Non-Invasive Characterization of Oxygen Transport in Sickle Cell Disease: A Pilot Study

Imoigele P. Aisiku*, Osama R. Kandalaft†, Wally R. Smith‡, Lynne T. Penberthy§, Raghu R. Seethala∥, Peter C. Hou¶ and Kevin R. Ward[¶]

1Assistant Professor, Department of Emergency Medicine Harvard Medical School, 75 Francis St Boston, MA 02115, USA
2Research Fellow, Department of Emergency Medicine Harvard Medical School, 75 Francis St Boston, MA 02115, USA
3Professor of Internal Medicine and Scientific Director of the VCU Center, Virginia Commonwealth University, Box 980306 Richmond, VA 23298, USA
4Associate Director, Division of Cancer Control and Population, Sciences National Cancer Institute, 9609 Medical Center Drive, Bethesda, MD 20892, USA
5Instructor, Department of Emergency Medicine Harvard Medical School, 75 Francis St Boston, MA 02115, USA
6Instructor, Department of Emergency Medicine Harvard Medical School, 75 Francis St Boston, MA 02115, USA
7Professor and Executive Director of the Michigan Center for Integrative Research in Critical Care (MCIRCC), Department of Emergency Medicine University of Michigan, NCRC: Bldg 10, Office A103 2800 Plymouth Rd, Ann Arbor, MI 48109, USA

*Corresponding author
Imoigele P. Aisiku, MD, MSCR, MBA
Assistant Professor
Department of Emergency Medicine
Harvard Medical School
75 Francis St Boston
MA 02115, USA
Tel. (617) 732-5640
Fax: (604) 828-4862
E-mail: aisiku@bwh.harvard.edu

ABSTRACT

Introduction: Vaso-occlusive (VOC) crisis is, in part, a result of microvascular ischemic insults to tissue causing pain in Sickle Cell Disease patients, which is a common presentation to the Emergency Department (ED). This study simultaneously measured and compared several global and regional indicators of oxygen transport in normal volunteers and subjects with Sickle Cell Disease (SCD).

Materials and Methods: Healthy African American volunteers were compared to SCD patients, assessed at states of clinical non-distress, referred to herein as “baseline”. All subjects underwent 10 minutes of non-invasive monitoring to measure cardiac output, oxygen consumption, arterial oxygen saturation (SpO2), and Cutaneous tissue saturation of oxygenation (CtSO2).

Results: Twenty one patients (9=healthy & 12=SCD baseline) were chosen. The median superficial CtSO2 (healthy vs. SCD baseline) was 72% (IQR=10.94) and 56% (IQR=26.86) with a p-value of 0.0011. Traditional measures of hemodynamic performance (heart rate, blood pressure, cardiac index) were not statistically significant between the two groups.

Conclusion: The study shows Sickle Cell Disease to share similarities with sub-clinical compensated state of shock on a microcirculatory level. The values obtained from the study can hopefully shed light into the intricacies of the baseline biophysiology of Sickle Cell Disease; with a foresight to further understand Vaso-occlusive crises pathological processes and sickled cells interactions with its surrounding environment.

KEYWORDS: Oxygen transportation; Microcirculation; Spectroscopy; Hemoglobin.

ABBREVIATIONS: VOC: Vaso-occlusive Crisis; ED: Emergency Department; SCD: Sickle Cell Disease; CtSO2: Cutaneous Saturation of Oxygen; OER: Oxygen Extraction Ratio; IQR: Interquartile ratios; SD: Standard Deviations; CI: Confidence Intervals.
INTRODUCTION

Sickle Cell Disease (SCD) is a disease manifestation of a set of genetic abnormalities primarily affecting patients of African and Mediterranean descent. It is caused by a substitution of valine for glutamic acid in the sixth position of the beta globin chain on chromosome 11. This alters the surface charge of the molecule and allows sickle hemoglobin (Hb S) tetramers to polymerize inside the red blood cell. The polymer can alter both the red cell shape and membrane properties leading to abnormal and complex interactions with the vascular endothelium. The combination of these effects produces a hemolytic anemia and suspected microvascular dysfunction, which results in severe pain. The mechanisms by which this occurs have not been well delineated, but are likely due in part to abnormalities in oxygen transport. Current concepts suggest several factors may impact oxygen transport including inflammation, neurohumoral responses, autonomic nervous system adaptations and abnormalities in vascular response. These factors may influence vasculature in SCD patients at baseline and during VOC.

