Sickle cell disease, pathophysiology and clinical complications
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Normal sublingual microcirculation during painful crisis in sickle cell disease

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Abstract

Obstruction of the microcirculation is the most important cause of painful crisis in sickle cell disease (SCD). Extensive microvascular obstruction has been observed in mouse models of SCD. A technique to determine the extent of the microcirculatory obstructions in humans may be helpful in the clinical setting and for research purposes. Therefore, we measured sublingual microcirculation longitudinally in patients with SCD admitted with painful crisis. Sublingual microcirculation was recorded with side-stream darkfield (SDF) imaging and semi-quantified with a microvascular flow index (MFI) on a range from 0 to 4 (arbitrary units; from 0 (no flow) to 4 (hyperdynamic flow)).

Thirteen consecutive adult sickle cell patients admitted with painful crises were included and provided 47 measurements of MFI in 14 episodes of painful crisis. Seven patients provided baseline measurements and seven healthy controls were studied. The mean (± standard error of the mean) MFI during painful crisis was 2.6±0.1 and did not change during the painful crisis. The mean MFI of patients with SCD during steady state (2.7±0.1) and the mean MFI of the controls (2.7±0.1) were not different from the mean MFI during painful crisis. During painful crisis irregular microvascular perfusion, expressed by the distribution width of the microvascular blood flow velocity, correlated negatively (r=−0.484; P=0.002) with hemoglobin concentration.

We conclude that sublingual microcirculatory blood flow velocity is not disturbed in sickle cell patients during painful crisis.
**Introduction**

Vaso-occlusive complications account for a major part of the morbidity suffered by patients with sickle cell disease (SCD) and has been demonstrated to be a major determinant of life expectancy. The most frequent vaso-occlusive complication is the painful crisis. In painful crises, widespread microcirculatory obstruction leads to ischemic bone pain, resulting in frequent hospital admissions for supportive care. Since objective tools to quantify the extent of the microcirculatory obstruction during the painful crises are not available, frequency and duration of painful crises is often used to assess the severity of crises and effect of therapy. A technique to determine the extent of the microcirculatory obstruction during crisis may therefore be very helpful in both clinical setting and for research purposes.

Despite the fact that the pain in vaso-occlusive crisis is mainly located in bones, animal models of SCD, which are frequently used to investigate the pathophysiology of SCD and painful crisis, have demonstrated that microcirculatory disturbances are not limited to the microvasculature of the bones.\(^1\)\(^-\)\(^5\) Previously, microcirculatory obstruction during painful crisis has been visualized in sickle cell patients at the vascular beds of the nailfold and conjunctiva but resulted in conflicting conclusions.\(^6\)\(^-\)\(^8\)

The Orthogonal Polarized Spectral imaging technique (OPS) is a new technique to visualize the microcirculatory blood flow velocity with better optical density on easy accessible mucosal membranes such as the sublingual tissue. This technique has been successfully used to quantify sublingual microcirculatory disturbances in different diseases.\(^9\)\(^-\)\(^14\)

In the present study, we explored the sublingual microcirculatory blood flow velocity in SCD during steady state and painful crisis using Side-stream Dark Field (SDF) imaging, an improved version of OPS.\(^15\)\(^-\)\(^17\)
Methods and materials

Patients
Consecutive sickle cell patients aged 17 years or older (HbSS, HbSC, HbSβ-thalassemia and HbSβ⁺-thalassemia) admitted with a painful crisis to the Academic Medical Center in Amsterdam, the Netherlands were included. A painful crisis was defined as the presence of typical bone pain which could not be explained otherwise. Sickle cell crises were divided in three artificial periods of admission defined as period I (day 1-3), period II (day 4-6) and period III (day 7-10). Patients with oral mucosal infections or trauma were excluded. Patients served as their own controls by assessment of the sublingual blood flow velocity at steady state, at least 4 weeks following the painful crisis. In addition, healthy individuals without SCD served as controls. The study was approved by the local medical ethics committee and all participating patients gave written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki.

Measurement of sublingual microcirculation
The sublingual microcirculation was analyzed using SDF imaging (Microscan, MicroVision, Amsterdam, The Netherlands) as described by others. In short, the SDF-probe, containing the camera and light source, is put gently under the middle of the tongue. SDF uses light emitting diodes (LED’s) emitting (green) light with a wavelength of 530 nm, which is completely absorbed by hemoglobin causing erythrocytes to appear as dark particles. The sublingual microcirculation was measured daily from admission until a maximum of 10 days thereafter. Intravital microscopy images were recorded using a digital video recorder (Sony SDR-20 P). The images had to be sufficiently sharp and the probe should not have exerted any pressure on the tissue examined. From each of these video recordings fragments were selected by a second investigator unaware of the clinical condition of the patient or control. These fragments had to consist at least 5 seconds of steady, clear and sharp images in order to measure Microvascular flow index (MFI) (see below). The fragments were edited using Microsoft® Windows ® Movie Maker® (version 5.1, service pack 2).

