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ORIGINAL RESEARCH ARTICLE

Microvesicle phenotypes are associated with transfusion requirements and mortality in subjects with severe injuries

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Background: Severe injury often results in substantial bleeding and mortality. Injury provokes cellular activation and release of extracellular vesicles. Circulating microvesicles (MVs) are predominantly platelet-derived and highly procoagulant. They support hemostasis and vascular function. The roles of MVs in survival after severe injury are largely unknown. We hypothesized that altered MV phenotypes would be associated with transfusion requirements and poor outcomes.

Methods: This single-centre study was approved by the Institutional Review Board. The study cohort consisted of patients with major trauma requiring blood product transfusion and 26 healthy controls. Plasma samples for MVs were collected upon admission to the emergency department (n = 169) and post-resuscitation (n = 42), and analysed by flow cytometry for MV counts and cellular origin: platelet (PMV), erythrocyte (RMV), leukocyte (LMV), endothelial (EMV), tissue factor (TFMV), and annexin V (AVMV). Twenty-four hour mortality is the outcome measurement used to classify survivors versus non-survivors. Data were compared over time and analysed with demographic and clinical data.

Results: The median age was 34 (IQR 23, 51), 72% were male, Injury Severity Score was 29 (IQR 19, 36), and 24 h mortality was 13%. MV levels and phenotypes differed between patients and controls. Elevated admission EMVs were found both in survivors (409/μL) and non-survivors (393/μL) compared to controls (23/μL, $p < 0.001$) and persisted over time. Admission levels of PMV, AVMV, RMV, and TFMV were significantly lower in patients who died compared to survivors, but were not independently associated with the 24 h mortality rate. Patients with low MV levels at admission received the most blood products within the first 24 h. AVMV and PMV levels either increased over time or stabilized in survivors but decreased in non-survivors, resulting in significantly lower levels at intensive care unit admission in non-survivors (1,048 vs. 1,880 AVMV/μL, $p < 0.00004$ and 1,245 PMP/μL vs. 1,866 PMP/μL, $p = 0.003$).

Conclusion: Severe injury results in endothelial activation and altered MV phenotypes. Significant differences in specific MV phenotypes or changes over time were associated with blood product requirements and the 24 h mortality rate.

Keywords: *extracellular vesicles*; *microvesicles*; *microparticles*; *transfusions*; *injury*; *trauma*; *coagulopathy*

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Microvesicles (MVs) are a heterogeneous population of extracellular vesicles released from a variety of cells with a size ranges from 100 to 1,000 nm (1–4). They circulate in healthy subjects providing physiological maintenance functions, but are also reported in a variety of pathologic conditions (5,6). They have complex biological and molecular properties, biodistribution, and functions (1). Generally, the phenotypes of MVs reflect their parent cells by retaining cell surface proteins from the cell of origin, along with cytosolic proteins, enzymes, RNA, and miRNAs. They play an important role in cellular communication, and their analysis in peripheral blood and other body fluids could serve as an important diagnostic and prognostic tool (7–9).

Traumatic injury remains the leading cause of death for younger populations ($\leq$44 years) worldwide and is the

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third leading cause of death in the United States (10). About one-quarter of all severe trauma patients experience acute, excessive, and uncontrolled haemorrhage that accounts for up to 30–50% of all trauma-related deaths/mortality (11–13). Haemorrhage from severe injury involves impaired coagulation, resulting in reduced clot formation and strength, and/or hyperfibrinolysis, and requires the transfusion of blood products to arrest bleeding. Injury provokes cellular activation and release of cell-derived MVs (14–23). However, despite recent advances in trauma research, the pathophysiology and mechanisms of haemostatic and cellular alterations of MVs in the early response to trauma and their roles in outcomes are not well characterized or understood.

Circulating platelets play a fundamental role in the haemostatic response to trauma by providing the surface for key reactions involved in clotting, and by generating highly procoagulant platelet microvesicles (PMVs). Although circulating MVs are predominantly of platelet origin and support hemostasis and vascular function, MVs can be released by various cell types including endothelial cells and circulating blood cells. Generally, MVs may express phosphatidylserine (PS), adhesion receptors, and tissue factor (TF), rendering them highly procoagulant. We and others have previously demonstrated that elevated procoagulant PMVs contribute to enhanced thrombin generation in healthy donors (24–26), and low PMVs are associated with trauma-induced coagulopathy (14). The role of MVs in the requirements for blood product transfusions and subsequent survival after severe injury is largely unknown. We hypothesized that altered MV phenotypes would be associated with transfusion requirements and poor outcomes. Thus, the aim of this study was to determine the potential association of MV counts and cellular origin (phenotypes) after trauma with blood product transfusions and the 24 h mortality rate.

Materials and methods

The study was conducted at the single Level 1 trauma centre in the US (Houston, Texas). The Institutional Review Board approved the protocols. The study included trauma patients meeting the criteria for the highest level trauma activation who were administered blood products. A total of 169 patients were selected. The study excluded prisoners; individuals under 16 years old; and patients who had been transferred from other hospitals. Finally, the study excluded any patient who had more than 5 min of cardiopulmonary resuscitation prior to or during the first 30 min of hospitalization. Twenty-six healthy controls of both genders (69% male, aged 18–59 years) were used as a comparison group.

