UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
****
POST-GRADUATE AND TRAINING SCHOOL OF LIFE SCIENCE, HEALTH AND ENVIRONMENT

UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT

DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES

LABORATORY OF ANIMAL PHYSIOLOGY
LABORATOIRE DE PHYSIOLOGIE ANIMALE

****

Immunological correlates of asymptomatic malarial parasitaemia in a *Plasmodium falciparum* hyper-endemic setting (Esse Health District, Center Region) in Cameroon

Thesis presented and defended in order to obtain the diploma of Doctorate/Ph.D in Biology of Animal Organisms

Option: **Animal Physiology**

by

FOGANG Balotin
MSc. in Biology of Animal Organisms
(Option: Animal Physiology)
Registration number**: 09Q0129**

Defended publicly on December 19, 2022, in front a jury constituted as follows:

President : TAN Paul VERNYUY, Prof., UYI

Rapporteurs : MEGNEKOU Rosette, Assoc Prof., UYI
AYONG Lawrence, DOR., CPC
LAMB Tracey, Assoc Prof., UoU

Members : ACHIDI Eric, Prof., UB
DJIOGUE Sefirin, Assoc Prof., UYI
GOUNOUE KAMKUMO Raceline, Assoc Prof., UYI

2022

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
****
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DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES

ATTESTATION DE CORRECTION

Nous soussignés, membres du jury de la soutenance de thèse de Doctorat/PhD en **Biologie des Organismes Animaux**, Option : **Physiologie Animale**, de Monsieur **FOGANG Balotin**, matricule 09Q0129, soutenance autorisée par la correspondance N° 220-2022/UY1/CRFD-SVSE/URFD-SV/ad du recteur de l'Université de Yaoundé I en date du 08 Décembre 2022 sur le sujet intitulé : « **immunological correlates of asymptomatic malarial parasitaemia in a *Plasmodium falciparum* hyper-endemic setting (Esse Health District, Center Region) in Cameroon** », attestons que les corrections exigées au candidat lors de cette évaluation, qui a eu lieu le Lundi 19 Décembre 2022 au Laboratoire 131 de Biologie et Physiologie Animale (2ème bâtiment extension) de la Faculté des Sciences, ont réellement été effectuées.

En foi de quoi, la présente attestation lui est délivrée pour servir et valoir ce que de droit.

Fait à Yaoundé, le ....20 JAN 2023...

L'Examinateur

R. Gounoue

Le Président du jury

Prof Tan Paul

Le chef de Département

UNIVERSITE DE YAOUNDE I
University of Yaounde I
Le Chef de Département
The Head of Department
Faculty of Sciences
Faculté des Sciences

Charles Félix Bilong Bilong
Professeur

LIST OF PERMANENT TEACHING STAFF

UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ACADEMIC YEAR 2021/2022

(by Department and by Grade)

LAST UPDATE 22nd June 2022

ADMINISTRATION

DEAN: TCHOUANKEU Jean Claude, *Associate Professor*
VICE-DEAN / DPSAA: ATCHADE Alex de Théodore, *Associate Professor*
VICE-DEAN / DSSE: NYEGUE Maximilienne Ascension, *Professor*
VICE-DEAN / DRC : ABOSSOLO Monique, *Associate Professor*
Head of Administrative and Financial Division: NDOYE FOE Marie C. F., *Associate Professor*
Head of Division of Academic affairs, Research and corporation / DAASR: AJEAGAH Gideon AGHAINDUM, *Professor*

1. DEPARTMENT OF BIOCHEMISTRY (BC) (39)			
N°	**NAMES AND FIRST-NAMES**	**GRADE**	**OBSERVATIONS**
1	BIGOGA DAIGA Jude	Professor	On duty
2	BOUDJEKO Thadée	Professor	On duty
3	FEKAM BOYOM Fabrice	Professor	On duty
4	FOKOU Elie	Professor	On duty
5	KANSCI Germain	Professor	On duty
6	MBACHAM FON Wilfried	Professor	On duty
7	MOUNDIPA FEWOU Paul	Professor	***Head of Department***
8	OBEN Julius ENYONG	Professor	On duty
9	ACHU Merci BIH	Associate Professor	On duty
10	ATOGHO Barbara Mma	Associate Professor	On duty
11	AZANTSA KINGUE GABIN BORIS	Associate Professor	On duty
12	BELINGA née NDOYE FOE M. C. F.	Associate Professor	***Head AFD/ FS***
13	BOUDJEKO Thaddée	Associate Professor	On duty
14	DJUIDJE NGOUNOUE Marcelline	Associate Professor	On duty
15	EFFA NNOMO Pierre	Associate Professor	On duty
16	EWANE Cécile Anne	Associate Professor	On duty
17	MOFOR née TEUGWA Clotilde	Associate Professor	***IA4/MINESUP***
18	NANA Louise épouse WAKAM	Associate Professor	On duty
19	NGONDI Judith Laure	Associate Professor	On duty
20	NGUEFACK Julienne	Associate Professor	On duty
21	NJAYOU Frédéric Nico	Associate Professor	On duty
22	TCHANA KOUATCHOUA Angèle	Associate Professor	On duty

23	AKINDEH MBUH NJI	Senior Lecturer	On duty
24	BEBEE Fadimatou	Senior Lecturer	On duty
25	BEBOY EDJENGUELE Sara Nathalie	Senior Lecturer	On duty
26	DAKOLE DABOY Charles	Senior Lecturer	On duty
27	DJUIKWO NKONGA Ruth Viviane	Senior Lecturer	On duty
28	DONGMO LEKAGNE Joseph Blaise	Senior Lecturer	On duty
29	FONKOUA Martin	Senior Lecturer	On duty
30	KOTUE KAPTUE Charles	Senior Lecturer	On duty
31	LUNGA Paul KEILAH	Senior Lecturer	On duty
32	MANANGA Marlyse Joséphine	Senior Lecturer	On duty
33	MBONG ANGIE M. Mary Anne	Senior Lecturer	On duty
34	Palmer MASUMBE NETONGO	Senior Lecturer	On duty
35	PECHANGOU NSANGOU Sylvain	Senior Lecturer	On duty
36	WILFRIED ANGIE Abia	Senior Lecturer	On duty
37	MBOUCHE FANMOE Marceline Joëlle	Senior Lecturer	On duty
38	OWONA AYISSI Vincent Brice	Senior Lecturer	On duty
39	FOUPOUAPOUOGNIGNI Yacouba	Assistant	On duty
2. **DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (A.B.P) (51)**			
1	AJEAGAH Gideon AGHAINDUM	Professor	***DAARS/FS***
2	BILONG BILONG Charles-Félix	Professor	***Head of Department***
3	DIMO Théophile	Professor	On duty
4	DJIETO LORDON Champlain	Professor	On duty
5	DZEUFIET DJOMENI Paul Désiré	Professor	On duty
6	ESSOMBA née NTSAMA MBALA	Professor	***Vice Dean/FMSB/UYI***
7	FOMENA Abraham	Professor	On duty
8	KEKEUNOU Sévilor	Professor	On duty
9	NJAMEN Dieudonné	Professor	On duty
10	NJIOKOU Flobert	Professor	On duty
11	NOLA Moïse	Professor	On duty
12	TAN Paul VERNYUY	Professor	On duty
13	TCHUEM TCHUENTE Louis Albert	Professor	***Coord. Progr MINHEALTH***
14	ZEBAZE TOGOUET Serge Hubert	Professor	On duty
15	ALENE Désirée Chantal	Associate Professor	***Head of the service/MINESUP***
16	BILANDA Danielle Claude	Associate Professor	On duty
17	DJIOGUE Séfirin	Associate Professor	On duty
18	JATSA BOUKENG Hermine épse MEGAPTCHE	Associate Professor	On duty
19	LEKEUFACK FOLEFACK Guy B.	Associate Professor	On duty
20	MBENOUN MASSE Paul Serge	Associate Professor	On duty
21	MEGNEKOU Rosette	Associate Professor	On duty
22	MONY Ruth épse NTONE	Associate Professor	On duty
23	NGUEGUIM TSOFACK Florence	Associate Professor	On duty
24	NGUEMBOCK	Associate Professor	On duty
25	TOMBI Jeannette	Associate Professor	On duty
26	ATSAMO Albert Donatien	Senior Lecturer	On duty
27	BASSOCK BAYIHA Etienne Didier	Senior Lecturer	On duty

28	DONFACK Mireille	Senior Lecturer	On duty
29	ESSAMA MBIDA Désirée Sandrine	Senior Lecturer	On duty
30	ETEME ENAMA Serge	Senior Lecturer	On duty
31	FEUGANG YOUMSSI Francois	Senior Lecturer	On duty
32	GONWOUO NONO Legrand	Senior Lecturer	On duty
33	GOUNOUE KAMKUMO Raceline	Senior Lecturer	On duty
34	KANDEDA KAVAYE Antoine	Senior Lecturer	On duty
35	KOGA MANG DOBARA	Senior Lecturer	On duty
36	LEME BANOCK Lucie	Senior Lecturer	On duty
37	MAHOB Raymond Joseph	Senior Lecturer	On duty
38	METCHI DONFACK Mireille Flaure Epse GHOUMO	Senior Lecturer	On duty
39	MOUNGANG Luciane Marlyse	Senior Lecturer	On duty
40	MVEYO NDANKEU Yves Patrick	Senior Lecturer	On duty
41	NGOUATEU KENFACK Omer Bébé	Senior Lecturer	On duty
42	NJUA Clarisse Yafi	Senior Lecturer	***Head of division UBA***
43	NOAH EWOTI Olive Vivien	Senior Lecturer	On duty
44	TADU Zephyrin	Senior Lecturer	On duty
45	TAMSA ARFAO Antoine	Senior Lecturer	On duty
46	YEDE	Senior Lecturer	On duty
47	YOUNOUSSA LAME	Senior Lecturer	On duty
48	AMBADA NDZENGUE Georgia Elna	Assistant	On duty
49	FOKAM Alvine Christelle Epse KEGNE	Assistant	On duty
50	MAPON NSANGOU Indou	Assistant	On duty
51	NWANE Philippe Bienvenu	Assistant	On duty
3. **DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (P. B. P) (33)**			
1	AMBANG Zachée	Professor	***Head of DAARS/UYII***
2	DJOCGOUE Pierre François	Professor	On duty
3	MBOLO Marie	Professor	On duty
4	MOSSEBO Dominique Claude	Professor	On duty
5	YOUMBI Emmanuel	Professor	***Head of Department***
6	ZAPFACK Louis	Professor	On duty
7	ANGONI Hyacinthe	Associate Professor	On duty
8	BIYE Elvire Hortense	Associate Professor	On duty
9	MALA Armand William	Associate Professor	On duty
10	MBARGA BINDZI Marie Alain	Associate Professor	***DAAC/UDla***
11	NDONGO BEKOLO	Associate Professor	***CE / MINRESI***
12	NGODO MELINGUI Jean Baptiste	Associate Professor	On duty
13	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	***CT / MINRESI***
14	TONFACK Libert Brice	Associate Professor	On duty
15	TSOATA Esaïe	Associate Professor	On duty
16	ONANA JEAN MICHEL	Associate Professor	On duty
17	DJEUANI Astride Carole	Senior Lecturer	On duty
18	GOMANDJE Christelle	Senior Lecturer	On duty
19	GONMADGE Christelle	Senior Lecturer	On duty
20	MAFFO MAFFO Nicole Liliane	Senior Lecturer	On duty
21	MAHBOU SOMO TOUKAM. Gabriel	Senior Lecturer	On duty

22	NGALLE Hermine BILLE	Senior Lecturer	On duty
23	NNANGA MEBENGA Ruth Laure	Senior Lecturer	On duty
24	NOUKEU KOUAKAM Armelle	Senior Lecturer	On duty
25	NSOM ZAMBO Apse PIAL Annie Claude	Senior Lecturer	***On secondment/UNESCO Mali***
26	GODSWILL NTSOMBAH NTSEFONG	Senior Lecturer	On duty
27	KABELONG BANAHO Louis-Paul-Roger	Senior Lecturer	On duty
28	KONO Léon Dieudonné	Senior Lecturer	On duty
29	LIBALAH Moses BAKONCK	Senior Lecturer	On duty
30	LIKENG-LI-NGUE Benoit C	Senior Lecturer	On duty
31	TAEDOUNG Evariste Hermann	Senior Lecturer	On duty
32	TEMEGNE NONO Carine	Senior Lecturer	On duty
33	MANGA NDJAGA Jude	Assistant	On duty
4. DEPARTMENT OF INORGANIC CHEMISTRY (I.C.) (31)			
1	AGWARA ONDOH Moïse	Professor	**Head of Department**
2	Florence UFI CHINJE épouse MELO	Professor	***Rector Univ.Ngaoundéré***
3	GHOGOMU Paul MINGO	Professor	***Ministre Chargé de Miss.PR***
4	NANSEU Njiki Charles Péguy	Professor	On duty
5	NDIFON Peter TEKE	Professor	***CT MINRESI***
6	NDIKONTAR Maurice KOR	Professor	***Vice-Dean Univ. Bamenda***
7	NENWA Justin	Professor	On duty
8	NGAMENI Emmanuel	Professor	***Dean FS Univ. Ngaoundéré***
9	NGOMO Horace MANGA	Professor	***Vice Chancelor/UB***
10	ACAYANKA Elie	Associate Professor	On duty
11	EMADACK Alphonse	Associate Professor	On duty
12	KAMGANG YOUBI Georges	Associate Professor	On duty
13	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	On duty
14	KENNE DEDZO GUSTAVE	Associate Professor	On duty
15	KONG SAKEO	Associate Professor	On duty
16	MBEY Jean Aime	Associate Professor	On duty
17	NDI NSAMI Julius	Associate Professor	On duty
18	NEBAH Née NDOSIRI Bridget NDOYE	Associate Professor	***CT/MINPROFF***
19	NJIOMOU C. épse DJANGANG	Associate Professor	On duty
20	NJOYA Dayirou	Associate Professor	On duty
21	NYAMEN Linda Dyorisse	Associate Professor	On duty
22	PABOUDAM GBAMBIE AWAWOU	Associate Professor	On duty
23	TCHAKOUTE KOUAMO Hervé	Associate Professor	On duty
24	BELIBI BELIBI Placide Désiré	Senior Lecturer	***CS/ ENS Bertoua***
25	CHEUMANI YONA Arnaud M.	Senior Lecturer	On duty
26	KOUOTOU DAOUDA	Senior Lecturer	On duty
27	MAKON Thomas Beauregard	Senior Lecturer	On duty

28	NCHIMI NONO KATIA	Senior Lecturer	On duty
29	NJANKWA NJABONG N. Eric	Senior Lecturer	On duty
30	PATOUOSSA ISSOFA	Senior Lecturer	On duty
31	SIEWE Jean Mermoz	Senior Lecturer	On duty
5. DEPARTMENT OF ORGANIC CHEMISTRY (O.C.) (38)			
1	DONGO Etienne	Professor	***Vice-Dean/FSE/UYI***
2	NGOUELA Silvère Augustin	Professor	***Head of Department UDS***
3	NYASSE Barthélemy	Professor	On duty
4	PEGNYEMB Dieudonné Emmanuel	Professor	***Director at MINESUP/*** **Head of Department**
5	WANDJI Jean	Professor	On duty
6	MBAZOA née DJAMA Céline	Professor	On duty
7	Alex de Théodore ATCHADE	Associate Professor	***Vice-Dean / DPSAA***
8	AMBASSA Pantaléon	Associate Professor	On duty
9	EYONG Kenneth OBEN	Associate Professor	On duty
10	FOLEFOC Gabriel NGOSONG	Associate Professor	On duty
11	FOTSO WABO Ghislain	Associate Professor	On duty
12	KAMTO Eutrophe Le Doux	Associate Professor	On duty
13	KENMOGNE Marguerite	Associate Professor	On duty
14	KEUMEDJIO Félix	Associate Professor	On duty
15	KOUAM Jacques	Associate Professor	On duty
16	MKOUNGA Pierre	Associate Professor	On duty
17	MVOT AKAK CARINE	Associate Professor	On duty
18	NGO MBING Joséphine	Associate Professor	***Head Cellule MINERESI***
19	NGONO BIKOBO Dominique Serge	Associate Professor	***C.E/ MINESUP***
20	NOTE LOUGBOT Olivier Placide	Associate Professor	***DAAC/Uté Bertoua***
21	NOUNGOUE TCHAMO Diderot	Associate Professor	On duty
22	TABOPDA KUATE Turibio	Associate Professor	On duty
23	TAGATSING FOTSING Maurice	Associate Professor	On duty
24	TCHOUANKEU Jean-Claude	Associate Professor	***Dean /FS/ UYI***
25	YANKEP Emmanuel	Associate Professor	On duty
26	ZONDEGOUMBA Ernestine	Associate Professor	On duty
27	NGNINTEDO Dominique	Senior Lecturer	On duty
28	NGOMO Orléans	Senior Lecturer	On duty
29	OUAHOUO WACHE Blandine M.	Senior Lecturer	On duty
30	SIELINOU TEDJON Valérie	Senior Lecturer	On duty
31	MESSI Angélique Nicolas	Senior Lecturer	On duty
32	TCHAMGOUE Joseph	Senior Lecturer	On duty
33	TSAMO TONTSA Armelle	Senior Lecturer	On duty
34	TSEMEUGNE Joseph	Senior Lecturer	On duty
35	MUNVERA MFIFEN Aristide	Assistant	On duty
36	NONO NONO Eric Carly	Assistant	On duty
37	OUETE NANTCHOUANG Judith Laure	Assistant	On duty
38	TSAFFACK Maurice	Assistant	On duty
6. DEPARTMENT COMPUTER SCIENCES (CS) (22)			
1	ATSA ETOUNDI Roger	Professor	***Head Div.MINESUP***

2	FOUDA NDJODO Marcel Laurent	Professor	***Head Dpt ENS/Head IGA.MINESUP***
3	NDOUNDAM Réné	Associate Professor	On duty
4	TSOPZE Norbert	Associate Professor	On duty
5	ABESSOLO ALO'O Gislain	Senior Lecturer	***Assistant director/MINFOPRA***
6	AMINOU Halidou	Senior Lecturer	***Head of Department***
7	DJAM Xaviera YOUH – KIMBI	Senior Lecturer	On duty
8	DOMGA KOMGUEM Rodrigue	Senior Lecturer	On duty
9	EBELE Serge Alain	Senior Lecturer	On duty
10	HAMZA Adamou	Senior Lecturer	On duty
11	JIOMEKONG AZANZI Fidel	Senior Lecturer	On duty
12	KOUOKAM KOUOKAM E. A.	Senior Lecturer	On duty
13	MELATAGIA YONTA Paulin	Senior Lecturer	On duty
14	MONTHE DJIADEU Valery M.	Senior Lecturer	On duty
15	OLE OLE Daniel Claude Delort	Senior Lecturer	***Deputy Director Enset. Ebolowa***
16	TAPAMO Hyppolite	Senior Lecturer	On duty
17	BAYEM Jacques Narcisse	Assistant	On duty
18	EKODECK Stéphane Gaël Raymond	Assistant	On duty
19	MAKEMBE. S . Oswald	Assistant	On duty
20	MESSI NGUELE Thomas	Assistant	On duty
21	NKONDOCK. MI. BAHANACK.N.	Assistant	On duty
22	NZEKON NZEKO'O Armel Jacques	Assistant	On duty
7. DEPARTMENT OF MATHEMATICS (MA) (31)			
1	AYISSI Raoult Domingo	Professor	***Head of Department***
2	EMVUDU WONO Yves S.	Professor	***Inspector MINESUP***
3	KIANPI Maurice	Associate Professor	On duty
4	MBANG Joseph	Associate Professor	On duty
5	MBEHOU Mohamed	Associate Professor	On duty
6	MBELE BIDIMA Martin Ledoux	Associate Professor	On duty
7	NOUNDJEU Pierre	Associate Professor	***Head service of programs & Diplômes/FS/UYI***
8	TAKAM SOH Patrice	Associate Professor	On duty
9	TCHAPNDA NJABO Sophonie B.	Associate Professor	***Director/AIMS Rwanda***
10	TCHOUNDJA Edgar Landry	Associate Professor	On duty
11	AGHOUKENG JIOFACK Jean Gérard	Senior Lecturer	***Head Cellule MINPLAMAT***
12	BOGSO Antoine Marie	Senior Lecturer	On duty
13	CHENDJOU Gilbert	Senior Lecturer	On duty
14	DJIADEU NGAHA Michel	Senior Lecturer	On duty
15	DOUANLA YONTA Herman	Senior Lecturer	On duty
16	KIKI Maxime Armand	Senior Lecturer	On duty
17	MBAKOP Guy Merlin	Senior Lecturer	On duty
18	MENGUE MENGUE David Joe	Senior Lecturer	***Head Dpt/ENS Uté Maroua***
19	NGUEFACK Bernard	Senior Lecturer	On duty
20	NIMPA PEFOUKEU Romain	Senior Lecturer	On duty
21	OGADOA AMASSAYOGA	Senior Lecturer	On duty

22	POLA DOUNDOU Emmanuel	Senior Lecturer	On duty
23	TCHEUTIA Daniel Duviol	Senior Lecturer	On duty
24	TETSADJIO TCHILEPECK M. E.	Senior Lecturer	On duty
25	BITYE MVONDO Esther Claudine	Assistant	On duty
26	FOKAM Jean Marcel	Assistant	On duty
27	LOUMNGAM KAMGA Victor	Assistant	On duty
28	MBATAKOU Salomon Joseph	Assistant	On duty
29	MBIAKOP Hilaire George	Assistant	On duty
30	MEFENZA NOUNTU Thierry	Assistant	On duty
31	TENKEU JEUFACK Yannick Léa	Assistant	On duty
8. DEPARTMENT OF MICROBIOLOGY (MB) (22)			
1	ESSIA NGANG Jean Justin	Professeur	***Head of Department***
2	NYEGUE Maximilienne Ascension	Professeur	***VICE-Dean / DSSE***
3	NWAGA Dieudonné M.	Professeur	On duty
4	ASSAM ASSAM Jean Paul	Associate Professor	On duty
5	BOUGNOM Blaise Pascal	Associate Professor	On duty
6	BOYOMO ONANA	Associate Professor	On duty
7	KOUITCHEU MABEKU Epse KOUAM Laure Brigitte	Associate Professor	On duty
8	RIWOM Sara Honorine	Associate Professor	On duty
9	SADO KAMDEM Sylvain Leroy	Associate Professor	On duty
10	BODA Maurice	Senior Lecturer	*In a position of irregular absence*
11	ESSONO OBOUGOU Germain G.	Senior Lecturer	On duty
12	NJIKI BIKOÏ Jacky	Senior Lecturer	On duty
13	TCHIKOUA Roger	Senior Lecturer	On duty
14	ESSONO Damien Marie	Senior Lecturer	On duty
15	LAMYE Glory MOH	Senior Lecturer	On duty
16	MEYIN A EBONG Solange	Senior Lecturer	On duty
17	NKOUDOU ZE Nardis	Senior Lecturer	On duty
18	TAMATCHO KWEYANG Blandine Pulcherie	Senior Lecturer	On duty
19	TOBOLBAÏ Richard	Senior Lecturer	On duty
20	MONI NDEDI Esther Del Florence	Assistant	On duty
21	NKOUE TONG Abraham	Assistant	On duty
22	SAKE NGANE Carole Stéphanie	Assistant	On duty
9. DEPARTMENT OF PHYSICS (PH) (43)			
1	BEN- BOLIE Germain Hubert	Professor	On duty
2	DJUIDJE KENMOE épouse ALOYEM	Professor	On duty
3	EKOBENA FOUDA Henri Paul	Professor	***Vice-Rector. Uty Ngaoundere***
4	ESSIMBI ZOBO Bernard	Professor	On duty
5	NANA ENGO Serge Guy	Professor	On duty
6	NANA NBENDJO Blaise	Professor	On duty
7	NDJAKA Jean Marie Bienvenu	Professor	***Head of Department***
8	NJANDJOCK NOUCK Philippe	Professor	On duty
9	NOUAYOU Robert	Professor	On duty
10	PEMHA Elkana	Professor	On duty

11	SAIDOU	Professor	***Head Center/IRGM/MINRESI***
12	TABOD Charles TABOD	Professor	***Dean FS Univ/Bda***
13	TCHAWOUA Clément	Professor	On duty
14	WOAFO Paul	Professor	On duty
15	ZEKENG Serge Sylvain	Professor	On duty
16	BIYA MOTTO Frédéric	Associate Professor	***DG/HYDRO Mekin***
17	BODO Bertrand	Associate Professor	On duty
18	ENYEGUE A NYAM épse BELINGA	Associate Professor	On duty
19	EYEBE FOUDA Jean sire	Associate Professor	On duty
20	FEWO Serge Ibraïd	Associate Professor	On duty
21	HONA Jacques	Associate Professor	On duty
22	MBINACK Clément	Associate Professor	On duty
23	MBONO SAMBA Yves Christian U.	Associate Professor	On duty
24	NDOP Joseph	Associate Professor	On duty
25	SIEWE SIEWE Martin	Associate Professor	On duty
26	SIMO Elie	Associate Professor	On duty
27	VONDOU Derbetini Appolinaire	Associate Professor	On duty
28	WAKATA née BEYA Annie	Associate Professor	***Director/ENS/UYI***
29	ABDOURAHIMI	Senior Lecturer	On duty
30	CHAMANI Roméo	Senior Lecturer	On duty
31	EDONGUE HERVAIS	Senior Lecturer	On duty
32	FOUEDJIO David	Senior Lecturer	***Head Cell. MINADER***
33	MELI'I Joelle Larissa	Senior Lecturer	On duty
34	MVOGO ALAIN	Senior Lecturer	On duty
35	WOULACHE Rosalie Laure	Senior Lecturer	*Absente since January 2022*
36	AYISSI EYEBE Guy François Valérie	Senior Lecturer	On duty
37	DJIOTANG TCHOTCHOU Lucie Angennes	Senior Lecturer	On duty
38	OTTOU ABE Martin Thierry	Senior Lecturer	On duty
39	TEYOU NGOUPOU Ariel	Senior Lecturer	On duty
40	KAMENI NEMATCHOUA Modeste	Assistant	On duty
41	LAMARA Maurice	Assistant	On duty
42	NGA ONGODO Dieudonné	Assistant	On duty
43	WANDJI NYAMSI William	Assistant	On duty
10. DEPARTMENT OF EARTH SCIENCES (E.S.) (41)			
1	BITOM Dieudonné	Professor	***Dean / FASA / UDs***
2	FOUATEU Rose épse YONGUE	Professor	On duty
3	NDAM NGOUPAYOU Jules-Remy	Professor	On duty
4	NDJIGUI Paul Désiré	Professor	***Head of Department***
5	NGOS III Simon	Professor	On duty
6	NKOUMBOU Charles	Professor	On duty
7	NZENTI Jean-Paul	Professor	On duty
8	ABOSSOLO née ANGUE Monique	Associate Professor	***Vice-Dean / DRC***
9	BISSO Dieudonné	Associate Professor	***Director/Projet Barrage Memve'ele***
10	EKOMANE Emile	Associate Professor	On duty

11	FUH Calistus Gentry	Associate Professor	***Sec. D'Etat/MINMIDT***
12	GANNO Sylvestre	Associate Professor	On duty
13	GHOGOMU Richard TANWI	Associate Professor	***Head Dpt/Uté Maroua***
14	MOUNDI Amidou	Associate Professor	***CT/ MINIMDT***
15	NGO BIDJECK Louise Marie	Associate Professor	On duty
16	NGUEUTCHOUA Gabriel	Associate Professor	**CEA/MINRESI**
17	NJILAH Isaac KONFOR	Associate Professor	On duty
18	NYECK Bruno	Associate Professor	On duty
19	ONANA Vincent Laurent	Associate Professor	***Head service Maintenance & Material/UYII***
20	TCHAKOUNTE J. épse NOUMBEM	Associate Professor	***Head.cell / MINRESI***
21	TCHOUANKOUE Jean-Pierre	Associate Professor	On duty
22	TEMGA Jean Pierre	Associate Professor	On duty
23	YENE ATANGANA Joseph Q.	Associate Professor	***Head Div. /MINTP***
24	ZO'O ZAME Philémon	Associate Professor	***DG/ART***
25	ANABA ONANA Achille Basile	Senior Lecturer	On duty
26	BEKOA Etienne	Senior Lecturer	On duty
27	ELISE SABABA	Senior Lecturer	On duty
28	ESSONO Jean	Senior Lecturer	On duty
29	EYONG JOHN TAKEM	Senior Lecturer	On duty
30	MAMDEM TAMTO Lionelle Estelle	Senior Lecturer	On duty
31	MBESSE CECILE OLIVE	Senior Lecturer	On duty
32	MBIDA YEM	Senior Lecturer	On duty
33	METANG Victor	Senior Lecturer	On duty
34	MINYEM Dieudonné-Lucien	Senior Lecturer	***CD/Uté Maroua***
35	NGO BELNOUN Rose Noël	Senior Lecturer	On duty
36	NOMO NEGUE Emmanuel	Senior Lecturer	On duty
37	NTSAMA ATANGANA Jacqueline	Senior Lecturer	On duty
38	TCHAPTCHET TCHATO De P.	Senior Lecturer	On duty
39	TEHNA Nathanaël	Senior Lecturer	On duty
40	FEUMBA Roger	Assistant	On duty
41	MBANGA NYOBE Jules	Assistant	On duty

Distribution of permanent lecturers in the faculty of science according to departments

	NUMBER OF LECTURERS				
Department	**Professors**	**Associate Professors**	**Senior Lecturers**	**Assist. Lecturers**	**Total**
BCH	08 (00)	14 (10)	15 (05)	02 (01)	**39 (16)**
A. B. P.	14 (01)	11 (07)	22 (07)	04 (02)	**51 (17)**
P. B. P.	06 (01)	10(01)	16 (09)	01 (00)	**33 (11)**
I. C.	09(01)	14(04)	08 (01)	00 (00)	**31 (06)**
O. C.	06 (01)	20 (04)	08 (03)	04 (01)	**38(09)**
C. S.	02 (00)	02 (00)	12 (01)	06 (00)	**22 (01)**
MAT	02 (00)	08 (00)	14 (01)	07 (01)	**31 (02)**
MIB	03 (01)	06 (02)	10 (03)	03 (02)	**22 (08)**
PHY	15 (01)	13 (02)	11 (03)	04 (00)	**43 (06)**
E. S.	07 (01)	16 (03)	18 (04)	01 (00)	**42(08)**
Total	**72 (07)**	**114 (33)**	**134 (37)**	**32 (07)**	**352 (84)**

A total of……**352 (84)**, with :

Professors……**72 (07)**

Associate Professors……**114 (33)**

Senior Lecturers……**134 (37)**

Assistant Lecturers……..**32** (**07)**

( ) = Number of women…….**84**

DEDICATION

I dedicate this work to my mother **Matsingoum Martine** and my late father **Nzokou Boniface,** whatever I say and write, I cannot express my great affection and deep gratitude.

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LIST OF ABBREVIATIONS

ACT: Artemisinin-based combination therapy

ALu: Artemether-lumefantrine

AMA-1: Apical membrane antigen 1

AMV-RT: Avian myeloblastosis virus reverse transcriptase

APC: Antigen-presenting cells

ASAQ: Artesunate-amodiaquine

ASC: Antigen secreting cell

ASMQ: Artesunate-mefloquine

AS-SP: Artesunate-sulphadoxine–pyrimethamine

BAFF: B cell activating factor belonging to the TNF family

BCL-6: B-cell lymphoma 6 protein

BCR: B cell receptor

CelTOS: Cell traversal protein for *Plasmodium* ookinetes and sporozoites

CLAG: Cytoadherence linked asexual gene

CLR: C-type lectin receptor

CSA: Chondroitin sulphate A

CSP: Circumsporozoite protein

DAMP: Danger-associated molecular pattern

DC: Dendritic cell

DHAPQP: Dihydroartemisinin-piperaquine

DNA: Deoxyribonucleic Acid

EBA-175: Erythrocyte-binding antigen 175

EDTA: Ethylenediaminetetraacetic acid

EGF: Epidermal growth factor

ELISA: Enzyme-linked immunosorbent assay

FGF-2: Fibroblast growth factor 2

Flt-3L: Feline McDonough Sarcoma-like tyrosine kinase 3 ligand

FoB: Follicular B cell

FOXP3: Forkhead box P3

G6PD: Glucose6-phosphate dehydrogenase

GC: Germinal center

G-CSF: Granulocyte colony-stimulating factor

GIA: Growth inhibition assay

GLURP: Glutamate-rich protein

GM-CSF: Granulocyte-macrophage colony-stimulating factor

GPI: Glycosylphosphatidylinositol

GPS: Global positioning system

GRO: Growth-regulated oncogene

HRP: Horse radish peroxidase

ICOS: Inducible T cell co-stimulator

IFA: Immunofluorescence assay

IFN: Interferon

IL-: Interleukin

IP-10: IFN-γ induced protein

IRF: Interferon regulatory factor

IRF: interferon regulatory factor

LAMP: Loop-mediated isothermal nucleic acid amplification

LSA: Liver-stage antigen

MBC: Memory B cell

MCP: Monocyte chemoattractant protein

MDC: Macrophage-derived chemokine

MHC: Major Histocompatibility Complex

MIP: Macrophage inflammatory protein

MOI: Multiplicity of infection

MSP: Merozoite surface protein

MZB: Marginal zone B cell

NAD: Nicotinamide adenine dinucleotide

NADH: Nicotinamide adenine dinucleotide hydrogen

NASBA: Nucleic acid sequence-based amplification

NK: Natural killer

NKT: Natural killer T cells

NLR: NOD-like receptor

PAMP: Pathogen-associated molecular pattern

PBMC: Peripheral blood mononuclear cell

PCR: Polymerase chain reaction

PD1: Programmed cell death 1

***Pf*EMP-**1: *P. falciparum* erythrocyte membrane protein 1

PfSir2a: *P. falciparum* sirtuin 2a

PRR: Pattern recognition receptor

RAMA: Rhoptry-associated membrane antigen

RBC: Red blood cell

RDT: Rapid diagnostic test

RIG: Retinoic acid-inducible gene

RNA: Ribonucleic acid

RON: Rhoptry neck protein

ROS: Reactive oxygen species

RPMI: Roswell Park Memorial Institute

rRNA: Ribosomal RNA

SIAP: Sporozoite invasion-associated protein

ssrRNA: Small subunit ribosomal RNA

TCRγ: T-cell receptor gamma

TCRδ: T-cell receptor delta

TFh: Follicular T helper cell

TGF-α: Transforming growth factor alpha

TLR: Toll-like receptors

TNF: Tumor necrosis factor

TRAP: Thrombospondin-related anonymous protein

Treg: Regulatory T cell

VEGF: Vascular endothelial growth factor

WBC: White blood cell

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ABSTRACT

The incidence of malaria cases and the mortality rate have declined considerably since 2000 by 36% and 63%, respectively, along with a sharp increase in the proportions of asymptomatic infections, known to contribute significantly to the persistence of malaria transmission in most endemic countries. Evidence from malaria epidemiological and immunological data attributed asymptomatic plasmodial infection to several factors, including, host, parasite and environmental factors. However, in highly exposed persons, where the duration of chronic malaria infection could range from a few weeks to several years, the host factors that determine the persistence of asymptomatic parasitaemia remain poorly understood. This study aimed to identify the host immunological determinants of persistent asymptomatic malarial parasitaemia in a *Plasmodium falciparum* hyper-endemic zone in Cameroon, critical for the development of new antimalarial control tools. A household-based longitudinal survey was undertaken between November 2018 and February 2019 in a malaria hyper-endemic area in Cameroon (Esse Health District, Central Region, Cameroon) using multiplex nested PCR to detect plasmodial infections. With the agreement of each participant (by signing the informed consent), residents with asymptomatic parasitaemia were monitored over a four-month period with weekly measurements of axillary temperatures and antimalarial treatment at week 11 post-enrollment. Asymptomatic participants who became febrile before the antimalarial treatment at week 11 were classified as short-term asymptomatic (ST) carriers, whereas those who remained asymptomatic were defined as long-term asymptomatic (LT). Plasma antibody levels or avidity to some *P. falciparum* antigens (mix stage extract: TE, merozoite extract: ME, schizont extract: SE, or the recombinant proteins EBA-175, MSP-1, and MSP-4) and plasma levels of 38 cellular biomarkers (cytokines, chemokines and growth factors) were measured by indirect ELISA and Multianlyte platform assay (Luminex technology), respectively. These biomarker levels were compared between study groups (NI: non-infected, LT, ST, and SY: symptomatic) and monitored at various follow-up time-points in individuals with asymptomatic parasitaemia. Furthermore, the growth inhibitory activities of circulating anti-parasite antibodies were measured and compared between the groups using laboratory strains of *P. falciparum* and a Sybr Green-I based parasite growth inhibition assay. Of the 353 individuals tested by multiplex PCR, 328 (92.9%) were positive for malaria parasitaemia of whom 266 (81.1 %) were asymptomatic carriers. Multivariate analyses identified low parasitaemia and absence of fever in the preceding three months as independent risk factors of asymptomatic parasitaemia. Furthermore, risk analyse revealed female gender and anaemia at the time of enrolment as key predictors of the early development of febrile illness (<3 weeks post enrolment) amongst the asymptomatic individuals. Of the 143 asymptomatic individuals who completed the follow-up study, 78 (55.4%) remained asymptomatic (LT), whereas 64 (44.8%)

developed malaria-associated fever (ST) and 1 (0.7%) resolved the infection without known antimalarial treatment. Plasma antibody levels and avidities to all *P. falciparum* antigens tested were comparable between the study groups. However, median *P. falciparum* growth inhibition index was significantly higher in the LT asymptomatic group compared to either ST asymptomatic or SY groups. Plasma levels of the pro-inflammatory chemokine IL-8 were significantly higher in LT asymptomatic individuals compared to the ST asymptomatic individuals whereas levels of anti-inflammatory IL-10/pro-inflammatory cytokine ratios were lower in individuals with persistent asymptomatic parasitaemia (LT) compared to those with ST asymptomatic infections. Overall there was no significant change in IgG antibody levels over time, although a continued increase in antibody avidity to TE and EBA-175 levels was observed. Despite fluctuations in the levels of most cytokines, levels of IL-10/pro-inflammatory cytokine ratio, as well as levels of main regulatory cytokines (IL-10, TNF-α, IL-1RA, MCP-1, IP-10 and eotaxin), remained stable during persistent asymptomatic infection. The cytokine ratios, IL-10 and MCP-1 plasma levels as well as plasma levels and avidity of some anti-plasmodial antibody responses, significantly declined following antimalarial treatment of persistent asymptomatic parasitaemia at week 11. Taken together, the data establishes the implication of yet unidentified antiplasmodial antibody responses and various regulatory and pro-inflammatory cytokine responses in the maintenance of long-term asymptomatic malarial parasitaemia in the study area. Furthermore, our data reveal an extremely high prevalence of asymptomatic malaria parasitaemia and asymptomatic malaria anaemia in the study area, unveiling for the first time the association of asymptomatic malarial anaemia with early clinical conversion from asymptomatic to symptomatic infections.

