Longitudinal assessment of adhesion to vascular cell adhesion molecule-1 at steady state and during vaso-occlusive crises in sickle cell disease

Jennell White,1,2 Michael U. Callaghan,3 Xiufeng Gao,2 Ke Liu,2 Ahmar Zaidi,3 Michael Tarasev2 and Patrick C. Hines2,4
1Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI, 2Functional Fluidics, Children’s Hospital of Michigan, Detroit, MI, 3Division of Pediatric Hematology and Oncology, Children’s Hospital of Michigan, Detroit, MI, and 4Division of Pediatric Critical Care Medicine, Children’s Hospital of Michigan, Detroit, MI, USA

Received 30 May 2021; accepted for publication 28 October 2021
Correspondence: Patrick Hines, 440 Burroughs St., Suite 520, Detroit, MI 48202, USA.
E-mail: patrick.hines@functionalfluidics.com

Summary
Sickle cell disease (SCD) is characterized by frequent and unpredictable vaso-occlusive crises (VOCs). Sickle erythrocytes (SSRBCs) contribute to VOCs by participating in a series of adhesive events with blood cells and the vascular endothelium. Adhesion assays have been used to evaluate the relationship between SSRBC adhesion and SCD severity. We developed a standardized, clinical flow adhesion assay of whole blood to vascular cell adhesion molecule (FA-WB-VCAM). The objective of this study was to assess the variability and clinical predictive value of FA-WB-VCAM in a six-month longitudinal, observational study (ELIPSIIS) in SCD subjects during at-home, steady-state and self-reported VOCs, and following VOC resolution. We observed a strong relationship between FA-WB-VCAM and SCD severity. Adhesion indices were significantly lower in SCD subjects on hydroxycarbamide and increased during VOCs; at-home VOCs had significantly higher FA-WB-VCAM than steady-state and contact VOCs. SCD subjects with a high frequency of self-reported VOCs had a pro-adhesive phenotype at steady state and were stratified into a high-adhesive phenotype cohort; two years prospectively we observed a higher frequency of VOCs in the high-adhesion cohort. This study supports stratifying SCD subjects based on steady-state FA-WB-VCAM and suggests that FA-WB-VCAM may be a plausible surrogate end-point for SCD severity.

Keywords: adhesion, very late antigen-4, vascular cell adhesion molecule-1, vaso-occlusion, sickle cell disease.

Introduction
Sickle cell disease (SCD) is a complex multi-system organ condition that is characterized by frequent and unpredictable vaso-occlusive crises (VOCs) and significant phenotypic variability. Lack of reliable biomarkers to objectively define VOCs in SCD has hindered the development and FDA approval of SCD-modifying therapies. Therefore, there is a need to identify and validate blood-based prognostic biomarkers to similarly stratify individuals at highest risk for vaso-occlusive complications earlier in the disease progression to prevent acute VOC, vasculopathy, and other end-organ events.

More than 100 biomarkers have been identified in SCD although none have been shown to reliably predict VOCs.1-10 Pathologic adhesive interactions of sickle erythrocytes (SSRBCs) to the vascular endothelium contribute to VOCs, resulting in impaired microvascular blood flow, tissue ischemia, severe pain, and end-organ damage. Static and flow-based adhesion assays have been used for decades to describe adhesion in SCD since earlier studies demonstrated that SSRBCs are more adherent than non-sickle erythrocytes and observed a link between SSRBC adhesion and SCD severity.11-13 Since then, numerous adhesion receptor/ligand pairs, expressed on sickle bloods cells, endothelial cells, and the subendothelial matrix, mediating pathologic adhesion in SCD, have been identified.14-17 Of these, very late antigen-4 (VLA-4 or alpha4beta1) is among the most well-characterized adhesion receptors in SCD. VLA-4 is expressed on immature red blood cells which results in increased...
adhesiveness to the vascular endothelium, in large part, via vascular cell adhesion molecule-1 (VCAM-1).\textsuperscript{16,18} Adhesion to the endothelium is reversed by antibodies that block VCAM-1.\textsuperscript{19} Clinically, VLA-4 is highly expressed on reticulocytes (immature SSRBCs) from SCD patients with frequent VOCs and decreased in hydroxycarbamide (HU)-treated patients.\textsuperscript{20,21} Despite these findings, a standardized platform to assess adhesion in SCD is not available for clinical application.