In order to gain a deeper understanding of the pathophysiology of SCD and oxygen transportation, better mechanisms to identify the components of oxygen transport are needed. It is now possible to non-invasively monitor several components of oxygen transport. Some method involve measuring cardiac output by a number of means including impedance cardiography, oxygen consumption through indirect calorimetry, arterial hemoglobin oxygen saturation through pulse oximetry, and tissue hemoglobin oxygen saturation through differential absorption spectroscopy. We used these techniques along with conventional hemodynamic parameters such as heart rate and blood pressure to measure and compare whole body and regional tissue oxygen transport and traditional hemodynamic measures in SCD patients at baseline and patients without SCD.

The purpose of this study to begin to understand the relationship between oxygen transport abnormalities in normal, healthy controls and in patients with SCD. The ability to document such abnormalities may provide important diagnostic and therapeutic endpoints allowing for more objective treatments of SCD and VOC.

MATERIALS AND METHODS

Study Population

The study population consisted of two groups. The first group was composed of nine normal, healthy controls of African-American descent with no history of reported Sickle Cell Disease or trait. The second group consisted of twelve patients with a known history of Sickle Cell Disease classified as homozygous Hb SS or doubly heterozygous Hb SC, that at the time of evaluation, did not report pain. There were no statistical analysis of the control group and SCD group demographics due to the control groups being race and age matched to the SCD patients. The Institutional Review Board has approved this study. All patients signed a consent form prior to enrollment in the study as per IRB regulations.

Non-Invasive Hemodynamic and Oxygen Utilization Measurements

Cutaneous tissue oxygen saturation measurements (CtSO2): Differential absorption spectroscopy was used to measure the aggregate hemoglobin oxygen saturation in a selected volume of tissue. CtSO2 measurements were made with a spectrophotometric monitor using a combination of visible and near-infrared light (O2C: LEA, Inc., Gießen, Germany). A combination of white light and near infrared light was used to detect CtSO2. Oxygen saturation was determined by the differential absorption spectra of oxygenated and deoxygenated hemoglobin to the various light sources as they traverse a certain volume of tissue. The volume of blood in any tissue is approximately 70% venous, 20% capillary, and 10% arterial. The derived CtSO2 is indicative of mainly venous hemoglobin and thus, represents the post-extraction compartment of the tissue. This in turn is indicative of the adequacy of oxygen delivery at the tissue level. Each probe has sensors that can detect superficial as well as deep cutaneous measurements based on optode spacing and the character of light used. Superficial sensors monitor a depth of 2 mm and deep sensors monitor a depth of approximately 7 mm. Two flat probes were secured to the thenar aspect of each individual’s palmar surface while recording CtSO2 readings. This was done to minimize pigment interference with the probes while recording data. CtSO2 was measured continuously and values, reported as “percent saturation”, were recorded every 5 seconds for averaging over the 10 minute period.

Cardiac index (CI): In order to determine whole body oxygen delivery, cardiac output was measured using an impedance cardiography (Medis Medizinische Meßtechnik, Thuringen, Germany). Eight standard electrodes were placed on each subject; as directed by the manufacturer. Two of these electrodes were placed on each side of the neck and thorax. The electrodes used were standard continuous EKG monitoring electrodes. CI was measured every 5 seconds and these values were used to average CI over the 10 minute period.

Oxygen consumption (VO2): VO2 was measured by having patients breathe into a mouthpiece connected to a system that can measure both airflow and the differences between expired and inspired oxygen concentration (BioPac Systems, Gloleta, CA, USA). The patient was instructed to breathe through a disposable mouthpiece and corrugated tubing identical to those used to administer respiratory aerosol treatments. These measurements were made continuously and values taken every 5 seconds were used to average VO2 over the 10 minute time period.

Arterial hemoglobin oxygen saturation: Arterial hemoglobin
oxygen saturation (SpO₂) was determined with the use of a pulse oximeter (General Electric Procare Auscultatory 400). SpO₂ was used to substitute for true arterial hemoglobin oxygen saturation. SpO₂ was measured every 5 seconds and averaged over the 10 minute monitoring period.

**Oxygen delivery:** Oxygen delivery was calculated using the formula \( \text{DO}_2 = \left(1.34 \times Hgb \times \text{SO}_2 \right) + \left(PO_2 \times 0.0031\right) \). Hemoglobin was measured as part of the routine clinic visits or from Emergency Department visits. Control subjects did not have hemoglobin levels drawn. A standard hemoglobin value of 12 mg/dL was used for the control subjects. A Hemoglobin value of 12 mg/dL was chosen for calculating oxygen delivery because this number represents the lower range of normal hemoglobin levels and would therefore underestimate the oxygen extraction ratios when compared to sickle cell patients.

