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Platelet Aggregation After Blunt Trauma Is Associated with the Acute Respiratory Distress Syndrome and Altered by Cigarette Smoke Exposure

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Abstract

Background—The risk of the acute respiratory distress syndrome (ARDS) is increased in passive and active smokers after blunt trauma. However, the mechanisms responsible, including the role of platelet aggregation, for this association are unknown.

Methods—We analyzed 215 patients with severe blunt trauma from a prospective observational cohort at a Level 1 trauma center between 2010 – 2015. Subjects underwent impedance-based platelet aggregometry in response to platelet agonists arachidonic acid (ASPI), adenosine diphosphate (ADP), collagen (COL) and thrombin receptor activating peptide-6 (TRAP). ARDS within the first 8 days of admission was adjudicated using Berlin criteria. Plasma cotinine was measured to assess cigarette smoke exposure. Regression analyses were used to assess the relationship between 1) platelet aggregation and ARDS and 2) cigarette smoke exposure and platelet aggregation.

Results—At both 0 and 24 hours, impaired platelet aggregation was associated with increased odds of developing ARDS. Cigarette smoke exposure was associated with increased platelet aggregation upon arrival to the emergency department. However, at 24 hours, cigarette smoke exposure was associated with increased impairment in platelet aggregation, reflecting a statistically significant decline in platelet aggregation over the initial 24 hours following trauma.

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The relationship between this decline in platelet aggregation and ARDS differed by cigarette smoke exposure status, suggesting that impaired platelet activation differentially affects the risk of ARDS in those with cigarette smoke exposure (ASPI, *p* for interaction: 0.005, COL *p* for interaction: 0.02, ADP, *p* for interaction: 0.05).

Conclusions—Impaired platelet aggregation at 0 and 24 hours is associated with an increased risk of developing ARDS after severe blunt trauma. Cigarette smoke exposed patients are more likely to develop impaired platelet aggregation over the first 24 hours of admission, which may contribute to their increased risk of ARDS.

Keywords

Acute Respiratory Distress Syndrome; Blunt Trauma; Platelets; Tobacco

Background

In recent years, studies in a variety of populations have found an increased risk of the acute respiratory distress syndrome (ARDS) amongst smokers. In critically ill blunt trauma patients, active and passive cigarette smoke exposure have been associated with an increased risk of ARDS (1); likewise, smoking is associated with an increased risk of ARDS in non-pulmonary sepsis (2) and following blood transfusion (3), as well as with primary graft dysfunction (4), a form of ARDS that occurs within 72 hours of lung transplant. However, the mechanisms that predispose cigarette smokers to develop ARDS remain poorly characterized.

One potential pathway that may prime smokers to develop ARDS is via increased platelet activation. Platelets have been implicated in ARDS pathogenesis. Bronchoalveolar lavage fluid samples from patients with ARDS have increased levels of platelet specific alpha granules (5), suggesting increased platelet activity. This increase in platelet activation may promote inflammation through neutrophil recruitment and activation (6, 7), including the development of neutrophil extracellular traps, which are thought to contribute to ARDS pathogenesis (8). Notably, this increase in pro-inflammatory platelet activity may come at the expense of platelet hemostasis, as a small study found that patients with acute respiratory failure had increased platelet activation but decreased platelet hemostasis compared to critically ill patients without acute lung injury (9). Cigarette smoking has been associated with increased platelet aggregation in a number of clinical settings outside of trauma and critical illness (10–12). However, the effect of cigarette smoke on platelet aggregation in the setting of critical illness and whether this may predispose patients to develop ARDS is unknown.

Impedance-based platelet aggregometry measures platelet aggregation in response to platelet agonists via electrical resistance across sets of silver-coated copper electrodes in whole blood (13). These assays have been used to study platelets in healthy subjects, those on antiplatelet medications and more recently in the setting of critical illness, including trauma (14–16). Studies suggest that trauma patients frequently develop impaired platelet aggregation in response to platelet agonists such as arachidonic acid, adenosine diphosphate, collagen and thrombin receptor activating peptide-6. This impaired platelet aggregation

develops immediately following trauma and often persists for several days into ICU admission (15, 17). Although this impaired platelet aggregation has been associated with increased mortality after trauma (15, 17), additional studies are needed to better characterize whether platelet aggregation in the setting of trauma is associated with the development of ARDS.

