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Bayesian analyses demonstrate tissue blood volume is not decreased during acute sickle cell pain episodes: A preliminary study

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Abstract

Background—Pain is the most common complication of Sickle Cell Disease (SCD). Tissue oximetry properties in SCD during steady state and acute pain are not well described.

Methods—This was a cross sectional study of tissue oximetry properties in individuals with SCD during steady state, acute pain and healthy controls without SCD. A novel tissue oximetry device was used to better account for tissue pigmentation interference. We hypothesized that during acute SCD pain, blood volume to painful areas would be at least 10% less than steady state. Bayesian analyses of the data (with flat priors) were planned *a priori* because of the small projected sample size.

Results—The sample included 14 individuals (4 during crisis, 5 steady state, and 5 controls). In individuals with SCD, blood volume to the lower back was higher during crisis (0.18% of tissue volume vs. 0.14%). Bayesian analyses yielded a 3% probability that our hypothesis (that blood volume would decrease by 10%) was correct.

Conclusions—During acute SCD pain, blood volume to painful areas is not decreased. Bayesian analyses were useful for interpretation of small sample data and may have utility in early phase trials for rare diseases.

Keywords

Sickle cell disease; pain crisis; tissue oximetry

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1. Introduction

Sickle cell disease (SCD) is an inherited hemoglobinopathy that affects approximately 100,000 individuals in the United States [14]. In individuals with SCD, mutated hemoglobins polymerize upon deoxygenation, causing red blood cells to become sickled leading to a cascade of events that alter blood rheology and ultimately lead to the clinical manifestations of the disease [8]. While every organ is progressively damaged in SCD, the hallmark clinical manifestation is pain, which occurs for many on a daily basis [16–18]. More severe episodes of pain may also occur and are referred to as vaso-occlusive pain crises, or ‘crises’ [5]. Many aspects of the pathophysiology of SCD are well understood, especially the mechanisms by which SCD leads to organ damage, but the etiology of SCD pain has not been adequately explored.

The source of SCD pain is commonly assumed to be vaso-occlusion, as sickled cells adhere to the endothelium and block blood flow to tissues [9–11]. Vaso-occlusion does occur in SCD, however the relationship between pain and vaso-occlusion is less clear. Certain rheological parameters associated with vaso-occlusion have been found to fluctuate throughout the steady state, with no significant difference in the level of fluctuation between steady state and crisis [21]. Recently, tissue oximetry measurements at the thenar eminence demonstrated that microvascular oxygen consumption is elevated in SCD compared to controls, and further elevated during painful episodes [22]. While some patients demonstrate vaso-occlusion (as shown by an increased percentage of dense cells in the blood) concurrently with the onset of pain, others show similar blood characteristics with no pain [1]. In addition, patients often describe pain in bilaterally symmetric parts of the body (i.e. the left and right femurs), which would be unusual if the pain were directly caused by random events of microvascular occlusion [23].

The primary hypothesis of our study is that compared to individuals with SCD who are not experiencing acute pain, there will be a reduction in tissue blood volume of at least 10% in areas where pain is felt during acute SCD pain. In this cross sectional study, we used a novel tissue oximetry (StO₂) measurement device to measure these parameters.

The device engineered for this work was similar to previously developed diffuse light spectrometers [7, 12] with the exceptions that it used a novel algorithm in the fitting routine that enabled the blood volume fraction and oxygen saturation parameters to be extracted in the presence of heavy melanin concentration. Briefly, the free parameters in the fitting routine included the scattering of the tissue and a spectrum for melanin absorption that was a decaying exponential function of wavelength. The melanin fitting parameters were key in extracting accurate hemodynamic parameters, which were one-or-more orders of magnitude weaker than melanin absorption [7, 12]. Similar methods have been used in SCD, however most measured cerebral or thenar eminence oxygenation, rather than sites of acute pain [2, 19, 20, 22, 25, 28].

An additional novel aspect of this study is the *a priori* use of Bayesian data analyses. SCD is a rare disease, and the challenges of recruiting large sample sizes have been a barrier to progress in observational and therapeutic SCD research. Bayesian techniques can be

particularly useful in these situations. In this study, we use a very small cohort (14 participants) to demonstrate the value of Bayesian data analyses.

