Apolipoprotein A-I and serum amyloid A plasma levels are biomarkers of acute painful episodes in patients with sickle cell disease

Ashaunta Tumblin,[1] Anitaben Tailor,[1] Gerard T. Hoehn,[2] A. Kyle Mack,[3] Laurel Mendelsohn,[1] Lita Freeman,[1] Xiuli Xu,[1] Alan T. Remaley,[1] Peter J. Munson,[4] Anthony F. Suffredini,[2] and Gregory J. Kato[1,2]

[1]Pulmonary and [2]Vascular Medicine Branch, National Heart, Lung and Blood Institute; [3]Critical Care Medicine Department, Clinical Center; [3]Pediatric Oncology Branch, National Cancer Institute, and [4]Center for Information Technology; National Institutes of Health, Bethesda, MD, USA

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Correspondence: Gregory J. Kato, M.D., National Institutes of Health, 10 Center Drive, MSC 1476 Building 10-CRC, Room 5-5140, Bethesda, Maryland 20892-1476, USA. E-mail: gkato@mail.nih.gov

ABSTRACT

Background
Acute painful episodes are the clinical hallmark of sickle cell disease and have been linked to morbidity and mortality in the sickle cell population.

Design and Methods
We undertook exploratory proteomic studies on paired plasma samples collected from a cohort of 26 adult sickle cell patients during steady state and on the first day of an acute painful episode. We screened for changes in abundance of specific protein peaks via surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS), and confirmed the identify of candidate protein peaks by specific immunoassays.

Results
The levels of hemoglobin, hematocrit, total protein, and albumin were lower and the levels of lactate dehydrogenase and absolute reticulocytes higher during acute painful episodes than during the steady state. Surface-enhanced laser desorption/ionization time of flight mass spectrometry spectral analysis consistently showed a mass-to-charge peak at 11.7 kDa with elevated intensities during acute painful episodes, which correlated significantly with the serum amyloid A immunoassay. Serum amyloid A levels were significantly elevated during acute painful episodes, especially in four patients with marked end-organ complications of such episodes. A second, recurring peak, less abundant during acute painful episodes, was present at 28.1 kDa; this peak was correlated significantly with immunoassay measurements of apolipoprotein A1.

Conclusions
On the average, plasma serum amyloid A rises and apolipoprotein AI falls during acute painful episodes. The serum amyloid A/apolipoprotein AI ratio increased in 81% of the patients during acute painful episodes, potentially making it a useful objective marker of such episodes. We propose that these protein alterations, known to contribute to endothelial dysfunction in other settings, might do likewise acutely in acute painful episodes and present a new target for therapeutic intervention in sickle cell disease. (*ClincalTrials.gov Identifier: NCT00081523*).

Key words: acute painful episode, immunoassay, sickle cell disease.

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Introduction

Sickle cell disease (SCD) is an inherited disorder characterized in part by transient acute painful episodes, which occur with varying frequency and severity. A number of factors may lead to the development of an acute painful episode, including hypoxia and clinical dehydration, which promote hemoglobin S polymerization and vascular obstruction. The ensuing pain is believed to be nociceptive secondary to obstruction of the microcirculation and tissue ischemia.[1] Infection and inflammation may promote coagulation cascades, leukocyte activation, and endothelial activation, which may further contribute to vascular obstruction.[2,3] These painful crises are unpredictable, but may follow exposure to cold or, less commonly, emotional stress, exercise, or alcohol.[1,4] Hematologic risk factors include elevated hematocrit, known to be increased in the presence of concomitant alpha thalassemia, and low fetal hemoglobin levels.[5,6] The rise in fetal hemoglobin seen following the administration of hydroxyurea in SCD patients is believed to account for this drug's effect of reducing the frequency of acute painful episodes.[7] Despite the advances that have been made in the prevention of acute painful episodes through the increasing use of hydroxyurea and exchange transfusion, effective management of these episodes remains a challenge.[8,9]

