

THE LANCET Microbe

Supplementary appendix

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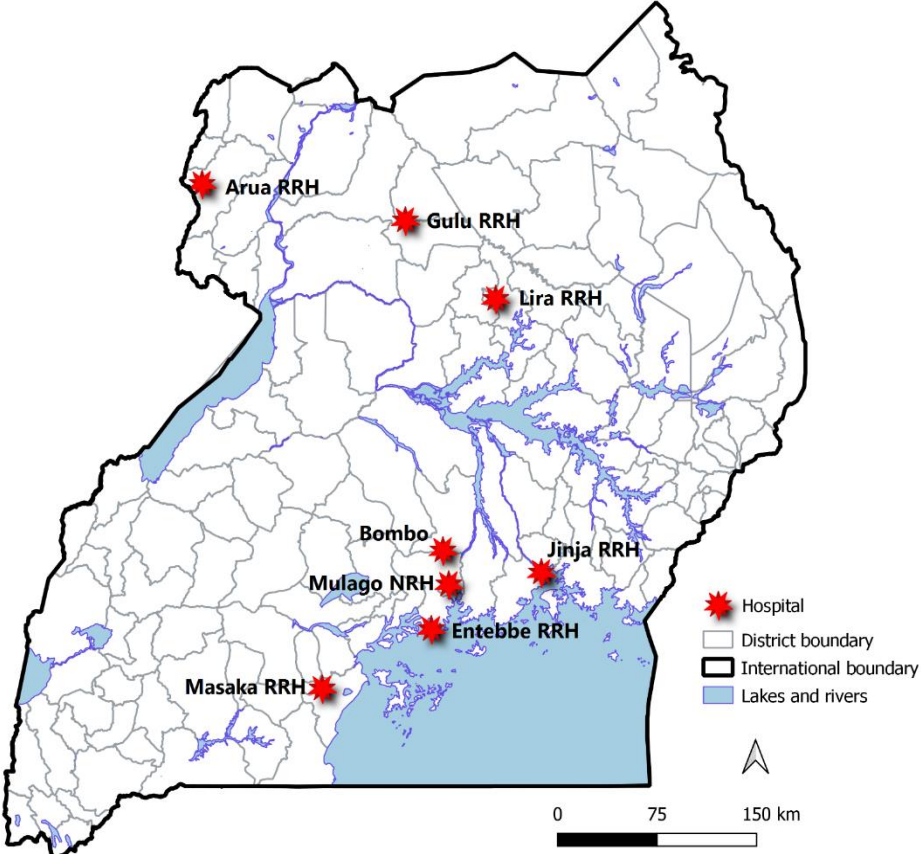
SUPPLEMENTARY APPENDIX

Title: Current malaria infection, previous malaria exposure and clinical profiles and outcomes of COVID-19 in a high malaria transmission setting: an exploratory cohort study in Uganda

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Figure S1. Map showing location of COVID-19 treatment centers and study sites in Uganda



Supplementary Appendix A: Molecular Detection of *P. falciparum*

Molecular detection of *P. falciparum* was done at the Molecular Laboratory at Makerere University in Kampala using established methodologies.¹ Briefly, three 6mm dried filter paper blood spots (DBS) were punched into a 96-well plate and digested in 20 µl of proteinase K and 180 µl of ATL tissue lysis buffer solution (Qiagen, Hilden, Germany). DNA extraction was done using the QIAamp 96 DNA QIAcube HT kit (Qiagen, Hilden, Germany) and Qiacube HT[®] robot. Extracted DNA was analysed with *varATS* qPCR using 5µL of extracted DNA per assay. For the analysis, the *var* gene acidic terminal sequence (*varATS*, 59 copies/genome) of *P. falciparum* (primer sequence: 5'- GTTC(A/T)A(A/G)TAC(C/T)ACTTC(A/T)AT(C/T)CCTGGT(A/G)CATATATATCATTAAATATCCAATTCTTCATA(C/T)TCACTTC(T/G)GA(A/T/G)GA-3') was amplified as previously described.¹ All PCR reactions included 10 standards prepared from tenfold serially diluted samples containing known numbers of infected erythrocytes diluted in whole blood. The limit of detection of the PCR assay is approximately 0.2 parasites/µl of blood.¹ The PCR output was analysed using the BioRad CFX Manager Software version 3.1. The sensitivity of the assay was approximately 0.02 parasites/µL of blood.