**Oxygen extraction ratio (OER):** OER can be determined by a number of means. Regional OER was determined by using the CtSO₂ and SpO₂ values (SpO₂·CtSO₂)/SpO₂. The Oxygen extraction ratio (OER) can be determined by a number of means.

**Vital signs:** Standard vital signs, Heart Rate, Blood Pressure, Temperature and Respiratory Rate, were measured by clinically accepted standards.

**Statistical Analysis**

Data entry and data analysis was performed using JMP 4.0 (SAS Institute, Cary, NC, USA). A non-parametric median Kruskal–Wallis test was performed to determine any significant differences between the study groups. Comparisons of hemodynamic and oxygen transport measures were made utilizing a non-parametric analysis and group medians. Inter-quartile ratios (IQR) ratios were substituted for Standard Deviations (SD) and Confidence Intervals (CI). The level of significance was set at an alpha of 0.05.

**RESULTS**

There were 9 self-reported, healthy African-American control subjects, and 21 SCD patients. The median age for the healthy controls was 29±6 years and the median age for the SCD patients was 34±11 years (Table 1). The majority of SCD patients were Hgb SS followed by Hgb SC (Table 1). The majority of the control subjects were male and there was a nearly even gender distribution in the SCD patients (Table 1). DO₂ and VO₂ measurements were measured for healthy control subjects and SCD patients at baseline. The DO₂ for the control subjects was 525.5 ml/O₂/min and 326.8 ml/O₂/min for the SCD group at baseline (Table 2). There were significant differences of DO₂ between the healthy control subjects vs. SCD patients at baseline (Figure 1 and Table 2). The VO₂ for the control group was 214.5 ml/min and 202.5 ml/min for the SCD group at baseline which showed no statistically significant differences in VO₂ (Table 2). Superficial and Deep CtSO₂ differences between the groups are shown in Figure 2 and Table 2. The median superficial CtSO₂ for the Control group and the SCD group was 72% (IQR=10.94) and 56% (IQR=26.86) respectively (p=0.0011). The median deep CtSO₂ (control vs. SCD baseline) was 73% (IQR=3.74) and 63% (IQR=12.1) respectively and was also significantly different (p=0.0033). Global measures of oxygen delivery (CO, CI, SV, SI and Hgb) were similar with no statistical differences existing between the groups except cardiac output (Table 3). Cardiac index and other global measures of cardiac function were not statistically significant therefore cardiac output was not interpreted to be clinically significant. We found no statistical difference in standard vital sign parameters (Blood Pressure, Heart Rate, Temperature, Respiratory Rate, and SpO₂) between healthy controls and sickle cell patients at baseline. There were significant differences in the OER between the Control group and the SCD group at baseline. The results of Regional Superficial OER were 26% and 42% respectively, with a p-value of 0.0013. The results of the Regional Deep OER were 25% and 34% respectively with a p-value of 0.0037 (Table 2).

**DISCUSSION**

The current study demonstrates that non-invasive oxygen transport monitoring is possible in SCD patients. Based on both CtSO₂ and VO₂ measurements in the SCD baseline state, it does not appear that SCD patients, despite the chronic nature of the disease, have down regulated their metabolism to compensate for this chronic decrease in DO₂. Due to the anemia that is commonly seen in SCD patients, despite full hemoglobin satura-
tion with oxygen, it is not surprising that their DO$_2$ is significantly lower than patients without SCD. What may not be as obvious is that there does not appear to be any significant increase in cardiac output to compensate for this reduction in hemoglobin content.

