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Circulating angiotensin-2 levels in the course of septic shock: relation with fluid balance, pulmonary dysfunction and mortality

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Abstract *Purpose:* To investigate whether angiotensin-2, von Willebrand factor (VWF) and angiotensin-1 relate to surrogate indicators of vascular permeability, pulmonary dysfunction and intensive care unit (ICU) mortality throughout the course of septic shock. *Methods:* In 50 consecutive mechanically ventilated septic shock patients, plasma angiotensin-2, VWF and angiotensin-1 levels and fluid balance, partial pressure of oxygen/inspiratory oxygen fraction and the oxygenation index as indicators of vascular permeability and pulmonary dysfunction, respectively, were measured until day 28. *Results:* Angiotensin-2 positively related to the fluid balance and pulmonary dysfunction, was higher in

non-survivors than in survivors and independently predicted non-survival throughout the course of septic shock. VWF inversely related to the fluid balance and pulmonary dysfunction throughout the course of septic shock, was comparable between survivors and non-survivors and predicted non-survival on day 0 only. Angiotensin-1 positively related to pulmonary dysfunction throughout the course, but did not differ between survivors and non-survivors. *Conclusions:* In contrast to VWF, plasma angiotensin-2 positively relates to fluid balance, pulmonary dysfunction and mortality throughout the course of septic shock, in line with a suggested mediator role of the protein.

Keywords Angiotensin · von Willebrand factor · Sepsis · Acute lung injury · Vascular permeability

Introduction

Excessive and sustained activation of the endothelium resulting in increased vascular permeability is a crucial process during sepsis [1, 2]. In the lung, the loss of plasma fluid into the interstitial space leads to pulmonary oedema, injury and dysfunction associated with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [1, 3]. Several clinical studies demonstrated that circulating levels of angiotensin-2 (Ang-2) are elevated

at the onset of critical illness in septic and non-septic patients with or at risk for ALI/ARDS, relate to vascular permeability and pulmonary dysfunction and are associated with intensive care unit (ICU) mortality [4–12]. It is not surprising that Ang-2 is a marker of those processes, since pre-formed Ang-2 can be released from the endothelial Weibel-Palade bodies during endothelial activation [13]. Another well-known marker of endothelial activation and dysfunction is von Willebrand factor (VWF), which is a major constituent of the Weibel-Palade body

[13, 14]. We previously showed that Ang-2 and VWF levels are simultaneously enhanced in the plasma of ALI/ARDS or sepsis patients on admission to the ICU or onset of sepsis, suggesting that they are released together upon activation of the endothelium [12]. This may explain why both Ang-2 and VWF levels are associated with vascular permeability and pulmonary dysfunction [4–6, 8, 11, 12], even though VWF may not be directly involved in permeability [15, 16].

Experimental studies demonstrated that angiotensin-1 (Ang-1) and Ang-2 bind to the endothelial Tie2 receptor [17–19]. Ang-1-induced activation of the Tie2 receptor results in vascular stabilisation [20–22]. Ang-2 provokes pulmonary inflammation and vascular leakage partly because of inhibition of the stabilising effect of Ang-1 by preventing Ang-1 from binding the Tie2 receptor [4, 5, 8, 10–12, 23, 24]. These experimental data together with clinical observations [4, 6, 7, 9, 11, 12] suggest that Ang-2 is not only a marker, but also a direct mediator in the pathogenesis of vascular permeability, pulmonary injury and dysfunction. This idea would be further reinforced if the relation between Ang-2, vascular permeability and pulmonary dysfunction persists throughout the course of critical illness in a homogeneous population of septic shock patients, more than VWF and Ang-1 levels. Up till now, the course and clinical correlates of Ang-2 have not been studied, and the predictive value of changes in Ang-2 during the course of septic shock for mortality is unknown. It has been hypothesised that the levels typically decrease in septic survivors and increase in septic patients that do not survive [5]. Only one study reported on the course of Ang-2 levels in multi-trauma patients [9], but clinical correlates were not studied.