Research blood samples for MVs were collected at admission to the emergency department (ED) ($n = 169$), of whom 42 had a post-resuscitation sample drawn upon admission to the intensive care unit (ICU). Blood was collected into sodium citrate (3.2%, 0.109 M) vacutainer tubes (Becton Dickson, Franklin Lakes, NJ) and processed immediately by double centrifugation, 15 min at 2,500 g, followed by 2 min at 13,000 g, at room temperature; and plasma was frozen at -80°C until use. Prior to analysis, samples were quickly thawed in a 37°C water bath. Cellular MV counts and phenotypes were analysed with Gallios flow cytometer (3 laser, Beckman Coulter, Inc., Miami, FL) and Kaluza analysis software v1.2. The MV analysis gate (< 1 μm) was set up with a blend of fluorescent beads (0.5, 0.9, and 3 μm), among which the 2-submicron beads covered the expected size range for MVs (0.5 to 1 μm) (Megamix, BioCytex, Marseille, France), according to the ISTH SSC First Collaborative Workshop (in which our laboratory participated) (27). MVs acquired within the pre-set MV scatter gate in the FS versus SS dot plot were further analysed in a specific fluorescence cytograms gate on this MV scatter gate (Fig. 1). MV phenotypes included platelet (PMV), red blood cell (RMV), leukocyte (LMV), endothelial MV (EMV), phosphatidylserine positive (AVMV), and tissue-factor-bearing MVs (TFMV), which were identified using the following monoclonal antibodies: CD41-APC (BioLegend, San Diego, CA, 6.25 μg/mL, 5 μL, IgG1k, clone HIP8), CD62P-PC5 (BD Pharmingen, San Jose, CA, 6 μg/mL, 5 μL, IgG1k, clone AK-4), CD235a-AF750 (Beckman Coulter, 50 μg/mL, 2.5 μL, IgG1, clone 11E4B-7-6 (KC16)), CD45-Pacific Blue (Beckman Coulter, 100 μg/mL, 2.5 μL, IgG1k, clone J.33), CD144-FITC (BD Pharmingen, San Jose, CA, 12.5 μg/mL, 5 μL, IgG1k, clone 55-7H1), annexin V-V500 and 10 × binding buffer (BD Horizon, 50 μg/mL, 2 μL) and CD142-PE (BD Pharmingen, 12.5 μg/mL, 5 μL, IgG1k, clone HTF-1). To remove antibody aggregates, for each panel, antibodies were combined and filtered using centrifugal filter units (Ultrafree-MC, 0.22 μm, Millipore, Billerica/Bedford, MA) prior to staining. The non-specific binding (background fluorescence) was set up by using plasma only (no antibodies), antibodies only in Isoflow (no plasma), and/or using fluorescence minus one staining. The procedure details: 25 μL of plasma was stained with appropriate premixed antibodies for 30 min at room temperature in the dark, diluted with 500 μL of Isoflow sheath fluid or with 1 × binding buffer for annexin V staining, and immediately analysed. Events were acquired at medium flow rates. Counting beads (25 μL, Flow-Count, Beckman Coulter) with known concentration were used for absolute MV counts. Acquired data were compared over time (ER admission versus ICU admission), and contrasted with clinical data.

Statistical analysis

Descriptive statistics are expressed as the median and interquartile range (IQR: Q1–Q3 in which Q1 and Q3 are the first and third quartiles) for continuous variables and proportions (%) for categorical variables. Parametric data were compared using a Student's t-test (unpaired or paired,

