Biological Research for Nursing
2022, Vol. 24(1) 24–30

DOI: 10.1177/10998004211027220
journals.sagepub.com/home/brn
SAGE

Severe Persistent Pain and Inflammatory Biomarkers in Sickle Cell Disease: An Exploratory Study

Mitchell R. Knisely, PhD, RN-BC, ACNS-BC[1], Paula J. Tanabe, PhD, MPH, RN, FAEN, FAAN[1], Julia K. L. Walker, PhD[1], Qing Yang, PhD[1], and Nirmish R. Shah, MD[2]

Abstract

Background: Severe pain is among the most common and deleterious symptoms experienced by individuals with sickle cell disease (SCD), of whom more than 50% report chronic pain. Despite this, the understanding of the biological contributors to persistent severe SCD pain is limited. This exploratory study sought to describe pain phenotypes based on frequency of severe pain experienced over 6 months and identify inflammatory biomarkers associated with pain phenotypes among individuals with SCD. **Methods:** This study used self-report and electronic health record data collected from 74 individuals enrolled in the Duke Sickle Cell Disease Implementation Consortium Registry. Plasma from previously collected blood specimens was used to generate inflammatory biomarker data using the Inflammation 20-plex ProcartaPlex™ panel. Descriptive statistics were used to describe the occurrence of severe pain over the past 6 months, and bi-variate analyses were used to evaluate the relationship between inflammatory biomarkers and pain phenotypes. **Results:** Among the 74 participants included in this study, 33.8% reported severe pain occurring never or rarely, 40.5% reported severe pain occurring sometimes, and 25.7% reported severe pain occurring often or always. Soluble E-selectin (sE-selectin) was the only inflammatory biomarker significantly associated with the pain phenotype groups ($p = 0.049$). Post hoc comparisons identified that participants in the often/always severe pain group had significantly higher plasma concentrations of sE-selectin compared to those in the sometimes severe pain group ($p = 0.040$). **Conclusions:** Our findings provide preliminary evidence of the frequent occurrence of severe pain and that sE-selectin may be an objective biomarker for the frequent occurrence of severe pain in this population.

Keywords

sickle cell disease, pain, biomarkers, inflammation

Pain is a common and debilitating symptom experienced across the lifespan by people with sickle cell disease (SCD). As few effective treatments for SCD pain exist, opioids remain a first-line treatment for this population (Han et al., 2018). Pain management is challenged by the substantial heterogeneity in SCD pain occurrence, severity, and disability (Dampier et al., 2002; Smith et al., 2008; Wilkie et al., 2010). Acute pain occurring during vaso-occlusive events (VOEs) are the hallmark of SCD. These events are abrupt and unpredictable, and the underlying pathophysiology of these events are complex. VOEs are caused by vaso-occlusion of the microcirculation, which includes elements of inflammation and abnormally shaped red blood cells adhering to blood vessel walls (Odievre et al., 2011; Telen, 2007). The number of acute episodes increases with age, and individuals can transition from acute episodes to persistent chronic SCD pain or pain lasting longer than 3 months (National Heart, 2014). Evidence suggests that more than 50% of adults with SCD experience chronic pain (Lanzkron et al., 2018; Smith et al., 2008).

The etiology of persistent SCD pain is unclear; however, inflammatory mechanisms likely contribute to its development (Gupta et al., 2018; Tran et al., 2017). Outside SCD, inflammatory mechanisms such as dysregulation of cytokines have been implicated in the development of persistent pain syndromes (e.g., fibromyalgia, neuropathic pain; Ji et al., 2018; Kraychete et al., 2009; Sturgill et al., 2014). The complex pathophysiological processes of SCD that lead to VOEs involve decreased oxygenation of tissues and ischemic-reperfusion injury (Gupta et al., 2018) and can eventually cause permanent tissue damage and trigger the release of

[1] Duke University School of Nursing, Durham, NC, USA
[2] Duke University School of Medicine, Durham, NC, USA

Corresponding Author:
Mitchell R. Knisely, PhD, RN, ACNS-BC, PMGT-BC, Duke University School of Nursing, 307 Trent Drive, DUMC 3322, Durham, NC 27710, USA.
Email: mitchell.knisely@duke.edu

inflammatory mediators, such as cytokines and endothelial cell adhesion molecules, leading to chronic vascular inflammation and damage (Conran & Belcher, 2018; Conran et al., 2009; Proença-Ferreira et al., 2014).