Their heterogeneous expression makes acute painful episodes difficult to diagnose accurately and manage systematically. The principal manifestation of an acute painful episode is pain, which is a subjective phenomenon, and its traditional definition has relied upon the patient's report. The start of an acute painful episode is often defined as the time of onset of pain severe enough to warrant hospitalization and the administration of opioid analgesia.[10] However, there is not a reliable objective marker of an acute painful episode. Such a marker would be extremely useful in clinical research of acute painful episodes and would enable observation of treatment effects during acute painful episodes and their resolution.[11] Rheological studies showed minor fluctuations in poorly deformable dense cells during steady state SCD and larger increases during overt acute painful episodes.[10,12] Several acute phase reactants have been found to exhibit similar patterns of transient low-level increases during steady state disease and more significant elevations during acute painful episodes. Such fast responding reactants include C-reactive protein and serum amyloid A (SAA). The slower responding proteins include fibrinogen, orosomucoid, sialic acid, and concanavalin-A binding protein.[10,11,13] Other possible markers of acute painful episodes include increases in erythrocyte sedimentation rate,[14] dense cells,[15] and plasma viscosity.[12,16]

We performed a broader high-throughput screening of the SCD plasma proteome using surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF MS) to screen for candidate proteins that might serve as biomarkers of acute painful episodes in SCD patients.

Design and Methods

Patients

Study participants were enrolled with informed consent on a research protocol approved by the institutional review board of the National Heart, Lung and Blood Institute. For inclusion, these sickle cell patients presented to the Clinical Center with an acute painful episode requiring hospitalization for pain management. Patients were excluded if they were on a chronic transfusion regimen, pregnant, breastfeeding or had hemoglobin SC disease. Patients who were clinically diagnosed at the time of hospital admission with bacterial infection, pneumonia, acute chest syndrome, or splenic sequestration were also excluded.

Plasma samples were collected and frozen in almost all cases during the first 24 hours of the acute painful episode, and matched with samples from the same patient at least 10 days before or 4 weeks after the episode. All patients had hemoglobin SS disease except one who had hemoglobin S-β^+-thalassemia. The patients' treatment at the time of the acute episode and during steady state was as follows: hydroxyurea only, 77% *versus* 69%; transfusion only within 30 days, 8% *versus* 0%; both hydroxyurea and transfusion within 30 days, 0% *versus* 12%; neither hydroxyurea nor transfusion, 15% *versus* 19%.

Standard laboratory investigations

Each of the 26 SCD patients underwent standard clinical laboratory investigations, performed at the National Institutes of Health Clinical Center Department of Laboratory Medicine, at baseline (during steady state disease) and during the admission for the acute painful episode. These investigations included evaluations of leukocyte count, hemoglobin concentration, hematocrit, platelet count, reticulocyte count, serum creatinine, total and direct bilirubin, lactate dehydrogenase, creatine kinase, aspartate transaminase, alanine transaminase, and alkaline phosphatase concentrations.

Proteomic screening

We performed SELDI-TOF MS screening and statistical analysis, previously described in great detail.[17] Briefly, each plasma sample was fractionated by ion exchange chromatography, and selected fractions were bound to specific affinity matrices and submitted to SELDI-TOF MS. Significant alterations in peak abundance were identified using the CiphergenExpress software application. Peaks with the lowest P values ($P<0.0001$) were selected for additional analysis. Preliminary identification of peaks was based on our published studies of peaks with similar charge-to-mass ratios.[17] Because of the matched-pair study design, no adjustments for gender or other demographic variables was necessary.

Measurements of apolipoprotein A1 and serum amyloid A

Plasma apolipoprotein A1 (apoA-I) levels were measured by Beckman Coulter IMMAGE rate nephelometry (Ramsey, MN, USA), according to the manufacturer's recommendations. SAA levels were measured using a standard immunoassay kit (US Biological, Swampscott, MA, USA).

