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GlycA is not a useful biomarker of inflammation in sickle cell disease

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Abstract

Introduction: Sickle cell disease (SCD) is a multisystemic disorder, the pathology being driven by recurrent inflammation particularly during a vaso-occlusive crisis. GlycA, a composite measure of protein glycation, is a sensitive biomarker for disorders associated with vascular inflammation. We determined the utility of GlycA as a biomarker of inflammation in SCD.

Methods: Stored plasma samples from patients with SCD recruited to two clinical studies were analyzed. One study encompasses 488 patient samples with SCD (HbSS, HbS β^0 and HbSC) at steady state and 52 race-matched, healthy controls. The other study included paired plasma samples during steady state and acute pain crisis from (HbSS) patients with SCD. Plasma GlycA was measured using a proton NMR on the Vantera[®] Clinical Analyzer. We performed analysis comparing patients with SCD, healthy controls, and paired samples analysis.

Results: The mean plasma GlycA level was lower in SCD compared with healthy controls ($324.6 \pm 70.4 \mu\text{mol/L}$ vs. $386.3 \pm 74.6 \mu\text{mol/L}$, $P < 0.0001$). Within the same patient, mean plasma GlycA during acute pain crisis was lower than steady state, although the difference was not significant ($300.5 \pm 36.3 \mu\text{mol/L}$ vs $314.2 \pm 34.8 \mu\text{mol/L}$, $P = 0.020$). Plasma GlycA correlated inversely with serum LDH ($P = 0.009$).

Conclusion: GlycA is not a suitable biomarker of inflammation in SCD. We surmise that its signal is confounded by hemolysis leading to a depletion of haptoglobin, one of the major plasma proteins included in the composite NMR signal. Hemolysis is further exacerbated during an acute pain crisis, hence the lower GlycA levels in crisis compared to steady state.

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Keywords

biomarker; GlycA; hemolysis; inflammation; sickle cell disease

1 INTRODUCTION

Sickle cell disease (SCD) is caused by the presence of an abnormal hemoglobin S (HbS) which results from a single base substitution in the beta-globin gene of hemoglobin. Polymerization of deoxygenated-HbS leads to “sickling” of red blood cells, resulting in chronic hemolytic anemia and recurrent episodes of acute vaso-occlusive pain. All patients have progressive end-organ damage and a reduced life expectancy. SCD is a multisystem disorder with multiple organ damage associated with recurring inflammation caused by tissue ischemia, reperfusion injury, and vascular damage.¹⁻⁵ Several markers of inflammation are elevated in SCD, including total white cell count, secretory phospholipase A2, interleukins, prostaglandin-E2, and Tumor Necrosis Factor- α .⁶⁻¹⁰ Acute phase reactants, proteins which rise or fall as a reaction to proinflammatory cytokines, include C-reactive protein (CRP) and ferritin. Platelets and haptoglobin are also elevated during inflammation.¹¹ However, the inherent variability of the plasma levels of these proteins challenges the ability to identify a biomarker with sufficient sensitivity and specificity for detecting acute crises. For instance, raised CRP levels in steady state are correlated with increased frequency of pain crisis and elevated levels are found during acute pain crises and acute chest syndrome.^{8,9,12} However, studies have found significant interindividual variability, a short half-life and a wide reference range for this marker.¹³ As inflammation is a key biological event accompanying acute vaso-occlusive crisis, a specific biomarker of inflammation would potentially be useful to determine disease activity in SCD.

GlycA shows promise as a biomarker of inflammation and disease severity in chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and systemic lupus erythematosus (SLE).¹⁴⁻¹⁷ GlycA is a composite signal derived from (¹H) nuclear magnetic resonance (NMR) spectra of the N-acetyl methyl group protons of the carbohydrate side chains of mainly four positive acute phase proteins: α 1-acid glycoprotein, haptoglobin, α 1-antitrypsin, and α 1-antichymotrypsin, and one negative acute phase protein, transferrin.^{13,18-21} Positive acute phase proteins increase, while negative acute phase proteins decrease, in response to inflammation. GlycA has relatively low interindividual variability compared with CRP and appears to have prognostic value as an indicator of treatment response and disease activity.¹⁹ We evaluated the plasma GlycA levels in a cross-sectional sample of patients with SCD and specifically tested its levels in patients experiencing an acute painful vaso-occlusive crisis (VOC).

