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Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients

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ABSTRACT

Background: Angiopoietin-2 and vascular endothelial growth factor (VEGF) may impair vascular barrier function, while angiopoietin-1 may protect. We hypothesised that circulating angiopoietin-2 is associated with pulmonary permeability oedema and severity of acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) during septic or non-septic critical illness.

Methods: In 24 septic and 88 non-septic mechanically ventilated patients, plasma levels of angiopoietin-1 and angiopoietin-2 were measured, together with the pulmonary leak index (PLI) for ^{67}Gallium-labelled transferrin and extravascular lung water (EVLW) by transpulmonary thermal-dye dilution as measures of pulmonary permeability and oedema, respectively. ALI/ARDS was characterised by consensus criteria and the lung injury score (LIS). Furthermore, plasma VEGF and von Willebrand factor (VWF) levels were assayed.

Results: Angiopoietin-2, VWF, PLI, EVLW, and LIS were higher in septic than in non-septic patients and higher in patients with ALI/ARDS (n=10/12 in sepsis, n=19/8 in non-sepsis) than without. VEGF was also higher in patients with sepsis than without. Patients with high PLI, regardless of EVLW, had higher angiopoietin-2 levels than patients with normal PLI and EVLW. Angiopoietin-2 correlated to the PLI, LIS, and VWF (minimum $r=0.34$, $P<0.001$), but not to EVLW. Angiopoietin-2 and VWF were of predictive value for ARDS in receiver operating characteristic curves (minimum area under the curve=0.69, $P=0.006$). Angiopoietin-1 and VEGF did not relate to the permeability oedema of ALI/ARDS.

Conclusion: Circulating angiopoietin-2 is associated with pulmonary permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic patients. The correlation of angiopoietin-2 with VWF suggests activated endothelium as a common source.

INTRODUCTION

Sepsis and major surgery are important risk factors for acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).[1] These syndromes are characterised by increased pulmonary permeability oedema,[1] the cause of which is still incompletely understood. Novel mediators that may be involved include vascular endothelial growth factor (VEGF) and the angiopoietins.[2-15]

In experimental models, administration of angiopoietin (Ang)-2 can provoke microvascular leakage in the lung and in other organs,[2, 7] while Ang-1 can protect against microvascular leakage induced by VEGF, Ang-2, or inflammatory agents.[3, 7-9] Ang-2 is released from endothelium,[11, 16] while Ang-1 is produced by various other tissue cells.[11] Both Ang-1 and Ang-2 bind to the Tie2 receptor, which is abundantly present in lung endothelium.[17] Ang-1-induced activation of the Tie2 receptor enforces endothelial barrier function via activation of Rac1, inhibition of RhoA, and consequently organisation of the cytoskeleton into a junction-fortifying arrangement,[3, 18] inhibition of VEGF-induced calcium influx,[19] and reduced VEGF-stimulated leukocyte adhesion.[20] In contrast, Ang-2 counteracts Tie2 receptor activation in many studies, although there are some exceptions.[2, 11, 21, 22] This results in impairment of endothelial barrier function,[2, 21] and an increase in the adhesion and migration of inflammatory cells.[7, 23]

In patients, circulating Ang-2, VEGF, and von Willebrand factor (VWF) are elevated during ALI/ARDS or sepsis.[2, 4-6, 10, 12-15, 24, 25] A high Ang-2 level correlated with impaired pulmonary gas exchange.[2] Plasma VEGF correlated in some, but not in all studies, to surrogate indicators of systemic permeability.[13-15] However, a direct correlation between Ang-2 or VEGF levels and pulmonary permeability oedema in ALI/ARDS of septic or non-septic origin has not yet been demonstrated. VWF is a marker of endothelial activation and injury and is associated with development and clinical outcomes of ALI/ARDS,[24, 25] although it may not be directly involved in permeability. Ang-2 and VWF reside in the same secretory organelle of endothelial cells, namely the Weibel Palade body and may be released together after activation of the endothelium.[16]

Radionuclide techniques,[26, 27] such as the pulmonary leak index (PLI), reflecting pulmonary permeability, can be used to differentiate between hydrostatic and permeability oedema.[26] The PLI represents the transvascular transport rate of ^{67}Gallium (^{67}Ga)-labelled transferrin and predicts and tracks the clinical course of ALI/ARDS.[26] Oedema is reflected by extravascular lung water (EVLW), which can be determined by transpulmonary thermal-dye dilution.[28-31]

We hypothesised that circulating Ang-2 levels are associated with 1) pulmonary permeability oedema, 2) occurrence and severity of ALI/ARDS, and that 3) Ang-2 and VWF are elevated simultaneously. To test these hypotheses, we measured the Ang-1, Ang-2, VEGF, and VWF levels in plasma together with the PLI, the EVLW, and the lung injury score (LIS), in critically ill, mechanically ventilated, septic or non-septic patients with or at risk for ALI/ARDS.

PATIENTS AND METHODS

This is a prospective observational study approved by the Ethical Committee of the VU University Medical Centre (Amsterdam, The Netherlands), involving 112 consecutive critically ill patients with or at risk for ALI/ARDS: 24 septic and 88 non-septic patients. The patients or their closest relatives gave informed consent. Patients took part of a prospective randomised clinical trial on the effect of resuscitation with various fluids in predefined and stratified groups of septic and non-septic mechanically ventilated patients in the intensive care unit (ICU). The data of this trial in cardiovascular surgery have been published.[28] The inclusion criteria, judged when the patient was enrolled, were absence of overhydration, defined as a pulmonary capillary wedge pressure (PCWP) ≤13 mm Hg in the presence of a pulmonary artery catheter (n=38) and proper wedging, or a central venous pressure (CVP) ≤12 mm Hg at positive end-expiratory pressure (PEEP) ≤15 cm H_2O, and ≤16 mm Hg when PEEP >15 cm H_2O in the presence of a central venous catheter (n=74), since PEEP elevates atmospheric pressure-referenced intrathoracic filling pressures, and a systolic arterial pressure <110 mm Hg in the absence of vasopressor therapy. Exclusion criteria were age ≥78 years, pregnancy, known anaphylactoid reaction to colloid fluids, and a life expectancy <24 h.