Statistical analysis

Paired statistics were used to calculate P values by Wilcoxon's signed-rank test. Spearman's correlation coefficients were calculated using Prism 4.01 (GraphPad Prism 4 software, San Diego, CA, USA). The level of statistical significance assumed for these standard statistical tests was a P value less than 0.05.

Results

Standard clinical laboratory variables

The paired analysis of the standard hematological and biochemical indices of the 26 patients in steady state and

during acute painful episode revealed several significant changes. Compared to during the steady state, SCD patients undergoing an acute painful episode had significantly lower levels of hemoglobin, hematocrit, red blood cells, total protein, and albumin as well as increased levels of lactate dehydrogenase, absolute reticulocytes, and leukocytes (Table 1), consistent with accelerated hemolysis and inflammatory changes during the acute painful episodes.

Surface-enhanced laser desorption/ionization time of flight mass spectrometry and immunoassay evaluations

Spectral analysis for the 26 patients was performed using the CiphergenExpress software program and intensity differences between the steady state and acute painful episodes were analysed. The peaks with the highest statistical significance were found in our previously published study.[17] Protein purification and high resolution mass spectroscopy identified similar peaks to be the vascular disease markers SAA and apoA-I at 11,757 and 28,133 kDa.[17] Repeated Random Forest and logistic regression modeling consistently showed higher abundance of an 11.7 kDa protein in samples from patients with an acute painful episode than in samples from the patients during steady state disease. To confirm the identity of this peak in the current study, we performed parallel immunoassays for SAA in the corresponding unfractionated plasma specimens. The SELDI-TOF MS peak intensities of the 11.7 kDa peak demonstrated a significant correlation with SAA immunoassay levels (r=0.85, $P<0.0001$), confirming the identity of the 11.7 kDa peak as SAA (Figure 1a), and consistent with our previous high-resolution mass spectroscopy identification of this peak.[17] This correlation remained equally strong even if the highest four or even ten outliers were excluded. More importantly, we found that the level of SAA was significantly higher during acute painful episodes than during the steady state (Figure 1b).

Table 1. Laboratory tests in sickle cell disease patients with steady state disease and acute painful episodes (APE).

Laboratory Test	Steady State	APE	*P* value
Leukocyte count ($\times10^9$/L)	8.8±0.6	13.1±1.3	<0.001
Erythrocyte count ($\times10^9$/L)	2.81±0.12	2.53±0.12	0.02
Hemoglobin (g/L)	96±2	88±3	0.003
Hematocrit (%)	28.9±0.7	25.2±0.9	0.006
Mean corpuscular volume (fL)	102±2	102±2	0.98
Platelets (10^9/L)	342±33	323±29	0.40
Absolute reticulocyte count ($\times10^9$/L)	201±23	252±15	0.01
Lactate dehydrogenase (U/L)	295±14	392±41	0.04
Alkaline phosphatase (U/L)	131±12	111±10	0.07
Total bilirubin (µmol/L)	43±3	80±21	0.08
Direct bilirubin (µmol/L)	9±2	12±2	0.31
Creatine kinase (U/L)	95±7	168±54	0.21
Aspartate aminotransferase (U/L)	42±4	65±17	0.19
Alanine aminotransferase (U/L)	34±6	41±8	0.50
Creatinine (µmol/L)	66±6	66±6	0.87

Values are mean ± standard error of the mean. Statistical significance is calculated by paired t-test.

