

Supplementary Methods

Blood collection and hematology

Patient peripheral venous blood was collected into 3 blood tubes in the following order; 2 mL BD Vacutainer® with no additives, 4.5 mL BD Vacutainer® containing a mixture of citrate, theophylline, adenosine and dipyridamole (CTAD), and a 10 mL BD Vacutainer® containing lithium-heparin. Blood remaining in the butterfly tubing was collected into a BD Microtainer® containing ethylenediaminetetraacetic acid to make blood smears and obtain a complete blood count on a Sysmex XS-1000i hematology analyzer (Hyogo, Japan). Patient blood smears were stained with 3% Giemsa and read in the research lab by two qualified microscopists to confirm hospital diagnoses and determine parasite counts (Obare Method Calculator). Patient platelet-free plasma from twice-centrifuged CTAD-coagulated blood and heparin plasma were collected and stored at -80°C. Longitudinal blood samples (except heparinized blood) were available for a subset of patients requiring hospital admission and had blood recollected upon hospital discharge.

Supplementary Materials

Table S1. Clinical features of severe malaria patients

Patient	Severe malaria criteria ^a	Symptoms ^b	Pulse (beats per min)	Respiratory rate (breaths per min)	Parasitaemia (parasites/ μ L)	Neutrophil activation (ng/mL)			NET count per μ L	
						NE	PR3	MPO	Giemsa	IF
1	Jaundice	F, H	81	20	60000	122	278	173	-	1050
2	Hyperparasitaemia	F, H	88	20	765000	212	438	289	-	58.2
3	Jaundice, AKI, hyperparasitaemia	F, H	120	28	346000	2660	2420	308	900	147
4	Jaundice, AKI, acidosis	F, H	95	28	1560	193	619	188	423	302
5	CM, hypoglycaemia, acidosis, ARDS	F, H, C, V, D, CR, W	118	41	221000	1240	2010	538	396	198
6	Jaundice	F, H, D	142	24	197000	2570	2240	483	252	108
7	Jaundice, hyperparasitaemia	F, H, C	90	24	988000	1560	1930	-	480	172
8	Hyperparasitaemia	F, H, V	117	24	683000	2050	2570	383	29.3	-

Footnotes:

^a AKI, severe acute kidney injury (creatinine > 265 $\mu\text{mol/L}$); CM, cerebral malaria; ARDS, acute respiratory distress syndrome.

^b F, fever; H, headache; C, cough; V, vomiting; D, diarrhoea; CR, crepitations; W, wheezing.

Table S2. Neutrophil activation and NETs in malaria patients

	Controls	<i>P. falciparum</i>		<i>P</i> -value ^b	UM <i>P. vivax</i>	UM <i>P. malariae</i>	<i>P</i> -value ^c
		UM	Severe				
Neutrophil elastase (ng/mL)	43.6 [36.9-53.1]	133.9****/ ## [87.5-219.2]	1398**** [197.7-2440]	<0.0001	120.8**** [81.8-155.7]	89.4*** [74.4-115.5]	<0.0001
	<i>n</i> =23	<i>n</i> =46	<i>n</i> =8		<i>n</i> =36	<i>n</i> =13	
Proteinase-3 (ng/mL)	36.6 [22.0-51.6]	213.9****/ ### [122.8-372.3]	1970**** [483.2-2374]	<0.0001	152.1**** [125.6-205.9]	111.1*** [89.1-159.5]	<0.0001
	<i>n</i> =23	<i>n</i> =46	<i>n</i> =8		<i>n</i> =36	<i>n</i> =13	
Myeloperoxidase (ng/mL)	51.8 [42.1-72.8]	67.0 ### [48.3-97.1]	308.2**** [188.2-482.9]	<0.0001	84.0 [50.1-111.9]	53.7 [39.5-73.8]	0.090
	<i>n</i> =23	<i>n</i> =47	<i>n</i> =7		<i>n</i> =37	<i>n</i> =14	
NETs per µL^a (Giemsa)	51.0 [20.8-70.8]	94.4*/ ## [66.1-168.9]	409.5* [243.7-584.6]	<0.0001	171.7** [78.7-315.1]	147.3** [84.4-228.7]	0.002
	<i>n</i> =12	<i>n</i> =29	<i>n</i> =6		<i>n</i> =16	<i>n</i> =12	
NETs per µL (IF)	10.9 [0-31.7]	39.7**/ # [30.4-76.1]	159.4**** [70.6-276.1]	<0.0001	43.8* [13.6-171.1]	48.7** [26.6-132.1]	0.009
	<i>n</i> =12	<i>n</i> =28	<i>n</i> =8		<i>n</i> =16	<i>n</i> =13	

Footnotes:

All values are median [interquartile range] unless otherwise indicated.

^a $n=19$ asymptomatic *P. falciparum* 102.0 [77.4-177.3], and $n=21$ asymptomatic *P. vivax* median 114.6 [82.7-145.8].

^b Kruskal-Wallis with Dunn's multiple comparisons test between controls, and uncomplicated and severe *P. falciparum*.

^c Kruskal-Wallis with Dunn's multiple comparisons test between controls, and uncomplicated *P. vivax* and *P. malariae*.

Significantly different to control: **** $p<0.0001$, *** $p<0.0005$, ** $p<0.005$, * $p<0.05$.

Significantly different to severe *P. falciparum*: ### $p<0.0001$, ## $p<0.0005$, # $p<0.05$ (Mann-Whitney test).

Abbreviations= NETs, neutrophil extracellular traps; IF, immunofluorescence; UM, uncomplicated malaria.

