Cytokine response during non-cerebral and cerebral malaria: evidence of a failure to control inflammation as a cause of death in African adults

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Background. With 198 million cases and 584,000 deaths in 2013, malaria remains one of the deadliest infectious diseases in tropical countries. Several species of the protozoan *Plasmodium* cause malaria. However, almost all the fatalities are due to *Plasmodium falciparum*, a species that causes the severest cases including cerebral malaria. Immune response to *Plasmodium falciparum* infection is mediated by the production of pro-inflammatory cytokines and chemokines whose actions are crucial for the control of the parasites. Following this response, the induction of anti-inflammatory hormones downregulates the inflammation thus preventing its adverse effects such as damages to various organs and death. **Methods.** We performed a retrospective, nonprobability sampling study using clinical data and sera samples from patients, mainly adults, suffering of non-cerebral or cerebral malaria in Dakar, Sénégal. Healthy individuals residing in the same area were included as controls. We measured the serum levels of 29 biomarkers including growth factors, chemokines, inflammatory and anti-inflammatory cytokines. **Results.** We found an induction of both pro- and anti-inflammatory biomarkers during malaria. The levels of pro-inflammatory biomarkers were higher in the cerebral malaria than in the non-cerebral malaria patients. In contrast, the concentrations of anti-inflammatory cytokines were comparable in these two groups. Additionally, six pro-inflammatory biomarkers were significantly increased in the deceased of cerebral malaria compared to the survivors. Regarding organ damage, kidney failure was significantly associated with death in adults suffering of cerebral malaria. **Conclusions.** Our results suggest that a poorly controlled inflammatory response determines a bad outcome in African adults suffering of cerebral malaria.

Cytokine Response during Non-cerebral and Cerebral Malaria: evidence of a failure to control inflammation as a cause of death in African adults

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Abstract

Background. With 198 million cases and 584,000 deaths in 2013, malaria remains one of the deadliest infectious diseases in tropical countries. Several species of the protozoan *Plasmodium* cause malaria. However, almost all the fatalities are due to *Plasmodium falciparum*, a species that causes the severest cases including cerebral malaria. Immune response to *Plasmodium falciparum* infection is mediated by the production of pro-inflammatory cytokines and chemokines whose actions are crucial for the control of the parasites. Following this response, the induction of anti-inflammatory hormones downregulates the inflammation thus preventing its adverse effects such as damages to various organs and death.

Methods. We performed a retrospective, nonprobability sampling study using clinical data and sera samples from patients, mainly adults, suffering of non-cerebral or cerebral malaria in Dakar, Sénégal. Healthy individuals residing in the same area were included as controls. We measured the serum levels of 29 biomarkers including growth factors, chemokines, inflammatory and anti-inflammatory cytokines.

Results. We found an induction of both pro- and anti-inflammatory biomarkers during malaria. The levels of pro-inflammatory biomarkers were higher in the cerebral malaria than in the non-cerebral malaria patients. In contrast, the concentrations of anti-inflammatory cytokines were comparable in these two groups. Additionally, six pro-inflammatory biomarkers were significantly increased in the deceased of cerebral malaria compared to the survivors. Regarding organ damage, kidney failure was significantly associated with death in adults suffering of cerebral malaria.

Conclusions. Our results suggest that a poorly controlled inflammatory response determines a bad outcome in African adults suffering of cerebral malaria.

Introduction

Despite a decade of sustained efforts that have substantially reduced mortality and morbidity due to malaria, this disease continues to represent an important heath concern in tropical countries (White et al. 2014). According to the World Health Organization (WHO), there were 198 million cases of malaria worldwide in 2013, which resulted in 584,000 deaths (WHO 2014). Ninety percent of the victims were from Africa, 78% being children under 5 years of age. Malaria is endemic in many sub-Saharan African countries. However, there are disparities between (WHO 2014) and even within countries (Espie et al. 2015) regarding the transmission of the disease. In many rural areas where the local environment favors the development of the mosquito vector and its interactions with humans, transmission of malaria is high and perennial (Trape et al. 2014). In contrast, in other areas including urban zones, the transmission of malaria is low to moderate and seasonal (White et al. 2014). Individuals living in regions of high and stable transmission progressively acquire immunity to the disease after experiencing and surviving to several infections (Olliaro 2008). This immunity protects against severe, life-threatening cases of malaria but does not confer a sterile protection (Doolan et al. 2009). In these areas, clinical malaria occurs in young children while healthy carriage of the parasite is common in adult. Adults who die of malaria typically are pregnant women or non-immune individuals from low transmission zones.