We developed a clinical bioassay to measure flow adhesion of whole blood to immobilized VCAM-1 (FA-WB-VCAM)\textsuperscript{22,23} and designed a longitudinal observational study (ELIPSIS)\textsuperscript{10} to assess adhesion in control (AA/sibling-matched trait-AS) and SCD (SS and Sbeta0) blood samples every three weeks at steady state and SCD blood samples within 24 h of a patient-reported pain crisis. Control samples and SCD samples were collected for three and six months, respectively. Adherent cells were quantified to generate an adhesion index (cells/mm\(^2\)). A daily electronic, patient-reported outcomes tool (ePRO) was utilized by SCD subjects to self-report steady-state and VOC pain status. The ELIPSIS SCD cohort was followed an additional two years after the original six-month longitudinal observation period to assess the frequency of vaso-occlusive end-organ events (VOEEs) in cohorts defined by FA-WB-VCAM (adhesive phenotype).

We documented the feasibility of monitoring out-of-hospital pain and patient-reported VOC days as end-points for clinical trials in SCD and the utilization of mobile phlebotomy to obtain blood samples to measure laboratory biomarkers to improve the identification and assessment of in-hospital and out-of-hospital VOCs in a recent publication.\textsuperscript{10} The objectives of this study were to report the longitudinal range of adhesion indices in control (AS/AA) and SCD (SS/Sbeta0) subjects at patient-reported steady-state, in-home study visits and during VOCs; VOCs were assessed in a home (home-VOC) or healthcare (contact-VOC) setting. We demonstrate the difference in FA-WB-VCAM levels between genotypes and self-reported baseline and VOCs, as well as the relationship between baseline FA-WB-VCAM and SCD severity.

**Methods**

**Study participants**

We designed a six-month longitudinal study to assess FA-WB-VCAM in sickle cell trait (HbAS; \( n = 10 \)), non-SCD healthy participants (HbAA; \( n = 4 \)), and SCD (\( n = 33 \)) subjects at steady-state home visits and self-reported SCD pain crisis. Steady-state samples were obtained every three weeks for six months in SCD subjects and for three months in control study subjects. VOC samples were collected within 24 h from report. This protocol was approved by the Wayne State University Human Investigation Committee Institutional Review Board and written informed consent was obtained from study participants, previously defined in detail.\textsuperscript{10} Participants who were being treated with HU must have been on a stable dose for at least eight weeks prior to visit 1.

**SCD severity index (SCDSI)**

A SCD severity index (SCDSI) is a representation of historical disease severity established by quantifying the total cumulative number of objectively documentable VOEEs, including acute chest syndrome/pneumonia, stroke, priapism, splenic sequestration, hepatic sequestration, and cholelithiasis, indexed over years of life (SCDSI = number of VOEEs/age in years). VOEEs were extracted from the patient’s medical records during the initial screening visit. Each individual VOEE was given equal weight in the scoring system. Additionally, VOEEs were extracted from the electronic medical record during a two-year period after completion of the initial six-month longitudinal study, including SCD-related ER visits and/or hospital admissions and transfusions.

**Clinical flow adhesion assay of whole blood to immobilized vascular cell adhesion molecule (FA-WB-VCAM)**

Flow adhesion assays were performed using pulsatile, shear flow (1.67 Hz, 1.0 dyne/cm\(^2\)) with a commercial well-plate micro-fluidic flow adhesion system, BioFlux 1000Z (Fluxion, San Francisco, CA USA) by modification of published methods.\textsuperscript{22,23} Microfluidic channels were coated with 0.02 mg/ml VCAM-1. Flow conditions mimic blood flow in the post-capillary venules where vaso-occlusive adhesive events are likely to occur. Images were acquired with a high-resolution CCD camera and analyzed with Montage imaging software (Molecular Devices, Downington, PA, USA). An adhesion index (cells/mm\(^2\)) was established for each sample by quantifying adherent cells within a standard viewing area. Steady-state composite adhesion indices were calculated by averaging FA-WB-VCAM adhesion indices from steady-state blood samples measured over six months.

