

Mechanisms and Laboratory Documentation of Adverse Events in Athletes with Sickle Cell Trait

ASCLS E&R Fund Member Grant Proposal

Background and Rationale

Sickle cell anemia was first discovered in 1910 by James B. Herrick, a physician practicing in Chicago, who described a 20-year-old black Caribbean dental student as having shortness of breath, palpitations, yellow eyes, and anemia.¹ Once the disease was characterized as a single gene mutation, homozygotes were described as having sickle cell disease (SCD) while heterozygotes were thought to simply be carriers and given the designation sickle cell trait (SCT). Much clinical information was collected regarding the symptoms, treatment, and prognosis of patients with SCD but it wasn't until the late 1970s and early 1980s that observations were published suggesting that individuals with SCT might better be classified as having a mild clinical condition.²⁻⁵ These conversations quickly waned but have recently resurfaced in both the clinical area and on the athletic field. Clinical complications with a "definite" connection to SCT have been described as: renal medullary carcinoma, hematuria, renal papillary necrosis, hyposthenuria, and splenic infarction. Many other clinical issues that have "probable" associations with SCT include: complicated hyphema, venous thromboembolism, fetal loss, and low birth weight. Still other clinical conditions with a "weak" association with SCT are: acute chest syndrome, asymptomatic bacteremia in pregnancy, and proliferative retinopathy.^{6,7} Recently, both the medical literature and the public news have reported cases of acute attacks and even sudden death in athletes associated with SCT.⁸⁻¹¹ The most recent reports in the medical literature have focused on certain physiological areas to include rhabdomyolysis,^{8-10,12} venous thromboembolism¹³⁻¹⁵, and renal complications.¹⁶⁻¹⁸

This study addresses the ASCLS E & R Fund by "demonstrating the value of laboratory services relating to the impact of test results in the diagnosis and treatment of patients" as well as "improving health by reducing disease incidence, morbidity and mortality." (See objectives)

Objectives

This study seeks to identify laboratory test results that can predict the onset of an acute attack in athletes with SCT under extreme physiological stress during training and competition. In doing so it will provide athletic trainers and team physicians tools to make early diagnoses and treatment decisions like when to pull an athlete from the field, when to call 911, or when to reinstate a pulled athlete. Improved decision-making will reduce the morbidity and mortality of athletes with SCT and will inform potential mechanisms of acute attacks to guide the direction of future research.

Methods/Design

Overview

Athletes from two universities (X and Y), representing two sports (basketball and soccer), involving both genders will be screened for SCT at their pre-athletic physical. All SCT athletes will be invited to be enrolled in the study (N is estimated at 5-10). An equal number of age and gender matched control subjects will be randomly selected and enrolled as follows: athletes from the same team who are homozygous normal (AA), non-athletes who are heterozygous for sickle cell (SCT).

Athletic directors and team physicians at both universities have been contacted and have given verbal permission to participate in the study. Written statements of participation are available upon request.

Subjects

Team physicians will list all athletes testing as sickle cell heterozygotes (SCT) and each will be approached for inclusion in the study. A recruitment flyer and verbal announcement will be made available by the AT to each team in which a SCT athlete has agreed to participate in the study. Once the number of athletes with SCT has been identified, the same number of athletes who are negative for sickle cell (AA) will be recruited for the study. The heterozygous non-athletes with SCT will be recruited from the student population of X and Y by posting flyers asking for students known to have SCT to volunteer as research subjects. Each respondent will be questioned to match age and gender and to ensure they are not a student athlete, regular recreational athlete, or a regular

Comment [Geo1]: Brief Hx of SS, straight to the point of the project.

Comment [Geo2]: Each specific claim is validated by citation.

Comment [Geo3]: Define unfamiliar terms.

Comment [Geo4]: These claims not validated by citation.

Comment [Geo5]: These two articles are reviews that support the prior two sentences.

Comment [Geo6]: Establishes timeliness and importance of the study.

Comment [Geo7]: Makes reference to stated purposes of the Member Grant program.

Comment [Geo8]: Objective stated clearly in simple expository sentences, includes indication of its impact.

Comment [Geo9]: Author obscures location when publishing results, however location is usually expressed in a proposal.

Comment [Geo10]: The small N is a weakness of the study. Most E&R Member Grants are pilot studies.

Comment [GF11]: This statement is unclear, does it mean two control arms, thus a total of 15-30 subjects? Answered in budget.