Several species of the protozoan *Plasmodium* causes malaria. However, almost all the deaths are due to *P. falciparum*, a species that causes the severest cases including cerebral malaria (Storm & Craig 2014). In response to *P. falciparum* infection, a robust immune inflammatory response takes place. An important component of this response is the production of inflammatory cytokines and chemokines whose actions are crucial for the control of the parasites (Deloron et

al. 1994; Lyke et al. 2004; Sarthou et al. 1997). This inflammatory response is rapidly followed by the production of anti-inflammatory cytokines that downregulate the inflammation preventing detrimental immune reactions (Kurtzhals et al. 1998; Peyron et al. 1994; Walther et al. 2005). Therefore, the immune response to *P. falciparum* infection is coordinated through a subtle balance of pro- and anti-inflammatory cytokines (Crompton et al. 2014; Frosch & John 2012). A rupture of this balance is at the basis of the events that lead to organ damage and death (Crompton et al. 2014). The pathogenesis of severe malaria and its associated mortality have been widely studied in children. In contrast, there are less investigations dealing with these aspects of the disease in adults, in particular from Africa (Olliaro 2008). In this study, we performed a retrospective analysis of the available clinical data and of the cytokine response of malaria patients, mainly adults, admitted at the Hôpital Principal de Dakar, Sénégal. Malaria is endemic in several areas in Sénégal. However, the capital city Dakar and its surroundings constitute a zone of low prevalence of malaria with a seasonal transmission. We report the analysis of data from control individuals and from patients suffering of non-cerebral (NCM) or cerebral malaria (CM). All the CM patients were adults and included deceased and survivors enabling to gain insights into the effect of the cytokine response in the outcome of the disease.

Materials and methods

Study population, ethics, consent and permissions

This study was performed on serum samples from patients diagnosed with malaria at the Hôpital Principal de Dakar, Sénégal between October 2012 and December 2014 (Torrentino-Madamet et al. 2014). The samples were taken after written consents from the patients or their accompanying family members. The controls corresponded to samples obtained from healthy volunteers residing in Dakar. This study was approved by the Université Cheikh Anta Diop de Dakar's

91 institutional research ethics committee (Protocol N° 001/2015/CER/UCAD). Venous blood
92 samples were collected in Vacutainer® ACD tubes (Becton Dickinson, Rutherford, NJ, USA)
93 prior to patient treatment. *Plasmodium* presence and density in blood samples were determined
94 by microscopic examination of thin blood smears stained with a 10% May-Grünwald Giemsa
95 solution (SigmaR, St-Louis, MO, USA). *P. falciparum* was the only species found. Blood
96 parameters were determined at the hospital's clinical laboratory. The following criteria were
97 used for enrollment into the two groups of Malaria patients. Life-threatening CM was
98 characterized by at least one of ten criteria defined by the WHO including cerebral symptoms,
99 coma, severe anemia, respiratory distress, liver dysfunction, renal impairment, lactic acidosis,
100 hypoglycemia, renal failure, and non-cardiogenic pulmonary edema. NCM cases were defined by
101 fever and presence of *P. falciparum* in blood smear, without other infections or symptoms of
102 severe malaria as defined by the WHO (WHO 2000). Malaria patients were treated according to
103 a protocol based on the Senegalese national recommendations that consisted to intramuscular
104 administrations of 20 mg/kg quinine every eight hours. Secondary samples analyzed in this study
105 corresponded to blood taken from survivors of CM before patient release from the hospital (1-15
106 days after admission).

107 **Biomarker measurement**

108 Serum biomarkers were measured using a Milliplex MAP kit for human cytokine/chemokine
109 magnetic bead panel (catalogue # HCYTMAG-60K-PX29, EMD Millipore Corporation,
110 Billerica, MA, USA) according to the recommendations of the manufacturer. The levels of 29
111 cytokines were measured in each sample including interleukin (IL)-1α, IL-1β, IL-1RA, IL-2, IL-
112 3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, interferon
113 (IFN)α2, IFNγ, IFN-inducible protein 10 (IP-10, CXCL-10), epidermal growth factor (EGF),

114 eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-
115 stimulating factor (GM-CSF), tumor necrosis factor (TNF)α, TNFβ, monocyte chemotactic
116 protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, and vascular endothelial
117 growth factor (VEGF). The measurements were performed using a Luminex 200™ system with
118 xPONENT™ software, version 3.1 (Luminex, Austin, TX, USA).

