a level known to minimize lung stress (15). Therefore, the chance to observe a statistical correlation between Gas6 level and V_T as a marker of lung stress was minimized. There was also a rather narrow spectrum of PEEP settings at day 0, which could explain at least in part the lack of correlation with Gas6 level at day 0. Unfortunately, we didn't measure in our patients transpulmonary pressures, which could have provided a better surrogate of the stress applied on the pulmonary vasculature.

The statistical difference between plasma Gas6 levels at day 0 and day 3 could be explained, at least in part, by a better modulation of the stress applied on the pulmonary vascular and endothelial bed in the "high PEEP" group, mainly by preventing cyclic reopening of collapsed pulmonary areas. Accordingly, we observed no significant variation in Gas6 level in the "low PEEP" group, in which there was no significant differences in PEEP level between day 0 and day 3. In contrast, we observed in the "high PEEP" group a significant decrease in Gas6 level in parallel with an increase in PEEP level from 8 cmH₂O (5-10) to 15 cmH₂O (10-17). Such differences according to ventilatory strategies have previously been reported for inflammatory mediators and apoptosis markers both at the pulmonary and at the systemic levels (12, 15). In

the same way, PEEP setting could have had an impact on plasma Gas6 levels in our ARDS patients-

We found elevated plasma levels of IL-8 at day 0 in accordance with previous series of ARDS patients (13-15, 17, 20-21). Given the implication of IL-8 as an important pro-inflammatory mediator associated with the development of ARDS and with poor outcomes (increase in mortality and decrease in ventilator-free days), the observed correlation with Gas6 suggest that Gas6 could act as an important co-factor participating to exacerbated inflammation in the context of ARDS

We found elevated plasma levels of VEGF at day 0, confirming previous results from Azamfirei (19). However, such an elevation was not observed in another series (22). Moreover, differential expression of VEGF at the pulmonary and plasma levels has previously been reported (19). Finally, the implication of VEGF in the early ARDS context is not fully established since it can promote an increase in vascular permeability but also exert a protective vascular effect (19, 22, 27). Therefore, the lack of correlation between plasma Gas6 and VEGF levels could reflect the fact that plasma VEGF levels probably poorly reflect local pulmonary inflammation.

In the ExPress study, the “high PEEP” strategy was associated with a clinical benefit in term of oxygenation, higher compliance values, less ventilator-free days and less organ failure-free days (12). In the present study, we observed in a representative subset of patients a decrease in Gas6 plasma levels at day 3 in the “high PEEP” group, unlike the “low PEEP” group. This was in parallel to better oxygenation and higher compliance values at day 3, suggesting a possible relationship between such clinical benefits and these biological findings. Results of extensive animal experimentations from Tjwa et al (4) can also support the hypothesis of a possible relationship between such biological findings and clinical benefits: the authors reported that Gas6 could be involved in the inflammatory process and could enhance the

inflammatory response in pathological conditions like sepsis or ARDS. Moreover, they suggested that the inhibition of Gas6 might warrant further consideration as a novel strategy for the treatment of sepsis. Importantly, such inhibition of Gas6 should not be interpreted as a decrease to information to cell survival but rather to a return to a controlled physiological state, therefore limiting the Gas6-induced exacerbation of inflammation.

We also found a decrease in plasma IL-8 levels between day 0 and day 3 in the “high PEEP” group, but not in the “low PEEP” group. Considering the key role of IL-8 in ARDS, such a result can also suggest a beneficial effect of the “high PEEP” strategy and confirms previous series demonstrating a link between respiratory settings and IL-8 levels (13, 15).

The first limitation to our study is that we cannot distinguish between the distinct effects of pulmonary insult and mechanical ventilation on the results of Gas6 plasma measurements from healthy subjects, since they cannot be subjected to a short course of mechanical ventilation. The generalization of the present results is also a critical point: measurements had been possible only in a limited number of centers participating to the ExPress study for technical reasons, and only during the last year of the study. The two groups were clinically comparable and similar to the whole population of the ExPress study, but given the relatively low number of patients, one can not formally exclude the possibility of type two errors with regard to baseline characteristics, and especially to the repartition of the patients between the 3 ARDS categories. Another limitation to our study is that the measurements were limited to the plasma compartment. Our hypothesis is that plasma Gas6 originates at least in part from the pulmonary compartment; however only BAL studies performed in ARDS patients could support it, and unfortunately such Gas6 BAL measurements were not possible planned during the ExPress study. Another limitation of the present study is the lack of measurement of Gas6 plasma level after day 3, precluding any conclusion on the implication of Gas6 in the end-stage ARDS. However, it's generally admitted that Gas6 acts as an acute phase reactant (28).