Sepsis was defined by two or more of the following: abnormal body temperature (>38 °C, <36 °C), tachycardia (>90/min), tachypnea (>20/min or partial pressure of CO_2 in arterial blood [P_aCO_2] <32 mm Hg), abnormal white blood cell counts (<4, >12 $x10^9$/l), and a microbiologically proven or clinically evident source of infection. ICU-acquired sepsis was defined as sepsis developing after two days in the ICU. The origin of sepsis was defined by clinical signs and symptoms and positive local and/or blood cultures. Non-sepsis involved cardiac surgery, major vascular surgery, other major surgery, and gastrointestinal bleeding. Cardiac surgery was defined as surgery for coronary artery bypass grafting (n=32), aortic valve replacement (n=8), or atrial septal defect (n=2). Major vascular surgery was defined as surgery for thoracoabdominal aortic (n=4), abdominal aortic (n=18), or mesenteric vascular disease (n=4). Other major surgery involved abdominal surgery (n=14), neurosurgery (n=4), and surgery for polytrauma (injury severity score >15, n=5). Patients were pressure-controlled (during sepsis) or volume-controlled (during non-sepsis) ventilated with an Evita 3 ventilator (Dräger; Lübeck, Germany). Patients with ALI/ARDS were pressure-controlled ventilated with a tidal volume (V_t) aiming not to exceed 8 ml/kg and resulting in an end-tidal CO_2 concentration between 4 and 5%, using an O_2-air mixture with an inspiratory O_2 fraction (F_IO_2) of 40% and a PEEP of 5 cm H_2O (inspiration:expiration 1:2) or more, when needed, guided by partial pressure of O_2 in arterial blood (P_aO_2; >60 mm Hg). Patients were otherwise treated by intensive care physicians not involved in the study according to institutional guidelines.

We characterised pulmonary dysfunction by the American-European Consensus Conference (AECC) criteria and the lung injury score (LIS), as commonly done before,[32, 33] since the definition for ALI/ARDS is not unequivocal. According to AECC criteria, ALI and ARDS are characterised by P_aO_2/F_IO_2 <300 or <200 mm Hg, respectively (regardless of PEEP), bilateral infiltration on frontal chest radiograph, and PCWP <18 mm Hg, or no clinical evidence of left atrial hypertension and congestive heart failure.[32] The LIS ranges from 0 to 4, with values ≥1 and <2.5, and ≥2.5 used as cut off values for ALI and ARDS, respectively.[28, 33] The LIS was also used to characterise the severity of the syndromes.[33]

Study protocol

Patients were included within 3 h after major surgery or gastrointestinal bleeding and 12 h after meeting sepsis criteria. Demographics and clinical data were recorded, including the Acute

Physiology and Chronic Health Evaluation (APACHE) II score. The F_IO_2, V_t, plateau inspiratory pressure, and PEEP were taken from the ventilator. Total respiratory dynamic compliance was calculated from V_t/(plateau pressure-PEEP). Arterial blood samples were obtained for determinations of P_aO_2, P_aCO_2, and O_2 saturations (Rapidlab 865, Bayer Diagnostics; Tarrytown, NY, USA, at 37 °C). Mixed or central venous blood, when a pulmonary artery catheter was in place, was taken simultaneously for measurement of gas pressures and saturations. Venous admixture was calculated according to standard formulae, with central venous substituting for mixed venous blood if unavailable. At the time of measurements, we also recorded CVP and inotropic/vasopressor treatment. Plasma samples were collected and stored at –80 °C until assayed.

A mobile probe system was used at the bedside to measure the PLI of ^{67}Ga-transferrin protein, as a measure of pulmonary permeability (n=108), as previously described.[26, 28, 29, 34] Technical problems precluded the PLI measurement in 4 patients. The reproducibility is within 10%.[26, 28] In brief, autologous erythrocytes were labelled with ^{99m}Technetium (^{99m}Tc, 11 MBq, physical half-life 6 h; Mallinckrodt Diagnostica; Petten, The Netherlands). Transferrin was labelled in vivo, following intravenous injection of ^{67}Ga-citrate (4.5 MBq, physical half-life 78 h; Mallinckrodt Diagnostica). Patients were in the supine position and two scintillation detection probes (Eurorad C.T.T.; Strasbourg, France) were positioned over the right and left lung apices. Starting at the time of ^{67}Ga-citrate injection, radioactivity was detected during 30 minutes and blood samples (2 ml aliquots) were taken every 4 min for 30 min. The ^{99m}Tc and ^{67}Ga counts were corrected for background radioactivity, physical half-life, spill-over, and expressed as counts per minute (CPM) per lung field. Each blood sample was weighted and ^{99m}Tc and ^{67}Ga counts were determined with a single well well-counter (LKB Wallac 1480 WIZARD, Perkin Elmer, Life Science; Zaventem, Belgium), 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. We choose PLI $\geq$14.7 x10^{-3}/min and $\geq$30.0 x10^{-3}/min as cut off values for ALI and ARDS, respectively, since the former is the upper limit of normal and PLI is typically elevated two-fold or more in ARDS.[26, 28] In order to judge oedema, EVLW was measured with help of transpulmonary thermal-dye dilution (n=94), as previously described.[28-31, 34] Technical problems precluded the EVLW measurement in 18 patients. We choose EVLW $\geq$10 ml/kg as cut off value for high EVLW, since the EVLW usually exceeds 10 ml/kg in case of pulmonary oedema (normal range 3-7 ml/kg).[31] The reproducibility of EVLW measurements is within 10%.[30] An anteroposterior chest radiograph was made with patients in supine position in order to calculate the LIS.[28, 33] The duration of mechanical ventilation was defined as the interval from intubation to extubation. Patients were followed until death or discharge from the ICU to record the ICU mortality rate.