Key features of inflammation in chronic pain include the production and secretion of cytokines (e.g., interleukin 6 (IL-6), interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α); Ji et al., 2018; Kiguchi et al., 2012). Cytokines are proteins that regulate inflammatory and immune processes and serve as excitatory mediators in pain modulation (Ramesh et al., 2013). Sustained increases in cytokine levels can induce hyperalgesia and allodynia, and promote the transition from acute to more persistent widespread pain (Ji et al., 2018; Slade et al., 2011). Pro-inflammatory cytokines serve as excitatory mediators in pain modulation, which underscores the bidirectional signaling between the nervous and immune systems in the development of pain (Huh et al., 2017; Ramesh et al., 2013). People with SCD in steady state have increased cytokines (e.g., IL-6, interleukin 10 (IL-10), TNF-α) and endothelial cell adhesion molecule (intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin) levels that are further increased during painful VOEs (Antwi-Boasiako et al., 2018; Francis & Haywood, 1992; Graido-Gonzalez et al., 1998; Hibbert et al., 2005; Pathare et al., 2004; Qari et al., 2012). Cytokines (e.g., interleukin 4 (IL-4), interleukin 8 (IL-8), IL-1β, TNF-α) are also significantly associated with experimentally induced pain severity in adults with SCD (Campbell et al., 2016). While evidence suggests that inflammatory biomarkers are elevated during acute painful episodes and induction of experimental pain, their relationship with persistent severe pain phenotypes in SCD remains unclear.

Pro-inflammatory mediators can activate the endothelium and promote the expression of cell adhesion molecules (Pober & Sessa, 2007; Zhang, 2008). Selectins are cell surface adhesion molecules categorized into P-, E- and L-selectins based on the tissues in which their expression was first found (platelets, endothelial cells and leukocytes; Miyagi & Yamaguchi, 2007). Under conditions of injury or infection the endothelial expression of E-selectins is naturally increased resulting in increased endothelial adhesivity for platelets and/or leukocytes. These cell-cell adhesions slow the circulating blood cells, an important step in clot formation and/or leukocyte diapedesis into the tissues (Harjunpää et al., 2019). Although healthy erythrocytes display relatively weak interactions with the endothelium and other blood cells (Wautier & Wautier, 2004), in diseases such as sickle cell disease and diabetes mellitus, red blood cells (RBCs) have increased adherence to other blood cells and the endothelium. Thus, there is evidence linking vaso-occlusive events in sickle cell disease with increased adhesive interactions of sickled RBCs, leukocytes, and the endothelium (Telen, 2007).

Endothelial activation has been a target of drug development and treatment of pain in SCD. For example, a pilot study of 19 SCD patients evaluated the efficacy of simvastatin (a statin medication that improves endothelial function through anti-inflammatory effects and increased nitric oxide production) for improving vaso-occlusion-related pain and reducing soluble inflammatory biomarkers (Hoppe et al., 2017). The investigators found that treatment with simvastatin caused a significant reduction in acute vaso-occlusive pain events; oral analgesic use; and circulating soluble E-selectin, ICAM-1, VCAM-1, and vascular endothelial growth factor (VEGF; Hoppe et al., 2017).

In this exploratory study, we sought to describe different pain phenotypes based on the frequency of severe pain experienced over the past 6 months and identify inflammatory biomarkers associated with different phenotypes of severe pain in individuals with SCD. This hypothesis-generating study will inform future research to improve our understanding and management of persistent severe pain in this population.

Materials & Methods

Design & Participants

This exploratory, cross-sectional, ancillary study leveraged recruitment and data collected for the Sickle Cell Disease Implementation Consortium (SCDIC) Research Registry at Duke University (U01HL133964) (DiMartino et al., 2018), a comprehensive registry that includes prospective, longitudinally collected patient-reported and electronic health record data (Glassberg et al., 2020). All participants signed a written consent prior to participation and were given the option to provide a blood specimen for biobanking and use in future research. Enrollment inclusion criteria for the Duke SCDIC Research Registry are (a) $\geq$15 years of age, (b) live in North Carolina, and (c) have a genetically-confirmed SCD diagnosis. An additional criterion for inclusion in this study was the availability of a blood specimen. A total of 74 participants met these inclusion criteria. The Duke University Institutional Research Board approved the research registry and determined that this ancillary study qualified as exempt.

Data Collection

Participant reports of pain were assessed using a modified item from the Adult Sickle Cell Quality of Life Measurement Information System (ASCQ-ME), a well validated measure of SCD quality of life (Keller et al., 2017). Specifically, pain phenotypes were determined using a modified ASCQ-ME question: "In the past 6 months, how often did you have very severe pain?" The question was modified by altering the timeframe for recall from 7 days to 6 months. This 6-month timeframe was determined by experts across the eight SCDIC study sites and is consistent with the proposed diagnostic criteria for chronic pain associated with SCD (Dampier et al., 2017). Possible responses were scored on a 5-point Likert scale: *Never* (0), *Rarely* (1), *Sometimes* (2), *Often* (3), and *Always* (4). Participants were classified into three pain phenotypes based on their responses on the occurrence of severe pain: *Never* or *Rarely* = never/rarely severe pain group, *Sometimes* =

sometimes severe pain group, and *Often* or *Always* = often/always severe pain group.

