Angiopoietin-2, marker and mediator of endothelial activation with prognostic significance early after trauma

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Angiopoietin-2, Marker and Mediator of Endothelial Activation with Prognostic Significance Early after Trauma ?

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MINI-ABSTRACT

Plasma levels of endothelial cell specific growth factors, Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) were measured immediately after trauma in 208 consecutive adult trauma patients. Levels of Ang-2, but not Ang-1 and VEGF correlated with injury severity, hypoperfusion, endothelial activation, coagulation abnormalities, inflammation and worse clinical outcome.

STRUCTURED ABSTRACT

Objective:

To measure plasma levels of Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) early after trauma and to determine their clinical significance.

Background:

Angiopoietins and VEGF play a central role in the physiology and pathophysiology of endothelial cells. Ang-2 has recently been shown to have pathogenetic significance in sepsis and acute lung injury. Little is known about the role of angiopoietins and VEGF early after trauma.

Methods:

Blood specimens from consecutive major trauma patients were obtained immediately upon arrival in the emergency department and plasma samples assayed for Ang-1, Ang-2, VEGF, markers of endothelial activation, protein C pathway, fibrinolytic system and

complement. Base deficit was used as a measure of tissue hypoperfusion. Data were collected prospectively.

Results:

Blood samples were obtained from 208 adult trauma patients within 30 min after injury prior to any significant fluid resuscitation. Plasma levels of Ang-2, but not Ang-1 and VEGF were increased and correlated independently with severity of injury and tissue hypoperfusion. Furthermore, plasma levels of Ang-2 correlated with markers of endothelial activation, coagulation abnormalities, activation of the complement cascade and were associated with worse clinical outcome.

Conclusions:

Ang-2 is released early after trauma with the degree proportional to both injury severity and systemic hypoperfusion. High levels of Ang-2 were associated with an activated endothelium, coagulation abnormalities, complement activation and worse clinical outcome. This data indicate that Ang-2 is a marker and possibly a direct mediator of endothelial activation and dysfunction after severe trauma.

INTRODUCTION

Trauma accounts for a significant proportion of death and disabilities worldwide and it is expected that trauma-related mortality will increase in the near future.[1] Uncontrollable hemorrhage and severe central nervous system injury are responsible for the majority of trauma-related death in the first 24 hours. After this initial period, critical care complications such as multi-organ failure replace hemorrhage as a major cause of trauma-related death.[2] Endothelial activation and dysfunction has been shown to play a major role in the development of organ injuries thereby representing an independent parameter for worse clinical outcome in critically ill patients.[3-6]

Angiopoietins and vascular endothelial growth factor (VEGF) are growth factors that have been shown to play an important role in the development, physiology and pathophysiology of endothelial cells.[7] Angiopoietin-1 (Ang-1) activates the endothelial Tie-2 receptor thereby maintaining a quiescent resting state of the endothelium. On the other side, Angiopoietin-2 (Ang-2) binds to the same site of the Tie-2 receptor but functions as an antagonist ligand of Tie-2.[8] Therefore, Ang-2 has been shown to destabilize blood vessels, to enhance vascular leakage, to induce vascular regression and to prime the endothelium to respond to VEGF and other angiogenetic and inflammatory cytokines.[9] Because Ang-1 and Ang-2 have agonist-antagonist properties on the endothelium, it has been proposed to determine the ratio between Ang-1 and Ang-2 to better describe the state of activation of the endothelium.[10] Like von Willebrand factor (vWF), Ang-2 is stored in endothelial cell specific storage granules (Weibel-Palade bodies) and rapidly released upon stimulation of the endothelium, serving as a potential marker of endothelial activation and dysfunction.[11] It has been recently shown that

plasma levels of Ang-2 are increased in patients with sepsis[12,13] and that Ang-2 plays an important pathogenetic role in acute lung injury.[14] However, little is known about the role of angiopoietins and VEGF in the early phase after trauma in humans.

The first aim of the present study was to measure plasma levels of Ang-1, Ang-2 and VEGF in a large number of trauma patients immediately after injury. We found that only Ang-2 is released early after trauma in degree proportional to both injury severity and global hypoperfusion. Levels of Ang-2 correlated with vWF and sTM, indicating that Ang-2 might be a marker of endothelial activation and dysfunction in trauma patients. It has been previously reported that thrombin[15] and hypoxia[16,17] induce the extracellular release of Ang-2 by endothelial cells. Furthermore, we have previously shown early after trauma in humans that systemic hypoperfusion is associated with a coagulopathy associated with an activation of the protein C pathway and the complement cascade.[18,19] Thus, the second aim of this study was to determine whether elevated plasma levels of Ang-2 were associated with the development of coagulopathy and complement activation early after severe trauma. The third aim was then to identify the prognostic significance of Ang-2, in particular its relationship to the development of organ failure and clinical outcome after severe trauma.

METHODS

The Institutional Review Board of the University of California at San Francisco approved the research protocol for this prospective cohort study and granted a waiver of consent for the blood sampling as a minimal risk intervention.

Patients

Consecutive major trauma patients admitted to the San Francisco General Hospital (level 1 trauma center) were studied. All adult trauma patients who met criteria for full trauma team activation were eligible for enrollment. Patients less than 18 years old or transferred from other hospitals were immediately excluded.