In this study, we tested (1) the association between platelet aggregation and ARDS, and (2) the association between cigarette smoke exposure and platelet aggregation in critically ill blunt trauma patients at risk for ARDS. We hypothesized that cigarette smoke exposure would be associated with abnormal platelet aggregation, which would be associated with an increased risk of developing ARDS. Some of the results of this study have been previously reported in the form of an abstract (18).

Methods

Subjects

We selected 215 critically ill blunt trauma patients who underwent impedance-based platelet aggregometry between 2010 – 2015 from a larger observational prospective cohort at San Francisco General Hospital, a Level 1 trauma center affiliated with the University of California, San Francisco (UCSF). The larger cohort was comprised of subjects meeting criteria for full trauma activation. A subset of these patients underwent impedance-based platelet aggregometry testing based on sample and staff availability. In our study, we included all patients who were admitted to the ICU and underwent platelet aggregometry testing, with the exception of those who had a history of anti-platelet use prior to admission (Figure 1). Data collection methods for this cohort have been described previously (19). Briefly, patients meeting criteria for full trauma activation underwent blood draw within 10 minutes of arrival to the emergency department. Blood samples from this timepoint underwent immediate processing, with plasma stored at -80°C for cotinine measurements when available. Additional whole blood was immediately used for platelet aggregation studies when available. A separate blood draw was performed at 24 hours for repeat platelet aggregation studies when feasible. Comprehensive demographic and clinical information were obtained until discharge or death. Injury Severity Score (ISS) was used to assess for severity of injury (20). Alcohol use history was obtained from chart review and, whenever possible, from patients or surrogates using the Alcohol Use Disorders Identification Test (AUDIT) (21). ARDS adjudication was performed by two physician review of all patient data during the first 8 days of hospitalization according to the Berlin definition (partial pressure arterial oxygen: fraction of inspired oxygen, $(\text{PaO}_2:\text{FiO}_2) < 300\text{ mm Hg}$) (22); physicians were not involved in the clinical care of the patients and were blinded to the smoking status of each patient and results of platelet aggregometry during this review. The Institutional Review Board of UCSF approved the research protocol and granted a waiver of consent for blood sampling as a minimal risk intervention. Informed consent was subsequently obtained from patients or their surrogates for continued study participation with the exception of subjects who died, who were included via a waiver of consent.

Measurements

Platelet Aggregation Studies—Platelet count was measured in the clinical lab, while platelet aggregation testing was assessed via point of care testing in a non-clinical research lab. Platelet aggregation was assessed using the Multiplate® multiple electrode aggregometer (Verum Diagnostica GmbH; Munich, Germany) immediately after sample collection as previously described (15). Specifically, 0.3mL of whole blood was diluted in warmed normal saline containing 3mM CaCl₂ and incubated for 3 minutes at 37°C with continuous stirring in a Multiplate® test cell. Each test cell contains two sets of 3 mm silver-coated copper wires, across which electrical resistance is measured at 0.57 second intervals. Platelet activation was induced by agonists arachidonic acid (ASPI, final concentration 0.5mM; via the cyclooxygenase pathway), adenosine diphosphate (ADP, final concentration 6.5μM; via P2 receptors), collagen (COL, final concentration 3.2μg/mL; via GpIa/IIa and GpVI receptors) or thrombin receptor activating peptide-6 (TRAP, final concentration 32μM; via PAR receptors). Platelet adhesion to the electrodes was detected as increasing electrical impedance, measured by duplicate sets of sensor wires in each test cell. Agonist responses are reported as area under the aggregation curve in units (U) over a 6-minute measurement period. Reference ranges were provided by the manufacturer based on studies of healthy control subjects. To study the relationship between ARDS and platelet aggregation, we dichotomized each aggregation test at the lower limit of normal, delineating impaired platelet aggregation, based on prior studies of impedance based platelet aggregometry in the setting of trauma (15).