Demographic and other clinical characteristics were obtained from either self-report data collection forms or the medical record to describe the sample. Study personnel completed a review of participants' medical records to abstract clinical data. All self-report and medical record data were entered and stored in an investigator-designed database.

Biomarker Data Collection

Blood specimens were collected at routine clinic appointments and immediately transported on ice to the laboratory. Specimens were centrifuged (10 minutes RCF 1,800 × *g* at 7 °C) immediately, and plasma was pipetted into cryotubes and stored at −80 °C until analyses. Using the Duke Immune Profiling Core, plasma inflammatory biomarker levels were analyzed using the Inflammation 20-plex ProcartaPlex™ Assay (Thermo Fisher, Waltham, MA) per manufacturer's protocol. This assay includes 20 pro- and anti-inflammatory cytokines and chemokines as well as other inflammatory mediators. The plates were run on a Luminex MAGPIX platform. All samples were run in duplicate and the average result between duplicate readings for each inflammatory biomarker was used in the analyses. Samples that were below the detectable limit of the assay for a particular inflammatory biomarker were excluded from analyses for that specific marker. Biomarkers (n = 4: GMCSF, IFN-γ, IFN-α, IL-4) that had ≥25% of participants who had concentrations below the detectable limits were excluded from the analyses.

Statistical Analyses

Descriptive statistics were used to summarize sample characteristics by each pain phenotype group. Differences in demographic and clinical characteristics across the three pain phenotype groups were assessed using χ^2 for categorial variables and one-way ANOVA tests for continuous variables. Distribution for each biomarker was assessed. When the distribution of the biomarker was not skewed, mean and standard deviation of concentrations of plasma markers of inflammation were presented and one way ANOVA was used to test the difference across the three pain phenotype groups. If the distribution of the biomarker was seriously skewed, we presented median and interquartile range (IQR) and used nonparametric Kruskal Wallis tests instead. When the omnibus test was statistically significant, post hoc analysis was conducted to determine which two or more groups were different. Given the exploratory nature of this study, a $p < 0.05$ was considered statistically significant without adjusting for any multiple testing.

Results

Among the 74 participants included in this study, 25 (33.8%) reported severe pain occurring never or rarely, 30 (40.5%) reported severe pain occurring sometimes, and 19 (25.7%) reported severe pain occurring often or always. Patient demographic and clinical characteristics across the three pain phenotype groups are shown in Table 1. Across all three pain phenotype groups, there was a significant difference in participants taking pain medications every day, χ^2 (2, 74) = 13.6, $p = 0.001$; number of pain attacks (i.e., VOE) in past 12 months, χ^2 (8, 74) = 27.6, $p = 0.001$; and time since most recent pain attack, χ^2 (12, 74) = 31, $p = 0.002$. There were no significant differences in all other characteristics across the three phenotypes.

Table 2 shows the concentrations of inflammatory biomarkers across the three pain phenotype groups. Soluble E-selectin, henceforth referred to as sE-selectin, was the only inflammatory biomarker significantly associated with the pain phenotype groups ($p = 0.049$). Post hoc comparisons indicated that participants in the often/always severe pain phenotype groups had significantly higher plasma concentration of sE-selectin compared to the sometimes severe pain phenotype group ($p = 0.040$).

Discussion

Our findings provide preliminary evidence describing the frequency of severe pain occurring over 6 months. While it is known that approximately 50%–70% of patients with SCD report chronic daily pain (Lanzkron et al., 2018; Smith et al., 2008), our findings provide a further contribution by suggesting that a majority of patients with chronic SCD pain likely experience pain that is severe. Our finding that more frequent reports of severe pain occurring often or always over 6 months were associated with occurrence of VOEs is consistent with the existing literature (Lanzkron et al., 2018; Ramsay et al., 2021). For example, Lanzkron et al. (2018) identified that the occurrence of chronic pain is a significant predictor for acute care utilization for VOEs. Additionally, approximately one-third of the participants in this study did not report experiencing severe pain in the past 6 months. These findings underscore the complex and interindividual variability in pain experiences for individuals with SCD and illuminate the need for future research to further differentiate characteristics of chronic pain and their effects on related outcomes (e.g., function, quality of life, and health care utilization) in this population.

This exploratory study is one of the first to provide insight into potential biomarkers associated with reports of persistent severe pain in SCD. Specifically, our findings that the sE-selectin biomarker is associated with often/always severe pain group suggests that endothelial activation likely contributes to the frequent occurrence of pain in this population. Endothelial cells coat blood vessel walls and are activated by stimuli such as pro-inflammatory cytokines, periods of hypoxia or oxidative stress, sickling of red blood cells, and infection. During endothelial cell activation, cell-surface molecules, such as E-selectin, are expressed and result in the recruitment and attachment of leukocytes to the blood vessel walls (Pober & Sessa, 2007). The activation of the endothelium by

Table 1. Sample Demographic & Clinical Characteristics.