In conclusion, we found that plasma Gas6 level is elevated at the onset of ARDS and that a “high PEEP” strategy was associated with a decrease in Gas6 plasma concentration over 3 days. Further studies are now warranted to confirm that the pulmonary compartment is a major contributor to the high plasma values of Gas6, to extend our knowledge about the kinetic of plasma Gas6 levels in the course of ARDS and finally to better delineate the prognostic and pathogenetic values of Gas6 as a biomarker in ARDS.

ACKNOWLEDGMENTS AND AUTHORS' CONTRIBUTIONS

The authors thank Véronique Remones for her excellent technical assistance.

JLD, JCMR, LB, AM, EG and DB contributed to the design of the study. J-LD, NC, CF, DO, GP, MS, AN, CG, EG, JR, JCMR, LB, AM collected the data. DB was responsible for biological measurements. JLD, NC and DB wrote the manuscript. All authors read and approved the manuscript.

FINANCIAL SUPPORT

The ExPress study was supported by the French Minister of Health (Programme Hospitalier de Recherche Clinique).

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to disclose.

REFERENCES

1. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *MolCell Biol* 1993; **13**:4976-85.
2. Hasanbasic I, Cuerquis J, Varnum B, Blostein MD. Intracellular signaling pathways involved in Gas6-Axl-mediated survival of endothelial cells. *Am J Physiol Heart Circ Physiol* 2004; **287**:H1207-13.
3. Borgel D, Clauser S, Bornstain C, Bièche I, Bissery A, Remones V, et al. Elevated growth-arrest-specific protein 6 plasma levels in patients with severe sepsis. *Crit Care Med* 2006; **34**:219-22.
4. Tjwa M, Bellido-Martin L, Lin Y, Plaisance S, Bono F, Delesque-Touchard N et al. Gas6 promotes inflammation by enhancing interactions between endothelial cells, platelets and leukocytes. *Blood* 2008; **111**:4096-105.
5. Camenisch TD, Koller BH, Earp HS, Matsushima GK. A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock. *J Immunol* 1999; **162**:3498-503.
6. Gibot S, Massin F, Cravoisy A, Dupays R, Barraud D, Nace L, et al. Growth arrest-specific protein 6 plasma concentrations during septic shock. *Crit Care* 2007; **11**:R8. doi: 10.1186/cc5158.
7. Ekman C, Linder A, Akesson P, Dahlback B. Plasma concentrations of Gas6 (growth arrest specific protein 6) and its soluble receptor sAxl in sepsis and systemic inflammatory responses syndromes. *Crit Care* 2010; **14**:R158 doi: 10.1186/cc9233.

8. Sather S, Kenyon KD, Lefkowitz JB, Liang X, Varnum BC, Henson PM, et al. A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation. *Blood* 2007; **109**:1026-33.
9. Scutera S, Fraone T, Musso T, Cappello P, Rossi S, Pierobon D, et al. Survival and migration of human dendritic cells are regulated by an IFN-alpha-inducible Axl/Gas6 pathway. *J Immunol.* 2009;**183**:3004-13.
10. Alciato F, Sainaghi PP, Sola D, Castello L, Avanzi GC. TNF-alpha, IL-6, and IL-1 expression is inhibited by GAS6 in monocytes/macrophages. *J Leukoc Biol* 2010 **87**:869-75.
11. Feng X, Deng T, Zhang Y, Su S, Wei C, Han D. Lipopolysaccharide inhibits macrophage phagocytosis of apoptotic neutrophils by regulating the production of tumour necrosis factor α and growth arrest-specific gene 6. *Immunology* 2011; **132**:287-95.
12. Mercat A, Richard JC, Vielle B, Jaber S, Osman D, Diehl JL, et al. Positive end-expiratory pressure setting in adults with acute lung injury and acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 2008 **299**:646-55.
13. Ranieri VM, Suter PM, Tortorella C, De Tullio R, Dayer JM, Brienza A, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 1999; **282**:54-61.
14. Bouros D, Alexandrakis MG, Antoniou KM, Agouridakis P, Pneumatikos I, Anevlavis S, et al. The clinical significance of serum and broncho-alveolar lavage inflammatory cytokines in patients at risk for acute respiratory distress syndrome. *BMC Pulmonary Medicine* 2004; **4** 6 doi:10.1186/1471-2466-4-6