Sample collection and measurement

The methodology has been described previously in detail.[18,19] Briefly, a 10 ml research sample of blood was drawn in citrated tubes within 10 minutes of arrival in the emergency department. The samples were immediately transferred to the central laboratory, centrifuged and the plasma extracted and stored at -80°C. In this study, we measured Angiopoietin-1 (Ang-1; Quantikine Ang-2 EIA, R&D Systems Inc., Minneapolis, MN), Angiopoietin-2 (Ang-2; Quantikine Ang-2 EIA, R&D Systems Inc., Minneapolis, MN), vascular endothelial growth factor (VEGF; Quantikine VEGF EIA, R&D Systems Inc., Minneapolis, MN), von Willebrand Factor Antigen (vWF; Asserachrom vWF, Diagnostica Stago Inc., Asniere, France), IL-6 and IL-10 (R&D Systems Inc., Minneapolis, MN) in addition to previously measured levels of the anticoagulatory thrombomodulin-protein C pathway (soluble thrombomodulin [sTM], protein C), fibrinolytic system (tissue plasminogen activator [t-PA], plasminogen activator inhibitor-1 [PAI-1], D-Dimers) and the complement cascade (Bb fragments, soluble C5b-9).[18,19] The normal value of Ang-2 was 641-2755 pg/ml and determined by testing 10 randomly selected healthy volunteers. All measurements were done in accordance with the manufacturer's instructions.

Data collection, outcome measures

Data were collected prospectively on patient demographics, the injury time, mechanism (blunt or penetrating) and severity, pre-hospital fluid administration, time of arrival in the trauma room and admission vital signs. The Injury Severity Score (ISS) was used as a measure of the degree of tissue injury.[20] An arterial blood gas was drawn at the same time as the research sample as part of the standard management of major trauma patients. The base deficit was used as a measure of the degree of tissue hypoperfusion. Admission base deficit is a clinically useful early marker of tissue hypoperfusion in trauma patients and an admission base deficit greater than 6 mmol/l has previously been identified as predictive of worse outcome in trauma patients.[21,22]

Outcome measures: Patients were followed until hospital discharge or death. For mortality analysis, patients surviving to hospital discharge were assumed to still be alive. Secondary outcome measures were also recorded for 28-day ventilator-free days, acute lung injury (American-European consensus conference definition[23]) and acute renal injury (Acute Dialysis Quality Initiative consensus conference definition[24]) and blood transfusions required in the first 24 hours.

Statistical analysis

Data analysis was performed by the investigators. Normal-quantile plots were used to test for normal distribution. Parametric data are expressed as mean ± 95% confidence intervals. Two-group analysis was performed with a two-tailed unequal variance Student's t-test. Correlation was assessed by Pearson's method and multiple regression was used to test for statistical independence. The χ test was used for dichotomous data analysis. A p-value of ≤ 0.05 was chosen to represent statistical significance.

RESULTS

Over a 15 month period, we enrolled 208 consecutive traumatized patients into the study. Median time from injury to blood sampling was 32 minutes, there was no vasopressor or colloid administration and patients received an average of 150 ± 100 ml of intravenous crystalloid prior to the blood specimen collection. Clinical characteristics of our severely injured patients are shown in **Table 1**.

Plasma levels of Ang-2, but not Ang-1 and VEGF were increased immediately after trauma and correlated with severity of injury as measured by Injury Severity Score (Figure 1A-C). Interestingly, female patients (n=53) had significantly higher plasma levels of Ang-2 than male patients (n=156) (3198±529 *vs.* 2570±284 pg/ml, $p < 0.05$). Similarly, blunt trauma patients had also significantly higher plasma levels of Ang-2 than penetrating trauma patients (2894±327 *vs.* 2245±217 pg/ml, $p < 0.05$). These differences are likely explained by the fact that both female patients and blunt trauma patients had significantly higher ISS than respectively male patients and penetrating trauma patients (female *vs*. male patients: 21.7±3.4 *vs.* 17.0±2.0, $p < 0.05$; blunt *vs*. penetrating trauma patients: 20.0±2.1 *vs*. 12.9±2.6, $p < 0.05$). Finally, the presence of head injury did not influence plasma levels of Ang-2 in our cohort of trauma patients (data not shown).

Ang-2 is stored in the same endothelial organelles like vWF (Weibel Palade bodies) and released in part by the same mechanism upon endothelial stimulation.[11] We therefore hypothesized that Ang-2 could be a marker of endothelial activation and dysfunction in trauma patients and compared Ang-2 plasma levels with those of vWF and sTM, known markers of endothelial activation.[25] Plasma levels of vWF (**Figure 1D**) and sTM (sTM: ISS <9: 35±4, ISS >25: 46±9 ng/ml, $p = 0.03$ – upper-lower quartile

comparison) were increased in patients with severe trauma and correlated with Ang-2 (**Figure 2**).

Hemorrhagic shock *per se* has been shown to compromise the endothelial cell function.[26] Furthermore, hypoxia induces the release of Ang-2 from endothelial cells.[16,17] We therefore examined the effects of systemic hypoperfusion on the release of endothelial cell specific growth factors and endothelial activation. Patients with a base deficit >7.6 had significantly higher plasma levels of Ang-2, vWF (**Figure 3A,D**) and sTM (BD <2.2: 35±3, BD >7.6: 57±15 ng/ml, p = 0.01 – upper-lower quartile comparison) compared to those with no base deficit (<2.2). In contrast, plasma levels of Ang-1 and VEGF (**Figure 3B,C**) were not affected by systemic hypoperfusion seen early after trauma. Furthermore, multiple regression analysis identified that the effects of injury severity and tissue hypoperfusion on the release of Ang-2 were statistically independent (r^2 = 0.17; ISS p < 0.001; BD p = 0.01).