2. Methods

2.1. Study design and setting

This was a cross-sectional convenience sample study. Data were collected in Mount Sinai Sickle Cell Clinic and the Mount Sinai Emergency Department (ED). For healthy controls, data were collected in the administrative offices of the Mount Sinai Department of Emergency Medicine. Participants were selected according to the times when the tissue oximetry device was available for use. This study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board. Written informed consent was obtained from all participants.

2.2. Recruitment

Potentially eligible individuals were approached for informed consent by a research coordinator. For each of the three groups **inclusion and exclusion criteria** were as follows. All participants were between the ages of 18–75 years. Healthy controls were required to have no known medical problems, and not be actively taking any medications other than oral contraceptives. Participants in the SCD steady state group were required to have HbSS confirmed by a hemoglobin electrophoresis and meet all criteria for the definition of ‘steady state’ as defined by Ballas et al. [4]. Participants in the crisis group were required to have HbSS, with documented pain in one or more areas of the body that 1) felt like prior episodes of SCD pain and 2) had no apparent cause other than SCD pain (e.g. infection, trauma etc.). In the ED, research coordinators identified potential participants by monitoring the ED real-time tracking board for patients with chief complaints related to SCD. When eligible patients were identified, the research coordinator approached the patient with permission from the treating physician.

2.3. Methods and measurement

Participants were placed in a private area and dressed in a gown. The research coordinator gathered basic demographic data and the locations where the patient was experiencing pain. This information was recorded in a comma separated variables (CSV) file using unique, de-identified codes for each participant. With the participant in a seated position, the tissue oximetry device was placed on the forehead, lower back and thigh for 30 seconds each (all patients in the ‘crisis’ group were experiencing pain in their lower backs at the site of measurement). Data collected by the tissue oximetry device were uploaded into a separate CSV file for data cleaning. At each location, 20 consecutive spectra were acquired taking 1.5 seconds per spectrum acquired. Each spectrum was fit to a model that minimized the least squares difference between the spectrum and a simulated result by varying the concentration of four chromophores (melanin, deoxyhemoglobin, hemoglobin and water). Varying the chromophores fine-tuned the simulated result to fit the experimental result. Therefore the resulting values for the chromophore concentrations after the fitting procedure specified the measured concentrations in the tissue. The fitting technique was derivative from our previous work [7] but with the addition of melanin in the model. Though melanin

was needed in the model to account for variations in skin pigmentation, the outputs reported here are restricted to oxyhemoglobin and deoxyhemoglobin as the vascular component is of interest. Ultimately, for each body location (forehead, lower back, thigh) two final values were generated: blood volume (expressed as a percent of total tissue volume) and oxygen saturation (also expressed as a percent to indicate the saturation of blood flowing through the area of measurement).

2.4. Outcomes and hypotheses

There were three outcomes for this study: a) blood volume, b) tissue oxygen saturation and c) oxygen delivery (computed as blood volume $\times$ oxygen saturation). The primary outcome for the study was blood volume because was felt to most directly reflect vaso-occlusion. Our hypothesis was that all three outcomes (blood volume, saturation and oxygen delivery) would be at least 10% lower in areas affected by pain in individuals with SCD who were experiencing acute pain.

2.5. Analyses

All analyses were performed using the statistical software, R. Demographic and clinical variables were expressed as medians and percentages with no assumption of normality because of the small sample size. For the main outcomes, we conducted both frequentist (non-parametric) and Bayesian analyses. Bayesian analyses were planned *a priori*. Mann-Whitney U test was performed to compare values between SCD steady state and crisis (the *a priori* primary hypothesis). As a secondary comparison across the three groups Kruskal-Wallis test was performed to assess for statistically significant differences in blood volume, saturation and oxygen delivery (*post hoc* testing was not performed because results were not statistically significant). Tissue blood volume data are presented in adjusted and unadjusted form [27].

In contrast to frequentist techniques (which generate the familiar *p*-values and confidence intervals), Bayesian analyses operate under the Bayesian interpretation of probability in which the investigator chooses their beliefs about a given situation prior to performing an experiment and then updates their beliefs based on the collected data. This allows for a quantitative description of results that is, in many ways, easier to interpret than a frequentist analysis especially when thresholds for statistical significance are not crossed.

A key element in Bayesian analyses involves the investigators description of their beliefs before the experiment, or 'choice of prior.' This 'choice of prior' is described in terms of a distribution where each possible result is given a probability. We performed Bayesian analyses using a uniform prior (where all plausible results are considered equally likely before the experiment is performed) because we could not find published data regarding tissue oximetry in SCD.