Plasma Cotinine—Plasma cotinine, a biomarker with a half-life of 16 hours that accurately quantifies cigarette smoke exposure (23), was measured on emergency department (0 hour) samples when available via liquid chromatography-tandem mass spectrometry (24, 25). The limit of quantification for plasma cotinine was 0.02 ng/ml.

Statistical Analysis

Normally distributed variables were compared using Student's t test and displayed as mean ± standard deviation. Non-normally distributed variables were compared using the Mann-Whitney U-test and displayed as median with interquartile range (IQR). Linear regression was performed to assess the association between plasma cotinine and platelet aggregation in response to platelet agonists. Logistic regression was used to assess the association between impaired platelet aggregation and the development of ARDS. Log transformation was used as needed to fulfill all assumptions required for regression. Propensity scores were used to adjust for potential confounders, which were selected based on known relevance to ARDS or platelet function. Propensity scores included age, sex, ISS, chest injury score, shock, alcohol abuse and platelet count for analyses at 0 hours with platelet transfusion also included for analyses at 24 hours. Regression analyses with an interaction term between platelet aggregation tests and cigarette smoke exposure for the outcome of ARDS were performed to assess whether the relationship between platelet aggregation testing and ARDS differs on the basis of cigarette smoke exposure and are presented with area under the receiver operating characteristic (AUROC) with 95% confidence intervals as a measure of model fit. For mediation analyses, we estimated and tested two multiple-mediator models of the relationship of cotinine to ARDS where the candidate mediators were platelet aggregation

tests using a weighted least squared minimum variance estimator. Bootstrapping using 5000 replications was used to estimate the standard error. A p value 0.05 was considered statistically significant. Statistical analyses were performed with STATA 13.1 (StataCorp LP, College Station, TX) and MPlus v8 (Muthén and Muthén, Los Angeles, CA).

RESULTS

Demographics

To account for potential sampling bias, we compared demographics and severity of illness (e.g. shock, defined as systolic blood pressure < 90 mm Hg and ISS) between patients in our sample and critically ill patients from the parent cohort who were not included in our sample. There was no difference in demographics or severity of illness between patients included in our sample and those that were not (Table Supplement 1), suggesting that our sample did not suffer from selection bias.

In our study sample of 215 patients, 171 patients had platelet studies performed at baseline. Of these, 64 patients had repeat platelet studies performed at 24 hours. An additional 44 patients had platelet studies performed at 24 hours only, but not at baseline. There were no differences in demographics or severity of illness between patients with and without platelet aggregation testing at 0h. Patients with platelet aggregation testing at 24 hours had higher ISS and ICU length of stay compared to those without (data not shown).

Patients who developed ARDS were older (51 years vs 43 years, $p = 0.01$) and had higher ISS (33 vs 18, $p < 0.0001$) compared to those who did not (Table 1). There was no difference in sex, body mass index, alcohol abuse or shock between patients with and without ARDS. The prevalence of chronic lung disease such as chronic obstructive pulmonary disease or asthma was < 2%. No subjects had a history of interstitial lung disease.

Plasma cotinine was measured in a subset of 180 patients with available plasma. Patients with cigarette smoke exposure, defined as plasma cotinine > 0.02 ng/mL, were younger (42 years vs 58 years, $p < 0.0001$) than those without exposure. There was no significant difference in sex, alcohol abuse, body mass index, ISS or shock upon admission between patients with and without exposure.

Platelet Aggregation & ARDS

41 subjects (19%) developed ARDS during the first 8 days of their ICU stay. At 0h, impaired platelet aggregation in response to ASPI (OR 2.6, 95% CI: 1.1 – 5.9, $p = 0.02$) and collagen (OR: 2.5, 95% CI: 1.1 – 5.8, $p = 0.03$) was associated with significantly increased odds of ARDS, with a similar trend in response to ADP (OR: 2.1, 95% CI 0.9 – 5.0, $p = 0.07$), but not TRAP (Table 2). Adjustment for potential confounders with propensity scores (Table 2) strengthened the association with ASPI, ADP and COL.