Coagulation abnormalities are common following major trauma and are directly related to worse clinical outcome.[27] We have recently shown that only patients who are severely injured and in shock are coagulopathic early after injury and that this is due to activation of the protein C pathway rather than consumption of coagulation factors.[18] We next thought to identify whether the release of Ang-2 in our patients was related to coagulation abnormalities. Patients with coagulation abnormalities (PT >15.2 sec, PTT >36.5 sec) had significantly higher levels of Ang-2 (**Figure 4A**). Increasing Ang-2 was associated with a rise in the levels of soluble thrombomodulin (**Figure 2B**) and a fall in protein C levels (**Figure 4B**), suggesting activation of the anticoagulant protein C pathway in patients with increased endothelial activation. Furthermore, levels of Ang-2 were positively correlated with d-dimer levels (**Figure 4C**), suggesting an increased

fibrinolytic activity in patients with endothelial activation. This finding was supported by measuring t-PA and PAI-1 levels: there was a significant positive and inverse correlation between t-PA and Ang-2 as well as PAI-1 and Ang-2, respectively (data not shown).

We have previously found in patients with severe trauma that activation of the complement system, particularly its alternative pathway, is related to increased rate of organ injuries and mortality.[19] To examine if endothelial cell specific growth factors are associated with the early inflammatory response in trauma, we measured Bb fragments, a marker of the activation of the alternative complement pathway and soluble C5b-9 (membrane attack complex), generated during the late phase of complement activation. Again, we found that levels of Ang-2, not Ang-1 or VEGF correlated with those of Bb and soluble C5b-9 (**Figure 5A,B**). Finally, we measured the plasma levels of two cytokines (IL-6 and IL10) that have been shown to be respectively markers of systemic inflammation and anti-inflammatory response. We found that that the plasma levels of both pro- and anti-inflammatory cytokines were significantly higher in trauma patients with hypoperfusion (base deficit greater than 6 mmol/l) compared to those without hypoperfusion (IL-6: 59.9 ± 3.9 *vs* 27.1 ± 1.0 pg/ml, $p < 0.05$; IL-10: 89.3 ± 6.9 *vs* 37.1 ± 0.9 pg/ml, $p < 0.05$).

Finally, to determine the clinical significance of these findings, we examined whether early Ang-2 release was associated with worse clinical outcome (**Table 2**). We found that there was a direct correlation between mortality rate and plasma levels of Ang-2 (**Figure 6A**). Non-survivors (n=26) had significantly higher plasma levels of Ang-2 compared to survivors (n=183) (4355±1426 *vs.* 2494±186 pg/ml, $p < 0.001$) (**Figure 6B**). Patients who later developed organ injury had significantly higher plasma levels of Ang-2. Acute renal failure was present in 5% in patients with normal Ang-2 levels but 28% of

those with high Ang-2 ($p < 0.001$). There was also a trend toward increased incidence of acute lung injury with elevated Ang-2 levels (19% *vs.* 11%, $p = 0.09$). High levels of Ang-2 were also associated with fewer ventilator-free days and increased blood transfusion requirements (**Table 2**).

DISCUSSION

The present study shows that Ang-2 is released within 30 min after trauma in patients with severe injury and systemic hypoperfusion. Levels of Ang-2 correlated with markers of endothelial activation. Furthermore, high levels of Ang-2 were associated with early coagulation abnormalities, complement activation and worse clinical outcome. This data indicate that Ang-2 is a marker and possibly a direct mediator of endothelial activation and dysfunction after severe trauma.

The first major finding of the present study is to demonstrate that Ang-2, but not Ang-1 and VEGF is increased in the plasma of patients early after trauma. The endothelial barrier is tightly regulated by angiopoietins.[28] Ang-1 and Ang-2 are the best characterized angiopoietins of the four currently known and bind to Tie-2 receptors (tyrosine kinase with immunoglobulin-like loop and epidermal growth factor homology domain), mostly expressed on endothelial cells.[29] Ang-1 is an agonist at the Tie-2 receptor, thereby controlling endothelial cell survival and vessel maturation and exerting anti-permeability and anti-inflammatory functions. In contrast, Ang-2 is a Tie-2 receptor antagonist blocking the Ang-1/Tie-2 signaling acting as a blood vessel destabilizing cytokine and disrupting the endothelial barrier.[12,30,31] Profiling studies have demonstrated that endothelial cells are the primary source of Ang-2.[32] Because Ang-1 and Ang-2 have

agonist-antagonist properties on the endothelium, it has been proposed to determine the ratio between Ang-1 and Ang-2 rather than the absolute levels of either ligand.[10] Our trauma patients with severe injury and shock had high plasma levels of Ang-2 but unchanged levels of Ang-1, i.e. a low Ang-1/Ang-2 ratio when compared with patients with minor or no injury indicating that these patients have an activated endothelial cell phenotype early after injury.

