PROTOCOL TITLE: Association between low bone density, vertebral fractures, and pain in sickle cell disease: an observational study

1) Protocol Title

Title: Association between low bone density, vertebral fractures, and pain in sickle cell disease: an observational study

Protocol Version *Date: 05/19/23*

2) Objective

To determine how low bone mineral density or vertebral compression fractures contribute to pain in a prospective cohort of adults with sickle cell disease

3) Background

Introduction

Sickle cell disease (SCD) is the most common inherited hemoglobin disorder in the world(1). SCD is considered an orphan disease in the United States (US) because it affects less than 200,000 people (2). SCD arises from a single nucleotide variant on chromosome 11 that encodes the β-globin gene. Under stressful physiologic conditions, red blood cells assume rigid, elongated shapes from sickle hemoglobin polymerization. Sickle erythrocytes, along with activated white cells and platelets, adhere to the vascular endothelium and impair blood flow downstream of the obstruction. Tissue ischemia and infarction ensues, eventually leading to end-organ damage (3). Therapeutic interventions like universal newborn screening, penicillin prophylaxis, vaccinations against encapsulated organisms, chronic red blood cell transfusions, and hydroxyurea therapy have transformed SCD from a genetic disorder with high childhood mortality to a chronic illness characterized by progressive pain and end-organ damage (4) .

SCD negatively impacts bone health. The skeletal system is repeatedly injured when recurrent hypoxia-reperfusion stress induces the release of cytokines that heighten bone resorption (5), while nutritional deficits, low muscle mass, delayed puberty and physical inactivity hinder bone formation (6-8). Skeletal complications of SCD (9) range from acute vasoocclusive bone pain, bone infarcts and osteomyelitis to more chronic manifestations, such as osteonecrosis, osteoporosis and vertebral deformities (10-13). Osteonecrosis, in particular, is a major cause of severe chronic SCD pain and significant permanent disability (14), which persist even after curative hematopoietic stem cell transplant (15).

Low bone density associates with osteonecrosis of the femoral head (or hip osteonecrosis) and pain in pediatric SCD. In collaboration with my co-investigators from the Sickle Cell Clinical Research and Intervention Program (SCCRIP), a lifespan prospective SCD cohort maintained at St. Jude Children's Research Hospital (16), we found that low bone density independently associates with hip osteonecrosis and chronic pain in pediatric SCD, see Table 1 (17). Generally, SCD complications progressively worsen with age. To support our hypothesis that therapies that improve bone health may also alleviate symptoms of hip osteonecrosis in adults, we first need to study the association between low bone density, vertebral fractures, and pain in a prospective cohort of adults with SCD.

	Total cohort		HbSS/Sβ^0-thalassemia subgroup	
Variable	**Adjusted OR (95% CI)**	***P***	**Adjusted OR (95% CI)**	***P***
Adolescent category	7.7 (1.94-30.20)	.0037	4.7 (1.23-18.17)	.023
Hip osteonecrosis	4.0 (1.02-15.63)	.046	3.7 (0.69-20.11)	.13
Chronic pain	10.4 (1.51-71.24)	.017	9.3 (0.97-88.70)	.053
Hemoglobin	0.74 (0.57-0.96)	.022	NA	NA
Indirect bilirubin	NA	NA	1.4 (0.99-1.90)	.055

CI, confidence interval; NA, variable not selected in final model.

Table 1. Multivariable regression model of low bone mineral density in the SCCRIP pediatric cohort, excerpted from **Adesina et al.** *Blood Adv*. 2019 May 14;3(9):1476-1488.

4) Inclusion and Exclusion Criteria

All adults with SCD who have received hematology care at UC Davis Medical Center (UCDMC) and non-UC Davis facilities will be assessed for potential eligibility. All eligible study participants will undergo a dual energy X-ray absorptiometry (DXA) scan to determine their baseline bone mineral density. The DXA instrument can also be programmed to obtain morphometric thoracolumbar spine X-ray to evaluate for the presence and severity of vertebral compression fractures. In addition to routine study labs, a small aliquout of fasting blood and urine samples will be collected from each study participant for biomarker analysis. Routine study labs can be drawn up to one week before or after the study visit. Fasting labs for biomarker analysis must be collected between 7:30-10am on the day of the study visit to account for changes due to the circadian rhythm.