At 24h, impaired platelet aggregation in response to ASPI (OR: 4.5, 95% CI: 1.2 – 16.1, $p = 0.02$), collagen (OR: 4.1, 95% CI: 1.1 – 14.8, $p = 0.03$) and TRAP (OR: 2.6, 95% CI: 1.1 – 6.7, $p = 0.04$) was associated with increased odds of developing ARDS, with a similar trend

in response to ADP (OR: 2.9, 95% CI: 0.9 – 9.3, $p = 0.07$) (Table 2). Adjustment for potential confounders with propensity scores (Table 2) attenuated these findings.

To ensure that our findings were not confounded by the inclusion of patients who already had ARDS at the time of platelet testing, we performed a sensitivity analysis excluding the 17 patients who developed ARDS within the 48 hours of admission. This sensitivity analysis showed a similar pattern to our full analyses, with impaired platelet aggregation both at 0 and 24 hours associated with an increase in the odds of developing ARDS (Table Supplement 2).

Cigarette Smoke Exposure & ARDS

Next, we examined the relationship between cigarette smoke exposure and ARDS in patients who had plasma cotinine measured. In a univariate logistic regression model, cigarette smoke exposure was associated with a trend toward increased odds of developing ARDS (OR: 2.1, 95% CI: 0.8 – 5.8, $p = 0.16$). After adjusting for age, sex, alcohol abuse, ISS, chest injury score and shock with propensity scores, cigarette smoke exposure was associated with significantly increased odds of developing ARDS (OR: 4.6, 95% CI: 1.2 – 17.2, $p = 0.03$).

Platelet Aggregation & Cigarette Smoke Exposure

Next, we studied the relationship between cigarette smoke exposure and platelet aggregation. There was no difference in platelet count at 0h in cigarette smoke exposed patients compared to those without cigarette smoke exposure (223 vs $202 \times 10^3/\mu\text{L}$, $p = 0.19$). At 0h, cigarette smoke exposure was associated with decreased odds of impaired platelet aggregation in response to ASPI (OR: 0.49, 95% CI: 0.30 – 0.80, $p = 0.005$), but not ADP, collagen or TRAP (Table 3), reflecting relatively increased platelet aggregation. Regression with propensity scores adjusting for confounders mildly attenuated these findings, although cigarette smoke exposure remained associated with decreased odds of developing baseline impaired platelet aggregation in response to ASPI (Table 3).

We then studied the relationship between cigarette smoke exposure and platelet aggregation at 24 hours. Overall, there was a decrease in platelet aggregation in response to all 4 agonists from 0 to 24 hours. Cigarette smoke exposure was associated with a greater decrease in platelet aggregation in response to ASPI ($p = 0.02$) and ADP ($p = 0.02$), with a similar trend in response to TRAP ($p = 0.07$) and collagen ($p = 0.13$) (Table 4) in patients with platelet testing at both 0 and 24 hours ($n = 62$). Linear regression adjusted with propensity scores did not attenuate this response (Table 4).

Thus, while cigarette smoke exposure was associated with decreased odds of impaired platelet aggregation at baseline, cigarette smoke exposure was associated with increased odds of impaired platelet aggregation at 24 hours in response to TRAP (OR: 2.2, 95% CI: 1.3 – 3.7, $p = 0.005$). Point estimates were similar in response to ASPI (OR: 1.6, 95% CI: 0.89 – 3.0, $p = 0.12$), ADP (OR: 1.5, 95% CI: 0.83 – 2.7, $p = 0.18$) and collagen (OR: 1.7, 95% CI: 0.90 – 3.7, $p = 0.11$) although the associations were not statistically significant (Table 3). Regression with propensity scores adjusting for potential confounders did not attenuate these findings (Table 3).