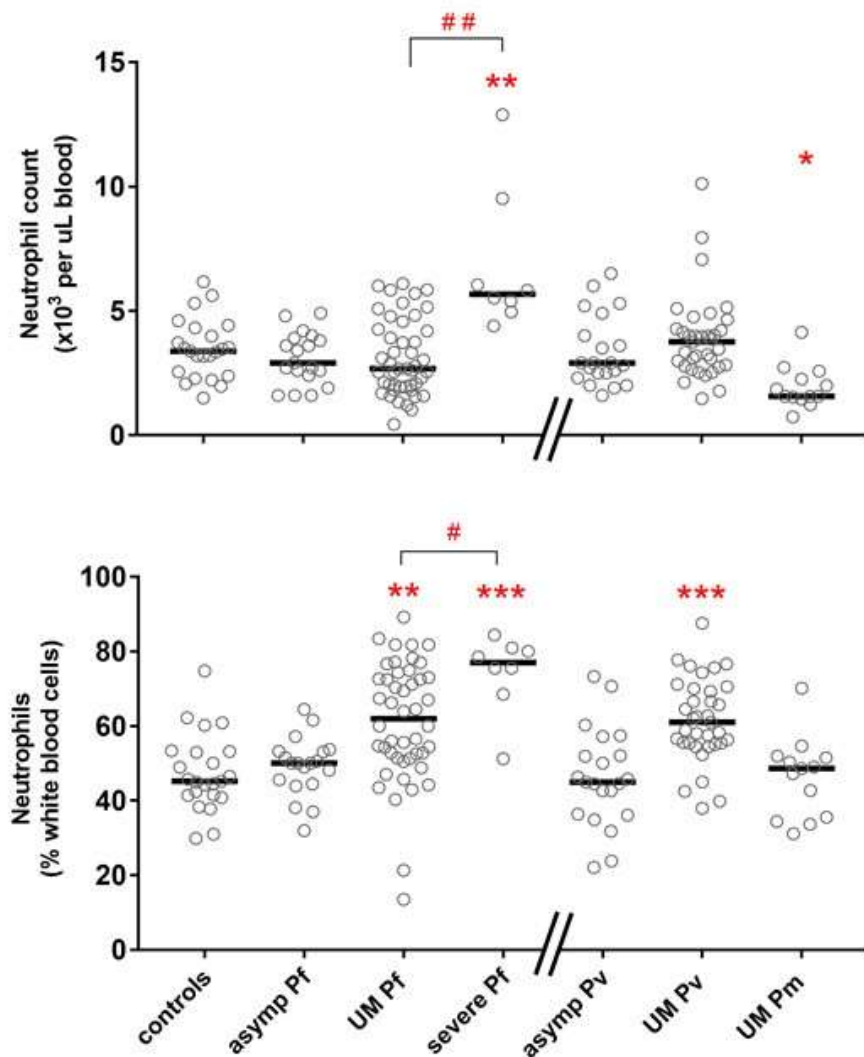


Figure S1. Distribution of neutrophils between infection groups. Neutrophil counts and percentages from automated blood counts in controls (n=23), individuals with asymptomatic *P. falciparum* (Pf) (n=19), uncomplicated malaria (UM) with Pf (n=46), severe Pf (n=8), asymptomatic *P. vivax* (Pv) (n=21), UM with Pv (n=35) and UM with *P. malariae* (Pm) (n=13). Plots show median with individual datapoints in each group. The Kruskal-Wallis with Dunn's multiple comparisons test was used for statistical comparisons to controls. Significant differences in all plots are signified by asterisks (*p<0.005, **p<0.0005, ***p<0.0001). The Mann-Whitney test was used for comparison between Um and severe Pf (#p<0.05, ##p<0.0001). Data presented in **Table 1**.

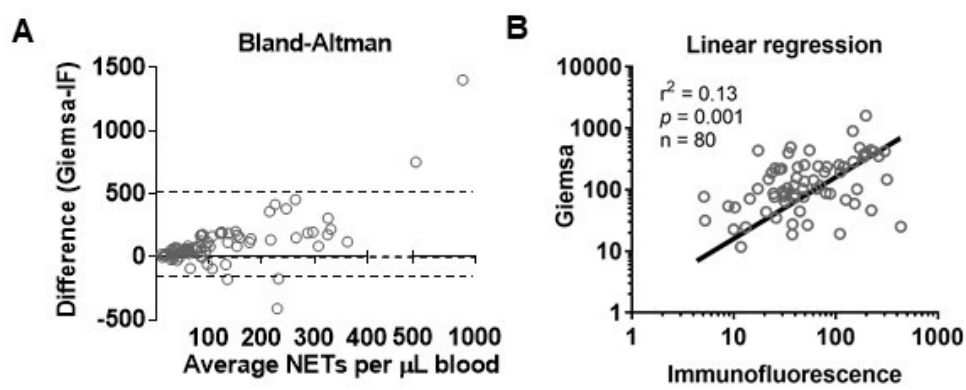


Figure S2. Comparison of Giemsa and immunofluorescent staining to count NETs. The Bland-Altman plot (A) and simple linear regression with robust standard errors (B) were used to compare NET counts per μL blood from 80 samples analyzed by Giemsa and immunofluorescent staining (IF). NET count data were combined from all patients and controls. The Bland-Altman plot shows the average NET count for each patient on the x-axis $([\text{Giemsa} + \text{IF}] \div 2)$, and the difference between NET counts for each patient on the y-axis $(\text{Giemsa} - \text{IF})$. Dotted lines represent the 95% confidence interval.