119 **Statistical analyses**

120 The statistical analyses were carried out using the IBM SPSS 22.0 software. Non-parametric
121 tests were used to compare the levels of biomarker and their correlation with other variables
122 across different groups. Mann-Whitney U test and Kruskal-Wallis one-way ANOVA were used
123 to compare data across two and three groups respectively. Wilcoxon matched pairs signed rank
124 test was used to compare biomarker levels in sera from CM patients at admission and at their
125 release from the hospital. Correlation tests were performed using Spearman's Rho rank test.
126 Pearson Chi-Square was used to test association of organ failure with outcome in CM patients.
127 For all the statistical analyses, a p value < 0.5 was considered as significant. The biomarker
128 profiles were determined as previously described (da Costa et al. 2014). In brief, the median
129 value in the global population (CT + NCM +CM) was calculated for each biomarker and used as
130 a cut-off to determine the percentage of "high" (above the median value) and "low" (below the
131 median value) producers in each group. An ascendant biomarker profile was then constructed in
132 the CT group by assembling the biomarkers from the one having the smallest percentage of high
133 producers to the one having the largest. The resulting ascendant curve was used as a reference to
134 visualize the variation of the percentage of high producers of biomarkers in the other groups
135 (Fig. 1).

136 **Results**

137 **Study population and clinical data**

138 We performed a retrospective, nonprobability sampling study using sera samples from healthy
139 individuals and from patients admitted at the Hôpital Principal de Dakar, Sénégal. The cohort
140 included 17 and 34 subjects diagnosed with NCM and CM respectively, and 18 healthy controls
141 (CT) (Table 1). The three groups of individuals were comparable in age and gender, and were
142 mainly composed of adults (Table 1). The only non-adults included six children aged 5-13
143 diagnosed with NCM and one 15-year old CM patient. Several clinical and blood parameters
144 existed but were not recorded for all the individuals preventing reliable statistical analyses.
145 Available data showed, as expected, hemoglobin levels comparable in the CT and NCM
146 individuals while significantly lower in the CM patients. Additionally, parasitemia was
147 comparable between the NCM and CM groups. Regarding organ defect in the CM group, kidney
148 failure was the most frequent (13/27) followed by liver, hematologic and respiratory
149 malfunction, while hemodynamic failure was rare (Table 1). All the NCM patients were
150 successfully treated, while 9/27 CM subjects died.

151 **Levels of inflammatory but not of anti-inflammatory biomarkers were higher in CM than**
152 **in NCM patients**

153 To analyze cytokine response during malaria, we measured the serum levels of 29 biomarkers
154 including growth factors, chemokines, inflammatory and anti-inflammatory cytokines. We
155 determined the ascendant biomarker profile of the CT group as previously described (da Costa et
156 al. 2014) and plotted the resulting curve on the profiles of the NCM and CM patients (Fig. 1).
157 Several cytokines were significantly higher in malaria patients (NCM and CM) than in CT
158 individuals (Figs. 1B and 1C). These biomarkers included most of the pro-inflammatory
159 cytokines tested (IL-1α, IL-6, IL-8, IL-15, IL-17A, IP-10, TNFα, IFNα2, IFNγ and MCP-1) and

the anti-inflammatory hormones IL-10 and IL-1RA. Additionally, we observed significant correlations between several biomarkers. Noticeably, in both NCM and CM patients, we detected a strong positive correlation between TNFα and IL-10 (Spearman's Rho coefficient of 0.855 in NCM and 0.867 in CM, $p < 0.001$) typical pro- and anti-inflammatory cytokines respectively. The induction of both inflammatory and anti-inflammatory cytokines that aims to respond to the infection while controlling the level of inflammation in order to prevent damages to host organs has been well documented in malaria patients (Frosch & John 2012). Next, we analyzed the difference of cytokine levels between the NCM and CM patients. Most of the biomarkers induced during malaria were significantly higher in CM than in the NCM individuals including the pro-inflammatory cytokines IL-6, IL-8, IL-15, TNFα, and MCP-1 (Fig. 2). These results indicate an inflammatory response of higher magnitude in CM compared to NCM patients as previously mentioned in several reports (Crompton et al. 2014). In contrast, the level of IL-10 was not significantly different between NCM and CM patients (Fig. 2). Additionally, the level of IL-5, a Th2 anti-inflammatory cytokine, was significantly lower in CM than in NCM patients (Fig. 2). These results suggest that the level of anti-inflammatory response did not match the strength of the inflammatory cytokine response in the CM patients.

