## Sickle cell patients are characterized by a reduced glycocalyx volume

**The glycocalyx is an important anti-inflammatory and anti-adhesive barrier at the luminal side of endothelial cells. Glycocalyx volume was significantly reduced in sickle cell patients (HbSS/HbSβ⁰–thalassemia median 0.47L, IQR 0.27-0.66, HbSC/HbSβ⁺-thalassemia 0.23L, 0.0-0.58) compared with controls (1×10⁹L, 0.52-1.77) ($p$=0.03). Reduced glycocalyx may be a new factor in the pathophysiology of sickle cell disease.**

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Endothelial activation and dysfunction play a central role in the pathophysiology of sickle cell disease (SCD) related vaso-occlusion.[1] The extent of endothelial activation was recently shown to be related to the presence of severe disease related manifestations such as pulmonary hypertension.[2]

New insights into endothelial biology have demonstrated that, in the quiescent state, the endothelium is shielded from circulating blood cells and proteins by the glycocalyx, a highly hydrated cell free mesh of membrane-associated proteoglycans, glycosaminoglycans, glycoproteins and glycolipids located at the endothelial surface.[3] With a thickness ranging from 0.5-3.0 μm, it exceeds the intra-luminal size of most endothelial adhesion molecules, thereby potentially preventing interactions of the endothelium with blood constituents.[4] Glycocalyx volume is regulated by numerous factors and rapid degradation has been observed during ischemia, hypoxia, and exposure to both tumor necrosis factor alpha and oxidized low density lipoproteins.[5-8] Therefore, in conditions of ischemia and inflammation, which occur continuously in SCD, a reduced glycocalyx volume may facilitate interactions of activated endothelial cells with blood cells and proteins. Given the important role of endothelial activation in the pathophysiology of SCD related vaso-occlusion, we set out to investigate whether the glycocalyx volume is reduced in patients with SCD.

Consecutive adult sickle cell patients (HbSS, HbSβ$^{0/+}$-thalassemia or HbSC, confirmed with high performance liquid chromatography) visiting the out-patient clinic of the Academic Medical Center in Amsterdam were eligible for inclusion. Exclusion criteria were a history of an acute vaso-occlusive episode (painful crisis, acute chest syndrome, stroke, splenic- or liver sequestration) or blood transfusion 4 weeks prior to sample collection; diabetes mellitus; hemorrhagic retinopathy; hemorrhagic disorders or hypertension (systolic and diastolic blood pressure >140 mm Hg or >90 mm Hg respectively). For data analysis patients with the most severe genotypes (HbSS and HbSβ⁰-thalassemia) were grouped together, as were patients with the relatively milder genotypes (HbSC and HbSβ⁺-thalassemia). Age, sex and race matched subjects, heterozygous for hemoglobin C or S (HbAS and HbAC, confirmed by high performance liquid chromatography), served as healthy controls. All patients and controls gave written informed consent and this study was approved by the Medical Ethics Committee of the Academic Medical Center in Amsterdam and carried out in accordance with the principles of the Declaration of Helsinki.

**Table 1.** Baseline characteristics.

| *Characteristics* | Genotype *HbSS/ HbSβ⁰-thal* | *HbSC/ HbSβ⁺-thal* | *Controls* |
|---|---|---|---|
| N | 12 (9/3) | 8 (6/2) | 10 |
| Age (y) | 33 (22-43) | 31 (26-39) | 40 (27-42) |
| Male/Female | 1/11 | 3/5 | 3/7 |
| Systole (mmHg) | 110 (104-121) | 114 (101-118) | 121 (112-139) |
| Diastole (mmHg) | 68 (65-78) | 70 (56-76) | 82 (77-92) |
| Body mass index | 21 (20-24) | 23 (22-26) | 27 (23-31) |
| Blood parameters | | | |
| Hemoglobin (mmol/L) | 5.5 (5.1-6.0) | 7.1 (6.3-7.8) | 8.5 (7.3-8.9) |
| Reticulocytes (%) | 8.8 (6.3-11) | 2.8 (2.1-2.9) | 1.1 (0.9-1.7) |
| Leukocyte count (x10⁹/L) | 8.0 (6.5-10.1) | 7.0 (5.7-8.2) | 7.2 (3.3-9) |
| Lactate dehydrogenase (U/L) | 385 (289-570) | 220 (181-235) | 171 (164-181) |
| Fetal hemoglobin (%) | 9.4 (4.2-14.7) | 2.4 (0.5-3.6) | 0.5 (0.5-0.5) |