We also noted that four subjects from the cohort of 26 patients showed extremely high levels of SAA during their acute painful episodes when compared to the rest of the group (median 6.5 *vs.* 413.5 µg/mL, $P=0.002$). Interestingly, these four patients with the highest SAA levels during their acute painful episodes had several laboratory findings and clinical characteristics distinguishing them from the rest of the patients. Considering steady state values, these four patients had lower mean corpuscular volume than the rest of the group (median 92.5 fL *versus* 105 fL, $P<0.003$), lower alanine tranasminase (median 18 IU *versus* 27 IU, $P<0.01$), and lower asparatate transaminase (median 24 IU *versus* 37 IU, $P<0.003$). During acute painful episodes, the same four patients had higher leukocyte counts compared to the remaining patients (median 18.3×10^9/L *versus* 11.0×10^9/L, $P<0.005$) and higher absolute reticulocyte counts (median 301×10^9/L *versus* 216×10^9/L, $P<0.03$). During their hospitalization for acute painful syndrome, two of these four patients developed multiple organ failure syndrome,[18] one developed acute chest syndrome, and the fourth developed acute cholecystitis, which might have been the triggering factor for the acute painful episode, but had not been clinically evident at the initial presentation of the episode. Thus particularly high SAA levels seemed to be an early indicator of clinically severe extra-osseous complications.

In addition to SAA, we also identified a peak at 28.1 kDa that was consistently less abundant in the samples

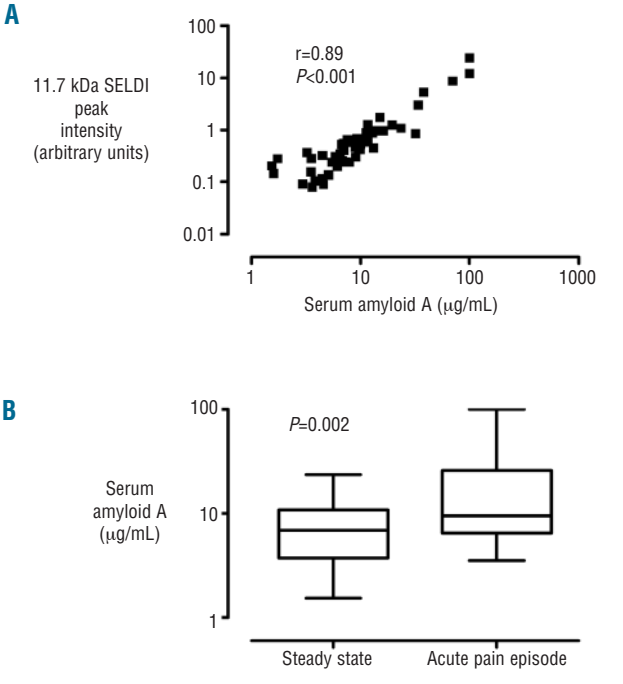

Figure 1. Serum amyloid A (SAA) levels in SCD in steady state *versus* acute painful episode. (A) Correlation of peak intensity of 11.7 kDa charge-to-mass ratio peak determined by SELDI TOF MS to SAA levels quantified by immunoassay in sickle cell patients (log-log scale). Data are representative of both steady state and acute painful episodes. The two values correlate significantly (r=0.89, $P=0.002$, Spearmans correlation). (B) Parallel immunoassay revealed significantly elevated SAA levels in patients during an acute painful episode compared to in the same patients at steady state ($P=0.002$, n = 26 paired observations)(box and whisker plot, logarithmic scale).

taken during an acute painful episode, for every fraction and affinity surface type evaluated. The 28.1 kDa peak intensities were significantly correlated with apoA-I levels measured by the immunoassay (r=0.49, $P<0.0002$) (Figure 2a); accordingly, immunoassay-measured apoA-I levels during acute painful episodes were significantly lower than those during steady state disease (Figure 2b). Assignment of the 28.1 kDa peak as apoA-I is consistent with our previous identification of this peak by high resolution mass spectroscopy.[17] Our data also demonstrated that the ratio of SAA/apoA-I rose significantly in acute painful episodes compared to steady state, increasing in 81% of the patients (Figure 3), suggesting that the two markers in combination may be a biomarker of acute painful episodes.