Inclusion Criteria

- Age ≥18 years with SCD (any genotype, confirmed by HPLC or hemoglobin electrophoresis)
- Ability to provide written informed consent
- Ability to lay on a DXA scanner
- Negative urine pregnancy test for women of childbearing potential at study entry

Exclusion Criteria

- Pregnant women
- Adults unable to consent
- Individuals who are not yet adults (infants, children, teenagers)
- Prisoners
- Hospitalizations (any cause) within 2 weeks of study entry

5) Study Timeline

This cross-sectional observational study involves obtaining a baseline DXA scan, vertebral fracture analysis (VFA) and pain assessment using the Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me). We plan to recruit 50 subjects and anticipate enrolling 4 adults with SCD per month from Nov 2022– Dec 2023. Our goal is to complete primary data anaylsis by Mar 2024.

6) Study Endpoints

- To determine the association between bone density Z-scores and ASCQ-Me pain impact scores in adults with SCD.
- To study the associaton between Spine Deformity Index scores (SDI, a proxy for vertebral fracture analysis) and ASCQ-Me pain impact scores in adults with SCD
- To assess the correlation between baseline hematological and biochemical laboratory parameters; including bone biomarkers, and skeletal morbidity in a prospective cohort of adults with SCD

7) Procedures Involved

We hypothesize that adults with SCD and low bone density or vertebral compression fractures—adjusted for sex, SCD genotype, vitamin D levels and SCD-modifying therapies—will report more severe pain than those with normal bone density or no vertebral fractures. ASCQ-Me is a validated patient-reported outcome measure of

physical, mental, and social health in adults with SCD (18, 19). For biomarker analyses only, 30cc of whole blood and 10cc of urine will be collected from each study participant once (at baseline), after an overnight fast.

Methods: We identified 113 adults with SCD (58% female) followed at UCDMC from Oct 2017- Jul 2021. Using the National Institutes of Health (NIH) Toolbox iPad App, we will electronically administer the ASCQ-Me pain domain questionnaire to 50 ambulatory adults with SCD and securely store their responses on the Research Electronic Data Capture System (REDCap).

ASCQ-Me uses a T-score metric with a mean score of 50 for the reference population and a standard deviation (SD) of 10. We hypothesize that ASCQ-Me pain impact scores will directly correlate with lumbar spine, hip, forearm, or whole body bone density Z-scores; and inversely correlate with SDI scores (Figure 1).

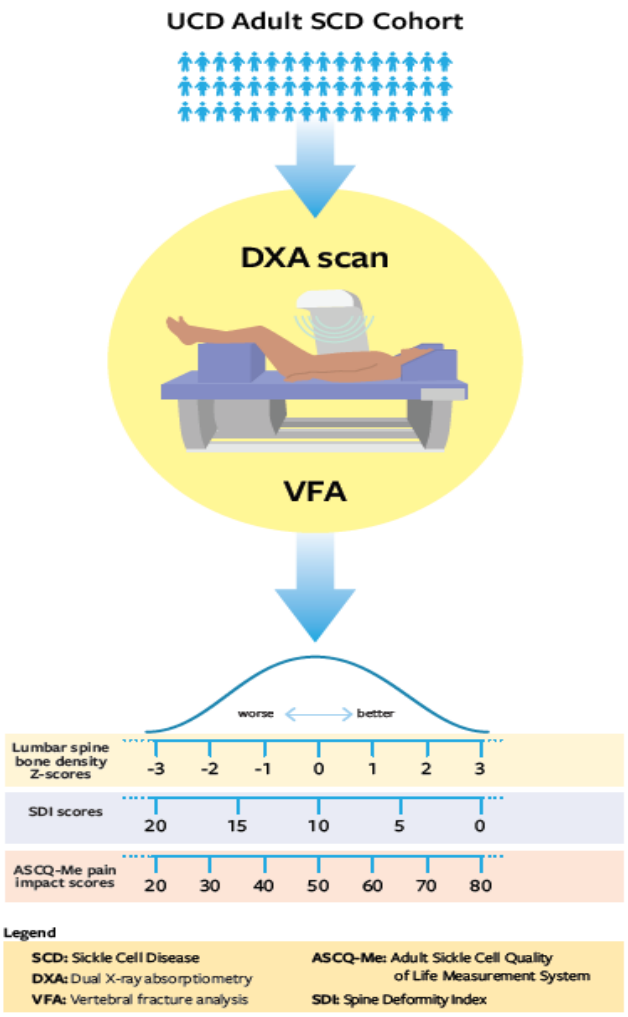

Figure 1. Study schema

Potential Problems/Alternative Strategies. Recruiting and retaining 50 adults with SCD is feasible within the study period. As previously noted, I am a co-investigator and co-chair the Bone Working Group in SCCRIP, the longitudinal study of 1300 children and adults with SCD maintained at St. Jude Children's Research Hospital. In the unlikely event that we are unable to recruit all 50 participants from UCDMC and non-UC Davis facilities, we will extend our study recruitment to include eligible adult participants from the SCCRIP cohort. For study participants who are not comfortable with using research iPads, we plan to administer the ASCQ-Me pain questions on paper in standard (13 questions) or short form (5 questions). A copy of the ASCQ-Me Pain Impact short form is excerpted below:

ASCQ-Me® v2.0 Pain Impact - Short Form

Pain Impact - Short Form

Please respond to each question or statement by marking one box per row.