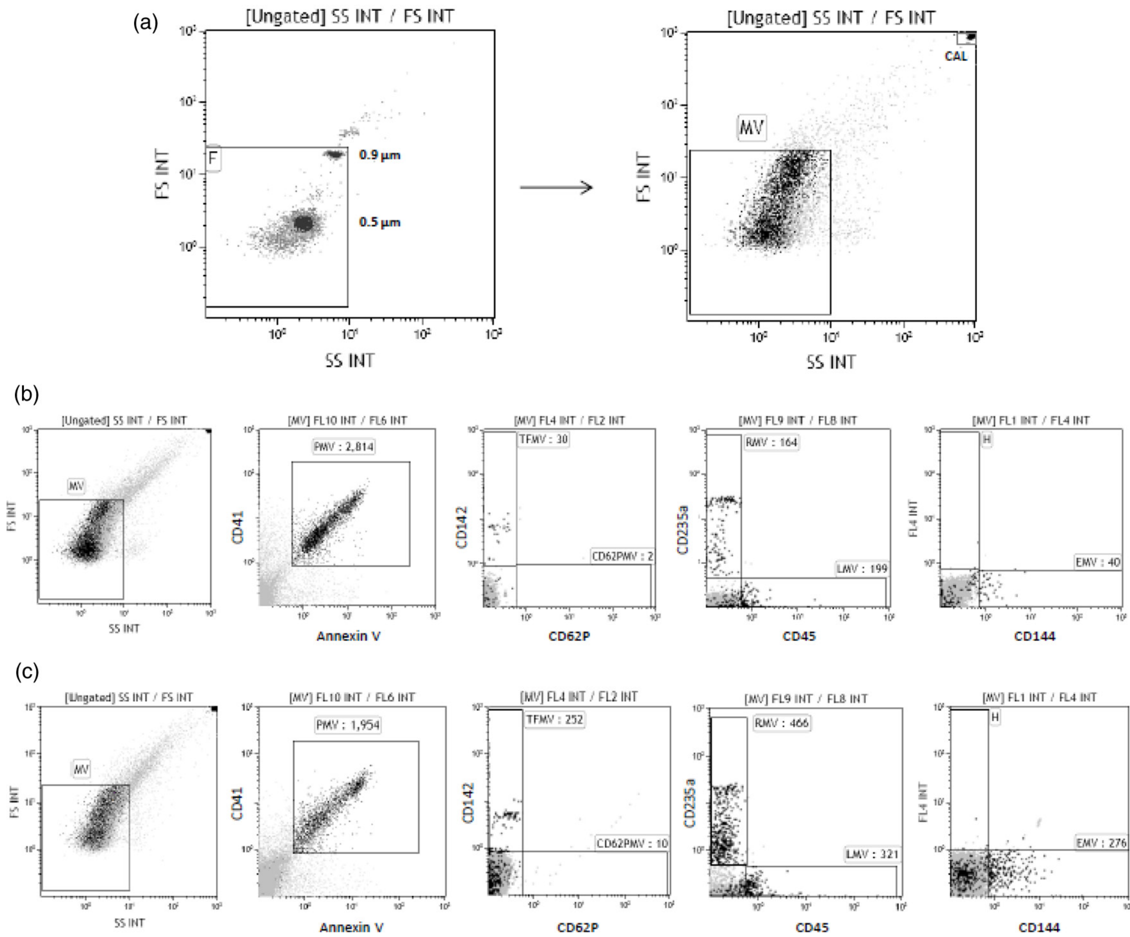

Fig. 1. Flow cytometry of microvesicles. (a) MV size gate setup with fluorescent beads; a representative cytograms of PMVs, AVMVs, EMVs, TFMVs, LMVs, RMVs of (b) healthy donor and (c) trauma patient.

2 tails), and non-parametric using the Kruskal–Wallis test. Correlation analysis was performed with Pearson's correlation test. Twenty-four hour mortality is the outcome measurement that was used to classify survivors versus non-survivors. An exploratory logistic regression modelling was performed to identify candidate associations between admission MV phenotypes and outcomes worthy of further research. Multiple-regression analyses were performed using Stata Software Version 12.1 (College Station, TX). Mortality rate at 24 h and total blood products transfused were selected as the outcomes of interest (dependent variables), with cell-derived MVs acting as independent variables, while controlling for age and platelet count. P-values of 0.05 or less were considered a crude indicator of potential significance.

Results

Patient characteristics

Patient demographics and injury characteristics are presented in Table I. These patients represent a severely injured and primarily blunt cohort. During the first 24 h of admission, all 169 patients were transfused. The 24 h mortality rate was 13% (22/169).

Table I. Trauma patients characteristics (n = 169)

	Median (IQR)	N (%)
Age (years)	34 (23, 51)	
Male		122 (72)
ISS	29 (19, 36)	
Penetrating		47 (28)
Platelet count, 10^9/L	231 (185, 276)	
INR	1.3 (1.1, 1.5)	
24 h transfused, total blood products, U	11 (4, 21)	169 (100)
24 h RBC, U	5 (2, 10)	169 (100)
24 h FFP, U	6 (3, 11)	154 (91)
24 h platelets, U	12 (6, 18)	46 (27)
24 h mortality		22 (13)

ISS, Injury Severity Score; INR, International Normalized Ratio.

Microvesicle phenotypes

In comparison to healthy controls, both the MV levels and their relative phenotypic distributions were different in trauma patients and controls. Injured patients at admission had significantly higher levels of EMVs, RMVs, LMVs, and TFMVs, while PMV and AVMV levels were lower but failed to reach statistical significance, most likely as a result of the high variance in the patient cohort (Table II), confirming our previous results (14). Phenotypic distributions of MVs were different in trauma patients and healthy controls, for which 87% of all MVs were of platelet origin in controls, but only 63% in patients, while EMVs were less than 1% in controls but 12% in patients. Figure 1a–c shows MV size gate settings with fluorescent beads as well as a representative example of cytograms for PMVs, AVMVs, EMVs, TFMVs, LMVs, and RMVs in a healthy donor and an injured subject, respectively. There were no gender-specific differences in any of the MV phenotypes studied, nor between blunt and penetrating injuries. The Injury Severity Score (ISS) correlated weakly with PMVs and AVMVs ($r = -0.226$, $p < 0.05$ and $r = -0.181$, $p = 0.02$), and platelet counts with PMVs ($r = 0.219$, $p < 0.05$).

Transfusions and MVs

When analysed by transfusion requirements, patients with the lowest admission levels of TFMVs ($p = 0.001$) were transfused with the highest volumes of total blood products (RBC + FFP + PLT) within the first 24 h (Fig. 2a); for the AVMVs, the only significant difference in the number of blood products received was between patients with the very lowest (Q1) and the very highest (Q4) MV counts ($p = 0.026$), most likely as a result of the high variance in AVMVs among patients (Fig. 2b). Similar trends were observed for PMVs ($p = 0.061$) and RMVs ($p = 0.057$). At the same time, there was a significant correlation ($p = 0.011$) between LMVs and higher volumes of transfused blood products, that is, the highest levels of LMVs were associated with a significantly higher volume of transfused blood products (Fig. 2c). In order to determine the contribution of MV phenotypes to patient transfusion requirements, multiple-regression analyses were performed while controlling for age and platelet count. Analysis showed that only low TFMV was a significant, independent predictor of total blood product transfusions (Correlation coefficient -0.056, $p < 0.05$, 95% Confidence Interval (-0.09, -0.03)).

