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Serial assessment of laser Doppler flow during acute pain crises in sickle cell disease

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Abstract

Changes in basal laser Doppler flowmetry (LDF) of skin blood flow in sickle cell disease are reported to have pathophysiologic relevance in pain crisis. This is the first study to strictly control for LDF variability in determining the value of serial, basal (unprovoked) skin LDF as a practical method to assess resolution of acute pain crisis in sickle cell patients. Daily LDF measurements were repeated on the exact same skin areas of the calf and forehead throughout each of 12 hospital admissions for uncomplicated acute pain crisis.

A progressive increase in perfusion was observed in the calf throughout hospitalization as pain crisis resolved, but measurement reproducibility in the calf was poor. Reproducibility in the forehead was better, but no significant trend over time in perfusion was seen. There was no significant correlation between perfusion and pain scores over time. There was also no significant pattern of LDF oscillations over time.

In conclusion, only perfusion units and not oscillatory pattern of LDF has probable pathophysiological significance in sickle cell disease vaso-occlusion. The reproducibility of basal skin LDF specifically in sickle cell disease needs to be confirmed.

Keywords

acute pain crisis; laser Doppler flowmetry; microcirculation; sickle cell disease

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Introduction

There is little data in the field of sickle cell disease (SCD) regarding practical, objective methods of clinical microvascular blood flow measurement. Objective measures would be extremely useful for acute pain crisis resolution, since current measures such as pain ratings, amount of opioid usage, and time to hospital discharge may be affected by factors other than physiologic vaso-occlusion. Laser Doppler fluxmetry (LDF) is a non-invasive technology that uses the change in wavelength magnitude and frequency of laser light striking moving red blood cells to measure red blood cell flux (product of velocity and concentration of moving blood cells within the measuring volume)[1]. This paper presents a case series of 12 hospital admissions in which LDF was assessed daily throughout the hospital stay, under the hypothesis that red blood cell flux would increase over time coincident with pain crisis resolution.

Microvascular (primarily postcapillary venular) occlusion is accepted to be a major component of the pathophysiology in SCD [2; 3]. Although publications on the use of skin LDF assessment in SCD are relatively sparse (14 publications total), evidence suggests that microvaso-occlusion in SCD is present in the cutaneous circulation [4; 5; 6]. Using a standard probe fiber separation (0.25 mm) and wavelength source (633 or 780 nm), skin LDF measures at a depth of 0.32–0.35 mm, corresponding to a microvascular papillary or reticular dermis location [7]. Macrovascular arteries and veins lie in the deeper hypodermis and are therefore not measured.

Previous studies of LDF in SCD have mostly focused on evaluating patients at steady state (4 weeks past a crisis). LDF in SCD was first described in 1984 [8], where resting measurements in the forearms of steady state SCD patients showed the presence of unique periodic oscillations (~ every 8 seconds or 0.12 Hz) in the measured flux, not observed in normal and β^+ thalassemia controls. Oscillations were hypothesized to be related to the more rigid rheology of sickle red cells and subsequent increased intraluminal pressure [8]. Only two studies have measured skin LDF during acute pain crises, both without provocation. The first [9] noted that 4 of 5 patients during an acute pain episode exhibited these oscillations. The second examined three patients during and after crisis [10] and during crisis found increased blood flow and, in contrast to the first study, absent oscillations, hypothesized as due to peripheral shunting to arteriovenous anastomoses during crisis. Variability of these studies could be related to lack of confirmation that precisely the same spatial area in the forearm was measured each time, with time intervals between measurements up to 2–3 weeks. Such confirmation is crucial given that the major source of variability with skin LDF measurement is spatial variability of skin blood flow in regions as small as 2.5 mm apart [11; 12].

No study in SCD, to our knowledge, has yet used daily basal (i.e. without provocation) LDF measurements within a single acute pain crisis admission to study correlation with crisis resolution. Basal flow measurements were used based on easy applicability, expected intolerance of patients in crisis for provocative measures, and previous report in healthy volunteers that, if the same spatial area is measured, basal flow measurements are reproducible over months [13; 14; 15].