**Statistical analysis**

FA-WB-VCAM adhesion indices were summarized using descriptive statistics. Box-and-whiskers plots were utilized to depict the range of FA-WB-VCAM adhesion indices. Inter-quartile range (IQR) was used to measure the variability of longitudinal samples. Geometric means were reported to account for the effect of data outliers. Mixed-model analyses, accounting for repeated measurements, were utilized to compare the geometric means of adhesion indices among varied genotypes and disease states. Linear mixed-model regression analyses determined relationships between FA-WB-VCAM adhesion indices and haematologic lab values and VOEEs. Data were analyzed using R software (R Foundation for
Statistical Computing, Vienna, Austria; version 4.0). A P value < 0.05 was considered statistically significant.

Results

Steady-state adhesion indices in normal (AA), trait (AS), and SCD (SS, Sbeta0) subjects

The longitudinal range of steady-state FA-WB-VCAM for normal (AA, n = 20, mean = 65 cells/mm², range = 20–294 cells/mm²), trait (AS, n = 49, mean = 57 cells/mm², range = 9–382 cells/mm²), Sbeta0 (n = 47, mean = 372 cells/mm², range = 75–192 cells/mm²), and SS (n = 231, mean = 260 cells/mm², range = 19–1654 cells/mm²) blood samples was established. Like previous reports, 11-13 SCD samples were found to be significantly more adherent than non-SCD samples (AA vs SCD, P < 0.001 and AS vs SCD, P < 0.001; Fig 1A). There was no significant difference between control (AA vs AS; P = 0.72) and SCD (Sb0 vs SS; P = 0.21) groups. Steady-state adhesion indices varied in individual study subjects (Fig 1B); however, adhesion levels did not exceed 400 cells/mm² in control subjects (Fig 1C).

We assessed the relationship between steady-state FA-WB-VCAM (n = 278) and haematologic lab values in SCD blood samples (Table I). Unfortunately, parallel haematologic lab values were not available for each sample; discrepancies in missing values were reported below (e.g. n = 271). Uric acid (n = 267, r = 0.27, P = 0.004), haematocrit (Hct; n = 271, r = −0.30, P = 0.003), white blood cell count (WBC ct; n = 271, r = 0.14, P = 0.02), and lactose dehydrogenase (LDH; n = 265, r = 0.15, P = 0.046) were weakly (2%–9% explanatory significance) associated (n = 220, r = −0.3913, P = 0.0002) with steady-state adhesion indices; however, fetal haemoglobin (HbF; n = 218, r = −0.39, P = 0.0002), and percent reticulocyte levels (Retic %; n = 265, r = 0.45, P < 0.001) showed a moderately strong correlation (15% and 20% explanatory significance, respectively). C-reactive protein (CRP; n = 264, r = 0.10, P = 0.05), haemoglobin S (HbS; n = 39, r = 0.21, P = 0.22), and platelet counts (n = 270, r = −0.02, P = 0.79) did not correlate with steady-state adhesion in this study. A multivariant mixed-effects regression model revealed that SCD subjects with a lower haematocrit (Hct) and higher reticulocyte percent (retic %) had significantly more adherent cells at steady state (r = 0.49; P = 0.009).

Fig 1. Steady-state adhesion indices in normal (AA), trait (AS), and sickle cell disease (SCD; SS, Sb0) patients. Serial blood samples were collected from AA, AS, and SCD patients at steady state. (A) SCD subjects adhered significantly higher than control subjects. (B) Steady-state adhesion varied in individual study subjects. (C) Flow adhesion assay of whole blood to vascular cell adhesion molecule (FA-WB-VCAM) adhesion indices in control subjects did not exceed 400 cells/mm². [Colour figure can be viewed at wileyonlinelibrary.com]
Composite steady-state FA-WB-VCAM in SCD subjects