Discussion

An acute painful episode in SCD includes an inflammatory response, thought to be mediated by macrophages infiltrating ischemic tissues, and is associated with an increase in acute phase reactants.[15] In our study, we found that standard clinical laboratory markers of hemolytic anemia were more pronounced during acute painful episodes, consistent with previously published data from hyperhemolysing SCD patients undergoing acute painful episodes.[19] In addition to hemolysing erythrocytes, ischemic bone marrow and other organs likely release lactate dehydrogenase during acute painful episodes,[20] and injured muscle tissue releases creatine kinase.[21]

To our knowledge, our study is the first application of proteomic screening to the clinical problem of acute painful episodes. The SELDI-TOF platform is well-positioned to undertake high throughput analyses and identify candidate markers for further investigation by more validated assays, as in this study. Direct use of the peak patterns themselves as diagnostic markers would be much more questionable, due to the trade-off of precision *versus* high throughput, and we chose to use SELDI-TOF MS solely as a screening modality. The use of hospitalization as a variable to dichotomize pain severity might be questioned, since some investigators have documented that sickle cell pain is frequently an issue outside of hospitalizations,[22] and prodromal pain can also occur as an outpatient.[23] However, use of hospitalization for pain as an outcome measure was the basis of the most successful therapeutic trial in the history of SCD, the Multicenter Study of Hydroxyurea, which validated hospitalization for pain as a useful clinical indicator in patients with SCD.[24]

SAA is a classic positive acute phase reactant known to increase within 6 to 10 hours of tissue injury,[25,26] ultimately reaching levels 1000-fold baseline.[27] In this study, the elevation of an 11.7 kDa peak during acute painful episodes had the highest statistical significance in both univariate and multivariate analyses. In another study of markers of pulmonary hypertension in SCD, we found a very similar peak, which, upon purification and high resolution mass spectroscopy, was SAA, confirmed by immunoassay.[17] In the present study, we find that the similar 11.7 kDa peak once again correlated very significantly with the SAA immunoassays of unfractionated plasma from the same specimens.

Of interest, in our study we also noted that, by principal component analysis, four patients showed distinctive characteristics from the rest of the patients. After their initial presentation with acute painful episode, these four patients went on to develop clinically severe painful

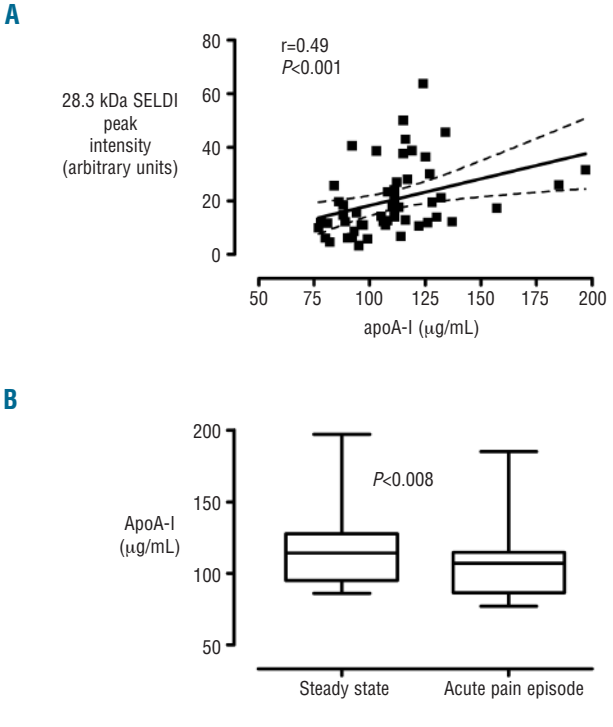

Figure 2. Apolipoprotein A-I (apoA-I) levels in sickle cell disease at steady state *versus* acute painful episode. (A) Correlation of intensity of a 28.3 kDa charge-to-mass ratio peak determined by SELDI-TOF MS with apoA-I levels quantified by immunoassay in sickle cell patients. Data are representative of both steady state and acute painful episodes. Peak intensity analysis showed a difference in levels of the 28.3 kDa peak between steady state and acute painful episode. (B) Immunoassay demonstrated a significant decrease in apoA-I levels in patients during acute painful episode when compared to during steady state ($P<0.008$). Data are representative of 26 matched pairs.