24 h mortality rate and MVs

Twenty-two patients (13%) died within 24 h of admission. Admission levels of AVMVs, PMVs, RMVs, and TFMVs were significantly lower in patients who died within 24 h of admission compared to survivors (all $p < 0.005$) (Fig. 3). There was no difference in age, gender, type of injury (penetrating, blunt), or admission platelet counts between patients who died and those who survived, however deceased patients were more severely injured (ISS 36 vs. 29, $p = 0.002$). There was a weak correlation between platelet count and MVs in survivors ($r = 0.197$, $p < 0.05$). In order to determine the contribution of MVs to 24 h mortality rate, multiple-regression analyses were performed while controlling for age and platelet count; MV levels were not independently associated with 24 h mortality rate.

Time effect (ED admission to ICU admission) and MVs

Forty-two patients had blood samples collected at two time points: pre-resuscitation (at admission to the ED) and post-resuscitation (upon the admission to ICU). Demographics are presented in Table III. The 24 h mortality rate for this cohort was 17% (7/42). When MV levels were compared over time, AVMV and PMV levels were either increased or remained at the ED levels in patients who survived, but decreased in non-survivors, resulting in significantly lower levels at ICU admission in non-survivors (1,048 vs. 1,880 AVMV/μL, $p < 0.00004$; 1,245 versus 1,866 PMV/μL, $p = 0.003$) (Fig. 4a and b). Percentage changes in MVs from ER to ICU in survivors versus non-survivors were calculated. In patients who survived, PMV and AVMV counts were not significantly changed; in non-survivors, PMV and AVMV levels decreased by 39% ($p < 0.05$) and 41% ($p < 0.01$), respectively.

Table II. MVs in controls and injured patients

	Controls (n = 26)		Injured patients (n = 169)		
	Median (IQR)	% of total MVs	Median (IQR)	% of total MVs	*p*
Phosphatidylserine, annexin V (AVMV)/μL	3,282 (2,424–5,364)		2,529 (1,504–4,152)		NS
Platelet (PMV)/μL	2,624 (1,765–4,332)	86.87	2,371 (1,372–3,465)	63.18	NS
Erythrocyte (RMV)/μL	191 (148–254)	4.27	302 (110–399)	9.52	< 0.001
Leukocyte (LMV)/μL	185 (151–328)	6.40	304 (162–456)	10.46	< 0.05
Endothelial (EMV)/μL	23 (19–39)	0.64	399 (286–525)	11.95	< 0.00001
Tissue factor (TFMV)/μL	67 (55–87)	1.82	227 (163–285)	4.89	< 0.00001

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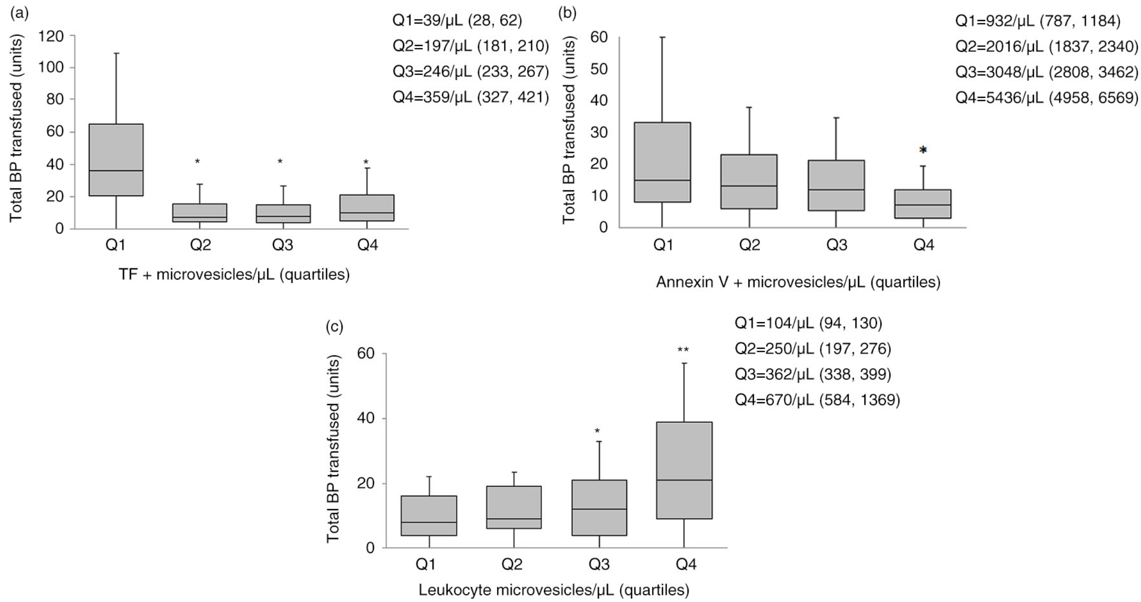

Fig. 2. Admission TFMV, AVMV, and LMV levels and total blood products transfused within the first 24 h. Differences in blood product transfusion by (a) TFMV, (b) AVMP, and (c) LMV level quartiles were determined by the Kruskal–Wallis test. (a) *Denotes $p < 0.0001$ compared to Q1; (b) *Denotes $p < 0.005$ compared to Q1; (c) *Denotes $p < 0.05$ compared to Q1;**Denotes $p < 0.001$ compared to Q1.