Cigarette Smoke Exposure, Platelet Aggregation & ARDS

To assess whether the relationship between cigarette smoke exposure and platelet aggregation was related to the increased risk of ARDS observed in smokers, we performed mediation analysis and interaction testing. In both 0h and 24h models, none of the tests of mediation were statistically significant at $p < 0.05$ (Table Supplement 3). However, at 24h, regression with an interaction term between cigarette smoke exposure and platelet aggregation testing with ARDS as the outcome showed a statistically significant interaction between cigarette smoke exposure and ASPI ($p = 0.005$, AUROC = 0.76, 95% CI: 0.65 – 0.86), COL ($p = 0.02$, AUROC = 0.73, 95% CI: 0.61 – 0.84) and ADP ($p = 0.05$, AUROC = 0.70, 95% CI: 0.59 – 0.81) but not TRAP ($p = 0.40$, AUROC = 0.68, 95% CI: 0.56 – 0.80). These findings suggest that the relationship between impaired platelet aggregation at 24 hours and ARDS differs based on cigarette smoke exposure, with impaired platelet aggregation associated with a higher risk of ARDS in patients with exposure compared to those without. We next studied the relationship between the change in platelet aggregation from 0 to 24 hours and cigarette smoke exposure for the development of ARDS. Regression models adjusting for confounders using propensity scores with ARDS as the outcome showed a statistically significant interaction between cigarette smoke exposure and the change in ASPI ($p = 0.008$, AUROC = 0.76, 95% CI: 0.65 – 0.86) with a similar trend for COL ($p = 0.10$, AUROC = 0.78, 95% CI: 0.66 – 0.91) and TRAP ($p = 0.06$, AUROC = 0.77, 95% CI: 0.64 – 0.90) but not ADP ($p = 0.79$, AUROC = 0.72, 95% CI: 0.58 – 0.86). These results reflect that the changes in platelet aggregation over the first 24 hours of admission are associated with an increased risk of ARDS to a greater extent in those with cigarette smoke exposure than in non-smokers, potentially contributing to the increased risk of ARDS observed in smokers.

Discussion

This study reports that impaired platelet aggregation, both upon arrival to the emergency room and 24 hours later, is associated with increased odds of developing ARDS following severe trauma. Furthermore, cigarette smoke exposure had a significant effect on platelet aggregation in the setting of severe trauma. Cigarette smoke exposure was initially associated with increased platelet aggregation; however, over the first 24 hours of admission, cigarette smoke exposure was associated with a statistically significant decline in platelet aggregation. We noted a statistically significant interaction between this decline in platelet aggregation over the first 24 hours of admission and cigarette smoke exposure for the development of ARDS, indicating that the relationship between impaired platelet aggregation and ARDS differs by cigarette smoke exposure, with a greater risk of ARDS in patients with cigarette smoke exposure. These findings suggest a potentially significant role for platelets in the development of ARDS in patients with cigarette smoke exposure.

Platelet aggregation as assessed by impedance-based aggregometry has been studied in critical illness, but to our knowledge has not been previously studied in relation to ARDS. Prior studies in the setting of sepsis (26, 27) and trauma (15, 17) have found that impaired platelet aggregation in response to platelet agonists is associated with increased mortality. Although the mechanisms underlying this finding are not known, impaired platelet

aggregation has been associated with platelet mitochondrial dysfunction in the setting of sepsis (28). Notably, this platelet mitochondrial dysfunction is thought to occur in response to systemic inflammation and has independently been associated with worse outcomes in sepsis (29). Thus, the impaired platelet aggregation observed in our trauma patients may similarly be related to platelet mitochondrial dysfunction in the presence of overwhelming systemic inflammation. Our findings could also reflect the phenomenon of “exhausted” platelets, which have already undergone activation and release of pro-inflammatory compounds, which may in turn promote microvascular thrombosis, and thus demonstrate decreased aggregation when stimulated with additional platelet agonists (30). Thus, the presence of impaired platelet aggregation may reflect increased platelet activation and platelet-related inflammation, mitochondrial dysfunction, and/or microvascular thrombosis, which may ultimately increase the risk of developing ARDS and mortality. Further studies combining platelet aggregometry testing with testing of platelet surface markers of activation are needed to provide mechanistic insights into the pathogenesis of ARDS after trauma.