Endothelial activation and dysfunction occurs early after trauma hemorrhage.[26] To assess the endothelial integrity, measurement of different endothelial-related markers with different characteristics like vWF and sTM have been proposed.[25] Pre-made vWF and Ang-2 protein is typically stored in Weibel-Palade bodies (subcellular organelles) together with other proteins allowing the endothelium to immediately release these mediators upon stimulation.[11] The exocytosis of Weibel-Palade bodies can be induced by multiple factors like hypoxia,[16,17] oxidative stress,[33] thrombin,[34] complement[35] and VEGF[36] – many of those being present in severely injured patients. Furthermore, we and others have previously reported that on admission to the hospital, increased plasma levels of sTM are observed in trauma patients with hypoperfusion and acidosis.[3,18] Thrombomodulin (TM) is a cell surface glycoprotein that complexes thrombin, thereby converting protein C to activated protein C. Several mechanisms like oxidative stress and proteolytic cleavage by activated leukocytes have been proposed by which TM is being shed from the cell surface indicating that sTM is another marker for endothelial activation and dysfunction.[37] Here we reported that the increase in plasma levels of Ang-2 correlated with those of vWF and sTM indicating that Ang-2 is a marker of endothelial activation and dysfunction in trauma patients.

VEGF is another endothelial cell specific growth factor and considered a key mediator in the control of vascular permeability acting in concert with angiopoietins.[38] Several studies measured VEGF levels in animals and humans and reported some controversial findings. For example, plasma levels of VEGF have been shown to be increased in septic patients and were related to disease severity and mortality.[39,40] In contrast in the present study, plasma VEGF levels measured 30 min after injury were unrelated to injury severity, systemic hypoperfusion and patient outcome. This finding is in accordance with previously published studies on trauma and burn patients where VEGF levels on admission were unrelated to the extent of the initial injury and not different from a healthy control group.[41,42] However, it may be premature to conclude that VEGF cell signaling is not activated in the early post-injury period. As shown previously in rat models of ischemia and reperfusion (I/R), VEGF cell signaling has been implicated in the I/R injury in the lung[43] and kidney[44] without any increase in VEGF protein release or expression.

Clinical significance. High levels of circulating Ang-2 have been recently reported in critical care patients with acute lung injury and sepsis, clinical situations associated with increased vascular leakage.[12-14] Orfanos *et al.* reported in 61 septic patients that levels of Ang-2 correlated with the intensity of the inflammatory response and disease severity.[13] Another study on 22 septic patients with acute lung injury by Parikh *et al.* showed a correlation of Ang-2 with impaired pulmonary gas exchange.[12] The present study is the first to report that plasma levels of Ang-2 on admission to the hospital have clinical relevance as they were associated with early coagulation abnormalities, complement activation and worse clinical outcome after severe trauma in humans. Although our study is observational, there are several lines of evidence indicating that

Ang-2 levels may play a critical role in the pathogenesis of an activated endothelium with increased vascular permeability observed early after trauma. For example, Parikh *et al.* showed that serum of septic patients with acute lung injury was able to disrupt the endothelial architecture *in vitro*, an effect that correlated with the serum levels of Ang-2. This effect could be reversed by adding Ang-1, thus increasing the Ang-1 signaling through the Tie-2 receptor.[12] Furthermore, Bhandari *et al.* recently showed with Ang-2 knockout and siRNA treated mice that Ang-2 is a key mediator responsible for the hyperoxia-induced acute lung injury.[14] Despite these results, it should be pointed out that the development of organ failure in severely traumatized patients is multifactorial. Several factors have been identified including, but not limited to old age (>55 years), ISS >24 and transfusion of more than 6 units of blood.[45,46] The data of our study confirm these findings, as trauma patients who later developed acute lung injury had a significantly higher blood transfusion requirement during the first 24 hours after admission to the hospital than those who did not develop lung dysfunction (data not shown). Interestingly, there seems to be a decrease in the incidence, severity and attendant mortality of post injury multiple organ failure over the last 10 years that is been attributed to improvement in trauma and critical care as well as the decreased use of blood transfusion during resuscitation.[47] Taken together, these data indicate that Ang-2 is not only a marker of endothelial activation and dysfunction, but may also have pathogenetic significance after severe trauma.

In summary, we have shown that Ang-2 is being released early after trauma and that plasma levels of Ang-2 correlated with severity of injury and hypoperfusion in humans. High levels of Ang-2 were associated with coagulation abnormalities, increased complement activation and worse clinical outcome after major trauma, as it has been

previously reported for acute lung injury, cerebrovascular accidents, diabetic retinopathy and neoplasms.[14,48-50] These findings indicate that Ang-2 may not only be a marker of endothelial cell activation but also be a mediator of injury and tissue edema. Further studies are required to define the exact role of Ang-2 after trauma and to determine whether interfering with the angiopoietin Tie-2 pathway could be therapeutically useful.

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TABLES

Table 1. Clinical characteristics of trauma patients.

Demographic data	
Age, yrs	41 (27-63)
Sex, Female / Male	n = 53 (25%) / n = 155 (75%)
Characteristics on injury	
Injury Severity Score	17 (9-26)
Penetrating injury	n = 53 (25%)
Severe head injury (AIS head > 3)	n = 59 (30%)
Physiology	
Heart rate > 100 /min	n = 84 (41%)
Systolic blood pressure <100 mmHg	n = 38 (18%)
Base deficit > 6 mmol/l	n = 56 (27%)

Total number of patients included is n = 208. Data are presented as median (inter-quartile range) and numbers (%).

Table 2. The relationship of elevated Angiopoietin-2 levels to clinical outcome.

	Odds Ratio *(95% CI)*	*P* value
Mortality	**4.0** *(1.6-10.2)*	0.001
Ventilator free days, ≤26	**3.0** *(1.5-5.7)*	<0.001
Acute lung injury	**2.0** *(0.8-4.7)*	0.096
Acute renal failure	**7.4** *(2.7-20.7)*	<0.001
Transfusion, ≥2 Units of PRBC	**2.7** *(1.3-5.4)*	0.003

CI = confidence interval, PRBC = packed red blood cells. Normal range for Angiopoietin-2 was 641-2755 pg/ml. Ventilator free days were calculated at 28 days. *P*-value: χ^2 analysis.