15. Parsons P, Eisner MD, Thompson BT, Matthay M, Ancukiewicz M, Bernard GR, et al. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005; **33**:1-6.
16. Medford ARL, Ibrahim NBN, Millar AB. Vascular endothelial growth factor receptor and coreceptor expression in human acute respiratory distress syndrome. *J Crit Care* 2009; **24**:236-243.
17. Hildebrand F, Stuhmann M, van Griensven M, Meier S, Hasenkamp S, Krettek C, et al. Association of IL-8-251A/T polymorphism with incidence of acute respiratory distress syndrome (ARDS) and IL-8 synthesis after multiple trauma. *Cytokine* 2007; **37**: 192-199.
18. Wei-Chieh W, Chiou-Feng L, Chia-Ling C, Chang-Wen C, Yee-Shin L. Prediction of outcome in patients with acute respiratory distress syndrome by bronchoalveolar lavage inflammatory mediators. *Exp Biol Med* 2010; **235**:57-65.
19. Azamfirei L, Gurzu S, Solomon R, Copotoiu R, Jung I, Tilinca M, et al. Vascular endothelial growth factor: a possible mediator of endothelial activation in acute respiratory distress syndrome. *Minerva Anestesiol* 2010; **76**: 609-616.
20. Ware LB, Koyama T, Billheimer D, Wu W, Bernard GR, Thompson T, et al. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. *Chest* 2010; **137**:288-296.
21. Agrawal A, Zhuo H, Brady S, Levitt J, Steingrub J, Siegel MD, et al. Pathogenic and predictive value of biomarkers in patients with ALI and lower severity of illness: results from two trials. *Am J Physiol Lung Cell Mol Physiol* 2012; **303**:L634-L639.
22. Wada T, Jesmin S, Gando S, Yanagida Y, Mizugaki A, Sultana SN, et al. The role of angiogenic factors and their soluble receptors in acute lung injury (ALI)/acute

- respiratory distress syndrome (ARDS) associated with critical illness. *Journal of Inflammation* 2013; **10**:6.
23. Clauser S, Peyrard S, Gaussem P, Crespin M, Emmerich J, Aiach M, et al. Development of a novel immunoassay for the assessment of plasma Gas6 concentrations and their variation with hormonal status. *Clin Chem* 2007; **53**:1808-13.
 24. Smadja DM, Borgel D, Diehl JL, Gaussem P. Vascular endothelial growth factor, as compared with placental growth factor, is increased in severe sepsis but not in organ failure. *J Thromb Haemost* 2012; **10**:974-976.
 25. Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, et al. The Berlin definition of ARDS: an expanded rationale, justification, and supplementary material. *Intensive Care Med* 2012;**38**:1573-1582.
 26. Orfanos SE, Mavrommati I, Korovesi I, Roussos C. Pulmonary endothelium in acute lung injury : from basic science to the critically ill. *Intens Care Med* 2004; **30**:1702-14.
 27. Medford ARL, Ibrahim NBN, Millar AB. Vascular endothelial growth factor receptor and coreceptor expression in human acute respiratory distress syndrome. *J Crit Care* 2009, **24**:236-242.
 28. Hurtado B, Garcia de Frutos P. Gas6 in systemic inflammatory diseases : with and without infection. *Crit Care* 2010;**14**:1003 doi: 10.1186/cc9263.

Legend for Figure

Values for plasma Gas6 concentration measured by ELISA at day 0 and day 3 in the “low PEEP” and “high PEEP” groups. A significant difference was found between Gas6 plasma levels at day 0 and day 3 in the “high PEEP” group but not in the “low PEEP” group.

TABLE 1

Characteristics at inclusion in the “low PEEP” and “high PEEP” groups

	Low PEEP n = 24	High PEEP n = 28	<i>p</i>
age (years)	64 (47-72)	61 (47-76)	0.99
ODIN	3 (2-4)	3 (2-4)	0.45
SAPS II	47 (39-57)	46 (39-63)	0.97
infection (n)	17	16	0.46
mild ARDS (n)	1	6	0.11
moderate ARDS (n)	17	13	
severe ARDS (n)	6	9	
PaO ₂ /FiO ₂	142 (105-169)	122 (90-195)	0.83
Compl. (mL/cmH ₂ O)	34 (29-40)	34 (26-38)	0.78
PEEP (cmH ₂ O)	8 (5-11)	8 (5-10)	0.65
V _T (mL)	450 (415-510)	450 (425-505)	0.71
V _T (mL/kg IBW)	6.9 (6.3-7.6)	7.0 (6.4-7.8)	0.76
iNO (n)	4	3	0.53
prone position (n)	3	1	0.23

