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The Acute Phase Inflammatory Response to Maximal Exercise Testing in Children and Young Adults with Sickle Cell Anaemia

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Summary

Although individuals with sickle cell anaemia (SCA) have elevated baseline inflammation and endothelial activation, the acute phase response to maximal exercise has not been evaluated among children with SCA. We measured the acute phase response to maximal exercise testing for soluble vascular cell adhesion molecule (sVCAM) as well as interleukin 6 (IL6), total white blood cell count (WBC), C-reactive protein (CRP) and D-dimer in 60 children with SCA and 30 controls at baseline, immediately after, and 30, 60 and 120 min following exercise. Despite higher baseline levels of all biomarkers except CRP, the acute phase response from baseline to immediately after exercise was significantly greater in subjects versus controls for CRP (2.1 vs. 0.2 mg/l, $p = 0.02$) and D-dimer (160 vs. 10 µg/l , $p < 0.01$) only. Similar between-group trends were observed over time for all biomarkers, including sVCAM, IL6, total WBC, CRP and D-dimer. Lower fitness, defined by peak oxygen consumption (VO_2), was independently associated with greater acute phase responses to exercise for sVCAM. Our results suggest maximal exercise may not be associated with any greater escalation of endothelial activation or inflammation in SCA and provide preliminary biomarker evidence for the safety of brief, high-intensity physical exertion in children with SCA.

Keywords

Sickle cell disease; fitness; inflammation; biomarkers; exercise testing

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Introduction

Sickle cell anaemia (SCA) is characterized by endothelial dysfunction and a pro-inflammatory state, which contribute to disease pathophysiology and complications (Brittain and Parise 2007, Hebbel, *et al* 2004). Surrogate markers of endothelial dysfunction and inflammation, including pro-inflammatory cytokines (e.g., interleukin (IL)1β, IL6, IL8, tumour necrosis factor (TNF)-α and interferon (INF)-γ) and markers of endothelial activation (e.g., endothelin (ET)-1, adhesion molecules and selectins), may be elevated at baseline and during complications (Hebbel, *et al* 2004, Hoppe 2014). Of the various biomarkers studied in SCA, soluble vascular cell adhesion molecule (sVCAM) has been consistently shown to correlate with clinical severity (Dworkis, *et al* 2011). Specifically, sVCAM is elevated at baseline, and increases in plasma levels of sVCAM are observed in association with such complications and outcomes as vaso-occlusive pain, acute chest syndrome, end organ damage and mortality (Duits, *et al* 1996, Kato, *et al* 2005, Sakhalkar, *et al* 2004, Schnog, *et al* 2003).

Acute exercise is also associated with a transient increase in circulating pro- and anti-inflammatory mediators and markers of endothelial activation in the general population (Bartzeliotou, *et al* 2007, McMurray, *et al* 2007, Ploeger, *et al* 2009, Suzuki, *et al* 2002). Greater baseline levels of C-reactive protein (CRP) and other pro-inflammatory biomarkers (e.g., IL6, white blood cell (WBC) count, TNF-α and fibrinogen) are associated with lower cardiopulmonary fitness and decreased levels of habitual physical activity (Kullo, *et al* 2007, Panagiotakos, *et al* 2005). Higher baseline levels of these acute phase reactants, including CRP, IL6 and fibrinogen, also predict a heightened risk of cardiovascular disease in large-scale epidemiological studies (Emerging Risk Factors Collaboration 2012, Zakai, *et al* 2007). In contrast, aerobic exercise training is associated with an attenuation of the acute phase response to exercise in the general population (Chen, *et al* 2014, Kasapis and Thompson 2005). Reductions in the acute phase response to exercise may underlie the mechanism by which regular exercise confers its cardiovascular protective benefits.

Although SCA and acute exercise both result in endothelial activation and stimulation of pro-inflammatory pathways, the acute phase response to maximal, high-intensity exercise has not been previously evaluated among children and young adults with SCA. Complications of SCA, including cardiopulmonary disease, have a significant impact on overall physical functioning and result in decreased cardiopulmonary fitness among affected individuals (Callahan, *et al* 2002, Liem, *et al* 2015, Panepinto, *et al* 2005). However, the relationship between baseline fitness and the acute phase response to maximal exercise also has not been examined in SCA. The objective of this study was to characterize the acute phase response to exercise of sVCAM and other biomarkers, including total WBC, IL6, CRP and D-dimer, among children and young adults with SCA undergoing maximal cardiopulmonary exercise testing (CPET). Given the endothelial activation and pro-inflammatory state observed at baseline with SCA, we hypothesized that exercise challenge is associated with a greater acute phase response among individuals with SCA when compared to that observed among controls.