The aims of our study were (1) to investigate whether Ang-2, VWF and Ang-1 relate to surrogate indicators of vascular permeability and pulmonary dysfunction throughout the course of septic shock and (2) to study the course of Ang-2, VWF and Ang-1 levels in survivors and non-survivors and their predictive value for ICU mortality. Therefore, we measured plasma levels of Ang-2, VWF and Ang-1, and recorded fluid balance as surrogate indicator of vascular permeability [25–28] and partial pressure of oxygen in arterial blood/inspiratory oxygen fraction (P_aO_2/F_iO_2), oxygenation index [29], pulmonary compliance and ventilator-dependency [30] as surrogate indicators of pulmonary dysfunction, respectively, throughout the course of septic shock.

Materials and methods

The local ethics committee approved this observational study and waived the need for informed consent. Fifty consecutive mechanically ventilated critically ill patients with community or ICU-acquired septic shock admitted to

the ICU of the Radboud University Medical Centre (Nijmegen, The Netherlands) between January 2005 and September 2007 were included within 12 h after meeting septic shock criteria. Sepsis was defined by two or more of the following: abnormal body temperature (<36 or $>38.5^\circ\text{C}$), tachycardia ($>90/\text{min}$), tachypnea [$>20/\text{min}$ or partial pressure of CO_2 in arterial blood ($P_a\text{CO}_2$) <32 mmHg], abnormal white blood cell counts (<4 or $>12 \times 10^9/\text{l}$) and a microbiologically proven or clinically evident source of infection [29]. Septic shock was defined as sepsis complicated by acute circulatory failure characterised by persistent arterial hypotension unexplained by other causes [31]. Hypotension was defined by systolic arterial pressure <90 mmHg or mean arterial pressure (MAP) <70 mmHg for at least 1 h despite adequate fluid resuscitation or requirement of vasopressor support to maintain MAP [31]. ICU-acquired sepsis was defined as sepsis developing after 2 days in the ICU. The origin of septic shock was defined by clinical signs and symptoms and positive local and/or blood cultures. Patients were pressure-controlled or volume-controlled (after surgery) ventilated with a Galileo (Hamilton Medical AG; Rhäzüns, Switzerland) or a Servo ventilator (Maquet, Inc.; Bridgewater, NJ). 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 a $F_i\text{O}_2$ of 40% and a positive end-expiratory pressure (PEEP) of 5 cmH $_2\text{O}$ (inspiration:expiration 1:2) or more, when needed, guided by $P_a\text{O}_2$ (>60 mmHg). Patients were followed until death or discharge from the hospital. The 28-day ICU mortality, total ICU mortality and hospital mortality were recorded. Patients were treated by intensive care physicians not involved in the study according to institutional guidelines. Platelets were infused when the platelet count had dropped below 10 – $20 \times 10^9/\text{l}$, possibly as a result of diffuse intravascular coagulation, and when there were foci with a bleeding tendency.

On the inclusion day (day 0), demographics and clinical data were recorded including the Acute Physiology and Chronic Health Evaluation (APACHE) II score. The number of failing organs was scored. On day 0, day 7 ± 1 days, day 14 ± 2 days and day 28 ± 3 days, arterial blood samples were obtained for determinations of pH, $P_a\text{O}_2$ and $P_a\text{CO}_2$, white blood cell counts and creatinine. The respiratory frequency, tidal volume (V_t), $F_i\text{O}_2$, plateau pressure (P_{plat}), mean airway pressure (P_{mean}) and PEEP were taken from the ventilator. The oxygenation index, which is a measure of oxygenation impairment for the intensity of mechanical ventilation, was calculated from $(P_{\text{mean}} \times F_i\text{O}_2 \times 100)/P_a\text{O}_2$ [29]. Total respiratory dynamic compliance was calculated from $V_t/(\text{plateau pressure} - \text{PEEP})$. Ventilator-free days were defined as follows: ventilator-free days = 0 if the patient dies before ICU day 28; ventilator-free days = $28 - x$ if the patient is successfully

weaned from mechanical ventilation within 28 days, where x is the number of days spent on mechanical ventilation; ventilator-free days = 0 if the patient requires mechanical ventilation for 28 days or more [30]. Days at which a patient was ventilator-dependent were recorded. ALI and ARDS were characterised by $P_aO_2/F_iO_2 \leq 300$ or ≤ 200 mmHg, respectively, and absence of clinical evidence of left atrial hypertension and congestive heart failure, according to the American European Consensus Criteria [32]. Vasopressor/inotropic treatment and body temperature were recorded. The fluid balance was calculated by dividing total fluid input minus total fluid output by the pre-ICU patient weight in kilograms [25–28]. Circulating Ang-2, VWF and Ang-1 were measured as described in the electronic supplementary material (ESM). The statistical analysis is described in the ESM.

