

1 **Haematological parameters and plasma levels of 8-iso-prostaglandin F2 α in malaria-sickle**
2 **cell co-morbidity: A cross sectional study**

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22 **Abstract**

23 **Introduction**

24 Malaria and sickle cell disease (SCD) co-morbidity have previously been reported in Ghana.
25 However, there is paucity of data on haematological profiles and oxidative stress in co-
26 morbidity states. This study identified novel inflammatory biomarkers associated with malaria
27 in SCD and analyzed the levels of 8-iso-prostaglandin F₂α oxidative stress biomarker in malaria-
28 SCD co-morbidity in Ghanaian patients.

29 **Methods**

30 Blood (5ml) was collected from malaria patients into K₃-EDTA tube. Malaria parasites speciation
31 and quantification were then done according WHO guidelines. All eligible samples were assayed
32 for haematological profile, sickle cell phenotyping, infectious markers (hepatitis B, hepatitis C,
33 syphilis and HIV 1&2) and plasma levels of 8-epi-prostaglandin F₂α. .

34

35 **Results**

36 Prevalence of malaria in SCD (malaria-SCD) was 13.4% (45/335). Male: female ratio was 0.8:1
37 ($X^2=1.43$, $p=0.231$). Mean ages for malaria in normal haemoglobin type (malaria-HbAA) and
38 malaria-SCD were 12.79 ± 4.91 and 11.56 ± 3.65 years respectively ($p=0.048$). Geometric mean of
39 parasite density was higher in malaria-HbAA (20394 parasites/ μ l vs. 9990 parasites/ μ l, $p=0.001$)
40 whilst mean body temperature was higher in malaria-SCD ($39.0\pm 0.87^\circ\text{C}$ vs. $37.9\pm 1.15^\circ\text{C}$,
41 $p=0.001$). Mean leukocytes, lymphocytes, eosinophils, monocytes, platelets and platelet indices
42 values were significantly elevated in malaria-SCD. Significant reduction in RBC and RBC indices

43 in malaria-SCD were also observed. Eosinophils-to-basophils ratio (EBR) and monocytes-to-
44 basophils ratio (MBR) were novel cellular inflammatory biomarkers which could predict malaria
45 in SCD. The sensitivities of cut-off values of EBR>14, MBR>22 and combined use of EBR>14 and
46 MBR>22 were 79.55%, 84.09% and 91.11% respectively. Mean 8-iso-prostaglandin F2 α was
47 338.1pg/ml in malaria-HbAA and 643.8pg/ml in malaria-SCD ($p=0.001$). 8-iso-prostaglandin F2 α
48 correlated with parasite density ($r=0.787$, $p=0.001$), temperature ($r=0.566$, $p=0.001$) and
49 leucocytes ($r=0.573$, $p=0.001$) and negatively correlated with RBC ($r=-0.476$, $p=0.003$),
50 haemoglobin ($r=-0.851$, $p=0.001$) and haematocrit ($r=-0.735$, $p=0.001$).

51

52 **Conclusion**

53 *Plasmodium falciparum* parasitaemia increases oxidative damage and causes derangement
54 haematological parameters. Cut of values of EBR>14 and MBR>22 could predict malaria in SCD.

55 **Keywords:** 8-epi-prostaglandin F2 α , oxidative stress, haematological profile, malaria-SCD co-
56 morbidity, eosinophils-to-basophils ratio, monocytes-to-basophils ratio

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59 **Introduction**

60 The asexual stages of *Plasmodium falciparum* are intra-erythrocytic thus inducing
61 hematological alterations such as anemia, thrombocytopenia and neutrophilia [1-4]. Parasites
62 density influence severity of the hematological changes. These changes depend on factors such

63 as level of malaria endemicity, presence of haemoglobinopathies, nutritional status and level of
64 malaria immunity [5, 6]. Malaria is meso-endemic in Ghana with recent nationwide prevalence
65 of 43.4% [7]. Sickle cell disease (SCD) resulting from two haemoglobin S (HbS) haplotypes
66 (HbSS) and heterozygote sickle cell phenotype resulting from one HbS haplotype and one
67 haemoglobin C (HbC) haplotype (HbSC) is also prevalent in Ghana [8]. Sickle cell haplotype HbS
68 leads to polymerization of deoxygenated sickle haemoglobin within inelastic red blood cells
69 which cause occlusion of microvasculature, resulting in acute complications, chronic organ
70 damage, high rate of morbidity and mortality [9]. These mechanisms have adverse effect on the
71 quality and quantity of formed blood cells in affected individuals. SCD has been found to be
72 associated with anemia, low RBC count, low packed cell volume (PCV), low mean cell volume
73 (MCV), low mean cell hemoglobin (MCH) [10, 11] and leukocytosis [12]. The trends in the
74 haematological profiles associated with SCD and malaria are similar. There is evidence of
75 altered hematopoiesis which affect all the three hematological cell lines in SCD and malaria.