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FIGURE LEGENDS

Figure 1. Effects of injury on plasma levels of endothelial cell specific growth factors and von Willebrand factor. Blood samples were obtained from 208 consecutive major trauma patients immediately upon admission to the hospital. **Panels A-C.** Plasma levels of Angiopoietin-2 (Ang-2), but not Angiopoietin-1 (Ang-1) and vascular endothelial growth factor (VEGF) correlated with the Injury Severity Score (ISS). **Panel D.** Plasma levels of von Willebrand factor (vWF), a marker of endothelial cell activation and dysfunction correlated with ISS. Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile.

Figure 2. Markers of endothelial activation and dysfunction. Panels A/B. Plasma levels of von Willebrand factor (vWF) and soluble thrombomodulin (sTM), markers of endothelial activation and dysfunction were measured in 208 trauma patients on admission to the hospital and correlated significantly with levels of Angiopoietin-2 (Ang-2). Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile.

Figure 3. Effects of systemic hypoperfusion on plasma levels of endothelial cell specific growth factors and von Willebrand factor. Blood samples were obtained from 208 consecutive major trauma patients immediately upon admission to the hospital. **Panels A-C.** Plasma levels of Angiopoietin-2 (Ang-2), but not Angiopoietin-1 (Ang-1) and vascular endothelial growth factor (VEGF) correlated with base deficit, a measure of the degree of tissue hypoperfusion. **Panel D.** Plasma levels of von Willebrand factor (vWF), a marker of endothelial cell activation and dysfunction correlated with base deficit. Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile.

Figure 4. High plasma levels of Angiopoietin-2 are associated with coagulation abnormalities in trauma patients. Panel A. Trauma patients with coagulation abnormalities (PT >15.2 sec, PTT >36.5 sec) had significantly higher levels of Angiopoietin-2 (Ang-2). *$p \leq 0.05$. **Panel B.** Ang-2 levels rise as protein C levels decrease, suggesting activation of the anticoagulant protein C pathway in patients with increased endothelial activation. **Panel C.** High Ang-2 levels are related to increased fibrinolytic activity, expressed as levels of d-dimers. Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile.

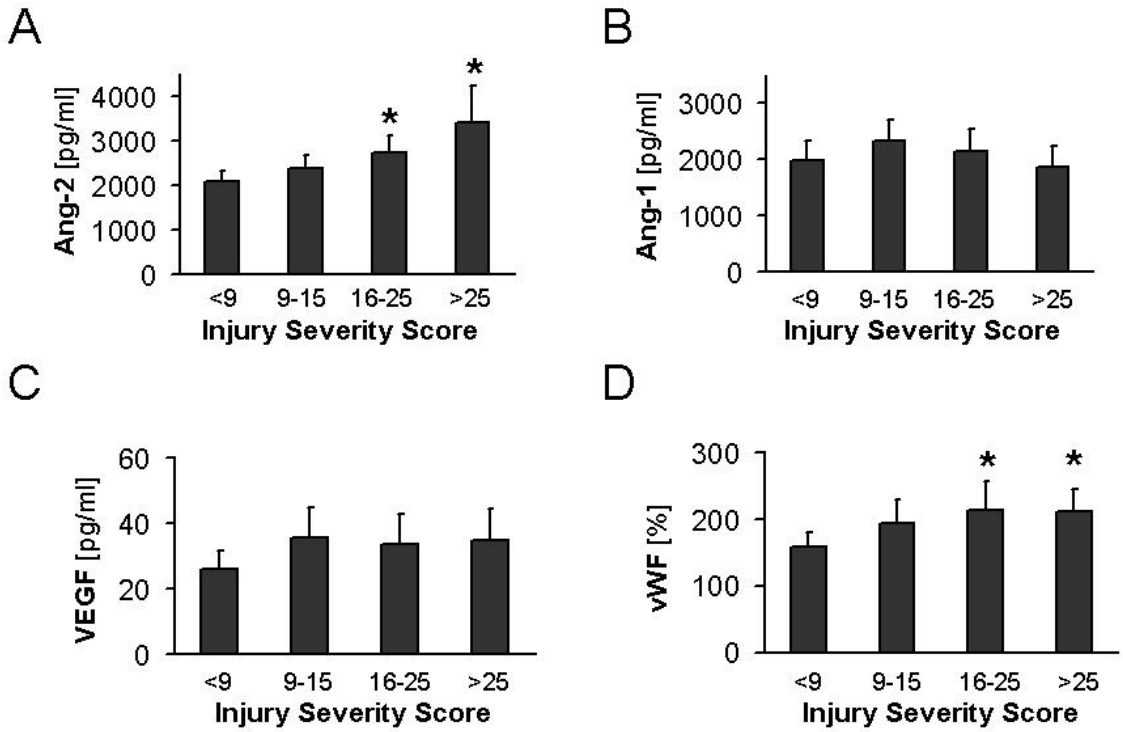
Figure 5. Plasma levels of Angiopoietin-2 correlate with early complement activation in trauma patients. Panel A/B. High plasma Ang-2 levels are associated with increased complement activity. Bb fragments, a marker of the activation of the alternative complement pathway and soluble C5b-9 (MAC), generated during the late phase of complement activation were measured. Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile.