Comment [Geo12]: All proposals now require Institutional Review Board approval, or application pending, citing no harm to subjects. Verbal agreement can be withdrawn.

Comment [GF13]: Standard grammar, spelling, syntax, and logical progression. What may be a valid study could be sunk by misspellings, sloppy composition and editing.

Comment [Geo14]: But avoid passive sentences.

participant in intramural sports. Those who qualify and agree to participate will be tested to verify they have SCT. For each sport, the athletes will be tested at four defined intervals according to the following schedule:

1. Baseline measurements will be taken at rest during the mandatory pre-season physical.
 - a. Electrolytes and serum protein are routinely performed but the tests below will need ordered:
 - i. Microalbumin
 - ii. Haptoglobin
 - iii. D-dimer
 - iv. Fibrinopeptides A and B
2. Measurements will be taken at the peak of the most difficult, early, pre-season practice session.
3. Measurements will be taken at the end of an early-season game in which the subject participated
4. Measurements will be taken at the end of a late-season game in which the subject participated

In addition, blood & urine will be collected in the event a subject is removed from practice or a game due to clinical evidence of a possible acute event.

Dependent Variables

Clinical measurements will be collected by the AT on duty (for measurements above 2-4).

1. Blood pressure
2. Pulse rate
3. Oxygen saturation by pulse oximetry
4. Eye exam for vision impairment or hyphema

Laboratory measurements for rhabdomyolysis

1. Complete urinalysis (PI)
 - a. Blood (PI - dipstick and microscopic)
 - b. Protein (PI - dipstick)
 - c. Microalbumin (PI – lateral flow immunochromatography)
2. Haptoglobin (X hospital lab)

Laboratory measurements for hydration

1. Urine specific gravity (PI—refractometry)
2. Hematocrit (PI—microhematocrit)
3. Electrolytes (X hospital lab)
4. Serum total protein (X hospital lab)

Laboratory measurement for venous thromboembolism

1. D-dimer (X hospital lab)
2. Fibrin monomer (PI—hemagglutination)

Blood and urine samples will be collected at predefined times and will be immediately transported back to the research lab of the PI for testing and processing for send out testing to X hospital lab. All samples will be tested within 5 hours, refrigerated if tested in less than 24 hours, or frozen if testing cannot occur within 24 hours of collection.

Analysis Procedures

Complete urinalysis will be performed by the PI using standard methods and the microalbumin will be performed using a lateral flow immunochromatography system that detects the presence of microalbumin above a threshold of 20ug/mL. Fibrin monomer assay will be performed by the PI using hemagglutination. The detection of any fibrin monomers indicates increased blood clot formation. The PI manufactures and performs sickle cell screening in his research lab. Hemoglobin electrophoresis will be performed using the alkaline gel electrophoresis QuikGel system manufactured by Helena Laboratory. Procedures performed by X hospital lab will use existing procedures listed in the budget table.

As a pilot study, we anticipate the subject N to be below the number needed to perform parametric statistics. Therefore, a separate line graph will be used to plot mean values for each parameter with 95% CV error bars to compare the three groups (separate lines) at each of the four time intervals (baseline, practice, early game, late game).

Comment [Geo15]: The review committee will ask if the variables are appropriate to the purpose, state of the art, in regular use, and readily available.

Comment [Geo16]: Awkward syntax.

Comment [Geo17]: Principle investigator will have trouble finding Fp A and B, may not be feasible.

Comment [Geo18]: PI should provide limits, indicating what is defined as normal, abnormal.

Comment [Geo19]: Fibrin monomer may be difficult to find, PI should set limits to the number of variables that can be measured.

Comment [Geo20]: Provide specific specimen management and storage conditions. Frozen at what temperature, for instance.

Comment [GF21]: Statistics are appropriate to the small N. Reviewers check appropriateness of statistics.