*All numbers are medians (interquartile range).*

The glycocalyx volume was estimated by comparing circulating blood volume with the intravascular distribution volume of a glycocalyx-permeable tracer such as neutral dextran 40 (molecular weight 40 kDa).[9] To determine the intravascular distribution volume of dextran 40, the concentration of dextran 40 at the time of injection was estimated by exponential fitting of the measured dextran 40 concentrations. The intravascular distribution volume of labeled, autologous erythrocytes was used to quantify circulating blood volume.[10] Labeled erythrocytes were measured using a flowcytometry (FACSCalibur; Becton Dickinson, Mountain View, CA, USA), during which at least 100,000 cells were counted. Circulating plasma volume was calculated from circulating erythrocyte volume (Vrbc) and systemic hematocrit (Hsys) by the following formula:

*circulating plasma volume = ([1 – Hsys] x Vrbc)/Hsys.*[10]

Data were expressed as medians with corresponding interquartile ranges (IQR). Between group differences were tested with the Kruskal Wallis Test. For correlation studies the Spearman Rank correlation coefficient was determined. $p$-values ″0.05 were considered statistically significant. Statistical analysis was performed using the SPSS 12.0.2 (SPSS Inc, Chicago, IL, USA). Twenty sickle cell patients (9 HbSS, 3 HbSβ⁰-thalassemia, 6 HbSC and 2 HbSβ⁺-thalassemia) and 10 healthy controls (9 HbAS, 1 HbAC) were included in the study. Patient and control characteristics and laboratory data are presented in Table 1. Four patients in the severe genotype group used hydroxyurea.

The glycocalyx volume was statistically significantly lower in patients compared with controls (Figure 1). There were no statistically significant differences in blood volume, plasma volume and dextran distribution volume between the groups. Glycocalyx volume did not differ between patients that used hydroxyurea and those who did not.

New insights into endothelial biology have demonstrated an important role of the glycocalyx in vascular homeostasis.[3] Loss of glycocalyx by acute hyperglycemia in healthy controls results in endothelial dysfunction and is associated with microvascular damage in type 1 diabetes.[9,11] In the present study, we demonstrated that the glycocalyx volume is significantly reduced in patients with SCD and is comparable to that reported in patients with diabetes mellitus type 1.[9,11] Contrary to expectations, the median glyco-

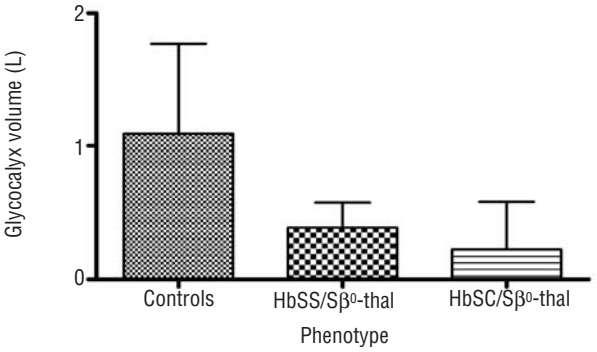


**Figure 1.** Glycocalyx volume in sickle cell patients and healthy controls. The glycocalyx volume differed significantly between the HbSS/HbS,0-thal group [median 0.47 liters (L), IQR 0.27-0.66], the HbSC/HbSβ$^0$-thal group (0.23L, IQR 0.0-0.58) and healthy controls (1.09L, IQR 0.52-1.77) (Kruskal Wallis $p$=0.025). Bars represent medians and error bars the 75$^{th}$ percentile.

calyx volume was slightly higher (non-significant) in HbSS/HbSβ$^0$-thal patients compared with HbSC/HbSβ$^+$-thal patients. This may be caused by the relatively low number of HbSC/HbSβ$^+$-thal patients and/or by the inclusion of relatively severely affected HbSC/HbSβ$^+$-thal patients.

Studies in humans and animals have shown that the glycocalyx volume is reduced after episodes of experimental ischemia, hypoxia and after exposure to oxidized low-density lipoproteins and oxygen radicals. Acute hyperglycemia, which is associated with the formation of reactive oxygen species, results in a severe loss of glycocalyx volume in healthy volunteers.[12] Continuous tissue ischemia and reperfusion is associated with the formation of reactive oxygen species in SCD which may result in a reduced glycocalyx volume. A reduction of the glycocalyx could have several important consequences in SCD. Adhesive interactions between sickle red blood cells, leukocytes and the activated endothelium play a key role in the initiation and propagation of SCD-related vaso-occlusion. A reduced glycocalyx volume may facilitate such interactions.

In conclusion, we demonstrate a reduced glycocalyx volume in sickle cell patients during the steady state suggesting a disrupted endothelial environment. We hypothesize that glycocalyx perturbation could be a new factor of bi-directional importance in the complex pathophysiology of SCD, since glycocalyx volume reductions can both result from and contribute to vaso-occlusion.

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