Role of Pro-Coagulant Platelet Microparticles in the Complications and Outcome of Acute Liver Injury/Acute Liver Failure (ALI/ALF).

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ABSTRACT

Background. Microparticles (MP), membrane fragments of 0.05-1µm, are derived from many cell types by an enzymatically-catalyzed mechanism in response to systemic inflammation. ALF is a prototypical syndrome of intense systemic inflammatory response (SIRS) associated with defective hemostasis, and paradoxically, a pro-coagulant state despite an elevated INR. **Hypothesis.** Patients with ALI/ALF develop increased pro-coagulant MP's in proportion to the severity of the SIRS, multi-organ system failure (MOSF), and adverse outcome. **Methods.** 50 patients with severe ALI (defined as INR ≥1.5), 78% of whom also had hepatic encephalopathy (HE; ALF), were recruited consecutively. The etiology of ALI/ALF was acetaminophen overdose in 50%. Platelet-poor plasma and other laboratory parameters were collected on admission to the study, and at day 3/4. MP's were characterized by ISADE (Invitrox, Inc), a light scattering device that can enumerate and size MP's as small as 0.15µm, and flow cytometry. Pro-coagulant activity was assessed by a functional MP-tissue factor (MP-TF) assay. **Results.** 16/50 (32%) patients died and 27/50 (54%) recovered without liver transplantation (LT). The presence of SIRS on admission was associated with a mean decline in platelets of $43x10^3$/L at day 3/4, while the absence of SIRS was associated with an increase of $12x10^3$/L (P=0.007). Decreasing platelets after admission was directly associated with laboratory predictors of death/LT (higher venous ammonia [r=0.49; P<0.001], lower arterial pH and bicarbonate [r=0.46 and 0.52; both P<0.001], higher lactate [r=0.39; P<0.01], and higher phosphate [r=0.30; P<0.05]), as well as progression to grade 3/4 HE (P<0.001) and death/LT (P<0.01). MP's of 0.15-1.0µm were detected in all patients, and were CD41+ by flow cytometry in 27/31 (87%), indicating platelet origin. Concentrations of MP's in the 0.36-0.64µm range increased in proportion to the decrease in platelet count after admission (r=0.27; P=0.07), and also correlated with laboratory predictors of death/LT (lower admission bicarbonate [r=0.44; P=0.001], higher phosphate [r=0.52; P=0.0001], and higher creatinine [r=0.31; P=0.03]). Concentrations of 0.36-0.64µm MP's were also increased in direct proportion to the number of SIRS components (P<0.001) and grade of HE on admission (P<0.001), and were significantly lower in patients who spontaneously recovered vs. those who died or underwent LT (4.5E7 vs. 2.1E8/ml, respectively; P=0.0001). MP-TF assays revealed a high degree of procoagulant activity on admission for ALI/ALF (mean 9.05±8.82 pg/ml [normal 0.1-0.3pg/ml]). **Conclusions.** A decline in platelet count after admission for ALI/ALF connotes platelet fragmentation into pro-coagulant MP's, and is associated with the SIRS and poor outcome. In patients with ALF, pro-coagulant platelet MP's may play a role in maintaining re-balanced hemostasis despite the elevated INR, but also may participate in exacerbating the MOSF.

Patients and Methods

Study population: 50 consecutive patients with ALI (evidence of liver injury with INR ≥1.5 in the absence of previous liver disease) and ALF (ALI with hepatic encephalopathy) were recruited from Virginia Commonwealth University as part of the ALF Study Group Registry. Baseline clinical characteristics, laboratories (Table 1), and platelet poor plasma was isolated on admission and at day 3/4 after admission.

Microparticle enumeration and sizing:

Microparticle tissue factor assays:

Microparticle phenotyping:

Feature	Entire Group (N = 51)	Spontaneous Survivors (N = 29)	Death or OLT (N = 22)
Age (years)	43.1 ± 14.7	40.3 ± 15.0	46.7 ± 13.8
Female Gender (%)	61	59	64
Caucasian Race (%)	65	66	64
Etiology of ALI/ALF (N [%]):			
Acetaminophen	22 [43]	17 [59]	5 [23]**
Autoimmune hepatitis	7 [14]		
Hepatitis B	7 [14]		
Idiosyncratic drug	6 [12]		
Indeterminate	4 [8]		
Hepatic ischemia	2 [4]		
Mushroom poisoning	1 [2]		
Heat stroke	1 [2]		
Malignant infiltration	1 [2]		
Hepatic encephalopathy (ALF) (%)	73	55	96**
Number of SIRS	1.6 ± 1.2	1.2 ± 1.0	2.1 ± 1.3**
Creatinine (mg/dL)	1.0 [0.4-8.1]	0.9 [0.4-7.5]	1.5 [0.5-8.1]
Total bilirubin (mg/dL)	6.5 [0.3-44.2]	4.7 [0.9-29.4]	21.0 [0.3-44.2]**
Venous ammonia (µmol/L)	80 ± 38	71 ± 36	91 ± 38
Lactate (mmol/L)	3.4 [0.4-21.4]	2.5 [0.4-6.6]	5.6 [0.7-21.4]**
Phosphate (mg/dL)	3.6 ± 2.4	2.8 ± 1.3	4.8 ± 3.1**
INR	3.4 ± 1.7	3.0 ± 1.3	4.0 ± 1.9*

Table 1. Demographic and laboratory characteristics of study population according to outcome of ALI/ALF.

*P < 0.05, **P ≤ 0.01, ***P < 0.001, ****P ≤ 0.0001 indicates significant difference between spontaneous survivors and those who died or underwent OLT.