Discussion

This study demonstrates that severe injury induces alterations in circulating MV phenotypes and that low-admission MV levels are associated with 24 h transfusion requirements and MV changes over time with death at 24h. Phenotypic distributions of MVs were different in trauma patients when compared to healthy controls. Injured patients had significantly higher EMV, RMV, TFMV, and LMV levels, and a trend toward lower admission levels of PMVs and AVMVs, although not of statistical significance, confirming our previous results (14). There were no age- or gender-specific differences in the MV counts and phenotypes of injured subjects in our study, although they were reported to be different in healthy people (28). The finding that PMVs and AVMVs were not significantly different between the controls and the patients is most likely due to the high variance in both populations, particularly in the levels of the predominant PMVs. The levels of different circulating MV types in injured patients were reported to be both decreased (17), and elevated (15,16,18,20). The majority of circulating MVs in trauma patients were generated by platelets and expressed phosphatidylserine (annexin V-positive staining), indicating their procoagulant phenotype. We and others have reported that MVs circulate in healthy subjects and contribute to thrombin generation (24–26), and it has been reported that PMV counts analysed by flow correlate with their functional activity (29). A trend toward lower admission levels of PMV and AVMV may indicate that, after trauma, either the procoagulant response to injury is impaired or procoagulant MVs are already engaged in clotting processes and interactions with other cells or MVs, and therefore cannot be detected as single vesicles in circulation. The continued release of procoagulant MVs (from platelets) would be beneficial for the maintenance of procoagulant activity.

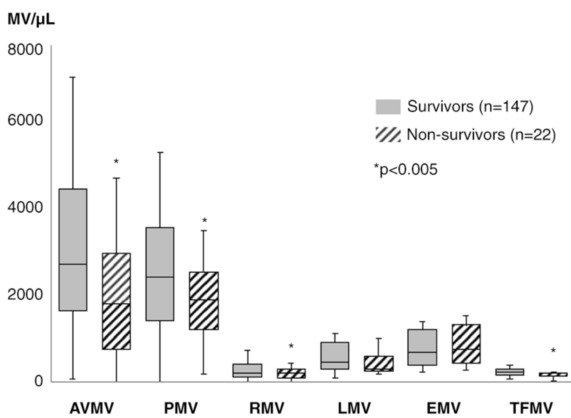

Fig. 3. Admission MV levels in trauma patients, survivors versus non-survivors. Box plots depict differences in MV levels for each cell of origin between patients who were alive versus non-survivors 24 h after admission. *Denotes $p < 0.005$ compared to alive.

Our results showed a weak correlation between PMVs and platelet count at admission. It is recognized that circulating platelet counts often decrease after severe injury, which may be accompanied by the loss of platelet function. Activated platelets become exhausted and refractory and are not capable of producing MVs, or are cleared from circulation. The continuing release of MVs

Table III. Demographics of injured patients with blood sample collected at ED and ICU (n = 42)

	Survivors (n = 35)		Non-survivors (n = 7)		
	Median (IQR)	N (%)	Median (IQR)	N (%)	p
Age (years)	34 (23, 51)		24 (21, 41)		NS
Male		24 (69)		5 (71)	NS
ISS	29 (25, 36)		41 (35, 47)		0.047
Penetrating		10 (29)		2 (28)	NS
Platelet count, 10^9/L	246 (182, 291)		195 (178, 260)		NS
Transfused, TBP, U	26 (11, 46)	35 (100)	21 (9, 83)	7 (100)	NS
24 h RBC, U	11 (7, 15)	35 (100)	12 (4, 31)	7 (100)	NS
24 h FFP, U	11 (5, 19)	33 (94)	9 (5, 27)	7 (100)	NS
24 h platelets, U	6 (6, 18)	17 (49)	30 (24, 33)	3 (43)	NS

ISS, Injury Severity Score; INR, International Normalized Ratio; TBP, total blood products.

from platelets after injury would be beneficial for the maintenance of procoagulant capacity. In line with this, the presence of procoagulant MVs was inversely correlated with transfusion requirements, further reflecting an impaired or suppressed procoagulant response to major trauma. Our previous report demonstrated lower MV levels in severely injured patients with coagulopathy and substantial bleeding (14). Similar findings of a correlation of MVs with transfusions were reported in two other trauma studies (15,16). However, unlike Windelov and colleagues, who did not detect any tissue-factor-positive MVs in their trauma patients, we report here that patients with the lowest admission TFMVs were transfused with the highest volumes of blood products, emphasizing important role of procoagulant MV phenotype in the course of resuscitation. Interestingly, Curry et al. reported a correlation between the number of TFMVs at admission and the number of RBCs transfused within the first 24 h, and significantly higher TFMVs in the RBC recipients compared to trauma patients who did not receive RBCs (15). It is important to note that although elevated circulating levels of TFMVs have been reported in a variety of disorders (30,31), it has also been recognized that the immunodetection of TFMVs is highly impacted by the TF-antibody clones used for detection as well as the presence of non-specific fluorescent particles in antibody solutions (5,32–43). In line with this, our TFMV results should be interpreted with caution when compared with studies that apply different TF clones.

In regard to blood product transfusions, it is important to note that all patients in our study were transfused. One of the criteria for inclusion in the study was that patients would receive blood products, resulting in an all-transfused patient cohort. Therefore, our patient population