Composite FA-WB-VCAM was calculated to represent steady-state adhesion by averaging the total number of steady-state FA-WB-VCAM adhesion indices obtained over the six-month period. Composite steady-state FA-WB-VCAM varied from patient to patient and was correlated with our SCDSI \( (r = 0.58, P = 0.005); \) Fig 2A), accounting for approximately 33% of the SCDSI variability. SCD subjects were stratified into two groups, defined as high and low adhesion phenotypes, based on their steady-state composite FA-WB-VCAM measuring above and below the 75th percentile (427 cells/mm\(^2\), Fig 2B) respectively. We also calculated the interquartile range \( (\text{IQR}) \), a reliable measure of variability, for steady-state FA-WB-VCAM in each SCD subject over the six-month period. The high-adhesive phenotype demonstrated higher variability in steady-state FA-WB-VCAM over the six-month period when compared to SCD subjects with a less adhesive phenotype \( \text{(high-adhesion phenotype: IQR = 344 ± 77.6 vs low adhesion phenotype: IQR = 175 ± 137.5, P = 0.009); Fig 2C} \). The high-adhesion cohort also experienced a significantly greater number of VOEIs, ER visits, and hospitalizations during a two-year follow-up period \( \text{(Fig 2D)} \).

Adhesion indices and SCD clinical state

Steady-state FA-WB-VCAM was significantly lower in SCD subjects receiving HU compared to non-HU SCD subjects \( \text{mean}_{\text{HU}} = 230 \text{ vs mean}_{\text{non-HU}} = 337 \text{ cells/mm}^2, \) \( P = 0.047; \) Table II). Also, FA-WB-VCAM from blood samples collected from SCD subjects at steady state \( \text{(non-VOC; n = 278, mean = 276 cells/mm}^2, \text{ range = 19–1654 cells/mm}^2 \) \) were lower than in blood samples collected during VOC \( \text{(n = 55, mean = 340 cells/mm}^2, \text{ range = 61–957 cells/mm}^2, \) \( P = 0.055) \) although statistical significance was not reached. However, FA-WB-VCAM in SCD subjects with at-home self-reported VOCs \( \text{(n = 29, mean = 412 cells/mm}^2, \text{ range = 74–957 cells/mm}^2 \) \), as opposed to those managed in a healthcare-setting \( \text{(Contact-VOC; n = 26, mean = 276 cells/mm}^2, \text{ range = 61–629 cells/mm}^2 \) \), exhibited significantly higher FA-WB-VCAM adhesion indices than steady-state \( \text{(P = 0.016) and Contact-VOC samples (P = 0.032; Table II}. \)

Discussion

Vasculopathy has been implicated in stroke, priapism, pulmonary hypertension, and leg ulceration and suggested in other chronic organ dysfunctions in SCD.\(^{24}\) Adhesive interactions of SSRBCs with the vascular endothelium have long been recognized as important mechanisms of pathologic vasulopathy in SCD. These "pro-adhesive" VOCs have been associated with frequent and unpredictable vaso-occlusive pain and other downstream microcirculatory pathologies. Heterogeneous research-based flow adhesion assays have been traditionally used to measure the adhesiveness of isolated SSRBCs to immobilized adhesive substrates and endothelial monolayers. Despite the established importance of SS RBC adhesive properties in SCD pathology and SCD therapies, there is not a universal accepted standard for the clinical assessment of adhesion in SCD. In this study, we utilized a previously validated standardized, adhesion bioassay \( \text{(FA-WB-VCAM)}^{22,23} \) to define the pro-adhesive phenotype that distinguishes whole blood samples based on genotype, steady-state vs self-reported VOC status, HU treatment status, and SCD severity \( \text{(SCDSI and VOEEs)}. \)

A longitudinal steady-state range is critical in order to establish a standard for an adhesion biomarker that can be used in the clinical management of SCD. We establish the longitudinal steady-state ranges and composite averages for FA-WB-VCAM in control \( \text{(22–168 cells/mm}^2 \) \) and SCD \( \text{(84–917 cells/mm}^2 \) \) subjects. This allows FA-WB-VCAM data collected in a clinic setting or in the context of a clinical trial to be contextualized against a similar population. We also report that SCD subjects on stable HU therapy have lower adhesion indices than SCD subjects not on HU. The difference in FA-WB-VCAM in subjects at steady state and during VOC did not achieve significance; however, a subanalysis comparing the difference in FA-WB-VCAM during home vs contact \( \text{(ER or hospital)} \) VOCs showed home VOCs were