Levels of inflammatory biomarkers were lower in survivors than in deceased of CM

Failure to control inflammation is proposed as one of the mechanisms leading to CM, which is consistent with the difference we observed between NCM and CM patients. To further analyze the effect of the inflammatory cytokines, we compared the levels of biomarker between the survivors ($n = 18$) and the deceased ($n = 9$) of CM. Interestingly, there were six biomarkers whose levels significantly differed between the two groups. All were pro-inflammatory cytokines (Eotaxin, IL-6, IL-8, IL-15, MCP-1 and TNFα**)** that were significantly lower in the survivors

than in the deceased of CM (Fig. 3). These results suggest that the cause of death involved an inflammatory response of high magnitude that was not properly controlled. To analyze possible effects of the inflammatory response in tissue damage, we compared the failure of different organs between survivors and deceased CM patients. Kidney failure was significantly associated with patient's death (χ^2 [1, N = 27] = 8.98, p = 0.003; Effect Size = 0.58) while the occurrence neurological, respiratory, liver, hematologic and hemodynamic failures were comparable between the two groups. We further attempted to correlate the biomarker levels with organ failure. Kidney failure showed significant moderate to strong positive correlations with several chemokines and pro-inflammatory cytokines (Table 2), while respiratory, hematological and liver failure displayed weak positive correlations with 5, 5 and 1 biomarkers respectively (not shown).

Variation of biomarker levels before and after cerebral malaria treatment

Analysis of biomarker profiles in malaria patients before and after treatment provides valuable information on cytokines that are induced during malaria. We compared the levels of cytokine between the times of emergency admission and of hospital release in 14 CM patients (Table 3). Wilcoxon rank test showed 10 biomarkers (GM-CSF, G-CSF, IL-10, IL-1α, IL-6, IL-8, IP-10, MCP-1, MIP-1β, TNFα) that were significantly different between the two time points. All these hormones were lower in the second samples confirming the induction of different types of cytokine including growth factor (GM-CSF, G-CSF), inflammatory (TNFα, IL-1α, IL-6, IP-10), anti-inflammatory (IL-10) and chemokines (IL-8, MCP-1, MIP-1β) during immune response to malaria (Table 3).

Discussion

Inflammation and outcome of cerebral malaria

In this study, we performed a retrospective analysis of 18 controls, and of 17 and 27 NCM and CM patients respectively. The CM patients included 18 survivors and 9 (30%) deceased subjects, a proportion similar to the highest mortality rates reported for CM. Beside neurological defect, Kidney failure was the most frequent organ malfunction in CM patients and was correlated with death. Analysis of the cytokine response showed a strong induction of pro- and anti-inflammatory biomarkers in malaria patients. However, the magnitude of this response was significantly higher in CM than in NCM patients for inflammatory biomarkers while it was comparable in the two groups for the anti-inflammatory cytokines. Additionally, comparison of the biomarkers in the survivors versus the deceased of CM showed six pro-inflammatory cytokines that were significantly higher in the deceased patients. Altogether, our results suggest a scenario in which a strong inflammatory response that was not properly contained led to organ failure and death during CM. The mechanism of CM pathogenesis is not fully understood. There is an old ongoing debate opposing two schools regarding the primary cause of CM (Berendt et al. 1994; Clark & Rockett 1994). One school proposes that sequestration of parasite-infected red blood cells in the brain capillary vessels and agglutination with uninfected red blood cells cause an obstruction that prevents blood flow leading to damages of brain tissues (Storm & Craig 2014; White et al. 2013). The other school primarily attributes CM to a strong production of inflammatory cytokines that results in the recruitment of immune cells in the brain (Clark et al. 2008). A recent theory proposes a conjunction of liver failure with a disruption of the blood brain barrier as sufficient to account for CM (Martins & Daniel-Ribeiro 2013). However, since < 30% (8/27) of our patients displayed liver failure, this theory might explain some but not all CM cases. Another recent hypothesis proposes severe hemolysis of infected red blood cells (RBC) as the primary cause of CM (Eisenhut 2015a; Eisenhut 2015b). In this hypothesis, cell-free

