Longitudinal Evaluation of a Standardized P-Selectin Flow Adhesion Bioassay: Potential Role for the Assessment and Prediction of Vaso-Occlusive Episodes in Sickle Cell Disease

Ke Liu[1], Xiufeng Gao[1], Jennell White[2], Michael Callaghan[3], Patrick Hines[1]

[1]Functional Fluidics, Detroit, MI [2]Wayne State University School of Medicine, Detroit, MI [3]Detroit Medical Center, Children's Hospital of Michigan, Detroit, MI

INTRODUCTION

- Sickle cell disease (SCD) is characterized by vaso-occlusive pain episodes (VOEs).
- VOEs are subjective, thus difficult to use as a primary clinical endpoint in SCD.
- P-selectin supports pathological adhesive interactions critical mechanism in VOEs.[1]
- Inhibition of P-selectin in sickle mice lowered leukocyte-endothelial interaction and reversed acute vascular occlusion.[2, 3]
- Transgenic sickle mice deficient in either P-selectin developed fewer vascular occlusions.[4]
- Crizanlizumab, an anti-P-selectin monoclonal antibody, reduced the annual rate of vaso-occlusive crises prospectively in a recent Phase 2 clinical trial.[5]
- Rivipansel, a pan-selectin inhibitor, decreased the length of active sickle crises.[6]
- A standardized P-selectin adhesion bioassay may be a useful prognostic biomarker to for VOEs in SCD.

STUDY OBJECTIVES

- Determine the baseline variability whole blood (WB) and isolated white blood cell (WBC) P-selectin adhesion indices in individuals with SCD over time (6-months).

- Establish the relationship of whole blood (WB) and isolated white blood cell (WBC) P-selectin adhesion indices with self-reported baselines and VOEs in individuals with SCD.

METHODS

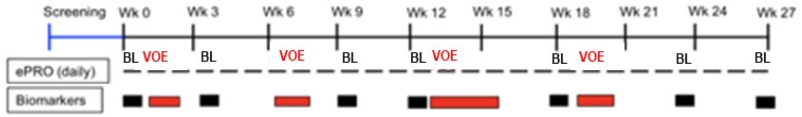

Figure 1. Schematic of study design.

- 6-month observational cohort study in 35 SCD subjects (Fig. 1)
- Clinical baseline or VOE was determined using an electronic patient-reported (ePRO) tool.[7]
- In-home baseline blood samples were collected by a mobile phlebotomist every 3 weeks
- VOE sample were collected within 24 and 48 hours of self-report VOE (home, ED, or hospital), followed by another collection 2 days after VOE resolution, and baseline blood draw was resumed 2 weeks afterwards.
- Hematologic lab tests were conducted for all blood samples (Table 1).
- A standardized microfluidic flow-based P-selectin adhesion assay was performed on all blood samples at physiological flow conditions (1 dyne/cm^2, 1.67 Hz pulsatile flow, 37ºC).
- Adhesion index was defined as the number of cells adhering within a fixed viewing area (cells/mm^2).
- Linear regression and mixed model were used for statistical analysis by R software. $p \leq 0.05$ was deemed statistically significant.

RESULTS

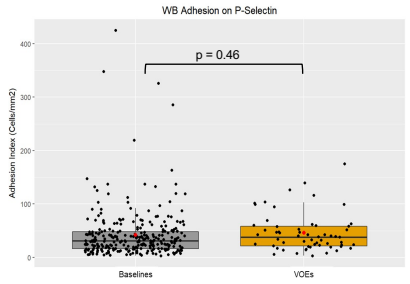

Figure 2A. No significant difference between WB P-selectin adhesion indices at baseline (n = 288, mean = 42 cells/mm^2) vs. VOE (n = 59, mean = 46 cells/mm^2) were observed based on mixed model analysis.

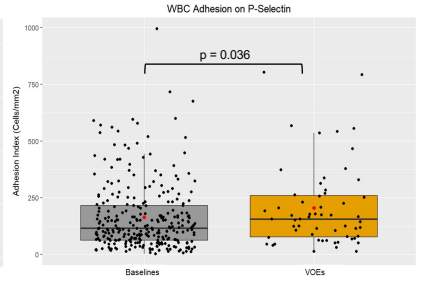

Figure 2B. WBC P-selectin adhesion indices were significantly different at baseline (n = 282, mean = 163 cells/mm^2) vs. VOE (n = 59, mean = 205 cells/mm^2) based on mixed model analysis.