Keywords: *Plasmodium falciparum*, Asymptomatic malaria, antibodies, cytokines, high malaria transmission setting

RESUME

L'incidence du paludisme et le taux de mortalité lié au paludisme ont considérablement diminué depuis 2000, de 36 % et 63 % respectivement, en parallèle à une augmentation accrue des proportions d'infections asymptomatiques, connues pour contribuer de manière significative à la persistence de la transmission du paludisme dans la plupart des pays endémiques. Les données épidémiologiques et immunologiques sur le paludisme ont permis d'attribuer l'infection plasmodiale asymptomatique à plusieurs facteurs, notamment des facteurs lié à l'hôte, au parasite et à l'environnement. Cependant, chez les personnes fortement exposées, où la durée de l'infection palustre chronique peut aller de quelques semaines à plusieurs années, les facteurs de l'hôte qui déterminent la persistance de la parasitémie asymptomatique restent inconnu. Cette étude avait pour but d'identifier les déterminants immunologiques de la persistance du paludisme asymptomatique dans une zone hyper-endémique à *Plasmodium falciparum* au Cameroun, critique pour le développement de nouveaux outils de lutte contre le paludisme. Une étude longitudinale a été entreprise entre Novembre 2018 et Février 2019 dans une zone hyper-endémique à *P. falciparum* au Cameroun (District de Santé d'Esse, Région du Centre, Cameroun) en utilisant la PCR multiplex nichée pour détecter les infections plasmodiales. Les participants présentant une parasitémie asymptomatique (infection sans fièvre) ont été suivis sur une période de quinze semaines avec des prises hebdomadaires des températures au niveau axillaires et un traitement antipaludique à la semaine 11. Les participants asymptomatiques qui ont développés la fièvre avant le traitement antipaludique à la semaine 11 ont été définis comme les porteurs asymptomatiques du parasite à court terme (ST), tandis que ceux qui sont restés asymptomatiques ont été définis comme des porteurs asymptomatiques du parasite à long terme (LT). Les taux plasmatiques d'anticorps et l'avidité des anticorps dirigés contre différents antigènes de *P. falciparum* (extrait de stade mixte : TE, extrait de mérozoïte : ME, extrait de schizonte : SE, ou les proteines recombinates EBA-175, MSP-1 et MSP-4) et les niveaux plasmatiques de 38 biomarqueurs cellulaires (cytokines, chimiokines et facteurs de croissance) ont été mesurés par les methodes ELISA indirect et Luminex, respectivement. Les taux de ces biomarqueurs ont ensuite été comparés entre les groupes d'étude (NI : non infecté, LT, ST et SY : symptomatique) et suivis au fil du temps chez les individus avec une parasitémie asymptomatique. De plus, les activités inhibitrices de croissance des anticorps antiparasitaires circulants ont été évaluées et comparées entre les groupes en utilisant des souches de laboratoire de *P. falciparum* et un test d'inhibition de la croissance parasitaire basé sur Sybr Green-I. Sur les 353 individus testés par PCR multiplex, 328 (92,9 %) étaient positifs à *Plasmodium*, parmi lesquelles 266 (81,1 %) étaient des porteurs asymptomatiques. Les analyses multivariées ont identifié une faible parasitémie et l'absence de fièvre dans les trois mois précédents l'inclusion comme facteurs de risque

indépendants du portage de la parasitémie asymptomatique. De plus, les analyses des facteurs de risques ont révélé que le sexe féminin et l'anémie au moment de l'inclusion étaient des prédicteurs clés du développement précoce de la fièvre (<3 semaines après l'inscription) chez les individus asymptomatiques. Sur les 143 individus asymptomatiques qui ont terminé la période de suivi, 78 (55,4%) sont restés asymptomatiques (LT), tandis que 64 (44,8%) ont développé la fièvre associée au paludisme (ST) et 1 (0,8%) a résolu l'infection sans un traitement antipaludique connu. Les taux d'anticorps et l'avidité des anticorps contre tous les antigènes de *P. falciparum* testés étaient comparables entre les groupes d'étude. Cependant, l'indice médian d'inhibition de la croissance de *P. falciparum* était significativement plus élevé dans le groupe des individus avec le paludisme asymptomatique a long-terme que dans les groupes de ceux avec le paludisme asymptomatique à court-terme ou le paludisme symptomatique. Les niveaux plasmatiques de la chimiokine pro-inflammatoire IL-8 étaient significativement plus élevés chez les individus asymptomatiques à LT que chez les individus asymptomatiques à ST, tandis que les niveaux des ratios anti-inflammatoires IL-10/cytokines pro-inflammatoires étaient plus faibles chez les individus présentant une parasitémie persistante que chez ceux présentant des infections asymptomatiques à ST. Dans l'ensemble, chez les individus avec le paludisme à long-terme, aucun changement significatif dans niveaux d'anticorps IgG n'a été observé au fil du temps, bien qu'une augmentation continue de l'avidité des anticorps contre les antigènes TE et EBA-175 ait été observée. Malgré les fluctuations des niveaux de la plupart des cytokines, les niveaux du rapport IL-10/cytokine pro-inflammatoire, ainsi que les niveaux des principales cytokines régulatrices (IL-10, TNF-α, IL-1RA, MCP-1, IP-10 et Eotaxin), sont restés stables au cours de l'infection asymptomatique persistante. Les ratios de cytokines anti-/pro-inflammatoires, les taux plasmatiques d'IL-10 et de MCP-1 et l'avidité des anticorps contre certains antigènes, diminuait significativement après traitement des infections asymtomatiques persistentes à la semaine 11. Dans l'ensemble, les données de cette étude établissent l'implication des réponses d'anticorps antiplasmodiale encore non identifiées et de diverses réponses de cytokines régulatrices et pro-inflammatoires dans le maintien de la parasitémie asymptomatique à long terme dans la zone d'étude. De plus, ces données révèlent une prévalence extrêmement élevée de parasitémie asymptomatique dans la population, ainsi que la preuve d'une parasitémie asymptomatique persistante dans la zone d'étude, dévoilant pour la première fois l'association entre l'anémie palustre avec la conversion clinique précoce d'une infection asymptomatique en une infection symptomatique.

Mots clés : *Pasmodium falciparum,* paludisme asymptomatique, anticorps, cytokines, zone de forte transmission du paludisme

INTRODUCTION

Despite tremendous efforts made in the past decades, malaria remains a world health problem, causing an estimated 241 million cases and 627000 deaths in 2020 (WHO, 2021). The WHO African Region bears the largest burden of malaria morbidity, with over 93% of global malaria cases. The incidence of malaria cases and mortality rate have decreased considerably since 2000 by 36 % and 63 %, respectively (Cibulskis *et al.*, 2016). This, however, has been paralleled by high increases in the proportions of asymptomatic infections, known to contribute significantly to persistent malaria transmission in most endemic countries (Galatas *et al.*, 2016; Drakeley *et al.*, 2018).

Cameroon is one of the ten highest malaria burden African countries where the entire population is at risk of the infection (WHO, 2021). Malaria is endemic in most regions of Cameroon, with a predominance of *P. falciparum,* although the level of endemicity varies considerably from one geo-epidemiological transmission zone to another(Antonio-Nkondjio *et al.*, 2019). As in most central African countries, there is a lack of data on asymptomatic parasitaemia in Cameroon. Microscopy-based cross-sectional studies in the South-West and in the Centre Region of Cameroon showed a high prevalence of asymptomatic malaria varying from 17.8 to 74.1 % (Nyasa *et al.*, 2015; Tientche *et al.*, 2016; Roman *et al.*, 2018). However, there is no published data from community-based studies on the burden of asymptomatic *Plasmodium* infection and associated risk factors of asymptomatic parasitaemia in Cameroon. As the goal of all elimination strategies is to reduce the global incidence and death rate of malaria by at least 90 % by 2030 (WHOMPAC, 2016), understanding the impact and epidemiological patterns of asymptomatic malaria parasite carriage is essential to achieving such ambitious goals.

Although there is no standard definition of asymptomatic plasmodial infection, asymptomatic parasitaemia is generally defined as the presence of circulating *Plasmodium*-infected red blood cells for an extended period (weeks to years) and the absence of obvious clinical symptoms (Lindblade *et al.*, 2013; Chen *et al.*, 2016). Much of the available data on asymptomatic infections is from community cross-sectional surveys, which does not take into account the infection outcome. The asymptomatic carriage of malaria parasites may represent 1) recent infection on its way to patent/symptomatic infection and often lasting between 1 and 3 weeks depending on age in semi-immune populations (Pinkevych *et al.*, 2014; Slater *et al.*, 2019), 2) a resolving infection following antimalarial medication, or 3) a chronic asymptomatic infection (Ashley and White, 2014; Drakeley *et al.*, 2018). Some epidemiological data reveal that untreated *P. falciparum* infection occurs in a wide range of malaria endemicity, on an average of 6 months, but its stability and duration is likely context-specific (Landier *et al.*, 2017; Nguyen *et al.*, 2018; Drakeley *et al.*, 2018). Asymptomatic malaria infections generally correlate with low parasite density (Bousema *et al.*, 2014; Björkman,

2018), below the detection limit of conventional diagnostic tools (rapid diagnostic test: RDT and microscopy) which have been proved to be inadequate for a community screening of asymptomatic *Plasmodium* carriers (Mwingira *et al.*, 2014; Cook *et al.*, 2015; Ayong *et al.*, 2019). Therefore, to determine the burden and extent of asymptomatic malarial parasitaemia, highly sensitive diagnostic tools (molecular diagnostic platforms) are required to provide accurate epidemiological information (Britton *et al.*, 2016).

Evidence mainly from malaria immune-epidemiological data attributed asymptomatic plasmodial infection to several factors, including environmental, host and parasite factors (Doolan *et al.*, 2009; Laishram *et al.*, 2012; Galatas *et al.*, 2016). Asymptomatic parasitaemia is frequently associated with elderly people living in endemic areas, as they are probably more exposed to the *Plasmodium* infection and its vector, thus acquiring partial immunity (Ladeia-Andrade *et al.*, 2009; Mendonça *et al.*, 2013). Likewise, people who have had several episodes of symptomatic malaria are more likely to become asymptomatic upon a new infection (Andrade *et al.*, 2009; Barbosa *et al.*, 2014).

The host determinants of asymptomatic malaria infections are thought to involve two main immunological processes: anti-parasite immunity, involving processes that directly suppress parasite growth, thereby, limiting their presence to low infection densities; and anti-disease immunity involving processes that prevent overt clinical manifestations of the infection despite high infection loads (Galatas *et al.*, 2016; Ademolue and Awandare, 2018). Anti-disease immunity is a less understood phenomenon, but it is defined as any endogenous mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a non-injurious response (Riley *et al.*, 2006; Ademolue and Awandare, 2018). Such tolerance is suggested to be multi-factorial, including: (i) the neutralization of parasite toxins and other virulence factors; (ii) immuno-regulatory processes that control the excessive inflammatory responses of the host immune system; and (iii) cellular and systemic adaptive responses that regulate the adverse effects associated with the stress imposed by pathogens and/or host immunity (Galatas *et al.*, 2016). As cytokines mediate cellular responses or are responsible for the symptoms and pathological alterations during disease, the outcome of infection depends on the regulation of pro-inflammatory responses leading to protection or pathogenesis (Andrade *et al.*, 2011; Rovira-Vallbona *et al.*, 2012). Key amongst the mediators of tolerance during asymptomatic malaria infections are the pro-inflammatory cytokines IFN-γ, TNF-α, IL-1, IL-12, IL-18, and the regulatory cytokines IL-10, TGF-β, and IL-13 (Luty *et al.*, 1999; Wilson *et al.*, 2010; Andrade *et al.*, 2011; Gonçalves *et al.*, 2012; Guiyedi *et al.*, 2015). Furthermore, the outcome of the infection also depends on the balance between anti- and pro-inflammatory responses (Andrade *et al.*, 2011; Rovira-Vallbona *et al.*, 2012).

Anti-parasite immunity, on the other hand, known to be acquired with age or exposure (Doolan *et al.*, 2009; Griffin *et al.*, 2015; White and Watson, 2021) is mediated mainly by anti-plasmodial antibody responses but could also involve regulated cytokine responses that together suppress parasite growth, limiting parasitaemia below the pyrogenic threshold and protecting against clinical disease (Galatas *et al.*, 2016; Ademolue and Awandare, 2018). Antibodies may control parasite replication and clearance via a number of mechanisms, including inhibition of merozoite egress and invasion, antibody-dependent cellular inhibition, complement-mediated lysis or opsonic phagocytosis and antibody-dependent respiratory burst activity by polymorphonuclear neutrophils (Bouharoun-Tayoun *et al.*, 1990; Thuilliez *et al.*, 2010; Duncan *et al.*, 2012; Osier *et al.*, 2014; Boyle *et al.*, 2015b). Anti-parasite immunity, particularly antibody responses increase with maturation of the immune system and elicited during cumulative clinical bouts of malaria, but this response is not adequately induced at levels that would confer protection in young children. By adulthood, after infection, parasite loads often remain at very low levels, usually undetectable by light microscopy, and most infected adults do not exhibit clinical symptoms. Development of partial immune responses to clinical malaria may be antigen dependent, and mediated by exposure to genetically distinct parasite subpopulations (clones). In areas of high malaria transmission, persons who are regularly exposed to a high diversity of parasite clones, are likely to develop anti-disease immunity and asymptomatic infections (Magesa *et al.*, 2002). However, in either case how anti-plasmodial acquired antibodies and cytokine responses are maintained over time to continuously protect against clinical malaria remains enigmatic.

In asymptomatic individuals, it has been suggested that persistent parasitaemia is essential for the maintenance of antimalarial immunity, the disruption of which could result in increased host susceptibility to severe disease (Sergent, 1950; Baird, 1995; Smith *et al.*, 1999). However, a recent study showed that chronic asymptomatic *P. falciparum* infection during dry season predicted decreased clinical malaria risk during the subsequent malaria season but treatment of these infections did not alter this reduced risk (Portugal *et al.*, 2017). The effect of treating persistent asymptomatic infections on the host's anti-parasitic and anti-disease immune responses is unclear.

Research question

Do host anti-parasite and/or anti-disease immune responses contribute significantly to the maintenance of asymptomatic parasitaemia in highly exposed individuals?

Hypothesis

Host anti-parasite and anti-disease immunity contribute to the maintenance of asymptomatic infections in highly exposed individuals.

Study objectives

General objective

Investigate the contribution of anti-parasitic and anti-disease immune responses in the maintenance of asymptomatic parasitemia in individuals in a *Plasmodium falciparum* hyper-endemic zone (Esse Health District, Central Region) in Cameroon

Specific objectives

1. Determine the prevalence and identify associated risk factors of asymptomatic *P. falciparum* parasitaemia in the Esse Health District, Central Region of Cameroon
2. Assess the contribution of host antibody responses to the maintenance of asymptomatic parasitaemia in highly exposed individuals
3. Identify the cytokines potentially involved in asymptomatic parasitaemia in highly exposed individuals

CHAPTER I: LITERATURE REVIEW

I.1. Epidemiology of malaria

Malaria is a parasitic infection caused by a protozoan of the genus *Plasmodium* and transmitted mainly to humans by the bite of a female *Anopheles* mosquito. Malaria is one of the main public health problems, with almost a third of the world's population at risk, particularly in tropical and poor regions of the world. This parasitic disease led to an estimated 241 million clinical cases and 627 000 deaths in 2019 (WHO, 2021). The incidence of malaria, which takes into account population growth, is estimated to have decreased by 37 % between 2000 and 2015 (Cibulskis *et al.*, 2016), but the global incidence rates did not decline from 2015 to 2019 (WHO, 2020). This vector-borne parasitic tropical disease is found in 91 countries worldwide. The sub-Saharan African Region bears the largest burden of malaria morbidity, with 228 million cases (95%) in 2020, followed by the South-East Asia Region (2%) and the East Mediterranean Region (1.7%) (WHO, 2021) (Figure 1). Almost 96% of all malaria cases globally were in 29 of the 85 countries that were malaria endemic, including 28 African countries and India. Disease incidence depends on the environmental suitability for local vectors in terms of altitude, climate, vegetation, and implementation of malaria control measures, and hence is linked to poverty, natural disasters, and war (Ashley *et al.*, 2018).

Cameroon is one of the 11 African countries that bears 70% of the global estimated case burden and 71% of global estimated deaths, with more than 25% of the population infected and the whole country at risk of infection (Massoda-Tonye *et al.*, 2018; WHO, 2019) . In 2020, the WHO estimated 5.6 million cases of malaria in Cameroon, causing 4121 deaths. Cameroon has different geographic and ecological zones, that generate three epidemiologic facets of malaria transmission (Massoda-Tonye *et al.*, 2018; Antonio-Nkondjio *et al.*, 2019) 1) the dry Sahelian in the Far North Region where malaria transmission period is between 1–3 months and ranging from 1 to 100 infected bites per man per year (ib/per/year); 2) Sudano- Sahelian in the North Region and the highlands of Adamawa where malaria transmission period is between 6–9 months and ranging from 100 to 200 infected bites per man per year (ib/per/year); 3) the equatorial forests which include Centre, West, East and part of South Regions, where the transmission is stable; and the Atlantic coastal covering the Littoral and a part of the South and South-West Regions where the malaria is perennial with seasonal variations ranging from 100 to 400 infected bites per man per year (ib/per/year) (Figure 2).

Several obstacles have been identified to limit the fight against malaria, including the spread of parasites resistant to antimalarial drugs, the lack of tools for effective diagnosis of infection, and the predominace of asymptomatic malaria infections (Guyant *et al.*, 2015; Ferreira and Castro, 2016; Cibulskis *et al.*, 2016).

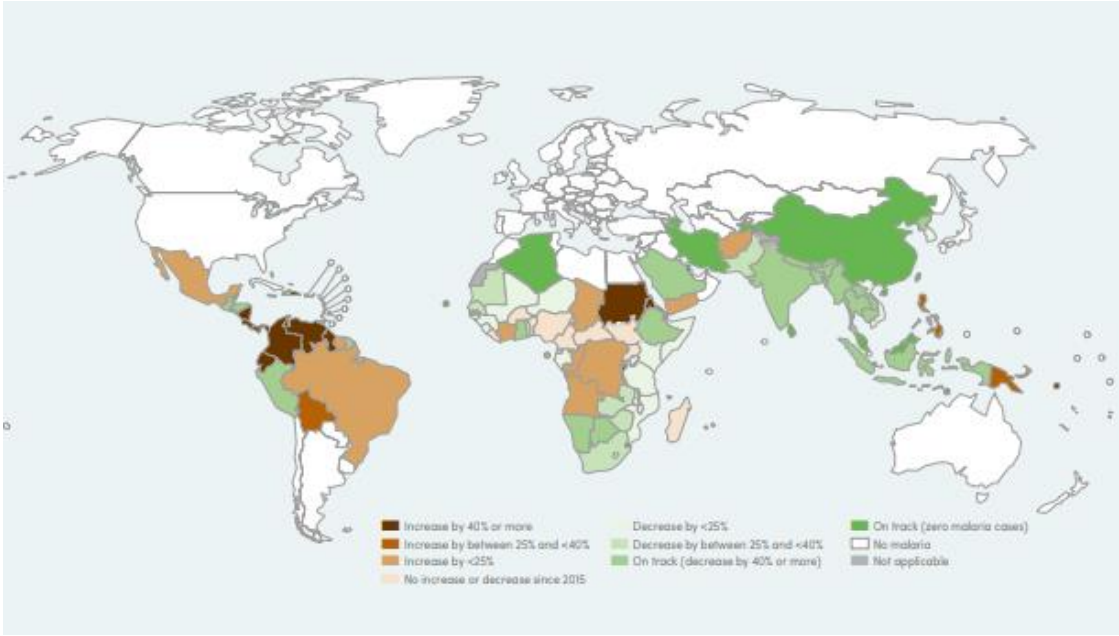

Figure 1: Map of malaria case incidence rate (cases per 1000 population at risk) by country. (WHO, 2021)

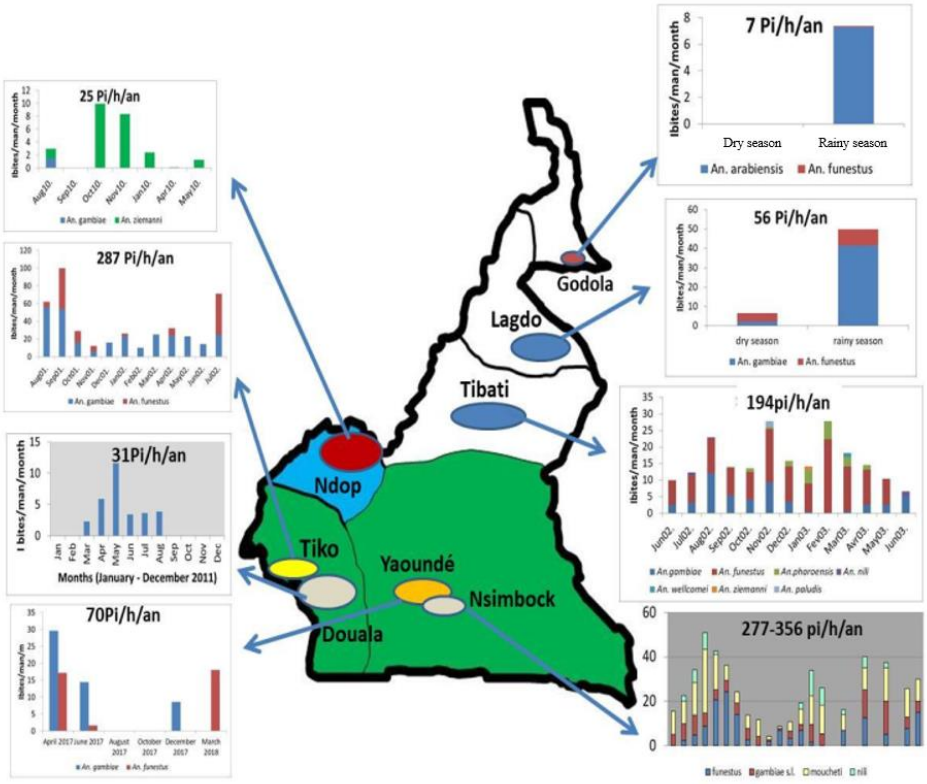

Figure 2: Cameroon eco-climatic facies and malaria epidemiology (PNLP, 2020)

I.2. Transmission of malaria

The malaria parasite (*Plasmodium*) is transmitted from one infected human to another human by the bite of the female *Anopheles* mosquito. Some less common transmission routes exist: from mother to child, or via blood transfusion which are rare occurrence in non-endemic countries, but represent a significant risk in resource-poor settings (Abdullah and Karunamoorthi, 2016).

I.2.1. Malaria vector: *Anopheles* mosquito

Human malarial parasite are transmitted by mosquitoes of the genus *Anopheles*, which includes 465 identified species and more than 50 unnamed members of complex species (Sinka *et al.*, 2012). Approximately, 70 of the *Anopheles* species are known to have the capacity to transmit human *Plasmodium* parasites (Sinka *et al.*, 2012) and 41 are considered to be dominant vector, capable of transmitting malaria at a level of major concern to public health (Hay *et al.*, 2010). In Cameroon, up to 52 anopheline species have been reported so far, with 16 recognized as main or secondary vectors (Antonio-Nkondjio *et al.*, 2006). Six of the *Anopheles* species are among the most efficient vectors in sub-Saharan Africa, including, *An. gambiae,* An. *coluzzii, An. arabiensis, An. funestus, An. nili and An. moucheti* (Antonio-Nkondjio *et al.*, 2006).

Several environmental, behavioural, cellular, and biochemical factors determine the ability of a mosquito to transmit malaria. These factors include physical conditions such as geographic colocalization with the parasite, temperature, and humidity as well as characteristics of the mosquitos, including, host preference, feeding rate and behaviour, density of the mosquito population, and the mosquito's susceptibility to infection by *Plasmodium* (Cohuet *et al.*, 2010). Different species show major differences in vectorial capacity. For example, some anopheline species, such as *An. gambiae*, are efficient vectors of malaria in part because they are anthropophilic; others, such as *An. albimanus*, are more zoophilic and prefer to feed on animals over humans, making them less efficient vectors of malaria (Sinka *et al.*, 2010). Most female *anopheles* mosquitos prefer to bite indoors and mostly nocturnal with pick biting periods between 11 pm and 2 am.

I.2.2. Malaria parasite: *Plasmodium* ***ssp***

I.2.2.1. Taxonomy

Plasmodia are eukaryotic unicellular organism and belong to the phylum apicomplexa. The genus *Plasmodium* is subdivided into sub-genus *Plasmodium* and *Laverania* under the sub-order Haemosporidiidea (Table I) (Antinori *et al.*, 2012). Among more than 120 *Plasmodium* species infecting mamalians, birds, and reptiles, only six are known to infect human beings regularly, including *Plasmodium falciparum, vivax, malariae, ovale*, *Knowlesi* and *cynomolgi* (Singh *et al.*, 2004; Ta *et al.*, 2014). *P. falciparum* and *P. vivax* are the predominant *Plasmodium* species worldwide. The vast majority of *P. falciparum* malaria occurs in sub-Saharan Africa where transmission remains extremely high in many locations, although there is considerable variation in incidence within and between countries or settings (WHO, 2019). *P. falciparum* is globally the deadliest *Plasmodial* species and is the most widespread in Africa. In Asia and Oceania, the number of malaria cases are generally lower, and the proportions caused by *P. vivax* and *P. falciparum* are similar. However, in America, *P. vivax* cases exceed *P. falciparum* by more than two times (WHO, 2019). *P. malariae* and *P. ovale* are distributed

worldwide but incidence is low with *P. ovale* found mainly in Africa and Southeast Asia. Although the macaques are the natural hosts of *P. Knowlesi*, significantly high proportion of *P. Knowlesi* infections have been identified in Malaysian individuals (Singh *et al.*, 2004). Human infections with other simian malaria parasites such as *P. cynomolgi* and *P. simium* have also been reported in Asia (Ta *et al.*, 2014; Brasil *et al.*, 2017)

Table I: Classification of human protozoa of the genus *Plasmodium*

Domain	Eukaryota
Kingdom	Chromalveolata
Superphylum	Alveolata
Phylum	Apicomplexa
Class	Aconoidasida
Order	Haemosporida
Sub-order	Haemosporidiidea
Family	*Plasmodiidae*
Genus	*Plasmodia*
Sub-genus	*Plasmodium; Laverania*
Species	*P. falciparum*
	P. vivax
	P. malariae
	P. ovale
	P. Knowlesi
	P. cynomolgi

I.2.2.2. Life cycle

Plasmodium parasites have a very complex life cycle (Figure 3), with sporozoites transmitted by the *Anopheles* mosquito during the blood meals to the human host. The injected sporozoites cross layers of the skin and enter the bloodstream, reaching the liver within hours follow the mosquito bite and then invade the hepatocytes, replicating and differentiating into schizonts. In *P. vivax* infection, some sporozoites turn into dormant forms know as hypnozoites, which can be activated after a long period of latent parasitic infection. As a result of hepatocyte rupture, merozoites are released into the bloodstream and infect erythrocytes (*P. falciparum* parasites) or reticulocytes (*P. vivax* parasites), thus initiating the asexual blood stage of the cycle. These blood parasitic forms undergo several cycles of multiplication and differentiation, through rings, trophozoites, schizonts, therefore, increasing the parasitaemia in the circulation. Whereas the newly-released merozoites can keep re-invading the new erythrocytes, a small fraction of them differentiate into gametocytes, giving rise to the sexual blood

stage. Gametocytes (male and female) are ingested by the mosquito vector during the blood meal and fuse with each other within the mosquito's digestive tract, forming a zygote. The zygote differentiates into an ookinete, and then into an oocyst which bursts and releases infectious sporozoites which migrate to the mosquito's salivary glands, thus completing the life cycle. When the infected *Anopheles* mosquito takes a blood meal on another individual, saliva containing sporozoites are injected into skin dermi and the cycle begins again. The *Plasmodium* parasite life cycle is shown in Figure 3.

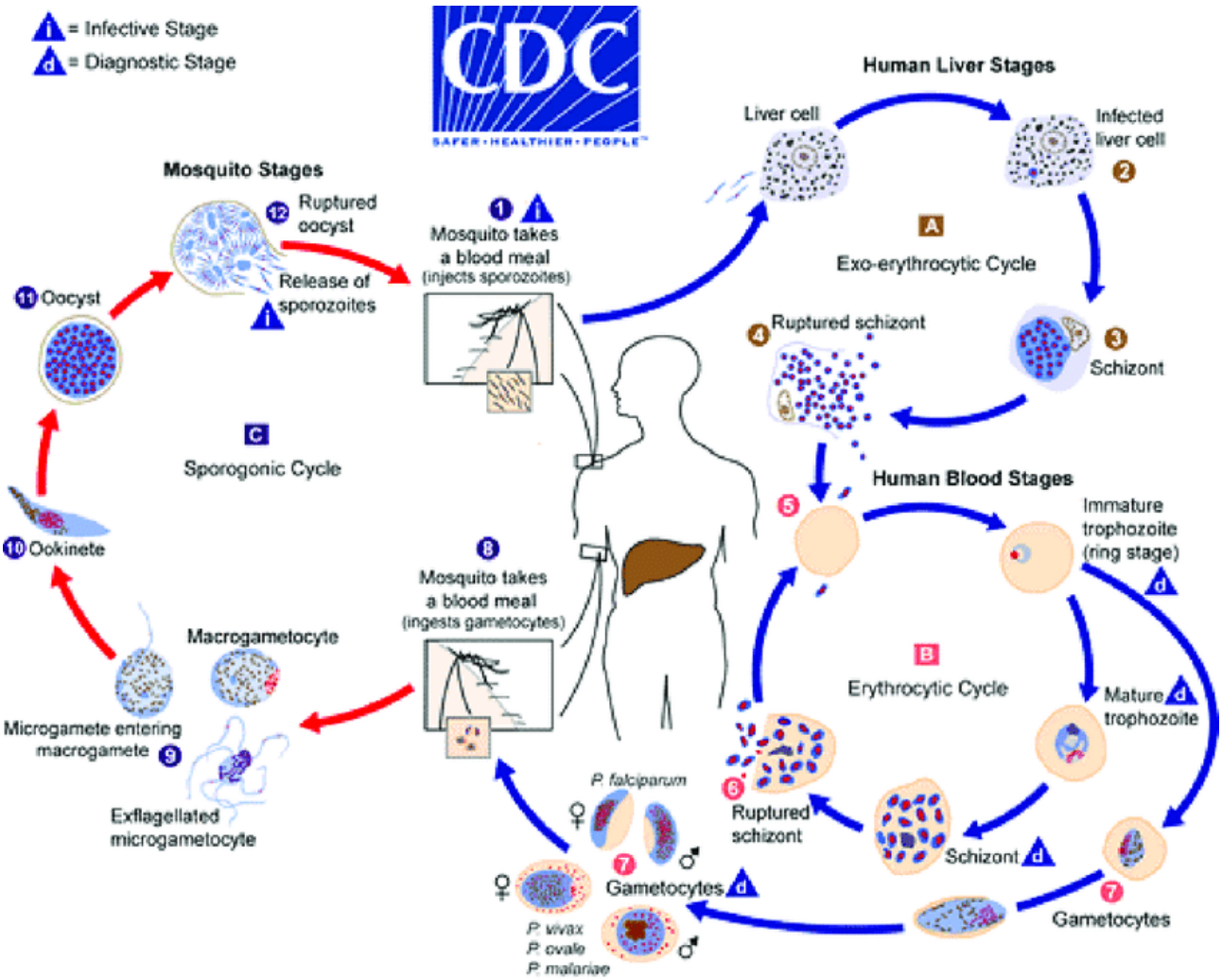

Figure 3: *Plasmodium* **parasite life cycle**.

During a blood meal, a malaria-infected female Anopheles mosquito inoculates sporozoites into the human host[1]. Sporozoites infect liver cells[2] and mature into schizonts[3], which rupture and release merozoites[4]. After this initial replication in the liver (exo-erythrocytic schizogony [A]), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony [B]). Merozoites infect red blood cells[5]. The ring stage trophozoites mature into schizonts, which rupture releasing new merozoites[5]. Some parasites differentiate into sexual erythrocytic stages (gametocytes)[7]. The gametocytes, male and female, are ingested by an *Anopheles* mosquito during a blood meal[8]. The parasites' multiplication in the mosquito is known as the sporogonic cycle[C]. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes[9]. The zygotes in turn become motile and elongated (ookinetes)[10] which invade the midgut wall of the mosquito where they develop into oocysts[11]. The oocysts grow, rupture, and release sporozoites[12], which make their way to the mosquito's salivary glands. Inoculation of the sporozoites[1] into a new human host perpetuates the malaria life cycle (Source CDC, 2017).

I.3. Clinical manifestation and pathophysiology of malaria

Infection with malaria parasites results in a wide range of clinical manifestations, from mild or uncomplicated to severe, and the poorly understood asymptomatic infections.

I.3.1. Asymptomatic infection

I.3.1.1. Definition of asymptomatic infections

After the pre-erythrocytic stage of *Plasmodium* infection, which usually takes between 11 and 15 days, the initial blood stage multiplication is mainly characterized by a recurrent cycle of fever and chills due to the release of merozoites into the bloodstream, as the result of the rupture of *Plasmodium* schizont. In some cases, these symptoms are not noticed, and the infection is described as asymptomatic in individuals without any recent history of antimalarial medication (Lindblade *et al.*, 2013; Galatas *et al.*, 2016). However, there are no standard criteria for defining the asymptomatic malaria infections (Laishram *et al.*, 2012; Lindblade *et al.*, 2013; Galatas *et al.*, 2016). The most basic definition seems to be the presence of *Plasmodium* in the bloodstream (regardless of the parasite load) and the absence of malaria symptoms, mainly fever (axillary temperature $\leq 37.5^{\circ}C$). This definition is ambiguous and several studies have modified it by incorporating some strict criteria, such as suggesting the use of longitudinal follow-up of initial asymptomatic cases, quantifying parasite rather than reporting its presence or absence, and the use of sensitive molecular test (PCR or LAMP) to identify low-density asymptomatic infections in a population (Laishram *et al.*, 2012). However, there is no common consent on the duration of the fever history and it ranges from weeks to months (Almelli *et al.*, 2014; Tran *et al.*, 2016; Drakeley *et al.*, 2018; Wamae *et al.*, 2019). The longitudinal follow-up after the diagnosis of the plasmodial infection reduce the chances of misclassified asymptomatic infections that are defined during *P. falciparum* incubation phase toward a clinical form of the infection. Studies that define asymptomatic status on the basis of a particular time point, may misclassify as asymptomatic certain infections that are at an early stage of progression toward symptomatic disease or in the process of being cleared after anti-malarial medication (Galatas *et al.*, 2016). In addition, it has been shown that, when initially infected, a person has a period of sub-patent parasitaemia after liver stage before they multiply to the detection limit of the microscopy. This takes longer in semi-immune adults (average ~3 weeks, sometimes >63 days) when compared to young children (average 1 week) (Pinkevych *et al.*, 2014), and indeed some infections may never reach microscopically detectable levels (Slater *et al.*, 2019).

The method used for the diagnosis of asymptomatic parasitaemia is also important. Microscopy, with a detection limit of ~50 parasites/µl (10-100 parasites/µL), may miss sub-patent infections, whereas molecular tests such as PCR or LAMP that are more sensitive can detect parasitaemia below one parasite/µl (Kemleu *et al.*, 2016; Ayong *et al.*, 2019). In addition, the use of molecular tools has been shown to identify significantly increase burdens of asymptomatic infections in most malaria endemic

areas. This suggests that, the most sensitive diagnostics tools (such as LAMP or PCR) are essential for detecting asymptomatic individuals in malaria endemic settings.

I.3.1.2. Prevalence and duration of asymptomatic infections

Asymptomatic malaria is the major outcome of *Plasmodium* infections in semi-immune individuals from endemic areas (Lindblade *et al.*, 2013), with prevalences four to five times higher than clinical infections. There is a link between asymptomatic malaria prevalence and high endemicity as well as high transmission intensity. Indeed high prevalences of asymptomatic infections have been reported in several African countries, ranging from 20% to 97% in areas of high transmission compared to less than 10% in low transmission areas (Laishram *et al.*, 2012; Lindblade *et al.*, 2013).

Most data on asymptomatic infections are from community cross-sectional surveys. The very few studies that have followed the asymptomatic infections over time report a shift to symptomatic infections in only a small proportion of infections. However, determining the infection duration in endemic areas is complicated because individuals are frequently infected with multiple *P. falciparum* clones (superinfection). A longitudinal study in Ghana showed that naturally occurring asymptomatic infections last on average 5–6 months (Felger *et al.*, 2012). In Myanmar, an area with low malaria infection (transmission approaching elimination levels), *P. falciparum* parasite carriage for 6–9 months was observed (Landier *et al.*, 2017). A study in Cambodia, which followed monthly 24 asymptomatically *P. falciparum*-infected adults, found that 13% carried parasites between 2–4 months, whereas 87% had cleared their parasitaemia after 1 month (Tripura *et al.*, 2016). In a recent cohort study in Vietnam, nearly 10% of asymptomatic infected individuals carried parasites for more than 4 months (Nguyen *et al.*, 2018). Finally, mathematical modeling of case reports, accidental malaria infections during blood transfusion, and other sporadic malaria cases in non-endemic countries have suggested that asymptomatic *P. falciparum* infections may persist for up to a decade or longer (maximum confirmed 13 years) (Ashley and White, 2014). Based on the available data, the mean duration of asymptomatic infection is hard to estimate because generally, studies vary in the design, however, the published data demonstrate that chronic parasite carriage occurs in different malaria endemicities, although the burden and duration are likely context-specific (Drakeley *et al.*, 2018).

I.3.1.3. Determinants of asymptomatic infections

Asymptomatic malaria manifestations may result from a combination of several factors, including host, parasite, and environmental factors.

a) Host determinants

Host immunity

The protection from clinical malaria can be attributed to two key immune processes: 1) anti-parasite immunity, which involves all the immune processes that directly suppress parasite multiplication and result in effective parasite densities control below the pyrogenic threshold, and 2)

anti-disease immunity, which would involve immune processes that altogether prevent the manifestation of clinical symptoms (i.e. immunopathology), despite a high parasitaemia (Ademolue and Awandare, 2018). The anti-parasite immune response, particularly mediated by antibody responses, is induced during progressive clinical bouts of malaria and can control parasite load below the pyrogenic threshold resulting in the control of disease manifestations, which leads to asymptomatic infection. Both innate and adaptive immunity seem to play major roles in asymptomatic *Plasmodium* infections. Host immunity to asymptomatic malaria is discussed further in the subsequent sections.

Host genetic factors

Genome-wide association studies (GWAS) have indicated some genetic variants provide at least some protection against severe malaria (Damena *et al.*, 2019), suggesting the possibility of coevolution between parasites and the host. These genetic variants include several RBC polymorphisms, like glucose 6-phosphate dehydrogenase deficiency (G6PD) (Ruwende *et al.*, 1995; Mombo *et al.*, 2003), haemoglobin variants (Modiano *et al.*, 2001; Chotivanich *et al.*, 2002), ABO blood group antigen (Hill, 1992), ovalocytosis (Genton *et al.*, 1995), and polymorphisms in complement receptor 1 or cytokine receptors (IL-12 and IL-23 receptors) (Huang *et al.*, 2000; Cockburn *et al.*, 2004). Such protective roles are less clear for mild or uncomplicated malaria as well as for asymptomatic malaria (Migot-Nabias *et al.*, 2006). There is strong evidence that blood group O provides protection against severe malaria by a mechanism of reduced rosetting and sequestration (Rowe *et al.*, 2007). In addition, a high proportion of blood group O was found in individuals with asymptomatic infections (Alemu and Mama, 2016). Significantly fewer studies have directly addressed the role of host genetic factors in asymptomatic infections, reporting inconclusive results probably due to the low power to detect modest effects (Laishram *et al.*, 2012).

Pregnancy

Pregnancy has been identified as a possible large source of chronic asymptomatic parasite infections (Khan *et al.*, 2014; Carmona-Fonseca and Arango, 2017). Similarly, in areas with intense malaria transmission, infected pregnant women have been identified to be commonly asymptomatic (Khan *et al.*, 2014). These observations could be due to the immunomodulation caused by the pregnancy. Studies on cytokine and growth factors levels in asymptomatic pregnant women showed an association with increased plasma concentrations of regulatory biomarkers IL-10 and G-CSF (Wilson *et al.*, 2010).

Comorbidity

Given that co-infections are common in developing countries, co-infecting organisms, thereby complicate the understanding of plasmodial infection outcomes. Individuals co-infected with *Plasmodium* and hepatitis B virus infection were more likely to be asymptomatic and to have lower parasite densities, possibly the result of an increase in the production of IFN-γ induced by the viral

infection that can contribute to *Plasmodium* clearance in the liver (Andrade *et al.*, 2011). Co-infection with *Plasmodium* and human immunodeficiency virus (HIV) is thought to decrease control of parasites by viral impairing lymphocyte function, inducing asymptomatic malaria (Noormahomed *et al.*, 2012; Okonkwo *et al.*, 2016). In Indonesia, one study reported that soil-transmitted helminth infections were associated with increased levels of *P. vivax* parasitaemia in asymptomatic individuals (Burdam *et al.*, 2016). Co-infection with the trematode *Schistosoma hematobium* in a co-endemic area of Mali was associated with the protection against the development of malaria fevers leading to a higher prevalence of asymptomatic infections (Doumbo *et al.*, 2014).

b) Parasite determinants

The differences noted between *P. falciparum* species and strains in terms of maximum parasitaemia, pyrogenic thresholds, multiplication rates, and cytoadhesion properties leading to sequestration in vital organs suggest that there might be parasite factors that contribute to the development of asymptomatic infections.

Pyrogenic Threshold

Clinical symptoms of malaria infection are not only due to the asexual blood-stage parasites. Rupture of infected red blood cells releases not only merozoites but also parasite by-products such as haemozoin, glycosylphosphatidylinositol (GPI), and other toxic factors into the bloodstream that trigger the production of pyrogenic inflammatory mediators and cytokines by innate immune cells which stimulate the increase in body temperature (Oakley *et al.*, 2011). High parasite densities lead to a high amount of toxins and pyrogenic mediators and then the increased body temperature and the risk of fever. The parasite densities threshold has been considered as a potential indicator of the risk of symptomatic malaria in an infected individual (Smith *et al.*, 2006). The ability of the parasites to control fever may be important to regulate its growth (Oakley *et al.*, 2011), thereby establishing chronic infection. The pyrogenic threshold of *P. falciparum* infections in non-immune individuals has been shown to vary according to transmission intensity, age, or host ethnicity (Gaston & Cheng, 2002). In high malaria transmission areas, clinical illness is not often associated with a *P. falciparum* parasitaemia below 10000 parasites/µl (Smith *et al.*, 2004). However, this has not been observed in areas with intense seasonal or low-transmission (Boisier *et al.*, 2002). Moreover, pyrogenic thresholds have been shown to be significantly different between different *P. falciparum* strains (Gaston & Cheng, 2002), suggesting that some strains may induce a febrile response in the host at lower parasite densities compared with other strains.

Multiplication rate

The parasite multiplication rate has been associated with the severity of *P. falciparum* infections (Chotivanich *et al.*, 2000), and depends on the balance between the intrinsic susceptibility of the host

erythrocytes, the level of immunity to malaria, and the ability of the parasites to multiply at high densities (Pinkevych *et al.*, 2014). The first *P. falciparum* epigenetic factor identified involved in transcriptional control of antigenic variation was the conserved NAD-dependent deacetylase Sir2 [*P. falciparum* sirtuin 2a (*Pf*Sir2a)] which regulates the transcription of *Plasmodium* rDNA (Duraisingh *et al.*, 2005). High temperature known to be associated with febrile illnesses during *Plasmodium* infection is suggested to modulate *Pf*Sir2a activity (Oakley *et al.*, 2011), balancing the malaria parasite's energy status with the synthesis of rRNA and parasite multiplication. Therefore, the epigenetic environment may play a role in the establishment or maintenance of asymptomatic infections and modulate the changes between asymptomatic and febrile infection in response to the host or as part of the parasite developmental program (Merrick *et al.*, 2012). Moreover, a study shows that regulation of genes involved in DNA replication and the resulting protein response could potentially lead to dormant phenotypes in *P. falciparum* malaria, and though to contribute to the resistance of parasites to artemisinin (Mok *et al.*, 2015).