	Total Sample (N = 74)	Never/Rarely Severe Pain Group (n = 25)	Sometimes Severe Pain Group (n = 30)	Often/Always Severe Pain Group (n = 19)
Demographic characteristics				
Age (years)				
Mean [SD]	29.9 (7.8)	29.6 (7.6)	29.3 (6.9)	31.4 (9.4)
Range	19–46	20–44	20–45	19–46
	n (%)	**n (%)**	**n (%)**	**n (%)**
Sex (female)	47 (63.5)	16 (64)	17 (56.7)	14 (73.7)
Marital status (never married)	57 (77)	18 (72)	24 (80)	15 (78.9)
Education				
High school graduate or less	17 (23)	6 (24)	5 (16.7)	6 (31.6)
Some college	32 (43.2)	11 (44)	13 (43.3)	8 (42.1)
College graduate	18 (24.3)	6 (24)	10 (33.3)	2 (10.5)
Graduate/Professional degree	7 (9.5)	2 (8)	2 (6.7)	3 (15.8)
Employment				
Student	13 (17.6)	6 (24)	2 (6.7)	5 (26.3)
Employed	31 (41.9)	13 (52)	12 (40)	6 (31.6)
Unemployed	30 (40.5)	6 (24)	16 (53.3)	8 (42.1)
Clinical characteristics				
SCD genotype				
Hb SS or $S\beta^0$	50 (67.6)	16 (64)	21 (70)	12 (63.2)
Hb SC	17 (23)	5 (20)	7 (23.3)	6 (31.6)
Other (Hb $S\beta^+$, S/HPFH, SE, SO, SD)	7 (9.5)	4 (16)	2 (6.7)	1 (5.6)
Taking pain medications every day*	38 (51.3)	6 (24)	17 (56.7)	15 (78.9)
Number of pain attacks (VOEs) in past year*				
0	9 (12.2)	7 (28)	2 (6.7)	0 (0)
1	7 (9.5)	4 (16)	2 (6.7)	1 (5.3)
2	10 (13.5)	6 (24)	4 (13.3)	0 (0)
3	11 (14.9)	5 (20)	4 (13.3)	2 (10.5)
4 or more	37 (50)	3 (12)	18 (60)	16 (84.2)
Time since most recent pain attack (VOE)*				
<1 week ago	9 (12.2)	3 (12)	1 (3.3)	5 (26.3)
1–3 weeks ago	13 (17.6)	0 (0)	8 (26.7)	5 (26.3)
1–6 months ago	16 (21.6)	2 (8)	8 (26.7)	6 (31.5)
7–11 months ago	23 (31.1)	10 (40)	11 (36.7)	2 (10.5)
1–5 years ago	3 (4.1)	2 (8)	0 (0)	1 (5.3)
5+ years ago	9 (12.2)	7 (28)	2 (6.7)	0 (0)
Never had a pain attack	1 (1.4)	1 (4)	0 (0)	0 (0)

*Indicates characteristics that were significantly different across the three pain phenotype groups (p's ≤ 0.002).

pro-inflammatory mediators can also lead to endothelial dysfunction, which occurs when there is a decreased availability or activity of endothelium-derived nitric oxide (Zhang, 2008). Importantly, nitric oxide plays an essential role in regulating inflammatory and immune responses, and increased nitric oxide levels can protect against the expression of cell-surface molecules and inflammation (Cyr et al., 2020; De Caterina et al., 1995). Previous studies identified that patients with SCD have decreased levels of NO which are inversely related to occurrence of acute painful events (Antwi-Boasiako & Campbell, 2018; Mack & Kato, 2006; Morris et al., 2000).

Our findings underscore the potential role that these endothelial adhesion molecules likely play in mediating frequently occurring pain in this population. Although research examining the role of adhesion molecules, such as selectins, in the development of pain is very limited, our findings are consistent with previous research. In an exploratory study examining the pathogenesis of nonspecific low back pain (LBP) among 42 patients and 21 asymptomatic controls, sE-selectin was significantly elevated in patients with chronic LBP but not in patients with acute LBP (Teodorczyk-Injeyan et al., 2018).

The processes of endothelial activation and resultant dysfunction are particularly pertinent in SCD as these mechanisms contribute to the multifactorial pathology of the disease and can lead to long-term complications such as blood vessel and organ damage (Sundd et al., 2019; Telen, 2007). sE-selectin is a glycoprotein commonly used as a marker of systemic endothelial dysfunction (Balta, 2021; Odegaard et al., 2016; Page & Liles, 2013). Notably, elevated levels of sE-selectin have been

Table 2. Comparison of Inflammatory Biomarker Plasma Concentrations Across Severe Pain Phenotype Groups.