### Table I. Correlation between steady-state flow adhesion assay of whole blood to vascular cell adhesion molecule (FA-WB-VCAM) adhesion indices and hematologic lab values in sickle cell disease (SCD) blood samples.

| Parameter                  | n    | R\(^2\) | t    | P value |
|----------------------------|------|---------|------|---------|
| C-reactive protein (CRP, mg/ml) | 264  | 0.0102  | 0.1010 | 0.0536  |
| Hematocrit (Hct, %)         | 271  | 0.0908  | −0.3012 | 0.0001* |
| Hemoglobin F (HbF, %)       | 218  | 0.1531  | −0.3913 | 0.0002* |
| Hemoglobin S (HbS, %)       | 39   | 0.0449  | 0.2119 | 0.2153  |
| Lactate dehydrogenase (LDH, units/l) | 265  | 0.0237  | 0.1540 | 0.0460  |
| Platelet count (K/mm\(^3\)) | 270  | 0.0003  | −0.0164 | 0.7887  |
| Reticulocytes (%)           | 265  | 0.2027  | 0.4502 | <0.0001* |
| Uric acid (mg/dl)           | 267  | 0.0729  | 0.2700 | 0.0004* |
| White blood cell (WBC, K/mm\(^3\)) | 271  | 0.0193  | 0.1389 | 0.0217* |

*Statistically significant values \( (P < 0.05). \)
significantly higher than both steady-state and contact VOCs. The likely explanation for the lower contact-VOC adhesion is that subjects generally receive therapeutic interventions such as RBC transfusions, fluid bolus, and non-steroidal anti-inflammatory drugs (NSAIDS), which can reduce systemic inflammation. The limitation of the study is that the study was not designed to collect information on acute interventions or to ensure blood was consistently taken before interventions, so a future study will determine this definitively.

We stratified SCD subjects into high- (>75th %) or low- (<75th %) adhesion phenotypes according to their composite steady-state FA-WB-VCAM (six-month longitudinal average of steady-state adhesion). There was a significant correlation between composite steady-state FA-WB-VCAM and historical SCDSI, demonstrating that FA-WB-VCAM reflects historical disease control in SCD. More importantly, subjects with a "high" adhesive phenotype had significantly greater variability in steady-state adhesion indices over time (e.g. IQR) and
VOEES two years following the original six-month longitudinal study. These data suggest that blood-based adhesion biomarkers obtained during steady state can stratify individuals with SCD into high and low risk categories based on their risk of future VOEES. Ongoing studies will determine whether adhesive phenotypes are stable over time by retesting composite steady-state FA-WB-VCAM in “low” and “high” cohorts.

High levels of very late antigen-4 (VLA-4) expression on reticulocytes, levels of soluble VCAM-1 and its expression on endothelium, have been associated with response to SCD-modifying therapy, vaso-occlusive adhesive events, and severe disease phenotypes.5,19,20,21,25,26,27 Soluble VCAM-1 (sVCAM-1) and endothelial VCAM-1 adhesive interactions are elevated in SCD plasma and during VOC and decreased during HU therapy.5,25,26,27 SSRBC adhesion to TNF-alpha-treated endothelium and plasma TNF-alpha is also increased in SCD.27 VLA-4 supports avid interactions between SSRBCs and endothelial VCAM-1.19,28,29,30 SSRBC adhesion to the endothelium and SSRBC-WBC aggregates are reversed by anti-VCAM-1 antibodies in vivo.19,31 Clinically, VLA-4 levels are higher in SCD subjects with frequent VOCs and decreased in SCD patients treated with HU.20,21 We have reported the utility of a VCAM-1-induced endothelial substrate in prior adhesion studies;32 however, cultured endothelial cells are not feasible for a standardized, scalable clinical assay. Assessment of adhesion to an immobilized VCAM-1 substrate with FA-WB-VCAM will directly assess this critical adhesive interaction in a much more physiologic context and is therefore expected to reflect current and historical SCD control and severity.