Results

Patient characteristics

Patients were grouped as ICU survivor or non-survivor (Table 1, ESM Table 1). All ICU non-survivors, except one (death on day 68), died before ICU day 28. Five ICU survivors died in the hospital at a median 24 (range 2–60) days after discharge from the ICU. ICU survivors and non-survivors were comparable with respect to baseline demographics and with respect to clinical characteristics, except for APACHE II score and hospital stay, which were higher and shorter, respectively, in non-survivors compared to survivors. Twenty-two survivors and 14 non-survivors suffered from ALI/ARDS (Table 1). Haemodynamic, biochemical and respiratory characteristics are reported in the ESM.

Angiotensin and VWF levels, fluid balance and pulmonary dysfunction

Patients had higher Ang-2 and VWF levels on day 0 [median (interquartile range) 2,350 (1,400–4,280) pg/ml and 289 (166–411)% of reference, respectively] than controls [113 (27–287) pg/ml, $P < 0.001$ and 93 (87–113)% of reference, $P < 0.001$, respectively]. Patients had lower Ang-1 levels on day 0 [330 (114–1,020) pg/ml] than controls [2,407 (969–3,730) pg/ml, $P < 0.001$]. Ang-2 levels related positively to the fluid balance throughout the course of septic shock after exclusion of one non-survivor who had extremely high Ang-2 levels ($\beta = 0.20$, $P = 0.043$ in GEE, $r = 0.30$, $P = 0.005$). Furthermore, Ang-2 levels related to indicators of pulmonary dysfunction throughout the course of septic shock, as demonstrated by an inverse relation with the

P_aO_2/F_iO_2 ratio ($\beta = -0.25$, $P < 0.001$ in GEE; $r = -0.25$, $P = 0.012$), a positive relation with the oxygenation index ($\beta = 0.37$, $P < 0.001$ in GEE; $r = 0.26$, $P = 0.017$), a weak inverse relation with the compliance ($\beta = -0.09$, $P = 0.004$ in GEE; $r = -0.05$, $P = 0.645$) and a positive relation with ventilator-dependency ($P = 0.043$ in GEE). In contrast, VWF inversely related to the fluid balance ($\beta = -0.21$, $P = 0.045$ in GEE; $r = -0.27$, $P = 0.014$), positively related to the compliance ($\beta = 0.21$, $P = 0.011$ in GEE; $r = 0.17$, $P = 0.134$) and did not relate to the other respiratory variables throughout the course of septic shock. Ang-1 levels did not relate to the fluid balance, but related to indicators of pulmonary dysfunction throughout the course of septic shock, as demonstrated by an inverse relation with the P_aO_2/F_iO_2 ratio ($\beta = -0.26$, $P = 0.022$ in GEE; $r = -0.21$, $P = 0.034$) and a positive relation with the oxygenation index ($\beta = 0.37$, $P = 0.005$ in GEE; $r = 0.28$, $P = 0.014$) throughout the course of septic shock. The fluid balance related to indicators of pulmonary dysfunction throughout the course of septic shock, as demonstrated by an inverse relation with the P_aO_2/F_iO_2 ratio ($\beta = -0.24$, $P < 0.001$ in GEE; $r = -0.29$, $P = 0.006$), a positive relation with the oxygenation index ($\beta = 0.22$, $P = 0.006$ in GEE; $r = 0.22$, $P = 0.068$) and an inverse relation with the compliance ($\beta = -0.26$, $P = 0.006$ in GEE; $r = -0.29$, $P = 0.014$).