Assays

Ang-1 and Ang-2 were measured in duplicate in ethylenediaminetetraacetic acid (EDTA) plasma samples from 112 patients and 15 healthy volunteers (controls, age 56 [45-73] years, 12 male, 3 female) using the human Ang-1 and Ang-2 DuoSet ELISA Development kits (R&D systems, Inc.; Minneapolis, Minnesota, USA). The Ang-2/Ang-1 ratio was calculated. VEGF was measured in duplicate in EDTA plasma samples from all septic patients, 12 non-septic patients

(samples from 4 cardiac surgery, 4 major vascular surgery, and 4 other major surgery patients were randomly chosen) and 4 healthy volunteers (randomly chosen) using the human VEGF Quantikine ELISA kit (R&D systems, Inc.). VWF was measured in duplicate in EDTA plasma samples from 112 patients and 15 healthy volunteers using rabbit anti-human VWF polyclonal antiserum and rabbit anti-human VWF peroxidase-conjugate (Dako Netherlands B.V.; Heverlee, Belgium). VWF is expressed as percentage of a pooled plasma control reference which is standardized against the 1st International VWF Standard. The detection limits for Ang-1, Ang-2, and VEGF were 0.02 ng/ml, 0.03 ng/ml, and 3.9 pg/ml, respectively. The intra-assay coefficients of variation were <7%, <4%, <7%, and <8% for Ang-1, Ang-2, VEGF, and VWF, respectively. The inter-assay coefficients of variation were <5%, <8%, and <8% for Ang-1, Ang-2, and VWF, respectively.

Statistical analysis

All data, except the APACHE II scores, tidal volumes, P_aO_2/F_IO_2 ratios, and VWF levels were non-Gaussian distributed. To obtain Gaussian distributions, data were logaritmically transformed prior to the statistical analysis. Afterwards, data were transformed back to the original scale. One-way analysis of variance (ANOVA) was used to evaluate whether continuous variables were different and to evaluate whether the Ang-1, Ang-2, VEGF, and VWF levels showed a per-category trend from non-ALI to ARDS according to AECC or LIS criteria, from normal PLI to elevated PLI, and from normal PLI and EVLW to elevated PLI and EVLW, as determined by PLI $<14.7 \times 10^{-3}$/min to PLI $\geq 30.0 \times 10^{-3}$/min, and by PLI $<30.0 \times 10^{-3}$/min and EVLW <10 ml/kg to PLI $\geq 30.0 \times 10^{-3}$/min and EVLW ≥10 ml/kg, respectively. If the one-way ANOVA indicated significance, the Student's t-test was used to further evaluate differences. The Bonferroni Holm method was used to adjust for multiple testing. The Pearson correlation coefficient (r) (95% confidence interval) was used to express relations. Standard multiple linear regression was performed to evaluate whether predictor variables Ang-2 and VEGF correlated to the outcome variable PLI. Areas under the receiver operating characteristic (ROC) curve (AUC) (95% confidence interval) were calculated to evaluate the predictive values of variables. The X^2 test or Fisher's exact test were used to compare categorical variables. A $P<0.05$ was considered statistically significant. Data are expressed as mean (95% confidence interval), or as number of patients (percentage). Differences are expressed as arithmetic mean difference (95% confidence interval), P-value (Gaussian distributed data) or geometric mean ratio (95% confidence interval), P-value (non-Gaussian distributed data). Exact P values are given if $P>0.001$.

RESULTS

Characteristics of septic and non-septic patients

Septic and non-septic patients were comparable with respect to age and sex (supplementary table 1A). The APACHE II scores, the use of vasopressor/inotropic therapy and the mortality rates were higher in septic than in non-septic patients. Septic patients also had a higher occurrence of ALI/ARDS, higher LIS, lower P_aO_2/F_IO_2 ratio, higher PLI and EVLW, more often a PLI $\geq$30.0 x10^{-3}/min and EVLW $\geq$10 ml/kg, and longer duration of mechanical ventilation (table 1).

Mediators in controls, septic, and non-septic patients

Ang-1 levels were lower in patients than in controls (1.8 [0.3, 11.3] ng/ml Ang-1 in controls; geometric mean ratio 4.55 [2.40, 8.64], P<0.001) and comparable between septic and non-septic patients. Ang-2 and VWF levels were higher in patients than in controls (0.1 [0.0, 1.2] ng/ml Ang-2 in controls; 95 [62, 128] % of reference VWF in controls; geometric mean ratio Ang-2 0.16 [0.06, 0.41], P<0.001; arithmetic mean difference VWF 65 [46, 83] % of reference, P<0.001). VEGF also tended to be higher in patients than in controls (13.6 [5.0, 36.9] pg/ml VEGF in controls; geometric mean ratio 0.48 [0.13, 1.81], P=0.268). Ang-2, VEGF, and VWF levels were higher in septic than in non-septic patients (table 1).

Table 1. Respiratory characteristics and mediators in septic and non-septic patients.

	Sepsis n=24	Non-sepsis n=88	Mean difference or ratio (95% CI), P-value
Respiratory characteristics			
ALI*	10 (42)	19 (22)	61 (46, 76), <0.001
ARDS*	12 (50)	8 (9)	
LIS†	2.14 (1.04, 4.41)	0.93 (0.28, 3.05)	2.30 (1.89, 2.79), <0.001
P_aO_2/F_IO_2 (mm Hg)*	207 (104, 311)	310 (88, 533)	103 (71, 135), <0.001
PLI (x10^{-3}/min)†	59 (15, 228)	20 (5, 87)	2.89 (2.08, 4.01), <0.001
PLI $\geq$30.0 x10^{-3}/min*	21 (88)	17 (19)	68 (53, 84), <0.001
EVLW (ml/kg)†	8.6 (2.3, 31.7)	6.1 (2.6, 14.2)	1.41 (1.04, 1.91), 0.027
EVLW $\geq$10 ml/kg*	9 (38)	10 (11)	26 (6, 47), 0.012
Duration of mechanical ventilation (h)†	281 (50, 1578)	19 (1, 295)	14.44 (7.90, 26.41),<0.001
Mediators			
Ang-1 (ng/ml)†	0.5 (0.0, 58.1)	0.4 (0.0, 32.4)	1.17 (0.42, 3.30), 0.759
Ang-2 (ng/ml)†	4.1 (0.6, 27.3)	0.4 (0.0, 10.4)	10.66 (6.31, 18.03), <0.001
VEGF (pg/ml)†	63.6 (5.0, 807.2) n=24	20.7 (2.7, 160.1) n=12	3.07 (1.30, 7.24), 0.012
VWF (% of reference)*	271 (124, 418)	130 (1, 258)	141 (111, 172), <0.001