76 Oxidative stress is the overproduction of free radicals beyond the physiological detoxification
77 ability of the body [13]. A principal consequence of Plasmodium infections is the development
78 of anemia [14]. Malaria infections release reactive oxygen species (ROS) as a result of activation
79 of the immune system of the body. Plasmodium infections and sickle cell disease are associated
80 with oxidative stress due to production of ROS which result in haemoglobin degradation [15-
81 17]. Oxidative stress is suspected to play a key role in disease pathogenesis, complications and
82 mortality [18-19] consequently, a number of studies have focused on the measurement of
83 oxidative stress, many of which are through specific biomarkers that indicate the oxidative
84 damage [20]. Lipid, protein and DNA biomolecules are the main targets of free radicals that

85 subsequently transformed into the reactive species reflecting oxidative stress in the
86 corresponding molecules. Malondialdehyde (MDA), a product of lipid peroxidation, has been
87 widely used as an indicator of oxidative stress. However, MDA measured by thiobarbituric acid
88 assay overestimates actual MDA levels by more than 10-fold due probably to cross-reactivity
89 with other aldehydes [21]. The isopentane, 8-iso-prostaglandin F₂α (8-iso-PGF₂α), is a more
90 stable product of lipid peroxidation [22] and has been described at the gold standard
91 biomolecule for assessing oxidative stress [23-24]. Measurement of 8-iso-PGF₂α is a reliable
92 tool for the identification of subjects with enhanced rates of lipid peroxidation [25].

93 In Africa, malaria and SCD are prevalent [26] and their co-morbidity have been reported in
94 Ghana [27]. However, there is paucity of data on clinical manifestations, haematological profiles
95 and degree of oxidative stress in SCD and malaria co-morbidities. Therefore, the aim of this
96 study was to evaluate the variability of haematological parameters in malaria and SCD co-
97 morbidities, identify novel cellular inflammatory biomarkers for predicting malaria in SCD and
98 levels of 8-iso-PGF₂α oxidative stress biomarker in Ghanaian sickle cell patients infected with
99 malaria parasites.

100 **Materials and methods**

101 **Study site**

102 This multi-center study took place in 3 district hospitals and 3 health centers in the Greater
103 Accra region of Ghana. The hospitals (latitude, longitude) were Ga West Municipal Hospital,
104 Amasaman (5.7020708, -0.2992889), Ashaiman Polyclinic (5.6856, -0.0398) and Ada East

105 District Hospital (5.8956754, 0.5340865). The health centres (latitude, longitude) were Mayera
106 Health Centre (5.720578, -0.2703561), Oduman Health Centre (5.64171, -0.3302) and Obom
107 Health Centre (5.7361, -0.4395).

108 **Study design**

109 A cross-sectional study conducted in malaria suspected patients from November 2017-August,
110 2018. The following clinical information; age, sex, temperature, body weight and clinical
111 presentations was collected from each patient before sample collection. Maximum of three
112 samples were collected each day from the study sites. 5ml of whole blood was collected from
113 each consented patient. Specimens were transported from study sites to Ga West Municipal
114 Hospital laboratory daily. Haematological profile was carried out on same day of sample
115 reception. Blood films were prepared in triplicate. Screening for sickle cell and infectious
116 makers were done, whole blood spun and plasma stored below -30°C. Haemolysate was
117 prepared from concentrated red cell and kept frozen till ready for analysis.

118 **Study subjects, sample size and sample processing**

119 Figure 1 details participant recruitment plan used for the present study. Patients recruited for
120 the study were physician suspected malaria cases. Sample size was determined based on single
121 population formula using confidence interval of 95% and estimated proportion of 1 in 4 malaria
122 infections occurring in sickle cell disease. The sample size was estimated to be 289.
123 Measurement of 8-epi-prostaglandin F2 α biomarker was done in age and sex matched patients
124 (normal controls n=40, malaria-HbAA n=40, SCD control n=40 and malaria-SCD n=40).

125 **Figure 1: Flow chart for sample collection and analysis.** Total of 2272 suspected malaria patients
126 consented to participate in the study; microscopy did not detect malaria in 1858 (81.78%) of the
127 patients while *Plasmodium falciparum* parasites were detected in 414 (18.22%) of the patients. Of the
128 414 malaria patients, 12 (2.9%) had hepatitis B surface antigen (5), hepatitis C antibody (1), Salmonella
129 typhi IgM (8) and HIV I&II antibodies (3) detected in their plasma. 5 triple infections were identified. Of
130 the 402 malaria mono-infected patients, 112 (27.8%) were sickle cell positive; 67(60.0%) and 45(40.0%)
131 were malaria in haemoglobin AS and haemoglobin SS/SC respectively. Prevalence of SCD and sickle cell
132 trait (SCT) with malaria were 11.2% (45/402) and 16.6% (67/402) respectively. Excluding malaria in SCT
133 from the study, the prevalence of malaria in SCD was computed to be 13.4% (45/335).
134

135 **Inclusion and exclusion criteria**

136 Patients included in the study were microscopy diagnosed malaria patients, aged 0-20 years,
137 who consented or whose parents consented to be part of the study. Individuals who were
138 known SCD patients or visited the health centre on account of sickle cell crisis and malaria
139 patients co-infected with hepatitis B virus, hepatitis C virus, syphilis and HIV 1&2 were
140 excluded. Also samples with malaria and sickle cell trait were excluded.

141 **Laboratory analysis**

142 **Malaria detection and quantification**

143 Thick and thin blood film was done for each specimen, in triplicate, on the same glass slide. The
144 dried thin film was fixed in absolute methanol briefly, air dried and stained with 10% Giemsa.
145 The dried smear was then examined for presence of Plasmodium parasites. The parasites were
146 subsequently identified to the species level and quantified per μL of blood and percentage RBC
147 infected according WHO guidelines. Parasites were quantified per 200 WBCs counted using the
148 patients' total WBC per μL of blood. A total of 500 WBCs were counted in negative infections

149 [28]. Each slide was double checked by a blinded certified malaria microscopist and in cases of
150 discordant results; a third opinion was final.