Methods

Patient population

Subjects were 8 patients with SCD, well known to the investigators, with SS or S β thalassemia genotype who were admitted to The Mount Sinai Hospital (New York, NY) or Montefiore Medical Center (New York, NY) through the emergency room with a total of 12 uncomplicated acute pain episodes from January 2009 to February 2011. Subjects had pain severe enough to have failed their outpatient prescription of as needed oral opioids, and had signed informed consent for an IRB-approved Phase I randomized, double-blind, dose-escalation (100 to 400 mg/kg) trial of single-dose intravenous immunoglobulin (IVIG, Gamunex[®]-C, Grifols, Clayton, NC) versus normal saline placebo for acute pain crisis [16]. IVIG or saline placebo was administered upon hospital admission, and otherwise subjects received standard care with fluids and opioid pain control (intravenous hydromorphone or morphine). Subjects had no hypertension, smoking, cardiovascular disease, leg ulcers, or alcohol use during admission, all of which can affect LDF readings [17]. Pain ratings were obtained at baseline and on a daily basis using either FACES or numeric rating scales.

LDF instrumentation

The LDF systems used were from Perimed (Stockholm, Sweden) and were calibrated on a monthly basis per manufacturer instructions. Laser light is delivered by a fiber-optic probe affixed to the skin surface, and a fraction of this light is scattered by moving red blood cells. The system detects and processes this Doppler-shifted light into a continuous voltage output which is related to the red blood cell flux (the concentration of moving blood cells times their average velocity). This voltage is expressed as arbitrary perfusion units (PU). For the first 4 crises, the LDF system used was a PF3 (633 nm Helium-Neon laser tube) with a probe (Model # 313) averaging 7 different measurement sites, thereby reducing PU variability due to spatial variation. For the following 8 crises, the LDF unit was updated to a PF5000 (780 nm laser diode) and the probe switched to one with heating capacity (Model #457) in order to reduce PU variation due to skin temperature, since patient room temperature was not controllable. The measurement depth is similar for the two wavelengths [7]. The probe was heated to 34 °C for all measurements, slightly higher than normal skin temperature of 33 °C, to factor in the potential for low-grade fever with pain crises. Both probes had the standard fiber separation of 0.25 mm; the heating probe heats a 1 cm diameter surrounding the laser source and measures a single rather than 7 sites.

LDF measurements

Critically, the precise same area on the calf or forehead was measured over the length of the hospital stay by outlining the probe circumference on the skin with a water-resistant skin marker, which was refreshed daily. LDF was assessed at least daily from hospital baseline (prior to study drug administration) until hospital discharge in all crises. Bias was controlled by “blinding” operators from reviewing previous measurements with the current measurement. Two anatomical sites were measured at each assessment by trained operators (the investigator, research nurse, or research fellow). Since the measurement volume of our instrument specifications [7] was only 0.14–0.19 mm³ and rotations of the probe angle can affect measurement reproducibility [11], consistent LDF technique was ensured through

training all operators to a standard recommended protocol (Perimed, Stockholm, Sweden). The anteromedial calf was chosen due to acceptable day-to-day calf reproducibility in healthy controls [14], relative lack of hair [18] and arteriovenous anastomoses, and clinical relevance with regard to leg ulcers. The forehead was also chosen due to its relatively high day-to-day reproducibility in healthy controls [15]. Of note, the forearm used in previous SCD studies is reported to have poor reproducibility in healthy controls [19] and LDF reproducibility at any site has not been formally tested in SCD. Sites were required to avoid visible veins, lesions, and body hair, and have a baseline flow > 5 PU.

Subjects were required to rest in their hospital bed for 30 minutes in a supine position 30–60° from horizontal immediately prior to measurements [12]. Motion artifact was limited by using double-sided tape (Perimed, Stockholm, Sweden) to fix the probe to the skin and immediately repeating any recording interrupted by subject movement. The LDF was recorded for at least 1 continuous minute with a sampling frequency of 32 Hz and a time constant of 0.2. Perimed's software program (Perisoft, Stockholm, Sweden) was used to calculate a mean PU value over that time interval.