229 hemoglobin released from disrupted red blood cells is oxidized and scavenges endothelial nitric
230 oxide (NO) obliterating its protective effects. This leads to an increased sequestration of
231 parasitized RBC together with a vasospasm (Eisenhut 2015a). Additionally, continuous release
232 of parasites from lysed RBC may increase the production of pro-inflammatory cytokines. This
233 hypothesis has the advantage of reconciling the sequestration and the inflammation theories.
234 However, it needs to be documented in more human cases of CM (Shabani et al. 2015).
235 Regarding the inflammatory response, although it might not be the primary cause of CM, its
236 importance in the outcome of the disease has been widely reported. Several studies in humans
237 attempted to correlate individual biomarkers to the severity and the outcome of malaria.
238 However, these studies have identified different biomarkers depending on the patients analyzed
239 and the cytokines tested. For example, IP-10 has been identified as a predictor of fatal CM in at
240 least two independent studies (Armah et al. 2007; Wilson et al. 2011) and its importance in CM
241 outcome was further suggested by animal experiments showing that inactivation of its encoding
242 gene conferred a protection against experimental CM fatality (Campanella et al. 2008; Wilson et
243 al. 2013). In contrast, Jain et al identified Angiopoietin-1 and -2 as predictor of CM outcome
244 (Jain et al. 2011) and Prakash et *al*. detected IL-1β, Il-12 and IFNγ as discriminators between
245 CM and severe NCM (Prakash et al. 2006). Another study found the anti-inflammatory cytokine
246 IL-10 as a key factor whose decrease promoted severe malaria (Mahanta et al. 2015). In this
247 study, we found that the pro-inflammatory biomarkers TNFα, eotaxin, IL-6, IL-8, IL-15, and
248 MCP-1 were significantly elevated in deceased CM patients compared to survivors. These
249 differences are not surprising given that the levels of circulating cytokines measured ex-vivo may depend
250 on several factors including host genotype (Basu et al. 2012; Jha et al. 2013; Pereira et al. 2015;
251 Zhang et al. 2012), the parasite strain (Pattaradilokrat et al. 2014), the intrinsic stability of the

cytokines, and the processing of the samples. However, beyond the particular biomarkers highlighted, the mentioned studies are consistent in showing a link between severe malaria and a fatal outcome of the disease to high production of inflammatory and/or low induction of anti-inflammatory cytokines. Given the complexity of CM pathogenesis that is shaped by multiple interactions between host and parasite factors, a more powerful approach to elucidate the mechanism and the influence of the inflammatory response in this disease would simultaneously analyze various parameters including the biomarker profiles and the genetic polymorphisms of the sequences and the regulatory elements of host and parasite genes encoding important pro- and anti-inflammatory cytokines and virulence factors respectively (Tran et al. 2012).

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384

Table 1(on next page)

Demographic, clinical, and disease outcome data.

1

		CT	NCM	CM	Total
Gender	Male	11	11	22	44
	Female	7	6	5	17
Age	Range	23-57	5-74	15-80	5-80
	Median	28.5	18	26	26
HB	Normal	18	17	11	46
	Low	0	0	16	16
Outcome	Survived	18	17	18	53
	Deceased	0	0	9	9
Organ Failure	Neurological	0	0	27	27
	Respiratory	0	0	6	6
	Kidney	0	0	13	13
	Liver	0	0	8	8
	Hematologic	0	0	7	7
	Hemodynamic	0	0	2	2

CT, control individuals; NCM, non-cerebral malaria patients; CM, cerebral malaria patients; HB, hemoglobin (Low, < 100 g/L; Normal, > 100 g/L).

2

Table 2(on next page)

Biomarkers correlated with kidney failure in cerebral malaria patients.

1

	ρ (*p* value)		ρ (*p* value)		ρ (*p* value)
Eotaxin	0.514 (0.006)	**IL-10**	0.457 (0.017)	**IL-1α**	0.593 (0.001)
G-CSF	0.500 (0.008)	**IL-12p70**	0.445 (0.020)	**IP-10**	0.533 (0.004)
GM-CSF	0.714 (<0.001)	**IL-15**	0.621 (0.001)	**MCP-1**	0.581 (0.002)
IFNα2	0.525 (0.005)	**IL-17A**	0.401 (0.038)	**TNFα**	0.542 (0.003)
IFNγ	0.529 (0.005)	**IL-1RA**	0.390 (0.044)		

ρ, Spearman's Rho coefficient.

2

Table 3(on next page)

Variation of biomarker levels between admission and release from hospital in cerebral malaria patients.