References

Hofmann N, Mwingira F, Shekalaghe S, Robinson LJ, Mueller I, Felger I. Ultra-sensitive detection of *Plasmodium falciparum* by amplification of multi-copy subtelomeric targets. *PLoS Med.* 2015; **12**:e1001788. doi: 10.1371/journal.pmed.1001788.

Figure S2. Study flow chart

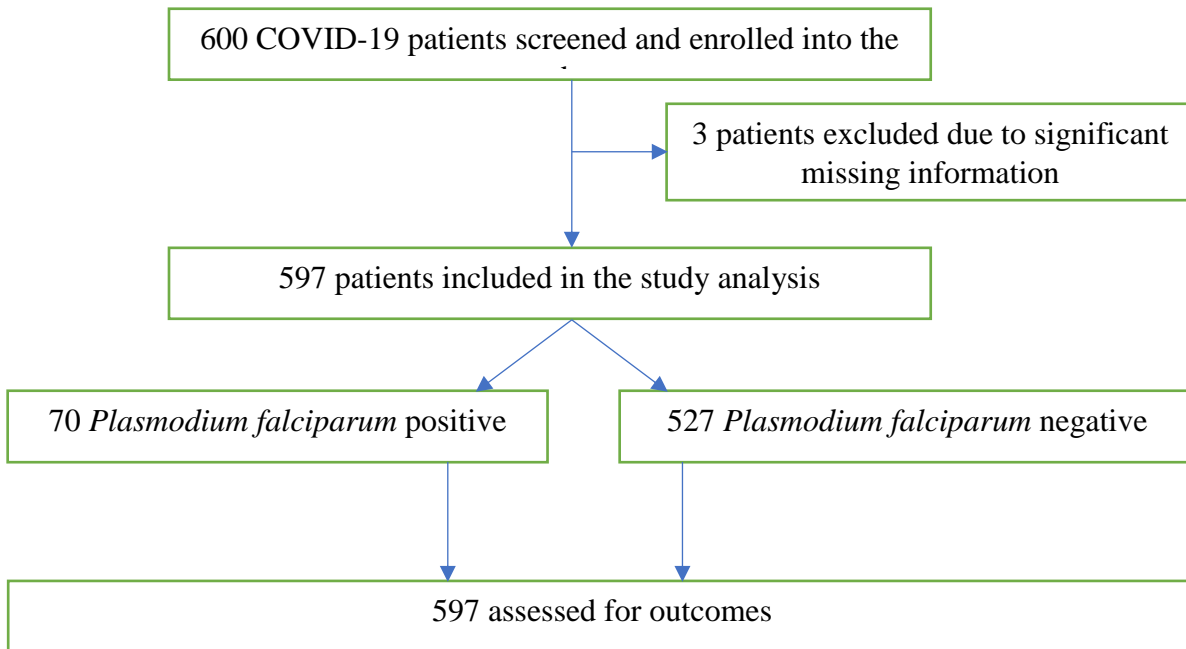


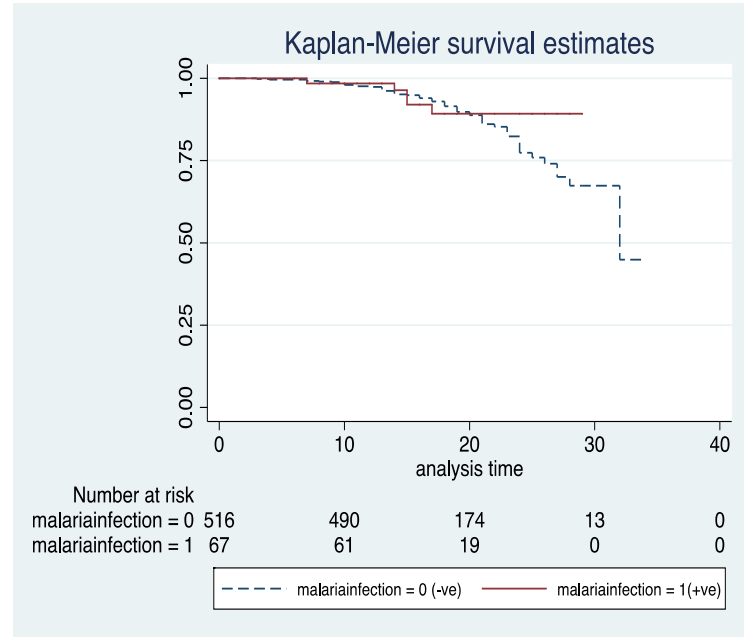
Table S1. Laboratory parameters of Covid-19 patients by *P. falciparum* infection status

