Lactate dehydrogenase to carboxyhemoglobin ratio as a biomarker of heme release to heme processing is associated with higher tricuspid regurgitant jet velocity and early death in sickle cell disease

To the Editor:
Chronic hemolysis is a hallmark of sickle cell disease (SCD). Prior estimates of intravascular hemolysis as a proportion of the total hemolytic rate ranged from less than 10% to more than 30%. Chronic intravascular hemolysis is associated with severe vasculopathic complications including pulmonary hypertension and early mortality in SCD. Intravascular hemolysis leads to release of cellular free hemoglobin and heme. Normally, these are scavenged by respectively haptoglobin and hemopexin. However, in SCD patients the availability of scavenging proteins in plasma is typically decreased because of rapid consumption by the prodigious amount of intravascular hemolysis. Both cellular free hemoglobin and heme, devoid of anti-oxidant buffering mechanisms normally present in red cells, are recognized as erythroid damage-associated molecular pattern molecules (e-DAMPs) and contribute to development of chronic vasculopathy, platelet activation and pulmonary hypertension in SCD. Intravascular heme release is associated with elevated levels of serum lactate dehydrogenase (LDH). All heme metabolized either via extravascular hemolysis or cleared from the plasma via scavenger proteins, will lead to endogenous carbon monoxide (CO) production by heme-oxygenase 1 (HO-1). In contrast to heme, CO has many protective effects including inhibition of polymerization of sickle hemoglobin, increased red blood cell hydration, anti-oxidative and anti-inflammatory effects. Theoretically, the balance of detrimental intravascular hemolysis to beneficial CO production (total hemolysis) could be a prognostic biomarker in SCD. Because the vast majority of endogenous CO is cleared by exhalation, end-alveolar CO (EACO) in morning first breath is an accepted proxy marker of overall hemolysis. Before exhalation, CO is transported primarily as the conjugate carboxyhemoglobin (HbCO).

Here we explore whether plasma HbCO can be a convenient surrogate marker of endogenous CO production. In addition, we hypothesize that the ratio of LDH to CO may give an estimate of the relative contribution of intravascular heme release to total heme processing and relates to clinical endpoints, and therefore could be a new readily available biomarker of interest in SCD.

We investigated the relationship between EACO to HbCO plasma levels (n = 37; cohort [A]). After establishing a good correlation, we evaluated the relation of the ratio of LDH/HbCO to echocardiography parameters and mortality rates in adults with SCD at the NIH, Bethesda, USA (n = 157; cohort [B]). [NCT01547793; NCT0001648].
A thorough description of study population, trial conduct and statistical analysis is provided in supplemental section 1.

An overview of the cohorts’ demographics, hematological and hemolytic parameters is provided in Table S1. We confirmed the expected correlation among surrogate biomarkers of intravascular heme release (LDH, AST).

We identified a clear positive correlation of HbCO with EACO (r = 0.66, 95% CI [0.36, 0.81]) (Figure S1). However, in both cohorts, there was no consistent correlation of EACO or HbCO with either LDH or AST. On the other hand, in cohort (A), both EACO and HbCO were significantly, positively correlated with absolute reticulocyte counts (Table S2). Additionally, we explored variability in CO production in individual patients (Figure S2). The observations suggest that every patient has a finite HbCO range with minor variation over time and not significantly associated with changes in LDH in this limited dataset. Thus, the serum level of LDH (known to reflect intravascular hemolysis and other tissue injury, but not extravascular hemolysis) appears to be heterogeneous among SCD patients and does not directly represent total hemolysis as represented by HbCO. This interpretation led us to formulate that the fraction of total hemolysis that is intravascular might more directly correlate with vascular health outcomes.

Next, we investigated whether an increased fraction of intravascular hemolysis, that is an increased ratio of LDH to HbCO, was related to clinical endpoints (n = 92 patients; 86 HbSS, three HbSt+, three HbSC; median age 30 years IQR 22; 40). Previously, tricuspid regurgitation velocity (TRV) ≥3.0 m/s was associated with the highest risk of development of pulmonary hypertension and increased mortality in SCD. Patients in this high-risk category (n = 13) had a significantly higher LDH/HbCO ratio compared to the other patients (p = 0.02) (Figure S3A). There was no significant difference in LDH...
values between groups. Individual median LDH/HbCO ratios and TRV were significantly correlated ($r = 0.38$, 95% CI [0.12, 0.60]). However, LDH and TRV were not correlated ($r = 0.22$, 95% CI [-0.10, 0.52]) (Figure S3B). Of interest, a cutoff value of LD/HbCO ratio of 1200 could be used to exclude patients with high risk TRV values: all patients ($n = 25$) with LDH/HbCO ratio $< 1200$ had a TRV $< 3.0$ m/s. The LDH/HbCO ratio was greater than 1200 in 15/16 patients (94%) with catheterization-proven pulmonary hypertension. (supplemental section 2).

Median follow-up was 12.1 years (IQR 10.3, 16.2). During follow-up, 25% (23/91) of the patients died (median age of death 42 years IQR 35, 58); 48% (11/23) were initially diagnosed with pulmonary hypertension based on pulmonary artery catheterization.