1

	Admission (pg/ml) MN ± SD / MD	Release (pg/ml) MN ± SD/ MD	*P* value
G-CSF	651 ± 1869 / 163	116 ± 111 / 99	0.007
GM-CSF	128 ± 450 / 12	37 ± 110 / 8	0.030
IL-10	1848 ± 1749 / 464	99 ± 165 / 40	0.002
TNFα	90 ± 101 / 60	24 ± 14 / 20	0.002
IL-1α	121 ± 165 / 76	57 ± 80 / 28	0.013
IL-6	161 ± 274 / 57	53 ± 77 / 18	0.044
IL-8	213 ± 417 / 66	58 ± 152 / 14	0.001
IP-10	5668 ± 6297 / 2496	1757 ± 2191 / 892	0.002
MCP-1	1303 ± 1754 / 508	409 ± 678 / 242	0.005
MIP-1β	182 ± 276 / 72	95 ± 112 / 70	0.023

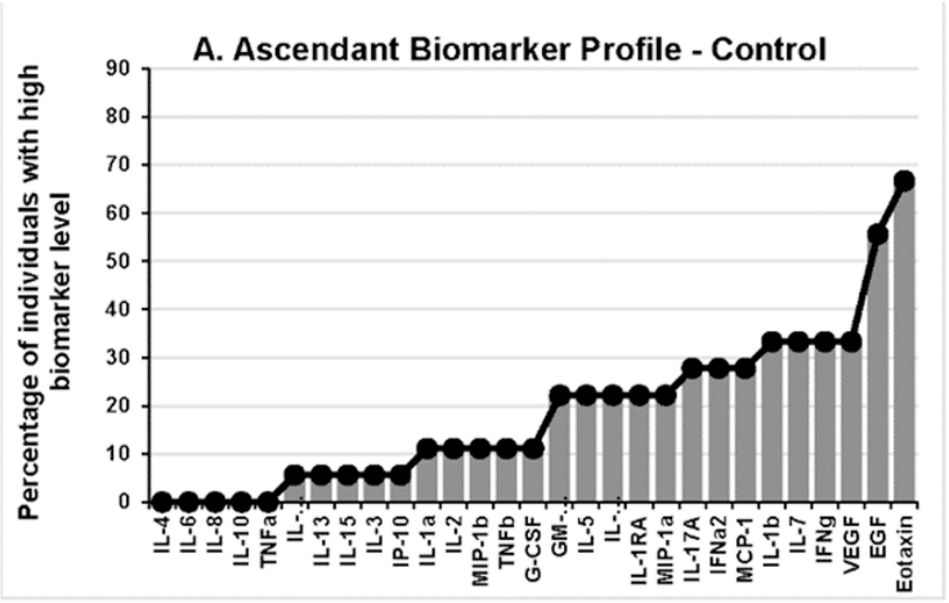
Admission, biomarker levels at the time of hospital admission of CM patients; Release, biomarker levels at the time of release of CM patients from hospital; *P* value, two-tailed *p* value of a Wilcoxon Rank test; MN, mean; SD, standard deviation; MD, median.