The adhesion bioassay utilized in this study provides clinicians an objective measure of adhesion in blood samples collected from SCD subjects. We show that a pro-adhesive phenotype is not only associated with having a high-level adhesion over time but that fluctuations of cell adhesion to VCAM-1 may allow for the initiation of adhesion-mediated vaso-occlusive events to occur. The results of this study suggest that SSRBC adhesion under physiologic flow in the context of whole blood, as assessed by FA-WB-VCAM, may be a surrogate for clinical disease severity. We recently demonstrated the ability of our flow-based adhesion biomarkers, including flow adhesion of whole blood to VCAM-1 (FA-WB-VCAM), flow adhesion of whole blood to P-selectin (FA-WB-Psel), flow adhesion of white blood cells to VCAM-1 (FA-WBC-VCAM) and flow adhesion of white blood cells to P-selectin (FA-WBC-Psel), to predict the risk of recurring VOEs in SCD subjects.33 Additional studies in a larger prospective cohort are underway to definitively establish the predictive value of FA-WB-VCAM for impending VOEs, identifying SCD subjects with severe disease phenotypes, and to establish its role in therapeutic monitoring of SCD-modifying therapies.

Acknowledgements

The authors acknowledge all SCD subjects and individuals who contributed to this study. We thank David Beidler and Debra D. Pittman for their role in the design and initiation of the study. We also thank Sanguine Biosciences, Clinical Ink, and CRF Health for their expertise and efforts to make this study possible.

Funding information

This study was sponsored by Pfizer Inc.

Author contributions

PCH contributed to study design, data analysis, and manuscript preparation. MUC contributed to study design and data analysis. AZ contributed to study design and data analysis. XG performed all flow adhesion assays and contributed analysis of biomarker data, and manuscript preparation. KL contributed to the flow adhesion data analysis. JW participated in flow experiments, analysis of biomarker data, clinical data collection, and manuscript preparation. MT contributed to the data analysis and manuscript preparation. All authors contributed to interpretation of the data, participated in the critical review and revision of the manuscript, and provided approval of the final manuscript. All authors have access to the data and assume responsibility for the completeness and accuracy of the data and data analyses.

Conflicts of interest

PCH, KL, XG, JW and MT are employees and shareholders of Functional Fluidics. MC has received grants and personal fees from Bayer, Biomarin, Global Blood Therapeutics, Hema Biologics, Kedrion, Octapharma, Pfizer, Roche/Genentech, Sanofi/Bioverativ, Spark Therapeutics and Takeda.
Data availability statement

The authors confirm that the full data supporting the findings of this study are available within the article.

References

1. Adewoeye AH, Kling E, Farber HW, Palaima E, Bausero MA, McMahon L, et al. Sickle cell vasculo-occlusive crisis induces the release of circulating serum heat shock protein-70. *Am J Hematol.* 2005;78(3):240–2.

2. Adisa OA, Hu Y, Ghosh S, Aryee D, Osunkwo L, Olori-Acuah SF. Association between plasma free haem and incidence of vasculo-occlusive episodes and acute chest syndrome in children with sickle cell disease. *Br J Haematol.* 2013;162(5):702–5.

3. Duits AI, Schnog JB, Lard LR, Saleh AW, Rojer RA. Elevated IL-8 levels during sickle cell crisis. *Eur J Haematol.* 1998;61(5):302–5.

4. Goncalves MS, Queiroz IL, Cardoso SA, Zanetti A, Strapazoni AC, Adorno E, et al. Interleukin 8 as a vasculo-occlusive marker in Brazilian patients with sickle cell disease. *Br J Med Biol Res.* 2001;34(10):1309–13.

5. Hatziantelis ES, Pana ZD, Gombakis N, Taparkou A, Tzimouli V, Kleta D, et al. Endothelial activation and inflammation biomarkers in children and adolescents with sickle cell disease. *Int J Hematol.* 2013;98(2):158–63.

6. Krishnan S, Setty Y, Betal SG, Vijender V, Rao K, Dampier C, et al. Role of Lu/BCAM in abnormal adhesion of sickle red blood cells to vascular endothelium. *Transfus Clin Biol.* 2008;15(6):872–6.