2 MATERIALS AND METHODS

2.1 Study definitions

An “acute pain vaso-occlusive crisis” was defined as a visit to the NIH Clinical Center for acute pain without evidence of an alternative etiology, for which the patient requires hospitalization and treatment with parenteral narcotic medication. “Steady state” was

defined as the period when patients were in their usual state of health and excluded the 8-week time period prior to or after an acute pain crisis. Patients with SCD included HbSS, HbSBeta⁰ thalassemia, and HbSC genotypes from Groups 1 and 2. For analysis purposes, HbSS and HbSBeta⁰ thalassemia were considered as one phenotypic group of sickle cell anemia (SCA) due to similarity of clinical disease severity.

2.2 Study participants

Group 1 consisted of a cross-sectional sample of 488 patients with SCD (392 HbSS; 15 HbSBeta⁰ thalassemia; 81 HbSC) not experiencing an acute pain crisis and who reported themselves to be in their usual state of health “steady state.” These patients were prospectively enrolled from 2001 to 2015 (NCT00011648). Twenty-three patients (21 HbSBeta⁺ thalassemia; 1 HbSD; 1 HbSO Arab) and one sample with an undetectable GlycA level were excluded from analysis. Group 1 additionally included 52 healthy African Americans enrolled as healthy controls.

A second group (Group 2) included paired samples obtained from patients with SCD in steady state and again during an acute pain crisis (NCT03049475). For analysis, this consisted of paired samples from 12 HbSS patients. Steady state samples obtained from Groups 1 (n = 488) and 2 (n = 12) were analyzed as a SCD cross-sectional group (n = 500; Figure 1). Both studies were approved by the Institutional Review Board of the National Institutes of Health and written informed consent was obtained from all participants.

2.3 Sample processing

Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood samples were collected and centrifuged for 10 minutes at 1200 *g*. The resulting supernatant plasma was removed by pipetting, transferred to Sarstedt Micro tubes, snap-frozen in liquid nitrogen, and stored at -80°C until use.

Hematological and biochemical parameters were obtained from routine hospital laboratory measurements at steady state and during acute pain crisis.

2.4 GlycA measurements

Aliquots of frozen plasma (500 µL) were thawed at 4°C for 24 hours. Subsequently, 450 µL was transferred to a cell-strainer capped Falcon™ Round-Bottom Polypropylene (Thermo Fisher Scientific, Hampton, NH, USA) test tubes on the day of analysis. Using previously described methodology, plasma GlycA was quantified in these samples with a 400 MHz proton (¹H) NMR spectrometer (Vantera® Clinical Analyzer, LipoScience Inc., NC, USA).
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2.5 Statistical analysis

Variables with a Gaussian distribution (*t* test or one-way analysis of variance) were aggregated into means with standard deviations (SD). Intraindividual paired samples were compared using the paired *t* test. Correlations between GlycA and markers of inflammation and hemolysis were assessed using the Pearson test. The conventional threshold of <0.05

was used for statements about statistical significance. All statistical analysis was performed using GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla, CA, USA).

3 RESULTS

3.1 Baseline characteristics

Five hundred patients with SCD from Group 1 and Group 2 were included in our study as detailed in Figure 1. The mean age was 34 ± 12.7 years and 236 (47%) were female. Two hundred and two of the 500 (40%) of these patients were receiving hydroxyurea. During steady state, the mean hemoglobin and white cell count were 91 ± 1.9 g/L and $10.6 \pm 4.2 \times 10^9$ /L, respectively. During acute pain crisis, the mean hemoglobin and mean white cell count were 90 ± 1.1 g/L and $9.8 \pm 6.0 \times 10^9$ /L, respectively. Ongoing hemolysis was reflected by elevated steady state levels of serum lactate dehydrogenase (LDH) mean 6.4 ± 19.4 μ kat/L and an elevated absolute reticulocyte count, 255.7 ± 143.5 cells/mm³. Evidence for the presence of inflammation was supported by an elevated CRP (48.6 ± 9.7 μ g/L; Table 1). Patients sampled during an acute vaso-occlusive crisis demonstrated similar LDH values (6.4 ± 22.5 μ kat/L) and absolute reticulocyte count (226.3 ± 107.5 cells/mm³) but had slightly higher CRP values (69.5 ± 9.0 nm/L; Table 2).