Methods

Subject selection

Sixty subjects (mean age 15.1 years, 50% females) with SCA (haemoglobin SS or S/beta[0] thalassaemia) from the Comprehensive Sickle Cell Program at Ann & Robert H. Lurie Children's Hospital and 30 age-, sex- and race-matched controls without SCA or sickle cell trait (mean age 14.6 years, 50% females) were included in this study. Subjects on chronic monthly transfusions were excluded from the study, but subjects on hydroxycarbamide were not.

Exercise Protocol

Maximal CPET was performed in all subjects and controls following a graded, symptom-limited cycle ergometry protocol (Godfrey, *et al* 1971). Subjects underwent testing at least 2 weeks after any vaso-occlusive pain episode requiring hospitalization and at least 3 months after any packed red blood cell transfusion. We used an electronically braked VIAsprint 150p Ergometric Bicycle (Carefusion Corp., San Diego, CA) with paediatric configurations and breath-by-breath gas exchange data was collected using a Vmax Encore 29C metabolic cart (CareFusion Corp.). Initial workload and workload increments were based on subject height, and workload increments occurred by continuous ramping at 1-min intervals until volitional exhaustion. Exercise testing was completed when subjects and controls could no longer maintain a cadence of 60 revolutions per minute after maximal effort, defined as having achieved a respiratory exchange ratio (RER) > 1.1. We calculated peak oxygen consumption (VO_2) from 20-s averages of data points as the highest weight-adjusted value achieved in the last minute of exercise prior to recovery.

Blood sampling and analysis

Following admission to the Clinical Research Unit, each subject and control underwent placement of a peripheral intravenous (IV) line through which all samples were drawn. Blood was drawn at baseline before CPET (Pre), immediately after (T0) and 30 (T30), 60 (T60) and 120 (T120) min after exercise. Samples were analysed for total white blood cell (WBC) count, absolute neutrophil and monocyte counts, platelet count, C-reactive protein (CRP) and D-dimer in the metabolic core laboratory at Lurie Children's Hospital. Circulating levels of sVCAM and interleukin (IL6) were measured using standard enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, MN, DVCOO and HS600B) following manufacturer instructions. All ELISA samples were run in duplicate.

Statistical considerations

Standard descriptive analysis was performed and the normality of data distribution was assessed. Only subjects and controls with all available time points of biomarker collection required of each described analysis were included. Soluble VCAM and IL6 values with coefficient of variation (CV) 25% between duplicates on ELISA testing were excluded. Continuous variables were compared between groups by Student t-test or Mann Whitney Wilcoxon Test where appropriate. Repeated measures analysis of variance (ANOVA) was conducted to determine if acute phase responses were similar in shape over time between

groups. Our primary outcome was the acute change in sVCAM from baseline to immediately after CPET (T0–Pre). Regression analysis was used to evaluate for independent predictors, including peak VO_2, of the acute phase response for sVCAM and other biomarkers. A *p* value < 0.05 was considered statistically significant.

Results

Subject Characteristics and Baseline Biomarkers

All 60 subjects with SCA and 30 controls completed CPET without any major adverse events. Among subjects, 20/60 (33%) were on hydroxycarbamide at the time of CPET, most commonly for recurrent pain episodes and acute chest syndrome. All had been on long-term treatment (mean 59±30 months) with a stable dose (mean 29±5 mg/kg/day) for at least 2 months prior to testing. We have previously shown that subjects with SCA demonstrate significant reductions in average total exercise time (5.6 vs. 7.8 min, *p* < 0.01) and weight-adjusted peak VO_2 (26.9±6.9 vs. 37.0±9.2 ml/kg/min, *p* < 0.001) when compared to controls (Liem, *et al* 2015). All 60 (100%) subjects and 22/30 (73%) controls met RER criterion for a maximal test, and testing was terminated due to excessive participant fatigue in all cases. Variability in sample sizes available for analysis of each biomarker reflects either unsuccessful IV placement or IV malfunction during sampling. When compared to controls at baseline, subjects with SCA demonstrated higher sVCAM and IL6 levels, WBC, monocyte and neutrophil counts, platelet count and D-dimer but not CRP (Table I).