Budget

The budget was calculated based on the following parameters:

1. Calculated based on 7 potential athletes with SCT
2. Equal number of AA athletes (7) and non-athletes with SCT (7) = 21 total subjects
3. 21 subjects x 4 testing time points = 84
4. Pre-season mandatory physical (7 AS athletes and 7 AA athletes) includes the following lab tests:
 - i. Urine dipstick
 - ii. CBC (includes hematocrit)
 - iii. CMP (includes electrolytes, and total protein)
 - iv. Sickle cell testing

Analyte	Manufacturer	Description	Cost/Unit	# of Units	Total
Microalbumin	Dialab GmbH	>20ug/mL	\$100/25	3	\$300
Dipsticks	Bayer	10 test strip	\$60/100	1	\$60
Specific gravity	N/A	Refractometer	N/A (on hand)	N/A	\$0
Hematocrit	Ram (crit tubes)	Plane tubes	N/A (on hand)	N/A	\$0
Sickle cell screen	Raw materials for kits	Solubility test	\$30 each	7 (AA controls)	\$210
Hb Electrophoresis	Helena Labs	Alk electrophoresis	\$50 each	7 (AA controls)	\$350
Fibrin Monomer	Stago	Hemagglutination	\$10 each	84 (all subjects)	\$840
Electrolytes	X hospital	Na, Cl, C02	\$11 each	28 (AA controls)	\$308
Haptoglobin	X hospital	Nephelometric	\$20 each	84 (all subjects)	\$1,680
Total protein	X hospital	Dye Binding	\$6 each	28 (AA controls)	\$168
D-dimer	X hospital	Quantitative	\$16 each	84 (all subjects)	\$1,344
Subjects who have an acute attack		Cost of all tests	\$68/subject	2	\$136
TOTAL					\$5,396

Comment [Geo22]: The basis for the budget is made clear. Reviewers know budgets are best estimates.

Comment [Geo23]: Reviewers tend to deny payment of research subjects, though not strictly forbidden.

Comment [Geo24]: Grants are confined to purchase of instruments and materials not readily available in a research or a clinical laboratory.

Comment [Geo25]: Grants may pay for services but not for direct salary compensation or stipends.

Appendix #1

Investigators

Appendix #2

Responsibilities of each investigator

Principle Investigator:

1. Design study in collaboration with the co-investigators and write all associated documents
2. Coordinate activities among investigators
3. Write grant to fund laboratory assessment portion of the study
4. Write IRB and interact with IRB office
5. Conduct the laboratory portion of the study
 - a. Blood and urine collection and analysis necessary to fulfill study design
 - c. Other duties as deemed necessary
6. Perform analysis of the results in collaboration with the co-investigators
7. Write manuscript in collaboration with co-investigators (appear as first author)
8. Develop follow-up study in collaboration with co-investigators

Comment [GF26]: Names suppressed for purpose of public presentation.

Comment [GF27]: IRB conclusion is required before the grant is provided.

Co-investigator (Athletic Trainer):

1. Work with PI in study design
2. Write accompanying grant to fund AT portion of the study
 - a. Remuneration for subjects
 - b. Other costs in accordance with study design funded by accompanying grant
3. Conduct and coordinate the AT portion of the study
 - a. Ongoing contact and coordination with athletes
 - b. Procurement, evaluation & implementation of AT procedures for SCT athletes at both colleges
 - c. Collect BP, O₂ saturation, pulse rate, eye exam

- d. Document adverse events encountered with ALL athletes enrolled in the study
 - e. Other duties as deemed necessary
4. Work with PI to analyze data and write manuscript

Co-investigator (Physician):

1. Work with PI in study design
2. Write accompanying grant to fund physician portion of study
 - a. Overlap with AT portion
 - b. Additional clinical data collection at any intervention time point according to study design
 - c. Other costs in accordance with study design
3. Conduct physician portion of the study
 - a. Perform initial (baseline) exam on all enrolled subjects and document findings
 - 1) Perform current physician exam
 - 2) Additional physician findings according to study design of accompanying grant
 - b. Work with AT to evaluate current AT and other clinical procedures
 - c. Work with AT to evaluate and document all adverse events that occur with ALL study subjects
 - d. Apply CURRENT policies and procedures to release inactivated athletes back to active status
 - e. Perform clinical assessment at each intervention or other duties
4. Work with PI to analyze data and write manuscript

Appendix #3

References

1. Herrick JB. Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia. *Arch Intern Med* 1910;6:517-521.
2. Sears DA. The morbidity of sickle cell trait: a review of the literature. *Am J Med* 1978; 64:1021-1036.
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16. Kiryluk K, Jadoon A, Gupta M, et.al. Sickle cell trait and gross hematuria. *Kidney Int* 2007;71:706-710.
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Comment [GF28]: References use correct Rutgers (NIH) format, do not use APA format. There is no room for creativity in reference citations. References appear in sequence as citations appear in text.

Comment [Geo29]: Always provide inclusive page numbers for easy reference. Reviewers check references for accuracy.