To generate the prior, a distribution of the mean of all values (control, steady state and crisis) was generated using bootstrapping with a sample size of 2 values and 100,000 trials. Because the final values for the posterior of the difference between steady state and crisis involved solutions that were not likely of closed form, Markov Chain Monte Carlo

simulation was used to generate posterior distributions for the SCD pain and SCD steady state groups. Markov Chain Monte Carlo Simulation was then used to generate a posterior distribution of the difference between the two groups.

3. Results

3.1. Characteristics of participants

A total of 14 participants (5 controls, 5 SCD steady state and 4 SCD crisis) were enrolled and completed the study. Demographic and clinical characteristics of the cohort are listed in Table 1.

3.2. Main results

3.2.1. Frequentist analyses—Results of total blood volume, oxygen saturation and oxygen delivery are presented in Fig. 1. In comparing the two SCD groups (crisis vs. steady state), blood volume appeared higher during crisis (0.18% of tissue volume vs. 0.14%), however this difference was not statistically significant ($p = 0.19$). Adjustment for steady state hemoglobin also showed a trend towards higher blood volume during crisis (1.7% blood per mg/dL of Hb vs. 2.3% during crisis, $p = 0.20$). For oxygen delivery there was also an unexpected trend towards higher oxygen delivery during SCD crisis (0.06% oxygenated blood per tissue volume vs. 0.05%) ($p = 0.55$). Three-group comparisons (crisis vs. steady state vs. healthy control) were not statistically significant.

3.2.2. Bayesian analyses—Figure 2 shows the results of a Bayesian interpretation of the blood volume data. The *a priori* hypothesis was that blood volume would decrease 10% or more during crisis; however, as stated above, the data suggested the opposite. Based on the small amount of data collected, a Bayesian calculation of the probability that our hypothesis was correct (that there was at least a 10% decrease in blood volume during crisis) was 3%. Furthermore the probability that blood volume actually increases during crisis (i.e. the opposite of our hypothesis) was 84% (a suggestive but not definitive trend which is consistent with the fact that frequentist tests were not statistically significant at an alpha level of 0.05). Similar Bayesian calculations of the probability that our hypotheses were correct for oxygen delivery and oxygen saturation were 16% and 17% respectively.

4. Discussion

4.1. Introduction

With this study, to our knowledge, we present for the first time the characteristics of tissue blood volume and oxygen saturation at painful sites during steady state and acute SCD pain. Counter to our hypothesis, there appeared to be an increase in oxygen delivery and blood volume during acute SCD pain. We also demonstrated the utility of Bayesian analyses for small data, which has important implications for early-stage research in SCD and other rare diseases where challenges of recruiting large samples have been a barrier to progress.

A recent study by Rowley et al., using a tissue oximetry device at the thenar eminence of the hand found results consistent with ours. While blood volume was not explicitly measured by their device, microvascular oxygen consumption was elevated in SCD and further elevated

during painful crisis. This is consistent with our finding of a trend towards increased blood volume and oxygen delivery in subcutaneous tissue at painful sites (opposite to our *a priori* hypothesis) [22]. Blood flow has been explored in SCD using other modalities. Most notably, transcranial Doppler ultrasonography demonstrated elevations in cerebral blood flow in SCD with higher velocity associated with stroke [6]. Using laser Doppler fluximetry, L'Esperance et al. demonstrated increased peripheral blood flow in children with SCD and further increases during breath holding however measurements were taken at the finger instead of lower back and thigh (areas more frequently affected by pain) [15]. Bachir et al. demonstrated delayed recovery of laser Doppler flux metering after induced ischemia, but also did not take measurements during episodes of SCD pain [3]. Shi et al. measured skin laser Doppler flow to the calf during SCD pain crisis, but measurements were hampered by poor reproducibility (stability of measurement is one advantage of tissue oximetry over laser Doppler flow) [24]. Two landmark descriptive studies of laser Doppler velocimetry in SCD showed high variability of flow patterns and proffered that higher flow states may be in part a response to smaller areas of compromised flow [13, 21]. Our tissue oximetry data demonstrate that in addition to elevated velocity, blood volume (and possibly O₂ delivery) also appears to be increased in SCD. While the device used in this study quantified delivery of oxygen (the product of blood volume and oxygen content of the blood), in no way were we able to measure extraction by the tissues or take measurements deep enough to penetrate bone. Ultimately our findings fit with previous blood velocity data, that peripheral blood flow is increased in SCD, but with the additional novel finding that volume is further increased during crisis. More importantly our data are not inconsistent with the vaso-occlusive model where post capillary venular occlusion is the precipitant of SCD pain; however, it appears that vaso-occlusion was not occurring at the exact tissue sites measured by the oximetry device [10, 26].