3.2 Plasma GlycA levels in SCD

The mean plasma GlycA level was lower in SCD steady state patients from Groups 1 and 2 compared with healthy control patients (324.6 ± 70.4 μ mol/L vs 386.3 ± 74.6 μ mol/L; $P < 0.0001$). When analyzed by genotype, both the SCA patients from Groups 1 and 2 and the HbSC patients from Group 1 had lower plasma GlycA levels compared with healthy controls (321.4 ± 70.0 μ mol/L vs 386.3 ± 74.6 μ mol/L; $P < 0.0001$ and 341.4 ± 70.0 μ mol/L vs 386.3 ± 74.6 μ mol/L; $P = 0.001$, respectively; Figure 2). Patients with the clinically more severe SCA genotype were found to have decreased plasma GlycA levels compared with the less clinically severe HbSC genotype (321.4 ± 70.0 μ mol/L vs 341.4 ± 70.0 μ mol/L; $P = 0.0905$), but the results did not reach statistical significance.

The plasma GlycA level was further lowered during an acute pain crisis when compared with the steady state GlycA level for paired HbSS patients from Group 2 (300.5 ± 36.3 μ mol/L vs 314.2 ± 34.8 μ mol/L; $P = 0.020$). Similar findings were shown when the plasma GlycA level of HbSS patients during an acute pain crisis (Group 2) was compared with SCA patients (Group 1 and Group 2) at steady state (300.5 ± 36.3 μ mol/L vs 321.4 ± 70.0 μ mol/L; $P = 0.305$; Table 2, Figure 3).

3.3 Correlation of plasma GlycA levels with hemolysis markers

There was a negative correlation between plasma GlycA in SCD steady state and LDH ($P = 0.0089$). Correlations with other hemolysis markers did not reveal significant findings.

4 DISCUSSION

Several recent studies of diseases involving chronic vascular inflammation, namely psoriasis¹⁴ and SLE,¹⁷ have found that plasma GlycA levels are higher compared with

healthy controls. As SCD is a known proinflammatory condition, we hypothesized that the plasma GlycA levels would also be similarly be elevated among SCD patients compared with healthy controls.^{1,11,13,15,25} We also hypothesized that the plasma GlycA level would be further elevated during VOCs when compared with its level during the steady state, reflecting the increased inflammation accompanying acute pain in SCD. On the contrary, we found (a) reduced levels of plasma GlycA in a cross section of patients with HbSS/HbS β^0 thalassemia and HbSC disease compared with healthy control patients; (b) lower plasma GlycA levels in the HbSS/HbS β^0 thalassemia genotypes compared with HbSC; (c) further reduction in plasma GlycA levels in patients with SCD experiencing an acute painful crisis compared to GlycA levels in the same patient and in the SCD pooled cohort of patients in steady state; and (d) a significant negative correlation of GlycA with LDH, a plasma marker of hemolysis.

Ongoing inflammation as a result of recurrent painful vaso-occlusive crisis with reperfusion injury is thought to mediate in part the vascular pathobiology of SCD.^{1,26–28} While elevated CRP levels provided biochemical evidence for inflammation in our patients with SCD, the GlycA values obtained were paradoxically lower than healthy controls and also lower than reported in other inflammatory diseases.^{8,9,11,13} For example, studies have shown the plasma GlycA level in rheumatoid arthritis and SLE was 353.8 ± 65.2 $\mu\text{mol/L}$ and 408.0 ± 75.4 $\mu\text{mol/L}$, respectively, compared with 324.6 ± 70.4 $\mu\text{mol/L}$ in our patients with SCD.^{14,16} We attributed these seemingly contradictory results to hemolysis observed in the patients with SCD, which is not observed in the other two diseases.²⁹ It would be interesting to compare the GlycA levels in other diseases involving hemolytic anemia where haptoglobin is depleted and there is a less pronounced inflammatory response, such as pyruvate kinase deficiency or hereditary spherocytosis.