Figure 6. High plasma levels of Angiopoietin-2 are associated with increased mortality in patients with severe trauma. Panel A. Non-survivors after severe trauma have higher plasma levels of Angiopoietin-2 (Ang-2). Data are presented in quartiles, *$p \leq 0.05$ compared with lowest quartile. **Panel B.** Scatter-gram of individual values of Ang-2 in survivors (n=183) and non-survivors (n=26) after major trauma; *$p \leq 0.05$ compared with survivors.

Figure 7. Schematic diagram of the proposed mechanism. Injury and hypoperfusion induce the extracellular release of Angiopoietin-2 by the vascular endothelium and activate the thrombomodulin-protein C pathway. Injury and

hypoperfusion cause the exocytosis of Weibel-Palade bodies (WPB) containing several inflammatory mediators including Angiopoietin-2 (Ang-2) and von Willebrand factor (vWF). Ang-2 binds to the same site of the Tie-2 receptor like Angiopoietin-1 (Ang-1) but functions as an antagonist ligand of Tie-2. Therefore, Ang-2 destabilizes blood vessels, enhances vascular leakage, induces vascular regression and primes the endothelium to respond to VEGF and other angiogenetic and inflammatory cytokines. vWF causes platelet activation and aggregation. Furthermore, injury and hypoperfusion activate the thrombomodulin-protein C pathway resulting in the de-activation of the coagulation Factors V and VIII and a de-repression of the fibrinolysis. T = thrombin, TM = thrombomodulin, PC = protein C, aPC = activated protein C, ePCR = endothelial protein C receptor.

Figure 1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Ang-2 [pg/ml]
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3000
2000
1000
0
*
*
<9
9-15
16-25
>25
Injury Severity Score
B
Ang-1 [pg/ml]
3000
2000
1000
0
<9
9-15
16-25
>25
Injury Severity Score
C
VEGF [pg/ml]
60
40
20
0
<9
9-15
16-25
>25
Injury Severity Score
D
vWF [%]
300
200
100
0
*
*
<9
9-15
16-25
>25
Injury Severity Score

Figure 2

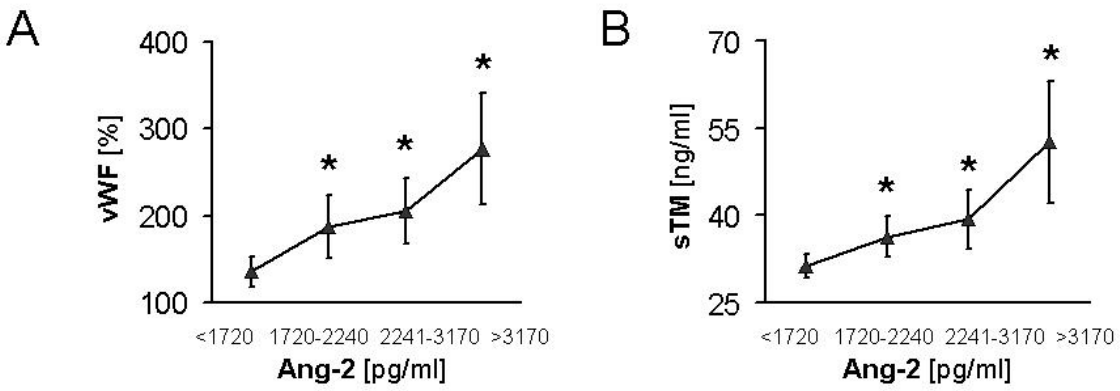
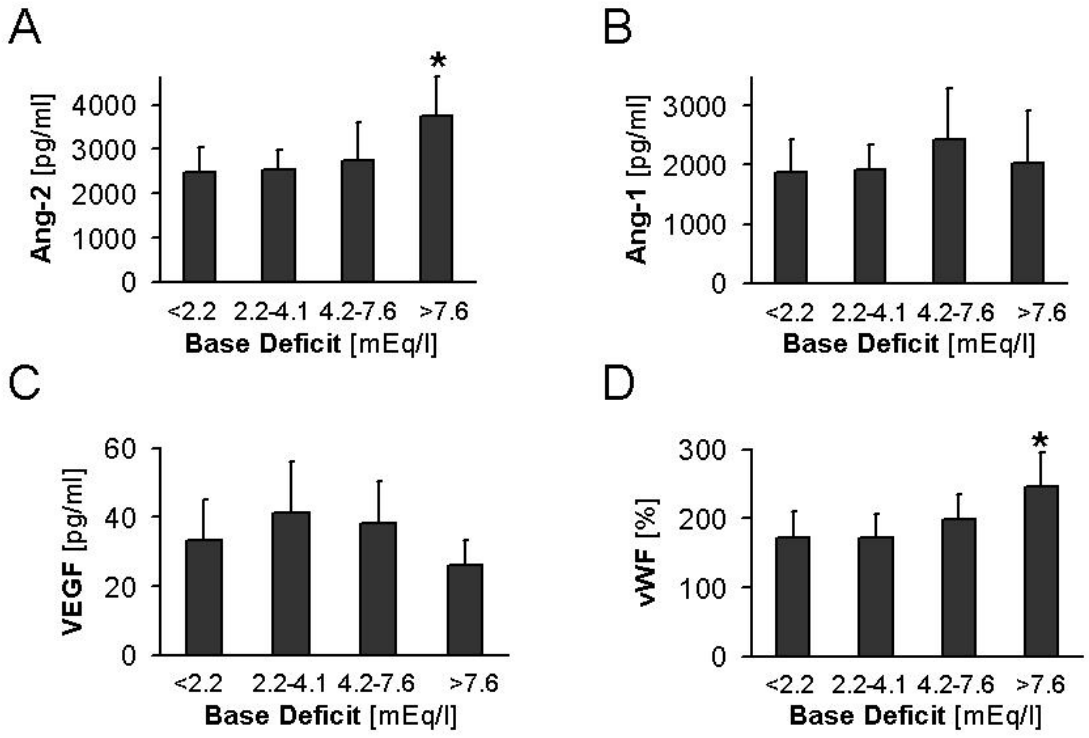