The Bayesian analyses performed here have important implications for clinical research in rare diseases. In this study, frequentist hypothesis testing did not yield statistically significant results. The Bayesian results however allowed for a more granular probabilistic quantification of sub-scenarios of the overall hypothesis. For example, the probability that our hypothesis was correct (we hypothesized that oxygen delivery would decrease by at least 10%) was 3%. We felt this calculation was sufficient to reject our original hypothesis. Thus, even with this small sample and frequentist results that were not statistically significant, we were able to draw meaningful conclusions about our data. Such analyses could have particular utility in early phase studies of therapeutic interventions for SCD to help identify treatments that justify the expenditures necessary to conduct a large-scale, definitive trial. For example, in a pilot study of a novel SCD therapy, Bayesian analyses can quantify individual probabilities of various scenarios (e.g. the probability that the therapy is 10 percent or 20 percent (or any other difference) better than placebo) and can calculate the predictive probability that the trial will succeed for any desired difference. Frequentist tests generally provide only a binary assessment of whether or not the results are statistically significant. Thus, Bayesian analyses may improve efficiency for identifying promising therapies in small studies.

The most important limitation of our study is small sample size. A larger sample would have allowed for a more detailed exploration of the data (e.g. comparisons between different locations on the body or adjustment for other clinical characteristics). Participants were identified on a convenience rather than consecutive basis which could have introduced a selection bias. This does not affect the validity of our results because we have no reason to believe that this would create a directional bias.

In conclusion our data demonstrate that during acute SCD pain, blood volume in subcutaneous tissue is not decreased. The data suggest that delivery of oxygen may in fact be increased during periods of acute pain, however; the sample was too small to explore the discriminatory value of tissue oximetry in identifying SCD pain. We were able to draw these conclusions with a small sample using Bayesian analytics, which have broad implications for therapeutic and observational research in SCD and other rare diseases.

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Fig. 1.

Blood Volume, Saturation and Oxygen Delivery to the lower back during crisis, steady state and in healthy controls. The three graphs depict tissue oximetry characteristics in each of the three groups (error bars indicate 95% confidence intervals). The left depicts mean tissue blood volume expressed as a percent of overall tissue volume. The middle graph depicts oxygen saturation in the tissues (values range from zero to one). The left graph depicts oxygen delivery (expressed as the product of blood volume and oxygen saturation). These results suggested a trend towards increased blood volume and oxygen delivery during sickle cell crisis which was tested with Bayesian and frequentist statistical approaches.

Fig. 2. Bayesian Analyses of Blood Volume During Steady State and SCD Crisis. The tissue oximetry device measures the fraction of tissue occupied by blood (expressed as a percent). The top graph depicts our beliefs about blood volume during steady state in individuals with SCD. The green line indicates our beliefs before we collected data (the prior distribution), the blue line depicts the data we collected (likelihood function) and the pink line depicts our updated beliefs about blood volume based on the data collected (the posterior distribution). The middle graph depicts the same three things about blood volume during SCD crisis. The bottom graph depicts our beliefs about the difference between steady state and SCD crisis. This graph is created by subtracting the difference between random samples from the two posterior distributions above (Markov Chain Monte Carlo simulation). In the bottom graph, the majority of the distribution is above zero indicating that blood volume likely increases during crisis (82% probability). The a priori hypothesis was that blood volume would drop by at least 10% during crisis. Based on the data collected, the probability that our hypothesis was correct is 3% thus we rejected our hypothesis.

Table 1
Demographic and clinical characteristics of the cohort

	Healthy controls (<i>n</i> = 5)	SCD steady state (<i>n</i> = 5)	SCD acute pain (<i>n</i> = 4)
Age (median with IQR *)	28 (25–36)	24 (23–49)	31 (27–34)
Gender (percent male)	0%	60%	50%
Hydroxyurea (y/n)	NA	60%	75%
History of Stroke	NA	20%	0%
History of Acute Chest Syndrome	NA	20%	75%
History of Pneumonia	NA	20%	75%
History of Avascular Necrosis	NA	20%	50%
History of Skin Ulcers	NA	0%	0%
History of Priapism (males only)	NA	0%	0%

* Interquartile range.