Statistical analysis

The mean daily LDF was used for analysis when > 1 daily measurement was available. Each crisis was treated as an independent observation due to their separation in time of at least 1 month. Two patients were enrolled more than once, with 4 and 2 admissions, respectively. Pearson correlation coefficients were used to examine strength of relationships and generalized estimating equation modeling (autoregressive correlation structure) was conducted to examine effect of time on LDF measures with $\alpha=0.05$. Data was analyzed using Microsoft Excel version 2010 (Microsoft, Redmond, WA) and SAS version 9.3 (SAS Institute, Cary, NC).

Results

Subject characteristics are described in Table 1. Of note, there were no significant differences between IVIG and control groups

The mean number of daily LDF readings for each subject averaged 1.9 ± 0.2 , and the mean LDF recording time for each subject averaged 2 minutes, 21 seconds in the calf and 2 minutes, 23 seconds in the forehead. There was a significant trend of increasing LDF in the calf but not the forehead with pain crisis resolution (Figure 1). Four subjects (6 crises) had baseline LDF measurements performed within 1–2 hours of each other, during which time the pain score was unchanged. The correlation coefficient between the 2 measurements was thus calculated as a measure of reproducibility of LDF measurements (Figure 2A). Only the forehead and not the calf had a meaningful correlation coefficient, although it was not statistically significant. Furthermore, there was no meaningful correlation over time between LDF readings and pain scores (Figure 2B).

In contrast to previous reports in the forearm [10], there was no significant trend towards appearance of periodic microcirculatory flow (oscillations) with pain crisis resolution, with variable synchronous concurrence of oscillations in the calf and forehead (Figure 3a). The

forehead had a significantly higher percentage of measurements exhibiting oscillations than the calf (Figure 3b). Oscillations varied from 3–25 second periodicity, were of variable amplitude, and could transiently appear or disappear during a single recording period without evident change in patient clinical status or pain score (Figure 4). Periodicity and amplitude also varied within individual subjects over time.

Discussion

This is the first case series in patients with SCD to perform daily measurement of unprovoked LDF over the course of hospitalization for acute pain episodes. Basal LDF measurements in the forehead, fingers, and calf of healthy volunteers are reported to be reproducible over time when the same spatial area is measured.[13; 14; 15] Strictly controlling for spatial variability[7; 11], we hypothesized that LDF would increase over the course of hospitalization due to improving vaso-occlusion. We also controlled for skin temperature (with the second probe), alcohol intake, physical activity level, and body position [20]. Although a significant trend of increasing LDF in the calf was observed, the validity of this result is questionable due to the poor reproducibility observed in the calf.

One possible factor contributing to the lack of meaningful trends is that, unlike in normal volunteers, in SCD patients LDF readings may not be reproducible. Another possible factor may be that, given oscillatory variability over time, a longer time period than 2 minutes needs to be measured [18]. Another possible contributing factor is suboptimal choice of the anatomical site for measurement. The anatomical sites assessed by LDF in previous SCD studies are the ventral forearm[8; 9; 10], medial and lateral calf [21; 22], finger pulp[23], and dorsum of foot[24], however no sites have been validated specifically in SCD. Reproducibility may be problematic in the forearm [19], therefore the calf and forehead were chosen due to reported high reproducibility.[14; 15] We confirmed acceptable reproducibility only in the forehead. Our lack of reproducibility in the calf may be related to measurement on the medial rather than lateral side of the calf as in the Kvernebo et al. study.

Another possible factor contributing to our results may be suboptimal probe placement. Literature suggests capillaries and post-capillary venules may be distinguished from arterioles by an LDF pattern of minimal cardiac pulsation and oscillations [25; 26; 27]. Therefore, since vaso-occlusion is thought to occur primarily in the post-capillary venules, our probe placement perhaps should have targeted areas with minimal cardiac pulsation and oscillation. As demonstrated in Figure 4, however, a site may exhibit only transient oscillations, and thus be identified as a post-capillary venule/capillary when actually an arteriole.

Suboptimal control of all environmental factors may be another explanation for the lack of meaningful trends [17]. Although subjects resided in the same room with a set temperature (~20°C) throughout their hospital stay, room temperature and humidity [28; 29] were not formally controlled. Ambient light level and circadian rhythm may influence LDF [30; 31; 32], and measurements were not necessarily taken at the same time of day. Although sporadically up to 2 different operators in addition to the PI performed LDF readings over the hospitalization duration in individual patients, all were trained and verified competent by

the principal investigator to the standard Perimed protocol. Therefore operator variability was probably not an issue.

