A Longitudinal Study to Identify and Assess Adhesion Indices during Vaso-Occlusive Crises in Adults and Adolescents with Sickle Cell Disease

Jennell White[1,2,3], Ke Liu[3], Xiufeng Gao[3], Michael U. Callaghan[2], Patrick Hines[3,4,5]

Department of Pharmacology[1], Carman and Ann Adams Department of Pediatrics[2], Wayne State University School of Medicine, Detroit, MI; Functional Fluidics, Detroit, MI[3]; Division of Critical Care Medicine[4], Children's Hospital of Michigan, Detroit, MI; Department of Physiology, Wayne State University School of Medicine[5]

BACKGROUND

- Sickle cell disease (SCD) is characterized by frequent and unpredictable vaso-occlusive complications (VOCs)
- Sickle erythrocyte adhesion contributes to microvascular occlusion VOCs.
- VOC leads to pain, chronic organ damage, and decreased life expectancy
- The decision to seek medical contact for VOCs varies (contact VOCs), and most self-reported VOCs are self-managed at home (Home VOCs).
- Validated biomarkers for VOCs are needed to understand the clinical phenotype of VOCs and ensure the individuals with SCD receive the most appropriate therapy.
- Standardized adhesion indices may serve as useful biomarkers to asses the contribution of adhesin to an individual patient's VOCs.

Contact VOC (ER/hospitalization)

Home VOC (Hidden Burden of Disease)

Objective

- To determine the relationship between self-reported clinical status and standardized erythrocyte adhesion indices

Study Design

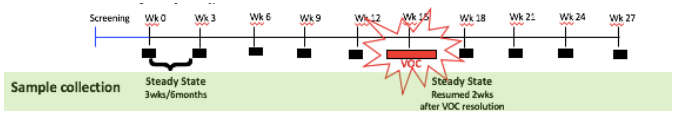
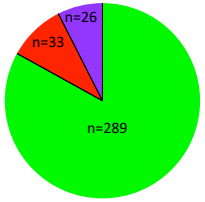


Figure 1. Longitudinal, observational study. SCD patients (n=35) were evaluated over a 6-month period. Steady state and VOC status was defined by self-report using an ePRO tool. Steady state samples were collected by a mobile phlebotomist every 3 weeks at home. VOC samples were collected within 48 hours from a self-reported VOC. VOC management was decided by the patient. VOC samples were collected at home (Home-VOCs) or during medical contact (Contact-VOCs). Steady state samples resumed 3wks after resolution of VOC. Adhesion and clinical lab data was captured at each blood draw.



Steady State
Home-VOC
Contact-VOC

Figure 2. Steady State vs. VOC Study Samples Data shown represents a total of 348 samples collected over 6-months in 35 SCD subjects. 289 samples were collected at steady state and 59 samples during patient-reported VOC (33 Home VOCs, 26 Contact VOCs).

METHODS

Patient Recruitment

Informed consent protocols were performed following institutional review board (IRB) approval by Wayne State University. Blood samples from SCD donors were drawn by venipuncture into anti-coagulated vacutainer tubes containing sodium citrate.

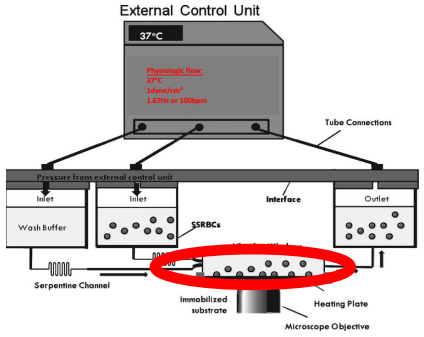


Figure 3. Standardized, Flow Adhesion Assays. Flow adhesion assays were performed with a commercial well-plate micro-fluidic flow adhesion system, BioFlux 1000Z (Fluxion, San Francisco, CA). Blood samples were perfused through VCAM-1-coated microchannels at standard physiologic flow conditions (1dyne/cm2, 1.67Hz). Images were acquired with a high-resolution CCD camera and analyzed with Montage imaging software (Molecular Devices, Downington, PA). An adhesion index was established by quantifying adherent cells within a standard viewing area (cells/mm^2), and could be obtained within 6-9 min.

Statistics

Linear regression and mixed model analyses (R software) were used for statistical analysis. P value < 0.05 was considered significant.