The course of the angiotensin and VWF levels in survivors and non-survivors

Ang-2 levels were higher in non-survivors than in survivors throughout the course of septic shock (Fig. 1). Ang-2 levels decreased in survivors from day 0 to the last ICU day, while they did not change in non-survivors (Figs. 1, 2). The course of VWF levels differed between survivors and non-survivors (Fig. 1), since VWF levels increased in survivors from day 0 to the last ICU day, while they did not change in non-survivors (Fig. 2). Ang-2 levels related to VWF levels on day 0, but the relation was lost throughout the course of septic shock (Fig. 3). Ang-1 levels were comparable between survivors and non-survivors (Fig. 1) and did not change from day 0 to the last ICU day (Fig. 2). The predictive value of angiotensin and VWF levels for ICU mortality is described in the ESM.

Discussion

This study demonstrates that circulating Ang-2 positively relates to the fluid balance, pulmonary dysfunction and

Table 1 Demographic and clinical characteristics of survivors and non-survivors

	Survivors (n = 35)	Non-survivors (n = 15)	P value
Age, year	68 (21–87)	65 (30–102)	0.427
Sex, male	21 (60)	13 (87)	0.099
Surgery prior to sepsis	17 (49)	9 (60)	0.459
Source of sepsis			0.529
Pulmonary	15 (43)	9 (60)	
Abdominal	15 (43)	5 (33)	
Urogenital	1 (3)	0	
Pressure sores	1 (3)	0	
Unknown	5 (14)	2 (13)	
Culture results			0.241
Blood gram –	2 (6)	1 (7)	
Blood gram +	0	2 (13)	
Local ^a gram –	5 (14)	3 (9)	
Local ^a gram +	7 (20)	1 (7)	
Leukocytes day 0, $\times 10^9/l$	11.9 (2.8–86.0)	2.7 (0.4–74.8)	0.661, 0.209
Leukocytes day 7, $\times 10^9/l$	15.6 (5.6–54.5)	11.3 (1.7–243.1)	
Leukocytes day 14, $\times 10^9/l$	12.8 (7.5–33.8)	18.8 (4.1–101.0)	
Temperature day 0, °C	37.7 (34.4–41.2)	38.0 (34.3–40.4)	0.288, 0.600
Temperature day 7, °C	37.5 (35.6–39.8)	38.2 (34.0–40.0)	
Temperature day 14, °C	38.1 (35.5–39.3)	38.1 (37.2–39.9)	
Number of failing organs	2 (1–6)	2 (1–7)	0.425
Severity of lung injury			0.869
Non-ALI	3 (9)	1 (7)	
ALI	9 (26)	3 (9)	
ARDS	23 (66)	11 (73)	
ICU-acquired sepsis	4 (12)	1 (6)	1.000
APACHE II score	20.5 (9.0–34.0)	22.0 (18.0–53.0)	0.014
Ventilator-free days	14 (0–27)	0	<0.001
ICU stay, days	13 (2–86)	6 (1–68)	0.056
Hospital stay, days	53 (6–146)	10 (1–83)	0.002

Variables are expressed as median (range) or as number of patients (percentage) with *P* value (survivor vs. non-survivor and interaction between day and survivor vs. non-survivor)

Non-ALI non-acute lung injury defined as partial pressure of O_2 in arterial blood/inspiratory O_2 fraction (P_aO_2/F_iO_2) at day 0 >300 mmHg, *ALI* defined as P_aO_2/F_iO_2 at day 0 >200 and

≤ 300 mmHg, *ARDS* acute respiratory distress syndrome defined as P_aO_2/F_iO_2 at day 0 ≤ 200 mmHg, *ICU* intensive care unit, *APACHE* Acute Physiology And Chronic Health Evaluation

^a Local when blood-culture negative

mortality throughout the course of septic shock. This is in line with the suggested mediator role of Ang-2 in those patients. In contrast, VWF was not positively related to the fluid balance and pulmonary dysfunction and predicted mortality only at the day of ICU admission, suggesting that VWF plays a marker role only in the early phase of septic shock. Indeed, circulating VWF and Ang-2 were related at day 0, but not throughout the course of septic shock.