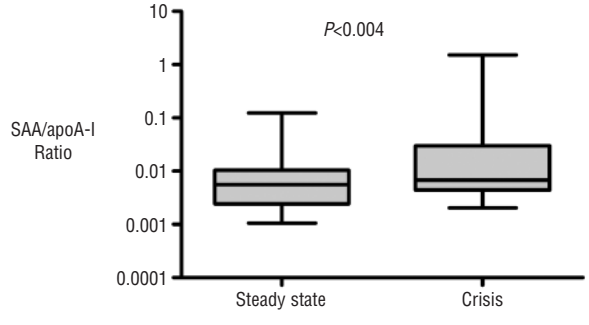

Figure 3. The SAA/Apo A-I ratio is significantly higher during acute painful episodes than during the steady state. Ratios were derived for each patient from the immunoassay data in the previous figures. Presented on a logarithmic scale, the ratio is significantly higher in SCD patients during acute painful episodes ($p<0.004$, box and whisker plot). Data are representative of 26 matched pairs.

episodes with end-organ damage. These patients were distinguished by having very high levels of SAA, and this was correlated with the largest increments in leukocyte count during the acute painful episodes. This subgroup of four patients also had a particularly significant increase in reticulocyte count during their acute painful episodes, suggesting accelerated hemolysis. During steady state, these four patients had smaller mean corpuscular volumes than those of the other patients, consistent with less hydroxyurea use and/or concurrent alpha thalassemia trait, both known risk factors for more frequent and potentially more symptomatic acute painful episodes.[28,29]

We also found that a 28.1 kDa peak was decreased during acute painful episodes in every fraction-surface combination, correlating strongly with the findings of the apoA-I immunoassay. During the general acute phase response, total cholesterol, high density lipoprotein (HDL), and apoA-I are known to be diminished.[30,31] Furthermore, a published study from our group has linked low levels of a similar peak to vasculopathy and pulmonary hypertension in SCD, with the peak having been confirmed by high-resolution mass spectroscopy and immunoassay to be apoA-I.[17]

Alterations in these markers have been reported even during the steady state of SCD. It has previously been reported that apoA-I levels are lower in sickle cell patients than in the general population.[32] Singhal *et al.* found SAA levels above 5 mg/L, and also C-reactive protein levels elevated above 10 mg/L, in 18% of symptom-free homozygous SCD patients, 17% of patients with SC disease, and 1% of healthy controls.[13] Subclinical changes in SCD markers appear to exist before overt pain symptoms develop.[2,33] Finally, our own work indicates higher SAA and lower apoA-I levels during the steady state in SCD patients with high pulmonary arterial pressures.[17] This subclinical inflammatory and ischemic "steady state" appears to become exacerbated during acute painful episodes.

SAA and apoA-I have vital functional roles in vascular biology beyond being simple markers of inflammation. ApoA-I is the principal apolipoprotein on the surface of HDL, but SAA is also an HDL-localized apolipoprotein. It is well established that apoA-I and HDL have cholesterol transport, anti-inflammatory, anti-oxidant, and vasculoprotective roles while low levels promote atherosclerosis. Furthermore, SAA-marked HDL inhibits reverse cholesterol transport and is a risk factor for atherosclerosis.[34,35] We observed that during acute painful episodes, the levels of both these lipoproteins were inversely correlated and that the SAA/apoA-I ratio is a more robust marker of acute painful episodes. This ratio may, therefore, be of use in clinical research as an objective marker of acute painful episodes. SAA may be especially useful as a marker of extensive inflammatory tissue damage during acute painful episodes, since we observed dramatic increases in SAA in the four patients distinguished by having the most clinically severe complications of acute painful episodes.