Inflammatory Biomarker	Total Sample	Never/Rarely Severe Pain Group	Sometimes Severe Pain Group	Often/Always Severe Pain Group	*p*-Value
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
MCP-1	70.8 (37.6)	67.1 (34.6)	65.9 (30.9)	83.2 (48.7)	0.248*
sE-selectin	17.6 (7.5)	17.2 (4.8)	15.8 (5.2)	21.1 (11.5)	0.049*
sP-selectin	93 (43.2)	98.3 (47.8)	90.9 (37.8)	89.3 (46.7)	0.754*
TNF-α	73.2 (42)	80.6 (41)	69.9 (45.1)	69.2 (39.2)	0.583*
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**×	
IL-1α	5.4 (6.8)	7 (9.3)	5.2 (5)	5.1 (6.6)	0.612
IL-1β	27.4 (30.1)	26.2 (36.3)	27.5 (26)	27.4 (25.4)	0.771
IL-6	70.1 (97.8)	75.1 (112.1)	69.5 (107)	77.8 (56.4)	0.985
IL-8	4.3 (5.7)	4.5 (5.8)	3.6 (10.1)	4.6 (12.7)	0.522
IL-10	9.7 (13.2)	10.1 (17.3)	9.2 (11)	9.5 (10.2)	0.975
IL-12p70	40.2 (31.2)	43.9 (37.3)	38.1 (5.7)	40.4 (30.6)	0.677
IL-13	6.5 (8.4)	7.7 (8.6)	5.7 (7.7)	5.7 (5.4)	0.645
IL-17A	16 (22.5)	20.2 (20.9)	14.9 (27)	15.9 (15.2)	0.507
IP-10	45.8 (42.1)	54.6 (38.7)	43.2 (42)	52.5 (59.6)	0.804
MIP-1α	18.9 (38.3)	18.8 (38)	20 (50.6)	17.3 (28)	0.558
MIP-1β	31.5 (36.8)	27.5 (33.5)	31.5 (34)	48.8 (44.2)	0.630
sICAM-1	39.9 (21.9)	41.8 (15.6)	33.5 (20.2)	46.1 (40.1)	0.342

Note. All concentrations are reported as pg/mL, except for sICAM-1, & sE-selectin, & sP-selectin which are reported as ng/mL plasma. Abbreviations: SD, standard deviation; IQR, interquartile range.
*p-value is calculated using ANOVA; all other p-values calculated using the non-parametric Kruskal-Wallis test.

found in steady state patients with SCD and are further increased in VOEs (Antwi-Boasiako et al., 2018). The important role for selectins in VOEs and associated sequalae is evidence by findings that treatment with rivipansel (GMI-1070), a pan-selectin inhibitor, reduced median levels of sE-selectin by 59%, shortened hospital stays and the time to opioid discontinuation in children and adults with SCD, if administered shortly after VOE onset (Dampier et al., 2020). In our current study, more than 94% of individuals in the often/always severe pain group had three or more VOEs in the past year, potentially leading to ongoing endothelial dysfunction and increased inflammation with ensuing experiences of frequently occurring severe pain.

Our findings should be interpreted in light of several limitations. First, the sample size was small and from one geographic location, resulting in limited generalizability of our findings. Second, this was a cross-sectional study that assessed only one dimension of pain (i.e., occurrence of severe pain) using only one survey item at one time point. Future research should phenotype persistent pain over time in this population more comprehensively and explore the relationships of pain trajectories with inflammatory biomarkers. Third, the pain item used in this study was modified from the ASCQ-Me measures. In particular, the SCDIC researchers included that recall of 6 months, rather than 7 days, to capture the occurrence of persistent pain in this sample. This modification can lead to recall bias; however, the 6-month timeframe is consistent with the proposed diagnostic criteria for chronic pain associated with SCD (Dampier et al., 2017). Fourth, as this was an exploratory, hypothesis-generating study, we did not correct for multiple testing. As a result, there is potential for type 1 error. Future research with a larger, geographically diverse sample is necessary to explore and validate our findings further.

Conclusion

To our knowledge, this study is one of the first to describe the occurrence of persistent severe pain and identify associations with inflammatory biomarkers in people with SCD. Our findings provide preliminary evidence of the frequent occurrence of severe pain as reported by the majority of patients in this study, and that sE-selectin may be an objective biomarker for severe pain occurring often or always in this population. Further research is necessary to explore these relationships and determine whether drugs that improve endothelial function reduce pain in this population. Furthermore, research exploring the occurrence and characteristics of chronic SCD pain and associations with inflammatory biomarkers, including endothelial adhesion molecules, is warranted. This line of research has the potential to identify individuals who are at greater risk for chronic SCD pain as well as potential targets for pharmacological and nonpharmacological therapies.