Experimental data [5, 19, 23] together with several observations in patient studies [4–9, 11, 12, 25] suggest that Ang-2 is a mediator in the pathogenesis of pulmonary vascular permeability and injury. This hypothesis is reinforced by several aspects of the present study. First, the relation between circulating Ang-2 and the fluid balance or pulmonary dysfunction persisted throughout the course of septic shock. Second, this longitudinal relation was Ang-2-specific, since VWF, which is also considered a marker of endothelial activation and dysfunction, did not reflect a positive fluid balance and pulmonary

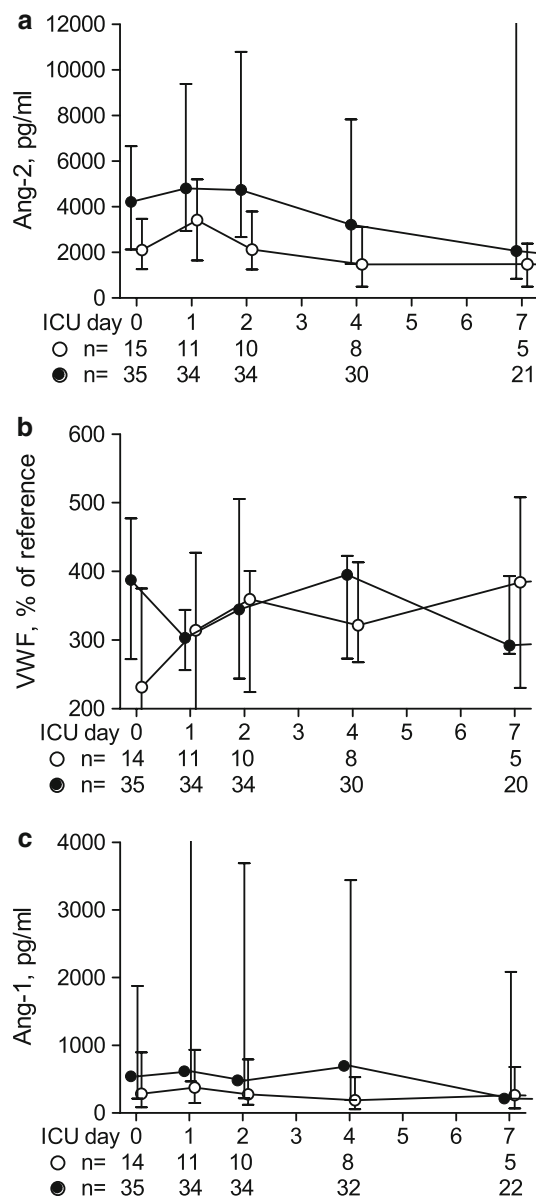
dysfunction. Third, the relation was observed within a relatively homogeneous group of septic patients with high Ang-2 levels and severe vascular permeability and pulmonary dysfunction, while previous studies focused on heterogeneous populations [4–7, 10–12]. In those heterogeneous populations, Ang-2, vascular permeability and pulmonary dysfunction may be related due to a group effect, because non-septic patients have low Ang-2 levels and moderate vascular permeability or pulmonary dysfunction, while septic patients have high Ang-2 levels and severe vascular permeability or pulmonary dysfunction. Nevertheless, *in vivo* Ang-2 blocking studies are necessary to formally confirm the mediator role of Ang-2.

Ang-2 levels at day 0 and throughout the course had independent predictive value for 28 day survival, in accordance with a previous study [10], since patients with Ang-2 levels <3,066 pg/ml had longer survival duration than patients with Ang-2 levels $\geq 3,066$ pg/ml, independent of the APACHE II score. Nevertheless, these cutoff values were selected post-hoc and should be validated