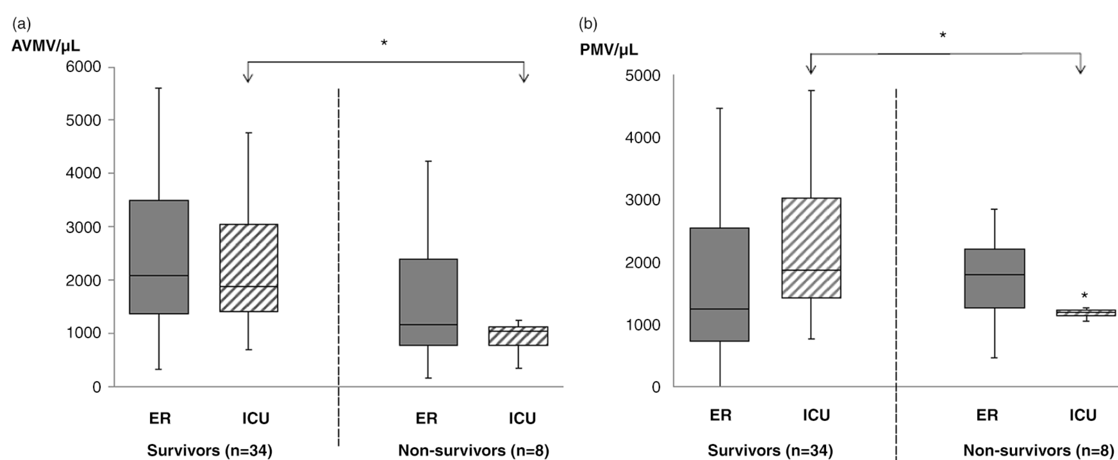

Fig. 4. PMV and AVMV levels in trauma patients at the ER and ICU admission, survivors versus non-survivors. Blood was collected on a subset of enrolled patients upon admission to the ICU. Box plots depict differences in (a) AVMV and (b) PMV levels upon admission to the ER versus ICU between patients who were alive or did not survive 24 h after initial admission. (a) *Denotes p = 0.00004 comparing AVMV levels in the ICU between survivors and non-survivors; (b) *Denotes p = 0.003 comparing PMV levels in the ICU between survivors and non-survivors.

is potentially different from those of other reported trauma studies. It would be useful to have a group of severely injured patients who did not receive blood transfusions and compare the MV response of a patient who does and does not require a post-trauma blood transfusion. Injury and transfusion might play distinct roles in the resulting MV profiles and/or functions, and it is likely that severely injured patients who require blood transfusions exhibit different MV profiles compared to injured patients who do not bleed and hence do not need blood products, as reported (17). A study by Windelov et al. reported lower levels of AVMVs at admission in trauma patients transfused with more than 10 units of RBCs, when compared with both patients who did not receive any transfusion, and those who received 1–10 units of RBCs (16), while Curry et al. reported higher admission levels of TF + PMVs in patients who received RBCs compared to non-transfused patients (15). The further difference between our study and other trauma studies is that we analysed MVs association with total volume of blood products (RBC, plasma, and platelets) compared to the transfused RBCs reported in other studies. Furthermore, we found a correlation between admission LMVs and the volume of total blood product transfused. It might be assumed that the inflammatory response to injury, reflected by increased LMVs, causes increased endothelial leak and dysfunction, requiring higher transfusion volumes. MVs are recognized for their participation in inflammatory responses (5,17), and might contribute to post-traumatic complications, which deserves further study.

In the current study, lower admission MV levels were found in patients who died within 24 h of admission, compared to survivors. However, after multivariate analysis, the lower levels were not independently associated with death at 24h, but MV changes over time (ER versus ICU) were associated with death at 24 h. There are controversial reports of an association between MVs and mortality rates. Windelov et al. found no difference in the PMV levels of survivors and patients who died within 24 h or 28 days (16). Jacoby et al. reported a 24 h mortality rate in trauma patients that was associated with higher levels of admission PMVs (35), while Curry et al. reported significantly lower PMVs in patients who died (15).

Conflicting reports on MV levels in various studies most likely arise from differences in the methodologies used (the selection and/or affinity and specificity of antibodies, and instrument sensitivity) or even differences in trauma populations and circulating blood cell counts over time during the course of trauma and transfusions. These discrepancies definitely open doors for new studies on the role and clinical significance of MVs in the course of trauma and later complications including, but not limited to, inflammation, sepsis, thrombosis, and multiple organ failure (17,35,36).

Importantly, by analysing plasma samples at two time points, pre- and post-resuscitation, we demonstrated that patients whose MV levels were either maintained or increased had positive outcomes compared to those whose levels declined. This is particularly important for AVMVs and TFMVs, two of the most procoagulant MV phenotypes (37). Interestingly, despite high volumes of transfused RBCs, there were no major changes in RMV levels post-resuscitation. Dhillon et al. recently reported that the transfusion of stored RBCs was not associated with increased RMVs (38). Potentially, RMVs might be smaller in size and undetectable by the standard flow cytometry, or their clearance is very fast and efficient. Although it has been reported that RMVs have procoagulant properties (39–41), circulating platelets may be the most important source of MVs involved in the body's response to severe injury.

Of note, apart from their well-recognized roles in hemostasis, PMVs also participate in endothelial repair. PMVs may modulate endothelial function in vitro by changing their receptor expression and secretome (42), and quickening endothelial repair in vivo. We detected significantly elevated EMVs after trauma and could speculate that, in the presence of elevated EMV levels, reflecting endothelial injury, low PMV levels in non-survivors may in fact contribute to poor endothelial repair. Thus, the maintenance or increase in PMV levels during resuscitation would be beneficial for endothelial repair and recovery. Some studies reported low levels of EMVs after injury (15,17,18), potentially indicating that the endothelium was not disrupted, or resulting from differences in the antibodies used to identify EMVs. There was neither a difference in circulating EMV levels pre- and post-resuscitation in our study subjects, nor an association with the 24 h transfusion or 24 h mortality rates, which suggests that, after trauma and transfusions, injured endothelium takes more time to be repaired. This is in line that with fact that vascular complications occur later in the course of trauma, beyond 24 h.

Upcoming results from our recently completed large multicentre trauma study (PROPPR), in which samples were collected at multiple time points post-injury, should provide some answers (44).