Acknowledgments

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors would like to thank Donnalee Frega, PhD for her editorial assistance and Cyrus Lacuesta and Akhil Hegde, PhD of the

Duke School of Nursing Biomarker Lab for their contributions to this research.

Author Contributions

Knisely, M.R. contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted manuscript, critically revised manuscript, gave final approval, agrees to be accountable for all aspects of work ensuring integrity and accuracy Tanabe, P.J. contributed to conception and design, contributed to acquisition and interpretation, critically revised manuscript, gave final approval, agrees to be accountable for all aspects of work ensuring integrity and accuracy Walker, J.K.L. contributed to conception and design, contributed to acquisition, analysis, and interpretation, drafted manuscript, critically revised manuscript, gave final approval, agrees to be accountable for all aspects of work ensuring integrity and accuracy Yang, Q. contributed to conception and design contributed to analysis and interpretation drafted manuscript critically revised manuscript gave final approval agrees to be accountable for all aspects of work ensuring integrity and accuracy. Shah, N.R. contributed to conception and design, contributed to acquisition, analysis, and interpretation, critically revised manuscript, gave final approval, agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Research reported in this publication was supported by the Duke University Clinical & Translational Sciences Institute's Special Populations Core Pilot grant, the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1TR002553, and the National Heart, Lung, and Blood Institute (U01HL133964).

ORCID iD

Mitchell R. Knisely https://orcid.org/0000-0002-2938-6125

References

Antwi-Boasiako, C., & Campbell, A. (2018). Low nitric oxide level is implicated in sickle cell disease and its complications in Ghana. *Vascular Health and Risk Management*, *14*, 199–204.

Antwi-Boasiako, C., Donkor, E., Sey, F., Dzudzor, B., Dankwah, G., Otu, K., Doku, A., Dale, C., & Ekem, I. (2018). Levels of soluble endothelium adhesion molecules and complications among sickle cell disease patients in Ghana. *Diseases (Basel, Switzerland)*, *6*(2), 29.

Balta, S. (2021). Endothelial dysfunction and inflammatory markers of vascular disease. *Current Vascular Pharmacology*, *19*(3), 243–249.

Campbell, C. M., Carroll, C. P., Kiley, K., Han, D., Haywood, C. Jr., Lanzkron, S., Swedberg, L., Edwards, R. R., Page, G. G., & Haythornthwaite, J. A. (2016). Quantitative sensory testing and pain-evoked cytokine reactivity: Ccomparison of patients with sickle cell disease to healthy matched controls. *Pain*, *157*(4), 949–956.

Conran, N., & Belcher, J. D. (2018). Inflammation in sickle cell disease. *Clinical Hemorheology & Microcirculation*, *68*(2-3), 263–299.

Conran, N., Franco-Penteado, C. F., & Costa, F. F. (2009). Newer aspects of the pathophysiology of sickle cell disease vaso-occlusion. *Hemoglobin*, *33*(1), 1–16.

Cyr, A. R., Huckaby, L. V., Shiva, S. S., & Zuckerbraun, B. S. (2020). Nitric oxide and endothelial dysfunction. *Critical Care Clinics*, *36*(2), 307–321.

Dampier, C., Ely, B., Brodecki, D., & O'Neal, P. (2002). Characteristics of pain managed at home in children and adolescents with sickle cell disease by using diary self-reports. *Journal of Pain*, *3*(6), 461–470.

Dampier, C., Palermo, T. M., Darbari, D. S., Hassell, K., Smith, W., & Zempsky, W. (2017). AAPT diagnostic criteria for chronic sickle cell disease pain. *Journal of Pain*, *18*(5), 490–498.

De Caterina, R., Libby, P., Peng, H. B., Thannickal, V. J., Rajavashisth, T. B., Gimbrone, M. A. Jr., Shin, W. S., & Liao, J. K. (1995). Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *Journal of Clinical Investigation*, *96*(1), 60–68.

DiMartino, L. D., Baumann, A. A., Hsu, L. L., Kanter, J., Gordeuk, V. R., Glassberg, J., Treadwell, M. J., Melvin, C. L., Telfair, J., Klesges, L. M., King, A., Wun, T., Shah, N., Gibson, R. W., & Hankins, J. S. (2018). The sickle cell disease implementation consortium: Translating evidence-based guidelines into practice for sickle cell disease. *American Journal of Hematology*, *93*(12), E391–e395.

Francis, R. B. Jr., & Haywood, L. J. (1992). Elevated immunoreactive tumor necrosis factor and interleukin-1 in sickle cell disease. *Journal of National Medical Association*, *84*(7), 611–615.