RESULTS

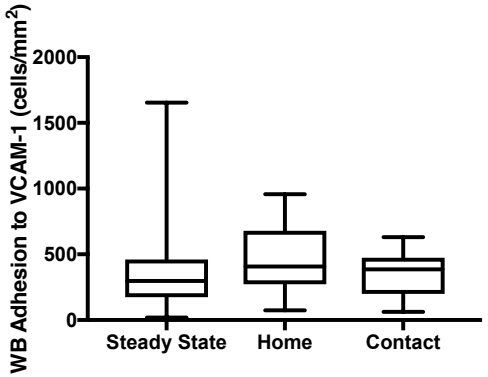


Figure 4. Comparison of adhesion indices at steady state and during ePRO-validated VOC Conditions. Steady state adhesion did not differ from either VOC groups although a comparison of steady state adhesion and home-VOCs approached significance. Home-VOC samples were significantly more adherent than Contact-VOC samples based on mixed model analysis.

**Functional Fluidics ownership interest*

RESULTS

	Steady State (n=289)		Total VOC (n=59)		Home VOC (n=33)		Contact VOC (n=26)	
	r	p value	r	p value	r	p value	r	p value
C-reactive protein (CRP)	0.1600	0.0139	0.0800	0.3602	0.0100	0.7115	0.3100	0.3084
White blood cell (WBC) count	0.2600	0.0010	0.0300	0.4801	-0.0400	0.9016	0.1500	0.5506
Hematocrit (Hct)	-0.1677	0.0046	-0.0845	0.9731	-0.1377	0.4824	-0.0845	0.9731
Reticulocyte %	0.4600	0.0000	0.3300	0.0000	0.4600	0.0074	0.3100	0.3084
Lactose dehydrogenase (LDH)	0.2300	0.0204	0.0400	0.9728	-0.0900	0.5465	0.0020	0.9314
Fetal hemoglobin (HbF)	-0.4400	0.0001	-0.4000	0.0367	-0.5400	0.0103	-0.2900	0.1516

Table 1. Hematologic Drivers of Adhesion During Steady State and VOC. Inflammatory state, RBC turnover, and HbF levels contribute to higher adhesion indices at steady state. Hematologic drivers of adhesion during VOC differ. Contact-VOCs were not related to hematologic lab values in this study.

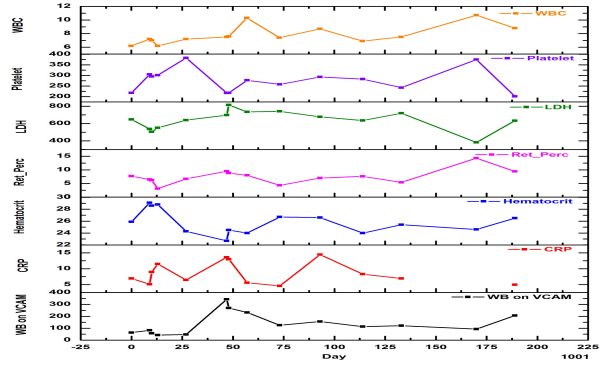


Figure 5. Representative Adhesion and Clinical Data. Patient 1001 received a blood transfusion on day 7 followed by an reported crisis on day 9. Adhesion was not elevated, likely due to contribution of nonadherent transfused AA RBCs. On day 47, a 2nd VOC was reported and adhesion was elevated. From day 47 to 53 and day 152 to 159 this patient visited the ER was admitted to the hospital. Adhesion was reduced immediately following admission. On day 91 (5/31/16), the patient experienced a short VOC, which likely contributed to the slight elevation of cell adhesion of the baseline on day 93 (6/2/16). Steady state adhesion was stable from day 73 to 170 and began to elevate at the end of study. The reason was not clear due to the completion of study.

CONCLUSION/DISCUSSION

- Study represents the largest longitudinal study of adhesion indices using a standardized adhesion bioassay.
- Data confirms the normal range and longitudinal variability of SCD adhesion indices at baseline and during VOC.
- Adhesion increased during patient-reported VOCs in a subpopulation of individuals with SCD.
- At-home VOCs are likely higher because ER-VOC indices are influenced by acute interventions (e.g. fluid boluses, blood transfusions, or anti-inflammatory therapy).
- Further studies are underway to determine if a clinical adhesion index can effectively monitor response to SCD-modifying therapies and prospectively predict disease progression.