Characteristic	Overall	<i>P. falciparum</i> positive	<i>P. falciparum</i> negative	P - value
HIV Positive [‡] , n	543 35 (6%)	64 7 (11%)	479 28 (6%)	0.12
Neutrophils, n	581 1980 (1230-2960)	67 1820 (1270-2750)	514 2000 (1220-2980)	0.48
Lymphocytes, n	580 2030 (1570-2560)	67 1920 (1490-2490)	513 2050 (1580-2580)	0.37
Hemoglobin*, n	583 15.0 (0.1)	67 14.6 (0.3)	516 15.1 (0.1)	0.06
Platelet count*, n	581 237,400 (89100)	66 216,400 (10500)	515 240100 (3900)	0.040
Aspartate aminotransferase (AST) U/L, n	505 24 (19.1-31.8)	53 21.3 (17.8-26.2)	452 24.1 (19.2-33.2)	0.015
Alanine aminotransferase (ALTI) U/L, n	542 26.1 (16.9-40.3)	57 21.7 (15.1-29.3)	485 26.8 (17.1-41.5)	0.020
Gamma-glutamyl transferase (GGT) U/L, n	514 35 (23.7-60)	58 27.5 (20.9-41.3)	456 37.3 (24.2-64.8)	0.0040
Total bilirubin, n	476 10.5 (7.5-14.8)	56 10.9 (7.9-16.0)	420 10.4 (7.5-14.4)	0.71
Direct bilirubin, n	541 4.6 (3.1-6.1)	63 4.8 (3.1-6)	478 4.6 (3.1-6.1)	0.87
Total protein, n	498 73.7 (68.7-78.8)	58 71.7 (65-77.6)	440 73.9 (69-79)	0.035
Albumin*, n	534 41.7 (0.4)	61 38.8 (1.4)	473 42.1 (0.4)	0.0080

n denotes number of participants in whom each parameter was measured (where n <597, parameters not measured due to insufficient sample)
 Values are median and IQR unless otherwise stated. * Mean (SD), [‡]proportion, U/L = units per litre; References ranges: AST- males: 17-59 U/L, females: 14-36 U/L; ALT – males: 21-72 U/L, females: 9-52 U/L, GGT – males: 15-73 U/L, females: 12-58 U/L

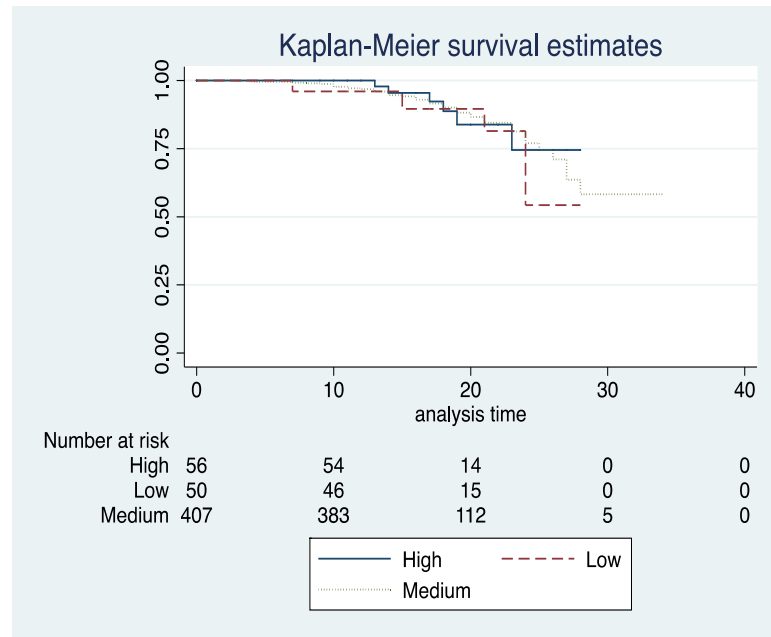
Figure S3. Kaplan-Meier survival analysis of *P. falciparum* infection, previous malaria exposure and COVID-19 clinical outcomes