7. Lum AF, Wun T, Staunton D, Simon SI. Inflammatory potential of neutrophils detected in sickle cell disease. *Blood Cells Mol Dis.* 2010;44(3):196,202–2.

8. Styles LA, Aarsman AJ, Vichinsky EP, Kuypers FA. Secretory phospholipase A(2) predicts impending acute chest syndrome in sickle cell disease. *Blood.* 2000;96(9):3276–8.

9. Pittman DD, Hines PC, Beidler D, Rybin D, Frelinger AL, Michelson AD, et al. Modulation of erythroid adhesion receptor expression by hydroxyurea in children with sickle cell disease. *Haematologica.* 2008;93(4):502–10.

10. Styles LA, Lubin B, Vichinsky E, Lawrence S, Hua M, Test S, et al. Decrease of very late activation antigen-4 and CD36 on reticulocytes in sickle cell patients treated with hydroxyurea. *Blood.* 1997;90(7):2554–9.

11. White J, Lancelot M, Gao X, McGraw BJ, Tabb C 2nd, Hines P. Cross-sectional analysis of adhesion in individuals with sickle cell disease using a standardized whole blood adhesion bioassay to VCAM-1. *Blood Cells Mol Dis.* 2020;61:102397.

12. White J, Lancelot M, Sarnaik S, Hines P. Increased erythrocyte adhesion to VCAM-1 during pulsatile flow. Application of a microfluidic flow adhesion bioassay. *Clin Hemorheol Microcirc.* 2015;60(2):201–13.

13. Searjent GR. The clinical features of sickle cell disease. *Baillieres Clin Haematol.* 1993;6(1):93–115.

14. Sakhalar VS, Rao SP, Weendon J, Miller ST. Elevated plasma sVCAM-1 levels in children with sickle cell disease: impact of chronic transfusion therapy. *Am J Hematol.* 2004;76(1):57–60.

15. Dworak DS, Klings ES, Soloviev N, Li G, Milton JN, Hartley SW, et al. Severe sickle cell anemia is associated with increased plasma levels of TNF-R1 and VCAM-1. *Am J Hematol.* 2011;86(2):220–3.

16. Verger E, Schoeavaert D, Carriavain P, Victor IM, Lapoumeroulie C, Elion J. Prior exposure of endothelial cells to hydroxyramamide alters the flow dynamics and adhesion of sickle red blood cells. *Clin Hemorheol Microcirc.* 2014;57(1):9–22.

17. Joneckis CC, Ackley RL, Orringer EP, Wayner EA, Parise LV. Integrin alpha 4 beta 1 and glycoprotein IV (CD36) are expressed on circulating reticulocytes in sickle cell anemia. *Blood.* 1993;82(12):3548–55.

18. Brittian JE, Knoll CM, Ataga KJ, Orringer EP, Parise LV. Fibronectin bridges monocytes and reticulocytes via integrin alpha beta 1. *Br J Haematol.* 2008;141(6):872–41.

19. Chaar V, Picot J, Renaud O, Bartolucci P, Negoatu R, Bachir D, et al. Aggregation of mononuclear and red blood cells through an [alpha]4 [beta]1-Lu/basal cell adhesion molecule interaction in sickle cell disease. *Haematologica.* 2010;95(11):1841–8.

20. Belcher JD, Mahaset H, Welch TE, Vilback AE, Sonbol KM, Kalambur VS, et al. Critical role of endothelial cell activation in hypoxia-induced vasocellular transmigration in transgenic sickle mice. *Am J Physiol Heart Circ Physiol.* 2005;288(6):H1275–25.

21. White J, Krishnamoorthy S, Gupta D, Lancelot M, Moore N, Sarnaik S, et al. VLA-4 blockade by natalizumab inhibits sickle reticulocyte and leukocyte adhesion during simulated blood flow. *Br J Haematol.* 2008;143(6):970–82.

22. Hines PC, Callaghan MU, Zaidi AU, Gao X, Liu K, White J, et al. Flow adhesion of whole blood to P-selectin: a prognostic biomarker for vasculo-occlusive crisis in sickle cell disease. *Br J Haematol.* 2021;194(6):1674–82.