Acute Phase Responses to Exercise

We found no significant difference in the mean acute sVCAM response to exercise challenge from baseline to T0 (T0–Pre) in subjects with SCA versus controls (87 vs 48 ng/ml, *p* = 0.15) (Table II). In general, the difference in sVCAM level between subjects with SCA and controls remained significant at each time point after CPET (Fig 1). However, repeated measures testing demonstrated that the mean difference in sVCAM between groups was constant over time (F(3.46, 221.22) = 1.81, *p* = 0.14), indicating the overall sVCAM response to CPET followed similar trends in both groups.

There was no consistent difference between subjects and controls in the acute phase response to maximal exercise for other biomarkers tested in this study. When compared to that observed in controls, mean acute phase responses from baseline to T0 (T0–Pre) for CRP (2.1 vs. 0.2 mg/l, *p* = 0.02) and D-dimer (160 vs. 10 μg/l,, *p* < 0.01) were significantly greater in subjects with SCA while the acute phase response for platelet count was lower (-2 vs 37 × 10^9/l, *p* < 0.01). Mean acute phase responses for IL6, WBC and absolute neutrophil and monocyte counts were not significantly different between groups. With the exception of that observed for platelet count (F(3.37, 256.11) = 8.93, *p* < 0.01), mean between group differences in IL6, WBC, neutrophil and monocyte counts, CRP and D-dimer did not change over time.

For sVCAM and most other biomarkers, hydroxycarbamide use generally did not affect the acute phase response to maximal exercise among subjects. We did find that subjects with SCA who were not on hydroxycarbamide demonstrated greater acute phase increases (T0–

Pre) in IL6 and absolute monocyte count with borderline significance, when compared to subjects not on hydroxycarbamide or controls. These differences, however, did not retain their significance after Bonferroni correction for multiple comparisons.

Acute Phase Response to Exercise and Fitness

We found no consistent relationship between baseline biomarker activity and cardiopulmonary fitness in our subjects with SCA. Lower peak VO_2 was significantly correlated with higher D-dimer (Spearman $\rho = -0.31$, $p = 0.02$) level but no other biomarkers at baseline, including sVCAM. We performed regression analysis to examine the relationship of peak VO_2 to the acute phase response (T0–Pre) for sVCAM and other biomarkers in our subjects with SCA. Age, sex, baseline haemoglobin and hydroxycarbamide status were included in the models as co-variates. We found that lower peak VO_2 was an independent predictor of greater acute phase responses for sVCAM and absolute monocyte count (Table III) but not for other biomarkers.

Discussion

This analysis represents the first published study of the acute phase response to maximal exercise challenge in children and young adults with SCA. Despite higher levels at baseline, we found no major difference in the immediate response of sVCAM, IL6 and other inflammatory mediators to exercise in subjects with SCA versus controls. The sampling profiles of sVCAM and other biomarkers demonstrated similar trends following exercise challenge up to 2 h of recovery. Importantly, we found that lower cardiopulmonary fitness, defined by peak VO_2, was independently associated with greater acute increases in sVCAM level to exercise.

The pro-inflammatory state associated with SCA has been well studied. SCA is characterized at steady state by marked leucocytosis, increased leucocyte recruitment and activation as well as elevations of various pro-inflammatory cytokines (Bourantas, *et al* 1998, Turhan, *et al* 2002). The pro-inflammatory state plays an important role in disease pathophysiology and, along with endothelial dysfunction, contributes to vaso-occlusion and other complications in SCA (Hatzipantelis, *et al* 2013, Krishnan, *et al* 2010, Solovey, *et al* 1997). Endothelial cell expression of VCAM, in particular, has been shown to play a major part in sickle cell pathophysiology. Plasma levels of sVCAM are elevated at baseline in SCA and further increase with acute complications, such as vaso-occlusive pain and acute chest syndrome (Blum, *et al* 2005, Sakhalkar, *et al* 2004). Although results have been variable, the pro-inflammatory cytokine IL6 has also been shown to be elevated in SCA at steady state and during pain episodes (Sarray, *et al* 2015). We included IL6 in this study of exercise responses because, in addition to its role as an inflammatory mediator, IL6 is a myokine produced by muscles under contraction during both aerobic and resistance exercise (Mendham, *et al* 2011, Steensberg, *et al* 2000).