The GlycA signal is a compilation of five acute phase proteins, four of which are elevated during inflammation, one of which is haptoglobin.^{13,18} However, haptoglobin is a scavenger of free hemoglobin and becomes rapidly depleted during intravascular hemolysis. The negative correlation between the plasma GlycA level and LDH, an indirect marker of hemolysis, supports this hypothesis.^{12,30–33} Our data, therefore, suggest that hemolysis in SCD nullifies GlycA as a valid biomarker of inflammation in SCD. Measuring GlycA in patients with SCD in their steady state also appears to be futile as ongoing chronic hemolysis renders haptoglobin immeasurable. The strengths of this study are the quantification of a novel plasma biomarker of inflammation in a large cohort of patients with SCD and the inclusion of paired samples obtained during steady state and an acute painful crisis. The Vantera[®] Clinical Analyzer is an automated NMR spectrometer with an inbuilt deconvolution algorithm designed to ease clinical utility. This design unfortunately limited our ability to interrogate the individual components of the GlycA signal to further investigate the effect of hemolysis on the GlycA measurement.²²

5 CONCLUSION

GlycA a composite signal derived from five acute phase reactants is not a useful biomarker of inflammation in SCD. Plasma GlycA measurements in patients with SCD appear to be

confounded by hemolysis. Further studies are warranted to identify a more specific biomarker of inflammation for this complex disorder.

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REFERENCES

1. Platt OS. Sickle cell anemia as an inflammatory disease. *J Clin Invest* 2000;106(3):337–338. [PubMed: 10930436]
2. Singhal A, Doherty JF, Raynes JG, et al. Is there an acute-phase response in steady-state sickle cell disease? *Lancet* 1993;341(8846):651–653. [PubMed: 7680738]
3. Pathare A, Al Kindi S, Alnaqdy AA, Daar S, Knox-Macaulay H, Dennison D. Cytokine profile of sickle cell disease in Oman. *Am J Hematol* 2004;77(4):323–328. [PubMed: 15551290]
4. Elmariah H, Garrett ME, De Castro LM, et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. *Am J Hematol* 2014;89(5):530–535. [PubMed: 24478166]
5. Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Subclinical ischaemic episodes during the steady state of sickle cell anaemia. *J Clin Pathol* 1992;45(10):902–906. [PubMed: 1385483]
6. Belcher JD, Bryant CJ, Nguyen J, et al. Transgenic sickle mice have vascular inflammation. *Blood* 2003;101(10):3953–3959. [PubMed: 12543857]
7. Bourantas KL, Dalekos GN, Makis A, Chaidos A, Tsiara S, Mavridis A. Acute phase proteins and interleukins in steady state sickle cell disease. *Eur J Haematol* 1998;61(1):49–54. [PubMed: 9688292]
8. Bargoma EM, Mitsuyoshi JK, Larkin SK, Styles LA, Kuypers FA, Test ST. Serum C-reactive protein parallels secretory phospholipase A2 in sickle cell disease patients with vaso-occlusive crisis or acute chest syndrome. *Blood* 2005;105(8):3384–3385. [PubMed: 15802550]
9. Krishnan S, Setty Y, Betal SG, et al. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vasocclusive crises. *Br J Haematol* 2010;148(5):797–804. [PubMed: 19995398]
10. Styles LA, Aarsman AJ, Vichinsky EP, Kuypers FA. Secretory phospholipase A(2) predicts impending acute chest syndrome in sickle cell disease. *Blood* 2000;96(9):3276–3278. [PubMed: 11050014]
11. Jain S, Gautam V, Naseem S. Acute-phase proteins: as diagnostic tool. *J Pharm Bioallied Sci* 2011;3(1):118–127. [PubMed: 21430962]
12. Rees DC, Gibson JS. Biomarkers in sickle cell disease. *Br J Haematol* 2012;156(4):433–445. [PubMed: 22122125]
13. Otvos JD, Shalaurova I, Wolak-Dinsmore J, et al. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem* 2015;61(5):714–723. [PubMed: 25779987]
14. Joshi AA, Lerman JB, Aberra TM, et al. GlycA is a novel biomarker of inflammation and subclinical cardiovascular disease in psoriasis. *Circ Res* 2016;119(11):1242–1253. [PubMed: 27654120]
15. Ormseth MJ, Chung CP, Oeser AM, et al. Utility of a novel inflammatory marker, GlycA, for assessment of rheumatoid arthritis disease activity and coronary atherosclerosis. *Arthritis Res Ther* 2015;17:117. [PubMed: 25956924]
16. Bartlett DB, Connelly MA, AbouAssi H, et al. A novel inflammatory biomarker, GlycA, associates with disease activity in rheumatoid arthritis and cardio-metabolic risk in BMI-matched controls. *Arthritis Res Ther* 2016;18:86. [PubMed: 27067270]
17. Chung CP, Ormseth MJ, Connelly MA, et al. GlycA, a novel marker of inflammation, is elevated in systemic lupus erythematosus. *Lupus* 2016;25(3):296–300. [PubMed: 26637290]