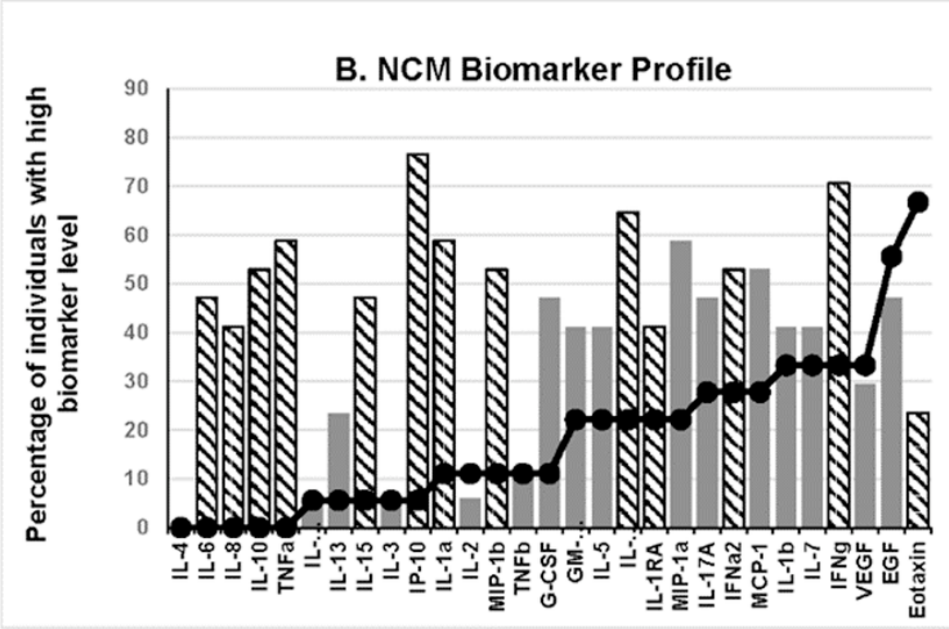
2

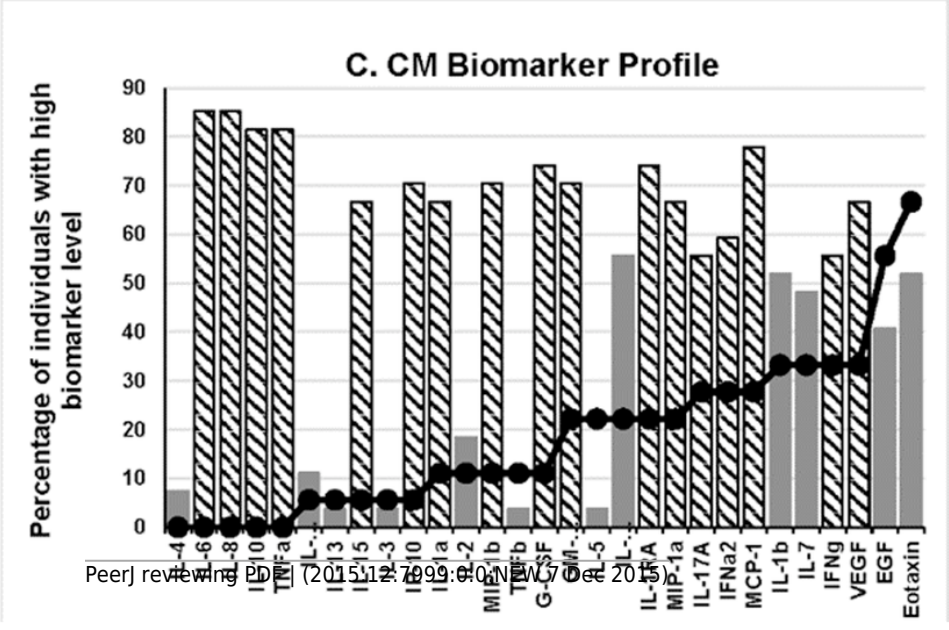
1

Serum cytokine response during malaria.

The levels of 29 cytokines were measured in control subjects (CT) and in non-cerebral (NCM) and cerebral (CM) malaria patients. The median value of each cytokine in the global population (CT + NCM + CM) was used as a cut-off value to determine the percentage of “high” (above median) and low (below median) biomarker producers in each group. The ascendant biomarker profile of the CT (A) was determined and the resulting curve used as a reference to visualize the variation of the percentage of high biomarker producers in the NCM (B) and CM (C) groups. Hatched bars represent biomarkers for which there was a significant difference with the CT reference group using Mann Whitney U test.


A. Ascendant Biomarker Profile - Control
Percentage of individuals with high biomarker level
0
10
20
30
40
50
60
70
80
90
IL-4
IL-6
IL-8
IL-10
TNFa
IL-...
IL-13
IL-15
IL-3
IP-10
IL-1a
IL-2
MIP-1b
TNFb
G-CSF
GM-...
IL-5
IL-...
IL-1RA
MIP-1a
IL-17A
IFNa2
MCP-1
IL-1b
IL-7
IFNg
VEGF
EGF
Eotaxin