Cigarette smoking has been associated with increased platelet aggregation in a variety of settings (12, 31, 32), providing face validity to our findings that cigarette smoke exposure was associated with increased platelet aggregation in response to platelet agonists at baseline, even in critically ill trauma patients. Notably, this association did not persist over time, as cigarette smoke exposure was associated with the development of impaired platelet aggregation over the first 24 hours of critical illness. These findings suggest that platelet function may differ in patients with and without cigarette smoke exposure in the setting of trauma. The exaggerated development of impaired platelet aggregation over the first 24 hours of critical illness in patients with cigarette smoke exposure, which may reflect exhausted platelets, may thus contribute to the increased incidence of ARDS in these patients, as evidenced by the statistically significant interaction we observed between the change in platelet aggregation and cigarette smoke exposure for the development of ARDS. However, further study of the role of platelets in ARDS pathogenesis is needed to determine the extent of abnormal platelet function in patients at risk for ARDS, and whether abnormal platelet function contributes to the increased risk of ARDS in smokers or merely reflects other processes and pathways that increase the risk of ARDS among smokers.

Our study has significant implications for clinical trials of antiplatelet agents in the setting of critical illness. As one example, there has been strong interest in the use of antiplatelet agents, such as aspirin, to potentially prevent ARDS in high-risk patients. This work is supported by observational studies that have reported decreased incidence of ARDS amongst patients who were already on antiplatelet therapy prior to admission (33, 34). However, a recent clinical trial of aspirin as preventative therapy for patients at risk for ARDS did not show any reduction in the risk of developing ARDS (35). We found that cigarette smoke exposure is associated with differences in response to platelet agonists in the setting of critical illness, including responses to arachidonic acid, reflecting the cyclooxygenase pathway that is targeted by aspirin. These findings, taken together with prior data that cigarette smokers have differential responses to aspirin therapy in other clinical settings,(10) suggests that trials of antiplatelet therapy should consider the potentially confounding effects of cigarette smoking both in their design and subsequent analyses.

Finally, in this study, impaired platelet aggregation immediately after trauma in samples drawn upon arrival to the emergency department was associated with increased odds of developing ARDS over the subsequent 8 days. Notably, the association between impaired platelet aggregation and ARDS persisted even after adjustment for standard measures of trauma severity, including ISS and shock. Given that platelet aggregation can be measured rapidly by point of care testing, impedance-based aggregometry may be useful to identify patients at increased risk of developing ARDS following trauma and enable the early implementation of supportive measures targeting ARDS, such as low tidal volume ventilation, in these patients, which may improve outcomes (36). However, additional studies of platelet aggregation are needed to assess whether these tests provide additional prognostic utility compared to current clinical prediction models and furthermore whether these associations are unique to trauma or are present in other settings of critical illness.

This study has several strengths. First, by obtaining samples immediately upon arrival to the emergency department, we were able to perform baseline platelet studies before the majority of medical care had occurred, thereby eliminating many process of care variables that could be potential confounders. Second, we used plasma cotinine as a biomarker of cigarette smoke exposure in the vast majority of our patients. Prior studies suggest that biomarkers of cigarette smoke exposure provide a more accurate assessment of true exposure in critically ill populations (23). Thus, by measuring plasma cotinine from samples obtained immediately upon arrival to the emergency department, we obtained a highly accurate assessment of recent cigarette smoke exposure in our population. Third, we used a standardized protocol with a two-physician review to assess all patients in our study for ARDS. This method of ARDS adjudication has been shown to be superior to simply relying on chest radiograph reports (37).

This study also has limitations. First, this was a single center study with a relatively homogenous population of trauma patients. Further studies are needed to assess whether our findings are generalizable to other populations of critically ill patients. Second, we were not able to obtain samples at both 0 and 24h for every patient in our study. Despite this, we identified statistically significant changes over time that were associated with cigarette smoke exposure. Third, given that the number of patients in our study with platelet function testing at two timepoints was relatively small, our power to conduct multivariate analyses in this subgroup was limited. To overcome this, we used propensity scores to assess for the presence of confounding. Finally, although we adjusted for several variables, we could not control for every process of care variable in these patients. However, our baseline samples were drawn before the majority of medical care was implanted, eliminating numerous confounders.