As discussed in the Methodology section of this paper, the values of CtSO$_2$ by differential absorption spectroscopy are representative of the venous hemoglobin oxygen saturation values, since venous blood dominates the majority of blood volume of the analyzed tissue. Thus, a measure of tissue hemoglobin oxygen saturation reflects the post-extraction compartment of tissue in terms of oxygen delivery and utilization. In our study, SCD patients compared to non-SCD controls exhibit evidence of increased regional oxygen extraction, even when not reporting a VOC. Patients in this cohort revealed that they have high extrac-

| Variable                | Control Group-Median (IQR) | SCD Group-Median (IQR) | P-Values |
|-------------------------|----------------------------|------------------------|----------|
| CtSO$_2$ Superficial (%)*| 72%(10.94)                 | 56%(26.86)             | 0.0011   |
| CtSO$_2$ Deep (%)*      | 73%(3.74)                  | 63%(12.1)              | 0.0033   |
| VO$_2$ (ml/min)         | 214.5(143.3)               | 202.5(88.75)           | 0.6742   |
| DO$_2$ (ml/O2/min)*     | 525.5(166.6)               | 326.8(239.3)           | <0.0001  |
| Regional Superficial OER (%)* | 26%                      | 42%                    | 0.0013   |
| Regional Deep OER (%)*  | 25%                       | 34%                    | 0.0037   |

*Significant at p-value<0.05.
SCD: Sickle Cell Disease; CtSO$_2$: Cutaneous Saturation of Oxygen; VO$_2$: Oxygen Consumption; DO$_2$: Delivery of Oxygen

Table 2: Comparison of Oxygen Delivery, Oxygen Consumption, Oxygen Extraction Ratio and Cutaneous Saturation of Oxygen.

![Figure 2: Superficial and deep CtSO$_2$: Control group (n=9) vs. SCD group (n=12).](image)

![Table 3: Comparison of global measures of oxygen delivery between control group and SCD group.](image)

| Variable | Control Group-Median (IQR) | SCD Group-Median (IQR) | P-Values |
|----------|----------------------------|------------------------|----------|
| CO (L/min) | 7.20(3.21)                  | 5.39(2.22)             | 0.0430   |
| CI (L/min/mm) | 3.34(1.06)                | 2.96(0.97)             | 0.0611   |
| SV (ml/min) | 97.49(28.23)              | 77.32(30.45)           | 0.7250   |
| SI (ml/min/mm) | 49.62(17.03)             | 41.87(19.30)           | 0.6560   |
| Hgb       | 12(N/A)                    | 9.4(2.8)               | -        |

SCD: Sickle Cell Disease; CO: Cardiac Output; CI: Cardiac Index, SV: Stroke Volume; SI: Stroke Index; Hgb: Hemoglobin

Table 3: Comparison of global measures of oxygen delivery between control group and SCD group.

*Significant at p-value<0.05. CI, SV and SI were not statistically significant, therefore CO was not interpreted to be clinically significant.
tion ratios at baseline. This decrease in microvascular delivery may in turn be caused by a combination of further rheologic and/or microvascular problems. Whether or not this can be termed “dysfunction” at the microvascular level is unclear, as this may represent appropriate compensation at this level. Given our data, sickle cell disease might be viewed as a sub-clinical compensated state of shock as defined by decreases in tissue oxygen delivery on a microcirculatory level.15-18 Similar to other states of shock, regional oxygen transport changes are possible without changes in global oxygen transport or vital signs.

Although it is unlikely that the subcutaneous tissues were dysoxic or ischemic, changes in CtSO₂ measured at this level are consistent with homeostatic changes seen in organ systems that are at risk of damage by states of shock, such as the splanchnic bed.19,20 Previous studies by Cheung, et al.21,22 have demonstrated the ability to use surrogate sites, such as conjunctival vessels, as a marker of active VOC. The study however, did not look at global measurements of oxygen transport and compare them to regional measurements of oxygenation. The study instead focused on the use of intravital microscopy to objectively quantify conjunctival vessels in SCD patients at baseline, during crisis, and post crisis.

Diverting blood flow from cutaneous tissue beds to more essential organ systems, to maintain oxygen delivery, is a known event in hemorrhagic, cardiogenic and at several stages of septic shock.23-25 With further investigative studies, the paradigm of SCD physiology may be shown to more closely resemble shock syndromes. The introduction of regional measurement techniques has highlighted the inadequacy of the information being garnered by global measurements of hemodynamic oxygenation such as DO₂, VO₂, and arterial hemoglobin oxygen saturation as well as traditional physical examination findings such as blood pressure and heart rate. Therefore, consideration should be given to emphasizing the underlying microcirculation,26,27 as reflected in tissue oxygenation as both a diagnostic and therapeutic endpoint.