18. Bell JD, Brown JC, Nicholson JK, Sadler PJ. Assignment of resonances for 'acute-phase' glycoproteins in high resolution proton NMR spectra of human blood plasma. *FEBS Lett* 1987;215(2):311–315. [PubMed: 2438159]
19. Connelly MA, Otvos JD, Shalurova I, Playford MP, Mehta NN. GlycA, a novel biomarker of systemic inflammation and cardiovascular disease risk. *J Transl Med* 2017;15(1):219. [PubMed: 29078787]
20. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A novel protein glycan biomarker and future cardiovascular disease events. *J Am Heart Assoc* 2014;3(5):e001221. [PubMed: 25249300]
21. Ritchie SC, Wurtz P, Nath AP, et al. The biomarker GlycA is associated with chronic inflammation and predicts long-term risk of severe infection. *Cell Syst* 2015;1(4):293–301. [PubMed: 27136058]
22. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. NMR measurement of LDL particle number using the Vantera Clinical Analyzer. *Clin Biochem* 2014;47(16–17):203–210. [PubMed: 25079243]
23. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. HDL particle number measured on the Vantera(R), the first clinical NMR analyzer. *Clin Biochem* 2015;48(3):148–155. [PubMed: 25438074]
24. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 2006;26(4):847–870. [PubMed: 17110242]
25. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340(6):448–454. [PubMed: 9971870]
26. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 2010;376(9757):2018–2031. [PubMed: 21131035]
27. Novelli EM, Gladwin MT. Crises in sickle cell disease. *Chest* 2016;149(4):1082–1093. [PubMed: 26836899]
28. Ballas SK, Smith ED. Red blood cell changes during the evolution of the sickle cell painful crisis. *Blood* 1992;79(8):2154–2163. [PubMed: 1562742]
29. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. *J Clin Invest* 2017;127(3):750–760. [PubMed: 28248201]
30. Damanhoury GA, Jarullah J, Marouf S, Hindawi SI, Mushtaq G, Kamal MA. Clinical biomarkers in sickle cell disease. *Saudi J Biol Sci* 2015;22(1):24–31. [PubMed: 25561879]
31. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood* 2006;107(6):2279–2285. [PubMed: 16291595]
32. Shih AW, McFarlane A, Verhovsek M. Haptoglobin testing in hemolysis: measurement and interpretation. *Am J Hematol* 2014;89(4):443–447. [PubMed: 24809098]
33. Nolan VG, Wyszynski DF, Farrer LA, Steinberg MH. Hemolysis-associated priapism in sickle cell disease. *Blood* 2005;106(9):3264–3267. [PubMed: 15985542]