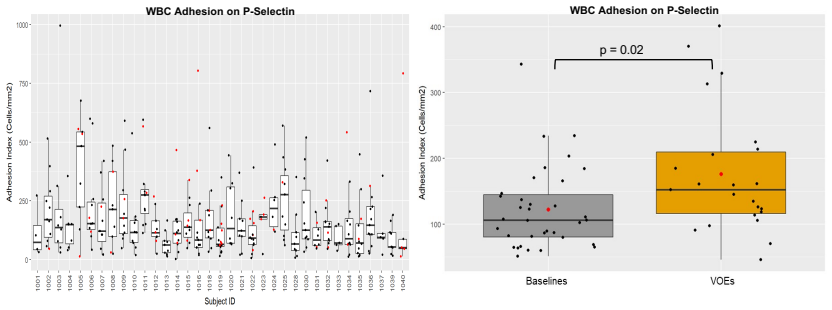

Figure 3. (L) Longitudinal baseline (black dots) and VOE (red dots) of WBC adhesion on P-selectin by subject showing inter- and intra-subject variability; (R) comparison (t-test) of individual patient geometric mean adhesion indices shows significantly higher adhesion indices at VOE (n = 23, mean = 148 cells/mm^2) than baseline (n = 35, mean = 109 cells/mm^2).

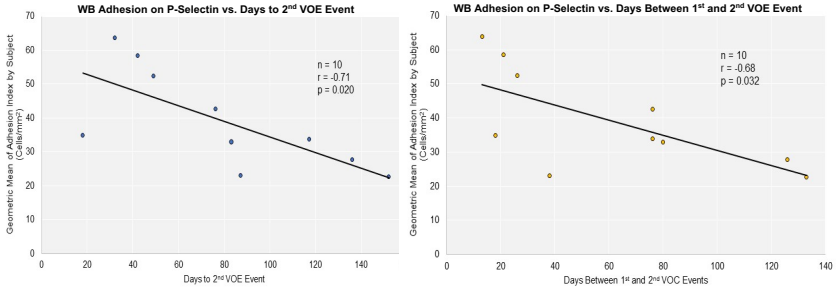

Figure 4. Significant negative correlations were found for individual patient adhesion index of WB on P-selectin with days to 2nd VOE event (L) and days between 1st and 2nd VOE event (R). Among the 35 SCD patients in this study, 10 patients reported > 2 VOEs during the 6-month study period. The geometric mean of adhesion index was based on all blood samples collected prior to the 2nd VOE event.

RESULTS

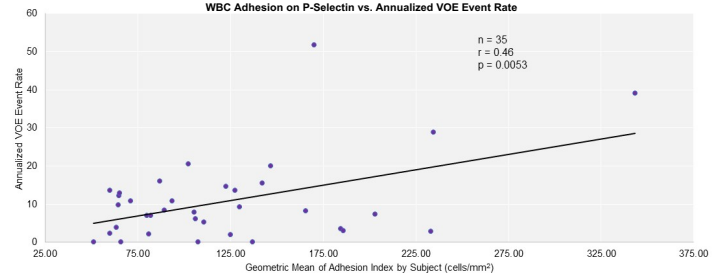

Figure 5. Significant correlation is observed between baseline WBC P-selectin adhesion index vs. annualized VOE rate.

Table 1. Summary of significant correlations between adhesion indices and hematologic parameters

	WB on P-selectin at Baseline	WBC on P-selectin at Baseline	WB on P-selectin at Home-VOE	WBC on P-selectin at Home-VOE	WB on P-selectin at Medical Contact-VOE
CRP	n = 276, r = 0.12 p = 0.047		n = 33, r = 0.44 p = 0.011		
WBC count		n = 277, r = 0.26 p < 0.0001	n = 33, r = 0.63 p < 0.0001		n = 26, r = 0.51 p = 0.0021
Hematocrit		n = 271, r = 0.15 p = 0.017		n = 33, r = -0.37 p = 0.027	
Reticulocyte Percent				n = 33, r = 0.44 p = 0.0058	

VOEs that were managed and treated at emergency department and hospital are categorized as medical-contact VOE

CONCLUSIONS

- The presented P-selectin adhesion assay identifies the unique adhesive phenotype of individuals with SCD.
- WBC adhesion indices on P-selectin were significantly higher during ePRO-validated VOEs compared to ePRO-validated baseline conditions.
- Individuals with higher adhesion indices on P-selectin were more likely to have a VOE event sooner compared to individuals with lower P-selectin indices.
- Adhesion indices of WB and WBCs on P-selectin may be useful prognostic biomarkers for the likelihood and frequency of impending VOEs in individuals with SCD.
- **Limitations**: The small number of subjects makes sub-group analyses challenging. These observations should be validated in a larger multi-center trial. Also, the annualized VOE rate was mathematically deduced rather than through patient observation. Patients should be followed for >12 month in future study.
- **Future Directions**: We are developing predictive model for impending VOEs incorporating the P-selectin adhesion index. Investigations are underway to determine the relationship of adhesion indices to the clinical response to SCD-modifying therapy. It is plausible that clinical adhesion indices may prove to be surrogate endpoints for VOEs and other primary clinical endpoints in SCD.

References

1. *Blood*, **2013**, *122*, 3892-3898
2. *Blood*. **2012**, *120*, 3862-3864
3. *Blood*. **2010**, *116*, 1779-1786
4. *PNAS*, **2002**, *99*, 3047-3051
5. *N Engl J Med*. **2017**, *376*, 429-439
6. *Blood*. **2015**, *125*, 2656-2664
7. *Blood*. **2017**, *130*, 973