It is fascinating that steady state levels of SAA and apoA-I, two well-established markers and mediators of vascular dysfunction and atherosclerosis, appear from our previous study to be markers of endothelial dysfunction and pulmonary hypertension[17] and, in the current study, become even more abnormal during APE. This suggests that these markers reflect and potentially even mediate a state of chronic vasculopathy in SCD that worsens acutely during acute painful episodes, consistent with our previous evidence that pulmonary hypertension worsens acutely during acute painful episodes.[36] We propose that the development of apoA-I-mimetic drugs or induction of higher apoA-I levels merits investigation for potential therapeutic utility in the acute vasculopathy of acute painful episodes or the chronic vasculopathy of SCD.[37]

Authorship and Disclosures

AsTu, AFS and GJK designed the study. AsTu, AKM and LM collected specimens. AsTu and GTH carried out the SELDI-TOF MS analysis under the supervision of AFS. AsTu and XL carried out the proteomics statistical analysis under the supervision of PJM. AnTa and LF carried out validation assays under the supervision of AR, with statistical supervision of GJK. AsTu, AnTa, and GJK wrote the manuscript.

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References

1. Ballas SK. Sickle cell disease: clinical management. Baillieres Clin Haematol. 1998;11(1):185-214.
2. Embury SH, Hebbel RP, Mohandas N, Steinburg MH. Sickle Cell Disease: Basic Principles and Clinical Practice. New York, NY: Raven Press; 1994.
3. Conran N, Franco-Penteado CF, Costa FF. Newer aspects of the pathophysiology of sickle cell disease vaso-occlusion. Hemoglobin. 2009;33(1):1-16.
4. Serjeant GR, Ceulaer CD, Lethbridge R, Morris J, Singhal A, Thomas PW. The painful crisis of homozygous sickle cell disease: clinical features. Br J Haematol. 1994; 87(3):586-91.
5. Bailey S, Higgs DR, Morris J, Serjeant GR. Is the painful crisis of sickle-cell disease due to sickling? Lancet. 1991;337(8743):735.
6. Platt OS, Thorington BD, Brambilla DJ, Milner PF, Rosse WF, Vichinsky E, et al. Pain in sickle cell disease. Rates and risk factors. N Engl J Med. 1991;325(1):11-6.
7. Charache S, Barton FB, Moore RD, Terrin ML, Steinberg MH, Dover GJ, et al. Hydroxyurea and sickle cell anemia. Clinical utility of a myelosuppressive "switching" agent. The Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Medicine (Baltimore). 1996;75(6):300-26.
8. Ballas SK, Lusardi M. Hospital readmission for adult acute sickle cell painful episodes: frequency, etiology, and prognostic significance. Am J Hematol. 2005;79(1):17-25.
9. Dunlop RJ, Bennett KC. Pain management for sickle cell disease. Cochrane Database Syst Rev2006(2):CD003350.
10. 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): 902-6.
11. Stuart J, Stone PC, Akinola NO, Gallimore JR, Pepys MB. Monitoring the acute phase response to vaso-occlusive crisis in sickle cell disease. J Clin Pathol. 1994;47(2):166-9.
12. Akinola NO, Stevens SM, Franklin IM, Nash GB, Stuart J. Rheological changes in the prodromal and established phases of sickle cell vaso-occlusive crisis. Br J Haematol. 1992;81(4):598-602.
13. Singhal A, Doherty JF, Raynes JG, McAdam KP, Thomas PW, Serjeant BE, et al. Is there an acute-phase response in steady-state sickle cell disease? Lancet. 1993;341(8846): 651-3.
14. Sabio H, McKie VC. Modified (mixed) ery-