Fig. 1 The course of angiopoietin and VWF levels in survivors and non-survivors. Median and interquartile range of **a** circulating angiopoietin-2 (Ang-2; pg/ml), **b** von Willebrand factor (VWF; % of reference) and **c** angiopoietin-1 (Ang-1) levels (pg/ml) in plasma of intensive care unit (ICU) survivors (*open circle*) and ICU non-survivors (*filled circle*) on day 0, 1, 2, 4 and 7. Days 14, 21 and 28 are not shown because of low numbers, but are included in the analysis. **a** Ang-2 levels tended to be higher in non-survivors than in survivors throughout the course of septic shock ($P = 0.199$ for survivor vs. non-survivor and $P = 0.028$ for interaction between day and survivor vs. non-survivor). One non-survivor had extremely high Ang-2 levels: day 0 125,100 pg/ml; day 1 149,000 pg/ml; day 2 114,300 pg/ml; day 4 115,550 pg/ml and day 7 116,800 pg/ml. Therefore, the upper limit of interquartile range at day 7 is 88,263 pg/ml. Analysis without this non-survivor yields a $P = 0.039$ for survivor versus non-survivor and $P = 0.016$ for the interaction between day and survivor versus non-survivor. **b** VWF levels did not differ between survivors and non-survivors throughout the course of septic shock ($P = 0.545$ for survivor vs. non-survivor and $P = 0.218$ for interaction between day and survivor vs. non-survivor). **c** Ang-1 levels did not differ between survivors and non-survivors throughout the course of septic shock ($P = 0.189$ for survivor vs. non-survivor and $P = 0.070$ for interaction between day and survivor vs. non-survivor)

prospectively. It has been proposed previously that circulating Ang-2 levels may better predict mortality of critically ill patients than the APACHE II score [7, 11]. For instance, Siner et al. [11] reported that there were no deaths among patients with Ang-2 levels less than 10,000 pg/ml, despite a predicted mortality of 16% based on the median APACHE II score. Indeed, the combination of Ang-2 and the APACHE II score had more predictive value than the APACHE II score alone. In our patient group, we did not observe a sharp increase in Ang-2 levels towards the day of death as was described for one patient by Parikh et al. [5]. Ang-2 was continuously higher in non-survivors compared to survivors. Interestingly, Ang-2 levels in survivors, which were already lower throughout the course, decreased from day 0 to the last ICU day. In this light, Ang-2 levels above a certain threshold may mediate the development of endothelial activation and dysfunction, associated with pulmonary dysfunction and mortality. Surprisingly, Ang-1 levels also positively related to pulmonary dysfunction. Nevertheless, Ang-1 levels in our septic shock patients were still much lower than Ang-1 levels in healthy controls according to the literature [10, 12], suggesting that the low Ang-1 levels did not offset the effects of high Ang-2 levels. Indeed, the Ang-2/Ang-1 ratio did not relate to the fluid balance or pulmonary dysfunction and mortality (data not shown).

VWF levels related inversely to the fluid balance and positively to the pulmonary compliance throughout the course of septic shock. This finding is in accordance with studies that did not find a predictive value for the development of ALI/ARDS [33, 34], while other studies reported that VWF levels at the onset of critical illness predicted the development of ALI/ARDS and related to pulmonary permeability [12, 35]. It is unclear how these



differences can be explained, but the predictive value of VWF might depend on the time at which the samples were taken during the course of sepsis or ALI/ARDS. Indeed, VWF had predictive value for mortality early in the course of sepsis and ALI/ARDS (day 0), as reported by most [15, 16, 33, 35], but not all prior studies [36], but lost its predictive value on the subsequent days. Although VWF may thus have an important early prognostic role, the data do not suggest a direct pathogenetic role of the factor, in contrast to Ang-2.

In our study, increased Ang-2 and VWF levels were related on day 0 as reported before [8, 12], suggesting that they were released together from the endothelial Weibel-Palade body, but not on the subsequent days. There are several explanations for the observed loss of relation