A



Y-axis indicates probabilities, Log rank = 0.55

B



Y-axis indicates probabilities, Log rank = 0.66

Table S2. Age distribution of Covid-19 patients in relation to previous malaria exposure

Variable	Previous Malaria exposure			Fisher's exact p-value [¶]
	Low (N=53)	Medium (N=418)	High* (N=56)	
Age Missing	0	0	3	
0-20	2 (4%)	17 (4%)	2 (4%)	
21-40	18 (34%)	265 (63%)	33 (62%)	
41-60	24 (45%)	108 (26%)	14 (26%)	
>60	9 (17%)	28 (7%)	4 (8%)	0.0030

^{*}Three with missing age, [¶]compares the distribution of previous malaria exposure categories in the different age groups.

Table S3A. Association between clinical parameters among Covid-19 patients with *P. falciparum* infection and previous malaria exposure among patients with comorbidities

Clinical parameter	Overall N = 108	Covid-19 patients by malaria category						
		Current malaria infection			Previous malaria exposure			
		Malaria N = 15	No malaria N = 93	Fisher's exact p- value	Low N = 20	Medium N = 67	High N = 7	Fisher's exact p- value
Presence of signs and symptoms								
Fever	37 (34%)	6 (40%)	31 (33%)	0.77	6 (30%)	35 (52%)	2 (29%)	0.82
Cough	61 (57%)	8 (53%)	53 (57%)	0.79	13 (65%)	40 (60%)	4 (57%)	0.94
Runny nose	20 (19%)	1 (7%)	19 (20%)	0.29	3 (15%)	12 (18%)	2 (29%)	0.74
Shortness of breath	52 (48%)	7 (47%)	45 (48%)	1.00	14 (70%)	37 (55%)	1 (14%)	0.047
Muscle pains	4 (4%)	1 (7%)	3 (3%)	0.46	0	4 (6%)	0	0.69
Confusion	7 (7%)	2 (13%)	5 (5%)	0.25	0	6 (9%)	0	0.45
Headache	20 (19%)	3 (20%)	17 (18%)	1.00	5 (25%)	8 (12%)	3 (43%)	0.07
Sore throat	8 (7%)	0	8 (9%)	0.60	2 (10%)	3 (5%)	1 (14%)	0.34
Chest pain	39 (36%)	5 (33%)	34 (37%)	1.00	9 (45%)	27 (40%)	2 (29%)	0.77
Diarrhea	7 (7%)	0	7 (8%)	0.59	2 (10%)	5 (8%)	0	0.81
Vomiting	0	0	0	-	0	0	0	-
Fatigue	5 (5%)	0	5 (5%)	1.00	1 (5%)	4 (6%)	0	1.00
Red Eyes	1 (1%)	0	1 (1%)	1.00	1 (5%)	0	0	0.29
Clinical outcomes								
Favorable	80 (74%)	11 (73%)	69 (74%)		12 (60%)	49 (73%)	5 (71%)	
Unfavorable	28 (26%)	4 (27%)	24 (26%)	1.00	8 (40%)	18 (27%)	2 (29%)	0.48
Covid-19 Severity								
Asymptomatic	25 (23%)	5 (33%)	20 (22%)		2 (10%)	12 (18%)	2 (29%)	
Mild	27 (25%)	2 (13%)	25 (27%)		4 (20%)	16 (24%)	3 (43%)	
Moderate	21 (19%)	1 (7%)	20 (22%)		4 (20%)	16 (24%)	0	
Severe/critical	35 (32%)	7 (47%)	28 (30%)	0.26	10 (50%)	23 (34%)	2 (29%)	0.55

Table S3B. Association between clinical parameters among Covid-19 patients with *P. falciparum* infection and previous malaria exposure among patients without comorbidities