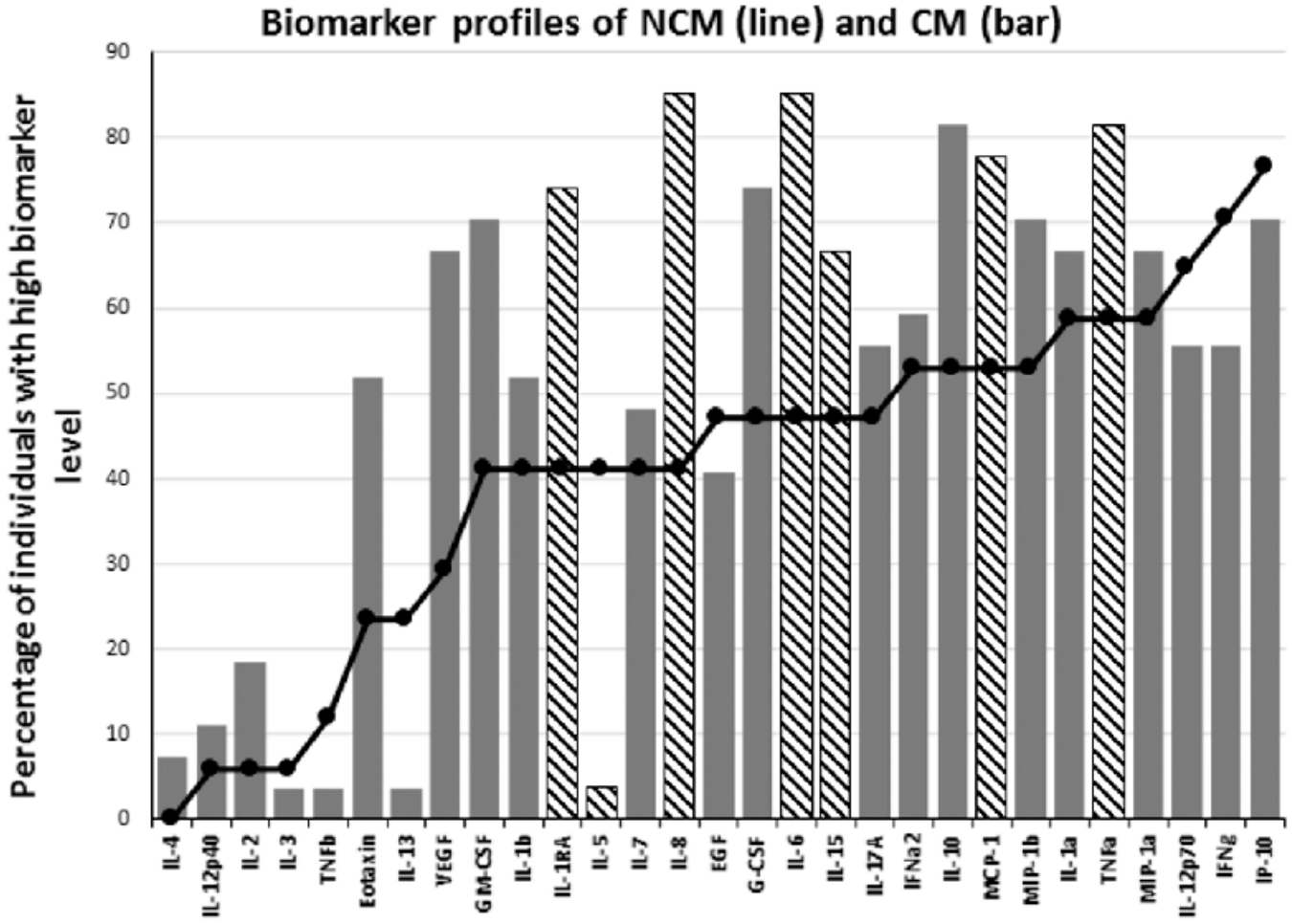

B. NCM Biomarker Profile
Percentage of individuals with high biomarker level
0
10
20
30
40
50
60
70
80
90
IL-4
IL-6
IL-8
IL-10
TNFa
IL-...
IL-13
IL-15
IL-3
IP-10
IL-1a
IL-2
MIP-1b
TNFb
G-CSF
GM-...
IL-5
IL-...
IL-1RA
MIP-1a
IL-17A
IFNa2
MCP-1
IL-1b
IL-7
IFNg
VEGF
EGF
Eotaxin


C. CM Biomarker Profile
Percentage of individuals with high biomarker level
0
10
20
30
40
50
60
70
80
90
IL-1RA
MIP-1a
IL-17A
IFNa2
MCP-1
IL-1b
IL-7
IFNg
VEGF
EGF
Eotaxin

2

Inflammatory cytokine response is stronger in cerebral than in non-cerebral malaria patients.

The ascendant biomarker profile curve of the NCM (line) was plotted on the CM graph (bars) to visualize the differences in the percentage of high biomarker producers. Hatched bars represent biomarkers for which there was a significant difference between the two groups using Mann Whitney U test.



3

Inflammatory cytokine response is stronger in deceased than in survivors of cerebral malaria.

The ascendant biomarker profile curve of the survivors (line) was plotted on the deceased's graph (bars) to visualize the differences in the percentage of high biomarker producers. Hatched bars represent biomarkers for which there is a significant difference between the two groups using Mann Whitney U test.