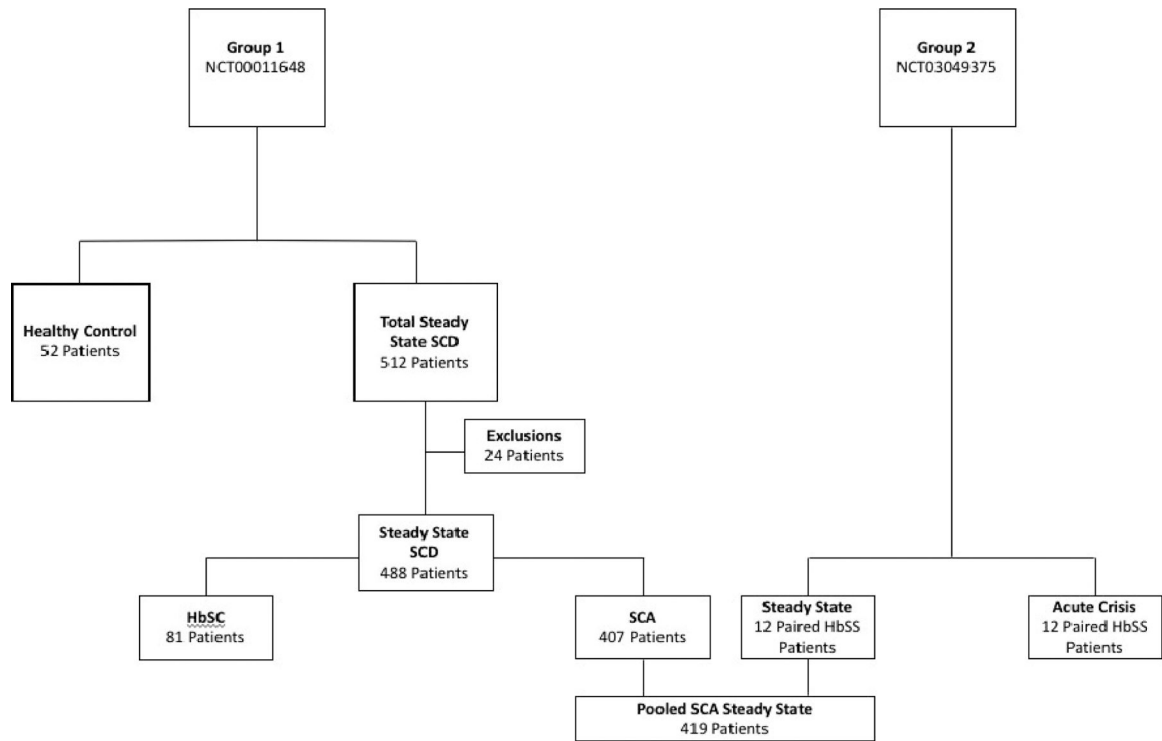


FIGURE 1. Study Population including SCD patients from Group 1 and Group 2 and healthy controls. SCD was defined as HbSS + HbS β^0 thalassemia + HbSC genotypes. SCA was defined as HbSS + HbS β^0 thalassemia