Parasite cytoadhesion

The virulence of *P. falciparum* has been explained by the expression on the surface of infected red blood cells (iRBCs) a large family of variant proteins called *P. falciparum* erythrocyte membrane protein 1 (*Pf*EMP-1) which can bind to several host receptors to mediate the sequestration of iRBCs in deep organs (Miller *et al.*, 2013). *Pf*EMP-1s are encoded by more than 60 variant (var) genes per parasite genome, which are expressed in an exclusive manner (one variant per clone of *P. falciparum*) to avoid simultaneous recognition by the immune system (Miller *et al.*, 2013). *Pf*EMP-1 is subdivided into three main groups A, B, and C based on motifs in noncoding sequences and chromosome/locus position, despite an extreme inter- and intra- genome variability, (Smith *et al.*, 2001). Previous studies have shown that group A var genes have preferentially expressed by parasites associated with severe malaria, while group C is expressed by parasites found in asymptomatic malaria cases (Rottmann *et al.*, 2006; Falk *et al.*, 2009). This differential expression of var gene suggests that once the initial var gene repertoire can be controlled by the immune system, var group C, or an alternative, unknown group of var gene variants that do not mediate cytoadherence in vital organs, might prevail in asymptomatic infections (Galatas *et al.*, 2016).

Multiplicity of Infection and Drug Resistance

Drug resistance or multiplicity of infection (MOI) has been suggested to differ between malaria clinical presentations. Some recent studies found a significantly higher prevalence of drug-resistant parasite strains among asymptomatic children compared to febrile children (Tukwasibwe *et al.*, 2014, 2017). This may be due to decreased virulence among drug-resistant parasites, as a result of, possible loss of parasite fitness mediating antimalarial resistance (Ord *et al.*, 2007). A study found a higher proportion of mixed genotype infections and a higher number of genotypes in individuals with

asymptomatic parasitaemia compared to those with symptomatic infections and that the diversity of infectious strains could increase the protection against clinical malaria (Sondén *et al.*, 2015). Additionally, there is evidence that multiplicity of genotypes could also favor the parasite by extending the duration of infections through the natural selection of genotypes that ensure parasite survival and transmissibility (Wargo *et al.*, 2007).

c) Environmental determinants

Since the prevalence, dynamics, and transmissibility of asymptomatic parasitaemia vary geographically, it is clear that environmental factors play an important role. The associated risk factors of asymptomatic parasite carriage, which may vary from one geo-epidemiological setting to the other, are also poorly understood. Studies have provided evidence that the individual- and household-level factors are associated with asymptomatic malaria infection in different endemic contexts (Sturrock *et al.*, 2013; Cotter *et al.*, 2013; Monteiro *et al.*, 2015; Zhao *et al.*, 2018). Indeed, studies found that an increased risk of asymptomatic infections in (1) males, (2) adults (≥ 15 years), (3) those who failed to use a bed net and IRS, (4) those who lived in poor houses, and (5) those who lived farther away from a clinic (Sturrock *et al.*, 2013; Cotter *et al.*, 2013; Monteiro *et al.*, 2015; Zhao *et al.*, 2018). Most of these factors are linked to exposure to mosquito bites and related to the fact that the level of anti-*Plasmodium* immunity is closely linked to the level of exposure and age.

I.3.1.4. Consequence of asymptomatic parasitaemia

Asymptomatic infection is sometimes considered beneficial to the host rather than no infection because asymptomatic infections are supposed to maintain immunity against malaria. However, persistent asymptomatic infections are often associated with recurrent episodes of symptomatic parasitaemia, chronic anaemia, susceptibility to non-invasive bacterial disease, cognitive impairment, maternal and neonatal mortality, and maintenance transmission of the parasite (Chen *et al.*, 2016). Chronic parasitaemia is also associated with splenomegaly in children arising from filtration, retention, and phagocytosis of parasitized erythrocytes, and erythrocyte debris from the associated inflammatory response (Buffet *et al.*, 2009). In extreme cases, hyper-reactive malarial splenomegaly can arise from chronic antigenic stimulation secondary to malaria parasitaemia, resulting in haemolytic anaemia, splenic rupture, and increased susceptibility to other acute infections (Leoni *et al.*, 2015). Asymptomatic infections and placental parasitaemia also have major consequences for mothers and their newborns. Placental malaria infection (generally asymptomatic) is associated with placental inflammation, fibrosis, and functional insufficiency, leading directly to miscarriage, preterm delivery, low birth weight, and peripartum haemorrhage and, thus, increased maternal and neonatal mortality (Tagbor *et al.*, 2008; Cottrell *et al.*, 2015).

At the community level, asymptomatic carriers usually do not seek medical attention, and thus serve as human reservoirs for malaria transmission. Such infections are recognized as an important obstacle to malaria elimination.

I.3.2. Uncomplicated malaria

All clinical symptoms of *Plasmodium* infections appear after hepatic merozoite invades red blood cells. All symptoms and signs of uncomplicated malaria are non-specific as they are common with other febrile conditions, and can occur early or later in the course of the infection (Grobusch and Kremsner, 2005). The most frequent clinical features of uncomplicated or mild malaria include fever, cephalgias, fatigue, malaise, and musculoskeletal pain. The initial blood stage multiplication of *Plasmodium* is mainly characterized by a recurrent cycle of fever and chills due to the rupture of the schizont stage and the release of merozoites or parasite byproducts (haemozoin, GPI, and other toxic factors) into the circulation. These immunological toxins (parasite byproducts) trigger the release of inflammatory cytokines such TNF, interleukin 1, and interleukin 6 from immune cells resulting in the increase body temperature by stimulating the thermoregulatory regions of the brain (Netea *et al.*, 2000) (Oakley *et al.*, 2011). Other uncomplicated malaria manifestations include chills and sweats, headache, vomiting, watery diarrhea, anaemia, jaundice, and splenomegaly, but do not generally have any of the characteristics identified in severe malaria.

I.3.3. Complicated malaria

In addition to the large prevalence of uncomplicated *Plasmodium* infections, malaria-associated mortality mainly results from complications of malaria which appear in approximately 0.2 % of the *Plasmodium*-infected individuals (WHO, 2019). The most common features of severe malaria are cerebral malaria (characterize by a coma), acute lung injury, which can progress to acute respiratory distress syndrome (in up to 25% of cases), acute kidney injury, typically presenting as acute tubular necrosis, and acidosis (Taylor *et al.*, 2012). Severe anaemia without any major organ dysfunction is a common presentation of severe malaria in children. As other differences in disease presentation in children compared to adults, children have more frequent seizures (in 60–80%), hypoglycaemia, and concomitant sepsis, and less frequent pulmonary oedema, and renal failure (Maitland, 2015; Ashley *et al.*, 2018). A non-exhaustive list of severe malaria symptoms and clinical manifestations are shown in Table II.

To avoid parasite clearance in the spleen, *P. falciparum* iRBCs sequester inside small and medium-sized vessels and cause host endothelial cell injury and microvascular obstruction. Cytoadherence is mediated by a clonally variant adhesion protein exported on the surface of iRBC named *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by the var gene family. PfEMP1 bind to different receptors on endothelial cells, but also on uninfected red blood cells

(rosetting). For example, PfEMP1 subtypes that can bond the intercellular adhesion molecule-1 and endothelial protein C receptor are associated with cerebral malaria (Turner *et al.*, 2013; Lennartz *et al.*, 2017). Taking advantage of the decrease in deformability and the expression of surface antigens, late schizonts easily accumulate in the microvasculature of the host and bind to the endothelium, a process named sequestration. The clinical consequence of iRBCs sequestration is associated with endothelial dysfunction which may depend on the organ(s) involved. In the brain, parasite sequestration contributes towards coma, in the lungs, it predisposes to respiratory distress, and in pregnancy associated malaria, parasite sequestration in the intervillous space of the placenta leads to placental malaria with a wide range of consequences both for mother and fetus such as maternal anaemia, low birth weight, preterm labour, and increased risk of abortion and stillbirth (Fried and Duffy, 2017; Moore *et al.*, 2017). The sequestration of the iRBCs in the placenta is mediated by binding to chondroitin sulphate A (CSA) by the PfEMP1 variant VAR2CSA, and the sad effects on the gravidity (Moore *et al.*, 2017).

Table II: Severe malaria diagnostic criteria ***(WHO, 2014).***

Clinical criteria

- Prostration
- Confusion or agitation (with Glasgow Coma Scale [GCS]>11)
- Coma (GCS ≤11 or Blantyre Coma Scale <3 in children)
- Respiratory distress (acidotic breathing)
- Convulsions
- Shock: prolonged capillary refill time (>2 s), with or without systolic blood pressure <80 mm Hg in adults (<70 in children)
- Pulmonary oedema (should be confirmed radiologically)
- Abnormal bleeding
- Jaundice
- Anuria
- Repeated vomiting

Laboratory criteria

- Haemoglobin <7 g/dL in adults, <5 g/dL in children
- Haemoglobinuria
- Hypoglycaemia (blood glucose <2.2 mmol/L or <40 mg/dL)
- Acidosis (ie, base deficit >8 meq/L or plasma bicarbonate <15 mmol/L or venous plasma lactate >5 mmol/L)
- Acute kidney injury (creatinine >3 mg/dL or urea >20 mmol/L)

- Asexual parasitaemia >10% of infected red blood cells (Note: national guidelines can vary according to malaria endemicity)

I.4. Host immune responses to the malaria parasite

There are multiple challenges in understanding immunity to *Plasmodium* infection and identifying the correlates of protective immune responses. During the complex life cycle of *Plasmodium* with multiple stages, the malaria parasite expresses not only a great variety of proteins at different stages, but these proteins also change with stage. As a result, natural infection with malaria parasites leads to only a partial and short-lived immunity that is unable to protect the individual against a new infection. This partial immunity is impacted by both age and frequency of infection on the balance between immune-pathogenesis and tolerance to disease. Immunity against plasmodial infection can be classified into two ams: natural or innate immunity and acquired or adaptive immunity.

I.4.1. Innate or natural immunity

In malaria, the innate immune system functions are the first line of defense by controlling parasite growth and regulating the development of adaptive immunity. Innate immunity is naturally present in the host, not dependent on the previous infection, and not specific to the malaria parasite. Innate immunity that is initiated in response to *Plasmodium* infection play key roles in both the development of acquired immunity and malaria pathogenesis (Stevenson and Riley, 2004a; Deroost *et al.*, 2016; Götz *et al.*, 2017b; Dunst *et al.*, 2017). Initial pro-inflammatory responses stimulate the development of anti-parasitic Th1 responses and promote effector cell function for efficiently clearing malaria parasites. Usually, with the progression of the infection, pro-inflammatory responses are gradually downregulated with a parallel increase in anti-inflammatory responses (Perry *et al.*, 2005). Generally, this leads to Th2 development, resulting in balanced pro-/anti-inflammatory and Th1/Th2 responses and resistance against pathogenesis (Gonçalves *et al.*, 2012).

I.4.1.1. Innate immune responses against the liver stage infection

During the liver stage of malaria infection, parasite-infected hepatocytes produce type I IFNs cytokine through cytosolic sensing by *Plasmodium* RNA (Gowda and Wu, 2018). The initial type I IFN cytokine released by hepatocytes might stimulate the production of chemokines, including IFN-γ-inducible protein 10 (IP-10), which in turn recruit to the site of infection cells expressing the chemokine receptor CXCR3, such as T cells, natural killer (NK), and NKT cells, which further contribute to limiting *Plasmodium* liver stage replication by IFN-γ secretion (Liehl *et al.*, 2014; Miller *et al.*, 2014) (Figure 4). An increase in the plasma concentration of IFN-γ prior to the detection of the blood stages of infection has been reported in controlled human *P. falciparum* infection (CHM) (Hermsen *et al.*, 2003). This cytokine response also contributes to the destruction of infected hepatocytes by NKT cells, exposing parasite components. The antigen-presenting cells (APC), primarily dendritic cells (DCs) and

inflammatory monocytes, can potentially recognize the exposed parasite factors. In addition, injected sporozoites that neither reached the blood circulation nor invaded hepatocytes die in the dermis and liver respectively. These dead parasites are likely phogocyted by DCs and inflammatory monocytes, leading to TLR- and inflammasome-mediated immune responses. Because in natural infections, the parasite density in the liver is very low, the innate immune responses are likely to be also very low. Thus, whether this first immune response in the liver reduces the initial parasite number released into the circulation, influencing the level of the initial blood stage-induced immune responses remains unknown. However, in hyper endemic areas, repetitive infections in humans may induce immune tolerance in APC that may modulate immune responses to the blood-stage infection to a certain extent.

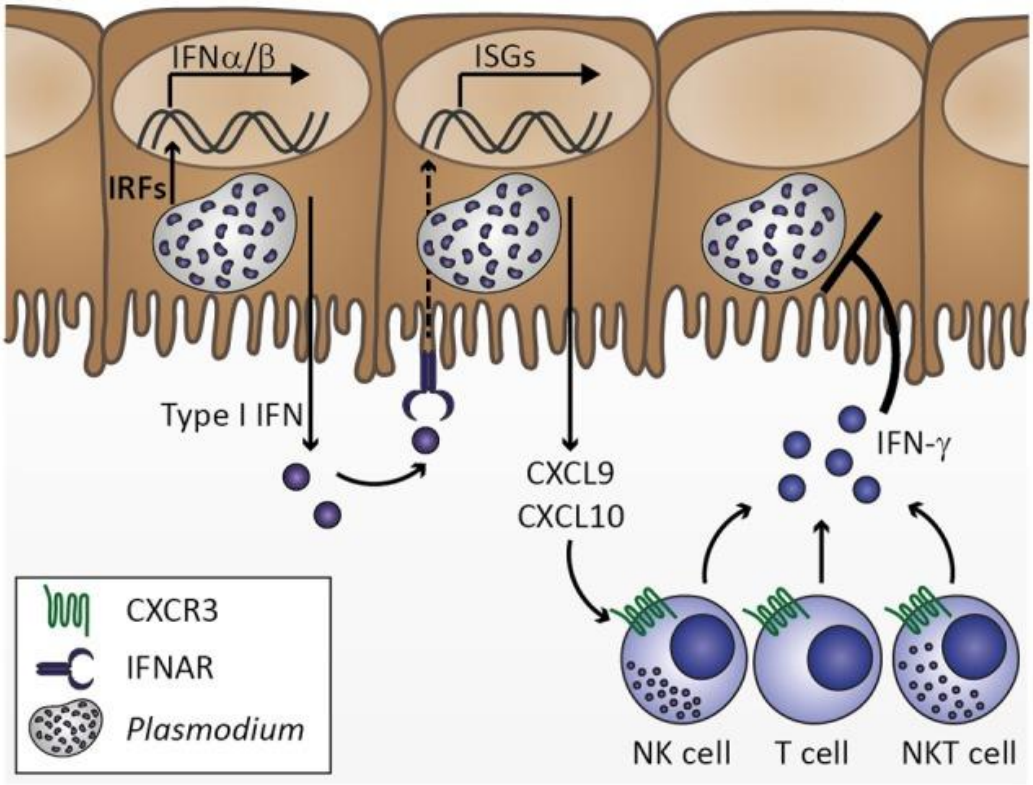

Figure 4: Innate immune responses to *Plasmodium* liver stage infection.

Infection of hepatocytes with *Plasmodium* activates interferon regulatory factors (IRF), which induce transcription of type I interferons (IFN) IFNα and IFNβ. Secretion of type I IFNs activates IFNα/β receptor IFNAR in an autocrine or paracrine manner. IFNAR signalling results in transcription of IFN-stimulated genes (ISGs), which includes chemokines, such as CXCL9 and CXCL10. Upon secretion from hepatocytes, these chemokines might recruit cells expressing the corresponding chemokine receptor CXCR3, including natural killer (NK), T cells, and NKT cells. Upon activation by type I IFN at the site of infection, these cell types could contribute to limiting *Plasmodium* liver stage expansion by IFN-γ secretion (Dunst *et al.*, 2017).

I.4.1.2. Innate immune responses against blood stage infection

The innate immune system represents the first line of defence against pathogens and mediates recognition and clearance of *Plasmodium* parasites (Figure 5). During blood-stage infection, unlike the liver stage, there is an efficient induction of innate immune responses because parasites grow exponentially through repetitive erythrocytic cycles, rapidly accumulating the parasite biomass. Furthermore, blood-stage infection is the exclusive cause of malaria symptoms, which is associated with

systemic inflammation and fever. Fever is a common and effective host defence against microbial pathogens and swiftly initiated upon the first host-pathogen interaction.

The febrile response is likely triggered through a universal mechanism, in which pyrogenic cytokines, such as the pro-inflammatory cytokines interleukin 1α (IL-1α), IL-1β, IL-6, or tumor necrosis factor (TNF), are secreted by innate immune cells upon recognition through pattern recognition receptors (PRRs) of pathogen-associated molecular patterns (PAMPs) or host-derived danger-associated molecular patterns (DAMPs) (Evans *et al.*, 2015). During *Plasmodium* infection, the recurrent fever episodes coincide with the rupture of infected erythrocytes at the mature schizont stage and the liberation of merozoite and parasite byproducts (Oakley *et al.*, 2011).

Innate immune cells include macrophages and dendritic cells (DCs) as well as non-professional immune cells such as endothelial cells and fibroblasts which express pattern recognition receptors (PRR). The PRR which recognize PAMPs and host-derived DAMPs, include Toll-like receptors (TLR), C-type lectin receptors (CLR), retinoic acid-inducible gene (RIG)-I-like receptors, and NOD-like receptors (NLR) (Takeuchi and Akira, 2010). The activation of PRR by the recognization of PAMPs and DAMPs initiates a signalling cascade including the adaptor protein MyD88 and the transcription factors NF-κB, AP-1, and interferon regulatory factor (IRF) leading to expression of genes encoding pro-inflammatory cytokines such as type I IFN, IFN-γ, IL-6, IL-12, and TNF, is induced (Eriksson, 2014; Gazzinelli *et al.*, 2014).

Several *Plasmodium*-derived byproducts have been recognized as malaria PAMPs based on their ability to stimulate cytokine released *in vitro* such as glycosylphosphatidylinositols (GPI) and haemozoin. An in vitro study found that, *P. falciparum* GPI can stimulate the production of pro-inflammatory cytokines TNF and IL-1β by murine macrophages (Schofield and Fiona, 1993; Tachado *et al.*, 1996). Haemozoin, an insoluble polymer formed inside the digestive vacuole to detoxify heme and its conjugated redox-active iron, is released during haemoglobin proteolysis (Sigala and Goldberg, 2014). Haemozoin becomes accessible after the rupture of iRBC or upon phagocytosis of infected erythrocytes and has been described to stimulate the production of pro-inflammatory cytokines, such as TNF and IL-1β, as well as some inflammatory chemokines by murine macrophages, human monocytes (Olivier *et al.*, 2014), or human monocyte-derived DCs (Bujila *et al.*, 2016). Haemozoin is recognized by endosomal TLR9 (Parroche *et al.*, 2007), cytoplasmic inflammasomes, or cytoplasmic sensors (Kalantari *et al.*, 2014).

In addition to inducing pro-inflammatory responses, recognition of parasites and *Plasmodium*-infected erythrocytes are crucial for the phagocytic uptake and thus removal of parasites from the circulation by macrophages and DCs (McGilvray *et al.*, 2000; Stevenson and Riley, 2004b). Interestingly, although, macrophages and DCs may both contribute to early pro-inflammatory cytokine responses *via* activation of PRR-mediated signalling, a recent study suggests that macrophage responsiveness is strongly compromised (inflammatory cytokine production) due to strong phagosomal

acidification following phagocytosis of *P. falciparum*- or *P. berghei*-infected erythrocytes or free merozoites (Wu *et al.*, 2015). Instead, DCs contribute a major source of pro-inflammatory cytokines during early-stage *Plasmodium* infection, including IL-6, IL-12p40, and TNF (Wu *et al.*, 2015).

In addition to the production of type I IFNs, DCs produce a wide range of pro-inflammatory cytokines, including TNF-α, IL-12, and IL-6, and chemokines, such as CXCL1, CXCL2, CCL2, CCL5, CXCL9, and CXCL10 in response to *Plasmodium* parasites, and then play a crucial role in malaria immunity and pathogenesis (Stevenson and Riley, 2004b; Wu *et al.*, 2010; Götz *et al.*, 2017a). Type I IFNs prime DCs for efficient cytokine and chemokine production and activate NK, NKT, γδ T, and T cells to induce IFN-γ and other inflammatory responses (McNab *et al.*, 2015), whereas IL-12 produced by DCs activates NK cells to induce the production of IFN-γ, which promotes Th1 and effector T cell responses (Walsh and Mills, 2013). The increased production of IFN-γ contributes to an efficient parasitaemia controlled by stimulating the phagocytic activity of monocytes and neutrophils and, thus, parasite clearance (Ing and Stevenson, 2009; King and Lamb, 2015).

Although IFN-γ stimulates parasite clearance, it also contributes to severe malaria complication (cerebral malaria or other severe malaria clinical manifestation) under certain conditions, such as parasite sequestration in vital organs (King and Lamb, 2015). On the other hand, chemokines stimulate the recruitment of immune cells at the site of the inflammation to promote an effective cell-mediated anti-parasitic effects (McGovern and Wilson, 2013). However, these immune responses also contribute to severe pathology (Ioannidis *et al.*, 2014; Dunst *et al.*, 2017). Overall, the initial innate immune responses are mainly aimed at controlling parasite growth by potentiating anti-parasitic cell-mediated immunity. However, pro-inflammatory responses contribute to pathogenesis (Deroost *et al.*, 2016; Götz *et al.*, 2017b; Dunst *et al.*, 2017). Overall, malaria protective immunity is promote by a balanced pro- and anti-inflammatory or Th1/Th2 responses that prevent the pathogenesis of *Plasmodium* infection (Riley *et al.*, 2006; Gonçalves *et al.*, 2012); imbalanced responses contribute to pathogenesis.

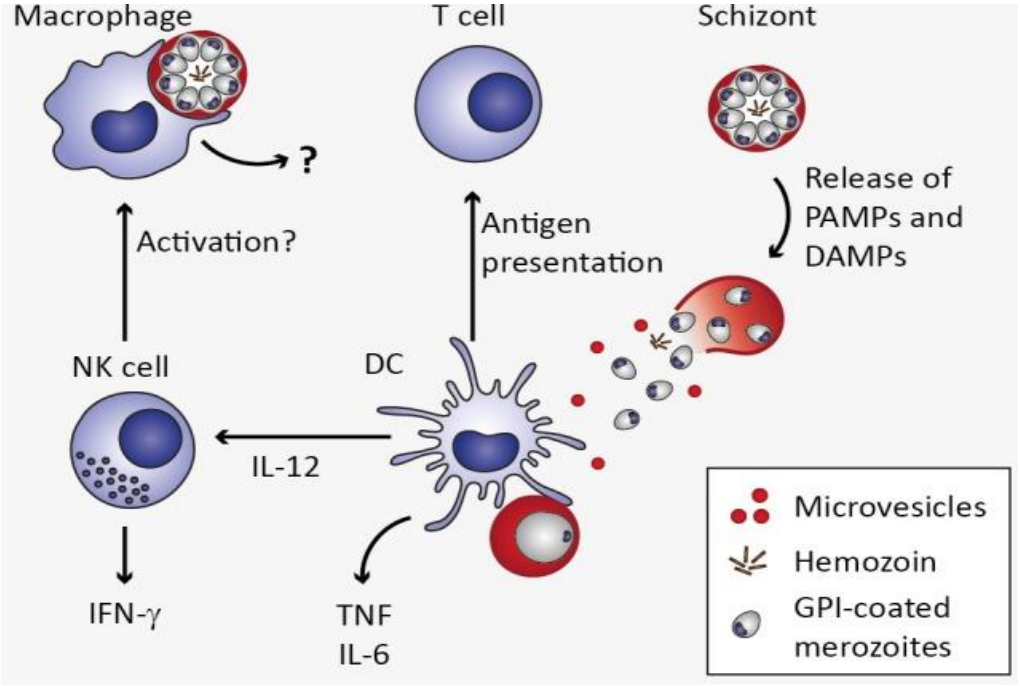

Figure 5: Innate immune responses to *Plasmodium* blood stage infection in the spleen.

Macrophages as well as dendritic cells (DC) remove infected erythrocytes from the circulation by phagocytosis. In macrophages, uptake of infected erythrocytes might not lead to secretion of pro-inflammatory cytokines due to phagosomal acidification *(Wu* et al., *2015)*. Upon rupture of infected erythrocytes, pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) are released, including microvesicles, hemozoin, and glycosylphosphatidylinositols (GPI). These potential PAMPs and DAMPs might be recognized by DC through pattern recognition receptors, resulting in the secretion of interleukin 12 (IL-12), tumor necrosis factor (TNF), and IL-6 *(Wu* et al., *2015)*. DC-derived IL-12 might activate natural killer (NK) cells, which in turn secrete interferon γ (IFN-γ) and could thereby activate macrophages *(Dunst* et al., *2017)*.

I.4.2. Acquired or adaptive immunity

Adaptive immunity develops after exposure to a pathogen. Acquired immunity to malaria is known to develop relatively slowly, is not sterile (named premunition) and is often said to decline quickly when immune adults leave malaria-endemic regions, suggesting that continued exposure to malarial antigens is required not only for the generation of memory cells and effector cells but also for their maintenance. Humoral immune response has been described as the most important for the establishment of protection in natural malaria infections.

I.4.2.1. Humoral immune responses to the malaria parasite

Regarding the mechanisms involved in naturally acquired malaria immunity, the humoral response has been described as the most important for the establishment of protection (Silveira *et al.*, 2018). B-cell and antibody responses to *Plasmodium* infection, are critical for control of parasitaemia and associated with immunopathology during malaria infection. In addition, antibodies may also provide protection to reinfection. Although humoral immune response has been shown to be critical to reduce malaria morbidity and mortality, immune protection mediated by antibody is usually take place after multiple parasitic exposures and may take even years to be established. During malaria infection, thousands of *Plasmodium* antigens are expressed in the different stages of the parasite life cycle (Roch

et al., 2004). However, due to low parasite densities during the liver stage, the malaria humoral responses preferentially lead to blood stage antigens rather than the liver stage antigens.

B cell Responses during malaria infection

A primary parasite exposure elicits the activation and differentiation of naive B cells into *Plasmodium*-specific memory B cells (MBCs) and antigen secreting cells (ASCs). Generally, upon any pathogen exposure, the antigen-specific antibodies are expected to be detected in the serum within 2 weeks. During this period, the naive B cells are activated upon B cell receptor (BCR) interaction with a parasite antigen in the periphery, eliciting cell proliferation and differentiation into multiple B cell subsets, including memory B cells (MBCs), follicular B cells (FoBs), or marginal zone B cells (MZBs) (Figure 6). All these B cell subsets express immunoglobulin (Ig) genes, but only the antibody-secreting cells (ASCs) secrete antibodies.

Follicular B cells (FoBs) form and maintain structures called the germinal centers (GCs) together with the follicular T helper cells (TFh), dendritic cells (DCs), cytokines such as IL-21, IL-6, and B cell activating factor (BAFF), and the critical participation of co-stimulatory molecules (CD40L and ICOS). During the germinal center formation, the GC B cells are activated and undergo several rounds of antigen selection before acquiring a mature status through the somatic hypermutations and class-switch in Ig genes. Then, signalling triggered by activation guides the FoB cells to exit the follicles and differentiate into high-affinity, atypical, or classical MBCs (Obeng-Adjei *et al.*, 2017) or short-lived class-switched ASCs. From a malaria murine model, there is evidence that high affinity, somatic hypermutated IgM+ MBCs dominate a recall response and could be differentiated either into IgM+ or IgG+ ASCs and MBCs (Krishnamurty *et al.*, 2016). The higher the frequency of the antigen-specific MBCs during that second encounter with the antigen, the higher the frequency of the antigen-specific ASCs generated. It is still not clear whether the switched or unswitched MBCs enter the GCs or are form the ASCs (Harms Pritchard and Pepper, 2018). Once generated, the ASCs migrate through the circulation to the bone marrow or secondary lymphoid organs. The physical contact of ASCs with bone marrow stroma cells and the recognition of cytokines described above lead to modifications in their transcriptome profile, upregulating preferentially the expression of anti-apoptotic genes. This process results in the transformation of short-lived ASCs into long-lived ASCs, the function of which results in increased circulating antibody titers

(Radbruch *et al.*, 2006).

Otherwise, malaria infection affects the generation of some critical cell subsets for humoral responses. It has been shown that repeated parasitic exposures drive the expansion and accumulation of atypical MBCs in individuals from *P. falciparum* malaria-endemic areas (Weiss *et al.*, 2009; Portugal *et al.*, 2015). Although atypical MBCs have been mostly associated with the impaired B cell responses in malaria infection context, some groups have stated that atypical MBCs present a similar function as

classical MBCs (Muellenbeck *et al.*, 2013). This hypothesis was demonstrated in an impaired GC response in an experimental severe malaria murine model explained by the inhibition of the differentiation of follicular T helper cells (TFh) (Ryg-Cornejo *et al.*, 2016). Noteworthy, the bone marrow ASCs have serious restrictions for sampling due to their location, being avoided in malaria clinical trials. This issue has impaired the complete understanding of the immune response triggered by malaria infection in humans.

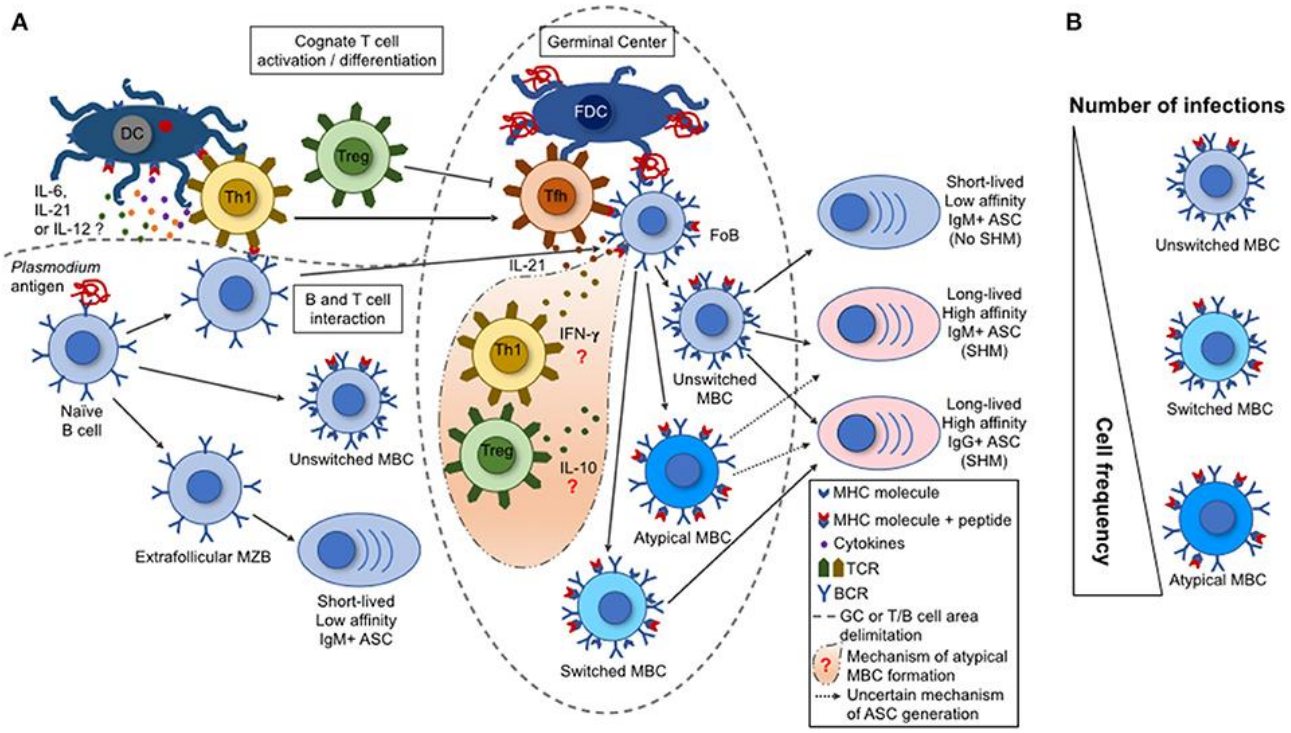

Figure 6: B cell response triggered by malaria infection.

(A) During a malaria infection, the naive B cells are activated by a *Plasmodium* antigen through the interaction with B cell receptors (BCR), leading to their differentiation into marginal zone B cells (MZB), follicular B cells (FoB), or unswitched memory B cell (MBCs). The switched and atypical MBCs are derived from the activation of FoBs within the germinal centers (GCs). Either the MZBs, or the unswitched, switched, or atypical MBCs can differentiate into antibody-secreting cells (ASCs). These ASCs range from short-lived, low-affinity, IgM+ to long-lived, high-affinity, IgM+ or IgG+. This variation depends on the type of interaction between a particular B cell with a T cell subset. The activated Th1 T cells migrate to the GCs, becoming follicular T helper cells (TFh) that help the GC reactions (acquisition of somatic hypermutations in V(D)J Ig genes and class switch by activated FoBs). Contrarily, the regulatory T cells (Tregs) have the potential to inhibit TFh cell differentiation and GC reactions. **(B)** A single parasite infection can induce the differentiation of multiple *Plasmodium*-specific B cell clones. However, the repeated parasite exposures shift the MBC frequencies with an increase for an atypical MBC over the unswitched or switched MBCs. This shift in cell frequency may interfere on the function of the secreted antibodies and, consequently, on the development of protective immunity. (Silveira *et al.*, 2018).

Antibody-dependent protective immunity to malaria infection

The malarial-specific antibody responses against *P. falciparum* antigens are usually transient as the titers decrease by the next malaria transmission season. After the initial induction of antimalarial antibodies, levels can be maintained at a higher magnitude than the respective levels detected during primary parasite exposure (Hviid *et al.*, 2015). Serological data against blood-stage antigens have determined a positive correlation between the antibody titers specific for *P. falciparum* MSP-2, MSP-3, and AMA-1 and protection from symptoms in the infected individuals. Indeed, increased breadth of

malaria antibody has been associated with a lower chance to experience a clinical or severe *P. falciparum* malaria (Osier *et al.,* 2008). Early studies demonstrated that passive transfer of immunoglobulin from adults with extensive malaria exposure to subjects with malaria could resolve symptomatic malaria infection (Cohen *et al.,* 1961). In addition, it has been shown that acquired immunity against severe malaria can occur after one or two malaria infections (Gupta *et al.,* 1999). On the other hand, it has been shown that in pregnancy-associated malaria, the anti-VAR2CSA antibody can protect against poor malaria pregnancy outcomes in both humans and mice (Staalsoe *et al.,* 2001; Megnekou *et al.,* 2005; Djontu *et al.,* 2020).

More rapid acquisition of immunity to severe malaria maybe because some level of immunity is sufficient to prevent severe malaria, or there may be specific immune mechanisms mediating protection against severe disease. Some studies have also suggested that host age is an important factor in susceptibility to severe malaria (Griffin *et al.,* 2015). In exposed populations to stable medium-high intensity malaria transmission, malaria is typically uncommon in older children and adults, and severe malaria is rare or largely restricted to young children (under 5 years), whereas severe malaria continues to occur later in childhood in settings where malaria transmission is lower (Carneiro *et al.,* 2010). However, there is no clear understanding of how much exposure is required for the development of clinical malaria immunity. It has been proposed that chronic parasitaemia or exposure to malaria parasite is required to maintain robust immunity (a phenomenon referred to as premunition). Infections may persist for many weeks to years in the absence of symptoms, named asymptomatic malarial infections that have been indicated to provide protection against clinical malaria (Färnert *et al.,* 1999; Ashley and White, 2014; Galatas *et al.,* 2016). Some longitudinal studies support the concept that persistent asymptomatic infections may be crucial for the maintaining of high antibody responses (Ibison *et al.,* 2012; Daou *et al.,* 2015; Rono *et al.,* 2015), but this has not been really investigated or clearly demontrated.

Antibodies targeting blood-stage malaria have a broad range of specificities, and several possible functions and these functional antibodies recognize specific antigens found on the parasite or the parasite-infected red blood cell. Antibodies may control parasite replication or clearance via a number of mechanisms, including inhibition of merozoite egress and invasion, antibody-dependent cellular inhibition, complement-mediated lysis or opsonic phagocytosis and antibody-dependent respiratory burst activity by polymorphonuclear neutrophils (Duncan *et al.,* 2012; Osier *et al.,* 2014; Boyle *et al.,* 2015a) (Figure 7 by (Teo *et al.,* 2016). Studies have shown that IgG subclass-specific responses to merozoite antigens are more strongly associated with protection, particularly the cytophilic ones (IgG1 and IgG3) (Megnekou *et al.,* 2005; Roussilhon *et al.,* 2007; Stanisic *et al.,* 2009; Richards *et al.,* 2010), suggesting that specific functional activities may be important in immunity. So far, tremendous progress has been made, and several antigens have been identified as vaccine candidates. Indeed, a vaccine based on a liver stage antigen, the circumsporozoite protein (CSP) which had shown the efficacy of 39-50 %,

is the most advance candidates and has made it all the way to licensure (RTS,S vaccine) (Mahmoudi and Keshavarz, 2017), while a handful of other antigens has been characterized and are in phases II and III clinical trials (MAHMOUDI and KESHAVARZ, 2018; Draper *et al.*, 2018).

Acquisition of malaria immunity depend on multiple host and parasite factors, which are reflected the different levels of acquired immune responses observed across populations living in the same or different malaria transmission areas. These factors include: 1) specific genetic traits the prevalence of which varies considerably between populations and influences susceptibility to malaria infection or clinical disease and potentially immune responses (Marquet, 2017; Damena *et al.*, 2019); 2) age, the acquisition of malaria immunity may depend on the age of first exposure and a study in transmigrants provided evidence that antibody responses are acquired quickly in adults than in children (Baird, 1995); 3) parasite diversity is also important and immunity may be acquired faster where genetic diversity is limited as many of the key targets of protective immunity are polymorphic (Sondo *et al.*, 2019).

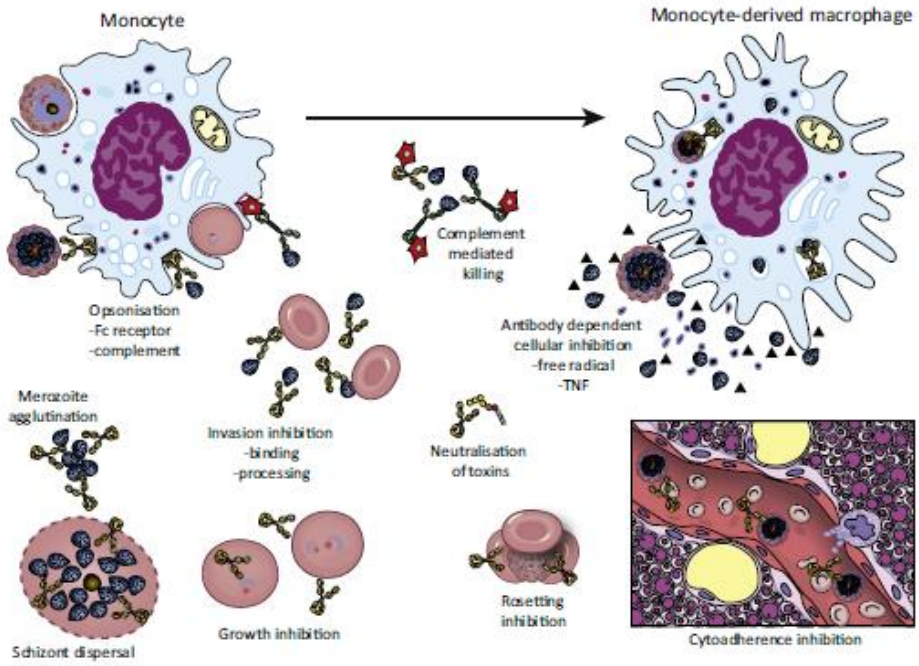

Figure 7: Schematic representation of proposed antibody-mediated mechanisms in immunity to blood-stages of ***Plasmodium falciparum*****.**

I.4.2.2. Cellular immune responses to the malaria parasite

Immunity to malaria has been linked to the availability and function of helper CD4+ T cells, cytotoxic CD8+ T cells and γδ T cells that can respond to *Plasmodium* infection in both the liver and the blood stages. However, T cell responses are modulated by regulatory T cells to avoid excessive responses. It had been shown that parasite-specific cytotoxic CD8+ T cells eliminate infected hepatocytes following recognition of parasite antigens presented on MHC class I molecules; whereas, CD4+ T cell-dependent antibody responses can prevent sporozoites invasion of hepatocytes. During the blood stage of malaria infection, *Plasmodium*-infected erythrocytes (which lack functional MHC

expression) are indirectly targeted by the CD4+ T helper (Th) cells and possibly γδ T cells that may orchestrate secreted antibody responses or the anti-parasitic phagocytic activity.

CD4+ T cells responses in malaria

CD4+ Th cells are activated by pathogen-specific markers presented on MHC class II molecules, and set-up key aspects of innate and adaptive immunity during malaria infection. The presence of *Plasmodium*-specific CD4 + T lymphocytes have been correlated with protective immunity following exposure to the *Plasmodium* parasite (Mordmüller *et al.*, 2017). One activated, CD4+ T cells have the capacity to differentiate into one of several functionally distinct subsets such as Th1, Th2, Tfh, Th17, and Treg among many others.

The presence of Th1 cells and elevated IFN-γ levels are signatures of both human and rodent malaria (Su and Stevenson, 2000; Thévenon *et al.*, 2010). The mechanisms by which IFNγ and effector Th1 cells contribute to protection during blood-stage malaria infection remain largely hypothetical (Riley and Stewart, 2013a), although experimental data suggest that IFN-γ is critical for activating macrophages (Bastos *et al.*, 2002; Blanchette *et al.*, 2003; Jaramillo *et al.*, 2003) and may tune class-switch recombination in *Plasmodium*-specific B cells (Su and Stevenson, 2000). IL-2, another Th1 cell-associated cytokine, is important for activating NK cells, which may participate in protective immune responses by direct cytolysis of *Plasmodium*-infected erythrocytes (Horowitz *et al.*, 2010). Th1 cell responses and IFN-γ secretion have also been associated with host resistance to *Plasmodium* infection at the liver-stage. Indeed, the presence of IFN-γ-expressing, circumsporozoite protein (CSP)-specific Th1 cells were associated with reduced parasite densities and disease severity in *Plasmodium* exposed individuals (Reece *et al.*, 2004).