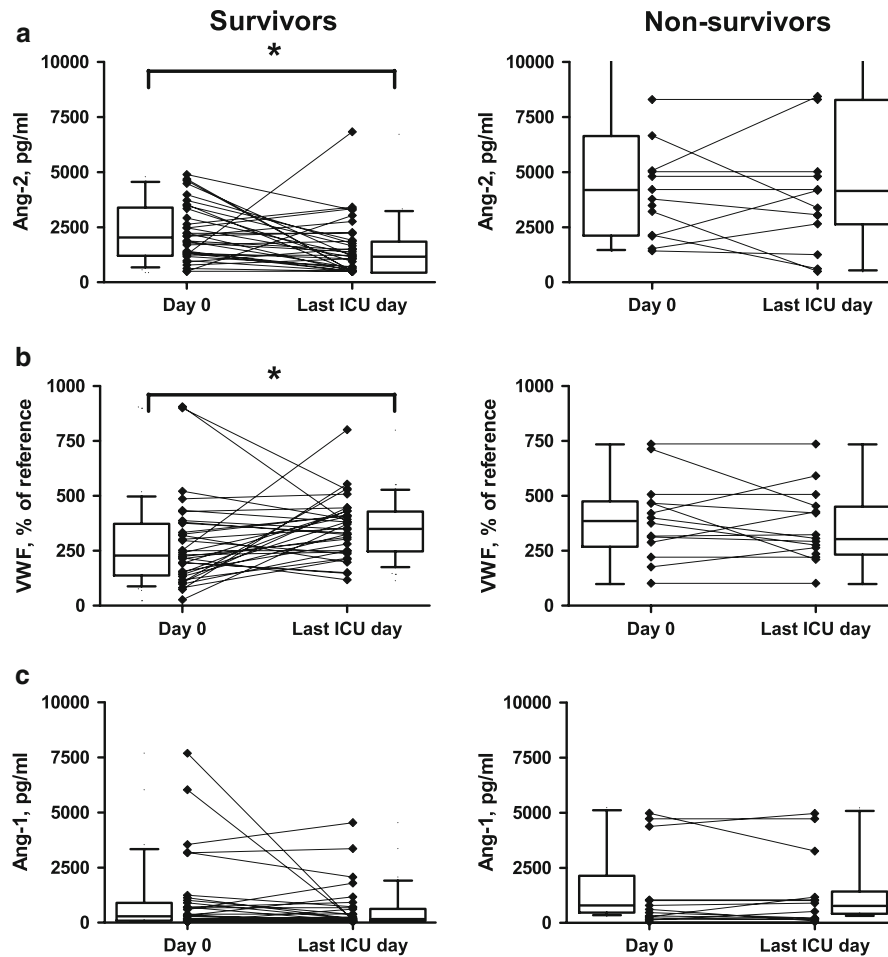


Fig. 2 Angiotensin and VWF levels on day 0 and the last ICU day in survivors and non-survivors. Individual levels and box plot of **a** circulating angiotensin-2 (Ang-2; pg/ml), **b** von Willebrand factor (VWF; % of reference) and **c** angiotensin-1 (Ang-1) levels (pg/ml) on day 0 and the last intensive care unit (ICU) day in ICU survivors and ICU non-survivors. Values in boxes are at 25th percentile, median and 75th percentile; whiskers are 10th and 90th percentile. **a** Ang-2 levels decreased from day 0 to the last ICU day in survivors ($*P = 0.0033$, $n = 35$ pairs), while they did not change in non-survivors ($P = 0.3910$, $n = 15$ pairs). Data not shown: Day 0

survivor: 25,090 pg/ml; day 0 non-survivor: 17,980 and 125,100 pg/ml; last ICU day non-survivor: 12,050 and 116,800 pg/ml; 90th percentile of non-survivors day 0: 60,828 pg/ml; 90th percentile of non-survivors last ICU day: 53,950 pg/ml. **b** VWF levels increased from day 0 to the last ICU day in survivors ($*P = 0.0057$, $n = 35$ pairs) and did not change in non-survivors ($P = 0.5070$, $n = 14$ pairs). **c** Ang-1 levels did not differ between day 0 and the last ICU day in survivors ($P = 0.1044$, $n = 35$ pairs) or non-survivors ($P = 0.7612$, $n = 14$ pairs)

between Ang-2 and VWF levels. First, the synthesis and subsequent storage of Ang-2 and VWF in de Weibel-Palade bodies may become differently regulated throughout the course of disease, so that the ratio between Ang-2 and VWF changed. Second, Ang-2 may not only be released from the Weibel-Palade body, but also by constitutive secretion from the cytoplasm. Indeed, Ang-2 is uniformly distributed in the cytoplasm of endothelial cells in the absence of VWF [13]. Third, the binding or consumption of VWF may be increased in more severely ill patients, for example, during diffuse intravascular coagulation.