In general, a single bout of aerobic and resistance exercise is associated with an acute phase response characterized by increases in WBC counts and various pro-inflammatory cytokines, including sVCAM, IL6, CRP and D-dimer (Kasapis and Thompson 2005, Signorelli, *et al* 2003, Smith, *et al* 2000). Several pro-inflammatory mediators have been

shown to increase in response to exercise in a dose-dependent manner, depending on exercise intensity or duration (Nieman, *et al* 2012, Pereira, *et al* 2012). Despite anecdotal concerns that acute exercise may trigger vaso-occlusive pain in SCA through exacerbation of underlying inflammation and endothelial activation, no previous studies have examined the acute phase response to high intensity exercise in children with SCA. Our results indicate that a brief bout of high intensity exercise in SCA is not associated with any greater exacerbation of circulating sVCAM or inflammatory mediators, including IL6, WBC counts, or WBC subsets up to 2 h following exercise. Subjects with SCA in our study did demonstrate a greater increase in CRP and D-dimer immediately following CPET. The physiological relevance of these findings, however, is not clear, because the overall trend of these biomarkers in the subsequent 2 h of recovery were similar between the groups. Although the rise in D-dimer immediately after exercise could suggest an increase in pro-coagulant activity, levels also remained in the normal range. These results complement our previous published findings that maximal CPET is safe and not associated with major adverse events in this population (Liem, *et al* 2015). There is also support for our findings in the literature for adults with SCA. Barbeau *et al* (2001) found that circulating inflammatory mediators, including CRP and IL6, were not affected by 3 days of exercise in 11 adult women with SCA. In a separate study of 11 adult men with SCA, sVCAM and soluble intracellular adhesion molecule (sICAM) levels increased significantly from baseline following 20 min of exercise by cycle ergometry (Faes, *et al* 2014). However, the prescribed exercise regimens were submaximal in intensity in both of these adult studies, thus making it difficult to compare their findings to those in this analysis.

Lower cardiopulmonary fitness and sedentary behaviour are associated with a heightened pro-inflammatory state at baseline as well as greater acute phase responses to exercise challenge in the general population (Kasapis and Thompson 2005, Kullo, *et al* 2007, Panagiotakos, *et al* 2005). The prognostic implications of inflammation as they relate to cardiovascular risk stratification, in particular with CRP, have been well studied (Pai, *et al* 2004, Shlipak, *et al* 2008). The cardiovascular benefits of regular exercise and conditioning are presumed to be mediated partly through reduction of inflammation, improvement in endothelial function and attenuation of the acute phase response of pro-inflammatory and endothelial biomarkers to exercise (Lara Fernandes, *et al* 2011, Mattusch, *et al* 2000, Palmefors, *et al* 2014, Roberts, *et al* 2006, Stewart, *et al* 2007, Tisi, *et al* 1997). Although there was not a consistent relationship between baseline biomarkers and cardiopulmonary fitness in our subjects, we did find that lower fitness levels were associated with a greater acute phase sVCAM response to exercise in our subjects. However, it remains unclear if this finding indicates children with SCA are less likely to be physically active, and therefore fit, because of a greater inflammatory response to physical exertion that may lead to adverse consequences. Alternatively, exercise training may be associated with attenuation of the acute phase response to exercise in SCA. This is plausible given that physical training is associated with reduced endothelial activation and oxidative stress in sickle cell mice exposed to hypoxia/reoxygenation conditions (Aufradet, *et al* 2014, Charrin, *et al* 2015). With the exception of the relationship observed with absolute monocyte count, cardiopulmonary fitness was not significantly associated with the acute phase response of any other biomarker measured in this study.

A few limitations of our study warrant discussion. Our analysis was limited to only a few biomarkers, which we selected based on prior findings in the literature that were relevant to our study aims. Based on our preliminary data, studying the impact of acute exercise on other biomarkers of inflammation, endothelial function and coagulation activation is justified. Also, we measured changes in biomarkers only in response to a relatively brief bout of high intensity exercise. In future studies, it would be interesting to assess biomarker responses to exercise stratified by both varying intensity and duration of exercise to gain a broader understanding of the safety of physical exertion in this population. Importantly, the equivalent rise in biomarker levels we observed in our subjects with SCA could still be physiologically more significant in this group. Both baseline and relative increases in biomarkers occurred at a higher level in our subjects. The maximal workload achieved by our subjects undergoing CPET was also lower than that measured in controls (data not shown) despite reaching maximal effort. This suggests that an equivalent rise in biomarkers in response to an overall lower workload in SCA may represent a "relatively" greater acute phase response, which could be more harmful. Although we evaluated peak VO_2 in our participants, we did not obtain data on daily physical activity, which may influence the acute phase response to exercise independent of cardiopulmonary fitness. Finally, our sample sizes were relatively modest, especially as some subjects and controls were dropped from our primary analysis due to challenges with collecting all required samples for biomarker profiling. Our sample size might also have limited our ability to accurately assess the impact of hydroxycarbamide use on study outcomes. Still, our sample sizes are comparable to many existing exploratory studies of biomarkers in the literature.