throcyte sedimentation rate in sickle cell anemia. Clinical hemorheology 1992;587: 587-92.
15. Stuart J. Acute-phase response and sickle crisis. Lancet. 1993;341(8846):664.
16. Laogun AA, Ajayi NO, Osamo NO, Okafor LA. Plasma viscosity in sickle-cell anaemia. Clin Phys Physiol Meas. 1980;1:145-50.
17. Yuditskaya S, Tumblin A, Hoehn GT, Wang G, Drake SK, Xu X, et al. Proteomic identification of altered apolipoprotein patterns in pulmonary hypertension and vasculopathy of sickle cell disease. Blood. 2009;113(5):1122-8.
18. Hassell KL, Eckman JR, Lane PA. Acute multiorgan failure syndrome: a potentially catastrophic complication of severe sickle cell pain episodes. The American journal of medicine. 1994;96(2):155-62.
19. Ballas SK, Marcolina MJ. Hyperhemolysis during the evolution of uncomplicated acute painful episodes in patients with sickle cell anemia. Transfusion. 2006;46(1):105-10.
20. Neely CL, Wajima T, Kraus AP, Diggs LW, Barreras L. Lactic acid dehydrogenase activity and plasma hemoglobin elevations in sickle cell disease. Am J Clin Pathol. 1969;52(2):167-9.
21. Hunt BJ, Korsah P, Eaton S, Brozovic M. Creatine kinase activity in sickle cell disease. J Clin Pathol. 1989;42(7):712-5.
22. Smith WR, Penberthy LT, Bovbjerg VE, McClish DK, Roberts JD, Dahman B, et al. Daily assessment of pain in adults with sickle cell disease. Ann Intern Med. 2008;148(2):94-101.
23. 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): 902-6.
24. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. N Engl J Med. 1995; 332(20):1317-22.
25. Cabana VG, Reardon CA, Feng N, Neath S, Lukens J, Getz GS. Serum paraoxonase: effect of the apolipoprotein composition of HDL and the acute phase response. J Lipid Res. 2003;44(4):780-92.
26. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv Immunol. 1983;34:141-212.
27. Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol. 2000;20(3):763-72.
28. Billett HH, Nagel RL, Fabry ME. Paradoxical increase of painful crises in sickle cell patients with alpha-thalassemia. Blood. 1995;86(11):4382.
29. Gill FM, Sleeper LA, Weiner SJ, Brown AK, Bellevue R, Grover R, et al. Clinical events in the first decade in a cohort of infants with sickle cell disease. Cooperative Study of Sickle Cell Disease. Blood. 1995;86(2): 776-83.
30. Cabana VG, Reardon CA, Wei B, Lukens JR, Getz GS. SAA-only HDL formed during the acute phase response in apoA-I+/+ and apoA-I-/- mice. J Lipid Res. 1999;40(6): 1090-103.
31. Hosoai H, Webb NR, Glick JM, Tietge UJ, Purdom MS, de Beer FC, et al. Expression of serum amyloid A protein in the absence of the acute phase response does not reduce HDL cholesterol or apoA-I levels in human apoA-I transgenic mice. J Lipid Res. 1999;40(4):648-53.
32. Sasaki J, Waterman MR, Cottam GL. Decreased apolipoprotein A-I and B content in plasma of individuals with sickle cell anemia. Clin Chem. 1986;32(1 Pt 1):226-7.
33. Murray N, May A. Painful crises in sickle cell disease--patients' perspectives. Bmj. 1988;297(6646):452-4.
34. Lowell CA, Stearman RS, Morrow JF. Transcriptional regulation of serum amyloid A gene expression. J Biol Chem. 1986;261(18):8453-61.
35. Pruzanski W, Stefanski E, de Beer FC, de Beer MC, Ravandi A, Kuksis A. Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. J Lipid Res. 2000;41(7):1035-47.
36. Machado RF, Mack AK, Martyr S, Barnett C, Macarthur P, Sachdev V, et al. Severity of pulmonary hypertension during vaso-occlusive pain crisis and exercise in patients with sickle cell disease. Br J Haematol. 2007;136(2):319-25.
37. Ou J, Ou Z, Jones DW, Holzhauer S, Hatoum OA, Ackerman AW, et al. L-4F, an apolipoprotein A-1 mimetic, dramatically improves vasodilation in hypercholesterolemia and sickle cell disease. Circulation. 2003;107(18):2337-41.