All-cause mortality predictability by LDH/HbCO ratio and LDH was analyzed in a Cox proportional hazard model (Table S3). The LDH/HbCO ratio was significantly associated with mortality in the unadjusted analysis ($p < 0.01$). Age, CRP and ferritin were previously reported to be related to early-mortality in SCD. In our cohort age ($p = 0.03$), CRP ($p = 0.05$) and ferritin ($p < 0.01$) were associated with mortality, as was TRV ($p < 0.01$). In the multivariate-adjusted analysis including age, CRP and ferritin, LDH/HbCO ratios remained significantly associated with all-cause mortality ($p = 0.02$). There was

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![Figure 1](image-url)

**FIGURE 1** Survival analysis. Kaplan-Meier estimates of overall survival for patients with LD/HbCO ratios $> 1200$ and patients with LD/HbCO ratios $< 1200$. Panel A Survival probability of all patients independent of tricuspid regurgitation velocity (TRV). In the group patients with LD/HbCO values $< 1200$ 5-year overall survival (OS) was 100%, 10-year OS 92.9% and 15-year OS 88.0%. In the group patients with LD/HbCO values $> 1200$ 5-year OS was 92.1%, 10-year OS 76.0% and 15-year OS 69.1%. Panel B. Survival probability in the group of patients with TRV values $< 3.0$ m/s. In the group patients with LD/HbCO values $< 1200$ 5-year and 10-year OS was 100%, 15-year OS was 94.4%. In the group patients with LD/HbCO values $> 1200$ 5-year OS was 97.9%, 10-year OS 87.1% and 15-year OS 84.1%
a clear trend toward association between LDH and all-cause mortality in the unadjusted analysis ($p = 0.10$), however, it did not reach statistical significance in our cohort. Kaplan–Meier survival probabilities for patients stratified by LDH/HbCO ratios greater than and less than 1200 are shown in Figure 1.

In summary, the LDH/HbCO ratio predicts survival probability independent of age, ferritin and CRP.

The products of intravascular red cell lysis have shown to be of major importance in the pathophysiology of vasculopathy in SCD. Infusion of high dose hemoglobin or heme in SCD mice induces lethal vaso-occlusive events. In contrast, low-dose heme and subsequent induction of HO-1 and production of the gasotransmitter CO is protective against vaso-occlusion in SCD mice. Therefore, it is biologically plausible that the LDH/HbCO ratio, representing an increase in the amount of CO, as product of heme processing, relative to the amount of intravascular heme release, provides useful information on characterization of hemolysis in SCD and its vasculotoxicity.

Excess cell free hemoglobin and heme, not cleared from the circulation via scavenger proteins, are excreted in the urine. Indeed, hemoglobinuria is detectable in many patients with SCD, and associated with biomarkers of intravascular hemolysis. This urinary excretion of heme somewhat confounds the interpretation of CO release as an indication of total hemolysis. However, the amount of excreted heme is limited compared to total red cell turnover. Therefore, we consider that HbCO is generally reflective of the total amount of CO production due to adaptive heme catabolism.

Serum LDH has been the most widely used marker of intravascular hemolysis associated with catheterization-proven pulmonary hypertension or elevated TRV in SCD cohorts. Elevated TRV in SCD was significantly associated with mortality in a recent meta-analysis, but an inconsistent association was observed between LDH and mortality. Notably, the current cohort on whom we have CO, LDH and mortality data is a subset with less statistical power than some of the prior datasets. As such, this present study may be underpowered to replicate prior LDH-mortality associations. Although all patients diagnosed with SCD were eligible for enrollment in these trials our cohort was enriched for SCD patients at risk for vasculopathic complications and/or early death. In this relatively small cohort, we show that the ratio of LDH to HbCO is superior to predict either elevated TRV or mortality as compared to LDH alone. These results suggest that the LDH/HbCO ratio may enhance the utility of LDH to identify patients at risk for vasculopathic complications and early death in SCD.

One limitation of our study is the presentation of HbCO values in relation to other hemolytic parameters in two cohorts that differ in regard to patient selection and size. The EACO cohort (A) was prospective and smaller; and, the HbCO cohort (B) was larger but retrospective and, therefore, may be biased toward a more acutely or chronically affected subpopulation for whom HbCO data were available. Altogether, HbCO results must be considered exploratory and in need of additional validation. The results here do not invalidate previously published correlations of serum LDH from larger studies with greater statistical power. Our new results highlight the potentially greater strength of the LDH/HbCO ratio.

In conclusion, a ratio of two readily available clinical laboratory markers, LDH and HbCO, representative of the intravascular fraction of total hemolytic rate, is promising as biomarker in SCD. Increased LDH/HbCO ratios are strongly associated with increased risk on pulmonary hypertension, the pathophysiologic complication of intravascular hemolysis, and all-cause mortality. And thereby may add to individual risk prediction in SCD patients. We suggest inclusion of these markers in future prospective trials in SCD for validation.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

A.J.V. analyzed and interpreted the data and wrote the manuscript; C.P.M. participated in the writing of the protocols, enrollment of subjects and analyzed data; L.M. participated in the writing of the protocols, enrollment of subjects and collection of the data; J.H.B. wrote clinical trial and laboratory protocols, participated in subject enrollment, collected and analyzed data (all EACO data); G.J.K. wrote clinical trial and laboratory protocols, supervised subject enrollment and laboratory data collection; E.J.B. interpreted the data, collected and analyzed data. All authors revised the paper critically and approved of the submitted version.

DATA AVAILABILITY STATEMENT

The aggregated data and analyses that support the findings of this study are available upon request from the corresponding author. Other data related to the patients are curated at the NIH, NHLBI, Sickle Cell Branch.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.