151 **Infectious marker screening**

152 The specimens were screened for hepatitis B virus, hepatitis C virus, syphilis and HIV I&II
153 pathogens to eliminate any possible effect on the haematological parameters. The
154 microbiological screening was done with rapid immunochromatographic test kits. HIVI&II and
155 syphilis were screened with First Response® test kit (Premier Medical Corporation Ltd, India)
156 whilst the hepatitis B and C were screened with FaStep Rapid Diagnostic Test (Houston, USA).

157 **Haematological profiling**

158 Haematological profiling was done using Urit 5200 (China) fully automated haematology
159 analyzer. The 5-part differential analyzer works on the principle of laser beam multi-
160 dimensional cell classification flow cytometry for white cell differentiation, white and red blood
161 cell estimation. Platelets were counted by optical and electrical impedance principles and
162 haemoglobin concentration was measured by cyanide-free colorimetric method. All other
163 parameters were calculated.

164 **Determination of leukocyte ratio cut-off values**

165 The 95% confidence interval (CI) was determined for each mean of the leukocyte ratios
166 recorded in malaria-SCD. The upper or the lower value of the CI that majority of the individual
167 values fell was taken as the cut-off value. The cut-off values were determined on ratio by ratio
168 basis.

169 **Sickle cell screening and phenotyping**

170 Sickle cell screening was done by the sodium metabisulphite reduction method as previously
171 described by Antwi-Baffour [29] Sickle cell phenotyping was done as described by
172 Cheesborough [30]. Haemolysate was separated using electrophoresis in alkaline medium (pH
173 8.6). Haemoglobin phenotyping was done alongside pooled HbA, HbS, HbC and HbF controls.
174 Electrical voltage of 250 V and current of 50 mA were employed to obtain complete separation
175 of haemoglobin variants for a maximum of 30 min. Results were read immediately against the
176 controls.

177 **Sandwich-ELISA for 8-epi-prostaglandin F2 α levels**

178 Reagents and consumables for 8-epi-prostaglandin F2alpha was obtained from SunLong Biotech
179 (Hangzhou, China, Catalogue Number: SL0035Hu). Measurement of 8-epi-prostaglandin
180 F2alpha was done according to manufacturer's protocol, with these modifications; incubation
181 of pre-diluted samples with coated anti-8-epi-prostaglandin F2 α and after addition of
182 Horseradish Peroxidase-conjugated antibody specific for human 8-epi-prostaglandin F2alpha
183 was done for 45 minutes at room temperature. Again, the chromogen solution was incubated
184 for 30 minutes at room temperature after dispensing into the microelisa wells. The optical
185 density (OD) was measured by Mindray MR-96A ELISA plate reader (Shenzhen, China) at a
186 wavelength of 450 nm. The concentration of 8-epi-prostaglandin F2 α was obtained by
187 comparing the OD of the samples to the standard curve.

188 **Statistical analysis**

189 Raw data were entered into Microsoft Excel 2010. Statistical analyses were done with Stata
190 15.0 statistical software (Stata Corp LLC, USA). Pearson Chi square was used to determine
191 differences in categorical data whilst differences in parametric variables were determined by t-
192 test. To compare more than two groups, one-way ANOVA was used with multiple comparison
193 undertaken using Tukey post hoc analysis. Correlation between variables was determined by
194 Pearson correlation test. P-value <0.05 was considered statistically significant. Receiver
195 operating characteristic (ROC) curve was used to estimate the sensitivity, specificity and
196 predictive values of eosinophils-to-basophils and monocytes-to-basophils ratios to predict
197 malaria in sickle cell disease.

198 **Results**

199 **Demographic, temperature and parasitemia in malaria and sickle cell** 200 **co-morbidity**

201 Patients with normal haemoglobin but infected with malaria (malaria-HbAA) formed the
202 majority (86.6%; 290/335) of participants compared to malaria-SCD (13.43%; 45/335). Majority
203 of the participants were females (55.8% females vs. 44.2% males). The mean age of malaria-
204 HbAA was significantly higher than that of malaria-SCD patients (12.79±4.91 years vs 11.56±3.65
205 years; p = 0.048). Body temperature was significantly higher in malaria-SCD than malaria-HbAA
206 (37.9±1.15 vs. 39.0±0.87; t=6.86, p=0.001). The geometric mean of malaria parasite density
207 was higher in malaria-HbAA (20394 parasites/μL, IQR 9519-51093) than malaria-SCD (9990
208 parasites/μL, IQR 6329-16945) (table 1).