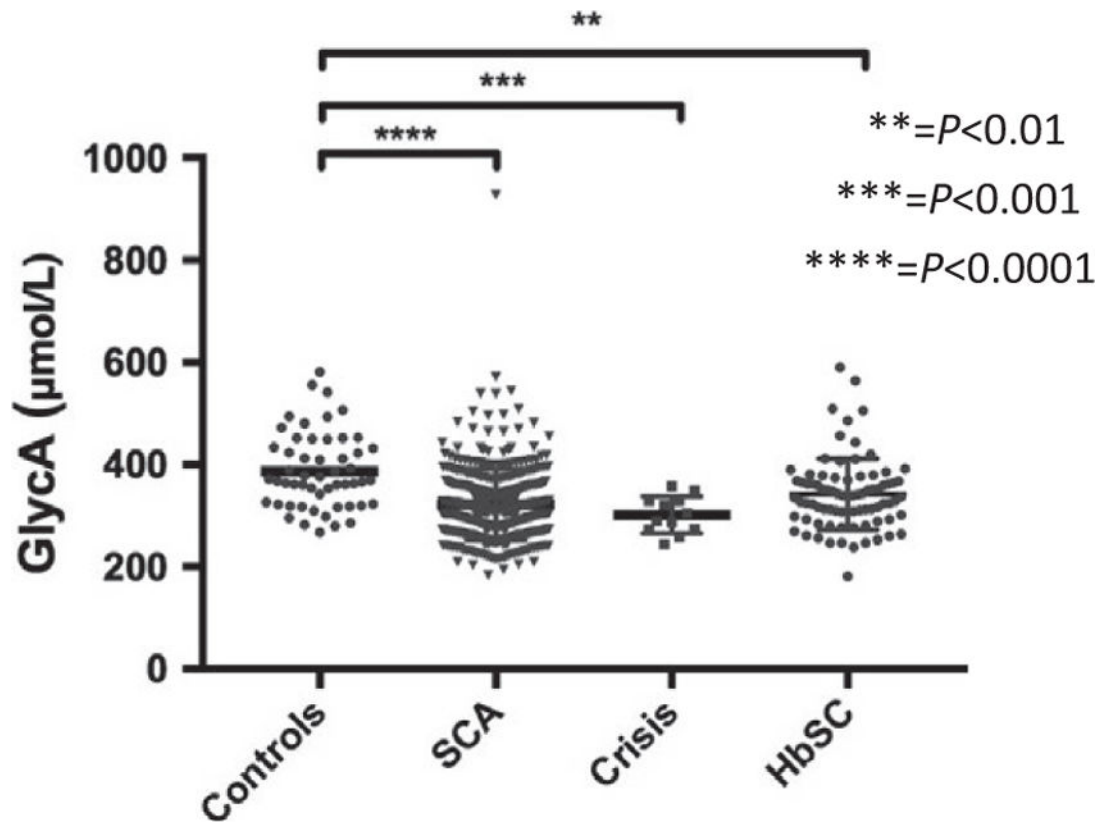


FIGURE 2.

GlycA levels in plasma (µmol/L): healthy control participants (circles); SCA steady state (SCA = HbSS + HbSβ⁰ thalassemia from Group 1 and Group 2, inverted triangles); SCA acute pain crisis (Group 2, squares); HbSC steady state (Group 1, circles). The plasma GlycA levels were lower in SCA and HbSC compared with healthy control and during an acute pain crisis compared with steady state

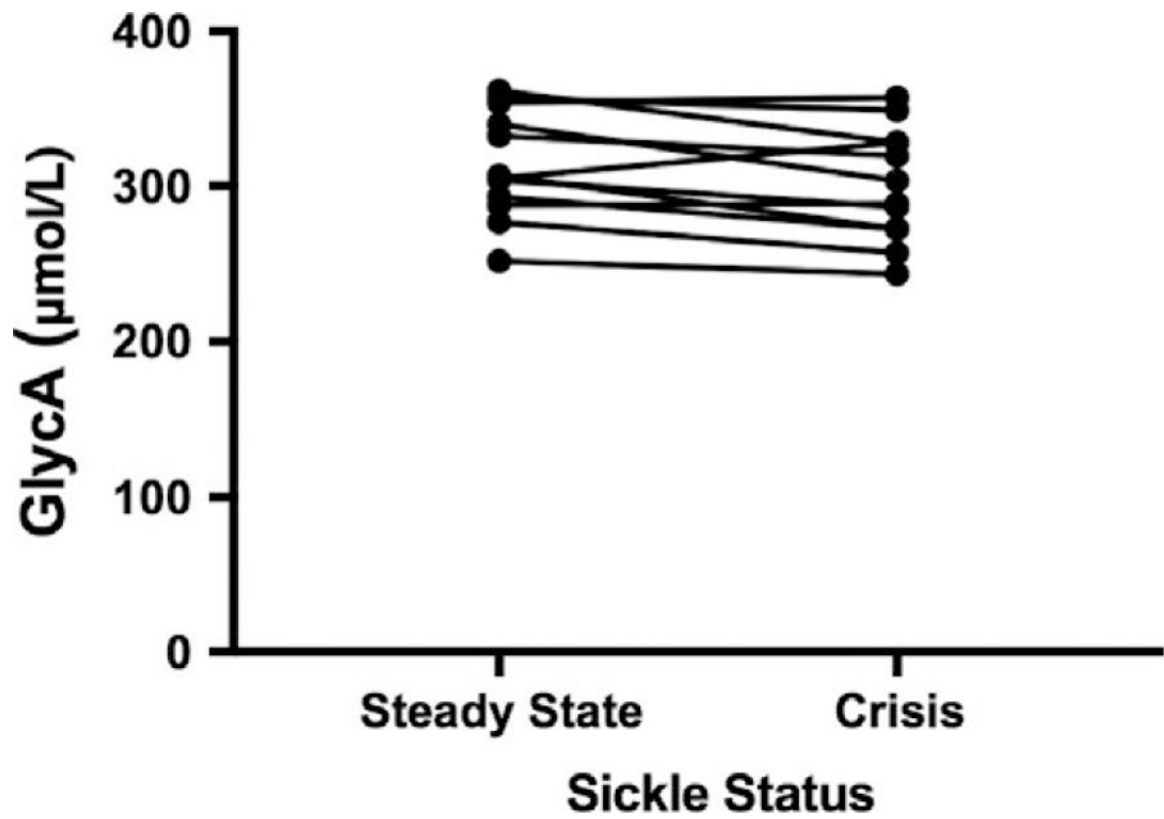


FIGURE 3.