The pathophysiology of sickle cell is complex and involves many organ systems as a result of episodic microcirculatory insults which are believed to result in end-organ ischemic damage and pain.28-30 Currently approved clinical tools are limited in their ability to detect localized changes in oxygen transport. The use of non-invasive tools will allow for increased understanding of microcirculatory oxygen delivery and utilization of SCD along with the many factors that are likely to impact it at this level in a clinical environment.11,12 For example, one could envision using this type of monitoring to explore the impact of such interventions as vasodilators or blood substitutes on their ability to improve regional oxygen delivery and to correlate this with the outward manifestation of pain. The effects of treatment on these parameters were not measured; however, future studies should incorporate these in a temporal fashion.

CONCLUSION

SCD patients have decreased levels of CtSO₂ at baseline when compared to healthy controls suggesting an increased rate of oxygen extraction, which may be secondary to decreases in tissue oxygen delivery, as represented by the DO₂ values of both study populations. SCD may share microvascular similarities to compensated shock and can be measured. Novel non-invasive techniques should be evaluated and may allow for further understanding of SCD microvasculature.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

This research article was funded in part by grants from the Office of Naval Research N00014-03-1-0253 and N00014-02-1-0642 and a grant from the United States Army Medical Research and Materiel Command W81K00-04-0998.

AUTHOR’S CONTRIBUTIONS

Dr. Imoigele P. Aisiku had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Study Concept and Design: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy and Kevin R. Ward.

Acquisition of Data: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

Analysis and Interpretation of Data: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy and Kevin R. Ward.

Drafting of Manuscript: Imoigele P. Aisiku, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.

Critical Review of Manuscript for Important Intellectual Content: Imoigele P. Aisiku, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.

Statistical Analysis: Imoigele P. Aisiku, Wally R. Smith, Lynn T. Penberthy, and Kevin R. Ward.

Obtained Funding: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

Administrative, Technical, or Material Support: Imoigele P. Aisiku, Osama R. Kandalaft, Wally R. Smith, Lynn T. Penberthy, Raghu R. Seethala, Peter C. Hou, and Kevin R. Ward.
Study Supervision: Imoigele P. Aisiku, Wally R. Smith and Kevin R. Ward.

CONSENT

No consent for publication is required as all patients signed a consent form to be part of the study and no identifying data is presented in the manuscript.

REFERENCES

1. Chies JA, Nardi NB. Sickle cell disease: a chronic inflammatory condition. Med Hypotheses. 2001; 57: 46-50. doi: 10.1054/mehy.2000.1310

2. Croizat H, Nagel RL. Circulating cytokines response and the level of erythropoiesis in sickle cell anemia. Am J Hematol. 1999; 60: 105-115.

3. Makis AC, Hatzimichael EC, Bourantas KL. The role of cytokines in sickle cell disease. Ann Hematol. 2000; 79: 407-413. doi: 10.1007/s002770000173

4. Moore CM, Ehlayel M, Leiva LE, Sorensen RU. New concepts in the immunology of sickle cell disease. Ann Allergy Asthma Immunol. 1996; 76: 385-400. doi: 10.1016/S1081-1206(10)63453-9

5. Graido-Gonzalez E, Doherty JC, Bergreen EW, Organ G, Telfer M, McMillen MA. Plasma endothelin-1, cytokine, and prostaglandin E2 levels in sickle cell disease and acute vaso-occlusive sickle crisis. Blood. 1998; 92: 2551-2555.

6. Kaul DK, Liu XD, Fabry ME, Nagel RL. Impaired nitric oxide-mediated vasodilation in transgenic sickle mouse. Am J Physiol Heart Circ Physiol. 2000; 278: H1799-H1806.

7. Lopez BL, Barnett J, Ballas SK, Christopher TA, Davis-Moon L, Ma X. Nitric oxide metabolite levels in acute vaso-occlusive sickle-cell crisis. Acad Emerg Med. 1996; 3: 1098-1103. doi: 10.1111/j.1553-2712.1996.tb03367.x

8. Nahavandi M, Wyche MQ, Perlin E, Tavakkoli F, Castro O. Nitric Oxide Metabolites in Sickle Cell Anemia Patients after Oral Administration of Hydroxyurea; Hemoglobinopathy. Hematol. 2000; 5: 335-339.

9. Dubois MJ, De Backer D, Creteur J, Anane S, Vincent JL. Effect of vasopressin on sublingual microcirculation in a patient with distributive shock. Intensive Care Med. 2003; 29: 1020-1023.

10. Rosse WF, Narla M, Petz LD, Steinberg MH. New Views of Sickle Cell Disease Pathophysiology and Treatment. Hematology (Am Soc Hematol Educ Program). 2000; 2-17.