A
400
300
200
100
vWF [%]
*
*
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]
B
70
55
40
25
sTM [ng/ml]
*
*
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]

Figure 3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4000
3000
2000
1000
0
*
<2.2
2.2-4.1
4.2-7.6
>7.6
Base Deficit [mEq/l]
B
Ang-1 [pg/ml]
3000
2000
1000
0
<2.2
2.2-4.1
4.2-7.6
>7.6
Base Deficit [mEq/l]
C
VEGF [pg/ml]
60
40
20
0
<2.2
2.2-4.1
4.2-7.6
>7.6
Base Deficit [mEq/l]
D
vWF [%]
300
200
100
0
*
<2.2
2.2-4.1
4.2-7.6
>7.6
Base Deficit [mEq/l]

Figure 4

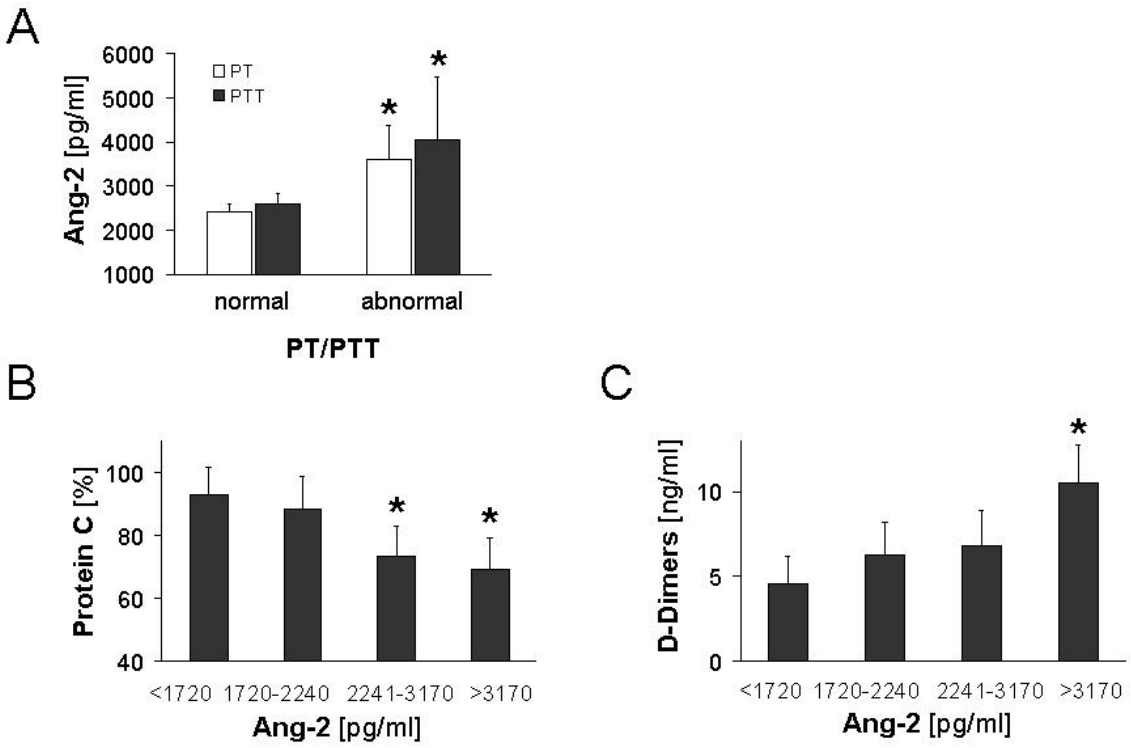
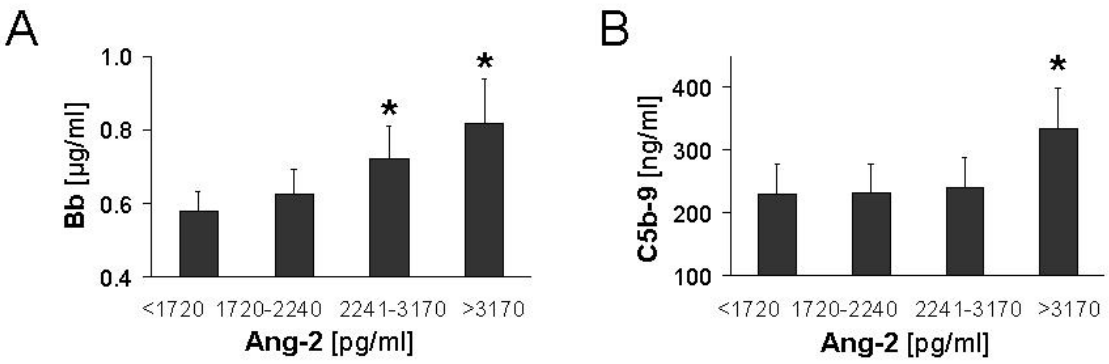