Microvascular flow index (MFI)
The microvascular flow index (MFI) is a previously described semi-quantitative analysis method to determine the blood flow velocity through the microvasculature of specific organs. Video fragments recorded using SDF were divided into quadrants (figure 1A). In each of the quadrants blood vessels were subdivided into the diameter categories small (6-15 μm), medium (15-25 μm) and large (25-50 μm) (figure 1B). For analysis of the microcirculation only the diameter categories
small and medium were studied. Flow velocity of erythrocytes in each individual blood vessel was estimated by trained observers and categorized as follows: 0: no flow, 1: intermittent flow, 2: stuttering flow, 3: normal flow and 4: hyperdynamic flow. To determine the average flow in both categories of blood vessels (small and medium sized blood vessels) the MFI was calculated in all quadrants per video fragment. Per measurement at least three video fragments were analyzed and averaged. All fragments were scored by the same observer. Since hyperdynamic flow in some vessels may compensate for limited or obstructed flow in other vessels resulting in a similar mean MFI, also the distribution width of blood flow velocity, defined as the standard deviation (SD) of the measured MFI, and the total number of occluded (0) and vessels with intermittent blood flow velocity (1) per fragment were assessed.

![Figure 1. Analysis video images.](image)

Captured video images are divided in four quadrants(I,II,III and IV) Classification of blood vessels by size; large(L3; >25μm), medium(M2; 15-25μm), small(S3; 6-15μm) blood vessels.

**Inter and intra observer variability**

Analysis of the SDF video fragments was tested for inter observer variability by randomly letting an experienced second observer analyzed a subset of SDF video fragments in a blinded fashion. Both observers were unaware of the other observers MFI estimations. Intra observer variability was tested by re-analyzing a random set of SDF video fragments three weeks after the first analysis by the same observer.
Chapter 4

Statistics
The average daily MFI and flow distribution during hospital admissions for painful crisis were compared with base-line measurements and controls using student’s T-test and ANOVA. The inter and intra observer variability was determined by calculating the correlation coefficient as described by Bland and Altman.19 All data are presented as means with standard errors and were statistically analyzed using SPSS 12.0.02 for Windows® (released 24 March 2004).
Results

Patients

Thirteen adult sickle cell patients (7 HbSS, 2 HbSC, 3 HbSβ+ -thalassemia 1 HbSβ⁰-thalassemia, mean age 37±13 years) provided 47 measurements of microcirculatory flow during 14 episodes of painful crises. Seven patients were reassessed during steady state condition to provide baseline measurements. Seven healthy controls were also included.

Figures 2 & 3. Flow measurement between groups and during painful crisis.

Figure 2: Mean Microvascular Flow Index (MFI) in patients with SCD during painful crisis (Crisis) and during steady state (Steady state) compared to healthy controls (Controls). No difference in MFI was demonstrated between the different groups. Figure 3: Mean Microvascular Flow Index (MFI) in patients with SCD during painful crisis. Day 1-3 represents the first 3 days after admission for painful crises, day 4-6 and day 7+ represent the days following. No difference in MFI was demonstrated between the clinical periods of the painful crises.

Microvascular Flow Index (MFI)

The microvascular blood flow velocity expressed as the MFI appeared to be similar in sickle cell patients during steady state (2.6 ± 0.1) and painful crisis (2.7 ± 0.1) (figure 2). In addition, no differences could be detected between the MFI in sickle cell patients and healthy controls (2.7±0.1). During painful crises the MFI measured on subsequent days did not change.(figure 3) Also the flow pattern, as presented in figure 4, and the distribution width of the microvascular flow velocity did not differ between the groups, excluding the possibility that hyperdynamic flow in some vessels may have compensated for the limited or obstructed flow in other vessels. During painful crisis, the distribution width of the microvascular blood flow velocity was negatively
correlated with hemoglobin level ($r=-0.484; P=0.002$) and positively correlated with leukocyte count ($r=0.438; P=0.014$) and lactate dehydrogenase plasma levels ($r=0.417; P=0.027$) and also the total number of blood vessels with no or intermittent flow was negatively correlated to hemoglobin concentration ($r=-0.367; P=0.028$). (Figure 5). Using a multivariate analysis to correct for genotype, the above mentioned correlations remained significant except for the correlation between LDH and distribution width. No difference in distribution width of the microvascular blood flow velocity was found between patients and controls.