Clinical parameter	Overall N = 489	Covid-19 patients by malaria category						
		Current malaria infection			Previous malaria exposure			
		Malaria N = 55	No malaria N = 434	Fisher's exact p- value	Low N = 33	Medium N = 351	High N = 49	Fisher's exact p- value
Presence of signs and symptoms								
Fever	84 (17%)	9 (16%)	75 (17%)	1.000	11 (33%)	67 (19%)	4 (8%)	0.019
Cough	137 (28%)	11 (20%)	126 (29%)	0.201	14 (42%)	110 (31%)	9 (18%)	0.06
Runny nose	109 (22%)	10 (18%)	99 (23%)	0.495	6 (18%)	91(26%)	8 (16%)	0.28
Shortness of breath	46 (10%)	6 (11%)	40 (9%)	0.624	9 (27%)	34 (10%)	2 (4%)	0.0050
Muscle pains	18 (4%)	2 (4%)	16 (4%)	1.000	0	16 (5%)	1 (2%)	0.49
Confusion	5 (1%)	2 (4%)	3 (1%)	0.098	1 (3%)	3 (1%)	0	0.33
Headache	92 (19%)	13 (24%)	79 (18%)	0.356	8 (24%)	73 (21%)	8 (16%)	0.60
Sore throat	41 (8%)	2 (4%)	39 (9%)	0.295	3 (9%)	35 (10%)	3 (6%)	0.78
Chest pain	45 (9%)	3 (6%)	42 (10%)	0.456	8 (24%)	34 (10%)	2 (4%)	0.018
Diarrhea	13 (3%)	4 (7%)	9 (2%)	0.046	1(3%)	11 (3%)	1 (2%)	1.00
Vomiting	9 (2%)	4 (7%)	5 (1%)	0.011	0	7 (2%)	2 (4%)	0.39
Fatigue	12(3%)	1 (2%)	11 (3%)	1.000	2 (6%)	9 (3%)	0	0.24
Red Eyes	3(1%)	0 (0%)	3 (1%)	1.000	0	3 (1%)	0	1.00
Clinical outcomes								
Favorable	444 (91%)	51 (12%)	393 (80%)		31 (94%)	316 (90%)	45 (92%)	
Unfavorable	45 (9%)	4 (1%)	41 (8%)	0.805	2 (6%)	35 (10%)	4 (8%)	0.90
Covid-19 Severity								
Asymptomatic	230 (47%)	31 (56%)	199 (46%)		11 (33%)	143 (41%)	30 (61%)	
Mild	206 (42%)	16 (29%)	190 (44%)		13 (39%)	167 (48%)	17 (35%)	
Moderate	24 (5%)	5 (9%)	19 (4%)		3 (9%)	19 (5%)	1 (2%)	
Severe/critical	29 (6%)	3 (6%)	26 (6%)	0.095	6 (18%)	22 (6%)	1 (2%)	0.015

Table S4a. Correlation of cytokine profiles with *P. falciparum* infection, previous malaria exposure and COVID-19 severity

Patient Characteristics	Interleukin 2 (pg/ml)			Interleukin 6 (pg/ml)			Interleukin 7 (pg/ml)			Interleukin 8 (pg/ml)		
	Mean (SD)	Coef [¶] (95% CI)	p-value	Mean (SD)	Coef [¶] (95% CI)	p-value	Mean (SD)	Coef [¶] (95% CI)	p-value	Mean (SD)	Coef [¶] (95% CI)	p-value
Covid-19 Severity												
Asymptomatic	19.6(5.5)	1		1.5(1.0)	1		6.4(4.7)	1		53.1(115.0)	1	
Mild	19.6(3.9)	-0.2 (-0.8 -0.8)	0.96	1.7(1.8)	0.3(-2.0-2.5)	0.82	6.6(4.8)	0.4(-0.8-1.6)	0.56	28.4(83.1)	-24.7(-43.2--6.3)	0.0090
Moderate	19.2(0)	-0.5 (-1.9 -1.0)	0.52	10.8(25.6)	9.3(5.5-13.1)	0.0010	12.4(10.8)	6.5(4.4-8.5)	0.0010	58.1(89.5)	4.9(-27.0-36.9)	0.76
Severe	19.2(0)	-0.5 (-1.7-0.7)	0.45	13.0(27.4)	11.5(8.2-14.7)	0.0010	13.9(10.8)	7.7(5.9-7.1)	0.0010	52.2(58.5)	-1.0(-28.0-26.1)	0.94
Malaria infection												
Negative	19.6(4.5)	1		3.9(12.9)	1		7.9(7.1)	1		42.5(94.2)	1	
Positive	19.2(0)	-0.4(-1.5-0.7)	0.48	2.3(3.3)	-1.5(-4.7-1.6)	0.34	7.7(5.7)	-0.2(-2.0-1.6)	0.85	42.1(104.5)	0.6(-24.3-25.6)	0.96
Previous Malaria												
Low	19.8(4.4)	1		3.6(5.8)	1		10.8(9.5)	1		30.3(34.6)	1	
Medium	19.6(4.6)	-0.2(-1.4-1.0)	0.73	4.0(13.6)	0.4(-3.1-4.0)	0.82	7.9(7.9)	-2.9(-4.9--0.9)	0.0040	41.9(41.9)	11.6(-13.7-36.9)	0.37
High	19.2(0)	-0.6(-2.2-1.0)	0.47	2.1(3.4)	-1.5(-6.1-3.1)	0.53	5.2(3.4)	-5.6(-8.2--3.0)	0.0010	41.4(41.4)	11.0(-22.0-44.1)	0.51
Clinical outcomes												
Good outcome	19.6(4.5)	1		2.8(9.4)	1		7.6(6.7)	1		40.0(97.1)	1	
Unfavorable outcome*	19.2(0)	-0.4(-3.0-2.2)	0.76	20.1(22.6)	17.3(11.2-23.3)	0.0010	9.1(8.3)	1.4(-2.5-5.3)	0.48	77.1(81.8)	37.0(-18.6-92.6)	0.19