209 **Table 1: Demographic, temperature and parasitaemia of the patients**

Variables	Malaria-HbAA (n=290)	Malaria –SCD (n=45)	Statistic	p-value
Age (mean±SD)	12.79±4.91	11.56±3.65	t=2.01	0.048 ^a
Males, n (%)	127 (43.80)	21 (46.70)	X ² =1.43	0.231 ^b
Females, n (%)	163 (56.20)	24 (53.30)		
Body temp range (mean ±SD)	37.9±1.15	39.0±0.87	t=6.89	<0.001 ^a
Parasite density range	223-620586	2492-112452		
GM of Parasite density	20394	9990	t=7.43	<0.001
Interquartile range	9519-51093	6329-16945		

210 ^a p-value determined by t-test, ^b p-value determined by Pearson Chi square, GM-Geometric
 211 mean

212 **Haematological parameters in malaria and sickle cell co-morbidity**

213 The haematological variables of the participants were also compared (table 2). Whereas TWBC
 214 (12.32±2.77 vs., 6.68±2.42 p=0.001), %lymphocytes (36.23±8.44 vs. 28.53±18.22, p=0.001),
 215 %eosinophils (4.77±0.99 vs. 2.19±1.79, p=0.001) and %monocytes (7.32±1.58 vs. 5.92±3.30,
 216 p=0.001) were significantly higher in malaria-SCD, %neutrophil (62.1 ±20.1 vs. 50.44±8.65,
 217 p=0.001) and %basophils (0.45± 0.24 vs. 0.32±0.07, p=0.001) were significantly higher in
 218 malaria-HbAA participants. Also, RBC count (4.22±0.78 vs. 3.87±0.69, p=0.001), haemoglobin
 219 (9.19±1.06 vs. 10.83±2.11, p=0.001), haematocrit (27.34±2.79 vs. 31.84±6.07, p=0.001), MCV
 220 (71.33 ±7.62 vs. 76.07±10.53, p=0.001) and MCH (24.53±4.09 vs. 25.89±3.78, p=0.041) were
 221 significantly lower in malaria-SCD than malaria-HbAA. Although MCHC and RDW_CV differed
 222 among the participants, the differences did not reach statistical significance. Moreover,

223 platelets (190.0 ± 55.30 vs. 138.71 ± 87.70 , $p=0.001$), MPV (10.78 ± 1.28 vs. 9.91 ± 1.40 , $p=0.001$),
 224 PDW (13.33 ± 1.89 vs. 12.66 ± 2.48 , $p=0.038$), P_LCR (30.21 ± 7.39 vs. 25.11 ± 8.06 , $p=0.001$) were
 225 significantly higher in malaria-SCD than malaria-HbAA.

226 **Table 2: Hematological parameters of malaria and sickle cell co-morbidity**

Hematological parameters	Malaria-HbAA (mean±SD)	Malaria-SCD (mean±SD)	T value	p-value
White blood cells	6.68±2.42	12.32±2.77	12.87	<0.001
Neutrophils %	62.1 ±20.1	50.44±8.65	6.65	<0.001
Lymphocytes %	28.53±18.22	36.23±8.44	4.66	<0.001
Eosinophils %	2.19±1.79	4.77±0.99	14.23	<0.001
Monocytes %	5.92±3.30	7.32±1.58	4.57	<0.001
Basophils %	0.45± 0.24	0.32±0.07	7.79	<0.001
Red blood cells	4.22±0.78	3.87±0.69	3.07	<0.001
Haemoglobin	10.83 ±2.11	9.19 ±1.06	8.13	<0.001
Haematocrit	31.84 ±6.07	27.34 ±2.79	8.20	<0.001
Mean Cell Volume	76.07 ±10.53	71.33 ±7.62	3.66	<0.001
Mean Cell Haemoglobin	25.89 ±3.78	24.53 ±4.09	2.10	0.041
MCHC	34.07 ±2.35	33.71±2.85	0.80	0.426
RDW_CV	14.29 ±1.78	14.48 ±1.68	0.70	0.485
RDW_SD	37.57 ±17.69	36.70 ±4.84	0.69	0.492
Platelets	138.71± 87.70	190.0± 55.30	5.28	<0.001
Mean Platelet Volume	9.91 ±1.40	10.78 ±1.28	4.20	<0.001

PDW	12.66±2.48	13.33±1.89	2.12	0.038
Plateletcrit	0.18±0.88	0.17±0.07	0.23	0.817
P_LCR	25.11±8.06	30.21±7.39	4.65	<0.001

227 MCHC=Mean cell hemoglobin concentration, RDW_CV=Red cell distribution width coefficient of
 228 variation, RDW_SD=Red cell distribution width standard deviation, L=Litre, fL=Fentolitre,
 229 pg=pictogram, Plt=Platelets, PDW=Platelet distribution width, PCT=Plateletcrit, P_LCR=Platelet
 230 large cell ratio
 231

232 **Variations in leukocyte ratios in malaria and sickle cell co-morbidities**

233 The mean leukocytes ratios observed in malaria-HbAA and malaria-SCD were significantly
 234 different from each other (table 3). Lymphocytes-to-basophils ratio (76.10±66.33 vs.
 235 125.19±59.30, p=0.001), eosinophils-to-monocytes ratio (0.43±0.65 vs. 0.68±0.21, p=0.001),
 236 eosinophils-to-basophils ratio (4.62±2.52 vs. 16.05±5.64, p=0.001), monocytes-to-basophils
 237 ratio (12.85±2.79 vs. 23.40±3.04, p=0.001) and platelets-to-neutrophils ratio (2.85±3.23 vs.
 238 3.82± 1.04, p=0.001) were significantly higher in malaria-SCD. However, neutrophils-to-
 239 lymphocytes ratio (3.82±3.86 vs. 1.51±0.55, p=0.001), neutrophils-to-eosinophils ratio
 240 (13.08±5.87 vs. 11.30±4.22, p=0.001), neutrophils-to-monocytes ratio (16.97±25.18 vs. 7.25±
 241 2.35, p=0.001), lymphocytes-to-eosinophils ratio (55.85±9.43 vs. 7.88±2.38, p=0.001),
 242 lymphocytes-to-monocytes ratio (8.32±8.48 vs. 5.33± 2.27, p=0.009) and platelets-to-
 243 lymphocytes ratio (7.16±6.80 vs. 5.63± 2.57, p=0.006) were significantly lower in malaria-SCD.