**Inter- en intra observer variability**

The calculated correlation coefficient for intra-observer variability of small and medium vessels were 0.965 ($P<0.001$) and 0.967 ($P<0.001$) respectively (corresponding Bland and Altman’s limits of agreement -0.31 to 0.21 and -0.56 to 0.35). The calculated correlation coefficient for inter-observer variability of small and medium vessels were 0.836 ($P = 0.001$) and 0.810 ($P<0.001$) respectively (corresponding Bland and Altman’s limits of agreement -0.38 to 0.30 and -0.77 to 0.57).

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**Figure 4 & 5. Flow distribution pattern and correlations during painful crisis.**

Figure 4: The total number of microvascular blood vessel with either: no flow (0), stattering flow (1), slow flow (2), normal flow (3) or hyperdynamic flow (4). “T” represents the mean of all flow patterns within the groups. Data are expressed as total number of blood vessels observed ± SD. No statistically significant difference was observed between the groups.

Figure 5: During painful crisis, the distribution width of the microvascular blood flow velocity was negatively correlated (Spearman rank test) with hemoglobin level ($r=-0.484; P=0.002$). *: The flow distribution width was defined as the standard deviation (SD) of the measured MFI.
**Discussion**

In this study, we measured sublingual microvascular blood flow velocity in patients with SCD during painful crisis and steady state with a new optical technique, called SDF imaging, to visualize the microcirculation.\textsuperscript{15-17} In addition, healthy controls were analyzed. No difference in sublingual microvascular blood flow velocity was observed between sickle cell patients during painful crisis and steady state or between sickle cell patients and healthy volunteers. Since also the distribution of blood flow velocity expressed by the MFI distribution width, was similar between all groups, our findings can not be explained by hyperdynamic blood flow in some microcapillaries compensating for stuttering flow in other microcapillaries. This confirms the observations of Lipowski et al. who did not find differences in blood flow velocity distribution between sickle cell patients in steady state, during crisis or healthy volunteers either.\textsuperscript{8}

Our findings are in contrast with the previous observations. Cheung et al. demonstrated a reduction in the bulbar microvascular blood flow velocity during painful crisis while the opposite was observed by Lipowski et al. in the vascular bed of the nailfolds.\textsuperscript{6,8} We considered a number of explanations for this observation. Firstly, a new technique of intravital microscopy was used in our study. However, it seems unlikely that this technique explains the difference since the SDF technique used in this study provides better image quality than conventional OPS intravital capillary microscopy.\textsuperscript{17,20} Secondly, the quantification methods of the microvascular blood flow may not have been sensitive enough to detect a disturbed microvascular flow in sickle cell patients. However, in the study of Cheung et al. microvascular flow velocities appeared to decrease 46% during crisis while in our study, the quantification method was powered to detect a difference in MFI of at least 0.3 representing a 10% reduction in the microvascular blood flow velocity.\textsuperscript{6} In addition, no difference in flow distribution in the microcapillaries was observed between patients in steady state and during sickle cell crisis. Thirdly, our results may be explained by the fact that sublingual microvascular blood flow is not hampered in SCD. Sublingual microvascular blood flow is considered to be a reliable estimation of systemic microvascular blood flow in septic patients and has demonstrated to predict survival in patients with severe heart failure.\textsuperscript{5,13,15} Lastly, our study may have missed actual changes in microcirculatory blood flow due to the relative small sample size. Interestingly, the distribution width of the microvascular blood flow velocity during painful crises appeared to be inversely related to hemoglobin levels indicating a more irregular microvascular blood flow pattern in patients with severe anemia. The elevated distribution width of the microvascular blood flow velocity of subjects with severe anemia may represent a degree of overcompensation for the rheological insult in capillaries that are occluded during painful crisis.\textsuperscript{21} The flow distribution on average however did not differ between patients and controls. This may be caused by normal physiological changes in flow through different capillaries at normal to high hematocrit which has also been observed by Lipowsky et al. and Cheung et al.\textsuperscript{7,8}
We conclude that sublingual microcirculatory blood flow velocity is not disturbed in sickle cell patients during painful crisis per se and is therefore not useful to quantify the severity of painful crisis in sickle cell disease.
Reference List