Several potential limitations are important for the interpretation of our exploratory analysis of the relationship between MV values and outcomes. First, and as are other trauma studies, ours is a single-centre experience; further work is needed to confirm and extend these findings. Second, a relatively small number of patients had a second sample collected, and a small number of study subjects died. Third, the size detection limits of standard flow cytometry are well known, and miss MVs of smaller sizes. Consequently, reported absolute MV counts may be underrepresented. Finally, the transfusion requirements results could potentially be affected by the number of patients who died soon after hospital admission

and did not live long enough to receive more blood products (45). Overall, the purpose of the current study was not to construct a comprehensive predictive model. The data presented here provide an informative description of MV phenotypes in resuscitated trauma patients. Further studies may identify novel molecular markers among MVs and potential therapeutic targets.

Conclusions

Severe injury results in alterations in MV counts and phenotypic distribution. Elevated MV levels reflect vascular and blood cells activation or injury and procoagulant phenotypes. Lower admission levels of procoagulant MVs are associated with higher transfusion requirements; MV decrease over time is associated with the 24 h mortality rate, while an increase or maintenance of MV levels during resuscitation is associated with survival. The monitoring of circulating MV levels during the course of trauma may potentially serve as a biomarker for the prediction of transfusions or survival or other complications.

Author's contributions

Contributions: NM designed the research, performed experiments, analysed and interpreted data, and wrote the manuscript. YWW performed experiments. JBH and KZ assisted in study design and the editing of the manuscript. JCC assisted with data analysis and the editing of the manuscript. CEW designed the research and assisted in data analysis and interpretation.

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Conflict of interest and funding

The authors declare no conflicts of interest.

References

1. van der Pol E, Boing AN, Harrison P, Sturk A, Nieuwland R. Classification, functions, and clinical relevance of extracellular vesicles. Pharmacol Rev. 2012;64:676–705.
2. Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borras FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015;4: 27066, doi: http://dx.doi.org/10.3402/jev.v4.27066
3. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200:373–83.
4. Gyorgy B, Szabo TG, Pasztoi M, Pal Z, Misjak P, Aradi B, et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. Cell Mol Life Sci. 2011;68: 2667–88.
5. Yuana Y, Sturk A, Nieuwland R. Extracellular vesicles in physiological and pathological conditions. Blood Rev. 2013; 27:31–9.
6. Lacroix R, Dubois C, Leroyer AS, Sabatier F, Dignat-George F. Revisited role of microparticles in arterial and venous thrombosis. J Thromb Haemost. 2013;11(Suppl 1):24–35.
7. van der Meel R, Krawczyk-Durka M, van Solinge WW, Schiffelers RM. Toward routine detection of extracellular vesicles in clinical samples. Int J Lab Hematol. 2014;36: 244–53.
8. Revenfeld AL, Baek R, Nielsen MH, Stensballe A, Varming K, Jorgensen M. Diagnostic and prognostic potential of extracellular vesicles in peripheral blood. Clin Ther. 2014;36: 830–46.
9. Piccin A, Murphy WG, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Rev. 2007; 21:157–71.
10. US Centers for Disease Control and Prevention. Injury prevention and control: data and statistics. 2012 [updated 2012; cited 2014 Dec 21]. Available from: http://webappa.cbc.gov/cgi-bin/broker.exe
11. Holcomb JB, Jenkins D, Rhee P, Johannigman J, Mahoney P, Mehta S, et al. Damage control resuscitation: directly addressing the early coagulopathy of trauma. J Trauma. 2007;62: 307–10.
12. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma. 2006;60: S3–11.
13. Kauvar DS, Wade CE. The epidemiology and modern management of traumatic hemorrhage: US and international perspectives. Crit Care. 2005;9(Suppl 5):S1–9.
14. Matijevic N, Wang YW, Wade CE, Holcomb JB, Cotton BA, Schreiber MA, et al. Cellular microparticle and thrombogram phenotypes in the Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study: correlation with coagulopathy. Thromb Res. 2014;134:652–8.
15. Curry N, Raja A, Beavis J, Stanworth S, Harrison P. Levels of procoagulant microvesicles are elevated after traumatic injury and platelet microvesicles are negatively correlated with mortality. J Extracell Vesicles. 2014;3:25625, doi: http://dx.doi.org/10.3402/jev.v3.25625
16. Windelov NA, Johansson PI, Sorensen AM, Perner A, Wanscher M, Larsen CF, et al. Low level of procoagulant platelet microparticles is associated with impaired coagulation and transfusion requirements in trauma patients. J Trauma Acute Care Surg. 2014;77:692–700.
17. Balvers K, Curry N, Kleinveld DJ, Boing AN, Nieuwland R, Goslings JC, et al. Endogenous microparticles drive the proinflammatory host immune response in severely injured trauma patients. Shock. 2015;43:317–21.
18. Park MS, Owen BA, Ballinger BA, Sarr MG, Schiller HJ, Zietlow SP, et al. Quantification of hypercoagulable state after blunt trauma: microparticle and thrombin generation are increased relative to injury severity, while standard markers are not. Surgery. 2012;151:831–6.
19. Fujimi S, Ogura H, Tanaka H, Koh T, Hosotsubo H, Nakamori Y, et al. Increased production of leukocyte microparticles with enhanced expression of adhesion molecules from activated polymorphonuclear leukocytes in severely injured patients. J Trauma. 2003;54:114–19; discussion 9–20.
20. Ogura H, Kawasaki T, Tanaka H, Koh T, Tanaka R, Ozeki Y, et al. Activated platelets enhance microparticle formation and platelet-leukocyte interaction in severe trauma and sepsis. J Trauma. 2001;50:801–9.
21. Nekludov M, Mobarrez F, Gryth D, Bellander BM, Wallen H. Formation of microparticles in the injured brain of patients with severe isolated traumatic brain injury. J Neurotrauma. 2014;31:1927–33.