244 **Table 3: Leukocyte ratios among malaria and malaria-sickle cell co-morbidities**

Leucocyte ratios	Malaria-HbAA	Malaria-SCD	T value	p-value
	(n=290)	(n=45)		

Neutrophils-to-lymphocytes ratio	3.82±3.86	1.51±0.55	9.59	<0.001
Neutrophils-to-eosinophils ratio	13.08±5.87	11.30±4.22	5.62	<0.001
Neutrophils-to-monocytes ratio	16.97±25.18	7.25± 2.35	6.39	<0.001
Lymphocytes-to-eosinophils ratio	55.85±9.43	7.88± 2.38	4.19	<0.001
Lymphocytes-to-monocytes ratio	8.32±8.48	5.33± 2.27	2.63	0.009
Lymphocytes-to-basophils ratio	76.10±66.33	125.19±59.30	4.95	<0.001
Eosinophils-to-monocytes ratio	0.43±0.65	0.68±0 .21	4.87	<0.001
Eosinophils-to-basophils ratio	4.62±2.52	16.05±5.64	13.38	<0.001
Monocytes-to-basophils ratio	12.85±2.79	23.40±3.04	21.88	<0.001

245

246 **Predictive novel cellular inflammatory biomarkers in malaria in SCD**

247 In table 4 the leucocyte ratios were further explored for their ability to predict malaria-SCD
 248 comorbidity by means of receiver operating characteristic (ROC). Neutrophils-to-monocytes
 249 ratio was the most sensitive (93.33%, 95% CI: 81.73-98.60) but only 56.90% specific (95% CI: 50.98–
 250 62.67) and had very low positive predictive value (PPV) (25.15%, 95% CI 22.37-28.15).
 251 Lymphocytes-to-eosinophils ratio and lymphocytes-to-monocytes ratio were 86.67% and
 252 80.00% sensitive respectively but not very specific (65.17% and 35.86% respectively) and had
 253 very low PPV (27.86% and 16.22% respectively). The sensitivity, PPV and ROC of lymphocytes-
 254 to-basophils ratio, neutrophils-to-eosinophils ratio and neutrophils-to-lymphocytes were
 255 comparatively low. Eosinophils-to-basophils ratio (EBR) and monocytes-to-basophils ratio
 256 (MBR) had relatively high predictive values. The cut-off values for EBR>14 and MBR>22
 257 associated with malaria-SCD. The sensitivity, specificity, positive predictive value (PPV),

258 negative predictive value (NPV) and ROC of EBR>14 were 79.55% (95% CI: 64.70-90.20), 97.11%
 259 (95% CI: 94.75-98.61), 77.78 % (95% CI: 65.11-86.78), 97.39 % (95% CI: 95.42-98.53) and 88.33%
 260 (95% CI: 79.73-94.40). The values obtained for MBR>22 in predicting malaria in SCD were 84.09
 261 % sensitive (95% CI: 69.93-93.36), 97.69 % specific (95% CI: 95.50-99.00), 82.22% PPV (95% CI
 262 69.73-90.28), 97.97% NPV (95% CI: 96.07-98.96) and ROC value 90.89% (95% CI: 82.72-96.18).
 263 When the combined performance of both EBR>14 and MBR>22 were analyzed, the obtained
 264 values were higher than using either ERB>14 or MBR>20 alone. The following indices were thus
 265 obtained for EBR>14-MBR>22; 91.11% sensitivity (95% CI: 78.78-97.52), 98.55% specificity (95%
 266 CI: 96.66-99.53), 89.13% PPV (95% CI: 77.37-95.16), 98.84% NPV (95% CI: 97.10-99.54) and ROC
 267 94.83% (95% CI 87.72-98.52). EBR and MBR were novel inflammatory markers found to be
 268 significantly associated with malaria-SCD.

269 **Table 4: Novel leukocyte ratios associated with malaria-SCD**

Inflammatory biomarker	Sensitivity	Specificity	PPV	NPV	ROC
and cut-off value	95% CI	95% CI	95% CI	95% CI	95%CI
EBR> 14	79.55%	97.11%	77.78 %	97.39 %	88.33%
	64.70-90.20	94.75-98.61	65.11-86.78	95.42-98.53	79.73-94.40
MBR>22	84.09 %	97.69 %	82.22 %	97.97 %	90.89%
	69.93-93.36	95.50-99.00	69.73-90.28	96.07-98.96	82.72-96.18
EBR>14-MBR>22	91.11%	98.55%	89.13%	98.84%	94. 83%
	78.78-97.52	96.66-99.53	77.37-95.16	97.10-99.54	87.72-98.52
LBR>107	60.00	78.28	30.00	92.65	69.14
	44.33-74.30	73.08-82.88	23.67-37.20	89.77-94.77	58.71-78.59