11. Wolff KD, Kolberg A, Mansmann U. Cutaneous hemoglobin oxygenation of different free flap donor sites. Plast Reconstr Surg. 1998; 102: 1537-1543.

12. Wolff KD, Marks C, Uekermann B, Specht M, Frank KH. Monitoring of flaps by measurement of intracapillary hemoglobin oxygenation with EMPHO II: experimental and clinical study. Br J Oral Maxillofac Surg. 1996; 34: 524-529. doi: 10.1016/S0266-4356(96)90250-8

13. Guyton A. The systemic circulation. Philadelphia: WB. Saunders, 1981.

14. Chittock DR RJ, Russell JA. Monitoring of oxygen transport and oxygen consumption. New York: McGraw-Hill, 1998.

15. Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med. 1999; 27: 1369-1377.

16. Labie D, Elion J. Molecular and cellular pathophysiology of sickle cell anemia. Pathol Biol (Paris). 1999; 47: 7-12.

17. Mentzer WC, Jr, Wang WC. Sickle-cell disease: pathophysiology and diagnosis. Pediatr Ann. 1980; 9: 287-296.

18. Noguchi CT, Schechter AN, Rodgers GP. Sickle cell disease pathophysiology. Baillieres Clin Haematol. 1993; 6: 57-91.

19. McKinley BA, Marvin RG, Cocanour CS, Moore FA. Tissue hemoglobin O2 saturation during resuscitation of traumatic shock monitored using near infrared spectrometry. J Trauma. 2000; 48: 637-642.

20. Varela JE, Cohn SM, Diaz I, Giannotti GD, Proctor KG. Splanchnic perfusion during delayed, hypotensive, or aggressive fluid resuscitation from uncontrolled hemorrhage. Shock. 2003; 20: 476-480.

21. Cheung AT, Chen PC, Larkin EC, et al. Microvascular abnormalities in sickle cell disease: a computer-assisted intravital microscopy study. Blood. 2002; 99: 3999-4005.

22. Cheung AT, Harmatz P, Wen T, et al. Correlation of abnormal intracranial vessel velocity, measured by transcranial Doppler ultrasonography, with abnormal conjunctival vessel velocity, measured by computer-assisted intravital microscopy, in sickle cell disease. Blood. 2001; 97: 3401-3404.

23. Dammers R, Wehrens XH, oude Egbrink MG, Slaff DW, Kurvers HA, Ramsay G. Microcirculatory effects of experimental acute limb ischaemia-reperfusion. Br J Surg. 2001; 88: 816-824. doi: 10.1046/j.0007-1323.2001.01794.x

24. De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL.
Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. *Am Heart J.* 2004; 147: 91-99. doi: 10.1016/j.ahj.2003.07.006

25. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary $O_2$ extraction in sepsis. *Am J Physiol Heart Circ Physiol.* 2002; 282: H156-H164.

26. Krejci V, Hiltebrand L, Banic A, Erni D, Wheatley AM, Sigurdsson GH. Continuous measurements of microcirculatory blood flow in gastrointestinal organs during acute haemorrhage. *Br J Anaesth.* 2000; 84: 468-475.

27. Zhao KS, Junker D, Delano FA, Zweifach BW. Microvascular adjustments during irreversible hemorrhagic shock in rat skeletal muscle. *Microvasc Res.* 1985; 30: 143-153. doi: 10.1016/0026-2862(85)90046-9

28. Fabry ME, Nagel RL. The effect of deoxygenation on red cell density: significance for the pathophysiology of sickle cell anemia. *Blood.* 1982; 60: 1370-1377.

29. Garrison RN, Spain DA, Wilson MA, Keelen PA, Harris PD. Microvascular changes explain the two-hit theory of multiple organ failure. *Ann Surg.* 1998; 227: 851-860.

30. Kaul DK, Hebbel RP. Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. *J Clin Invest.* 2000; 106: 411-420.

31. Quinn CT, Buchanan GR. Predictors of outcome in sickle cell disease. *J Pediatr Hematol Oncol.* 2002; 24: 244-245.

32. Zuzak KJ, Gladwin MT, Cannon RO, 3rd, Levin IW. Imaging hemoglobin oxygen saturation in sickle cell disease patients using noninvasive visible reflectance hyperspectral techniques: effects of nitric oxide. *Am J Physiol Heart Circ Physiol.* 2003; 285: H1183-H1189. doi: 10.1152/ajpheart.00243.2003