		Never	Rarely	Sometimes	Often	Always
PainImpactQ2	In the past 7 days, how often did you have pain so bad that you could not do anything for a whole day?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpactQ7	In the past 7 days, how often did you have pain so bad that you could not get out of bed?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpactQ8	In the past 7 days, how often did you have very severe pain?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpactQ10	In the past 7 days, how often did you have pain so bad that you had to stop what you were doing?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
PainImpactQ12	In the past 7 days, how often did you have pain so bad that it was hard to finish what you were doing?.....	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

8) Data and/or Specimen Management and Confidentiality

☒ I understand that if this study involves the use of the UC Davis Health Electronic Health Record (EMR/EPIC), which also contains the clinical data for Marshall Medical Center (MMC). I understand that MMC patient data cannot be accessed for research purposes and that I must take the necessary steps to ensure that MMC data is not accessed, used, or disclosed for UC Davis Health research purposes.

☐ I undersand that if this study involves use of UC Davis students' educational records (including records in the PI's own possession such as course exams/assignments), I must consult with the Registrar's office to see if all requirements of the Family Educational Rights and Privacy Act (FERPA) are satisfied. **N/A**

Data analysis plan and Power calculations:

To test the association between ASCQ-Me pain scores and bone density Z-scores, we will build a linear regression model with ASCQ-Me pain scores as the outcome of interest and bone density Z-scores as an independent variable. We will repeat the analysis using SDI scores and laboratory parameters as independent variables. We will further adjust for potential confounders, such as age, sex, SCD genotype, vitamin D levels and SCD-modifying therapies i.e, hydroxyurea use or chronic red blood cell transfusions. Then, we will add osteonecrosis in any joint (yes vs no) into the linear regression model to test the interaction between osteonecrosis and low bone density on ASCQ-Me pain scores, adjusted for the previously mentioned covariates. We will also test for the interaction between osteonecrosis and vertebral fractures on ASCQ-Me pain scores. For statistical power calculations only, we will categorize study subjects as being in group A (low bone density or vertebral compression fractures) and group B (normal bone density or no vertebral fractures) to better estimate the differences in pain scores observed between both groups. If we assume 50% prevalence of low bone density or vertebral fractures in adults with SCD ((12)18-20), then 50% of our study subjects will fall in group A and group B. If we assume mean pain scores of 40 in group A (i.e., one SD worse than the average population) and mean pain scores of 50 in group B (population mean), then enrolling 40 adults with SCD provides approximately 80% power to detect a statistically significant and clinically useful difference in ASCQ-Me pain impact scores between groups A and B (2-sided, alpha of 0.05; SD 10). We will enroll an additional 10 adults to account for study

discontinuations and to support exploratory endpoints e.g., interaction between low bone mineral density or vertebral compression fractures, osteonecrosis, and sickle cell pain.

Procedures for maintenance and confidentiality:

- This research study will include a Partial Waiver of HIPAA Authorization for participant identification and recruitment through medical chart review.
- A signed HIPAA Authorization will be required for access, use, or disclosure of identifiable private information for participation in research activities.
- All study data will be securely stored on password-protected REDCap system, which can only be accessed through institutional computers with encryption.
- The PI and research coordinator will complete a weekly audit to ensure all study data are collected/securely stored until study completion.

9) Data and/or Specimen Banking:

All patient data and biospecimens will be securely stored for at least 5 years after study completion to allow time for full data analyses.

10) Provisions to Monitor the Data to Ensure the Safety of Subjects

Every research team member will complete training in the Human Research Protections and Good Clinical Practice for Clinical Trials with Investigational Drugs and Biologics, through the Collaborative Institutional Training Initiative (CITI Program). The PI will monitor all research activities. The research will also be regulated by UC Davis's Institutional Review Board (IRB). The written consent forms include 24-hour physician contact information for any study-related emergencies. Any protocol deviations or study participants' safety concerns will be promptly reported to the UC Davis IRB and to the study sponsors.

11) Withdrawal of Subjects

The principal investigator (PI) is a board-certified hematologist with expertise in sickle cell disease, who will oversee all research activities and closely review all reported adverse events with the clinical research staff.