Variables are expressed as arithmetic* or geometric mean† (95% confidence interval), or as number of patients (percentage) with arithmetic mean difference or geometric mean ratio (95% confidence interval), P-value. Abbreviations: CI, confidence interval; ALI, acute lung injury; ARDS, acute respiratory distress syndrome (according to the AECC criteria); LIS, lung injury score; P_aO_2, partial pressure of O_2 in arterial blood; F_IO_2, inspiratory O_2 fraction; PLI, pulmonary leak index; EVLW, extravascular lung water; Ang, angiopoietin; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor. Control levels of mediators were 1.8 (0.3, 11.3) ng/ml Ang-1, 0.1 (0.0, 1.2) ng/ml Ang-2, 13.6 (5.0, 36.9) pg/ml VEGF, 95 (62, 128) % of reference VWF.

Characteristics of non-ALI, ALI, and ARDS patients according to the AECC criteria
ALI and ARDS patients had higher plateau pressure, lower total respiratory dynamic compliance, lower P_aO_2, higher F_IO_2, lower P_aO_2/F_IO_2 ratio, higher LIS, and longer duration of mechanical ventilation than non-ALI patients. The PLI was higher in ARDS than in non-ALI patients. The percentage of patients with PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg increased from non-ALI to ALI and ARDS (P=0.043 and P=0.030, respectively, X^2 test). ALI and ARDS patients also had higher venous admixture, and higher CVP associated with higher PEEP levels, than non-ALI patients. ARDS patients had lower P_aO_2, higher F_IO_2, lower P_aO_2/F_IO_2 ratio, higher LIS, and higher venous admixture than ALI patients (table 2 and supplementary table 2A).

Mediators in non-ALI, ALI, and ARDS patients
Ang-1 levels did not differ between non-ALI, ALI, and ARDS patients according to AECC criteria (table 2 and supplementary table 2A). Ang-2 levels and Ang-2/Ang-1 ratios were higher in ALI and ARDS than in non-ALI patients (fig 1A and supplementary fig 2A). Ang-2 levels were also higher in ALI and ARDS patients than in non-ALI patients and higher in ARDS than in ALI patients according to the LIS criteria (fig 1B). VEGF levels tended to be higher in ALI and ARDS than in non-ALI patients (geometric mean ratio 0.10 [0.18, 1.28], P=0.137 for non-ALI vs. ALI/ARDS). VWF levels were higher in ALI and ARDS patients than in non-ALI patients (table 2 and supplementary table 2A).

Mediators, pulmonary permeability oedema, and severity of ALI/ARDS
Ang-2 levels increased in parallel with a rise in PLI (fig 1C). Ang-1 levels did not correlate to the PLI (r=-0.07 [-0.26, 0.20], P=0.463), while Ang-2 and VWF levels did (r=0.34 [0.16, 0.49], P<0.001 and r=0.24 [0.05, 0.41], P=0.014, respectively). Ang-2 also correlated to the PLI in the smaller group of 36 patients in whom VEGF was measured (r=0.44 [0.13, 0.67], P=0.008), while VEGF did not (r=0.20 [-0.13, 0.50], P=0.233). Indeed, multiple linear regression analysis with PLI as outcome and Ang-2 and VEGF as predictor variables showed that Ang-2 predicted PLI (regression coefficient=0.41 [0.10, 0.65], P=0.014), while VEGF did not (regression coefficient=0.12 [-0.22, 0.43], P=0.463). Patients with PLI ≥30.0 $x10^{-3}$/min, regardless of EVLW, had higher Ang-2 levels than patients with PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg (fig 1D). The Ang-2/Ang-1 ratio was higher in patients with PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg than in patients with PLI <30.0 x 10^{-3}/min and EVLW <10 ml/kg (supplementary fig 2B). The percentage of patients with non-ALI or ALI according to LIS criteria decreased from normal (PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg) to permeability oedema (PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg), while the percentage of patients with ARDS increased (P=0.026, X^2 test, fig 1D). For AECC criteria there was a tendency for a similar distribution (P=0.090, X^2 test). None of the mediators positively correlated to EVLW (Ang-1: r=-0.10 [-0.30, 0.10], P=0.322; Ang-2: r=0.11 [-0.10, 0.30], P=0.314; VEGF: r=-0.39 [-0.65, -0.05], P=0.026; VWF: r=0.04 [-0.16, 0.24], P=0.682).

To further study clinical significance, we evaluated whether Ang-1, Ang-2, VEGF and VWF levels correlated to the LIS, the P_aO_2/F_IO_2 ratios, the compliance, the duration of mechanical ventilation, and the ICU mortality. Ang-2, VEGF, and VWF levels positively correlated to the LIS (r=0.43 [0.26, 0.57], P<0.001; r=0.33 [0.00, 0.60], P=0.049 and r=0.53 [0.39, 0.66], P<0.001, respectively). Ang-2 and VWF inversely correlated to P_aO_2/F_IO_2 ratios (r=-0.22 [-0.03, -0.39], P=0.022 and r=-0.43 [-0.26, -0.57], P<0.001, respectively). Ang-2, VEGF, and VWF inversely correlated to compliance (r=-0.44 [-0.28, -0.58], P<0.001; r=-

0.40 [-0.08, -0.65], P=0.017 and r=-0.46 [-0.30, -0.60], P<0.001, respectively). Ang-2 and VWF positively correlated to the duration of mechanical ventilation (r=0.62 [0.50, 0.73], P<0.001 and r=0.58 [0.44, 0.69], P<0.001, respectively). Ang-2 and VWF levels were higher in non-survivors than in survivors (geometric mean ratio 0.15 [0.08, 0.27], P<0.001 and arithmetic mean difference 71 [21, 121] % of reference, P=0.006, respectively). Ang-2 levels correlated to Ang-1 and VWF levels (r=0.29 [0.11, 0.45], P=0.002 and r=0.46 [0.29, 0.59], P<0.001, respectively).