A
6000
5000
4000
3000
2000
1000
Ang-2 [pg/ml]
PT
PTT
*
*
normal
abnormal
PT/PTT
B
100
80
60
40
Protein C [%]
*
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]
C
10
5
0
D-Dimers [ng/ml]
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]

Figure 5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0.8
0.6
0.4
Bb [µg/ml]
*
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]
B
400
300
200
100
C5b-9 [ng/ml]
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]

Figure 6

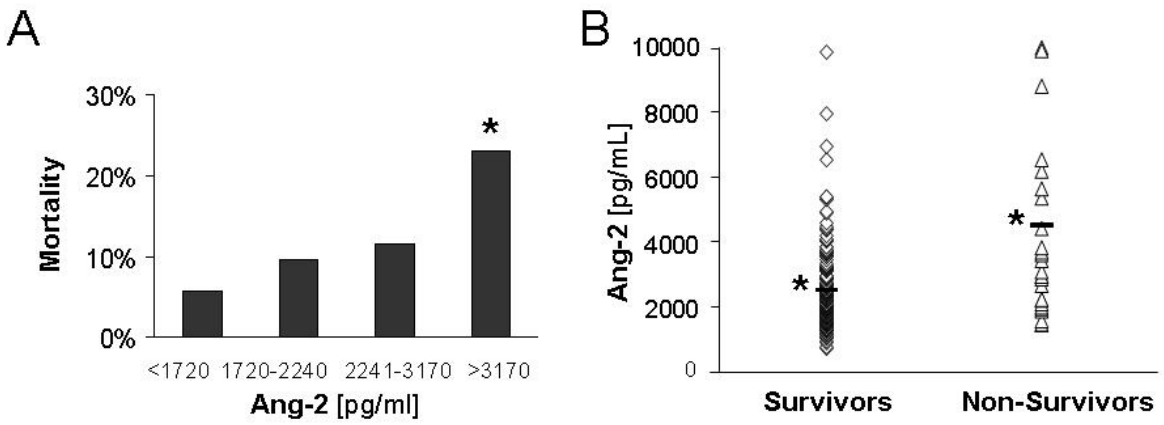
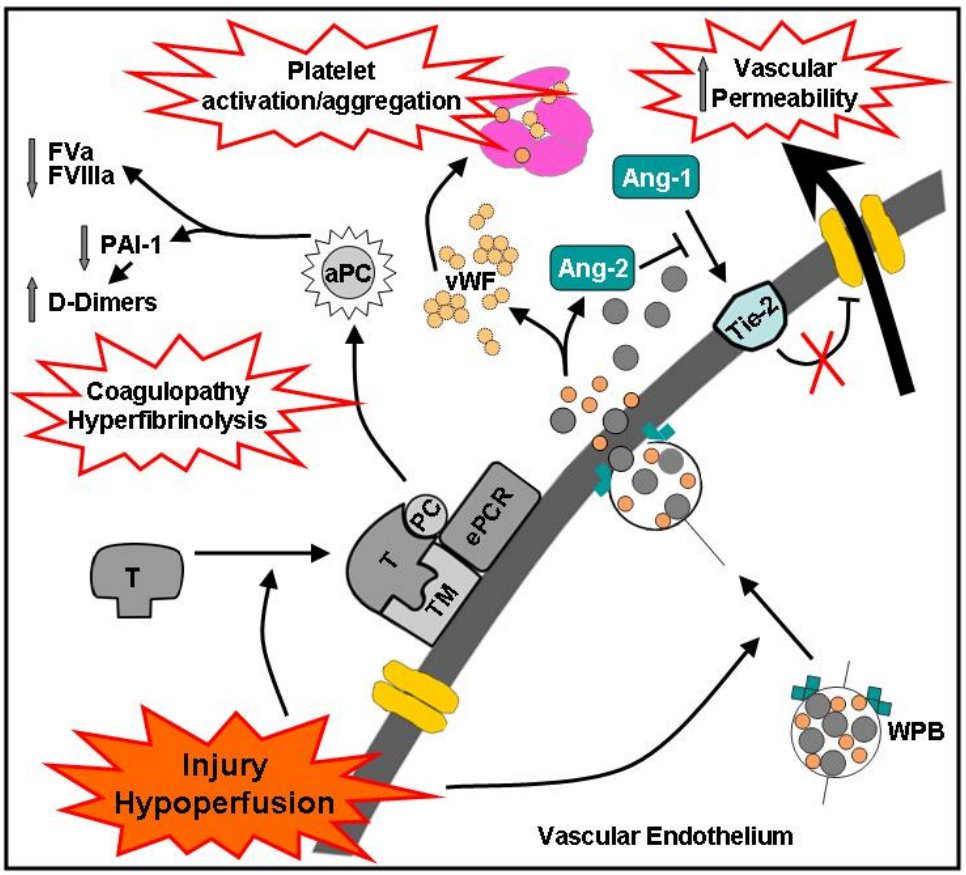

A
30%
20%
10%
0%
Mortality
*
<1720
1720-2240
2241-3170
>3170
Ang-2 [pg/ml]
B
10000
8000
6000
4000
2000
0
Ang-2 [pg/mL]
*
*
Survivors
Non-Survivors

Figure 7


Platelet
activation/aggregation
Vascular
Permeability
FVa
FVIIIa
PAI-1
D-Dimers
aPC
vWF
Ang-1
Ang-2
Tie-2
Coagulopathy
Hyperfibrinolysis
PC
ePCR
T
TM
T
WPB
Injury
Hypoperfusion
Vascular Endothelium