Introduction: Posttraumatic coagulopathy has been repeatedly associated with the pathogenesis of posttraumatic respiratory distress and the development of the fat embolism syndrome. Intravasation of bone marrow contents into the venous circulation and subsequent pulmonary embolization following intramedullary nailing procedures have been shown to be coupled with the activation of the coagulation and fibrinolytic cascades. The objective of this study was to use a recently developed rabbit model of fat embolism to assess the systemic hemostatic response to pulmonary fat embolism.

Material and methods: All animal procedures were approved and performed in accordance with the local animal care committee guidelines. Following anesthetic administration and preparation, 15 New Zealand White male rabbits were randomly assigned into one of two groups, control and fat embolism (FE). In FE group (n=8), we exposed both distal femoral condyles through a medial parapatellar approach to the right knee. After drilling into the intramedullary cavity in a retrograde fashion, we subsequently reamed the canal using 3.5, 4, and 4.5 mm diameter T-handle reamers. The intramedullary canal was pressurized via a standardized injection of 1-1.5 ml of low viscosity bone cement. The patella was then reduced, and the incision was closed. In the control group (n=7), a sham knee incision was made, exposing both femoral condyles, but was immediately closed without drilling, reaming or pressurization. All animals were mechanically ventilated for an additional monitoring period of four hours post-surgical closure.

Fibrinogen assay, D-dimer latex screen assay, one stage prothrombin time (PT) and activated partial thromboplastin time (aPTT) were analyzed in the usual manner in our institution’s coagulation laboratory. For flow cytometric evaluation of platelet activation, 0.5 ml of blood was mixed with CTAD anticoagulant at a final concentration of 1:10. Samples were stained with fluorescence-conjugated monoclonal antibodies against CD41 (FITC), CD62P (PE) and annexin V (FITC). Platelet events were identified by their characteristic CD41 staining and size; platelet microparticles were identified as CD41-positive events smaller than 0.8 μm. A minimum of 10,000 events was acquired for each sample using a FACSCalibur model flow cytometer equipped with a 488 nm argon ion laser and data was analyzed with CellQuest software. We used the two way repeated measures ANOVA to analyze data from sequential continuous variable measurements. When p<0.05, differences between groups at each time point were compared using unpaired t-tests. Differences between sequential time points within groups were compared using a one-way repeated measures ANOVA followed by Fisher’s PLSD post hoc test.

Results: Fibrinogen and PT levels remained stable throughout the experiment in both groups. Platelet count decreased significantly at 2 and 4 hours post knee manipulation in the FE group. There was also a significant prolongation in aPTT only in the FE group at 4 hours following knee manipulation as compared to baseline values. D-dimers (values ≥250) were only detected in three animals at 2 and 4 hours following knee manipulation in the FE group. In the control group, only one animal revealed D-dimers at 4-hours post sham knee incision. To assess platelet activation, we measured percent expression of CD62P (P-selectin), annexin V, and levels of platelet-derived microparticles (PMP). Platelet microparticles significantly increased at 2 hours post knee manipulation in the FE group. Annexin V expression increased in the FE group at 2 and 4 hours post knee manipulation. Lastly, CD62P expression only increased significantly in the FE group at 2 hours post knee manipulation.

Discussion: Our findings demonstrate platelet activation following forced liberation of bone marrow contents into the circulation in the FE group, as demonstrated by CD62P elevation (a marker of platelet degranulation), annexin V elevation, and increased levels of platelet-derived microparticles (markers of procoagulatory surface expression). Platelet activation coincided with significantly lower platelet counts in the FE group at 2 and 4 hours post embolism, suggesting platelet aggregation.

The implications of platelet activation following fat embolism are numerous. Upon activation platelets adhere, aggregate and secrete key components of both the coagulation and inflammatory cascades. They also actively participate in regulating thrombin production following blood vessel injury and release preformed mediators and generate eicosanoids that regulate hemostasis and inflammation. With increasing numbers of hypercoagulable surfaces (i.e. annexin V, PMP) following platelet activation, one could expect to see platelet aggregation with fat and leukocytes, and microthrombi formation. Aggregates and microvascular thrombi could further exacerbate the acute pulmonary hypertension and associated hemodynamic changes caused by fat embolism. The resulting mechanical obstruction and the release of vasoactive substances into the pulmonary circulation may lead to endothelial damage. Furthermore, PTT was significantly more prolonged in the FE group. This suggests activation of the intrinsic coagulation pathway, which is most likely caused by foreign (e.g. bone marrow debris) surface contact. Interestingly in the FE group, D-dimers (fibrin degradation products) were only detected in three cases with the most significant PT and PTT prolongations, changes resembling the biochemical scenarios of patients with DIC. The findings are consistent with clinical studies of patients undergoing invasive intramedullary procedures, which report considerable variation in the extent to which the coagulative response occurs. These findings therefore suggest that fat embolism does play a role in platelet activation and in the overall activation of the hemostatic system and further investigation should be pursued.