Table 2. Respiratory and haemodynamic characteristics and mediators: mean differences or ratios between non-ALI, ALI, and ARDS patients according to American-European consensus conference criteria.

	ALI vs. non-ALI	ARDS vs. non-ALI	ARDS vs. ALI
Respiratory and haemodynamic characteristics			
Pplat (cm H_2O)†	0.77 (0.68, 0.89), <0.001	0.65 (0.57, 0.76), <0.001	0.84 (0.70, 1.02), 0.081
PEEP (cm H_2O)†	0.77 (0.60, 0.98), 0.035‡	0.61 (0.52, 0.73), <0.001	0.80 (0.58, 1.10), 0.167
Compliance (ml/cm H_2O)†	1.24 (1.07, 1.42), 0.004	1.31 (1.10, 1.56), 0.003	1.06 (0.87, 1.30), 0.546
Tidal volume (ml/kg)*	0.05 (-0.67, 0.77), 0.878	1.15 (0.13, 2.18), 0.029‡	1.10 (0.00, 2.20), 0.051
P_aO_2 (mm Hg)†	1.21 (1.11, 1.32), <0.001	1.62 (1.44, 1.81), <0.001	1.34 (1.20, 1.48), <0.001
F_IO_2†	0.90 (0.86, 0.95), <0.001	0.71 (0.66, 0.75), <0.001	0.78 (0.72, 0.85), <0.001
P_aO_2/F_IO_2 (mm Hg)*	99 (72, 127), <0.001	200 (170, 229), <0.001	100 (82, 119), <0.001
LIS†	0.45 (0.36, 0.55), <0.001	0.31 (0.25, 0.39), <0.001	0.70 (0.58, 0.85), 0.001
PLI ($x10^{-3}$/min)†	0.91 (0.62, 1.32), 0.604	0.59 (0.39, 0.89), 0.013	0.65 (0.39, 1.09), 0.102
EVLW (ml/kg)†	0.86 (0.70, 1.05), 0.128	0.73 (0.49, 1.09), 0.117	0.85 (0.57, 1.29), 0.434
Duration of mechanical ventilation (h)†	0.24 (0.11, 0.53), 0.001	0.16 (0.08, 0.31), <0.001	0.65 (0.22, 1.90), 0.423
Venous admixture†	0.65 (0.54, 0.79), <0.001	0.41 (0.35, 0.48), <0.001	0.63 (0.52, 0.77), <0.001
CVP (mm Hg)†	0.67 (0.50, 0.91), 0.010	0.49 (0.38, 0.62), <0.001	0.72 (0.53, 1.00), 0.048‡
Mediators			
Ang-1 (ng/ml)†	0.77 (0.29, 2.06), 0.598	2.28 (0.69, 7.51), 0.173	2.96 (0.79, 11.10), 0.104
VEGF (pg/ml)†	0.54 (0.21, 1.41), 0.197	0.41 (0.12, 1.46), 0.158	0.76 (0.26, 2.20), 0.594
VWF (% of reference)*	63 (26, 100), 0.001	110 (64, 156), <0.001	47 (-6, 100), 0.080

Arithmetic mean differences* or geometric mean ratios† (95% confidence interval), P-value. Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome (according to the AECC criteria); Pplat, plateau pressure; PEEP, positive end-expiratory pressure; P_aO_2, partial pressure of O_2 in arterial blood; F_IO_2, inspiratory O_2 fraction; LIS, lung injury score; PLI, pulmonary leak index; EVLW, extravascular lung water; CVP, central venous pressure. Ang, angiopoietin; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor. ‡Rejected according to Bonferroni Holm method.

Predictive value of Ang-2 levels for permeability oedema and ARDS

The ROC curves for ARDS versus ALI and non-ALI characterised by the AECC (supplementary fig 3A) and the LIS criteria (supplementary fig 3B) showed that Ang-2 and VWF, in contrast to Ang-1 and VEGF (supplementary fig 3C and 3D), were of predictive value.

DISCUSSION

This study shows that circulating Ang-2 is associated with pulmonary permeability oedema and the severity of ALI/ARDS in septic and non-septic patients with or at risk for these syndromes. Furthermore, our study demonstrates that Ang-2 levels correlate to VWF levels, suggesting a similar source and stimulus for release.

In our patients, the level of circulating Ang-1 did not differ between non-ALI, ALI, or ARDS patients, whereas Ang-2 and the Ang-2/Ang-1 ratio were elevated in parallel with pulmonary permeability and ALI/ARDS. This supports the idea that a high Ang-2, which antagonises the protective role of Ang-1, is one of the mediators involved in the increase in pulmonary permeability leading to ALI/ARDS.[2, 4, 5, 7, 11, 21] Moreover, Ang-2 was specifically associated with pulmonary permeability, as it correlated to the PLI and not to the EVLW. Ang-2 levels also related to the severity of ALI/ARDS as reflected by the LIS and by the predictive value for ARDS in the ROC curve. These associations suggest a contributory role of Ang-2 in the pathogenesis of ALI/ARDS, but further studies, including serial plasma and alveolar compartment measurements of Ang-2 and blocking studies are necessary to confirm this.

The Ang-2 levels in our ARDS patients are comparable to those reported in severe sepsis and septic shock by Orfanos et al.[4] and mild sepsis in the study of Parikh et al.[2] However, Parikh et al.[2] reported higher Ang-2 levels in their severe septic patients. This discrepancy may be explained by a higher disease severity in their study, suggested by higher APACHE II scores.[2] Our study extends the observations of Parikh et al.[2] by demonstrating that circulating Ang-2 is associated with the, independently measured, pulmonary permeability oedema and severity of ALI/ARDS, regardless of the origin.

VEGF levels were higher in septic than in non-septic patients and tended to be higher in ALI and ARDS patients than in non-ALI patients, in agreement with the literature.[12-15] We could not establish an association between circulating VEGF and directly measured pulmonary permeability in agreement with some, but not all studies using surrogate indicators of systemic permeability.[13-15] This suggests that circulating Ang-2 may better reflect pulmonary permeability oedema of ALI/ARDS than circulating VEGF.