Th2 cells are primarily characterized by expression of the GATA3 transcription factor and by the production of IL-4 and IL-5 cytokines (Walker and McKenzie, 2018). The role of Th2 cells in malaria is relatively unknown because GATA3+CD4+ T cells are rare or absent during *Plasmodium* infections (Perez-Mazliah and Langhorne, 2014). However, IL-4 cytokine which is the major cytokine produced by the Th2 cells, can promote B cell class switching to IgE (von der Weid *et al.*, 1994; Shimoda *et al.*, 1996) and modulate macrophage responses (Kumaratilake and Ferrante, 1992) during *Plasmodium* infection, as well as enhanced humoral immunity in *Plasmodium*-exposed humans (Troye-Blomberg *et al.*, 1990).

T follicular helper cells are characterized by expression of the transcriptional repressor BCL-6, the chemokine receptor CXCR5 and the inhibitory receptor programmed cell death 1 (PD1) (Vinuesa and Cyster, 2011). Additionally, *Plasmodium*-specific Tfh cells may also express IL-21 (Pérez-Mazliah *et al.*, 2015) and inducible T cell co-stimulator (ICOS) (Sebina *et al.*, 2016), which induce maturation of *Plasmodium*-specific B cells GC and promote the production of long-lived plasma cells and memory B cells. CXCR5+PD1+ Tfh cell populations expand during blood-stage *Plasmodium* infections in both human and rodent and are essential for promoting protective antibody responses (Obeng-Adjei *et al.*,

2015; Ryg-Cornejo *et al.*, 2016; Figueiredo *et al.*, 2017; Pérez-Mazliah *et al.*, 2017). In addition, it has been shown that severe malaria infections impair GC responses by inhibiting Tfh cells differentiation (Ryg-Cornejo *et al.*, 2016).

In the presence of IL-6, IL-23, and TGF-β, naive CD4 + T cells can differentiate into Th17 cells that have been linked to orchestrating neutrophil recruitment and function during several scenarios of microbial infection (Sandquist and Kolls, 2018). A limited number of studies describe *Plasmodium*-induced Th17 cells. The first study described CD4+ T cells in Malian malaria-exposed individuals with the capacity to express IL-17A *in vitro* after stimulation (Metenou *et al.*, 2011). However, Megnekou et al. show that levels of IL-17A were higher in non-infected pregnant women (Megnekou *et al.*, 2015a). TH17 cells can also secrete IL-21 (Wei *et al.*, 2007), suggesting that Th17 cells may play a modest role in supporting GC reactions.

Treg cells are a class of CD4+ T cells characterized by their expression of the transcription factor FOXP3 and are known to regulate the immune system from damaging the host during *Plasmodium* infection, while their impact and mode of action have remained controversial and contentious (Yadav *et al.*, 2013). Longitudinal or cross-sectional studies in human subjects from endemic areas naturally or experimentally infected with *Plasmodium* consistently show that Treg cell populations expand in blood-stage malaria and the higher Treg cell frequencies are correlated with increased parasite densities and more severe disease outcomes (Torcia *et al.*, 2008a; Jangpatarapongsa *et al.*, 2008; Hansen and Schofield, 2010; Kurup *et al.*, 2017). Due to the inability to manipulate the human's immune system, it is difficult to know whether the expansion of Treg cell populations is a cause or consequence of increased parasite loads (Kurup *et al.*, 2019). Lower frequencies of functionally deficient Treg cells associated with lower parasite densities was observed in Malian Fulani people who are naturally more resistant to malaria infection or clinical malaria compared to sympatric Mossi group (Torcia *et al.*, 2008b), indicating that in human malaria, Treg cell expansion may be a consequence, but not the cause, of increasing parasite densities. In addition, higher Treg cell frequencies in healthy individuals were correlated with increased risk of subsequent febrile malaria (Todryk *et al.*, 2008).

CD8+ T cells responses in malaria

CD8+ T cells recognize pathogen-derived peptides bound to surface MHC class I molecules on antigen-presenting cells (APCs) or infected cells and contribute to the clearance and immune memory against many intracellular pathogens (Kurup *et al.*, 2019). *Plasmodium* parasite-specific CD8+ T cells have been found in the peripheral blood of exposed individuals in malaria endemic areas (Sedegah *et al.*, 1992; Doolan *et al.*, 1997) and in experimental malaria (Epstein *et al.*, 2011; Van Braeckel-Budimir and Harty, 2014).

The vital role of CD8 + T in protecting against hepatic stage malaria has been described, but it is thought to contribute little to the control of the blood stage of malaria infection due to the lack of MHC class I on erythrocytes (Miyakoda *et al.*, 2012). In addition, studies had suggested that immunity to *P. yoelii* or

P. vivax may be related to MHC class I expression on reticulocytes and found that CD8+ T cells alone transferred protection in immunodeficient mice (Imai *et al.*, 2013; Junqueira *et al.*, 2018). Activated CD8 + T cells can use different effector mechanisms to eliminate pathogens, divided into cytolytic and cytokine pathways (Harty *et al.*, 2000; Halle *et al.*, 2017). Among the multiple effector pathways and molecules involved in CD8+ T cell function, IFN-γ, TNF and perforin contributed to protective immunity, whereas Granzyme and FAS–FASL-mediated pathways appeared is dispensable for the CD8+ T cells effector functions to the malaria liver-stage in mice, although depending on the *Plasmodium* species and the host genetic background (Butler *et al.*, 2010; Nganou-Makamdop *et al.*, 2012). A malaria mouse model study found that IFN-γ produced by CD8+ T cells induced nitric oxide synthase (and hence nitric oxide) in the infected hepatocytes to help eliminate them (Seguin *et al.*, 1994). In *P. vivax* infection, which preferentially colonizes reticulocytes, CD8+ T cells are thought to eliminate parasitized reticulocytes by Granulysin-driven mechanism, possibly contributing to the protection (Junqueira *et al.*, 2018).

Although the role of CD8 + T lymphocytes in protecting against blood-stage malaria with some *Plasmodium* species may be limited, they are known to be highly involved in the pathogenesis of cerebral malaria in humans based on the murine model's study of malaria (Nitcheu *et al.*, 2003; Swanson *et al.*, 2016). In the ECM mouse model, the pathogenic role of CD8+ T cells is mediated directly by perforin and Granzyme B and indirectly by IFN-γ-driven accumulation of parasitized red blood cells in the brain (Claser *et al.*, 2011; Haque *et al.*, 2011). However, the functional mechanism of CD8+ T cells in orchestrating cerebral malaria in humans remains unclear.

γδ Tcells responses in malaria

γδ T cells are a subset of T cells that express distinct TCRγ and TCRδ chains and account for ~4% of all T cells in healthy adult humans (Holtmeier and Kabelitz, 2005; Chien *et al.*, 2014). The role of this cell population in anti-malarial immune responses remain poorly understood and controversial (Kurup and Harty, 2015). γδ T cell populations, specifically those expressing the Vγ9+Vδ2+ chains (which account for ~75% of all γδ T cells in humans), expand in primary *P. falciparum* or *P. vivax* infections and correlated with protection (D'Ombrain *et al.*, 2008; Teirlinck *et al.*, 2011). In human malaria, it is remarkable that Vγ9+Vδ2+ γδ T cell populations expand during acute primary infections, but possibly contract with each subsequent exposure to malaria, despite reactivation each time (Teirlinck *et al.*, 2011). The frequencies of Vγ9+Vδ2+ γδ T cells naturally increase with age (De Rosa *et al.*, 2004). However, progressive improvement in tolerance to clinical malaria with multiple exposures in endemic regions has been attributed to the decline of Vγ9+Vδ2+ γδ T cells (Jagannathan *et al.*, 2014b).

Although fundamental mechanisms of γδ T cells in the context of infections are also poorly understood, recently a studies showed that γδ T cells are reported to serve as a source of IL-21 that may

support Tfh cell responses (Inoue *et al.*, 2018), and γδ T cells helped control *Plasmodium* recrudescence in a TCR dependent manner, possibly by their production of M-CSF (Mamedov *et al.*, 2018). In humans, Vγ9+Vδ2+ γδ T cells may assist the control primary *Plasmodium* infections through the production of various immune cell mediators, such as IFN-γ, TNF or Granzyme B, in addition to possibly direct killing the merozoites in blood-stage malaria (Riley and Stewart, 2013b; Kurup *et al.*, 2017). In addition, in *P. chabaudi* blood-stage malaria in mouse model, Vδ6.3+ γδ T cells (also known as TRAV15N-1+) specifically undergo clonal expansion and exhibit a unique transcriptional and functional profile that contributes to the protection (Mamedov *et al.*, 2018). All these properties that differentiate γδ T cells of innate and adaptive immune cells make them functionally unique (Kurup *et al.*, 2019).

I.4.3. Host immune responses during asymptomatic plasmodial infections

Little is known about the immune mechanisms that can explain asymptomatic parasitaemia. During malaria infection, two mutually inclusive processes precede pathology: (i) parasitaemia, which leads to (ii) inflammation, including both local (as observed in cerebral malaria) and systemic inflammation. Protection from clinical malaria is thought to be achieved by two key processes: anti-parasite immunity, which involve immune responses that directly control parasite replication and maintain parasitaemia levels below the pyrogenic threshold; and anti-disease or clinical immunity, which involve processes that altogether prevent the manifestation of clinical symptoms despite a high parasitaemia (Ademolue and Awandare, 2018).

Antibodies are a critical component of anti-parasite immunity (Ademolue and Awandare, 2018) and work through a variety of functions that include opsonisation for antibody-dependent phagocytosis by phagocytic cells, antibody-dependent cytotoxicity by NK cells and invasion blocking (Teo *et al.*, 2016; Arora *et al.*, 2018). Antibody responses are develop in individuals slowly after repeated *Plasmodium* infection and considered the key in protection against high parasitaemia and clinical malaria (Gupta *et al.*, 1999; Kana *et al.*, 2018). Cross-sectional studies have used arrays with several recombinant proteins of the asexual *P. falciparum* blood-stage and have identified a considerable number of antibodies to higher proteins in asymptomatic individuals compared to symptomatic individuals (Torres *et al.*, 2015; Guiyedi *et al.*, 2015; Baum *et al.*, 2016; Uplekar *et al.*, 2017; Kanoi *et al.*, 2017; Lehmann *et al.*, 2017; Sakamoto *et al.*, 2018). These include both blood and pre-erythrocytic stage antigens such as MSP3, EBA-175, MSPDBL1, RON2, RON4, CLAG3.1, LSA3-C, RAMA, and GLURP, SIAP-2, TRAP, CelTO and among others. However, these responses are slowly acquired and not adequately induced in young children at the levels that could confer clinical protection (Ademolue and Awandare, 2018), suggesting that antibodies are vital to controlling parasite levels in asymptomatic carriers, but not in controlling the manifestation of the disease. On the other hand, the composition of the antibodies associated with symptomatic malaria varied greatly from one study to another, suggesting the need for further studies in other malaria-endemic settings.

Anti-disease immunity in malaria is attributed mainly to decrease levels of pro-inflammatory immune mediators or increase in anti-inflammatory responses leading to tolerance to the infection (Figure 8) (Jagannathan *et al.*, 2014a; Portugal *et al.*, 2014; Bediako *et al.*, 2016; Ademolue *et al.*, 2017). Based on the protective role of anti-inflammatory cytokines such as IL-10 and TGF-β in both mice and human severe malaria (Kurtzhals *et al.*, 1998; C *et al.*, 1999; Li *et al.*, 1999; Freitas do Rosario and Langhorne, 2012), it is assumed that asymptomatic carriers generally have lower levels of pro-inflammatory cytokines, including IFN- γ and TNF -α, and high levels of anti-inflammatory cytokines such as IL-10 and TGF-β compared to symptomatic individuals (Mendonça and Barral-Netto, 2015; Ademolue and Awandare, 2018; Kimenyi *et al.*, 2019). A study found that increased plasma levels of IL-10 were independently associated with asymptomatic malaria (Andrade *et al.*, 2011). However, higher plasma concentrations of TNF-α, IFN-γ and low levels of IL-10 as well as the low ratios of IL-10/ TNF-α, IL-10/IFN-γ and IL-10/IL-6 have been observed in asymptomatic *Plasmodium* individuals compared to those with symptomatic infections (Andrade *et al.*, 2011; Gonçalves *et al.*, 2012; Guiyedi *et al.*, 2015). In addition, high frequency of Fox-P3+ T regulatory cells, which secrete immunomodulatory cytokines such as IL-10 and TGF-β was associated with symptomatic malaria and high parasite burden (Frimpong *et al.*, 2018). Pregnant women with asymptomatic malaria have been shown to have higher levels of regulatory makers including IL-10 and G-CSF compared to healty control (Wilson *et al.*, 2010; Megnekou *et al.*, 2015b). In a low malaria transmission area, despite the very low sample size, PBMC from asymptomatic adult carriers secreted higher levels of both pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-2, IL-8, IL-17, GM-CSF and MIP-1β and regulatory IL-4 and IL-34 cytokine than PBMC from symptomatic parasite carriers (Lehmann *et al.*, 2017). These controversial data suggest the role of cytokine in both clinical disease and anti-parasite immunity. However, the protective role of these cytokines or pro/anti-inflammatory cytokine balances in the maintenance of asymptomatic malaria infections has never been explored.

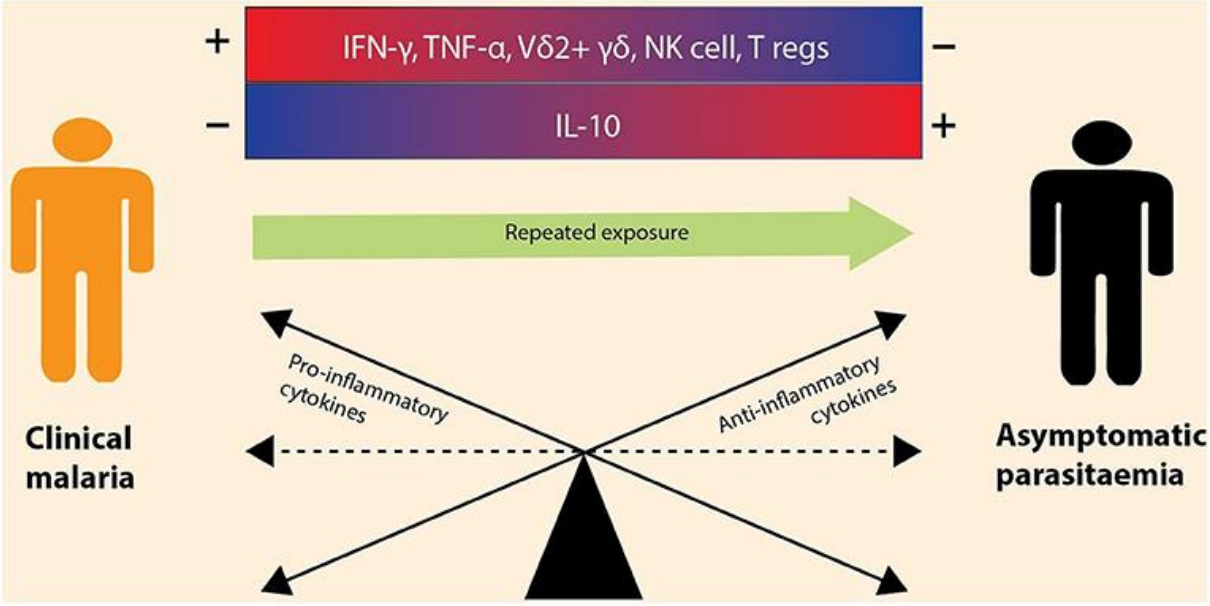

Figure 8: Immune modulation during asymptomatic vs. symptomatic malaria infections.

The outcome of a malaria infection depends on the balance between anti-inflammatory and pro-inflammatory cytokines. Clinical malaria is the result of increased (+) production of pro-inflammatory cytokines (e.g., IFN-γ, TNF-α) and increased levels of immune cells (e.g., $V\delta2^+$ γδ, NK cells, and T regs) and the downregulation (–) of anti-inflammatory cytokines (e.g., IL-10). However, with repeated malaria exposure, the immune balance shifts toward an increased production of anti-inflammatory cytokines, leading to asymptomatic infection. The cytokines are encoded by immune genes, thus differential expression of these genes depicts that there is a balance between anti-inflammatory and pro-inflammatory cytokines **(**Kimenyi *et al.*, 2019).

I.5. Diagnosis of malaria

Different diagnostic approaches are currently used in case management and/or epidemiological studies of malaria as reviewed by Ayong et al (Ayong *et al.*, 2019). Diagnosing malaria involves the use of one of several clinical, parasitological, molecular, and immunological methods to identify individuals with active infection by malaria parasites or those whose medical condition results from recent exposure to malaria parasites.

I.5.1. Clinical diagnosis of malaria

Clinical diagnosis is based on patients' clinical manifestations or symptoms and is routinely practiced by highly trained healthcare personnel (Ayong *et al.*, 2019). The clinical features of malaria are extremely variable and may include fever, headache, chills, anorexia, body itches, abdominal pain, diarrhoea, nausea, vomiting, anaemia, arrhythmia, general weakness, convulsion, and coma (Chandramohan *et al.*, 2002). The clinical diagnosis alone is only recommended in resource-limited countries where malaria transmission is high, and the laboratory diagnosis (lack of material or qualified personnel) may be non-existent. However, the clinical manifestation of plasmodial infection overlaps considerably with other common and potentially life-threatening diseases simultaneous found in malaria transmission areas, including several viral and bacterial infections. One of the main limitations of the clinical diagnosis is the inability to detect asymptomatic infections cases that often do not seek medical

care, thus serving as important human reservoirs of malaria transmission and necessitating the use of specialized diagnostic tools to ensure their identification.

I.5.2. Microscopy-based diagnosis

Microscopic examination of blood smears is considered the gold standard for detecting malaria parasites (Ashley *et al.*, 2018). In general, in most laboratories in malaria endemic areas, both thick and thin smears are prepared on a routine basis, with thick smears used to detect the presence of parasites, whereas thin smears are used to identify *Plasmodium* species.

For the preparation of the thin smear, approximately 2 microliters of peripheral blood are deposited in the center of a microscopy slide and spread using the rounded edge of the spreader slide. To prepare the thick smears, approximately 6 microliters of blood are deposited on a microscopy slide and spread in a circular pattern using the bevelled corner of a spreader slide until a diameter of about 12 mm is evenly covered (Ayong *et al.*, 2019). Upon drying the smears (about 1 h at 37–40 °C or overnight at room temperature), the thin smear is fixed for few seconds by dipping in absolute methanol and air-drying to eliminate water. Both the fixed thin smear and unfixed thick smears are then stained using one of the suitable stains (Giemsa, Wright or Field) which are variants of a mixture of different basic and acidic stains. The recommended and most widely used stain in malaria-endemic areas is Giemsa, which is composed of methylene blue (basic stain) and eosin (acidic stain). The methylene blue stains the parasite cytoplasm blue, while the eosin component stains the nucleus red. Following dye staining, the slides are washed in buffered water and air-dried for at least 30 min at room temperature prior to microscopic examination using an immersion oil 100X objective.

Parasitaemia is determined in the thick smear by counting the number of parasites against a recommended number of white blood cell count (WBC), and on the thin smear is by determining the proportion of iRBCs after examining at least 100 microscopic fields. The number of parasites per µl of blood (parasitaemia) is then calculated on the basis of the patient's own white cell count or using an estimated average human white blood cell count of 8000/µl.

However, microscopic diagnosis is accompanied by several shortcomings, the slide staining and reading processes is laborious, time-consuming and require considerable expertise. In addition, the method is subject to significant variability related to the reader and the sensitivity depends on the level of expertise of the microscopist. The estimated sensitivity limit of the microscopy is 5–10 parasites/µl for expert microscopists, 50–100 parasites/µl for average microscopists, and lower for the majority of microscopists in malaria-endemic areas.

I.5.3. Immuno-chromatographic methods

As microscopy is impractical in many areas, mainly in the field rapid diagnostic tests (RDTs) are now recommended as the first-line investigation (WHO, 2014) with a wide range of devices available

on the market. The principle of the immune-chromatographic-based methods for the detection of malaria parasites is based on the detection of parasite-specific proteins in infected peripheral blood using both soluble and immobilized antibodies on a chromatographic test strip. Given the distribution of *Plasmodium* species, in Africa, the only test based on the highly expressed histidine-rich protein 2 (PfHRP2) antigen is often used, as *P. falciparum* is the main species in Africa. The RDT based on the PfHRP2 can remain positive for several weeks after parasite elimination because of persisting of the protein in the deparasitized red blood cells (Ndour *et al.*, 2017). Otherwise, RDTs often incorporate both an HRP2-detecting strip (for sensitive *falciparum* detection) and a pan-species strip that detects the lactate dehydrogenase enzyme (LDH) of all human *Plasmodium*, although this is unable to detect *P. Knowlesi* (Ashley *et al.*, 2018). HRP2 gene deletions were identified in different malaria endemic areas, that significantly affect the sensitivity of HRP2-based tests (Gamboa *et al.*, 2010), and there is evidence of the emergence of this problem in Africa (Berhane *et al.*, 2017). In addition, very high *P. falciparum* parasitaemias (> 312000 parasites/µL) can also give negative results due to the prozone effect (Luchavez *et al.*, 2011). The detection limit of the RDT methods is approximately 100-200 parasites/µL for PfHRP2-based *P falciparum* RDTs (several times higher for non-*falciparum* RDTs).

I.5.4. Molecular diagnosis

Nucleic acid amplification techniques (NAAT) have been shown to be the most accurate and sensitive method for detecting *Plasmodium* parasites. These NAATs include PCR-based methods such as nested, real-time, multiplex, reverse transcription-based PCRs, and isothermal approaches methods such as nucleic acid sequence-based amplification (NASBA) and loop-mediated nucleic acid amplification (LAMP)-based methods (Britton *et al.*, 2016).

PCR is based on the amplification target DNA sequence from extracted parasite DNA using a DNA polymerase such as Taq polymerase, as well as a pair of sequence-specific primers. This approach involves different cycling temperatures of template denaturation, primer annealing, and strand synthesis steps. Detection of the resulting amplicon is carried out either by 2-5% agarose gel electrophoresis or by using a real-time approch in which a DNA-binding fluorescent dye or target-specific probe is added to the reaction mix and its fluorescence emission is monitored over time. PCR approaches may be used for infection detection (classical PCR), for quantifying infection loads (quantitative real-time PCR), or for parasite speciation (multiplex PCR). Otherwise, from the RNA templates, one of several known reverse transcriptase enzymes is used to generate the cDNA strands prior to conventional PCR. PCR-based techniques are extremely sensitive (detection limits of 0.002–5 parasites/µl) for RT-PCR and 0.7–10 parasites/µl for classical PCR) and specific when compared with standard routine method microscopy. However, PCR methods are limited by their high costs, the absolute need for electricity and expensive equipment, labour-intensiveness, and long turnaround times (2–4 h), as well as susceptibility to common

laboratory contamination (Khan *et al.*, 2013). The reason why PCR-based techniques are used only in reference laboratory settings and for research purposes.

Isothermal nucleic acid amplification techniques such as NASBA and LAMP are also used as approaches for the diagnostic of *Plasmodium* infection, as they are less costly, not required a thermocycler, are rapid (~1 h for NASBA and <30 min for LAMP), and are capable of detecting infections of <1 parasite/µl of whole blood. NASBA is a nucleic acid amplification method that involves the use of oligonucleotide primers and three enzymes avian myeloblastosis virus reverse transcriptase (AMV-RT), RNase H, and T7 RNA polymerase for target-specific amplification, and usually run at 41 °C constant temperature. The NASBA amplification approach generates very high numbers of RNA copies per cycle, leading to the generation of detectible amounts of RNA products in a shorter time frame than PCR-based methods. The detection of products can be achieved by probe-capture hybridization, electrochemiluminescence, or by real-time analysis using molecular beacons that fluoresce when bound to a target sequence. However, NASBA is also expensive, labour-intensive, and prone to frequent laboratory contamination and false-positive amplification.

Among the existing molecular tests for *Plasmodium* infection diagnosis, LAMP appears to be the most practicable in terms of its field applicability, cost-effectiveness, diagnostic performances, and rapidity (Chander *et al.*, 2014; Kemleu *et al.*, 2016). LAMP is a one-step amplification technique that is based on the use of strand displacement polymerases, notably Bst DNA polymerase to amplify target DNA under isothermal conditions (65 °C) with high sensitivity and specificity. The LAMP method necessitates four to eight specially designed oligonucleotide primers that recognize six distinct regions within the target DNA sequence.

Some alternative methods for malaria diagnosis include those that are based on fluorescence microscopy and antigen or antibody detection methods (ELISA, IFA, flow cytometry) and those that are based on detection of certain *Plasmodium* metabolites have been developed.

I.6. Treatment of malaria

Artemisinin-based combination (ACT) is the most effective antimalarial therapy that has been adopted as a strategy to mitigate multidrug resistance to antimalarial monotherapies (WHO, 2015). The principle behind ACT is based on the assumption that given short plasma half-life of artemisinins but high potency, combining them with a longer-acting drug would eliminate any chances of parasites not cleared within the few hours of the drug action and prevent the chances of developing resistance (Kavishe *et al.*, 2017). Currently, five ACTs are recommended by the WHO for treatment of malaria, including artemether-lumefantrine (AL), artesunate-mefloquine (ASMQ), artesunate-amodiaquine (ASAQ), dihydroartemisinin-piperaquine (DHAPQP), and artesunate-sulphadoxine–pyrimethamine (AS-SP) (World Health Organization, 2019). Although the exact mechanisms of action of artemisinins are not well understood, studies have proposed multiple cellular targets of artemisinins with the

involvement of reactive oxygen species (ROS) that induces membrane damage and eventually parasite death (Kavishe *et al.*, 2017).

However, there is documented evidence of resistance to ACTs across the malaria-endemic areas. Resistance to artemisinin was associated to kelch propeller domain (K13-propeller) polymorphisms, including K-13 polymorphisms C580Y, R539T, Y493H, M476I, and I543T (Ariey *et al.*, 2014). Such mutations and several others were found in Asia as well as in more than 18 countries in sub-the Saharan Africa region although in low frequencies (Ariey *et al.*, 2014).

I.7. Malaria vaccines

Immense efforts have been undertaken to develop vaccines as an additional tool to control malaria. Up to now, only a vaccine based on a liver stage antigen, the circumsporozoite protein (CSP), RTS, S vaccine passed the phase 3 clinical trial with an efficacy of 39-50 % (Mahmoudi and Keshavarz, 2017), while other vaccine candidates have been characterized and are in phases II and III clinical trials (Mahmoudi and Keshavarz, 2018; Draper *et al.*, 2018). However, based on results from an ongoing pilot programme in Ghana, Kenya and Malawi that has reached more than 800 000 children since 2019, World Health Organization (WHO) has recommended the widespread use of the RTS, S/AS01 (RTS, S) malaria vaccine among children in sub-Saharan Africa and in other regions with moderate to high *P. falciparum* malaria transmission.

I.8. Critical synthesis of the literature review

With the goal of reducing the global incidence and mortality rate of malaria by at least 90% by 2030, programs face the challenge of targeting all *Plasmodium* infections, not only symptomatic cases. Asymptomatic infections acting as silent reservoirs of malaria transmission because asymptomatic parasite carriers are unlikely to seek medical treatment and they are missed by passive surveillance although remaining infectious to mosquitoes. To achieve such ambitious goals in 2030, we need a deeper understanding of the underlying mechanisms promoting asymptomatic parasite carriage, which may involve both pathogen and host factors. Furthermore, Since the prevalence and dynamics of asymptomatic malarial parasitaemia vary geographically and are influenced by complex factors involving hosts, parasites and environments, the identification of these factors is particularly important for malaria control.

Much of the available data on asymptomatic infections is from community cross-sectional surveys, which does not take into account the infection outcome. The asymptomatic carriage of malaria parasites may represent 1) recent infection on its way to patent/symptomatic infection and often lasting between 1 and 3 weeks depending on age in semi-immune populations, 2) a resolving infection following antimalarial medication, or 3) a chronic asymptomatic infection. However, these single time-point data do not provide any information on the dynamics of asymptomatic infections over time. Asymptomatic malaria infections generally correlated with low parasite density, below the detection limit of

conventional diagnostic tools (RDT and microscopy) which have been proved to be inadequate for a community screening of asymptomatic *Plasmodium* carriers. Therefore, to determine the burden of asymptomatic malaria carriage, highly sensitive diagnostic tools (molecular diagnostic platforms) should help to provide accurate epidemiological information to guide the malaria control strategies. In addition, our longitudinal study using nested PCR as a *Plasmodium* diagnostic tool would provide more information on the dynamics and duration of asymptomatic parasitaemia in highly exposed individuals.

The host determinants of asymptomatic malaria infections are thought to involve two main immunological processes: anti-parasite immunity, involving processes that directly suppress parasite growth (mainly mediated by antibodies), thereby limiting their presence to low infection densities; and anti-disease immunity involving processes that prevent overt clinical manifestations of the infection despite high infection loads. In either case, the contributions of anti-parasite or anti-disease immune processes in the maintenance of persistent asymptomatic malaria infections remain unclear. Furthermore, in highly exposed persons, where the duration of chronic malaria infection could range from a few weeks to several years, the contribution of acquired immunity in the maintenance of asymptomatic parasitaemia remains poorly understood. Most malaria prevention research efforts principaly in the case of vaccine research have focused on antiparasite immunity, however the antidisease processes have attracted little to no attention. Given the key role of the inflammatory processes in the pathogenesis of *P. falciparum* malaria, regulation of these inlammatory processes appears to be a major mechanism for antidisease immunity during malaria infections. Further work is required to clearly elucidate the mechanisms of parasite tolerance leading to asymptomatic malaria. Monitoring immune responses (antibody and cytokine responses) in asymptomatic parasitemic individuals in this study would provide a potential answer to questions about the contribution of anti-parasite or anti-disease immune processes in maintaining persistent asymptomatic parasitaemia in highly exposed individuals.

As malaria elimination becomes within sight for more and more countries in Africa, understanding the contribution of the asymptomatic reservoir to disease persistence, particularly in high transmission zones, is relevant to public health because it will be essential to drive the development of more effective control and elimination strategies. We will undertake a population-based longitudinal study to define the contribution of host immunological and parasite factors in the establishment and the maintenance of asymptomatic malaria infection in an area with intense transmission of *Plasmodium falciparum* in Cameroon.

CHAPTER II: MATERIALS AND METHODS

II.1. Materials

II.1.1. Ethical clearance and administrative autorisations

II.1.1.1. Administrative approvals

The study protocol was reviewed and approved by Cameroon National Ethics Committee for Human Health Research (Ethical clearance N°: 2018/09/1104/CE/CNERSH/SP), an administrative authorisation was obtained from the Ministry of Public Health and administrative approvals obtained from Senior Divisional Officer (SDO) of Esse and chiefs from all the villages involved in the study. The study was conducted in accordance with national guidelines for human study as defined by the Human Health Research Division of the Ministry of Public Health.

II.1.1.2. Individual consent

The study information sheet and informed consent form were written in French, English, or interpreted into the local dialect for participants who could not read or speak French or English. The potential risks and benefits of the study were clearly explained to participants and their families prior to any application to participate in the study. All questions regarding the project, the sample collection process, and the handling of participant's personal information were answered prior to the agreement to participate in the study. As this was a longitudinal study, we made it clear to each participant that his involment in the study was voluntary and that he/she was free to withdraw from the study at any time during the follow-up period without any explanation. Individual informed consent was obtained from all participants over 19 years of age or from the parent/legal guardian of children under 20 years of age. The parental consent was obtained from all children less than 12 years of age. In addition assent was obtained from all children between 12 and 20 years of age.

II.1.1.3. Confidentiality of participant information

The data from participants in this study were strictly confidential and were encoded. Patient identities were encoded by a unique study identification number (ID number). Only the principal investigator had the key that links this ID number to the patient identity. Samples only contained ID number, samples date and type of samples. The participant's study information was not shared with anyone outside the project team except as necessary and under confidentiality agreements for independent monitoring, auditing, and inspection by competent authorities.

II.1.2. Study site and duration

This study was conducted in 5 selected villages (Afanétouana, Koutou, Meboé, Ondoundou and Tueson) within the Esse Health District in the Mefou-et-Afamba Division, Central Region of

Cameroon (Figure 9). The study area has been described elsewhere (Essangui *et al.*, 2019). Briefly, the health district of Esse is located approximately 20 Km from Yaounde (4° 05′ 00″ North, 11° 53′ 00″ East), it is an eco-geographically and epidemiologically homogeneous area consisting of 54 villages with inhabited zones mostly structured as clusters of houses. The vegetation in this area consists of equatorial forest degraded by the agricultural activities. Houses in these villages are mostly built with clay or plank walls and corrugated iron roofs. In addition, the houses have large eaves or crevices on the walls, which may favour the entry of mosquitoes. Like all the neighbouring communities of Yaounde, malaria transmission in this area is perennial and hyper-endemic with an entomological inoculation rate of 0.7–1.4 infected bites per person per month (Antonio-Nkondjio *et al.*, 2019), with *P. falciparum* as the predominant plasmodial species. A recent study carried out in two villages (Ongandi and Ngondi-Bele) of Esse by Essangui et al. showed a high prevalence of *P. falciparum* infection of 87.8% using Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) molecular based diagnostic (Essangui *et al.*, 2019). A non-probability convenience sampling method was used for selection of clusters. All the houses in the selected clusters were included in the study and all eligible and consenting individuals from each household were enrolled.

This was a longitudinal study conducted between November 2018 and February 2019 in the Central Region of Cameroon where malaria transmission is perennial with a peak during the rainy season (Antonio-Nkondjio *et al.*, 2019). The climate is equatorial with two rainy seasons (March-June and September-November) and two dry seasons (July-August and December-February) (Lienou *et al.*, 2008).

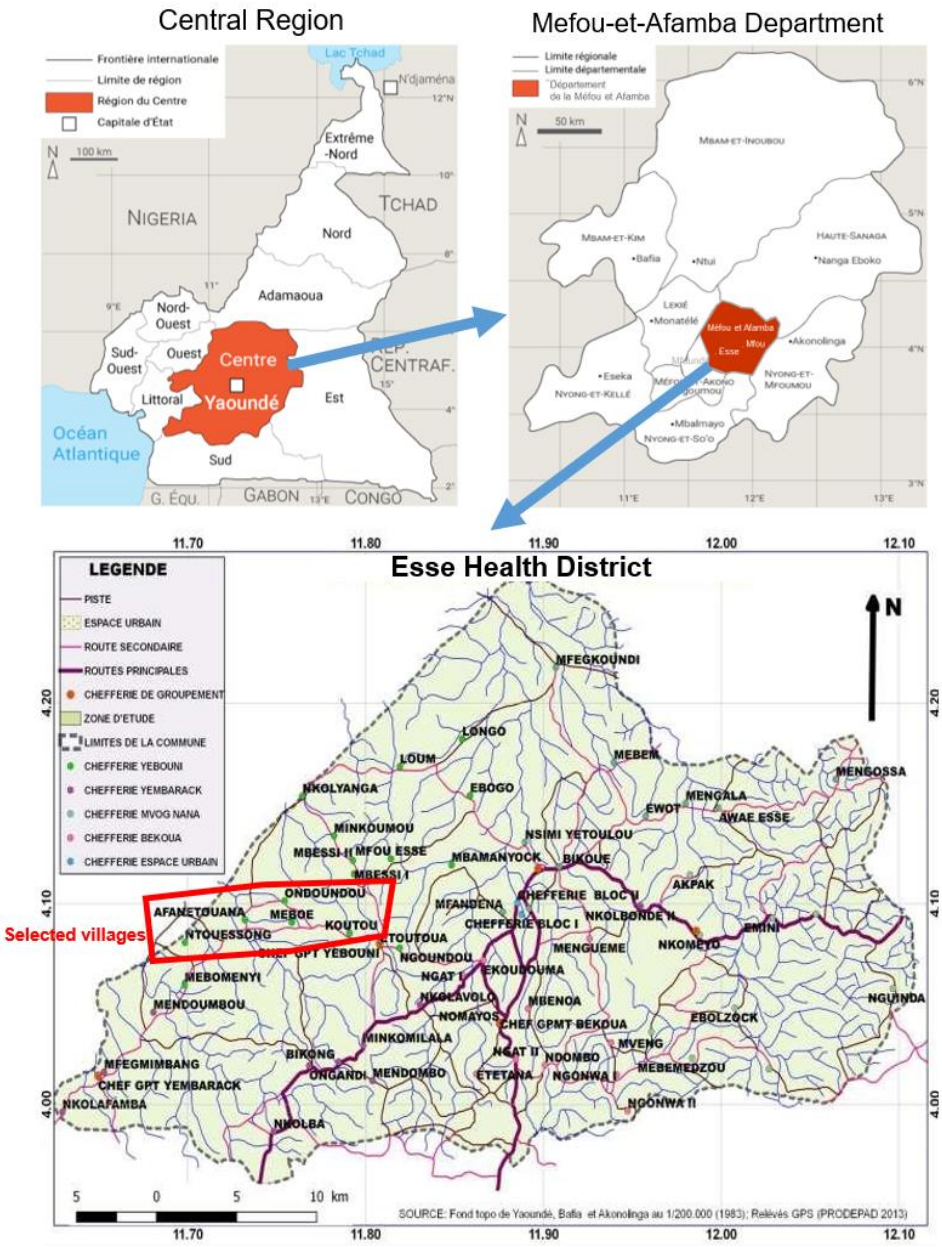

Figure 9: Map of the study site (Source: PLAN COMMUNAL DE DEVELOPPEMENT D'ESSE)

II.1.3. Study population

II.1.3.1. Inclusion criteria

The study population included permanent residents of Esse Health District of both sexes, aged at least two years. The inclusion criteria were; i) be at least two years old, ii) obtain the consent of each older participant or parent or legal guardian for the children, iii) permanently living in the area for at least 6 months before the inclusion in the study, and iv) not pregnant.

II.1.3.2. Exclusion criteria

The exclusion criteria for this study were: i) being ill at the time of inclusion in the study, ii) refusing to give consent, iii) showing symptoms of severe malaria.

II.1.4. Parasites used

Parasites used in this study for protein extraction and growth inhibition assay (GIA) were the laboratory adapted *P. falciparum* 3D7 strain (ATCC) parasites were obtained from the BEI Resources (MRA-102G, USA).

II.1.5. Sample size determination

A large-scale study in Cameroon carried out by the PALEVALUT program, the aim of which was the operational evaluation of the integrated fight against malaria, reported a 20% prevalence of asymptomatic malaria in the ESSE locality (unpublished data). Based on this data, the Lorentz formula was used to calculate the sample size for this study as follows:

$N= Z^2 \times p\ (1-p)/d^2$, where

N= Targeted sample size; Z= Standard normal variate (Z value is 1.96 for 95% confidence level); P= Estimated prevalence of asymptomatic malaria in the area: 20 %; d= Absolute error (5%)

$N= (1.96)^2 \times 0.2\ (1-0.2)/\ (0.05)^2 = 246$ samples

Given that the prevalence of asymptomatic infections in this area was determined by microscopy and knowing the low sensitivity of this technique compared molecular techniques, the sample size was increase by 30%. A recent study conducted in this area using molecular tools; RT-LAMP showed that 35.3% of infected subjects were identified as submicroscopic infections (Essangui *et al.*, 2019). The final target sample size was= 246 + 74 = 320 samples, giving a power sample size calculation of > 90%.

A non-probability convenience sampling method was used for selection of clusters. All the houses in the selected clusters were included in the study and all eligible and consenting individuals from each household were enrolled in the study.

II.2. Methods

II.2.1. Study design and case definitions

II.2.1.1. Study design

This was a longitudinal community and household-based study designed to determine the prevalence and associated determinants of asymptomatic malaria in a high malaria transmission area. The study was carried out from November 2018 to February 2019. Included houses were coded and

geolocated with a GPS (GARMIN GPSmap 62st, Taiwan) before the mass screening of the study population for *Plasmodium* infections. With the agreement of each participant, a prestructured questionnaire was used to collect individual (gender, age, fever history, bed net usage, medicinal plant consumption, and duration in the area) as well as household (household size, type of construction, presence of crevices in the house, type of vegetation, and presence of stagnant water around the house) information. During mass screening, participants were considered asymptomatic if positive for *P. falciparum* using multiplex nested PCR, with the absence of fever at least forty-eight hours prior to enrolment. Symptomatic malaria was determined based on either axillary temperature ≥ 37.5 ° C or history of fever 48 hours prior to participant enrolment. Confirmed symptomatic cases were treated with ACT-based drugs following recommended national guidelines.

Participants with asymptomatic infection were monitored weekly until they developed a fever, or until the end of the study at week 16 post-inclusion. Participant follow-up comprised of weekly interviews regarding the occurrence of fever during the past one week, measurement of axillary temperatures on each visit, and laboratory testing for malaria at weeks 1, 4, 11 and 16. All symptomatic cases occurring during the follow-up period were treated with ACTs as recommended and excluded from further follow-up. At week 11 post-inclusion all participants with persisting asymptomatic infections were further treated with ACTs and monitored for 5 additional weeks. Participants who developed malaria-associated symptoms prior to week 11 post-inclusion (betweem week1 and week 11) were considered as short-term carriers whereas those with persistent asymptomatic infections at week 11 post-enrollment were considered as long-term asymptomatic carriers. The flowchart of participant's follow-up is showed in Figure 10.