In conclusion, this study identifies differences in platelet aggregation associated with cigarette smoke exposure, which may reflect differences in underlying biology between smokers and non-smokers with critical illness that predispose smokers to developing ARDS. These differences have implications for clinical trials of antiplatelet therapies in patients at risk for ARDS. Additional studies are needed to assess the prognostic utility of platelet aggregation testing to identify patients at high risk of ARDS as well as to conduct

mechanistic analyses of platelets in patients with and without cigarette smoke exposure at risk for ARDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Overview of Study Population

Table 1

Patient Demographics

	No ARDS (n = 174)	ARDS (n = 41)	P value
Age (years)	43 ± 18	51 ± 25	0.01
Male (%)	78%	80%	0.80
BMI (kg/m ²)	26 ± 5	26 ± 5	0.61
Injury Severity Score	18 ± 14	33 ± 14	< 0.0001
Shock (%)	6%	8%	0.63
Alcohol Abuse			0.71
Yes	35%	28%	
No	48%	54%	
Unknown	17%	18%	

Table 2

Odds ratio for developing ARDS with impaired platelet aggregation in response to platelet agonists at 0h & 24 hours, univariate & multivariate models.

	0 hours (n = 171)			24 hours (n = 108)		
	Unadjusted (95% CI)	P value	Multivariate* (95% CI)	P value	Multivariate** (95% CI)	P value
ASPI	2.6 (1.1 – 5.9)	0.02	3.0 (1.2 – 7.7)	0.02	3.0 (0.8 – 11.8)	0.11
ADP	2.1 (0.9 – 5.0)	0.07	2.5 (1.0 – 6.2)	0.05	3.0 (0.8 – 11.2)	0.11
COL	2.5 (1.1 – 5.8)	0.03	3.3 (1.3 – 8.7)	0.02	3.2 (0.9 – 12.1)	0.08
TRAP	1.0 (0.3 – 2.8)	0.96	1.1 (0.4 – 3.2)	0.91	1.9 (0.7 – 5.1)	0.19

ASPI = Arachidonic Acid, ADP = Adenosine Diphosphate, COL = Collagen, TRAP = Thrombin Receptor Activating Peptide 6

*. Propensity score including age, sex, alcohol abuse, injury severity score, chest injury score, shock and platelet count

**. Propensity score including age, sex, alcohol abuse, injury severity score, chest injury score, shock, platelet count and platelet transfusion

Odds ratio for impaired platelet aggregation at 0h and 24 hours for each standard deviation increase in plasma cotinine, univariate and multivariate models.

ASPI = Aspirin, ADP = Adenosine Diphosphate, COL = Collagen, TRAP = Thrombin Receptor Activating Peptide 6

* Propensity score including age, sex, alcohol abuse, injury severity score, chest injury score, shock and platelet count

** Propensity score including age, sex, alcohol abuse, injury severity score, chest injury score, shock, platelet count and platelet transfusion

Table 4

Linear regression coefficients for the change in platelet aggregation between 0 and 24 hours for each SD increase in plasma cotinine. N = 62

24 hour	Univariate	P value	Multivariate	P value
ASPI	-7.7 (-14.1 – -1.4)	0.02	-7.4 (-14.0 – -0.9)	0.03
ADP	-7.8 (-14.6 – -1.1)	0.02	-8.3 (-14.8 – -1.7)	0.02
COL	-4.8 (-11.2 – 1.5)	0.13	-4.8 (-11.4 – 1.9)	0.16
TRAP	-7.1 (-14.8 – 0.6)	0.07	-6.7 (-14.7 – 1.2)	0.10

ASPI = Arachidonic Acid, ADP = Adenosine Diphosphate, COL = Collagen, TRAP = Thrombin Receptor Activating Peptide 6

* Propensity score including age, sex, alcohol abuse, injury severity score, chest injury score, shock, platelet count and platelet transfusion