Ang-2 and VWF were simultaneously enhanced in plasma of ALI/ARDS or septic patients. Although a common effect on their clearance cannot be excluded yet, it is likely that both are released together from the Weibel Palade body after activation of the endothelium. This may explain why VWF is a marker of endothelial activation and injury resulting in an association with pulmonary permeability, occurrence and severity of ALI/ARDS, and subsequent ICU mortality, while it may not be directly involved in permeability.[24, 25]

Our study carries some limitations. We cannot exclude that the relatively high tidal volumes used in some of our patients potentially harmed the lungs during ALI/ARDS. Furthermore, it is uncertain whether the source of Ang-2 is the systemic or pulmonary endothelium. If it is true that Ang-2 mainly acts in an autocrine manner,[16, 22] a pulmonary source is favoured. Finally, the observation that some patients had high PLI and a normal EVLW can be explained in part by inaccessibility of the thermal indicator to injured lung areas, thereby underestimating lung oedema, or to compensatory Starling forces and increased lymph flow, removing excess EVLW in spite of increased permeability.[29]

In conclusion, Ang-2 contributes to the pulmonary permeability oedema of ALI/ARDS in septic and non-septic patients with or at risk for these syndromes. VWF and Ang-2 may be simultaneously released from the endothelium, thereby explaining the previously reported marker function of VWF for endothelial activation associated with ALI/ARDS.

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COMPETING INTEREST STATEMENT
Neither of the authors had competing interests.

FIGURE LEGENDS

Figure 1. Ang-2 (ng/ml) in controls and patients with non-ALI, ALI, or ARDS according to AECC (A) or LIS criteria (B), in patients grouped according to PLI (C), or the combination of PLI and EVLW (D). Bars represent mean, whiskers represent standard error of the mean, *significant vs. control (A), non-ALI (B), PLI <14.7 $x10^{-3}$/min (C), or PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg (D), †significant vs. non-ALI (A), ALI (B), or PLI ≥14.7 and <30.0 $x10^{-3}$/min (C). Differences between means are expressed as mean ratio (95% confidence interval), P-value. For abbreviations see text.

A. For non-ALI (n=63), ALI (n=29), and ARDS (n=20) vs. controls (n=15): 0.28 (0.11, 0.72), 0.009; 0.07 (0.03, 0.19), <0.001; 0.08 (0.03, 0.23), <0.001, respectively. For ALI and ARDS vs. non-ALI: 0.26 (0.12, 0.55), 0.001; 0.28 (0.12, 0.67), 0.005, respectively. For ARDS vs. ALI: 1.08 (0.40, 2.90), 0.873. The Ang-2 level showed a per-category trend from control to ARDS (P<0.001) with a mean increase of 0.39 ng/ml Ang-2 per category.
B. For ALI (LIS ≥1 and <2.5, n=53) and ARDS (LIS ≥2.5, n=16) vs. non-ALI (LIS <1, n=43): 0.44 (0.21, 0.89), 0.023; 0.09 (0.05, 0.17), <0.001, respectively. For ARDS vs. ALI: 0.21 (0.11, 0.39), <0.001. The Ang-2 level showed a per-category trend from non-ALI to ARDS (P<0.001) with a mean increase of 1.52 ng/ml Ang-2 per category.
C. For PLI ≥30.0 $x10^{-3}$/min (n=38) vs. PLI ≥14.7 and <30.0 $x10^{-3}$/min (n=36) and PLI <14.7 $x10^{-3}$/min (n=34): 0.23 (0.10, 0.52), 0.001; 0.28 (0.12, 0.62), 0.002, respectively. For PLI ≥14.7 and <30.0 $x10^{-3}$/min vs. PLI <14.7 $x10^{-3}$/min: 1.23 (0.55, 2.73), 0.607. The Ang-2 level showed a per-category trend from PLI <14.7 $x10^{-3}$/min to PLI ≥30.0 $x10^{-3}$/min (P=0.002) with a mean increase of 0.57 ng/ml per category.
D. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (n=10), PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg (n=22) and PLI <30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (n=8) vs. PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg (n=50): 0.18 (0.06, 0.57), 0.004; 0.18 (0.08, 0.41), <0.001; 0.51 (0.15, 1.82), 0.296. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg and PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg vs. PLI <30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg: 0.36 (0.05, 2.39), 0.269; 0.35 (0.08, 1.54), 0.159. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg vs. PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg: 1.02 (0.27, 3.84), 0.972. The Ang-2 level showed a per-category trend from PLI <14.7 $x10^{-3}$/min and EVLW <10 ml/kg to PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (P=0.002) with a mean increase of 0.52 ng/ml per category.