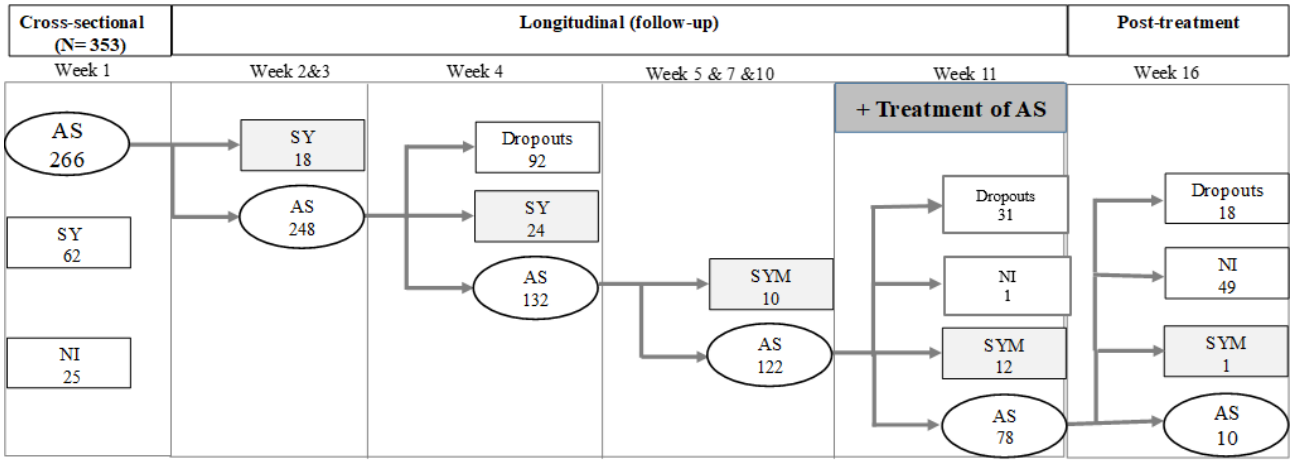

Figure 10: Sample collection flowchart for cross-sectional and longitudinal studies of asymptomatic malaria in the Esse district of Cameroon

Asymptomatic participants were followed for 10 weeks post recruitment, and again for five weeks following treatment at week 11. At each follow-up time point, only the asymptomatic individuals were retained for subsequent follow-up studies, whereas those with confirmed malaria symptoms were treated in accordance with national guidelines. Population follow-up comprised clinical diagnosis at each time point and laboratory diagnosis on weeks 1, 4, 11, and 16. Individuals with asymptomatic parasite carriage on week 1 who became symptomatic on or before week 11 were considered as short-term asymptomatic carriers whereas asymptomatic individuals on week 11 were considered to be long-term carriers. AS: Asymptomatic carriers, SY: Symptomatic carriers, NI: Non-infected individuals.

II.2.1.2. Case definitions for the study population

Participants were defined as asymptomatic if positive for malaria parasites by PCR in the absence of fever at least 48 hours prior to enrolment. Febrile malaria was determined based on RDT and/or light microscopy positivity for malaria parasites and axillary temperature ≥ 37.5 ° C or a history of fever in the past 48 hours. Short-term asymptomatic (ST) were defined as asymptomatic individuals who had developed fever associated with malaria during the 11-week infection follow-up period, whereas long-term asymptomatic (LT) or persistent asymptomatic represented the asymptomatic individuals who remained asymptomatic until anti-malarial treatment at the end of the infection follow-up period. The 11-weeks cut-off time chosen in this study was based on previous data showing that untreated *P. falciparum* infections persist on average for 6 months, with fluctuations of the parasite density over the course of an infection (Felger *et al.*, 2012; Landier *et al.*, 2017; Nguyen *et al.*, 2018; Slater *et al.*, 2019). Based on that, we decided to take half of that 6-month time as our limit. Participants were classified as anaemic based on World Health Organisation (WHO) classification guidelines (OMS, 2011). Early clinical converters were defined as asymptomatic individuals who developed fever in the presence of malaria parasites during the first 3-week follow-up period. The 3-weeks follow-up time limit for early clinical converters was based on known duration for pre-patent infections in semi-immune individuals, which ranges from 1 to 3 weeks depending on age (Pinkevych *et al.*, 2014; Slater *et al.*, 2019).

II.2.2. Blood sample collection and processing

Approximately 3-5 ml of venous blood were collected in EDTA-coated tubes and used to prepare dry blood spots, collect blood plasma and for haemoglobin levels measurement. Plasma was separated by high speed centrifugation (1,900 x g for 5 min) and stored at - 80 °C until used for immunological assays. Parasite density (parasites/µl) was determined by microscopy examination of Giemsa-stained thick blood smears. Polymerase chain reaction (PCR) was performed on all sample to identify submicroscopically infected individuals and for parasite speciation. Blood haemoglobin levels were assessed using a portable Mission Hb haemoglobinometer (ACON Laboratories, Inc, USA).

II.2.3. *Plasmodium* detection, speciation and genotyping

The diagnosis of plasmodial infections was done using both the reference method, microscopy and a molecular-based approach; multiplex nested PCR.

II.2.3.1. Diagnostic using light microscopy

Thick and thin blood film slides were prepared using 5 or 3 µl of blood and stained with 10 % Giemsa solution, and examined under a 100x oil immersion lens by two independent microscopists.

In brief, to prepare a thin smear, approximately 3 µl of blood was spotted on a central position of a glass slide and spread using the ground edge of the spreader slide. For thick smears, 5 microliters of peripheral blood are deposited on a glass slide and spread in a circular pattern using the bevelled corner of a spreader slide. Upon drying the smears for about 1 h at room temperature, the thin smear was fixed by dipping in absolute methanol for a few seconds and then air-dried to eliminate water artefacts.

Both the fixed thin and unfixed thick smears are stained with 10% Giemsa for 10 min. Following staining, the slides were washed by brief dipping in and out of water and air-dried for at least 30 min at room temperature prior to microscopic examination using an immersion oil 100X objective. A blood slide was considered positive when a concordant result was obtained by at least two independent microscopists and when a discrepancy occurred, a third microscopist was used for confirmation. A slide was considered negative if no parasite were detected after a count of 500 white blood cells. Parasite density was determined based on the number of parasites per at least 200 WBC count on a thick film, assuming a total white blood cell count of 8000 cells/µL of whole blood. The parasitemia was expressed in parasites/µL.

II.2.3.2. Parasite speciation using multiplex nestedPCR

II.2.3.2.1. DNA extraction

Genomic DNA was extracted from dry blood spots by the chelex method as described by Plowe et al. 1995 (Plowe *et al.,* 1995). In short, 3 mm of dry blood spot was incubated with 1 ml of 0.5% saponin overnight at 4°C. Following incubation, the contents of the tubes were centrifuged for 30 seconds at 2,500 x g and the supernatent was discarded. The spot was washed twice with 1ml PBS, then incubated in 150 µL of 6.7% Chelex at 95°C for 10 minutes using a heat block/ shaker. Tube was spun for 5 min at 2,500 x g and approximately 100 µL of the top layer was transferred to the new tube and stored at -20°C.

II.2.3.2.2. Multiplex nested PCR

The speciation of the *Plasmodium* parasites was carried out by multiplex nested PCR-based genotyping of the 18S ssrRNA genes of *Plamodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae, P. vivax* and *P. Knowlesi,* according to the protocol described by Snounou et al. with some modifications (Snounou *et al.,* 1993). For the first PCR reaction, 5 µL of genomic DNA were used in 20 µL reaction with 0.2 µM of *Plasmodium* genus-specific outer primers (Table III) and 10 µl of Platinum green Hot start PCR 2X master mix (Cat: 13000014, Invitrogen). Nested PCR was performed with 2 µL of the PCR product, 0.2 µM species-specific primers (Table III) and 10 µl of Platinum green Hot start PCR 2X master mix. Both PCR steps were done under the same cycle conditions, which included initial denaturation/enzyme activation at 95°C for 15 min, and 43 cycles

of 95°C for 45 seconds and 55°C for 1 min 30 seconds, and a final extension at 72°C for 5 min. The PCR assays were performed using a SimpliAmp thermal cycler (Applied Biosystems). PCR products were analysed by agarose gel electrophoresis and visualized by UV transillumination. *Plasmodium species* were identified on the basis of the amplified DNA band sizes.

Table III: Primer sequences for the speciation of *Plasmodium*

Species	Sequences	Reaction	PCR product
Plasmodium genus-specific	PUF1 : 5'AGTGTGTATCAATCGAGTTTC 3' PUR1 : 5'TAACTTTCTCGCTTGCGCG 3'	Nested 1	
P. ovale	PUF2 : 5'TGTTAGGGTATTGGCCTAAC 3' POR2 : 5'TCATTCCAATTACAAAACCATG 3'	Nested 2	240
P. malariae	PUF2 : 5'TGTTAGGGTATTGGCCTAAC 3' PMR2 : 5'CCAGACTTGCCCTCCAATTGCC 3'	Nested 2	278
P. falciparum	PUF2 : 5'TGTTAGGGTATTGGCCTAAC 3' PFR2 : 5'GAAAAGCTAAAATAGTTCCCC 3'	Nested 2	409
P. vivax	PUF2 : 5'TGTTAGGGTATTGGCCTAAC 3' PVR2 : 5'GTAACAAGGACTTCCAAGC 3'	Nested 2	506
P. Knowlesi	PUF2 : 5'TGTTAGGGTATTGGCCTAAC 3' PKR2 : 5'AAGGAAGCAATCTAAGAGTTC 3'	Nested 2	916

II.2.3.3. Genotyping of *Plasmodium falciparum* ***msp2*** gene

Genotyping of msp2 gene was performed by nested PCR on *P. falciparum* PCR positive samples as previously described (Ranford-Cartwright *et al.*, 1997). Two sets of primers specific for the polymorphic regions of msp2 (block 3) were used. The first PCR amplified the outer msp2 domain (forward, 5′-GAAGGTAATTAAAACATTGTC-3′; reverse, 5′-GAGGGATGTTGCTGCTCCACAG-3′), and the second PCR used the inner primers to identify different msp2 alleles (forward, 5′-GAGTATAAGGAGAAGTATG-3′; reverse, 5′-CTAGAACCATGCATATGTCC-3′). In brief PCR reactions were carried out in a final volume of 15 µL containing 3 µL of parasite DNA or 1.5 µL of amplicon, 200 nM of each primer and 7.5 µL of Platinum green Hot start PCR 2X master mix (Cat: 13000014, Invitrogen). Cycling conditions for the PCRs were as follows: initial denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 45 seconds, primer annealing at 45 °C for the first PCR or 55°C for the second PCR for 1 min 30 seconds. The final cycle involved an extension at 72 °C for 5 min. PCR reactions were incubated in a thermal cycler (SimpliAmp thermal cycler, Applied Biosystems). The PCR products were analysed by agarose gel electrophoresis and visualized on a UV transilluminator. The sizes of

the amplicons were determined using a 1000 bp DNA ladder. *msp2* alleles were defined on the basis of the amplified DNA band sizes. The multiplicity of infection (MOI), was defined as the number of msp-2 alleles (representing a *P. falciparum* clone) detected in each sample. Following determination of MOI, the *P. falciparum* infection was classified as monoclonal (MOI = 1) or multiclonal (MOI> 1).

II.2.4. Antiplasmodial antibody quantification and avidity assessment

Plasma IgG antibody levels and avidity were determined by indirect ELISA method using different *P. falciparum* soluble protein extracts from different parasite stages (Merozoites, schizontes or mixed stages parasite culture) or the recombinant proteins EBA-175, MSP-1c19 and MSP-4p20. Soluble antigens were extracted from laboratory maintained *P. falciparum* 3D7 strain parasites. The recombinant protein erythrocyte binding antigen EBA-175, F3 region was obtained from the BEI Resources (MRA-1162). *P. falciparum* Merozoite Surface Protein-1_{19} (MSP1c19) and *P. falciparum* Merozoite Surface Protein 4 p20 (MSP-4p20) were produced using a baculovirus-insect cell expression system (Bonnet *et al.*, 2006).

II.2.4.1. Parasite culture and protein extraction

3D7 *P. falciparum* clones were cultured at 3% haematocrit in RPMI 1640 medium containing glutamax and sodium bicarbonate, supplemented with gentamicin, HEPES, albumax II and hypoxanthine. Five mL of cultures were maintained at 37°C in a humidified atmosphere with 5% CO2 at <10% parasitaemia as described (Trager and Jensen, 1976). At parasitaemia of about 8-10%, cultures were harvested for merozoite and schizonte isolation or parasite protein extraction (Costa *et al.*, 2013; Quintana *et al.*, 2018).

To extract the soluble antigens from the merozoite and schizonte stages, the merozoites and schizontes were first purified as described previously (Quintana *et al.*, 2018). Briefly, early ring-stage (< 10 hpi) parasites were synchronized by sorbitol treatment (5%) and maintained in culture till they reached segmenters (46 hpi) and were further purified on a magnetic column (Miltenyi Biotec).
Cysteine protease inhibitor; E64 (E3132, Sigma, Conc= 1 mg/mL) was added to the purified segmenter-stage parasite culture at a final concentration of 10 mM. E64-treated schizonts were centrifuged at 1,900 x g for 5 min and pellet was stored at -80°C until needed for protein extraction. For merozoite isolation and protein extraction, the E-64 treated schizonts were re-introduced in culture for about 5–8 hours till mature segmenters were formed; fully mature sacks of merozoites were observed. E64-treated schizonts were centrifuged at 1,900 x g for 5 min and pellet was re-suspended in 1 mL of 0.1% saponin in PBS, incubated for 10 min at 4°C to lyse RBCs. The pellet was washed twice with ice cold PBS and stored at -80°C until used for protein extraction.

To extract the total soluble antigens from parasite culture with mixed-stages, 10% infected asynchronous red blood cells were lysed with 0.1% saponin in PBS for 10 min at 4°C. The free parasites were washed twice with ice-cold PBS and stored at -80 ° C until used for protein extraction. For the extraction of proteins, lysis buffer (0.1% Triton X, 120 mM NaCl, 50 mM Tris–HCl pH 7.8 and 1X protease inhibitor) was added to different parasite preparations (merozoite, schizont and mixed-stages), and homogenized thoroughly by vortexing. After two cycles of freeze-thaw fractionation, the mixtures were centrifuged at 15,300 x g at 5 ° C for 15 min. The supernatant was recovered and the protein concentrations were quantified using NANODROP 2000 spectrophotometer (Thermo Scientific, USA) at 280 nm and the expression mg/mL.

II.2.4.2. Anti-Plasmodial antibody quantification

Plasma IgG antibody levels or avidity were quantified by indirect ELISA method as previously described (Perraut *et al.*, 2017; Essangui *et al.*, 2019). In brief, 96-well microplates (F96 CERT-Maxisorp) were coated with each antigen (1 mg/mL of soluble protein extracts, 0.20 mg/mL of EBA-175, 0.5 mg/mL of MSP-1c19 and 0.5 mg/mL of MSP-4p20) in 0.1 M bicarbonate buffer (for protein extracts and EBA-175) or PBS (for MSP-1 and MSP-4) overnight at 4˚C. After incubation, the plates were washed twice with 250 µL/well of PBS then blocked with 1% BSA in PBS (pH 7.2) for one hour at room temperature. Plates were washed thrice with 300 µL/well of wash buffer PBS-T (PBS 0.05% Tween 20) and incubated with 100 µL/well of diluted plasma (1/250 in PBS-T/1% BSA) for one hour at room temperature. After incubation with plasma samples, the plates were washed three times. For detection of human total IgG anti-*P. falciparum* antibodies, the plate was incubated with 100 µL/well of horse radish peroxidase (HRP)-conjugated goat anti-human IgG diluted with PBS-T/1% BSA to 1:40000 (Invitrogen, USA). For subclass-specific mouse anti-human secondary antibodies (Thermo Scientific, USA) diluted in PBS-T/1% BSA at concentrations of 1:10000, 1:400, 1:10000, and 1:400 for IgG1, IgG2, IgG3, and IgG4, respectively, were added to all wells. Plates were incubated at room temperature for 1 h and then washed. Plates with subclass-specific antibodies were then incubated with 100 µL of 1:10000 goat anti-mouse horseradish peroxidase (HRP)-conjugated IgG tertiary antibody (novex by life technology, USA) for one hour at room temperature and then washed three times. The antigen-antibody complexes were revealed by adding 100 µL/well of substrate solution 3,3 ,5,5 –tetramethylbenzidine, TMB (Thermo Scientific, USA). Plates were placed in the dark for 10 min. The reaction was stopped with 50 µL/well of 1/10 diluted sulfuric acid, and optical densities (OD) were read by a MULTISKAN FC plate-reader (Thermo Scientific, USA) at 450 nm. Plasma from non-exposed European blood donors were used as negative controls whereas a pool of IgG fractions from adults living in high malaria endemic areas were used as positive controls. Results were expressed as optical-density ratios (OD sample/mean

OD naive control). The cut-off value was defined as OD ratio ≥ 2. Cytophilic ratios were calculated as the ratio of IgG1 + IgG3 / IgG2 + IgG4.

II.2.4.3. Anti-Plasmodial antibody avidity assessment

For avidity testing, 100 µL of 8 M urea in wash buffer (treated wells) or 100 µL of wash buffer (untreated wells) were added for 15 minutes at room temperature to the respective wells following primary antibody binding as previously described (Berry *et al.*, 2019). Avidity indices (AI) were calculated as the proportion of bound antibodies following urea treatment (Avidity index = [mean OD of treated wells /mean OD of untreated wells] × 100).

II.2.5. *P. falciparum* growth inhibition assay (GIA)

GIA was performed using plasma from endemic and non-endemic individuals as previously described (Duncan and Bergmann-Leitner, 2015). Plasma samples were heat inactivated at 56°C for 20 min and a 1:20 ratio of 50% RBC (from endemic individuals) was added to each sample and incubated at room temperature for 1 h to remove the RBC-reactive antibody. After spinning for 2 min at 13,000 x g to pellet the red blood cells, the supernatant was collected and transferred to a 96-well V-bottom plate.

Synchronized 3D7 *P. falciparum* late trophozoite or early schizont (30-40 h) cultures at 0.5% parasitaemia were incubated with the heat inactivated plasma for 48 h at 37°C (5% CO2, 5% O2, 90% N2) in a flat-bottom 96-well microtitre plate. Growth inhibition was assessed by the SYBR green based method. In brief, 80 µL of the parasite culture were transfered to a 96-well black plate and 40 µL of SYBR green-I lysis buffer (0.625 mM Tris-base, 7.5 mM EDTA, 0.1% Saponin, 1.2% Triton X 100 and 0.25x SYBR green 10000x, in H_2O) were added and incubated for 30 min at 37°C in the dark. Fluorescence corresponding to the amount of DNA in each well was obtained using a Fluoroscan plate reader at an excitation wavelength of 485nm and emission of 538nm. GIA indices (%) corresponding to the growth inhibition rate was estimated as = 100 x [1 - ((OD Immune sample - OD RBC) / (OD parasitized RBC - OD RBC))].

II.2.6. Plasma cytokine measurement

Plasma concentrations of 38 immune mediators including interleukin (IL)-1α, IL-1β, IL-1RA, IL-2 IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, Interferon (IFN)-α2, IFN-γ, tumor necrosis factor (TNF)-α, TNF-β, IFN-γ induced protein (IP-10), monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage inflammatory protein (MIP)-1α, MIP-1β, Eotaxin, Fractalkine, human growth-regulated oncogene (GRO), Macrophage-derived chemokine (MDC), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), sCD40L, FMS-like tyrosine kinase 3 ligand (Flt-3L), epidermal

growth factor (EGF), fibroblast growth factor 2 (FGF-2), transforming growth factor alpha (TGF-α) and vascular endothelial growth factor (VEGF) were determined by Luminex-based method using the Human Cytokine/Chemokine Magnetic Bead Panel MILLIPLEX MAP Kit (Cat. No. HCYTMAG-60K-PX38, EMD Millipore Corporation). Briefly, 25 µL of premixed magnetic capture beads were incubated with equal volumes of plasma or diluted standard in a 96-well microplate placed on a horizontal plate shaker for 2 hours. After washing using a magnetic plate, plates were further incubated with 25 µL/well of biotinylated detection antibodies for 1 hour at room temperature. The resulting antibody-cytokine complexes were further revealed by adding 25 µL of streptavidin-PE in each well and incubating for 30 minutes at room temperature. Median fluorescence intensities were read on a Luminex MAGPIX Analyser (XMAP Technology) equipped with xPONENT software version 4.2. The relative concentration of each cytokine (pg/mL) in each sample was determined based on the automatically generated standard curve for each analyte. Analyte amounts less than the limit of detection for each cytokine were attributed the value of the limit of detection.

II.2.7. Statistical analysis

Statistical analyses were performed using different software: Stata version 13.0, GraphPad Prism version 8.0, and RStudio version 3.5.2. For risk factor analysis, both univariate and multivariate logistic regression models were applied with proportions of the main outcome compared between the predictor groups. Explanatory variables were tested individually in univariate analysis, and all variables with $P < 0.05$ were selected for multivariate analysis. Logistic regression analyses were adjusted for clustering at the household level. The comparison of the proportions between groups was carried out using the chi-square test ($\chi 2$). Differences in median quantitative variables between two or more groups were determined using Wilcoxon signed rank test and Kruskall-wallis test, respectively. Differences in mean quantitative variables between two groups were investigated using the two-sample t test after assessment of normality (Anderson-Darling test). Spearman rank correlation tests or linear regression were used to determine the correlation between quantitative variables. Multivariate linear regression was used to determine independent factors associated with outcomes among quantitative variables. Association between age or parasitaemia and cytokine levels were analyzed using a factor analysis of mixed data (FAMD). In all cases, P values < 0.05 were considered statistically significant.

CHAPTER III: RESULTS

III.1. Prevalence and associated risk factors of asymptomatic parasitaemia in a *P. falciparum* hyper-endemic zone in Cameroon

III.1.1. Baseline characteristic of the study population

Of the 353 individuals screened for *Plasmodium* infections, 159 (45%) were children under 15 years of age with median age of 26 years, and 53.3% were females. Overall, 65 households spread across nine distantly located clusters (at least 0.5km apart) were included in the study giving a median household size of 4 per household (range: 1-15 persons per household). About 99.4% of the participants were of the low-to-middle income group as determined on the basis of type of houses (thatch, planks or blocks) and availability of source of electricity (generator or solar panel), as described previously (Guerra *et al.*, 2018). Overall, 18.4 % of the participants reported having a history of fever in the past 48 hours. Out of 236 participants whose haemoglobin were quantified, 38.6 % were considered as anaemic according to the WHO classification (OMS, 2011). For the preventive methods, 54.7 % of the participants used bed net or insecticides. Individual and household characteristics of the study population are summarized in Table IV. In each analysis, only participants with complete information on the study outcome (known parasitemia, MOI and hemoglobin levels) were considered, thus only 311 out of the 328 parasitaemic individuals, 250 individuals with known MOI out of the 316 *P. falciparum* infected subjects and 236 individuals with known haemoglobin levels out of 353 participants were included in this study.

Table IV: Characteristics of the study population, by village (N = 353)

Determinant	Afanétouana (n= 80)	Koutou (n= 49)	Meboé (n= 93)	Ondoundou (n= 50)	Tueson (n= 81)	Total (N= 353)
Number of households	12	7	15	12	19	65
Median household size (range)	8 (1-15)	8 (3-11)	5 (3-13)	4 (2-8)	4 (1-15)	4 (1-15)
Proportion of female, n (%)	46 (57.5)	27 (55.1)	42 (45.2)	27 (54)	46 (56.8)	188 (53.3)
Age groups (years), n (%)						
≤ 14	34 (42.5)	34 (69.4)	50 (53.8)	35 (70.0)	41 (50.6)	158 (45.0)
> 14	46 (57.5)	15 (30.6)	43 (46.2)	15 (30.0)	40 (49.4)	194 (55.0)
Bed net or insecticide use, n (%)	58 (72.5)	23 (46.9)	59 (63.4)	21 (42.0)	32 (39.5)	193 (54.7)
Socio-economic levels, n (%)						
High	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.5)	2 (0.6)
Middleclass	49 (61.3)	24 (49.0)	35 (37.6)	7 (14.0)	59 (72.8)	173 (49.0)
Low	31 (38.5)	25 (51.0)	58 (62.4)	43 (86.0)	20 (24.7)	178 (50.4)
Participants with a recent fever episode, n (%)	20 (25.0)	18 (36.7)	32 (34.4)	9 (18.0)	21 (25.9)	65 (18.4)
Fever on the day of enrolment, n (%)	8 (10.0)	7 (14.3)	20 (21.5)	2 (4.0)	14 (17.3)	51 (14.4)
Participants with anaemia*, n (%)	37 (57.8)	2 (33.3)	23 (39.0)	3 (11.5)	26 (32.1)	91 (38.6)

**Of the 353 participants enrolled based on their PCR status (positive or negative), information on blood Hb levels were lacking for 117 of the participants due to a breakdown with the haemoglobinometer and t*he prevalence of anaemia was calculated based on samples with known Hb levels (N=236).

III.1.2. Prevalence and extent of asymptomatic infections in the study area

Of the 353 participants tested for malaria parasite, 191 (54.1%), 235 (66.6%), 328 (92.9%) were positive for *Plasmodium* infection by RDT, light microscopy and multiplex nested PCR, respectively. Of the 328 infections 85.4% (280) were *P. falciparum* mono-infections, 11% (36) were mixed *P. falciparum* infections (8.5% *P. falciparum* + *P. malariae*, 1.8% *P. falciparum* + *P. ovale* and 0.6% *P. falciparum* + *P. malariae* + *P. ovale),* and 3.6% (11) were non-*falciparum* infections (2.1% *P. malariae* and 1.5% *P. ovale*). A total of 81.2% (266) of the infected participants were non-febrile and did not experience fever symptoms in the past forty-eight hours (asymptomatic infections) resulting in an overall prevalence of asymptomatic malarial parasitaemia of 75.4% (266) in the study area (Figure 11). 17.6% (62) of the study participants carried a symptomatic infection, while 7.1% (25) were not infected. In this study, the positivity was based on the nested PCR results.

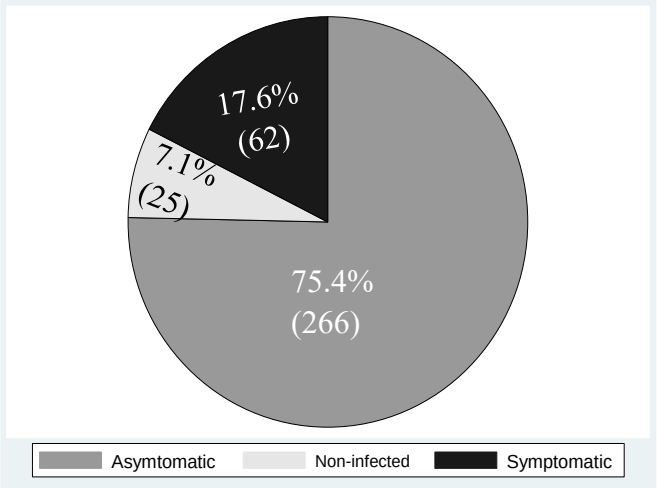

Figure 11: Poportion of infection status in the study area.
In this graph, the positive sample are based on the nested PCR.

Of 143 participants who completed the follow-up study as designed, 64 (44.8%) developed malaria-associated fevers within 10 weeks of follow-up, 1 (0.7%) resolved the infection in the absence of any known antimalarial treatment, whereas 78 (55.4%) remained asymptomatic until antimalarial treatment at week 11 post-enrolment. Based on the 10-week follow-up duration prior to antimalarial treatment, an asymptomatic to symptomatic malaria conversion rate was 4.5% per week in the study population. Together, the above findings indicate a high prevalence of asymptomatic malaria parasite carriage as well as a high proportion of persistent parasitaemia in the study area based on the 10-week follow-up duration.

III.1.3. Asymptomatic malaria and age

The prevalence of asymptomatic malaria was high in all age groups but higher in older children (6-14 years) or adults compared to younger children aged 0-5 years, although non-significant (Figure

12A). However, the prevalence of symptomatic infections significantly decreases with age (P= 0.0157 for >14 vs 0-5 years) (Figure 12A). In addition, the median age was significantly higher in asymptomatic individuals than in those with symptomatic infections (P= 0.0012) (Figure 12B), indicating an assoaciation between age and asymptomatic malaria.

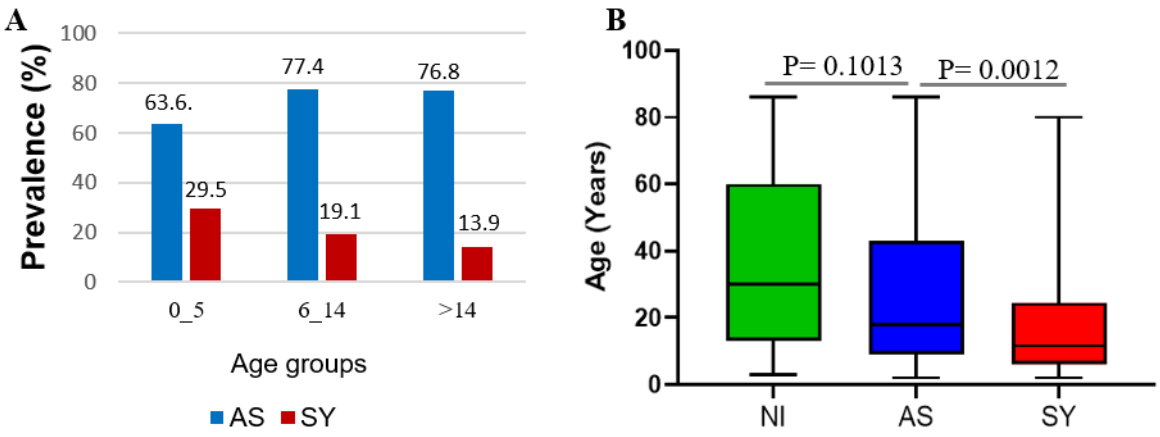

Figure 12: Association between age and infection status.

A) Prevalence of asymptomatic malaria according to age groups. B) Median age between study groups. Data in A are presented in bar chart representing the percentage of infection status in each age group. Data on B are presented in boxplot representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)) of age. Median age was compared between groups using Wilcoxon ran-sum test. NI: Non-infected, AS: Asymptomatic, SY: Symptomatic.

III.1.4. Asymptomatic malarial parasitaemia and parasite densities

It has been shown that, asymptomatic infections are associated with low parasite densities. Of the 328 infections detected by nested PCR in this study, 68.3% (224) were within the detection limit of light microscopy with a median parasite density of 327.3 parasites/µL. Of these microscopic infections, 178 were asymptomatic and 46 symptomatic. Although non-significant, median parasite densities were about 2-fold lower in asymptomatic individuals (268.1 parasites/µL) compared to symptomatic ones (511.7 parasites/µL) (P = 0.1178) (Figure 13A). Consistent with the hypothesis that host immunity increases with repeated exposure to malaria parasites, parasite densities decreased significantly with age (r= -0.49, P< 0.0001) (Figure 13B), only in individuals aged 20 years and younger (r= -0.39, P< 0.0001) (Figure 13C). Above 20 years, parasite densities appear stable. Furthermore, 75.7% of sub-microscopic infections were observed in adults (> 14 years old) and the median age was more than 2 times significantly higher in submicroscopic infected subjects than in those with microscopic infections (28 vs 12 years; P< 0.0001).

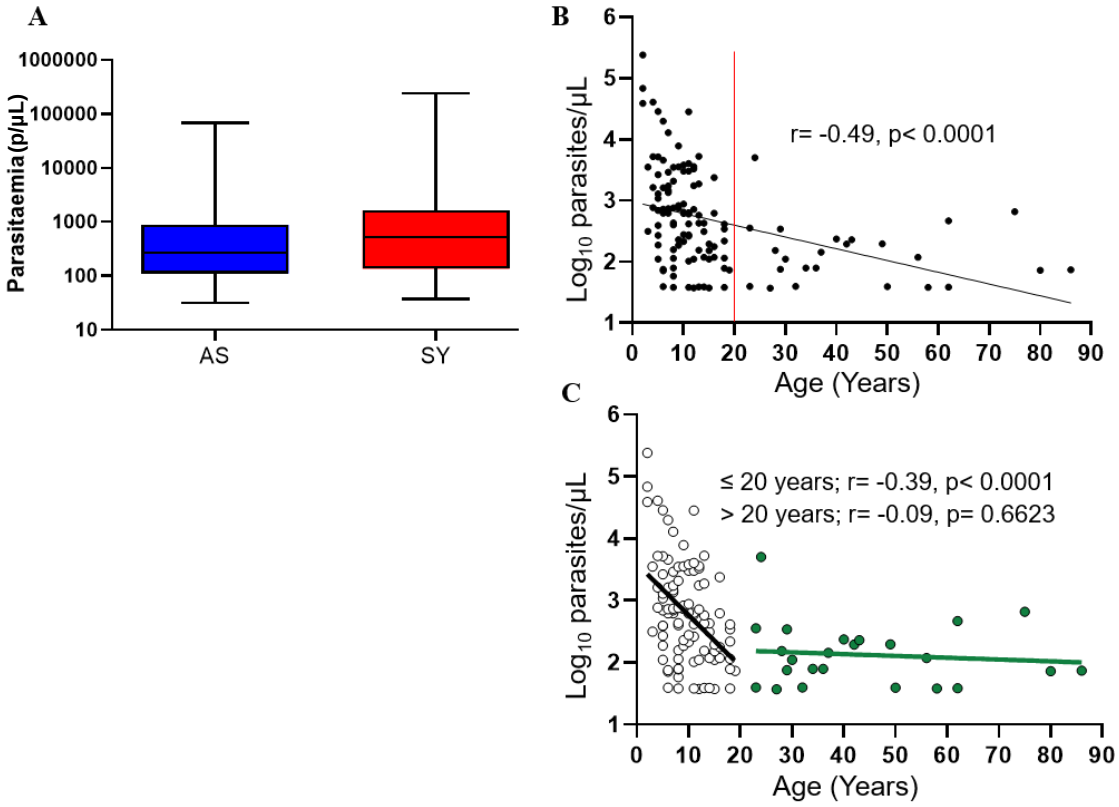

Figure 13: Parasite densities and asymptomatic malaria.

A) Parasitaemia between asymptomatic and symptomatic individuals. B and C) Correlation between parasitaemia and age in infected individuals. Data on A are presented in boxplot representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)) of log10 parasitaemia. Median parasitaemia was compared between groups using Wilcoxon ran-sum test. B is represented as Scatterplot of parasitaemia levels and age in AS or SY individuals. Correlations were performed using Spearman correlation test and presented as best of fit line with 95% confidence intervals. AS: Asymptomatic, SY: Symptomatic.

In persistent asymptomatic infected individuals (long-term asymptomatic), although not significant, the median parasitaemia decreased between weeks 1 and 4 (p= 0.4025), then increased at week 11 (p= 0.3864) (Figure 14A). Furthermore, the median parasitaemia was significantly higher at week 11 compared to week 1 (p= 0.0115). Individually, parasite densities in individuals with persistent asymptomatic parasitaemia fluctuated significantly over time during the follow-up period from ultra-low parasitaemia (submicroscopic) to high parasitaemia and vice versa (Figure 14B). Taken together, these data show important fluctuations in individual parasitaemia over time, suggesting a dynamic interaction between the parasite and host defence mechanisms during persistent asymptomatic infections.

Although parasitaemia fluctuated in persistent asymptomatically infected individuals (long-term asymptomatic), the median parasitaemia was comparable between individuals who progressed from asymptomatic to symptomatic (short-term asymptomatic) infections and those who remained

asymptomatic during the infection follow-up period (356.3 vs 336.1 parasites/µl; p= 0.9908), thus, indicating that, parasitaemia may not play a role in the clinical conversion.

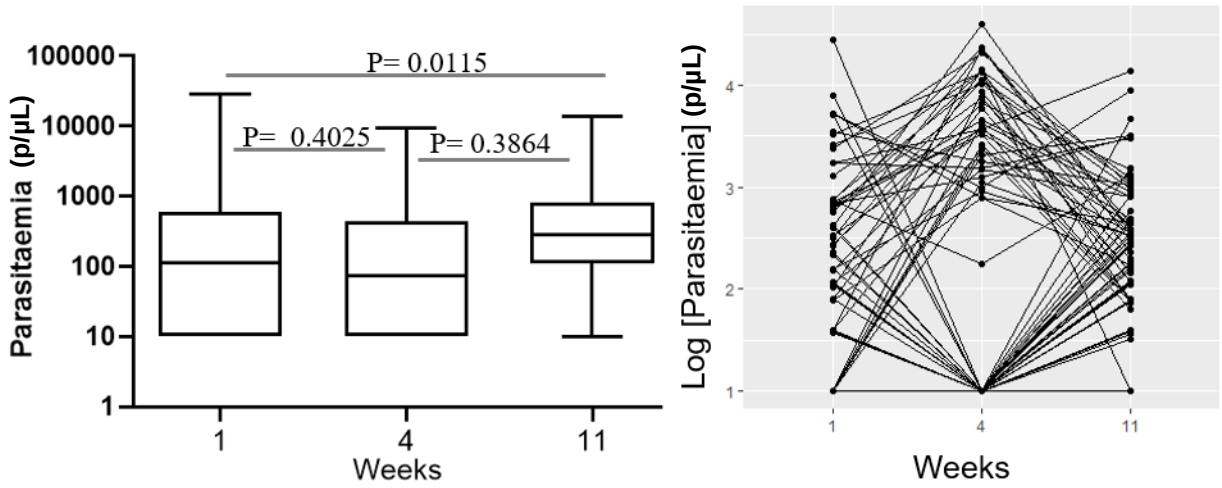

Figure 14: Kinetics of parasitaemia in asymptomatic malaria parasite carriers over time

A) Median parasite density over time in long-term asymptomatic parasite carriers. B) Individual parasite density over time in long-term asymptomatic carriers. Data on A are presented in boxplot representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)) of log10 parasitaemia. Median parasitaemia was compared between groups using Wilcoxon ran-sum test. On B, each line represents log10 parasitaemia in an individual at different time point. Weeks 1, 3 & 11 correspond to sampling time points before antimalarial treatment at Week 11.

III.1.5. Asymptomatic malaria and *P. falciparum* parasite clone

In an effort to assess the role of *P. falciparum* parasite clonality in asymptomatic malaria, the highly polymorphic merozoite surface protein 2 gene was use to discriminates between different *P. falciparum* genotypes and 79.1 % (250) *P. falciparum* parasite isolates were successfully amplified by nested PCR. About 30 msp2 alleles were identified in this study population, with an overall mean MOI of 1.7 (range: 1-8), suggesting a high diversity of parasite clones in this study area. The frequency of msp2 alleles in this population varied from 0.5 to 18.6% in asymptomatic individuals and from 0.0 to 21.6% in those with symptomatic infections (Figure 15A). Median MOI was comparable between isolates from asymptomatic individuals to those from symptomatic ones (P= 0.2321) (Figure 15B). However, some msp2 alleles (350,422, 546, 566, 580, 620, 633, 680, 800 and 830 bp) were only present in isolates from asymptomatic parasite carriers (Figure 15A). MOI was positively correlated with parasitaemia only in asymptomatic participants (P = 0.0470, r = 0.1732 and P = 0.5843, r = 0.0929 for asymptomatic and symptomatic, respectively) (Figure 15C). MOI was negatively correlated with age in asymptomatic parasite carriers (P< 0.0001, r = -0.3684), but not correlated with age in symptomatic individuals (P = 0.8467, r = -0.0278) (Figure 15D). Together, these data indicate the implication of some *P. falciparum* clones in the establishment of asymptomatic parasitaemia.

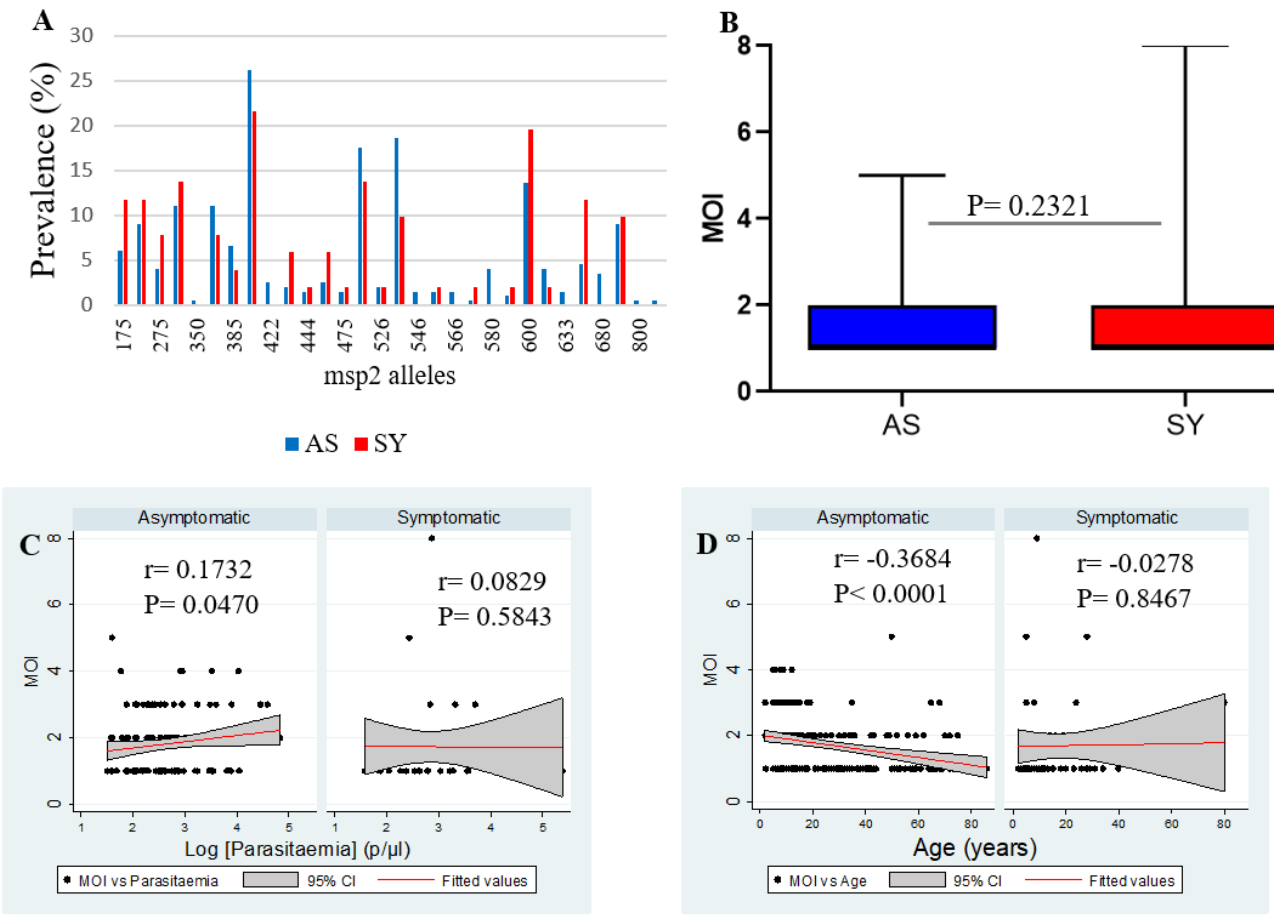

Figure 15: *Plasmodium falciparum* parasite clones and asymptomatic malaria.