22. Midura EF, Jernigan PL, Kuethe JW, Friend LA, Veile R, Makley AT, et al. Microparticles impact coagulation after traumatic brain injury. J Surg Res. 2015;197:25–31.
23. Morel N, Morel O, Petit L, Hugel B, Cochard JF, Freyssinet JM, et al. Generation of procoagulant microparticles in cerebrospinal fluid and peripheral blood after traumatic brain injury. J Trauma. 2008;64:698–704.
24. Matijevic N, Wang YW, Kostousov V, Wade CE, Vijayan KV, Holcomb JB. Decline in platelet microparticles contributes to reduced hemostatic potential of stored plasma. Thromb Res. 2011;128:35–41.
25. Matijevic N, Kostousov V, Wang YW, Wade CE, Wang W, Letourneau P, et al. Multiple levels of degradation diminish hemostatic potential of thawed plasma. J Trauma. 2011;70: 71–9; discussion 9–80.
26. Lawrie AS, Harrison P, Cardigan RA, Mackie IJ. The characterization and impact of microparticles on haemostasis within fresh-frozen plasma. Vox Sang. 2008;95:197–204.
27. Lacroix R, Robert S, Poncelet P, Kasthuri RS, Key NS, Dignat-George F, et al. Standardization of platelet-derived microparticle enumeration by flow cytometry with calibrated beads: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop. J Thromb Haemost. 2010;8:2571–4.
28. Nielsen MH, Beck-Nielsen H, Andersen MN, Handberg A. A flow cytometric method for characterization of circulating cell-derived microparticles in plasma. J Extracell Vesicles. 2014;3: 20795, doi: http://dx.doi.org/10.3402/jev.v3.20795
29. Ayers L, Harrison P, Kohler M, Ferry B. Procoagulant and platelet-derived microvesicle absolute counts determined by flow cytometry correlates with a measurement of their functional capacity. J Extracell Vesicles. 2014;3:25348, doi: http://dx.doi.org/10.3402/jev.v3.25348
30. Lechner D, Weltermann A. Circulating tissue factor-exposing microparticles. Thromb Res. 2008;122(Suppl 1):S47–54.
31. Owens AP, 3rd, Mackman N. Microparticles in hemostasis and thrombosis. Circ Res. 2011;108:1284–97.
32. Key NS, Mackman N. Tissue factor and its measurement in whole blood, plasma, and microparticles. Sem Thromb Hemost. 2010;36:865–75.
33. Basavaraj MG, Olsen JO, Osterud B, Hansen JB. Differential ability of tissue factor antibody clones on detection of tissue factor in blood cells and microparticles. Thromb Res. 2012; 130:538–46.
34. Aass HC, Ovstebo R, Troseid AM, Kierulf P, Berg JP, Henriksson CE. Fluorescent particles in the antibody solution result in false TF- and CD14-positive microparticles in flow cytometric analysis. Cytometry A. 2011;79:990–9.
35. Jacoby RC, Owings JT, Holmes J, Battistella FD, Gosselin RC, Paglieroni TG. Platelet activation and function after trauma. J Trauma. 2001;51:639–47.
36. Soriano AO, Jy W, Chirinos JA, Valdivia MA, Velasquez HS, Jimenez JJ, et al. Levels of endothelial and platelet microparticles and their interactions with leukocytes negatively correlate with organ dysfunction and predict mortality in severe sepsis. Crit Care Med. 2005;33:2540–6.
37. Joop K, Berckmans RJ, Nieuwland R, Berkhout J, Romijn FP, Hack CE, et al. Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms. Thromb Haemost. 2001;85: 810–20.
38. Mooberry MJ, Key NS. Microparticle analysis in disorders of hemostasis and thrombosis. Cytometry A. 2015. doi: http://dx.doi.org/10.1002/cyto.a.22647 [Epub ahead of print].
39. Dhillon SK, Houck ML, Jenkins DH, Rosedahl JK, Harmsen WS, Halling TM, et al. Transfusion of stored red blood cells in trauma patients is not associated with increased procoagulant microparticles. J Trauma Acute Care Surg. 2014;77:674–8.
40. Rubin O, Delobel J, Prudent M, Lion N, Kohl K, Tucker EI, et al. Red blood cell-derived microparticles isolated from blood units initiate and propagate thrombin generation. Transfusion. 2013;53:1744–54.
41. van Beers EJ, Schaap MC, Berckmans RJ, Nieuwland R, Sturk A, van Doormaal FF, et al. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. Haematologica. 2009;94:1513–9.
42. Koshiar RL, Somajo S, Norstrom E, Dahlback B. Erythrocyte-derived microparticles supporting activated protein C-mediated regulation of blood coagulation. PLoS One. 2014; 9:e104200.
43. Mause SF, Ritzel E, Liehn EA, Hristov M, Bidzhekov K, Muller-Newen G, et al. Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. Circulation. 2010;122:495–506.
44. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA. 2015;313:471–82.
45. Del Junco DJ, Bulger EM, Fox EE, Holcomb JB, Brasel KJ, Hoyt DB, et al. Collider bias in trauma comparative effectiveness research: the stratification blues for systematic reviews. Injury. 2015;46:775–80.