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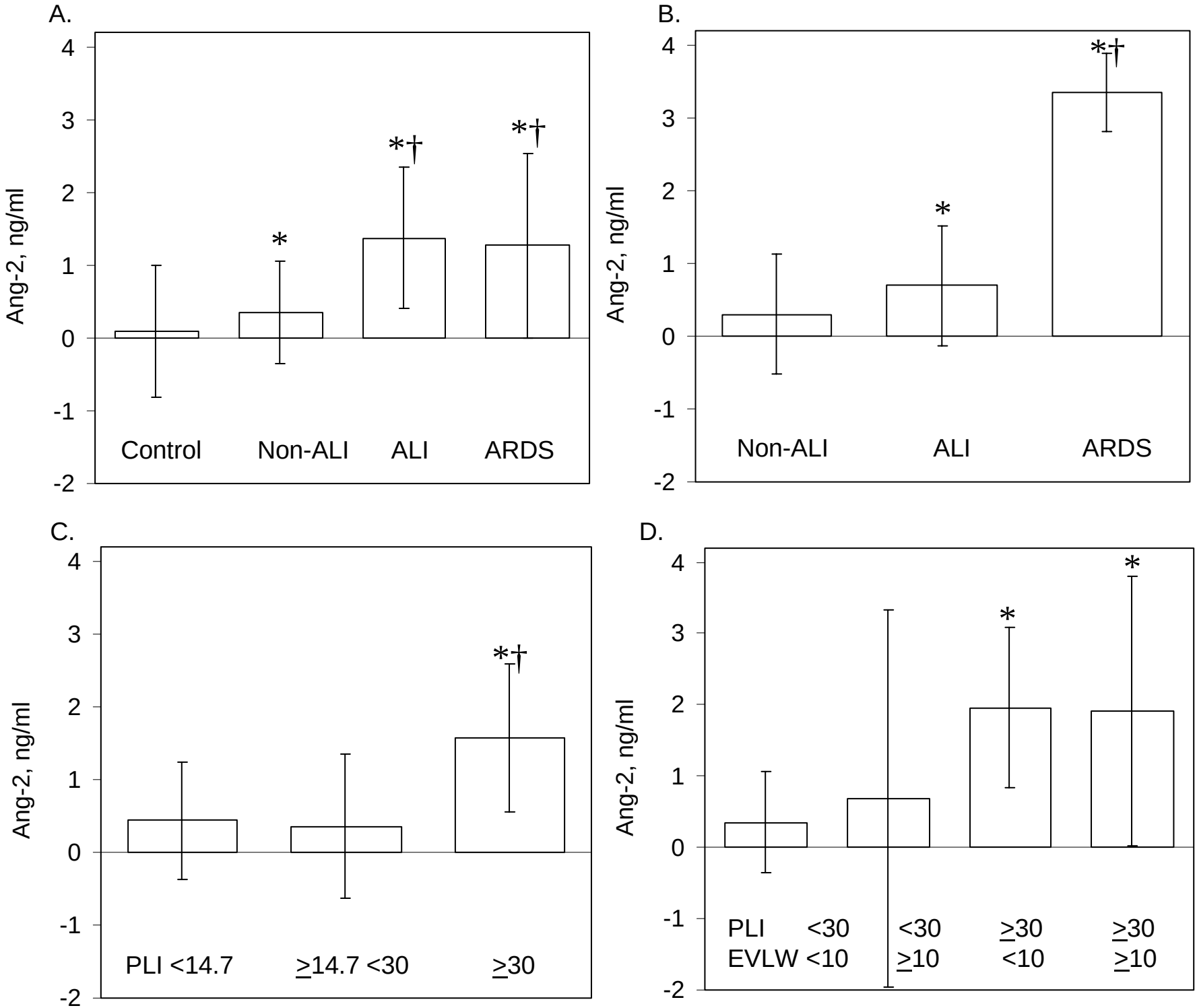
Figure 1.



DATA SUPPLEMENT

Table 1A. Patient characteristics.

	Sepsis n=24	Non-sepsis n=88	Mean or proportion difference (95% confidence interval), P-value
Age (year, range)	60 (35-77)	60 (22-77)	-1 (-6, 5), 0.540
Sex, M/F	18 (75)/6 (25)	68 (77)/20 (23)	-2 (-22, 17), 0.815
Underlying disease			
prior myocardial infarction	2 (8)	25 (28)	-19 (-33, -4), 0.051
acute pancreatitis	1 (4)	0 (0)	4 (-4, 12), 0.214
malignancy	7 (29)	19 (22)	8 (-13, 28), 0.436
chronic lung disease	3 (13)	2 (2)	10 (-3, 24), 0.065
Source of sepsis			
pneumonia	12 (50)		
abdominal	4 (17)		
other	8 (33)		
Type of non-septic critical illness			
cardiac surgery		38 (43)	
major vascular surgery		26 (30)	
other major surgery		23 (26)	
gastro-intestinal bleeding		1 (1)	
Culture results			
blood gram –	3 (13)		
blood gram +	5 (21)		
local* gram –	7 (29)		
local* gram +	6 (25)		
ICU-acquired sepsis	7 (29)		
APACHE II score	14 (5, 24)	9 (1, 17)	5 (3, 7), <0.001
Vasopressor/inotropic therapy	21 (88)	56 (64)	24 (7, 40), 0.025
Mortality in ICU	9 (38)	4 (5)	33 (13, 53), <0.001

Variables are expressed as arithmetic mean (95% confidence interval), or as number of patients (percentage) with arithmetic mean or proportion difference (95% confidence interval), P-value. ICU, Intensive Care Unit; APACHE, Acute Physiology and Chronic Health Evaluation. *local when blood-culture negative.

Table 2A. Respiratory and haemodynamic characteristics and mediators of non-ALI, ALI, and ARDS patients according to American-European consensus conference criteria.

	Non-ALI n=63	ALI n=29	ARDS n=20
Respiratory and haemodynamic characteristics			
Pplat (cm H_2O)†	17 (12, 25)	22 (11, 43)	26 (15, 46)
PEEP (cm H_2O)†	6 (4, 12)	8 (3, 28)	11 (5, 23)
Compliance (ml/cm H_2O)†	56 (30, 105)	45 (24, 85)	42 (21, 87)
Tidal volume (ml/kg)*	7.4 (5.1, 9.8)	7.5 (4.0, 10.9)	8.6 (4.6, 12.5)
P_aO_2 (mm Hg)†	141 (82, 242)	117 (85, 160)	87 (58, 132)
F_IO_2†	0.4 (0.3, 0.5)	0.5 (0.4, 0.6)	0.6 (0.4, 0.8)
P_aO_2/F_IO_2 (mm Hg)*	350 (148, 552)	251 (193, 308)	150 (80, 221)
LIS†	0.73 (0.27, 1.98)	1.65 (0.78, 3.47)	2.34 (1.42, 3.87)
PLI ($x10^{-3}$/min)†	23 (5, 112)	25 (4, 160)	39 (9, 174)
PLI $\geq$30.0 $x10^{-3}$/min*	16 (25)	11 (38)	11 (55)‡‡
EVLW (ml/kg)†	6.0 (2.6, 13.7)	7.0 (3.3, 14.8)	8.2 (1.8, 38.4)
EVLW $\geq$10 ml/kg*	6 (10)	6 (21)	7 (35)‡
Duration of mechanical ventilation (h)†	17 (1, 198)	71 (2, 3058)	109 (5, 2418)
Venous admixture†	0.15 (0.06, 0.35)	0.22 (0.10, 0.49)	0.36 (0.22, 0.56)
CVP (mm Hg)†	3 (1, 12)	5 (1, 21)	7 (3, 15)
Mediators			
Ang-1 (ng/ml)†	0.4 (0.0, 43.6)	0.6 (0.0, 28.1)	0.2 (0.0, 23.7)
VEGF (pg/ml)†	25.0 (3.0, 210.0) n=9	46.1 (5.3, 399.7) n=14	60.9 (2.6, 1453.1) n=13
VWF (% of reference)*	124 (0, 255)	187 (11, 363)	234 (47, 420)