12) Risks to Subjects

The proposed research study confers minimal risk of harm to the study participants. SCD patients enrolled in the study will undergo a DXA scan to measure their bone mineral density, which will expose them to small doses of ionizing radiation with attendant risk. While still in the DXA scanner, they will be repositioned to obtain a lateral thoracolumbar spine X-ray for vertebral fracture analyses (VFA). The amount of radiation exposure received from the DXA and VFA scans fall below levels that are thought to result in a significant risk of harmful effects.They will also be asked to give blood and urine samples for biomarker analysis, and fill out the ASCQ-Me pain impact questionnaire.

Dual energy X-ray absorptiometry (DXA): We will perform one DXA scan for this study. Study participants will be exposed to a very small amount of X-ray radiation (less than 0.01mSV) during the DXA procedure(20). There are no known risks associated with radiation exposure levels this low. There may be some side effects with repeated exposure to radiation, including an increased risk of cancer. However, exposure to doses several thousand times greater than that obtained from this study would be required before an increased risk of cancer could be detected. Thus, the radiation exposure from this procedure represents a negligible health risk. Pregnancy testing will be performed prior to the DXA scan.

Study subjects may experience discomfort while being positioned on the DXA scanner. The exercise physiologist conducting the study is well trained and will be instructed to position study subjects as quickly as possible to minimize any physical discomfort.

Vertebral fracture analysis (VFA): The DXA machine can be programmed to obtain a lateral spine X-ray of VFA scan. Similar to the DXA scan, patietns will be exposed to a very small amount of X-ray radiation (0.05 mSV) during the VFA scan (20). There are no known risks associated with radiation exposure levels this low. There may be some side effects with repeated exposure to radiation, including an increased risk of cancer. However, exposure to doses several thousand times greater than that obtained from this study would be required before an increased risk of cancer could be detected. Thus, the radiation exposure from this procedure represents a negligible health risk. As above, pregnant patients will not undergo a VFA scan. The exercise physiologist conducting the study will reposition study subjects in the DXA scanner as quickly and as comfortably as possible to minimize any discomfort from this additional scan.

Specimen collection: The needle stick from a venipuncture (blood draw) may hurt. There is a small risk of bruising and fainting, and a rare risk of infection. Some SCD patients have indwelling port catheters for vascular access, which may also be used for blood collection. Venipuncture or accessing a port may be temporarily uncomfortable. Urine sample collection might also be inconvenient for some patients.

13) Potential Benefits to Subjects

We do not anticipate any direct benefits to study participants. If any study participant is found to have osteoporosis (a severe form of bone loss) on the DXA scan, they will be referred for additional work-up and appropriate treatment.

14) Multi-Site Research: N/A

15) Community-Based Participatory Research: N/A

16) Sharing of Results with Subjects

Study results may be shared with subjects, upon request. Dr. Adesina (PI) will present preliminary and final results of all aspects of her proposed study at local and national meetings, including the NHLBI Annual Sickle Cell Clinical Research Meeting and the American Society of Hematology annual research meeting. She will publish her findings in peer-reviewed journals and share the results with the public through SCD advocacy groups and organizations.

17) Prior Approvals

This research is being funded by **the National Institutes of Health/National Heart, Lung and Blood Institute (NIH/NHLBI) and the Doris Duke Charitable Foundation (DDCF),** who will provide additional research oversight**.**

18) Provisions to Protect the Privacy Interests of Subjects

This study confers minimal risk of a confidentiality breach. We will apply appropriate data security measures to prevent unauthorized access to individually identifiable data

19) Compensation for Research-Related Injury

This study confers minimal risk of research-related injury as the DXA scan related to the study will only expose study participants to minimal radiation. Given the limited scope of this observational study, the University of California and the study Sponsors will cover any necessary medical treatment for study-related injuries, but they will not provide any other form of compensation for study-related injuries.

20) Economic Burden to Subjects

Study participants will be compensated $100 for their time and effort. They may be asked for their social security number for payment purposes. It will not be used for any other purpose without their permission. In addition to the $100 compensation, all study participants will be reimbursed for mileage (travel to and from study visit) parking, and other travel-related expenses for this research study visit. Since study participants will be fasting for the study, they will also be offered a meal voucher to use at the UCDMC cafeteria after their study visit.

21) Drugs or Devices

☐ I confirm that all investigational drugs will be received by the Investigational Drug Service (IDS). The IDS will store, handle, and administer those drugs so that they will be used only on subjects and be used only by authorized investigators. **N/A**

☐ I confirm that all investigational devices will be labelled in accordance with FDA regulations and stored and dispensed in such a manner that they will be used only on subjects and be used only by authorized investigators. **N/A**

22) Review Requirements

Are there any contractual obligations or other considerations that require IRB review of this research, or review at intervals other than those required by the Common Rule or FDA? If yes, check box:

☐ Yes

x No

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