The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe


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Online Supplementary Figure S1. Relative quantification of RBC microparticles comparing fresh and frozen plasma samples. RBC microparticles in platelet-poor plasma were enumerated using absolute count beads following ex vivo stimulation of whole blood obtained from a healthy volunteer at baseline (untreated), with LPS E. coli O111:B4 (10 ng/mL for 2 h), or calcium + ionophore (as positive control to induce microparticle formation). Microparticle counts are calculated according to their relative ratio to a known quantity of fluorescence micro beads spiked into each sample as depicted in the red and blue insets (% microparticles: % beads spiked into sample). Frozen and fresh plasma samples from the same healthy volunteer were assayed for microparticle counts in samples that were untreated or stimulated ex vivo with LPS, or CaCl2 ionophore as shown. Fresh and previously frozen plasma sample from the same healthy volunteer showed a similar relationship across the 3 conditions (untreated, LPS, CaCl2 ionophore). Two samples from the walk-PHaSST cohort (1) SCD1, patient with hyperhemolysis in hemolysis quartile 4 and (2) SCD2, patient with lower hemolysis quartile in quartile 1. SCD1 shows strikingly high MP counts (9,143/mL) compared with SCD2 patient with lower hemolysis quartile. SCD1 shows MP counts that are higher than baseline counts observed in the healthy volunteer (fresh, 159/mL vs frozen 87/mL). Red cell microparticles were defined as discrete, homogenous population of glycophorin A+ events in the forward scatter range at least 1 log range below the size of absolute count fluorescence microbeads (~7.6 microns in diameter) spiked into each sample and indicated in the blue inset. Note the log scale of the x-axis, indicating that the red cell microparticles (shown in the GPA+ inset, red rectangle) are less than 0.76 microns. Microparticle (MP) counts are shown below the x-axis. SCD1 and SCD2 represent frozen plasma samples from sickle cell disease patients that were thawed and assayed. Note the significant amount of glycophorin A+ events within the range of microparticles with SCD1 and SCD2 samples, compared to healthy volunteer. Events acquired with Beckman Coulter Gallios Flow Cytometer.

References