A) Allelic frequency in asymptomatic and symptomatic *P. falciparum* infected individuals. B) Median MOI in participants with asymptomatic or symptomatic *P. falciparum* infection. C) Correlation between MOI and parasitaemia. D) Correlation between MOI and age. Data on A are presented in bar chart representing the percentage of msp2 alleles in each infected group. Data on B are presented in boxplot representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)) of MOI. Median MOI was compared between groups using Wilcoxon ran-sum test. C&D are represented as scatterplot of MOI levels and parasitaemia or age in AS or SY individuals. Correlations were performed using Spearman correlation test and presented as best of fit line with 95% confidence intervals. AS: Asymptomatic, SY: Symptomatic.

As observed with parasite densities, the median MOI was comparable between short-term and long-term asymptomatic individuals (2 vs 1, p = 0.4317), suggesting that the maintenance of persistent asymptomatic parasitaemia may be independent of the parasite clonality. In addition, as parasite densities fluctuated over time during persistent asymptomatic parasitaemia, *P. falciparum* clones identified in these individuals also fluctuate over time (some clones disappear when some others appear over time) (Figure 16), indicating that parasite loads during persistent *P. falciparum* asymptomatic infections may depend on the types of clones.

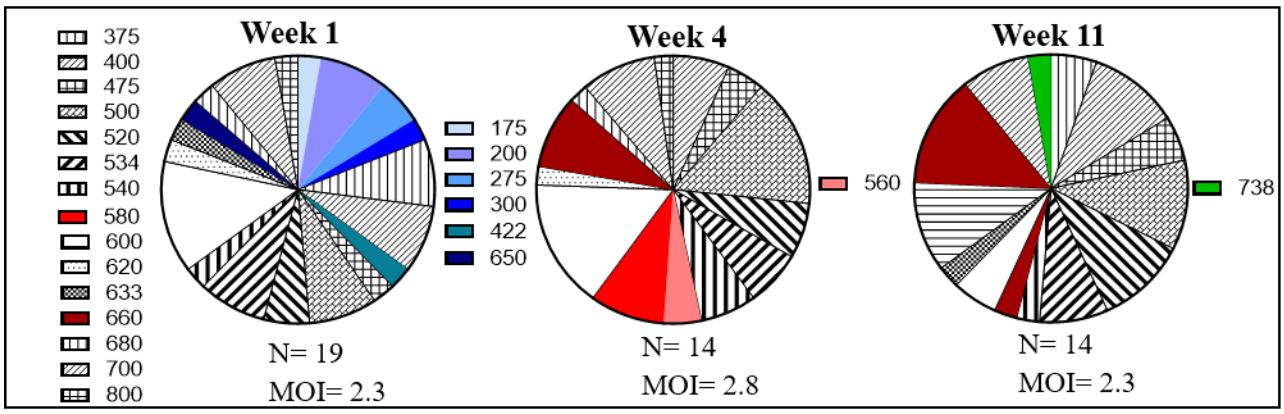

Figure 16: *Plasmodium falciparum* **parasite clones during persistent asymptomatic parasitaemia**.

Data are presented in pie chart representing the msp2 alleles determined by nested PCR in individuals with persistent asymptomatic infection per week. Each slice represents the proportion of one allele in the isolates. Blue slices represent the alleles only present at week 1. Blue slices represent the alleles only present at week 1. Black and white slices represent the alleles that were present at all three sampling timepoints. Green slices represent the allele only present at week 11. Red slices represent the alleles present at both weeks 4 and 11. N: Number of alleles per week, MOI: Multiplicity of the infection.

III.1.6. Asymptomatic malaria and blood haemoglobin levels

Blood haemoglobin levels were generally within the normal range in both the asymptomatic and non-infected groups, and the median haemoglobin level was significantly higher in the asymptomatic compared to the symptomatic group (P = 0.0182) (Figure 17A). Linear regression showed a significantly increased in haemoglobin levels with age in both asymptomatic (P < 0.0001, coef= 0.0737) and symptomatic individuals (P= 0.029, coef= 0.0716) (Figure 17B). In contract to the observed correlation with age, blood haemoglobin levels decreased with increased parasite densities in asymptomatic participants (P< 0.0001, r= -0.0310), but not in symptomatic ones (P= 0.094, r= -0.0312) (Figure 17C). Multivariate linear regression analysis, including parasitaemia and age as predictor variables showed that parasite densities and age independently predict the haemoglobin levels in asymptomatic persons (P= 0.003, coef= -0.0199 and P= 0.001, coef = 0.0478 for parasitaemia and age, respectively), indicating an intricate link between parasitaemia, age, and peripheral blood haemoglobin levels during asymptomatic malaria infections. However, age, but not parasitaemia, was an independent predictor of the haemoglobin levels in symptomatic individuals (P= 0.023, coef= 0.1162 and P= 0.898, coef= -0.0026, for age and parasitaemia, respectively). These data indicate that predictors of haemoglobin levels may vary depending on the clinical status of the infection.

To determine the effect of persistent asymptomatic parasitaemia on the carrier's health, blood haemoglobin levels were measured in long-term asymptomatic individuals at different time points during the infection follow-up period and after antimalarial treatment. Median haemoglobin levels in persistent asymptomatic parasite carriers decreased significantly from 12.5 g/dL to 11.5 g/dL after 3

weeks of follow-up (p = 0.0017), and remained stable until week 11 of follow-up (11.6 g/dL), but significantly increased after antimalarial treatment to 12.45 g/dL (P= 0.0051) (Figure 17D). These findings show the effect of asymptomatic infections on carrier's health.

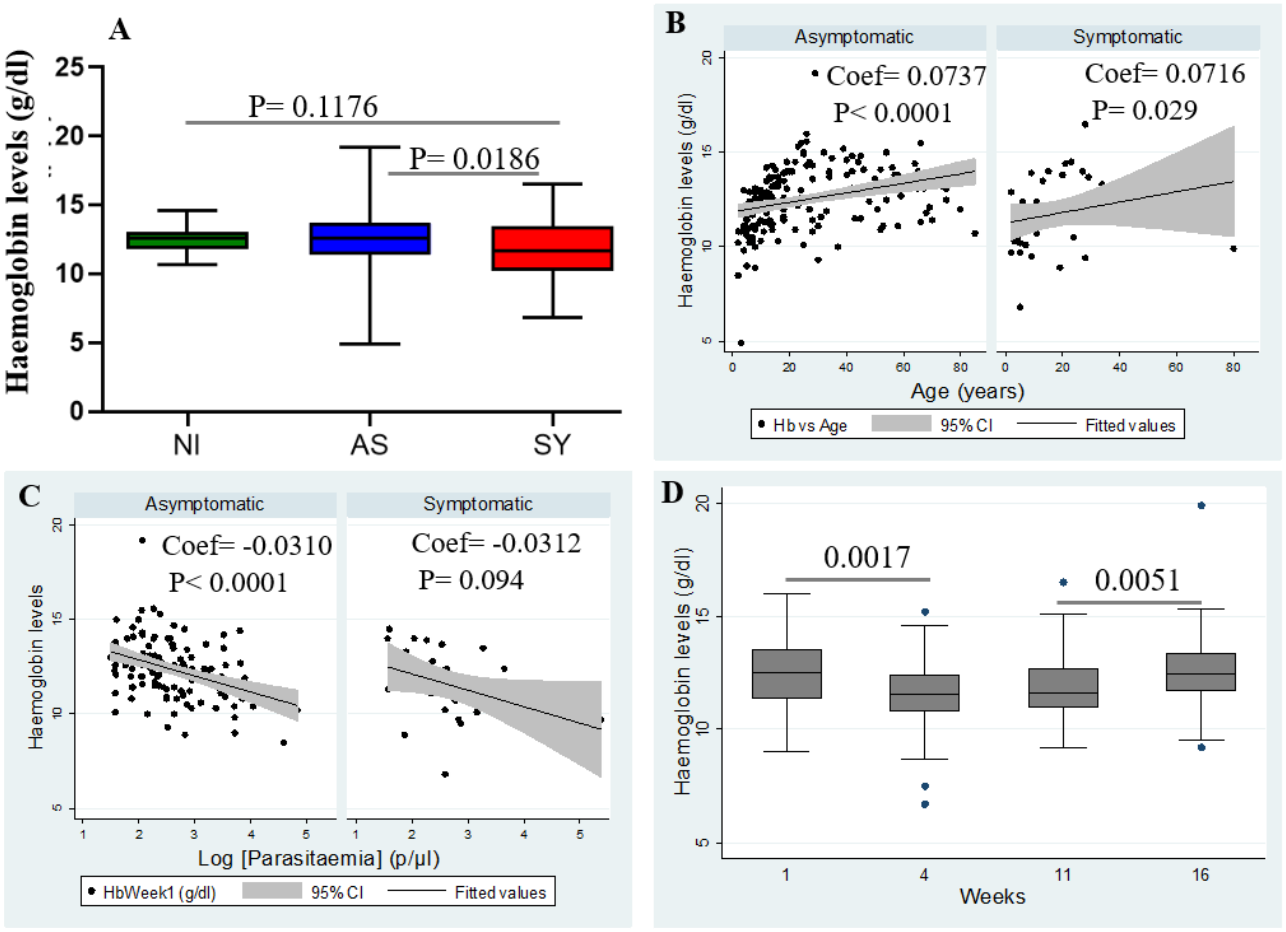

Figure 17: Blood haemoglobin levels and asymptomatic malaria.

A) Median haemoglobin levels between study participants. B) Correlation between haemoglobin levels and age in the infected groups. C) Correlation between haemoglobin levels and parasitaemia in the infected groups. D) Median haemoglobin levels over time during the follow-up period. Data on A&D are presented in boxplot representing interquartile ranges (25th, median and 75th percentile, whisker percentile (1st and 99th)) of haemoglobin levels. Median haemoglobin levels were compared between groups using Wilcoxon ran-sum test. B&C are represented as scatterplot of haemoglobin levels and age or parasitaemia in AS or SY individuals. Correlations were performed using Spearman correlation test and presented as best of fit line with 95% confidence intervals. AS: Asymptomatic, SY: Symptomatic, NI: Non-infected.

III.1.7. Risk factors of asymptomatic *Plasmodium* parasite carriage in the study population

To identify risk factors associated with asymptomatic malaria parasitaemia in the study area, both univariate and multivariate logistic analyses were performed using the asymptomatic group as the independent variable and only infected individuals were considered for the analysis. Factors investigated included those related to host susceptibility to febrile malaria (age, gender, health status, and use of medicinal plant), parasite factors (blood parasitaemia and MOI), as well as levels of exposure to the infection (bed net or insecticide use, duration in the study area, type of houses,

household size, presence of stagnant water, and/or thick vegetation in the vicinity). As shown in Table V, submicroscopic or low parasitaemia, not having a fever in the preceding three months, length of stay in the study area > 10 years and living in houses with less than 5 inhabitants increased the odds of being asymptomatic for malaria parasitaemia. Indeed, individuals with submicroscopic or low parasitaemia (<median parasitaemia in the study population) had more than twice the risk of carrying asymptomatic parasitaemia compared to those with high parasitaemia (OD= 2.109, P= 0.018 and OD= 2.285, P= 0.010 for submicroscopic and low parasitaemia, respectively). Not having a fever in the preceding three months or length of stay in the study area > 10 years increased the odds of having asymptomatic malarial parasitaemia (OD= 4.172, P< 0.0001 and OD= 1.009, P= 0.028 for fever and length of stay, respectively). Participants living in a household of less than 5 inhabitants had a three times higher risk of having asymptomatic infections than those living in a household of 6 to 9 inhabitants (OD= 3.491, P= 0.008).

Multivariate analyses of all associated factors from univariate analysis (ie with a P value < 0.05) revealed only the absence of fever in the preceding three months and low parasite densities as independently associated factors of asymptomatic malarial parasitaemia (Table V). Taken together, these data suggest that increased exposure to *Plasmodium* infection leading to the control of the parasite load may explain the establishment of asymptomatic parasitaemia in the study area.

Table V: Assessment of risk factors of asymptomatic malaria infection in highly exposed individuals

Predictive factors	Category	Asymptomatic, n* (%)	Univariate		Multivariate	
			OR [95% CI]	P value[1]	OR [95% CI]	P value[2]
Gender	Male	125 (81.2)	1			
	Female	141 (81.0)	1.008 [0.624 - 1.630]	0.971		
Age groups (years)	Children (<14 years)	117 (77.0)	1			
	Adults (> 14 years)	149 (84.7)	1.651 [0.968 - 2.815]	0.066		
Parasite density	**Submicroscopic**	**84 (84.8)**	**2.109 [1.136 - 3.915]**	**0.018**	**1.495 [1.022 - 2.186]**	**0.030**
	Low (< median)	**91 (85.8)**	**2.285 [1.222 - 4.271]**	**0.010**		
	High(≥ median)	77 (72.6)	1			
MOI	1	104 (76.5)	1			
	≥ 2	95 (83.3)	1.538 [0.818 - 2.895]	0.182		
Anaemia	Yes	63 (77.8)	1			
	No	117 (86.7)	1.857 [1.838 - 4.117]	**0.**127		
History of fever in the past 3 months	Yes	57 (63.3)	1			
	No	**209 (87.8)**	**4.172 [2.224 - 7.826]**	**< 0.0001**	**3.603 [1.954 - 6.644]**	**<0.0001**
Duration in the area (years)	< 2	31 (75.6)	1			
	2-10	93 (76.9)	1.071 [0.550 - 2.088]	0.839	1.033 [0.866 - 2.043]	0.193
	> 10	**137 (86.2)**	**1.009 [1.079 – 3.739]**	**0.028**		
Bed net or insecticide use	Yes	138 (77.9)	1			
	No	128 (84.8)	1.573 [0.822 – 2.007]	0.171		
Medicinal plants use	Yes	54 (78.3)	1			
	No	212 (81.8)	1.253 [0.718 - 2.186]	0.427		
Household size	**< 5**	**74 (91.4)**	**3.491 [1.382 - 8.822]**	**0.008**	1.239 [0.864 - 1.776]	0.244
	5-9	109 (75.2)	1	_		
	> 9	83 (81.4)	1.443 [0.647 - 2.216]	0.370		
House with crevices	Yes	62 (76.5)	1.454 [1.103 - 3.321]	0.292		
	No	204 (82.6)	1			
Stagnant water around the house	Yes	59 (75.6)	1			
	No	207 (82.8)	1.550 [0.748 - 2.214]	0.239		
Vegetation around of the house	Clean	4 (66.7)	1			
	Savannah	48 (81.4)	1.182 [0.742 - 6.413]	0.156		
	Forest	214 (81.4)	1.183 [0.979 - 4.872]	0.056		

*n refers to the number of cases, whereas the proportion of the asymptomatic in each category (eg male or female) is represented as percentages in parenthesis. Only infected participants (asymptomatic ad symptomatic) were included in this analysis. OR: odds ratio, CI: confidence interval. P value[1] was determined in a univariate logistic regression model. P value[2] was determined in the multivariate logistic regression model from variables with P[1] values < 0.5 in the univariate analysis

III.1.8. Risk factors of persistent asymptomatic malarial parasitaemia

To determine whether host susceptibility factors to febrile malaria, parasitic factors, as well as levels of exposure to infection may predict long-term asymptomatic carriage of the *Plasmodium* parasite, univariate and multivariate analyses were performed using the proportion of included asymptomatic participants who remained asymptomatically infected during the 10 weeks of follow-up as the independent variable. Of the 266 asymptomatic participants who were enrolled in the study, 143 (53.8%) completed the 11 weeks' follow-up study (Figure 11) and were considered for determination of the associated risk factors of persistent asymptomatic infections. Of these, 78 (54.5%) remained asymptomatically infected during the follow-up, indicating the evidence of long-term asymptomatic *Plasmodium* parasite carriage in this study area. However, none of the potential risk factors analysed, including age, gender, health status, use of herbal medicine, blood parasitaemia, MOI, bed net or insecticide use, duration in the study area, type of houses, household size, presence of stagnant water, and/or thick vegetation in the vicinity were associated with the persistent asymptomatic parasitaemia (Table VI). Taken together, these data indicate the need to study other host factors such as immune or genetic factors, as well as other parasite factors that may explain this persistent asymptomatic parasitaemia.

Table VI: Identification of risk factors of persistent symptomatic infections

Predictive factors	Category	Persistent cases [n (%)]	Univariate		Multivariate	
			OR [95% CI])	P value[1]	OR [95% CI]	P value[2]
Gender	Male	34 (59.6)	1.377 [0.699 - 2.716]	0.355		
	Female	44 (51.8)	1			
Age groups (years)	Children (<14 years)	46 (56.8)	1.191 [0.611 - 2.322]	0.608		
	Adults (> 14 years)	32 (52.5)	1			
Parasite density	Submicroscopic	25 (59.5)	1.348 [0.579 - 3.139]	0.489		
	Low (< median)	24 (52.2)	1			
	High(≥ median)	25 (53.2)	1.042 [0.461 - 2.351]	0.922		
MOI	1	29 (55.8)	1.418 [0.653 - 3.081]	0.377		
	≥ 2	24 (47.1)	1			
Anaemia	Yes	20 (52.6)	1			
	No	38 (64.4)	1.629 [0.710 - 3.736]	0.250		
History of fever in the past 3 months	Yes	13 (41.9)	1			
	No	65 (58.6)	1.956 [0.873 - 3.386]	0.103		
Duration in the area (years)	< 2	6 (50.0)	1			
	2-10	29 (50.9)	1.036 [0.298 - 3.597]	0.956		
	> 10	42 (59.1)	1.448 [0.425 – 3.938	0.554		
Bed net or insecticide use	Yes	38 (52.0)	1			
	No	40 (58.0)	1.270 [0.655 – 2.465]	0.479		
Medicinal plants use	Yes	10 (40.0)	1			
	No	68 (58.1)	2.082 [0.863 - 5.021]	0.103		
Household size	< 5	16 (51.2)	1.231 [0.510 - 2.969]	0.644		
	5-9	32 (50.0)	1	-		
	> 9	30 (61.2)	1.579 [0.742 - 3.360]	0.236		
House with crevices	Yes	66 (58.4)	1.989 [0.869 - 4.554]	0.104		
	No	12 (41.4)	1			
Stagnant water around the house	Yes	16 (50.0)	1			
	No	62 (56.4)	1.292 [0.587 - 2.843]	0.525		
Vegetation around of the house	Clean	1 (100.0)	-			
	Savannah	17 (63.7)	1.365 [0.587 - 3.174]	0.470		
	Forest	60 (53.1)	1			

*n refers to the number of persistent cases amongst asymptomatic individuals, whereas the proportion of the persistent in each category (eg male or female) is represented as percentages in parenthesis. OR odds ratio, CI confidence interval. P value[1] was determined in a univariate logistic regression model. P value[2] was determined in the multivariate logistic regression model from variables with P values < 0.5 in the univariate analysis.

III.1.9. Risk factors of early clinical conversion from asymptomatic to symptomatic infections

It has been shown that, the known durations for pre-patent infections in semi-immune individuals range from 1 to 3 weeks depending on age (Pinkevych *et al.*, 2014; Slater *et al.*, 2019). To determine the risk factors of early clinical conversion, individuals who reported having a fever during the first 3 weeks of follow-up were considered as the outcome. Of the 156 participants with known asymptomatic malaria status in the week 4, 24 (15.4%) reported development of a malaria-associated fever in the first 3 weeks of the follow-up indicating early conversion to symptomatic malaria. Univariate analyses identified gender and blood haemoglobin levels as associated risk factors of early conversion from asymptomatic to symptomatic infection (Table VII). Indeed, being a female or being classified as asymptomatic malarial anaemic at the initial enrolment into the study increased the odds of early conversion from asymptomatic to symptomatic infection by more than 3-fold. Furthermore, both female gender and anaemia were independently associated with early conversion from the asymptomatic to symptomatic infections revealed from multivariate analyses (Table VII). Median blood haemoglobin levels at enrolment were significantly lower among the converters than in the persistent asymptomatic non-febrile participants (P=0.0036). In this population, the proportion of female with anaemia was significantly higher than the proportion of male with anaemia (48.4 vs 27.3, P= 0.001). In addition, median haemoglobin levels were lower in female compared to male, both for converters and non-converters (P = 0.0473 and P = 0.0231 for converters and non-converters, respectively), indicating that the association between female gender and early clinical conversion may be indirectly related to the anaemia occured in women. Together the above findings suggest strong association between blood haemoglobin levels and febrile disease during asymptomatic *Plasmodium* infection.

Table VII: Identification of associated risk factors of clinical conversion

Predictive factors	Category	Converters, n* (%)	Univariate		Multivariate	
			OR [95% CI])	P value[1]	OR [95% CI]	P value[2]
Gender	Male	5 (7.6)	1			
	Female	**19 (21.1)**	**3.265 [1.141 - 9.341]**	**0.027**	**2.123 [1.092 -4.413]**	**0.027**
Age groups (years)	Children (≤14 years)	12 (14.5)	1	-		
	Adults (> 14 years)	12 (16.4)	1.164 [0.437 – 3.100]	0.761		
Parasite density	Submicroscopic	7 (15.6)	1.381 [0.517 - 3.695]	0.520		
	Low (< median)	6 (11.8)	1			
	High(≥ median)	9 (17.3)	1.570 [0.480 - 5.130]	0.445		
MOI	1	10 (16.2)	1			
	≥ 2	10 (18.5)	2.368 [0.604 - 9.288]	0.216		
Anaemia	**Yes**	**11 (27.5)**	**3.730 [1.326 - 10.491]**	**0.013**	**3.253 [1.117 - 9.474]**	**0.031**
	No	6 (9.2)	1			
History of fever in the past 3 months	Yes	6 (19.4)	1.427 [0.536 - 3.794]	0.476		
	No	18 (14.4)	1			
Duration in the area (years)	< 2	2 (13.3)	1			
	2-10	8 (14.4)	1.061 [0.195 - 5.762]	0.945		
	> 10	13 (15.8)	1.225 [0.253 - 5.930]	0.801		
Bed net or insecticide use	Yes	13 (17.8)	1.294 [0.500 - 3.349]	0.595		
	No	11 (13.2)	1			
Medicinal plants use	Yes	6 (23.1)	1.867 [0.615 - 5.663]	0.270		
	No	18 (13.8)	1			
Household size	< 5	6 (17.6)	1.309 [0.374 - 4.590]	0.677		
	5-9	9 (14.1)	1	-		
	> 9	9 (15.5)	1.122 [0.441 - 2.858]	0.809		
House with crevices	Yes	20 (15.4)	1			
	No	4 (15.4)	1.0 [0.323 - 3.965]	1.0		
Stagnant water around the house	Yes	6 (16.7)	1.133 [0.303 - 3.186]	0.812		
	No	18 (15.0)	1			
Vegetation around of the house	Clean	0 (0.0)	-			
	Savannah	3 (9.1)	1	-		
	Forest	21 (17.4)	2.1 [0.665 - 6.630]	0.206		

OR odds ratio, CI confidence interval. P value[1] was determined in a univariate logistic regression model. P value[2] was determined in the multivariate logistic regression model from variables with P values < 0.5 in the univariate analysis.

III.2. Assessessment of the contribution of host antibody responses to the maintenance of asymptomatic parasitaemia in highly exposed individuals

III.2.1. Demographic and parasitological characteristics of the study population

In this part of the study, which aimed to determine the association between antibody responses and persistent asymptomatic parasitaemia, 216 samples (out of 353 samples) were included and distributed as follows: 13 non-infected, 142 asymptomatic with 69 long-term and 63 short-term asymptomatic, and 61 symptomatic. These samples were selected based on information available during the 11-week infection follow-up period.

All the demographic and parasitological characteristics of the studied population are presented in Table VIII. Sex ratio, median age and median haemoglobin levels were comparable between study groups. Although non-significant, the median parasitaemia was almost 2-fold lower in the asymptomatic groups compared to the symptomatic group.

Table VIII: Demographic and parasitological characteristics of the study population

Variables	NI (13)	LT (69)	ST (63)	SY (61)	p value
Sex ratio (M:F)	0.3 (3:10)	0.86 (32:37)	0.54 (22:41)	0.90 (29:32)	0.6196
Median age (years)	56.5 (4-68)	12 (4–75)	13 (2–86)	11 (2–80)	0.1391
Median Hb (g/dl)	12.1 (10.7-14)	12.4 (9-16)	12 (8.5-15)	11.8 (6.8-16.6)	0.4919
Parasitaemia (parasites/µl)	-	112.7 (10-28253.7)	142.6 (10-68190.5)	269.2 (10-240457.1)	0.49798

NI: Non-infected, ST: Short-term asymptomatic, LT: Long-term asymptomatic, SY: Symptomatic. Individuals with submicroscopic parasitaemia were assigned a value of 10 parasite/µl relative to the limit of detection of light microscopy.

III.2.2. Seroprevalence and correlation between anti-plasmodial antibody levels in the study population

The Figure 18 shows a high prevalence of antibodies against all *P. falciparum* antigens tested ranging from 54.6% for MSP-1 to 85.6% for MSP-4. This high prevalence of responders against the different antigens incated the hogh transmission of malaria in this area.

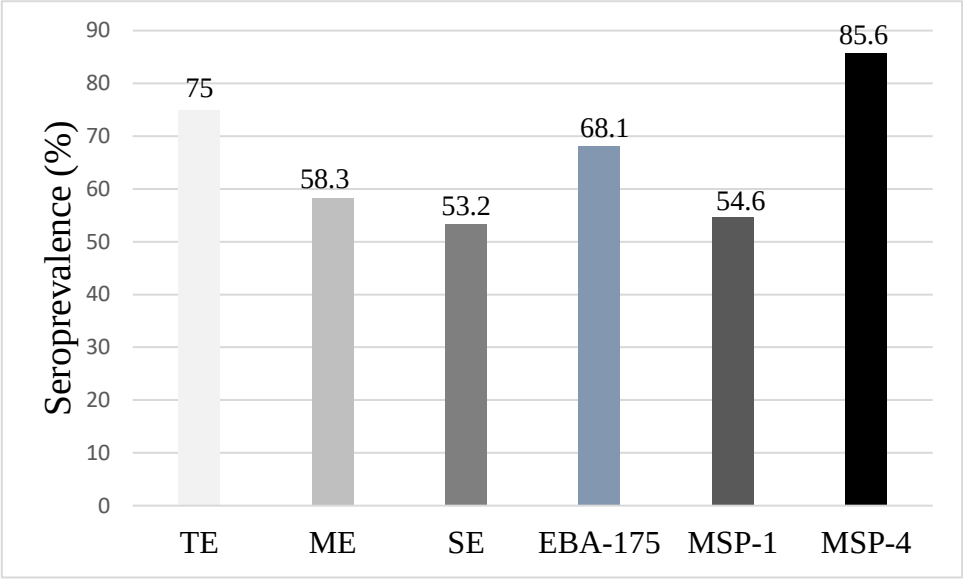

Figure 18: Prevalence of antibody responses to P. *falciparum* ***antigens***.

A sample was considered positive if IgG levels (OD ratio) were >2 i.e. OD of naive control + 2SD. TE: Total extract (mix stage parasite), ME: Merozoite extract, SE: Schizonte extract.

Table IX shows a strong correlation between the levels of anti-*Plasmodium* antibodies in this study population. The levels of antibody to crude extracts (TE, ME and SE) were the most positively correlated with each other (Spearman r> 0.6). However, anti-MSP-4 antibody levels were less negatively correlated with other antibody levels (Spearman r> -0.4).

Table IX: Correlation between anti-*P.* *falciparum* antibody levels

		TE	ME	SE	EBA-175	MSP-1	MSP-4
TE	r	1					
	P						
ME	r	0.68	1				
	P	<0.0001					
SE	r	0.70	0.91	1			
	P	<0.0001	<0.0001				
EBA-175	r	0.47	0.60	0.57	1		
	P	<0.0001	<0.0001	<0.0001			
MSP-1	r	0.59	0.65	0.66	0.44	1	
	P	<0.0001	<0.0001	<0.0001	<0.0001		
MSP-4	r	-0.05	-0.20	-0.15	-0.32	-0.12	1
	P	0.4623	0.0037	0.0296	<0.0001	0.0786	

TE: Total extract (mix stage parasite), ME: Merozoite extract, SE: Schizonte extract. r: Spearman correlation coefficient. P: P value

III.2.3. Anti-plasmodial antibody levels and age or parasitaemia in infected individuals

The effect of age and parasitaemia on the antibody levels in infected individuals was assessed by univariate and multivariate linear regression. Age was negatively correlated with parasitaemia (r= -0.4891, p< 0.0001), and had a significant effect on some antibody levels in infected individuals with

different patterns. Indeed, levels of antibody to TE, ME, SE and MSP-1 increased with age whereas anti-MSP-4 antibody levels decreased with age (Figure 19 & Table X).

As shown in Figure 20, levels of antibody to TE, ME, SE and MSP-1 were significantly higher in infected individuals with submicroscopic parasitaemia than in those with microscopic infection, whereas, anti-MSP-4 levels follow the opposite pattern. Likewise, univariate linear regression analysis showed that, levels of antibody to TE, ME and SE decreased with increased parasitaemia in individuals with microscopic parasitaemia, while anti-MSP-4 antibody levels increased with parasitaemia (Table IX). Multivariate analysis using age and parasitaemia as independent variables revealed that age independently predicted antibody levels to TE, SE and MSP-4, whereas, parasite densities independently predict antibody levels to TE, ME, SE and MSP-1. Taken together, these results show that antibody levels during *P. falciparum* infections are independendly predicted by age and parasite density.

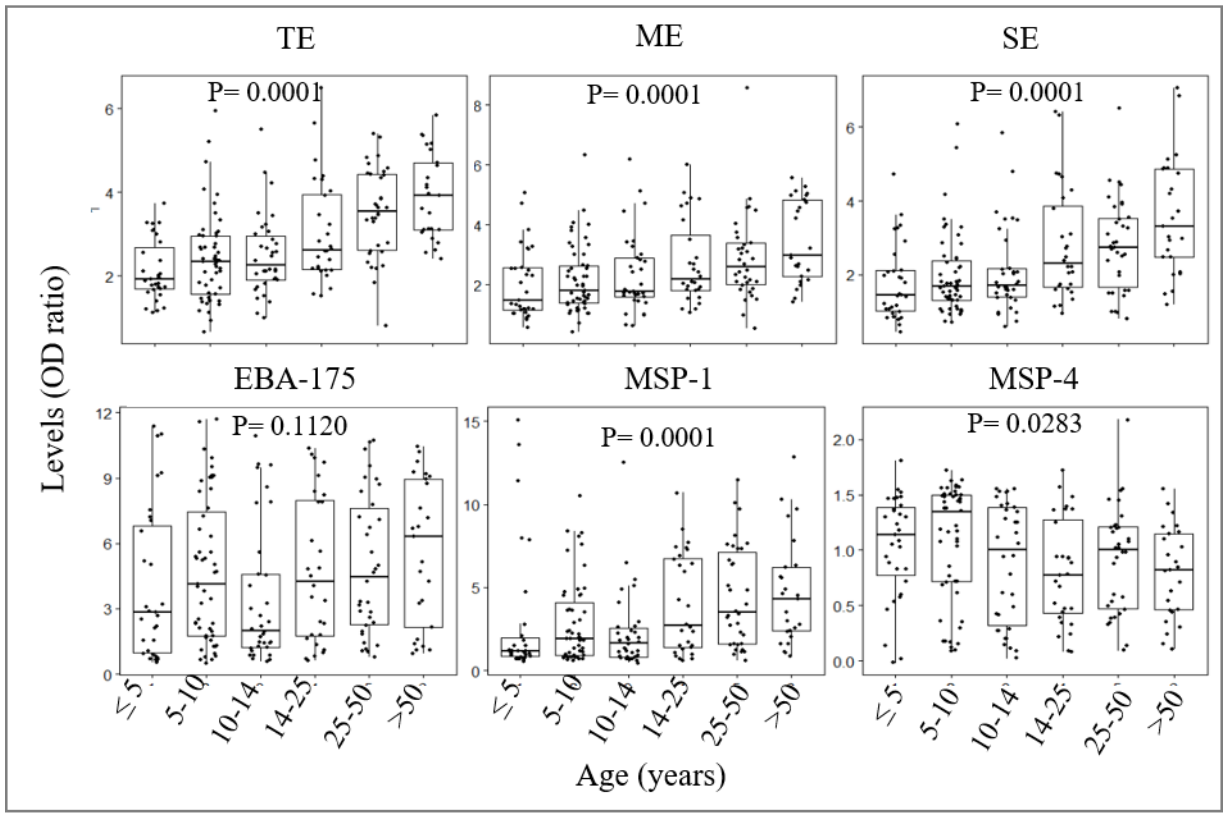

Figure 19: Difference in IgG levels against *Plasmodium* antigens in infected individuals according to age groups.

Box plots representing the median and interquartile range of IgG levels (OD ratio) measured in plasma of the included participants by ELISA. IgG levels between groups were compared by Kruskal Wallis test. TE: Total extract (mix stage parasite), ME: Merozoite extract, SE: Schizonte extract.

Table X: Effect of age and parasitaemia on anti-plasmodial antibody concentrations in infected individuals

Antibody to :	Univariate				Multivariate			
	Age		Parasitaemia		Age		Parasitaemia	
	Coefficient (CI)	P-value	Coefficient (CI)	P-value	Coefficient (CI)	P-value	Coefficient (CI)	P-value
Merozoite	1.151 (0.695, 1.607)	< **0.001**	-0.564 (-0.841, -0.288)	< **0.001**	0.734 (-0.022, 1.490)	0.057	-0.402 (-0.723, -0.081)	**0.014**
Schizonte	1.452 (1.007, 1.897)	< **0.001**	-0.614 (-0.879, -0.349)	< **0.001**	1.042 (0.329, 1.754)	**0.004**	-0.383 (-.069, -0.081)	**0.013**
TE	1.483 (1.117, 1.849)	< **0.001**	-0.636 (-0.861, -0.412)	< **0.001**	1.033 (0.436, 1.630)	**0.001**	-0.407 (-0.670 -0.154)	**0.002**
EBA-175	0.948 (-0.232, 2.128)	0.115	-0.935 (-1.639, -0.231)	**0.010**	-0.906 (-2.852, 1.041)	0.359	--1.136 (-1.96, -0.309)	**0.007**
MSP-1	1.866 (0.805, 2.927)	**0.001**	-0.779 (-1.410, -0.149)	**0.016**	1.292 (-0.442, 3.027)	0.143	-0.493 (-1.229, 0.243)	0.187
MSP-4	-1.866 (-2.132, -0.437)	**0.003**	0.755 (0.245, 1.264)	**0.004**	-1.602 (-2.987, -0.217)	**0.024**	0.400 (-0.188, 0.988)	0.181

The effect of age and parasite density on anti-plasmodial antibody levels was assessed through univariable and multivariable linear regressions for each antibody, with antibody concentration as outcome (pg/mL) and age or parasitaemia as the predictor variables. CI: confident interval. P< 0.05 are in bold.

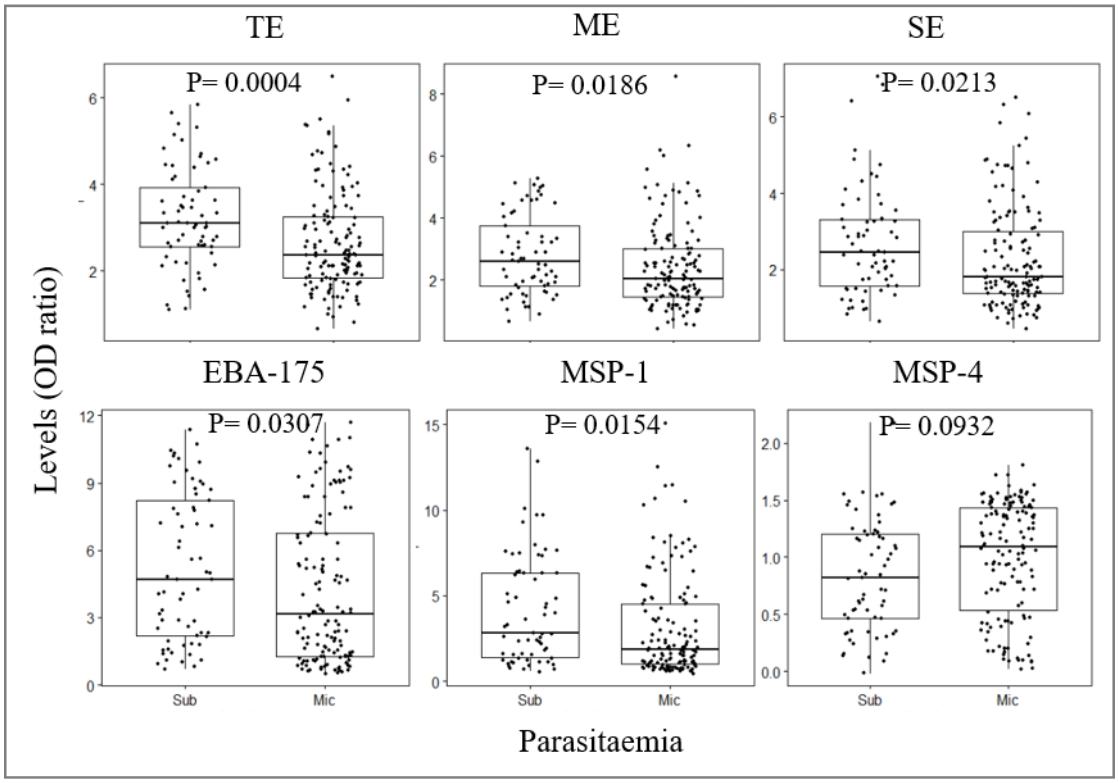

Figure 20: Difference in IgG levels between infected individuals with submicroscopic and microscopic parasitaemia.

Box plots representing the median and interquartile range of IgG levels (OD ratio) measured in plasma of the infected participants by ELISA. IgG levels between groups were compared by Wilcoxon ran-sum test. Sub: Submicroscopic; Mic: Microscopic.

III.2.4. Relationship between antiplasmodial antibody levels and asymptomatic malarial parasitaemia

To investigate the contribution of antibody levels in the protection against clinical conversion from asymptomatic to the symptomatic infections, plasma antiplasmodial antibody levels were compared between individuals with long-term asymptomatic, short-term asymptomatic, symptomatic malaria or not infected. As shown in Figure 21, no significant differences in median plasma antibody levels were observed between the infected groups, indicating that persistent asymptomatic parasitaemia may be independent of plasma levels of anti-plasmodial antibodies in highly exposed individuals. No significant differences in antiplasmodial antibody levels were observed between the non-infected and infected groups ($P > 0.05$), suggesting equivalent levels of exposure to the infection in the study population. Likewise, when the LT and ST groups were combined as an asymptomatic group, no significant difference was observed between the asymptomatic and symptomatic or uninfected groups, suggesting that antibody levels may not play a role in the establishment of asymptomatic parasitaemia.

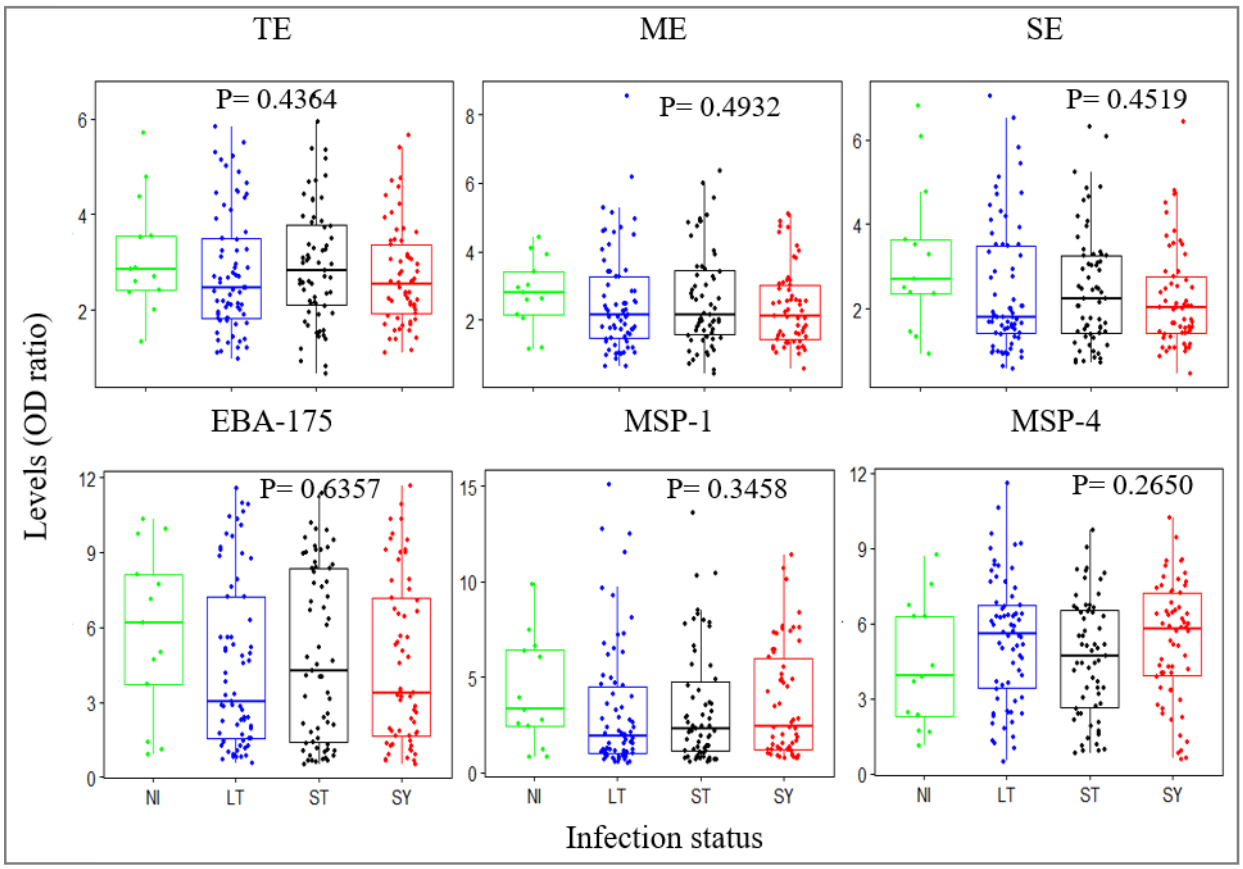

Figure 21: Levels of IgG levels against *Plasmodium* antigens between the study groups.