LER<9	86.67	65.17	27.86	96.92	75.92
	73.21-94.95	59.38-70.65	24.12-31.93	93.70-98.52	66.30-82.80
LMR<6	80.00	35.86	16.22	92.04	57.93
	65.40-90.42	30.34-41.68	14.04-18.65	86.33-95.48	47.87-66.05
NER>10	44.44	89.31	39.22	91.20	66.88
	29.64-60.00	85.17-92.62	28.81-50.70	88.83-93.10	57.40-76.31
NLR<1.7	53.33	72.41	23.08	90.91	62.87
	37.87-68.34	66.89-77.48	17.73-29.46	87.89-93.23	52.38-72.91
NMR<8.0	93.33	56.90	25.15	98.21	75.20
	81.73-98.60	50.98-62.67	22.37-28.15	94.83-99.40	66.36-80.64

270 CI-confidence interval; ROCV-receiver operating characteristic value; PPV-positive predictive
 271 value; NPV-negative predictive value, EBR-eosinophils-to-basophils ratio, LBR-lymphocytes-to-
 272 basophils ratio, LER-lymphocytes-to-eosinophils ratio, LMR-lymphocytes-to-monocytes ratio,
 273 MBR-monocytes-to-basophils ratio, NER-neutrophils-to-eosinophils ratio, NLR-neutrophils-to-
 274 lymphocytes ratio, NMR-neutrophils-to-monocytes ratio

275 **8-iso-prostaglandin F2 α levels and its correlates**

276 The oxidative stress associated with malaria infection and/or SCD inheritance were also
 277 assessed through estimation of 8-iso-prostaglandin F2 α (table 5). The mean levels of 8-iso-
 278 prostaglandin F2 α oxidative stress biomarker was significantly lower in control (no malaria-SCD
 279 negative participants) compared to all other groups [84.1pg/ml vs 129.1pg/ml (SCD patients) vs
 280 338.1pg/ml (malaria-HbAA) vs 643.8pg/ml (malaria-SCD); p = 0.001]. However, Tukey post hoc
 281 analysis indicated non-significance difference between malaria-SCD and malaria-HbAA (t=1.13,
 282 p=0.792). But pairwise post hoc analysis in the other groups were significant [(Malaria-SCD vs.
 283 normal control, t=41.12, p=0.001), (Malaria-SCD vs. SCD, t=36.5, p=0.001), (Malaria-HbAA vs.

284 normal control, $t=40.0$, $p=0.001$), (Malaria-HbAA vs. SCD, $t=35.38$, $p=0.001$) and (SCD vs. normal
 285 control, $t=44.95$, $p=0.001$)]. Moreover, whereas 8-iso-prostaglandin F2 α was significantly
 286 positively correlated with parasite density ($r=0.787$, $p=0.001$), temperature ($r=0.566$, $p=0.001$)
 287 and total WBC ($r=0.573$, $p=0.001$), it was inversely related to RBC ($r=-0.476$, $p=0.003$),
 288 haemoglobin concentration ($r=-0.851$, $p=0.001$) and haematocrit ($r=-0.735$, $p=0.001$).

289 **Table 5: Analysis of 8-iso-prostaglandin F2 α and its correlation with haematological and other**
 290 **parameters**

Variable	Control	SCD patients	Malaria-HbAA	Malaria-SCD	p-value
Age (years)	13.875(3.8)	14.125(3.7)	13.525(4.2)	13.575(3.7)	0.956 ^a
Gender					
Male	17(42.50)	20(50.00)	16(40.00)	18(45.00)	0.675 ^b
Female	23(57.50)	20(50.00)	24(60.00)	22(55.00)	
8-iso-PGF2 α	84.1(16.3)	129.1(18.8)	338.1(50.2)	643.8(54.54)	<0.001 ^a
8-iso-PGF2α levels correlates with significant variables				r	p-value
Parasite density				0.787	<0.001
Body temperature				0.566	<0.001
Red blood cells				-0.476	0.003
Haemoglobin				-0.851	<0.001
Haematocrit				-0.735	<0.001
Total WBC				0.573	<0.001

^cPost hoc comparison of mean levels of 8-iso-prostaglandin F2 α oxidative stress biomarker

8-iso-prostaglandin F2 α levels	Tukey	
Tukey pairwise analysis	t	p-value
Malaria-SCD vs. malaria-HbAA	1.13	0.792
Malaria-SCD vs. normal control	41.12	<0.001
Malaria-SCD vs. SCD	36.50	<0.001
Malaria-HbAA vs. normal control	40.00	<0.001
Malaria-HbAA vs. SCD	35.38	<0.001
SCD vs. normal control	4.62	<0.001

291 ^aone-way-ANOVA; ^bPearson chi square; ^cpost-hoc analysis; abbreviations: 8-iso-PGF2 α -8-iso-
292 prostaglandin F2 α ; SCD-sickle cell disease; Malaria-SCD-malaria-sickle cell disease co-morbidity

293 Discussion

294 In sub-Saharan Africa where inheritance of SCD is high and malaria infection is endemic, malaria
295 and SCD comorbidity is prevalent in the general population. Malaria has been demonstrated to
296 induce oxidant stress due to parasite multiplication as well as host immune response to the
297 parasite [31]. In this study we show that inheritance of SCD alone or *P. falciparum* infection
298 induces significant elevations in oxidant stress marker 8-iso-prostaglandin F2 α . Additionally, *P.*
299 *falciparum* infection and SCD co-morbidity leads to synergistic increase of this oxidant stress
300 biomarker in the peripheral blood of these patients.