Glassberg, J. A., Linton, E. A., Burson, K., Hendershot, T., Telfair, J., Kanter, J., Gordeuk, V. R., King, A. A., Melvin, C. L., Shah, N., Hankins, J. S., Epié, A. Y., & Richardson, L. D. (2020). Publication of data collection forms from NHLBI funded sickle cell disease implementation consortium registry. *Orphanet Journal of Rare Disease*, *15*(1), 178.

Graido-Gonzalez, E., Doherty, J. C., Bergreen, E. W., Organ, G., Telfer, M., & McMillen, M. A. (1998). Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. *Blood*, *92*(7), 2551–2555.

Gupta, K., Jahagirdar, O., & Gupta, K. (2018). APS sickle cell disease conference mini-review: Targeting pain at its source in sickle cell disease. *American Journal of Physiology*, *315*, R104–R112.

Han, J., Zhou, J., Saraf, S. L., Gordeuk, V. R., & Calip, G. S. (2018). Characterization of opioid use in sickle cell disease. *Pharmacoepidemiology & Drug Safety*, *27*(5), 479–486.

Harjunpää, H., Llort Asens, M., Guenther, C., & Fagerholm, S. (2019). Cell adhesion molecules and their roles and regulation in the immune and tumor microenvironment. *Frontiers in Immunology*, *10*(1078), 1–24.

Hibbert, J. M., Hsu, L. L., Bhathena, S. J., Irune, I., Sarfo, B., Creary, M. S., Gee, B. E., Mohamed, A. I., Buchanan, I. D., Al-Mahmoud, A., & Stiles, J. K. (2005). Proinflammatory cytokines and the

hypermetabolism of children with sickle cell disease. *Experimental Biology & Medicine*, *230*(1), 68–74.

Hoppe, C., Jacob, E., Styles, L., Kuypers, F., Larkin, S., & Vichinsky, E. (2017, May). Simvastatin reduces vaso-occlusive pain in sickle cell anaemia: A pilot efficacy trial. *British Journal of Haematology*, *177*(4), 620–629.

Huh, Y., Ji, R. R., & Chen, G. (2017). Neuroinflammation, bone marrow stem cells, and chronic pain. *Frontiers in Immunology*, *8*, 1014.

Ji, R. R., Nackley, A., Huh, Y., Terrando, N., & Maixner, W. (2018). Neuroinflammation and central sensitization in chronic and widespread pain. *Anesthesiology*, *129*(2), 343–366.

Keller, S., Yang, M., Treadwell, M. J., & Hassell, K. L. (2017). Sensitivity of alternative measures of functioning and wellbeing for adults with sickle cell disease: Comparison of PROMIS(R) to ASCQ-Me. *Health & Quality Life Outcomes*, *15*(1), 117.

Kiguchi, N., Kobayashi, Y., & Kishioka, S. (2012). Chemokines and cytokines in neuroinflammation leading to neuropathic pain. *Current Opinion Pharmacology*, *12*(1), 55–61.

Kraychete, D. C., Sakata, R. K., Issy, A. M., Bacellar, O., Jesus, R. S., & Carvalho, E. M. (2009). Proinflammatory cytokines in patients with neuropathic pain treated with Tramadol. *Brazilian Journal of Anesthesiology*, *59*(3), 297–303.

Lanzkron, S., Little, J., Field, J., Shows, J. R., Wang, H., Seufert, R., Brooks, J., Varadhan, R., Haywood, C. Jr., Saheed, M., Huang, C. Y., Griffin, B., Frymark, S., Piehet, A., Robertson, D., Proudford, M., Kincaid, A., Green, C., Burgess, L., Wallace, M., & Segal, J. (2018). Increased acute care utilization in a prospective cohort of adults with sickle cell disease. *Blood Advances*, *2*(18), 2412–2417.

Mack, A. K., & Kato, G. J. (2006). Sickle cell disease and nitric oxide: A paradigm shift? *International Journal of Biochemistry & Cell Biology*, *38*(8), 1237–1243.

Miyagi, T., & Yamaguchi, K. (2007). 3.17—Sialic acids. In H. Kamerling (Ed.), *Comprehensive glycoscience* (pp. 297–323). Elsevier.

Morris, C. R., Kuypers, F. A., Larkin, S., Vichinsky, E. P., & Styles, L. A. (2000). Patterns of arginine and nitric oxide in patients with sickle cell disease with vaso-occlusive crisis and acute chest syndrome. *Journal of Pediatric Hematology & Oncology*, *22*(6), 515–520.