Box plots representing the median and interquartile range of IgG levels measured by ELISA in plasma of the different study groups at the time of enrolment. IgG levels (OD ratio) between groups were compared by Kruskal Wallis test. TE: Total extract (mix stage parasite), ME: Merozoite extract, SE: Schizonte extract. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic.

III.2.5. Antiplasmodial antibody avidity and asymptomatic malarial parasitaemia

To determine whether the affinity of antibodies may play a role in protection against clinical malaria, antibodies avidity (strength of the antibody to bind to the target antigen) indexes were compared between asymptomatic and symptomatic groups or non-infected group. As observed with antibody levels, no significant difference in antibody avidity indexes were observed between the different groups (Figure 22), indicating that antibody qualities may not predict the protection against the clinical infection or absence of an infection.

For all antigens, avidity indices were significantly correlated with corresponding antibody levels (r= 0.2904, P= 0.0002; r= 0.7286, P< 0.0001; r= 0.4920, P< 0.0001 and r= 0.6469, P< 0.0001 for TE, EBA-175, MSP-1 and MSP-4 respectively), indicating that antibody levels may influence the antibody affinity. However, only the avidity of antibodies against MSP-4 correlated with age (r= 0.1549, P= 0.0490; r= 0.0293, P= 0.7240; r= -0.0270, P= 0.7715 and r= -0.3017, P< 0.0001 for TE, EBA-175, MSP-1 and MSP-4 respectively). Similarly, only the avidity of antibodies against MSP-4

was positively correlated with parasitaemia (r= -0.0172, P= 0.8715; r= -0.1046, P= 0.3435; r= -0.1904, P= 0.1317 and r= 0.2407, P= 0.0092 for TE, EBA-175, MSP-1 and MSP-4 respectively).

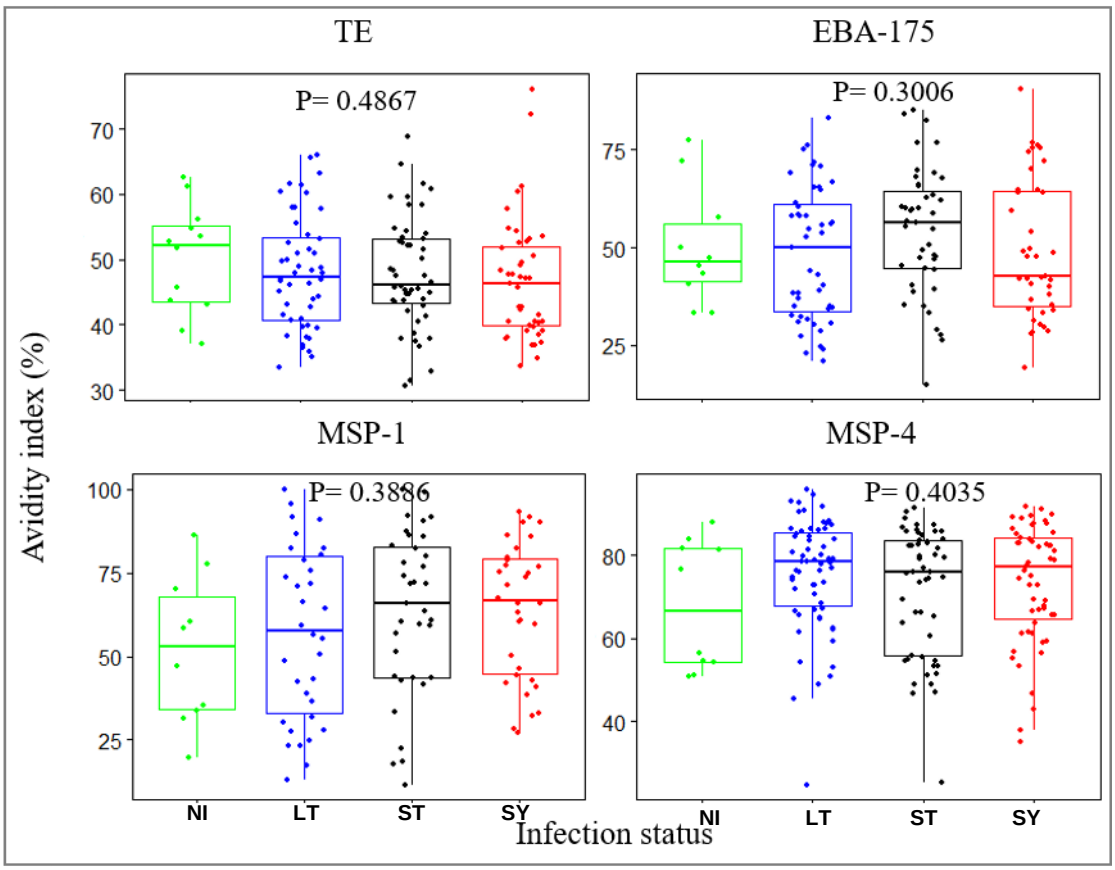

Figure 22: Plasma antiplasmodial antibody avidity indexes in the different study groups.

Box plots representing the median and interquartile range of IgG avidity indexes measured by ELISA in plasma of the different study groups at the time of enrolment. IgG avidity index levels (%) between groups were compared by Kruskal Wallis test. TE: Total extract (mix stage parasite). TE: total extract. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic.

III.2.6. Antiplasmodial antibody isotypes and asymptomatic parasitaemia

Figure 23A shows no significant difference in all IgG antibody subclass levels (Ig1, IgG2, IgG3 and IgG4) between infection groups, indicating that the composition of antibodies against the total protein extract may not play a role in the establishment and maintenance of asymptomatic parasitaemia. In addition, the cytophilic ratio which is the ratio of the level of cytophilic antibody to the level of non-cytophilic antibody (Ig1 + IgG3) / (IgG2 + IgG4) was also comparable between the study groups (Figure 23B).

With the exception of the IgG4 subclass, all levels of the other IgG subclasses (IgG 1, 2 and 3) as well as the cytophilic ratio were positively correlated with the total IgG antibody levels against the total protein extract (r= 0.4324, P< 0.0001; r= 0.4450, P< 0.0001; r= 0.5551, P< 0.0001, r= 0.1259, P= 0.0668 and r= 0.1825, P= 0.0072 for IgG1, Ig2, IgG3, IgG4 and cytophilic ratio, respectively).

Likewise, except IgG4 subclass levels, all anti-TE IgG antibody subclass levels were positively correlated with IgG antibody avidity against TE (r= 0.3802, P< 0.0001; r= 0.1574, P= 0.0455; r= 0.0128, P= 0.0128, r= 1275, P= 0.1058 and r= 0.2479, p= 0.0015 for IgG1, Ig2, IgG3, IgG4 and cytophilic ratio, respectively).

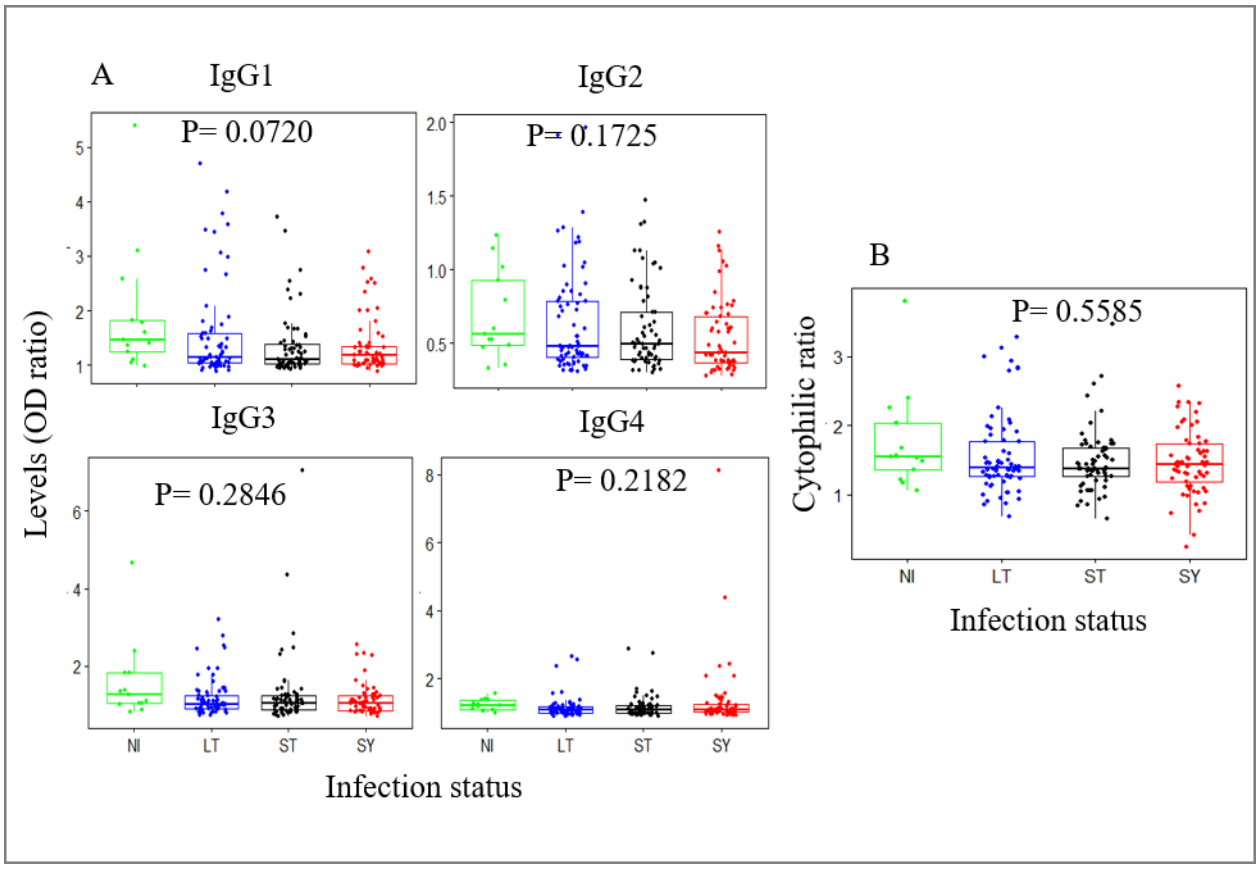

Figure 23: IgG subclass levels and cytophilic ratio in the study groups.

Box plots representing the median and interquartile range of IgG subclass levels or cytophilic ratio measured by ELISA. IgG levels (%) or cytophilic ratio between groups were compared by Kruskal Wallis test. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic. Cytophilic ratios were calculated as the sum of the IgG1 and IgG3 levels divided by the sum of the IgG2 and IgG4 levels.

III.2.7. Parasite growth inhibition activity and asymptomatic parasitaemia

The median *P. falciparum* growth inhibition index was significantly higher in the LT compared to ST asymptomatic (P= 0.011) and SY individuals (P= 0.001) (Figure 24), suggesting that antibody activity may play a role in protection against the development of the clinical diseases. In addition, the median *P. falciparum* growth inhibition index was significantly higher in the asymptomatic group at enrolment compared to symptomatic subjects (P= 0.044). No significant difference was observed in growth inhibition indices between the asymptomatic and non-infected individuals (Figure 24).

The *P. falciparum* growth inhibition index was negatively correlated with parasite densities (r= -0.2083, P= 0.0162), but not with age (r= 0.0610, P= 0.3722). The growth inhibition index was positively correlated with levels of anti-ME antibody (r = 0.1553, P = 0.0224), but not with levels of antibodies against other antigens (SE, TE, EBA-175, MSP-1 and MSP-4). In addition, the growth inhibition index was positively correlated with the levels of the anti-TE IgG3 antibody subclass (r = 0.2831, P< 0.0001), as well as with the cytophilic ratio (r = 0.2151, P= 0.0015).

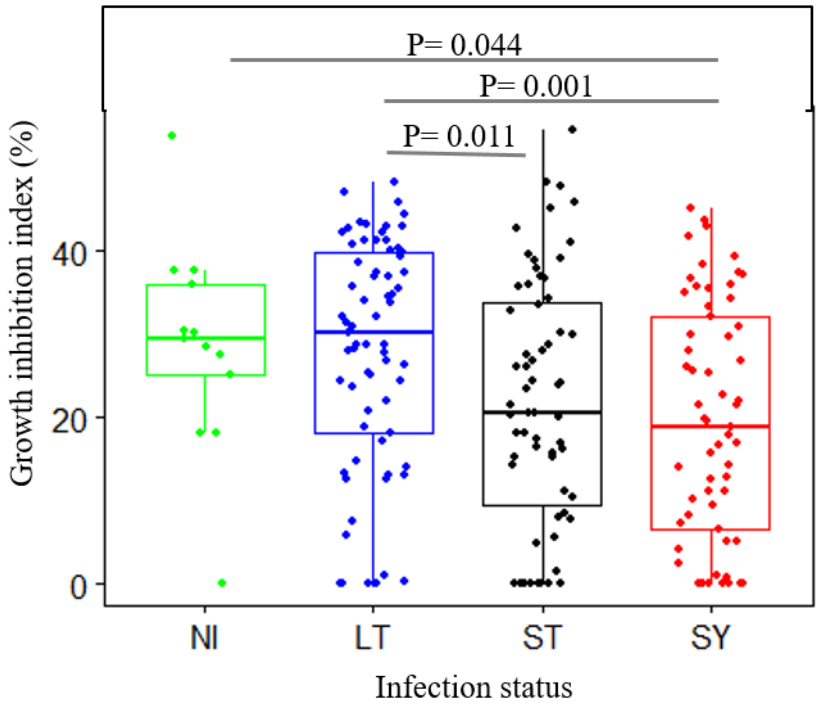

Figure 24: *P. falciparum* growth inhibition levels in the different study groups.

Box plots representing the median and interquartile range of parasite growth inhibition index. Growth inhibition index (%) were compared between groups by Wilcoxon ran-sum test. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic.

III.2.8. Profile of antibody levels and avidity during persistent asymptomatic parasitaemia and after antimalarial treatment

To determine the kinetic profiles of antibody responses during long-term asymptomatic parasite carriage in highly exposed individuals, anti-plasmodial antibody levels or avidity were monitored over time in individuals with persistent asymptomatic infections and after treatment. As shown in Figure 25, with the exception of anti-merozoite antibodies whose levels increased from week 4 to week 11, no significant differences were observed in median antibody levels during the two-month infection follow-up period, despite significant fluctuations in median parasitaemias. However, antibody avidity to EBA-175 and total extract increased over time during persistent asymptomatic infection (Figure 26). Antimalarial treatment at week 11 post-participant enrolment resulted in a significant decline in the levels of total extract (P= 0.0150), but not in antibody levels against ME and

SE as well as the candidate vaccine antigens EBA-175, MSP-1c19 and MSP-4p20 (p> 0.05) (Figure 25). Similarly, antibody avidity to TE and EBA-175 decreased significantly following antimalarial treatment (p= 0.0054 and p= 0.0068 for TE and EBA-175, respectively) (Figure 26). Together, these data suggest that persistent parasitaemia does not affect antibody levels, but may promote the production of high-affinity antibodies at the expense of low-affinity antibodies by B cells. Furthermore, persistent parasitaemia is crucial in maintaining higher levels or high affinity antiplasmodial antibodies in untreated asymptomatic parasite carriers.

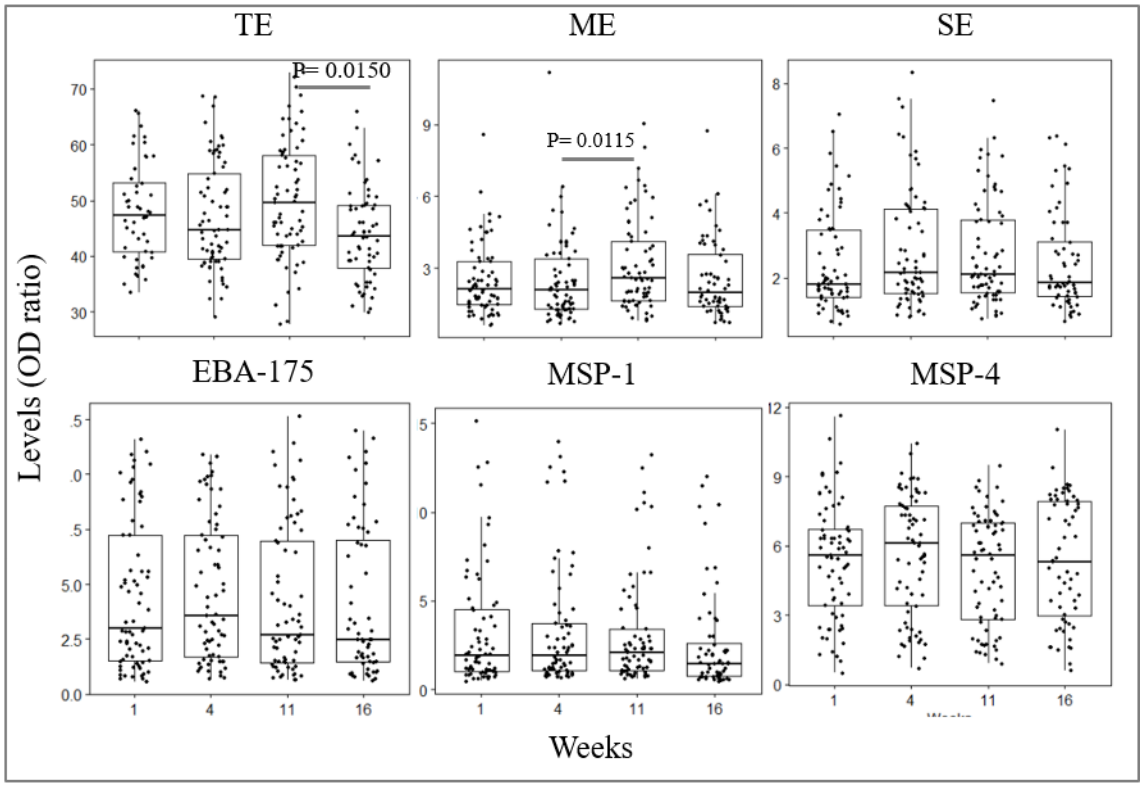

Figure 25: Kinetics of antiplasmodial antibody levels over time and following anti-malarial treatment.

Box plots representing the median and interquartile range of IgG levels measured by ELISA. IgG levels were compared between sampling time points by Wilcoxon ran-sum test. Weeks 1, 4 and 11 represent the sampling time before antimalarial treatment, while week 16 represents the sampling time following antimalarial treatment at week 11.

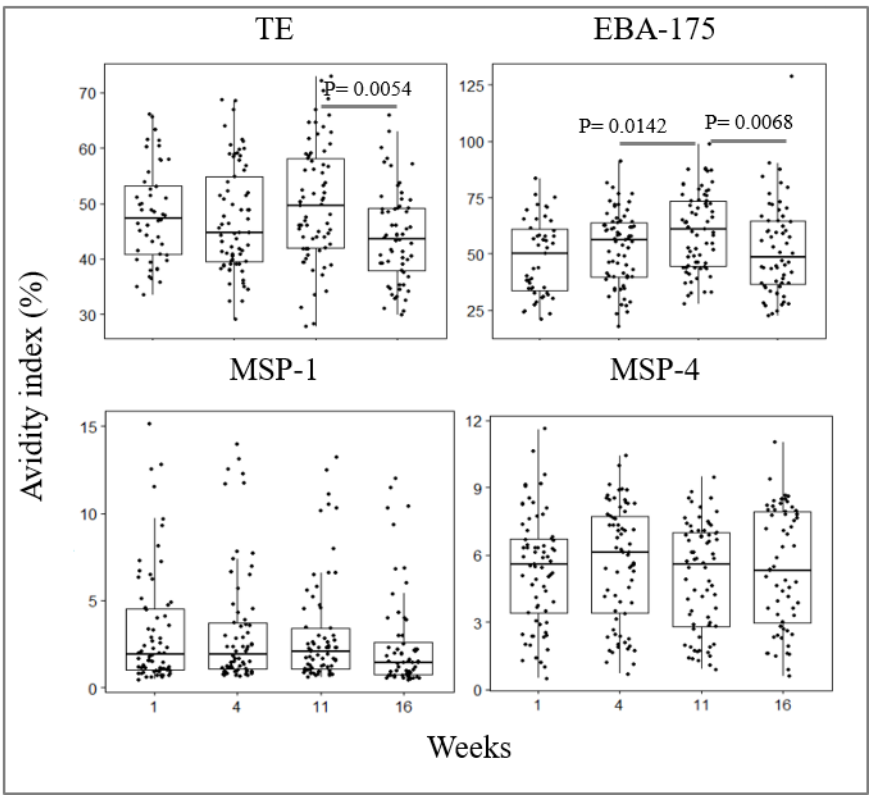

Figure 26: Kinetics of antiplasmodial antibody avidity over time and following anti-malarial treatment.

Box plots representing the median and interquartile range of IgG antibody avidity indexes measured by ELISA. Median IgG antibody avidity indexes were compared between sampling time points by Wilcoxon ran-sum test. Weeks 1, 4 and 11 represent the sampling time before antimalarial treatment, while week 16 represents the sampling time following antimalarial treatment at week 11. TE: Total extract (mix stage). TE: Total extract (mix staged parasites).

III.3. Identification of potential cellular immune biomarkers of persistent asymptomatic parasitaemia in highly exposed individuals

In this part of the study, the same study population that was used to determine the association between antibody responses and asymptomatic parasitaemia (see section III.2) was used, which included 216 samples (out of 353 samples) distributed as follows: 13 non-infected, 142 asymptomatic with 69 long-term and 63 short-term asymptomatic, and 61 symptomatic.

III.3.1. Association between cytokine, chemokine or growth factors and asymptomatic malarial parasitaemia

In order to assess the association between both pro-inflammatory and anti-inflammatory biomarkers with asymptomatic malaria or persistent asymptomatic parasitaemia, the levels of 38 biomarkers including cytokines, chemokines and growth factors were quantified by Luminex technology and compared between the different study groups (NI, AS, LT, ST and SY). Of 38 cytokines/chemokines/growth factors analysed (Figures 27 and 28), plasma levels of IL-8, IL-10, IL-1RA and MCP-1 were significantly different between the infected groups. Indeed, Plasma levels of the pro-inflammatory chemokine IL-8 were significantly higher in LT asymptomatic compared to the ST asymptomatic individuals ($P= 0.003$) (Figure 27). However, levels of IL-10, IL-1RA and MCP-1 were significantly lower in the LT asymptomatic compared to symptomatic participants ($P< 0.05$), but comparable between LT and ST asymptomatic individuals (Figure 27). Furthermore, levels of IL-10 and MCP-1 were significantly higher in infected groups (LT, ST and SY) compared to non-infected group ($P< 0.05$) (Figure 27). The above findings suggest the involvement of the pro-inflammatory chemokine IL-8 in the protection against the development of febrile *Plasmodium* infection, but the involvement of the anti-inflammatory cytokines IL-10 and IL-1RA in clinical disease.

Overall, IL-10 anti-inflammatory/pro-inflammatory cytokine ratios (IL-10 to TNF-α, TNF-β, IFN-γ and IL-6) were higher in the ST asymptomatic group compared to both the LT asymptomatic and non-infected groups (Figure 29). Furthermore, as observed with IL-10 and IL-1RA levels, IL-10/pro-inflammatory and IL-1RA/IL-1β ratios were highest in symptomatic malaria groups compared to the other study groups (Figure 29).

Otherwise, when combining LT and ST groups as asymptomatic groups, levels of both pro-inflammatory markers (IL-6 and MCP-1) and anti-inflammatory cytokines (IL-10 and IL-1RA) were significantly higher in individuals with symptomatic malaria compared to those with asymptomatic infections ($p< 0.05$) (Figure 30). Additionally, anti-/pro-inflammatory cytokine ratios (IL-10 to TNF-α, TNF-β, IFN-γ and IL-6; IL-1RA to IL-1β) were significantly higher in the symptomatic group

compared to the asymptomatic group (Figure 31), supporting the implication of anti-inflammatory cytokines IL-10 and IL-1RA in clinical malaria in the study population.

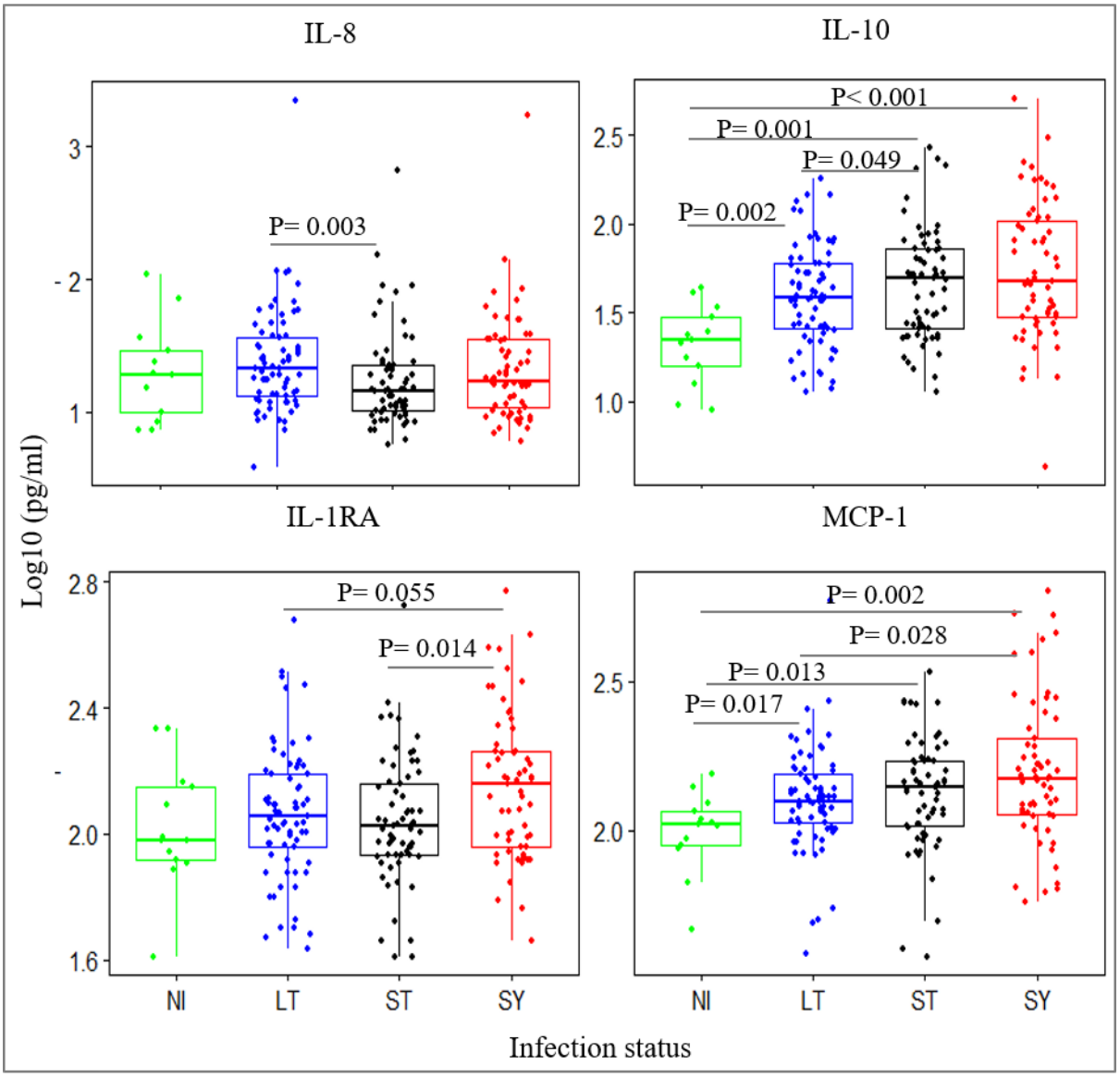

Figure 27: Plasma levels of cytokine in the different study groups.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Median cytokine levels were compared between groups using Wilcoxon ran-sum test. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic. Here we have presented the graphs that show significant differences between the infected groups and the rest are shown in Figure 28.

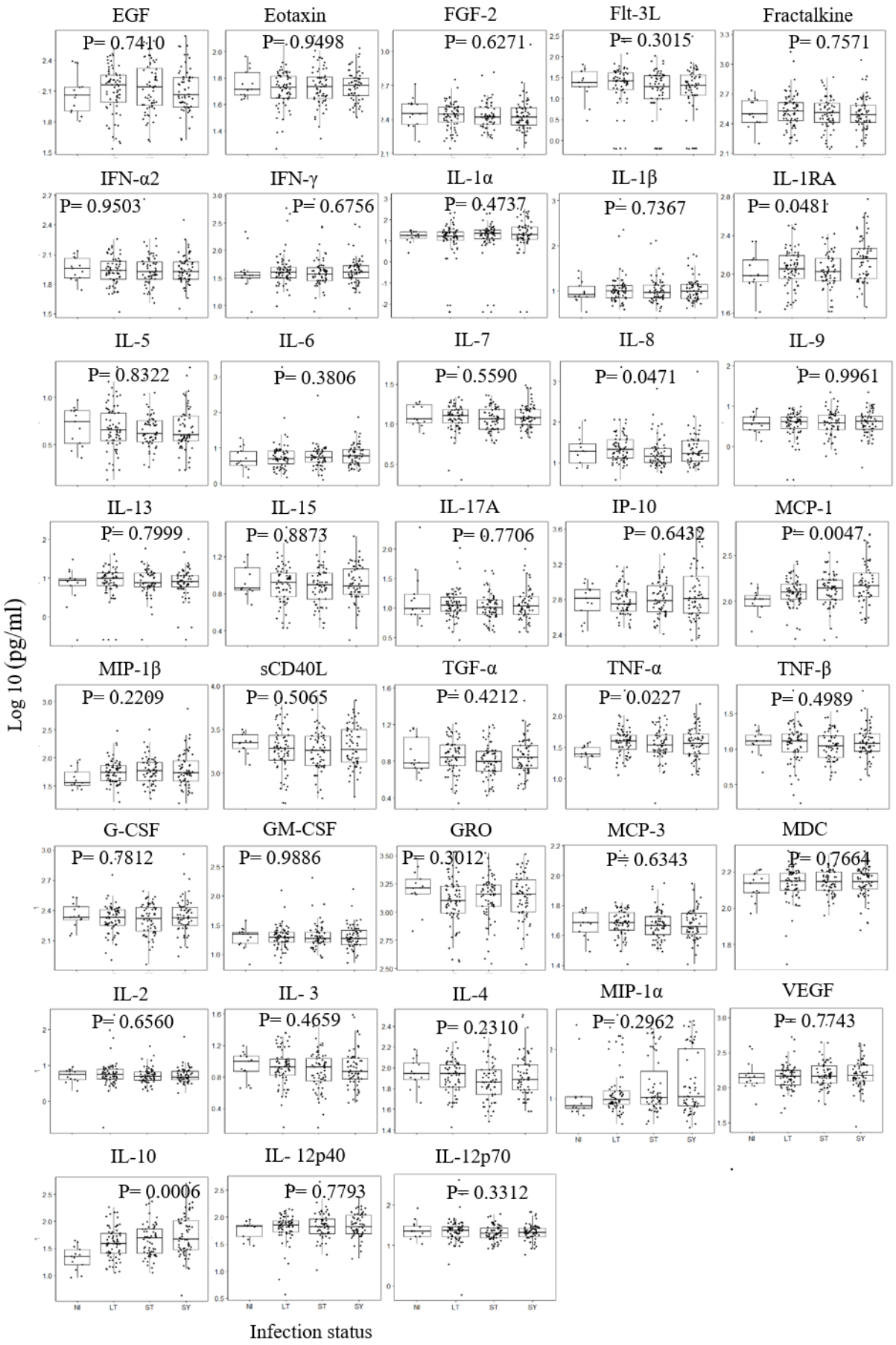

Figure 28: Plasma levels of cytokine, chemokine and growth factors between study groups.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Median cytokine levels were compared between groups using Kruskal-Wallis test. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic.

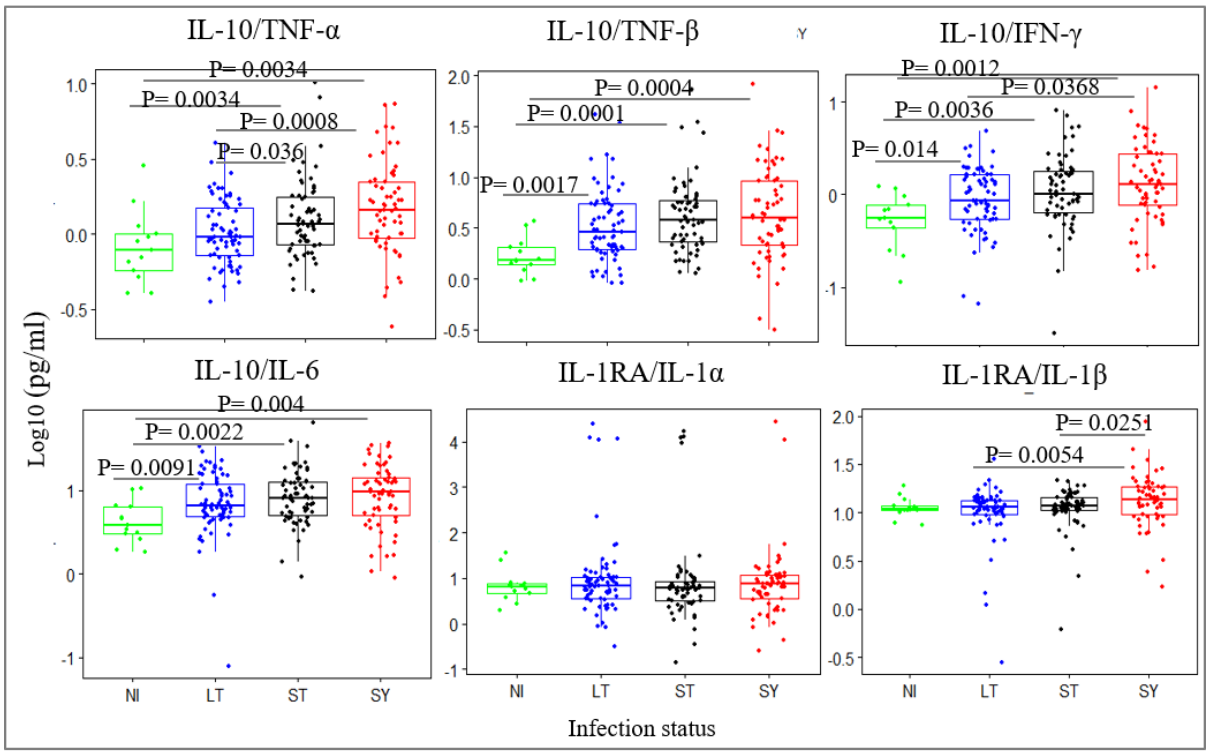

Figure 29: Anti-/pro-inflammatory cytokine ratios in the different study groups.

Box plots representing the median and interquartile range of anti-/pro-inflammatory cytokine ratios. Median anti-/pro-inflammatory cytokine ratios levels were compared between groups using Wilcoxon ran-sum test. NI: non-infected, LT: long-term asymptomatic, ST: short-term asymptomatic, SY: symptomatic.

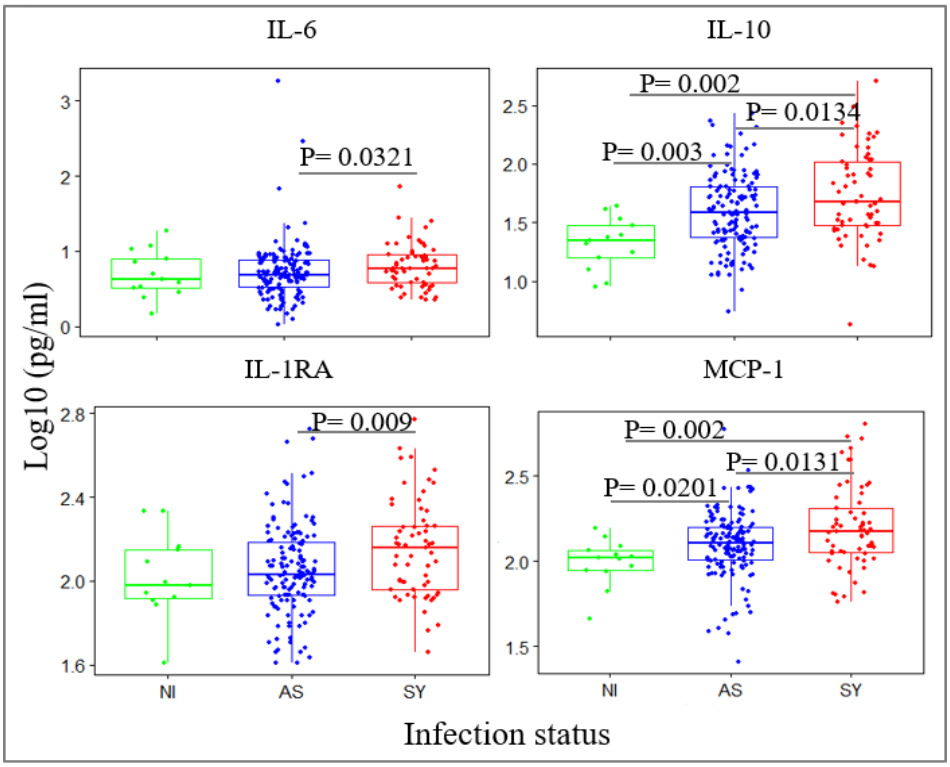

Figure 30: Circulating cytokine levels in the different infection groups.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Median cytokine levels were compared between groups using Wilcoxon ran-sum test. NI: non-infected, AS: asymptomatic, SY: symptomatic. Here we have presented the graphs that show significant differences between the infected groups.

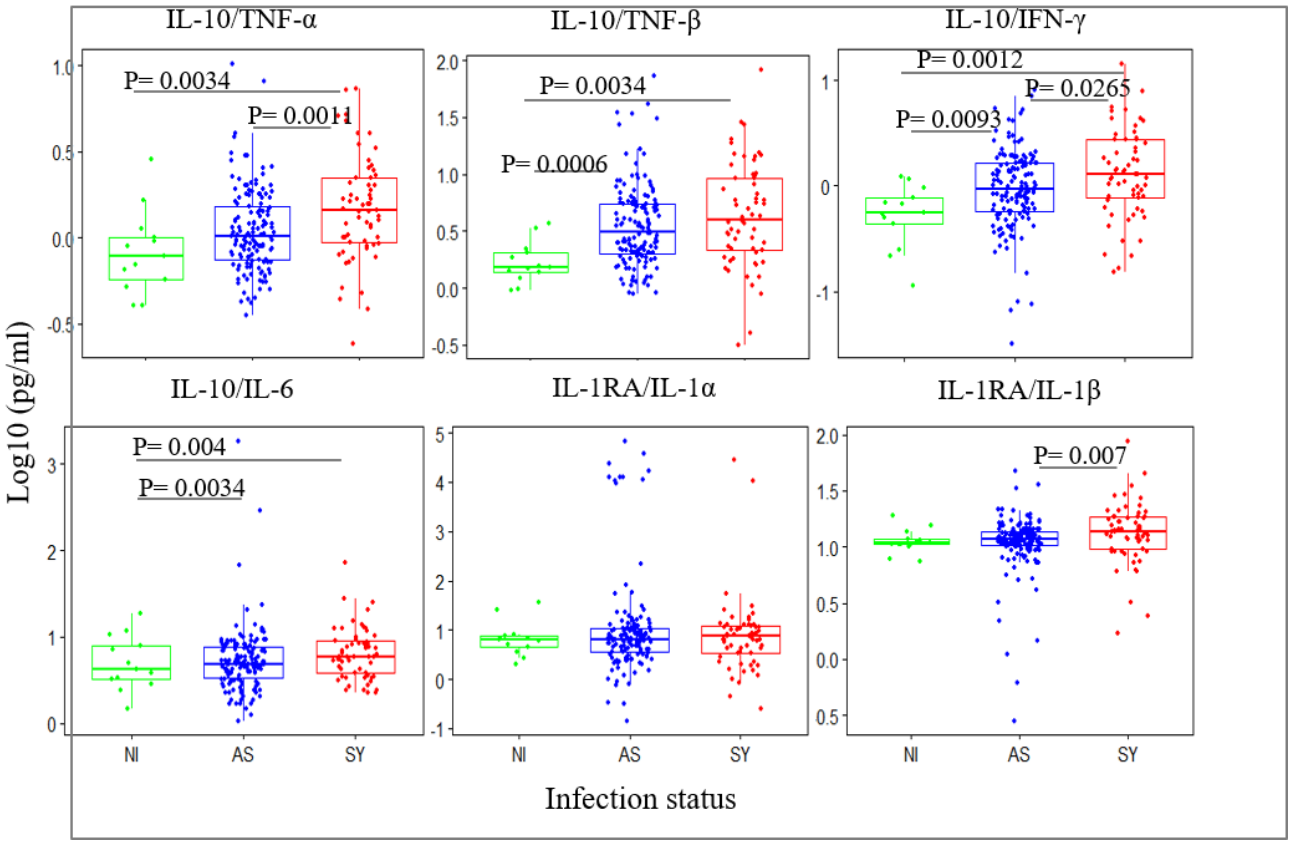

Figure 31: Anti-/pro-inflammatory cytokine ratios according to infection status.