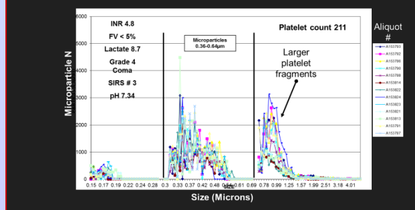

Figure 1. Enumeration and sizing of microparticles in PPP from patients with ALI/ALF by ISADE.

Representative tracing from a patient with severe ALF after acetaminophen overdose, with the indicated admission clinical characteristics. Microparticles of 0.36-0.64µm are indicated, and the focus of subsequent analyses.

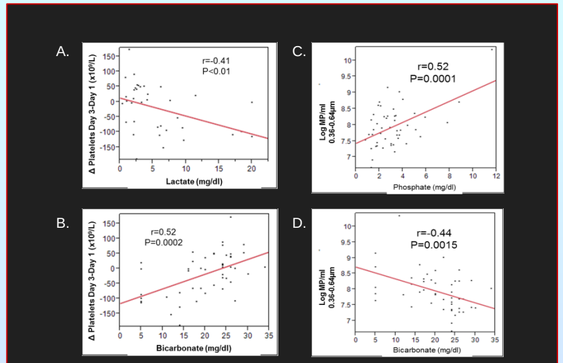

Figure 2. Relationship between change in platelet count and microparticle number on admission to laboratory markers of poor prognosis in patients with ALI/ALF.

(A, B), change in platelet count from day 1 to day 3 vs. day 1 lactate and bicarbonate, respectively. (C, D), microparticle number day 1 vs. phosphate and bicarbonate day 1, respectively.

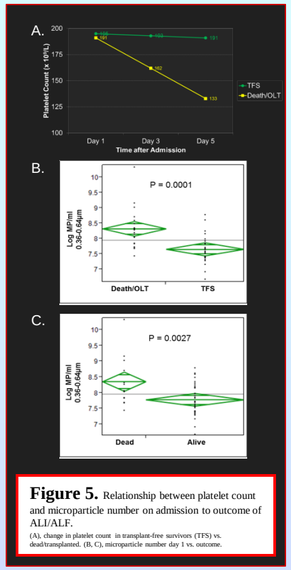

Figure 5. Relationship between platelet count and microparticle number on admission to outcome of ALI/ALF.

(A), change in platelet count in transplant-free survivors (TFS) vs. dead/transplanted. (B, C), microparticle number day 1 vs. outcome.

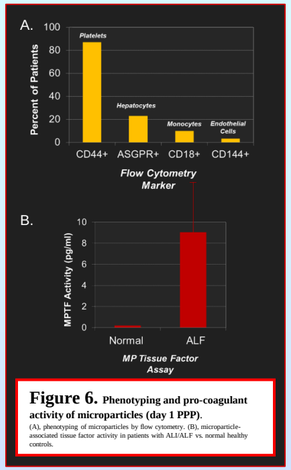

Figure 6. **Phenotyping and pro-coagulant activity of microparticles (day 1 PPP).**

(A), phenotyping of microparticles by flow cytometry. (B), microparticle-associated tissue factor activity in patients with ALI/ALF vs. normal healthy controls.

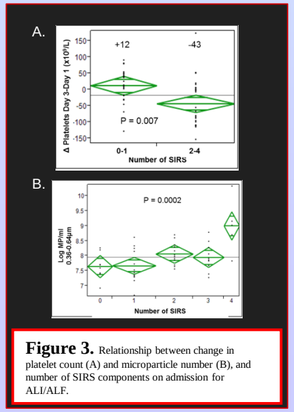

Figure 3. Relationship between change in platelet count (A) and microparticle number (B), and number of SIRS components on admission for ALI/ALF.

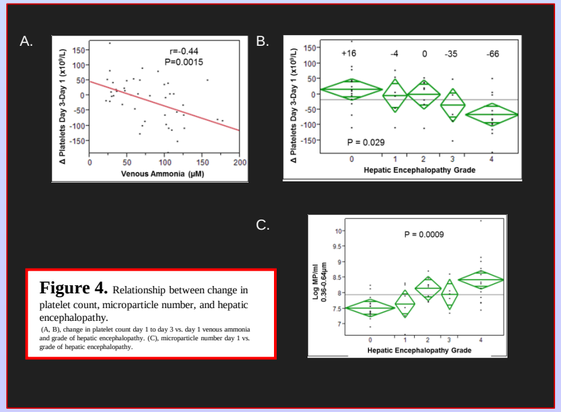

Figure 4. Relationship between change in platelet count, microparticle number, and hepatic encephalopathy.

(A, B), change in platelet count day 1 to day 3 vs. day 1 venous ammonia and grade of hepatic encephalopathy. (C), microparticle number day 1 vs. grade of hepatic encephalopathy.

Summary

1. A decrease in platelet count after admission for ALI/ALF is associated with laboratory markers of poor prognosis, the severity of the SIRS, poor outcome, and the appearance of platelet-derived microparticles.
2. The number of platelet-derived microparticles on admission for ALI/ALF is associated with laboratory markers of poor prognosis, the severity of the SIRS, and poor outcome.
3. Platelet-derived microparticles in plasma of patients with ALI/ALF are highly pro-coagulant.

Conclusions/Hypotheses

1. **In patients with ALI/ALF, fragmentation of platelets into pro-coagulant microparticles may participate in exacerbating the ALF syndrome[1,2].**
2. **Pro-coagulant microparticles may re-balance a hypocoagulable state in ALI/ALF, implied by the elevated INR.**

References

1. Ganey, PE, Luyendyk, JP, Newport, SW, et al. Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. Hepatology. 2007; 46: 1177-1186.
2. Lesurtel, M, Graf, R, Aleil, B, et al. Platelet-derived serotonin mediates liver regeneration. Science. 2006; 312: 104-107.