ODIN: organ dysfunction and infection score, SAPS II: simplified acute physiologic score, infection: clinically evident infection as defined by the ODIN score, PaO₂: partial pressure of arterial oxygen, FiO₂: fraction of inspired oxygen, Compl.: respiratory system compliance, PEEP: positive end-expiratory pressure. V_T: tidal volume. IBW: ideal body weight. iNO: inhaled nitric oxide treatment.

Results are expressed as median (IQR) or n (number of patients) as appropriate.

Table 2

Characteristics at day 3 and relevant physiological and clinical end-points until day 28.

	Low PEEP	High PEEP	
	n = 24	n = 28	<i>p</i>
ODIN	2 (2-3)	2 (1-2)	0.14
PaO ₂ /FiO ₂	161 (111-215)	241 (179-292)	0.001
Compl. (mL/cmH ₂ O)	29 (26-38)	38 (31-53)	0.01
PEEP (cmH ₂ O)	7 (5-8)	15 (10-17)	< 0.001
V _T (mL)	410 (365-450)	430 (380-455)	0.47
V _T (mL/kg IBW)	6.0 (6.0-6.1)	6.0 (6.0-6.1)	0.63
iNO until day 7 (n)	6	5	0.53
prone position until day 7 (n)	5	3	0.31
ventilator free-days until day 28 (n)	7 (0-16)	10.5 (0-16.5)	0.51
OF free-days until day 28 (n)	6.5 (0-15)	7.5 (0-16.5)	0.74
mortality at day 28	8	6	0.33

ODIN: organ dysfunction and infection score, PaO₂: partial pressure of arterial oxygen, FiO₂: fraction of inspired oxygen, Compl.: respiratory system compliance, PEEP: positive end-expiratory pressure. V_T: tidal volume. IBW: ideal body weight, iNO until day 7: inhaled nitric oxide treatment at any time from day 0 to day 7, prone position until day 7: prone position at any time from day 0 to day 7, ventilator free-days until day 28: number of alive

ventilator free-days from day 0 to day 28, OF free-days until day 28: number of alive organ failure free-days from day 0 to day 28.

Results expressed as median (IQR) or n (number of patients) as appropriate.

TABLE 3:

Plasma Gas6, IL-8 and VEGF levels at days 0 and 3 in the “high PEEP” and “low PEEP” groups.

p values are related to comparisons of plasma levels between days 0 and 3.

	day 0	day 3	<i>p</i>
Gas6 « high PEEP »	93 (73-123)	92 (70-110)	0.02
(ng/mL) « low PEEP »	121 (80-143)	102 (84-135)	0.83
IL-8 « high PEEP »	23 (3-102)	12 (0-39)	0.02
(ng/mL) « low PEEP »	51 (0-187)	12 (0-83)	0.63
VEGF « high PEEP »	111 (62-145)	110 (63-134)	0.57
(pg/mL) « low PEEP »	99 (53-148)	98 (55-202)	0.12

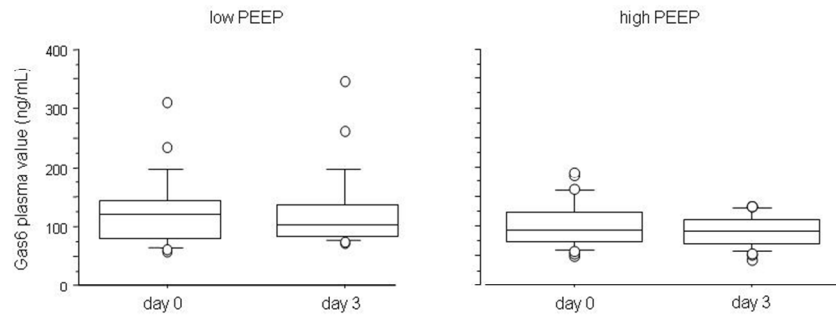


Figure 1: Values for plasma Gas6 concentration measured by ELISA at day 0 and day 3 in the "low PEEP" and "high PEEP" groups. A significant difference was found between Gas6 plasma levels at day 0 and day 3 in the "high PEEP" group but not in the "low PEEP" group.

254x190mm (96 x 96 DPI)