In summary, children and young adults with SCA, when compared to their peers, demonstrate similar trends in the acute phase response to maximal CPET for up to 2 hof recovery. This is observed despite elevated levels of biomarkers of endothelial activation and inflammation at baseline and following cessation of exercise. These results suggest that maximal exercise challenge may not be associated with any greater escalation of inflammation or endothelial activation in SCA. Given the absence of any adverse events associated with maximal CPET in our study, our results also provide preliminary biomarker evidence for the safety of brief, high intensity physical exertion in children and young adults with SCA. Further studies of maximal CPET and high intensity physical exertion are warranted to further elucidate the relationship among inflammatory and endothelial pathways, fitness and clinical outcomes in this population.

Acknowledgments

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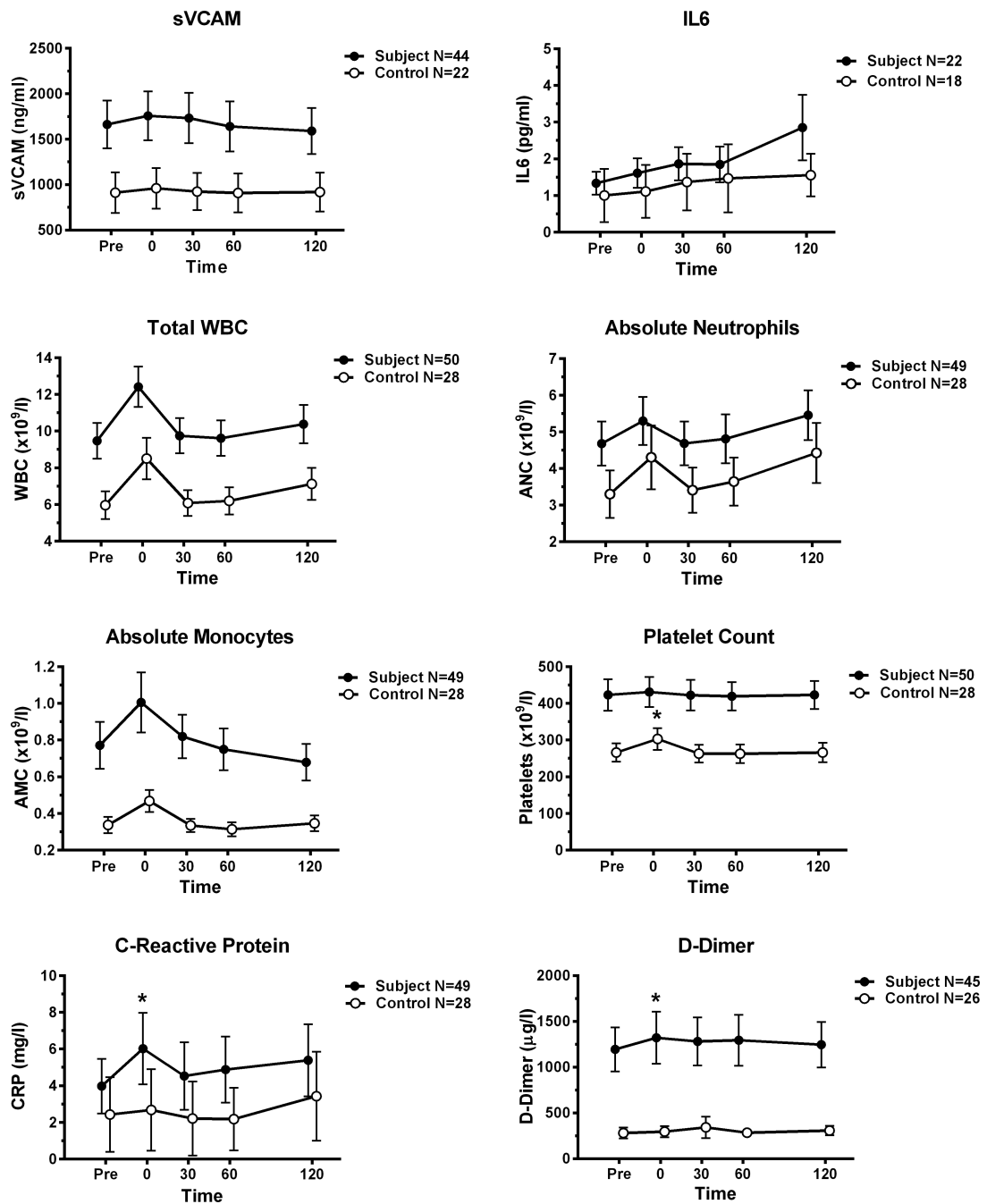
Fig 1.
Profiles of biomarkers from baseline through recovery following maximal cardiopulmonary exercise testing in subjects with sickle cell anaemia versus controls. The acute phase response from baseline (Pre) to immediately after exercise (T0) was significantly different (designated by *) in subjects versus controls for platelet count, CRP and D-dimer only. With the exception of the profile for platelet count, mean between group differences did not change over time for the other biomarkers. sVCAM, soluble vascular cell adhesion