GlycA levels in plasma ($\mu\text{mol/L}$) of the 12 paired HbSS patients who provided samples during steady state and acute pain crisis (Group 2). The steady state and crisis samples are each a single value. Mean plasma GlycA level in steady state was $314.2 \pm 34.8 \mu\text{mol/L}$ and during an acute pain crisis was $300.5 \pm 36.3 \mu\text{mol/L}$ ($P = 0.020$)

Descriptive data for Group 1 study cohort and healthy controls. Steady state values are given for hematological and biochemical parameters. Data were summarized by the mean \pm SD

TABLE 1

	SCD patients in steady state		Controls
	(HbSS/HbSp ⁰)	(HbSC)	(HbAA)
N	392	81	52
Male:female	202:205	36:45	22:30
Age, years (mean, SD)	33.1 \pm 12.1	38.3 \pm 14.4	36.4 \pm 11.7
WBC ($\times 10^9/L$)	11.3 \pm 4.2	7.7 \pm 2.4	5.9 \pm 1.5
Hemoglobin (g/L)	86 \pm 1.6	115 \pm 1.5	13.1 \pm 1.6
Hematocrit (fraction)	0.25 \pm 4.6	0.33 \pm 4.4	0.29 \pm 4.1
Absolute retic (cells/uL)	280.4 \pm 142.7	127.8 \pm 49.7	59.2 \pm 27.6
CRP (mm/L)	53.3 \pm 9.9	31.4 \pm 9.0	22.9 \pm 3.1
ALT (U/L)	36.2 \pm 49.3	24.6 \pm 13.3	27.3 \pm 14.1
AST (U/L)	50.1 \pm 87.2	26.7 \pm 17.2	20.1 \pm 8.1
Bilirubin, total (μ mol/L)	54.7 \pm 2.3	27.4 \pm 0.9	10.3 \pm 0.3
LDH (μ kat/L)	6.9 \pm 19.9	4.0 \pm 7.4	2.9 \pm 4.5
Haptoglobin (mg/L)	104.0 \pm 15.8	230.0 \pm 40.8	1132.0 \pm 65.2

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, c-reactive protein; LDH, lactate dehydrogenase; SCD, HbSS + HbSp⁰ thalassemia + HbSC genotypes; WBC, white blood cell count.

Descriptive data for Group 2 study cohort of paired HbSS patient samples in steady state and during an acute pain crisis. Data were summarized by the mean \pm SD

TABLE 2

	Steady state (HbSS; n = 12)	Acute pain crisis (HbSS; n = 12)	P-value
Age-yrs. 40.1 ± 10.5 y			
Female n = 8 (66.7%)			
Male n = 4 (33.3%)			
WBC ($\times 10^9/L$)	7.4 ± 3.4	9.8 ± 6.0	0.211
Hemoglobin (g/L)	97 ± 1.0	90 ± 1.1	0.002
Hematocrit (fraction)	0.27 ± 2.4	0.25 ± 2.7	0.003
Absolute retic (cells/ μL)	252.8 ± 160	226.3 ± 107.5	0.912
CRP (mm/L)	30.5 ± 2.4	69.5 ± 9.0	0.211
ALT (U/L)	25.3 ± 8.67	31.3 ± 23.8	0.420
AST (U/L)	34.8 ± 14.4	52.3 ± 45.5	0.342
Bilirubin, total ($\mu mol/L$)	35.9 ± 1.3	39.3 ± 1.3	0.977
LDH ($\mu kat/L$)	2.9 ± 16.4	6.4 ± 22.6	0.034

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; WBC, white blood cell count.