Our study has several limitations. First, the relation between Ang-2 and a positive fluid balance or pulmonary dysfunction was relatively modest ($r = 0.25\text{--}0.30$),

suggesting that the positive fluid balance and pulmonary dysfunction are only partly due to increased Ang-2 levels. Indeed, it has been reported that Ang-2 sensitizes the endothelium to inflammatory stimuli rather than acting alone in the vascular system [37]. Second, it was not feasible to measure the pulmonary vascular permeability in a more direct manner with the pulmonary leak index method as we did in our previous study [12]. Instead, we studied the relation with fluid balance as surrogate indicator of vascular permeability. The data suggest that differences and changes in fluid balance were not confounded by differences and changes in renal function as can be judged, albeit imperfectly, from serum creatinine levels. Therefore, changes in the fluid balance are likely explained, at least in part, by

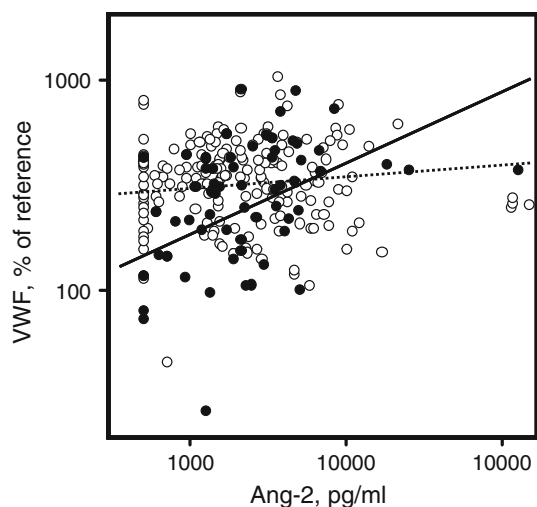


Fig. 3 The relation between Ang-2 and VWF levels. Angiopoietin-2 (Ang-2) levels (pg/ml) positively related to von Willebrand factor (VWF) levels (% of reference) at day 0 (filled circle, $r = 0.45$, $P = 0.001$, black line). Ang-2 levels throughout the course of septic shock did not relate to VWF levels (open circle day 1, day 2, day 4, day 7, day 14, day 21 and day 28, $r = 0.10$, $P = 0.1190$, dotted line)

changes in vascular permeability, even though not reflecting pulmonary vascular permeability directly. However, it is likely that the systemic and pulmonary vascular endothelium are simultaneously activated, since both are exposed to circulating inflammatory mediators. Furthermore, the fluid balance related to the P_{aO_2}/F_{iO_2} ratio and the oxygenation index, and we confirmed that a positive fluid balance is related to a worse outcome in ALI and septic shock [26, 27], suggesting that a positive fluid balance includes pulmonary injury-induced oedema. Third, the plasma levels of Ang-2, VWF and Ang-1 and the difference

in Ang-2 levels between survivors and non-survivors may have been underestimated due to fluid resuscitation and plasma dilution. This indicates that the difference in plasma levels of the measured proteins may be even greater and the relation with the fluid balance and pulmonary dysfunction even stronger. Fourth, in multivariate analysis, APACHE II score and Ang-2, but not fluid balance, P_{aO_2}/F_{iO_2} ratio, oxygenation index, PEEP and Ang-1 at day 0 remained significantly predictive factors of ICU mortality. Nevertheless, the value of this analysis is limited in our relatively small group of 50 patients.

In conclusion, in contrast to VWF levels, increased Ang-2 levels relate to a positive fluid balance and pulmonary dysfunction throughout the course of septic shock. While VWF levels only predict ICU mortality early in the course of septic shock, Ang-2 levels do so throughout the course. Pharmacological blockade of Ang-2 during the course of septic shock might reduce the degree of vascular permeability and pulmonary dysfunction and should be pursued in future studies for outcome benefits.

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