301 The utility of 8-iso-prostaglandin F2 α as oxidative stress biomarker indicated significant
302 increases in malaria-HbAA, malaria-SCD and sickle cell patients compared to control subjects. 8-
303 iso-prostaglandin F2 α oxidative stress biomarker levels increased by 1.53, 4.0 and 7.6 folds in
304 SCD, malaria-HbAA and malaria-SCD respectively. This finding is suggestive that 8-iso-
305 prostaglandin F2 α may be a useful oxidative stress biomarker in malaria and sickle cell patients.
306 Interestingly levels of 8-iso-prostaglandin F2 α in malaria-SCD comorbidity significantly
307 correlated positively with parasite density, total WBC and body temperature and negatively
308 with RBC, haemoglobin and haematocrit. As it has been demonstrated that increasing
309 parasitemia leads to increased red cell destruction, elevated body temperature of patients and
310 subsequent oxidant stress, the relationship between 8-iso-prostaglandin F2 α and *P. falciparum*
311 parasitemia reported herein is not surprising. The mean 8-iso-prostaglandin F2 α in malaria-SCD
312 patients was twice the mean 8-iso-prostaglandin F2 α in malaria-HbAA patients. The observed
313 differences could be attributed to cumulative effect of malaria infection in SCD. Previous study
314 in Kenya found SCD to increase the severity of malaria [32]. This study could link this finding to
315 excessive lipid peroxidation with the consequent elevation of 8-iso-prostaglandin F2 α . There is
316 limited data on the use of 8-iso-prostaglandin F2 α oxidative to assess oxidative stress in SCD
317 even though previous works have assessed oxidative stress using nitric Oxide (NO), superoxide,
318 peroxide, hydroxyl radicals [33, 34] and malondialdehyde (MDA) [33, 35-37]. The use 8-iso-
319 prostaglandin F2 α to assess oxidative stress in malaria is not popular, though 8-iso-
320 prostaglandin F2 α is currently the gold standard for the assessment of oxidative stress in
321 disease conditions [23, 24]. Oxidative stress due to reactive oxygen species (ROS) activity on
322 MDA has been implicated previously in the pathogenesis and complications in malaria. It has

323 been established that Falciparum infected human RBCs are under constant oxidative stress [38]
324 due to generation of ROS within erythrocytes infected cells and also from immune activation
325 [39, 40].

326 Previous study done in Ghana in patients below 20 years, reported that WBC and lymphocytes
327 in malaria patients were lower compared to control subjects. The other leucocytes sub-types
328 were not significantly different from control subjects [41]. However, the current study observed
329 significantly high leucocytes and leucocytes sub-types derangements in malaria-SCD except
330 neutrophils and basophils. Presentations of observed symptoms in malaria-SCD could be
331 related to the actions of pro-inflammatory cytokines with mononuclear cells having been
332 implicated as a key player [42]. This study found two novel cellular inflammatory biomarkers,
333 namely eosinophils-to-basophils ratio (EBR) and monocytes-to-basophils ratio (MBR), as being
334 associated with malaria-SCD. EBR>14 and MBR>22 and combination of the two, EBR>14-
335 MBR>22, could be used to predict malaria in sickle cell disease. The sensitivity of EBR>14-
336 MBR>22 was 11.56% and 7.02% higher than EBR>14 and MBR>22 when compared individually.
337 The specificity and negative predictive values of the novel biomarkers were greater than 90%;
338 this makes them very specific in excluding malaria in sickle cell disease. The ROCV obtained
339 makes the diagnostic use of EBR>14-MBR>22 better than EBR>14 and MBR>22. As leukocyte
340 ratios have been widely suggested to predict both communicable and non-communicable
341 diseases [43-45], our findings are suggestive of potential roles in *P. falciparum* pathophysiology.

342 In malaria-SCD, haemoglobin, hematocrit, mean cell haemoglobin and mean cell volume were
343 significantly reduced in a similar fashion as seen in microcytic and hypochromic anaemia. The

344 reductions in RBC and red cell indices associated with malaria-SCD could probably be due to
345 cumulative effect of increased rate of haemolysis during oxygenation and deoxygenation
346 process, reduced response to erythropoietin secretion in sickle cell anemia together with acute
347 malaria infections [46]. Significant elevations were observed in platelets, mean platelet volume,
348 platelet distribution width and plateletcrit in malaria-SCD patients. Relative thrombocytopenia
349 was seen in the malaria-HbAA patients. Malaria-related case vs. control thrombocytopenia has
350 been reported in several studies [6, 47-48]. Elevation in platelets and platelet indices suggests
351 efficient haemostasis in malaria-SCD than malaria infections in the absence of SCD.

352 **Conclusion**

353 EBR>14 and MBR>22 were novel cellular inflammatory biomarkers found to be associated with
354 malaria-SCD and can possibly be employed in the diagnosis of this co-morbidity. Additionally,
355 malaria-SCD levels of 8-iso-prostaglandin F2 α oxidative stress biomarker was twice as observed
356 in malaria-HbAA.