Several factors possibly influencing LDF could not be controlled for by study design. The menstrual cycle may influence LDF flow [33; 34] and 4 of 8 subjects were females with average hospitalization duration of 4.3 days. Neurogenically mediated increases in blood flow may occur secondary to pain itself [35], thereby negating discernment of an increasing LDF trend as pain resolves. Despite setting the probe temperature at high normal for skin (34°C), occasionally the patient's endogenous body temperature was higher than the set temperature. Finally, variability in intra-individual RBC concentration that occurred during the hospitalization is significant because the LDF value depends partly on the concentration of moving blood cells. Variations in RBC concentration were seen in non-transfused as well as transfused patients, which is typical of the course of acute pain episodes [36]

We did not observe any trend in oscillatory LDF pattern with pain crisis, as previously reported.[9; 10] Since oscillatory patterns have been reported to be characteristic of probe placement in relation to vessel type[25; 26; 27], and oscillations were variably present in previous SCD studies which did not report controlling for spatial variability over time, there is unlikely to be specific oscillatory trends with pain crisis resolution.

Conclusions

Oscillatory patterns are probably not pathophysiologically meaningful in unprovoked LDF measurements in SCD, other than as a guide to proper probe placement. That SCD patients have reproducible measurements over time needs to be confirmed. Even if confirmed, basal LDF measurements to assess resolution of acute pain episodes may not be feasible in a real-world hospital unit rather than a carefully controlled experimental setting, due to the multitude of variables influencing LDF readings[17]. Studies of skin LDF in SCD after provocations like transient ischemia[10; 23; 37; 38; 39], vasodilation [40], heat or cold stimulus[23; 41; 42], or veno-arteriolar reflex stimulation [21; 22; 24; 43] have not been used during pain crisis due to poor tolerability at baseline. Finally, alternative microvascular blood flow techniques such as conjunctival microscopy [23; 44; 45; 46], nailfold capillary microscopy [47; 48], or contrast-enhanced ultrasound (clinicaltrials.gov identifier NCT01566890) may correlate with and provide better estimates for pain crisis resolution in SCD.

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Figure 1.

Laser Doppler flow data. Each crisis (N=12) is identified with the same color in both graphs. Legend subject numbers (N=8) in the same color represent the same patient. **(A)** Normalized perfusion units, where each dot represents an individual subject's percent change from the mean of all his/her LDF measurements. P-values were obtained using generalized estimating equation models with autoregressive correlation structure to test the effect of hospitalization day on LDF measurements. **(B)** Means of daily absolute perfusion units as directly calculated by the device.

Figure 2.

(A) Reproducibility of LDF measurements. PU= perfusion units. Values in orange were measured 1 hour apart; values in blue were 2 hours apart. Data points outlined in red represent 1 patient during 3 separate crises. r-values were obtained using Pearson correlation. (B) Correlations over time between LDF measurements and pain scores. BL=baseline. Each time point is the mean of the variations from the mean for each patient at that time point. r-values were obtained using Pearson correlation.

Figure 3.

(**A**) Trend in oscillations over hospitalization duration. On the Y-axis, 1= oscillations present, 0= oscillations absent, with the 1st number representing the calf and the 2nd number the forehead. P-value was obtained using generalized estimating equations. Each crisis (N=12) is identified with the same colors as in Figure 1; legend subject numbers (N=8) in the same color represent the same patient. (**B**) Percent of measurements with oscillations present in each subject. P-value was obtained using a paired t-test comparing the mean proportion of forehead and calf oscillations.

Figure 4.
Examples of oscillatory patterns. Excerpts are taken directly from device tracings. Perfusion unit tracings are in blue.

Table 1

Subject characteristics^a

Age (years)	Gender	Baseline pain (0–10 scale)	Discharge pain (0–10 scale)	Morphine equivalents (mg/kg/day)	Length of stay (days)
23 ± 9	4 F, 4 M	7.3 ± 1.0	4.0 ± 1.3	0.9 ± 0.7	4.5 ± 2.0

^aData is presented as mean ± standard deviation. F=female, M=male.