*Defined as death, discharge with sequelae or requiring intensive care, [¶]change in cytokine levels between the different patient categories

Table S4b. Correlation of cytokine profiles with *P. falciparum* infection, previous malaria exposure and COVID-19 severity

Patient Characteristics	Cytokine profiles								
	TNF- α (pg/ml)			TGF- β 1 (pg/ml)			Interleukin 10 (pg/ml)		
	Mean (SD)	Coef [†] (95% CI)	p-value	Mean (SD)	Coef [†] (95% CI)	p-value	Mean (SD)	Coef [†] (95% CI)	p-value
Covid-19 Severity									
Asymptomatic	4.8(1.2)	1		162457(105430.3)	1		9.2(75.2)	1	
Mild	4.8(1.5)	0.1(-0.3-0.3)	0.83	154715(94974.1)	-7741.8(-27887.2-12403.6)	0.45	11.9(85.2)	2.7(-18-23.8)	0.80
Moderate	4.9(0.6)	0.1(-0.4-0.6)	0.68	173140(95712.1)	10682.8(-23960-59893.5)	0.55	6.8(15.6)	-2.4(-38.6-33.8)	0.90
Severe	5.4(2.5)	0.6(0.2-1.0)	0.0040	192485(145064.7)	30028.2(162.9-59893.5)	0.049	37.8(234.9)	28.6(-2.4-59.5)	0.07
Malaria infection									
Negative	4.8(1.1)	1		164147(104383.2)	1		14.1(115.4)	1	
Positive	5.4(3.1)	0.6(0.3-1.0)	0.0010	160068(121007.5)	-4079.3(-31759-23601.3)	0.77	7.6(18.1)	-6.5(-34-21.7)	0.65
Previous Malaria									
Low	4.7(0.7)	1		201746(130924.9)	1		5.8(10.9)	1	
Moderate	4.8(1.3)	0.1(-0.3-0.5)	0.64	166932(105540.2)	-34813.9(-65344.4- -4283.4)	0.026	15.9(123.3)	10.1(-21.4-41.7)	0.53
High	5.1(2.5)	0.4(-0.2-0.9)	0.17	116307(76013.8)	-50625 (-85439.2- -45470.8)	0.0010	4.4(8.1)	-1.4(-42-40.0)	0.95
Clinical outcomes									
Good outcome	4.8(1.4)	1		160808(100811.4)	1		10.9(77.6)	1	
Unfavorable outcome*	5.8(2.2)	0.9(0.1-1.8)	0.029	174395(137530.8)	13586.7(-44667-71840)	0.65	160.0(224.3)	149.1(84.5-213.6)	0.0010

*Defined as death, discharge with sequelae or requiring intensive care, [†] change in cytokine levels between the different patient categories