357 **List of abbreviations**

358 ANOVA-one way analysis of variance; EBR-eosinophils-to-basophils ratio; ELISA-enzyme lined
359 immuno-sorbent assay; EMR-eosinophils-to-monocytes ratio; HbS-Sickle cell hemoglobin; HbSS-
360 two HbS haplotypes; LBR-lymphocytes-to-basophils ratio; LER-lymphocytes-to-eosinophils ratio;
361 LMR-lymphocytes-to-monocytes ratio (LMR); malaria-HbAA- malaria in normal hemoglobin;
362 malaria-SCD-malaria in sickle cell disease; MDA-malondialdehyde; MBR-monocytes-to-basophils
363 ratio; MCH-mean cell hemoglobin; MCV-mean cell volume; NBR-neutrophils-to-basophils ratio;
364 NER-neutrophils-to-eosinophils ratio; NLR-neutrophils-to-lymphocytes ratio; NMR-neutrophils-
365 to-monocytes ratio; OD-optical density; PNR-platelet-to-neutrophils ratio; ROC-receiver
366 operating characteristic; SCD-Sickle cell disease; SCT-sickle cell trait; SSA-Sub-Saharan Africa;
367 TBAA-thiobarbituric acid assay

368 **Ethical approval**

369 Ethical approval for this study was granted by Ghana Health Service Ethics Review Committee
370 (Approval No: GHS-ERC002/03/18). Participant consent was sought for participant.
371

372 **Raw data**

373 All relevant data are within the paper.

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377 specimens for the study. We are also grateful to Nicholas Sowa for his immense contributions
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379 **Authors' contributions**

380 EA, PA, DOA, RKDE conceptualized, designed and coordinated the study. EA, EDT, PA performed
381 the statistical analysis and JB, EN participated in the sample collection and processing. AE, PA
382 drafted the manuscript, manuscript proofread by RKDE, AE-Y which was later approved by all
383 authors

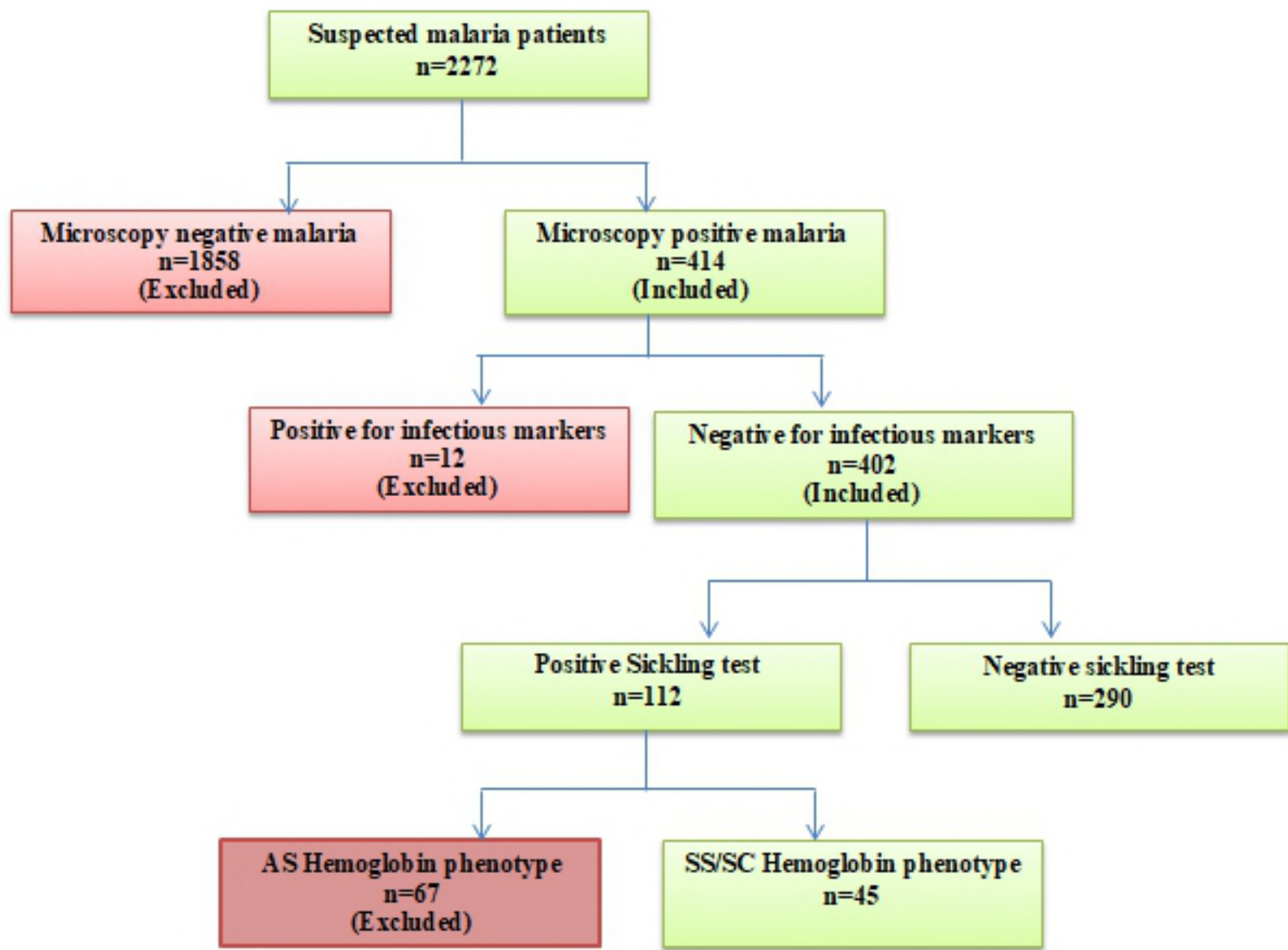
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Figure