National Heart, Blood, & Lung Institute. (2014). *Evidence-based management of sickle cell disease: Expert panel report*. https://www.nhlbi.nih.gov/health-topics/evidence-based-management-sickle-cell-disease

Odegaard, A. O., Jacobs, D. R. Jr., Sanchez, O. A., Goff, D. C. Jr., Reiner, A. P., & Gross, M. D. (2016). Oxidative stress, inflammation, endothelial dysfunction and incidence of type 2 diabetes. *Cardiovascular Diabetology*, *15*, 1–12.

Odievre, M. H., Verger, E., Silva-Pinto, A. C., & Elion, J. (2011). Pathophysiological insights in sickle cell disease. *Indian Journal of Medical Research*, *134*, 532–537.

Page, A., & Liles, W. (2013). Biomarkers of endothelial activation/dysfunction in infectious diseases. *Virulence*, *4*(6), 507–516.

Pathare, A., Al Kindi, S., Alnaqdy, A. A., Daar, S., Knox-Macaulay, H., & Dennison, D. (2004). Cytokine profile of sickle cell disease in Oman. *American Journal of Hematology*, *77*(4), 323–328.

Pober, J. S., & Sessa, W. C. (2007). Evolving functions of endothelial cells in inflammation. *Nature Reviews Immunology*, *7*(10), 803–815.

Proença-Ferreira, R., Brugnerotto, A., Garrido, V. T., Dominical, V., Vital, D., Ribeiro, M., dos Santos, M., Traina, F., Olalla-Saad, S., Costa, F., & Conran, N. (2014). Endothelial activation by platelets from sickle cell anemia patients. *PLoS One*, *9*(2), e89012–e89012.

Qari, M. H., Dier, U., & Mousa, S. A. (2012). Biomarkers of inflammation, growth factor, and coagulation activation in patients with sickle cell disease. *Clinical & Applied Thrombosis/Hemostasis*, *18*(2), 195–200.

Ramesh, G., MacLean, A., & Philipp, M. (2013). Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. *Mediators of Inflammation*, *2013*, 480739. https://doi.org/10.1155/2013/480739

Ramsay, Z., Bartlett, R., Ali, A., Grant, J., Gordon-Strachan, G., & Asnani, M. (2021). Sickle cell disease and pain: Is it all vaso-occlusive crises? *Clinical Journal of Pain*. Advance online publication. https://doi.org/10.1097/AJP.0000000000000949

Slade, G. D., Conrad, M. S., Diatchenko, L., Rashid, N. U., Zhong, S., Smith, S., Rhodes, J., Medvedev, A., Makarov, S., Maixner, W., & Nackley, A. G. (2011). Cytokine biomarkers and chronic pain: Association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness. *Pain*, *152*(12), 2802–2812.

Smith, W. R., Penberthy, L. T., Bovbjerg, V. E., McClish, D. K., Roberts, J. D., Dahman, B., Aisiku, I. P., Levenson, J. L., & Roseff, S. D. (2008). Daily assessment of pain in adults with sickle cell disease. *Annals of Internal Medicine*, *148*(2), 94–101.

Sturgill, J., McGee, E., & Menzies, V. (2014). Unique cytokine signature in the plasma of patients with fibromyalgia. *Journal of Immunology Research*, *2014*, 938576.

Sundd, P., Gladwin, M. T., & Novelli, E. M. (2019). Pathophysiology of sickle cell disease. *Annual Review of Pathology*, *14*, 263–292.

Telen, M. J. (2007). Role of adhesion molecules and vascular endothelium in the pathogenesis of sickle cell disease. *Hematology ASH Education Program*, *2007*, 84–90.

Teodorczyk-Injeyan, J., McGregor, M., Triano, J., & Injeyan, S. (2018). Elevated production of nociceptive CC chemokines and sE-selectin in patients with low back pain and the effects of spinal manipulation: A nonrandomized clinical trial. *Clinical Journal of Pain*, *34*(1), 68–75.

Tran, H., Gupta, M., & Gupta, K. (2017). Targeting novel mechanisms of pain in sickle cell disease. *Blood*, *130*(22), 2377–2385.

Wautier, J., & Wautier, M. (2004). Erythrocytes and platelet adhesion to endothelium are mediated by specialized molecules. *Clinical Hemorheology & Microcirculation*, *30*, 181–184.

Wilkie, D. J., Molokie, R., Boyd-Seal, D., Suarez, M. L., Kim, Y. O., Zong, S., Wittert, H., Zhao, Z., Saunthararajah, Y., & Wang, Z. J. (2010, Jan). Patient-reported outcomes: Descriptors of nociceptive and neuropathic pain and barriers to effective pain management in adult outpatients with sickle cell disease. *Journal of the National Medical Association*, *102*(1), 18–27.

Zhang, C. (2008). The role of inflammatory cytokines in endothelial dysfunction. *Basic Research in Cardiology*, *103*(5), 398–406.