Variables are expressed as arithmetic* or geometric† mean (95% confidence interval), or as number of patients (percentage). Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; Pplat, plateau pressure; PEEP, positive end-expiratory pressure; P_aO_2, partial pressure of O_2 in arterial blood; F_IO_2, inspiratory O_2 fraction; LIS, lung injury score; PLI, pulmonary leak index; EVLW, extravascular lung water; CVP, central venous pressure; Ang, angiopoietin; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor. X^2 test: ‡P=0.030, ‡‡P=0.043. Arithmetic mean differences or geometric mean ratios (95% confidence interval), P-values are shown in Table 2.

FIGURE LEGENDS

Figure 2. The Ang-2/Ang-1 ratio in controls and patients with non-ALI, ALI, or ARDS according to AECC criteria (A), and in patients grouped according to the combination of PLI and EVLW (B). Bars represent mean, whiskers represent standard error of the mean, *significant vs. control (A) or PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg (B), †significant vs. non-ALI (A). Differences between means are expressed as mean ratio (95% confidence interval), P-value. For abbreviations see text.

A. A. For non-ALI, ALI, and ARDS vs. controls: 0.07 (0.02, 0.26), <0.001; 0.02 (0.01, 0.08), <0.001; 0.01 (0.00, 0.03), <0.001, respectively. For ALI and ARDS vs. non-ALI: 0.34 (0.12, 0.96), 0.041 (rejected by Bonferroni Holm method); 0.12 (0.04, 0.43), P=0.001, respectively. For ARDS vs. ALI: 0.37 (0.11, 1.26), 0.108. The Ang-2/Ang-1 ratio showed a per-category trend from control to ARDS (P<0.001) with a mean increase of 2.21 ratio units per category.
B. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (n=10), PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg (n=22) and PLI <30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (n=8) vs. PLI <30.0 $x10^{-3}$/min and EVLW <10 ml/kg (n=50): 4.06 (0.83, 19.98), 0.083; 0.16 (0.05, 0.53), 0.003; 3.5 (0.62, 19.80), 0.151. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg and PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg vs. PLI <30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg: 1.16 (0.10, 13.62), 0.902; 1.81 (0.20, 16.35), 0.585. For PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg vs. PLI ≥30.0 $x10^{-3}$/min and EVLW <10 ml/kg: 0.64 (0.08, 4.96), 0.659. The Ang-2 level showed a per-category trend from PLI <14.7 $x10^{-3}$/min and EVLW <10 ml/kg to PLI ≥30.0 $x10^{-3}$/min and EVLW ≥10 ml/kg (P=0.004) with a mean increase of 0.76 ratio units per category.

Figure 3. Ang-2 and VWF predicted ARDS characterised by the AECC criteria (area under the curve [AUC] 0.69 [0.57, 0.82], P=0.006 and 0.80 [0.69, 0.91], P<0.001, respectively [A] and by the LIS criteria (0.85 [0.78, 0.92], P<0.001 and 0.85 [0.78, 0.92], P<0.001, respectively [B]). Ang-1 and VEGF did not predict ARDS characterised by the AECC (0.39 [0.19, 0.59], P=0.273 and 0.66 [0.44, 0.87], P=0.116, respectively [C]) or the LIS (0.31 [0.14, 0.51], P=0.057 and 0.65 [0.46, 0.85], P=0.132 [D]) criteria.

Figure 2.

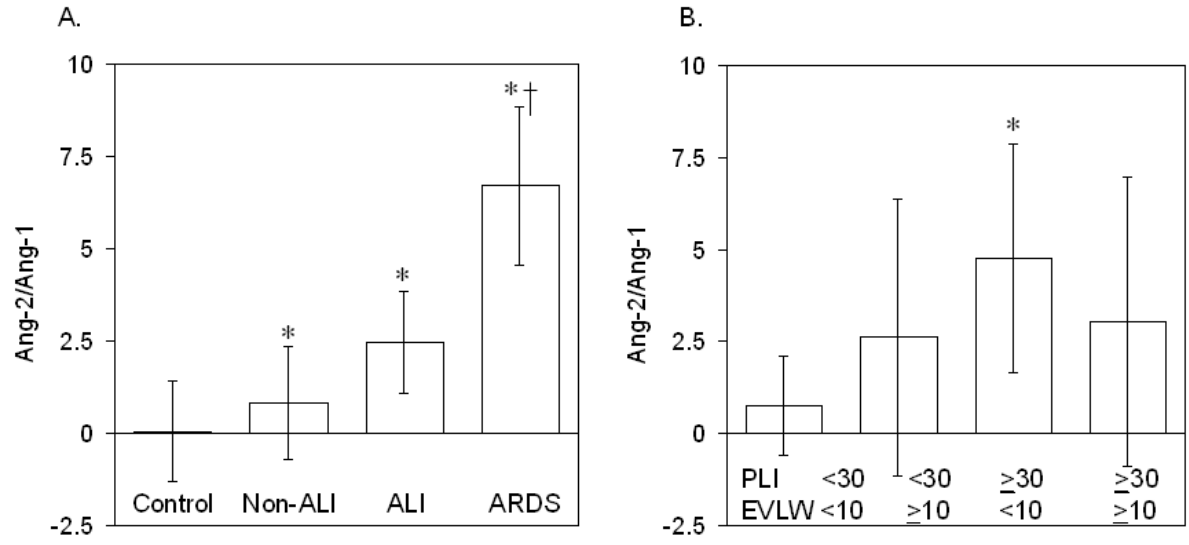


Figure 3.