molecule; IL6, interleukin 6; WBC, white blood cell count; ANC, absolute neutrophil count; AMC, absolute monocyte count; CRP, C reactive protein.

Table I

Baseline characteristics and biomarkers

	Subjects with SCA			Controls			
Variable	N	Mean	IQR	N	Mean	IQR	P value[*]
Age (years)	60	15.1	5.0	30	14.6	5.0	0.55
Haemoglobin (g/l)	53	87.6	22.0	28	129.0	21.0	<0.01
Platelet count ($\times 10^9$/l)	53	436	141	28	266	77	<0.01
sVCAM (ng/ml)	54	1586	1245	26	876	740	<0.01
IL6 (pg/ml)	31	1.45	1.54	19	0.95	0.65	0.01
WBC count ($\times 10^9$/l)	60	9.62	4.05	28	5.96	3.29	<0.01
Monocyte count ($\times 10^9$/l)	53	0.77	0.67	28	0.34	0.11	<0.01
Neutrophil count ($\times 10^9$/l)	53	4.74	2.74	28	3.30	2.49	<0.01
CRP (mg/l)	53	4.2	8.0	28	2.4	5.0	0.12
D-dimer (μg/l)	52	1530	1200	28	280	100	<0.01

SCA, sickle cell anaemia; IQR, interquartile range; sVCAM, soluble vascular cell adhesion molecule; WBC, white blood cell; CRP, C-reactive protein

[*] P value significant < 0.05.

Table II

Acute phase response (T0–Pre) in biomarkers

Variable	N	Mean	IQR	N	Mean	IQR	P value[*]
	Subjects with SCA			Controls without SCA			
Haemoglobin (g/l)	51	1.5	4.0	28	9.6	7.2	<0.01
Platelet count ($\times 10^9$/l)	51	−2	47	28	37	29	<0.01
sVCAM (ng/ml)	52	87	209	24	48	179	0.15
IL6 (pg/ml)	26	0.23	0.51	18	0.11	0.39	0.26
WBC count ($\times 10^9$/l)	51	2.93	2.13	28	2.54	1.55	0.31
Monocyte count ($\times 10^9$/l)	51	0.23	0.43	28	0.13	0.15	0.08
Neutrophil count ($\times 10^9$/l)	51	0.62	0.89	28	1.00	0.68	0.07
CRP (mg/l)	52	2.1	5.8	28	0.2	0.0	0.02
D-dimer (µg/l)	51	160	200	28	10	0.0	<0.01

SCA, sickle cell anaemia; IQR, interquartile range; sVCAM, soluble vascular cell adhesion molecule; WBC, white blood cell; CRP, C-reactive protein

[*]
 P value significant < 0.05

Table III

Predictors of acute phase response (T0–Pre) in subjects only

Variable	β Coefficient	Standard Error	P value [*]
Age (years)	−16.1	11.9	0.18
Female sex	−131.4	84.3	0.13
Haemoglobin (g/l)	13.8	26.3	0.60
Peak VO$_2$ (ml/min/kg)	−17.4	6.6	0.01
Hydroxycarbamide use	23.8	70.3	0.74

[*] P value significant < 0.05