Box plots representing the median and interquartile range of anti-/pro-inflammatory cytokine ratios. Median anti-/pro-inflammatory cytokine ratios levels were compared between groups using Wilcoxon ran-sum test. NI: non-infected, AS: asymptomatic, SY: symptomatic.

III.3.2. Profile of cytokine responses during persistent asymptomatic parasitaemia and after antimalarial treatment

In order to determine the kinetic of cytokine profiles during long-term asymptomatic parasite carriage in highly exposed individuals, plasma cytokine, chemokine and growth factors were monitored over the 16-week asymptomatic infection follow-up period and after treatment. Consistent with the fluctuating parasite densities over time, significant changes were observed in several pro-and anti-inflammatory cytokine levels over the 10-week follow-up period, with four different patterns (Figure 32 & 33):

i) Median levels of some cell activation/recruitment markers such as sCD40L, GRO, IL-3 and EGF significantly increased over time during persistent asymptomatic parasitaemia, indicating the continuous stimulation of immune cells during long-term asymptomatic parasitaemia;

ii) No significant changes were observed in levels of the key regulatory cytokines/chemokines TNF-α, IL-10, IL-1RA, IP-10, MCP-1 and Eotaxin over time;

iii) levels of IL-12p40, TNF-β, IL-6, IL-17A, IL-7, IL-15, IL-9, IL-5, IL-13, Fractaline, MIP-1α, MIP-1β, and G-CSF increased during the first three weeks and remained stable until week 11;

iv) levels of IL-12p70, IFN-γ, IL-1β, IL-2, IL-4, IL-1α , IFN-α2, GM-CSF, IL-8, MCP-3, Flt-3L , FGF-2 and TGF-α increasing in the first three weeks and decreasing at the week 11.

As the main regulatory cytokine levels, anti-/pro-inflammatory cytokine ratios (IL-10 to TNF-α, TNF-β, IFN-γ and IL-6; IL-1RA to IL-1α and IL-1β) also remained stable throughout the 10-week follow-up period (Figure 32 and 33), indicating that a balanced anti/pro-inflammatory cytokine response may be crucial for the maintenance of persistent asymptomatic parasitaemia.

As shown in Figures 32 and 34, antimalarial therapy on week 11 post-enrolment resulted in a significant decline in the IL-10 anti-/pro-inflammatory cytokine ratios, as well as in the levels of IL-10 and MCP-1, indicating the importance of persistent parasitaemia in their continuous release. However, levels of Th2 regulatory cytokine IL-4 significantly increased after anti-malarial treatment. Together, these data indicate that a balanced anti/pro-inflammatory cytokine response may be crucial for the maintenance of asymptomatic parasitaemia, whereas, the antimalarial treatment of persistent parasitaemia may perturb established cellular immune responses in highly exposed individuals.

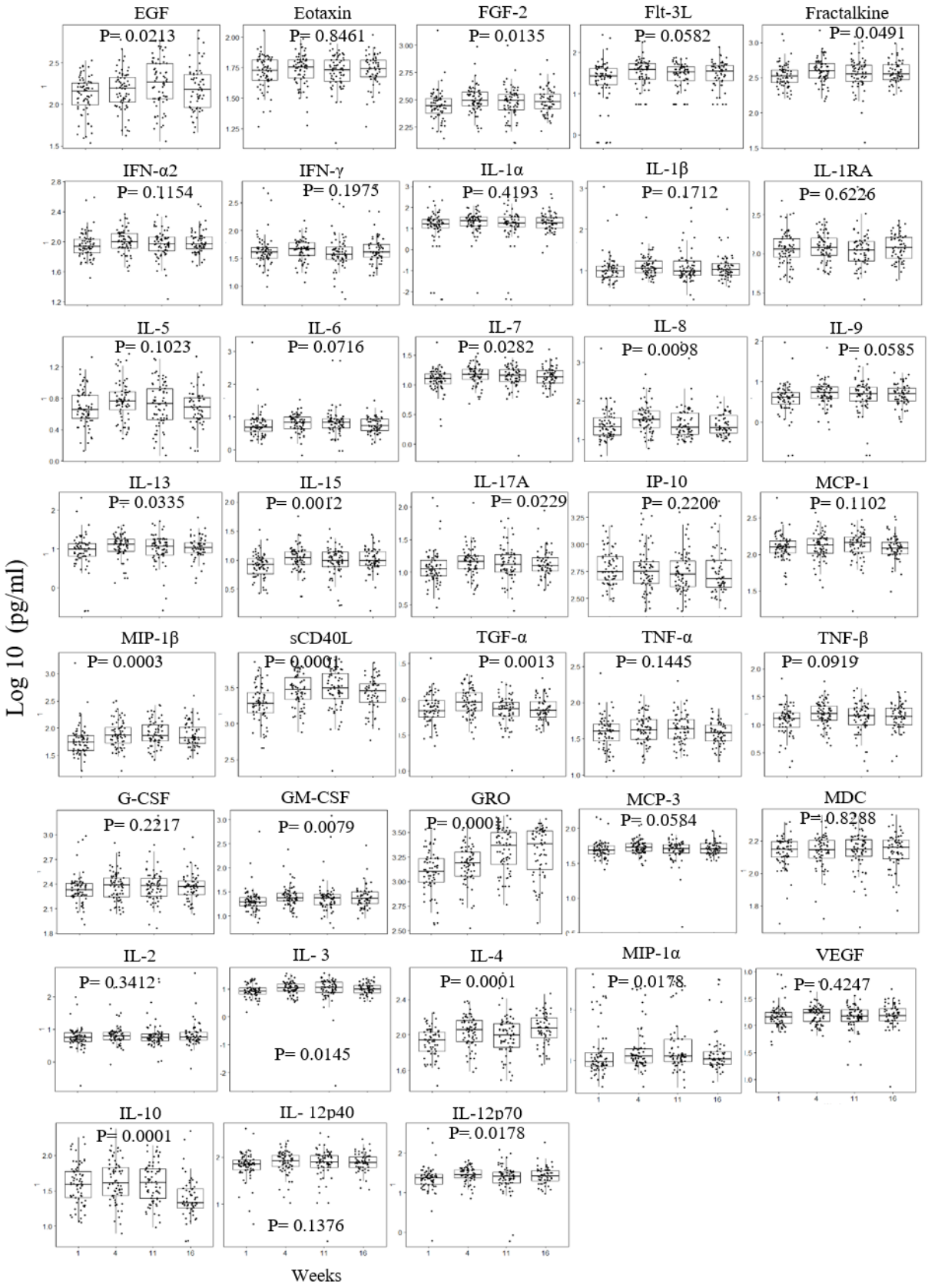

Figure 32: Kinetics of cytokine, chemokine and growth factors levels over time and following anti-malarial treatment.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Cytokine levels were compared between sampling time points by Kruskal-Wallis test. Weeks 1, 4 and 11 represent the sampling time before antimalarial treatment, while week 16 represents the sampling time following antimalarial treatment at week 11.

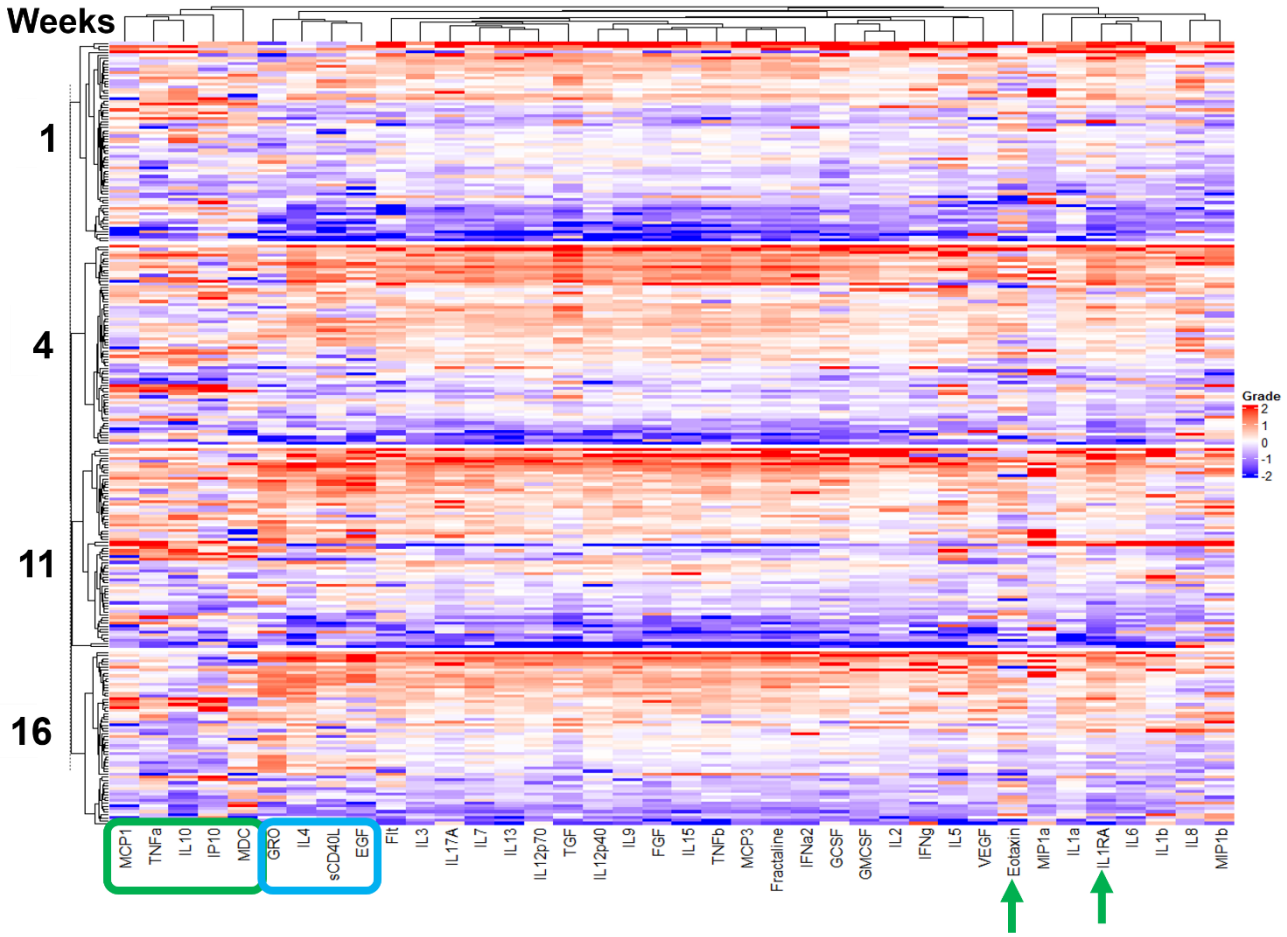

Figure 33: Heatmap of kinetics of cytokine levels over time and following anti-malarial treatment.

The heatmap representing the hierarchical clustering of the standardized least-square means of 38 differentially expressed cytokines/chemokines/growth factors across the four sampling time points during asymptomatic infection follow-up. Green box or ligne. The green box or arrow indicates the cytokines which levels remained stable during the 10 weeks of follow-up. The blue box indicates cytokines which levels increased significantly over time.

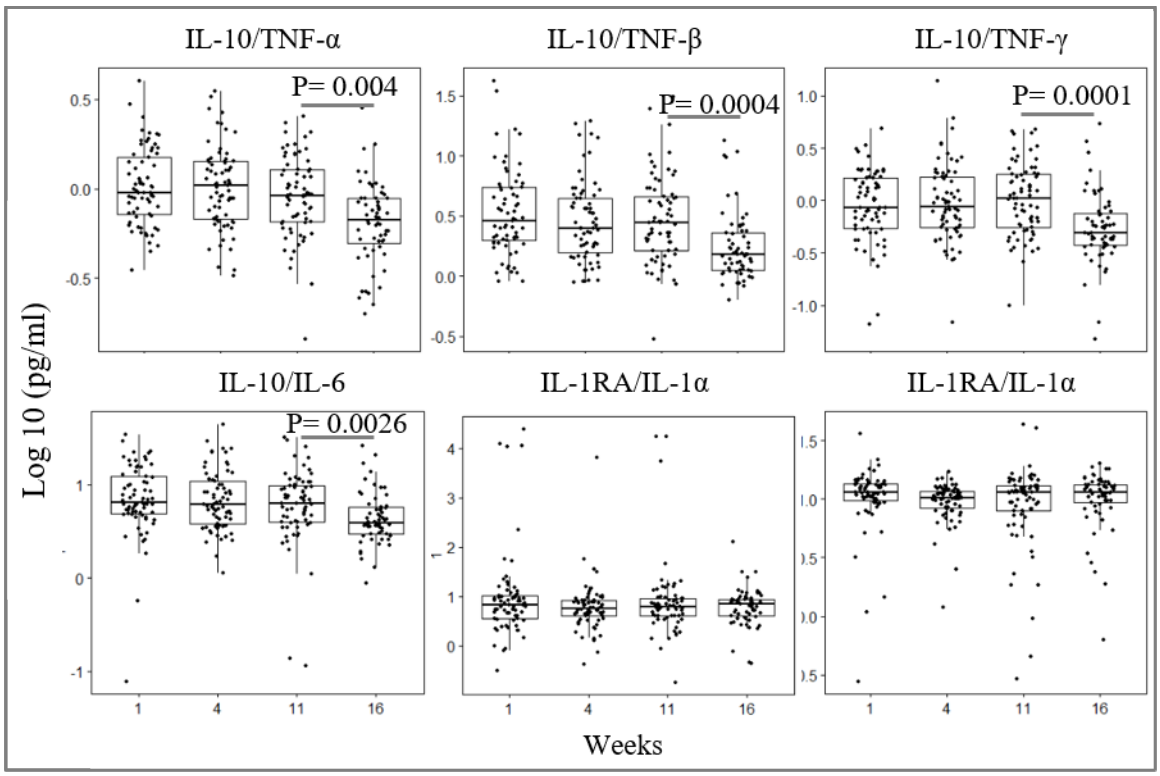

Figure 34: Kinetics of anti-/pro-inflammatory cytokine ratios levels over time and following anti-malarial treatment.

Box plots representing the median and interquartile range of cytokine ratio levels measured by Luminex technology. Cytokine ratios were compared between sampling time points by Wilcoxon ran-sun test. Weeks 1, 4 and 11 represent the sampling time before antimalarial treatment, while week 16 represents the sampling time following antimalarial treatment at week 11

III.3.3. Association between age or parasitaemia and plasma cytokine concentrations in infected individuals

The effect of age and parasitaemia on the cytokine, chemokine and growth factor responses in infected individuals was assessed by univariate and multivariate linear regression. Age had a statistically significant effect on the levels of some analyte in infected individuals. Univariate analysis showed that levels of some cytokines such as IL-10, TNF-α, IL-6, IL-12p70 and IL-1RA, chemokine, such as IL-8, MCP-1 and MIP-1β, and growth factors such as GRO, MDC, sCD40L, G-CSF and EGF significantly decreased with age (Figure 35 & Table XI). Although not significant, other biomarkers showed a decreasing trend with respect to age in infected individuals. These data suggest the possibility of a blunted cytokine response with age which could be associated to host tolerance due to repeated exposure.Among *P. falciparum*-infected subjects, parasite density had an effect on different biomarkers (Figure 36 & Table XI). As shown in Figure 34, levels of TNF-α, IL-10, IL-6, IP-10, MCP-1 and MI-1β were significantly higher in participants with microscopic parasitaemia than in those with submicroscopic infection. Furthermore, linear regression analysis showed increased levels of IL-10,

TNF-α, IL-6, IL-1RA, IP-10, MCP-1 and MIP-1β, but decreased GRO levels with increased parasitaemia in individuals with microscopic parasitaemia (Table XI).

Multivariate analysis using age and parasitaemia as the independent variables in individuals with microscopic parasitaemia showed that age independently predicted the levels of TNF-α, IFN-γ, IL-9, IL-8, GM-CSF, MDC and sCD40L (Table XI). Levels of cytokines including, IL-10, TNF-α, IFN-γ, IL-1RA, IP-10, MCP-1, GM-CSF and GRO were independently predicted by parasite densities (Table XI). Using an FAMD on cytokine data along with age and circulating parasite density, we found that levels of TNF-α, IL-10, MCP-1, and IP-10 along with age and parasite density explained most of the variation in the peripheral cytokine response in asymptomatic individuals (Figure 37). Taken together, these findings suggest that plasma cytokine levels during *Plasmodium* infection are predicted by age and parasite densities.

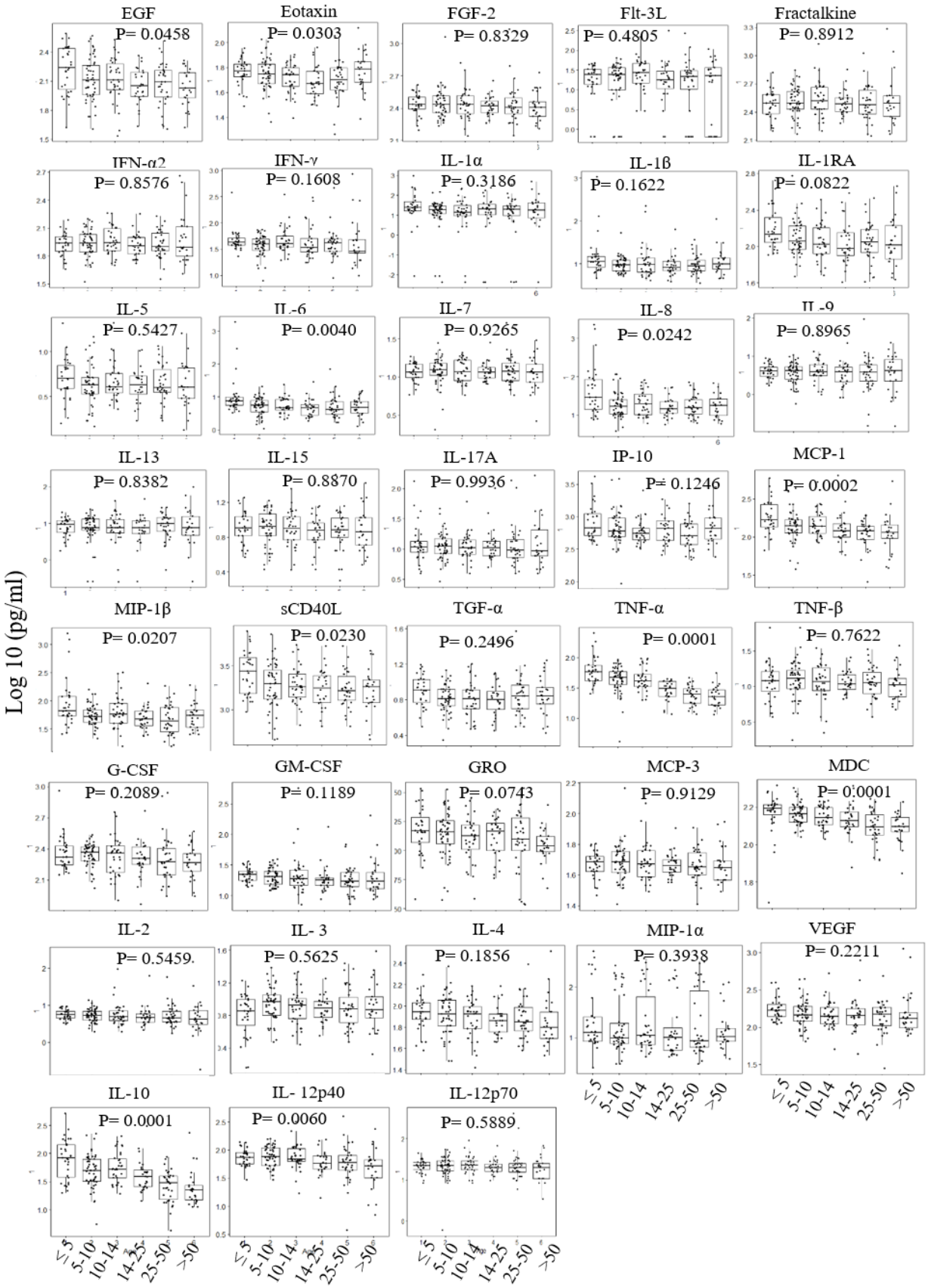

Figure 35: Plasma cytokine, chemokine and growth factors levels according to age groups in infected individuals.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Median cytokine levels were compared between age groups using Kruskal-Wallis test.

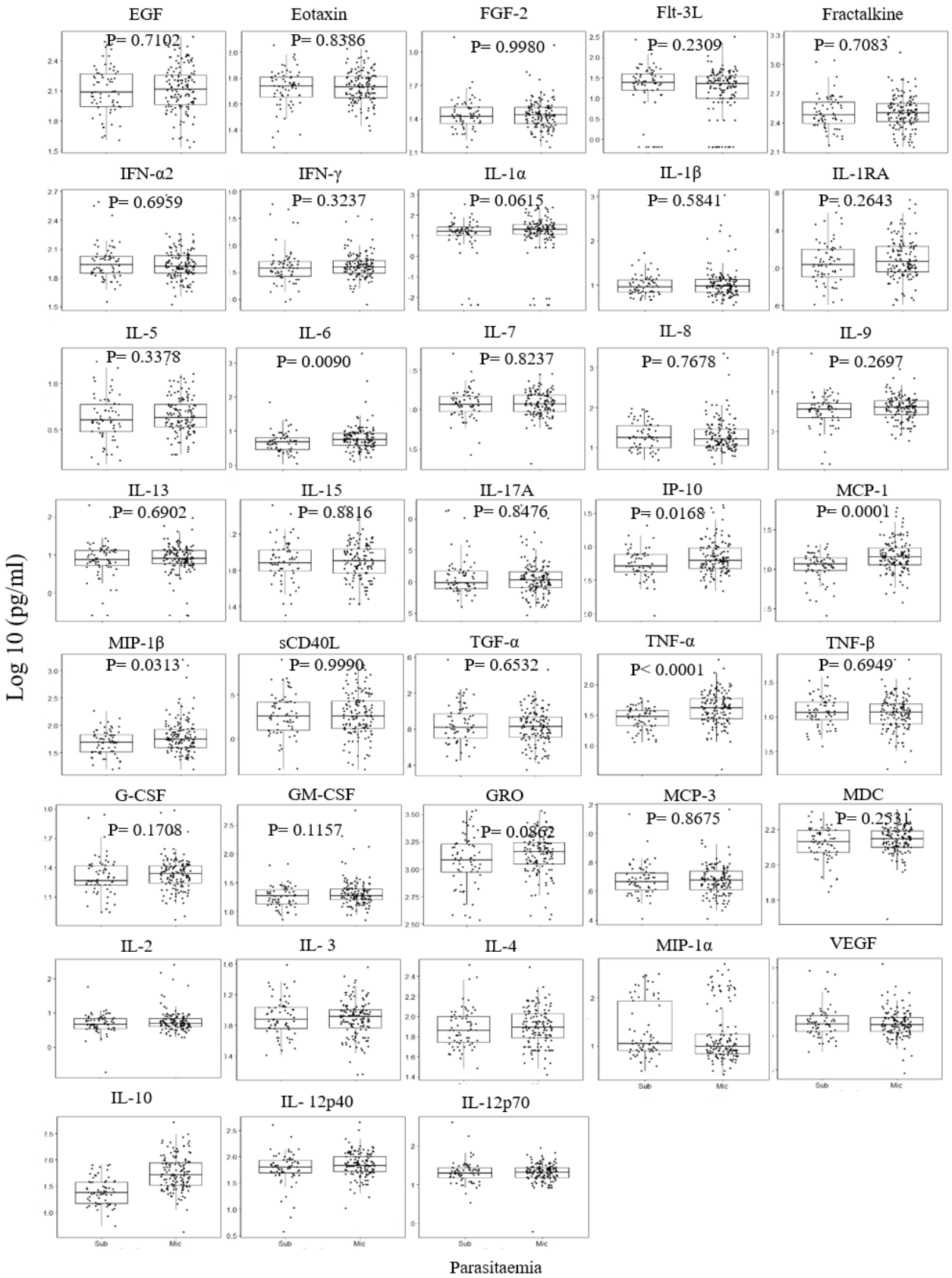

Figure 36: Plasma cytokine, chemokine and growth factors levels in infected individuals with submicroscopic and microscopic parasitaemia.

Box plots representing the median and interquartile range of cytokine levels measured by Luminex technology. Median cytokine levels were compared between parasitaemic groups using Wilcoxon ran sum test. Sub: Submicroscopic; Mic: Microscopic.

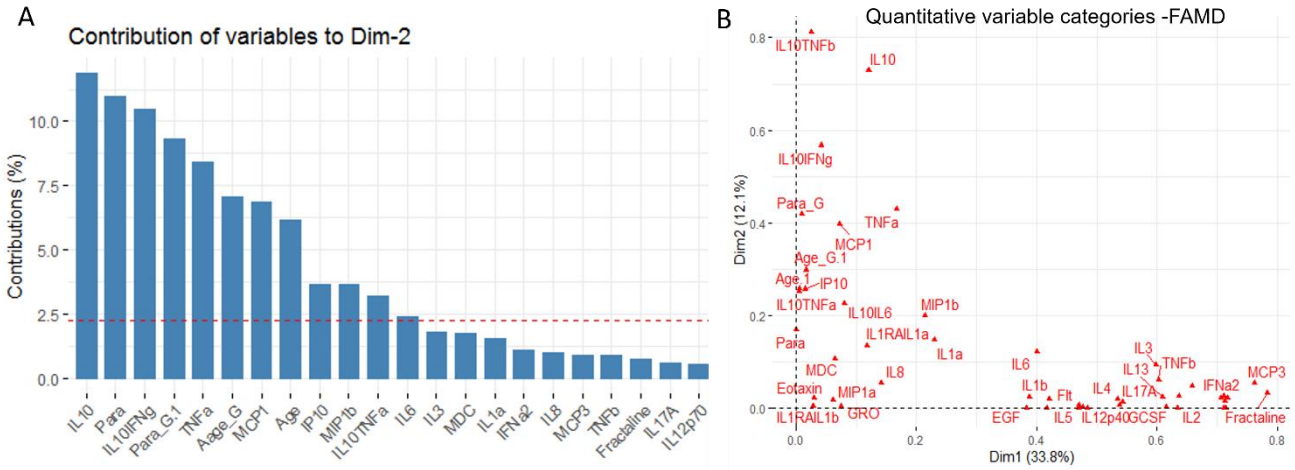

Figure 37. Cytokine responses are blunted with increased age and dependent on parasite densities.

A) Principal component of cytokines, age and parasitemia from FAMD (left graph) analysis. The graph of B shows the contribution of each variable on the principal component 2 (dimension 2).

Table XI: Effect of age and parasitaemia on cytokine, chemokine and growth factors concentrations in infected individuals

Biomarkers	Univariate				Multivariate			
	Age		Parasitaemia		Age		Parasitaemia	
	Coefficient (CI)	P-value	Coefficient (CI)	P-value	Coefficient (CI)	P-value	Coefficient (CI)	P-value
EGF	-0.114 (-0.194, -0.035)	**0.005**	0.005 (-0.041, .054)	0.784	-0.1 (-0.231, 0.031)	0.134	-0.015 (-0.071, 0.040)	0.583
FGF-2	-0.012 (-0.058, 0.035)	0.615	0.009 (-0.018, 0.036)	0.516	0.045 (-0.030, 0.120)	0.235	0.019 (-0.013, 0.051)	0.240
Eotaxin	-0.010 (-0.075, 0.019)	0.248	0.022 (-0.005, 0.048)	0.108	-0.006 (-0.080, 0.067)	0.864	0.020 (-0.012, 0 .051)	0.200
TGF-α	-0.010 (-0.079, 0.059)	0.775	-0.021 (-0.060, 0.019)	0.306	0.037 (-0.073, 0.147)	0.503	-0.012 (-0.059, 0.034)	0.601
G-CSF	-0.061 (-0.122, -0.001)	0.049	0.032 (-0.003, 0.067)	0.075	-0.022 (-0.112, 0.075)	0.651	0.027 (-0.014, 0.068)	0.200
Flt-3L	-0.138 (-0.355, 0.078)	0.209	-0.020 (-0.145, 0.104)	0.749	-0.093 (-0.438, 0.252)	0.594	-0.041 (-.187, 0.105)	0.582
GM-CSF	-0.040 (-0.119, 0.039)	0.320	0.033 (-0.016, 0.084)	0.186	0.141 (0.003, 0.278)	**0.046**	0.065 (0.007, 0.123)	**0.029**
Fractaline	0.008 (-0.054, 0.069)	0.810	-0.011 (0.047, 0.026)	0.566	0.067 (-0.035, 0.169)	0.194	0.004 (-0.039, 0.047)	0.851
IFN-α2	0.037 (-0.021, 0.096)	0.208	0.001 (-0.032, 0.033)	0.961	0.058 (-0.031, 0.148)	0.199	0.0138 (-0.024, 0.052)	0.476
IFN-γ	-0.005 (-0.107, 0.097)	0.925	0.023 (-0.032, 0.079)	0.409	0.190 (0.040, 0.341)	**0.013**	0.065 (0.002, 0.129)	**0.044**
GRO	-0.909 (-0.162, -0.020)	**0.013**	-0.038 (-0.076, -0.001)	0.047	-0.103 (-0.205, -0.001)	**0.049**	-0.061 (-0.104, -0.017)	**0.006**
IL-10	-0.398 (-0.507, -0.289)	**<0.001**	0.245 (0.191, 0.299)	**<0.001**	-0.130 (-0.278, 0.019)	0.087	0.216 (0.153, 0.279)	**0.000**
MCP-3	0.001 (-0.038, 0.039)	0.986	0.001 (-0.022, 0.024)	0.938	0.044 (-0.0195, 0.108)	0.172	0.011 (-0.016, 0.038)	0.435
IL-12p40	-0.164 (-0.255, -0.074)	**<0.001**	0.040 (-0.008, 0.089)	0.106	-0.104 (-0.238, 0.029)	0.125	0.017 (-0.039, 0.074)	0.556
MDC	-0.072 (-0.10 -0.044)	**<0.001**	0.012 (-0.004, 0.029)	0.140	-0.674 (-0.112, -0.023)	**0.003**	-0.002 (-0.021, 0.016)	0.796
IL-12p70	-0.016 (-0.107, 0.075)	0.727	0.001 (-0.050, 0.052)	0.976	0.0215 (-0.112, 0.163)	0.765	0.005 (-0.054, 0.065)	0.855
IL-13	0.050 (-0.094, 0.195)	0.493	-0.025 (-0.106, 0.055)	0.532	0.132 (-0.090, 0.354)	0.242	0.004 (-0.091, 0.098)	0.938
IL-15	-0.017 (-0.092, 0.057)	0.645	0.007 (-0.036, 0.051)	0.742	0.41 (-0.079, 0.161)	0.501	0.016 (-0.035, 0.067)	0.528
sCD40L	-0.125 (-0.210, -0.040)	**0.004**	-0.049 (-0.065, 0.035)	0.560	-0.167 (-0.304 -0.030)	**0.017**	-0.052 (-0.110, 0.006)	0.079
IL-17A	0.010 (-0.090, 0.108)	0.851	-0.003 (-0.059, 0.054)	0.924	0.078 (-0.079, 0.235)	0.330	0.014 (-0.052, 0.081)	0.669
IL-1RA	-0.112 (-0.189 -0.035)	**0.005**	0.062 (0.019, 0.106)	**0.005**	-0.47 (-0.167, 0.072)	0.434	0.051 (0.001, 0.103)	**0.046**
IL-1α	-0.134 (-0.476, 0.209)	0.440	0.164 (-0.035, 0.361)	0.101	0.021 (-0.524, 0.567)	0.939	0.169 (-0.063, 0.400)	0.151
IL-9	-0.0194 (-0.143, 0.104)	0.756	-0.008 (-0.071, 0.055)	0.804	0.245 (0.0756, 0.414)	**0.005**	0.046 (-0.026, 0.118)	0.205
IL-1β	-0.066 (-0.173, 0.042)	0.229	0.039 (-0.033, 0.110)	0.285	0.008 (-0.189, 0.207)	0.932	0.041 (-0.043, 0.125)	0.340
IL-2	-0.032 (-0.140, 0.076)	0.561	0.015 (-0.051, 0.081)	0.658	0.178 (-0.002, 0.358)	0.053	0.054 (-0.022, 0.131)	0.163
IL-3	0.059 (-0.024, 0.143)	0.160	-0.042 (-0.090, 0.007)	0.092	0.060 (-0.074, 0.194)	0.375	--0.028(0.085, 0.028)	0.327

IL-4	-0.058 (-0.123, 0.008)	0.084	-0.015 (-0.054, 0.023)	0.424	-0.104 (-0.208, 0.001)	0.051	-0.038 (-0.083, 0.006)	0.088
IL-5	-0.024 (-0.102, 0.054)	0.547	-0.006 (-0.052, 0.039)	0.780	0.039 (-0.086, 0.165)	0.538	0.002 (-0.051, 0.055)	0.933
IL-6	-0.184 (-0.306, -0.062)	0.003	0.084 (0.007, 0.162)	**0.033**	-0.143 (-0.356, 0.070)	0.187	0.953 (-0.038, 0.143)	0.253
IL-7	0.002 (-0.057, 0.061)	0.947	-0.004 (-0.037, 0.029)	0.819	0.013 (-0.078, 0.105)	0.778	-0.001 (-0.040, 0.038)	0.962
IL-8	-0.252 (-0.387, -.118)	< **0.001**	0.011 (-0.079, 0.102)	0.799	-0.387 (-0.628, -0.146)	**0.002**	-0.074 (-0.176, 0.028)	0.155
IP-10	-0.081 (-0.168, .007)	0.071	0.076 (0.024, 0.129)	**0.005**	0.005 (-0.141, 0.151)	0.942	0.777 (0.016, 0.139)	**0.015**
MCP-1	-0.159 (-0.231, -.087)	< **0.001**	0.092 (0.049, 0.135)	< **0.001**	-0.116 (-0.233, 0.001)	0.051	0.067 (0.017, 0.116)	**0.009**
MIP-1α	-0.109 (-0.311, 0.092)	0.286	0.114 (-0.007, 0.235)	0.065	-0.050 (-0.386, 0.285)	0.768	0.104 (-0.040, 0.245)	0.156
MIP-1β	-0.177 (-0.277, -0.078)	0.001	0.094 (0.031, 0.158)	**0.004**	-0.135 (-0.311, 0.041)	0.131	0.65 (-0.001, 0.139)	0.089
TNF-α	-0.368 (-0.439, -0.298)	< **0.001**	0.161 (0.115, 0.208)	< **0.001**	-0.307 (-0.424, -0.190)	< **0.001**	0.093 (0.044, 0.143)	< **0.001**
TNF-α	-0.025 (-0.108, 0.0598)	0.559	-0.012 (-0.065, 0.039)	0.631	-0.028 (-0.723, 0.117)	0.707	-0.019 (-0.080, 0.043)	0.546
VEGF	-0.046 (-0.122, 0.029)	0.230	0.024 (-0.019, 0.068)	0.271	-0.030 (-0.151, 0.091)	0.627	0.018 (-0.035 0.069)	0.494

The effect of age and parasite density on biomarker concentrations was assessed through univariable and multivariable linear regressions for each analyte, with analyte concentration as outcome (pg/mL) and age or parasitaemia as the predictor variables. CI: confident interval. P< 0.05 are in bold.

III.3.4. Correlation of cytokine, chemokine and growth factors with antibodies in infected individuals

The correlations of cellular markers with malaria antibodies in infected individuals were explored by univariate linear regression (Table XII). IL10, TNF-α, IL-3 MCP-1, Eotaxin and MDC levels negatively correlated with antibody to TE, ME and SE. Levels of IL-3, IFN-α2 and Flt-3L were negatively correlated with anti-EBA-175 antibody levels, whereas anti-MSP-1 antibody levels correlated negatively with IL10 and TNF-α levels. Contrary, to other antibodies, anti-MSP-4 antibody levels correlated positively with cellular markers IL10, TNF-α, IL-6, IL-5, IL-8, MCP-1, Flt-3L, MIP-1β, G-CSF, IL-12p40, FGF-2 and VEGF. Together, these data indicate the association between acquired humoral immune response and cellular immune response.

Table XII: Effect of IgG anti-plasmodial antibody levels on biomarker concentrations

Biomarkers	**Merozoite**		**Schizonte**		**TE**		**EBA-175**		**MSP-1**		**MSP-4**	
	Coefficient (CI)	**P-value**	**Coefficient (CI)**	**P-value**	**Coefficient (CI)**	**P-value**	**Coefficient (CI)**	**P-value**	**Coefficient (CI)**	**P-value**	**Coefficient (CI)**	**P-value**
EGF	-0.005 (-0.028, 0.017)	0.652	-0.016 (-0.037, 0.006)	0.150	-0.012 (-0.037, 0.014)	0.370	-0.002 (-0.011, 0.007)	0.727	-0.003 (-0.013, 0.007)	0.577	-0.003 (-0.015, 0.009)	0.640
FGF-2	-0.001 (-0.013, 0.013)	0.985	-0.003 (-0.015, 0.009)	0.641	0.001 (-0.014 0.016)	0.886	0.003 (-0.003, 0.008)	0.322	0.002 (-0.004, 0.007)	0.590	0.007 (0.001, 0.014)	**0.049**
Eotaxin	-0.012 (-0.025, 0.001)	0.078	-0.014 (-0.026, -0.002)	**0.027**	-0.188 (-0.033, -0.004)	0.013	-0.001 (-0.007, 0.004)	0.581	-0.002 (-0.008, 0.003)	0.408	0.005 (-0.002, 0.013)	0.139
TGF-α	0.011 (-0.009, 0.030)	0.279	0.003 (-0.015, 0.022)	0.723	0.003 (-0.019, 0.025)	0.774	0.004 (-0.004, 0.012)	0.323	0.001 (-0.007, 0.010)	0.723	0.009 (-0.001, 0.020)	0.083
G-CSF	0.003 (-0.014,0 .020)	0.707	-0.001 (-0.017, 0.015)	0.916	-0.015 (-0.034, 0.005)	0.134	0.002 (-0.005, 0.009)	0.604	-0.001 (-0.007, 0.007)	0.989	0.012 (0.003, 0.022)	**0.008**
Flt-3L	0.011 (-0.049,0 .072)	0.716	0.018 (-0.039, 0.075)	0.536	0.032 (-0.037, 0.100)	0.364	0.029 (0.006, 0.053)	**0.016**	0.021 (-0.005, 0.048)	0.110	0.044 (0.012, 0.077)	**0.008**
GM-CSF	-0.001 (-0.024, 0.021)	0.911	-0.006 (-0.027, 0.015)	0.583	-0.008 (-0.034, 0.017)	0.509	0.003 (-0.005, 0.012)	0.442	-0.002 (-0.012, 0.008)	0.664	0.010 (-0.002, 0.022)	0.114
Fractaline	0.009 (-0.008, 0.026)	0.310	0.002 (-0.014, 0.019)	0.794	0.005 (-0.015, 0.024)	0.635	0.006 (-0.001, 0.013)	0.083	0.001 (-0.007, 0.008)	0.808	0.008 (-0.001, 0.018)	0.089
IFN-α2	0.012 (-0.004, 0.029)	0.128	0.007 (-0.008, 0.023)	0.362	0.010 (-0.009, 0.028)	0.303	0.008 (0.001, 0.014)	**0.021**	0.001 (-0.006, 0.009)	0.703	0.006 (-0.003, 0.015)	0.178
IFN-γ	0.007 (-0.022, 0 .036)	0.633	-0.004 (-0.031, 0.024)	0.790	0.007 (-0.032, 0.034)	0.967	0.002 (-0.009, 0.014)	0.690	0.003 (-0.010, 0.016)	0.622	0.012 (-0.004, 0.028)	0.134
GRO	0.005 (-0.015, 0.026)	0.610	-0.004 (-0.024, 0.015)	0.654	-0.009 (-0.031, 0.015)	0.491	-0.001 (-0.010, 0.007)	0.745	-0.004 (-0.013, 0.004)	0.322	0.003 (-0.014, 0.008)	0.603
IL-10	-0.037 (-0.071, -0.002)	**0.036**	-0.46 (-0.079 -0.0134)	**0.005**	-0.057 (-0.096, -0.019)	**0.004**	-0.010 (-0.024, 0.004)	0.155	-0.016 (-0.031, -0.001)	**0.035**	0.033 (0.015, 0.052)	< **0.001**
MCP-3	0.001 (-0.010, 0.011)	0.969	-0.002 (-0.013, .008)	0.685	-0.001 (-0.013, 0.011)	0.859	0.002 (-0.002, 0.006)	0.407	-0.001 (-0.005, 0.004)	0.875	0.004 (-0.001, 0.010)	0.148
IL-12p40	-0.007 (-0.033, 0.019)	0.581	-0.006 (-0.031, 0.018)	0.614	-0.008 (-0.038, 0.021)	0.572	0.005 (-0.006, 0.015)	0.367	-0.005 (-0.016, 0.007)	0.413	0.017 (0.003, 0.031)	**0.015**
MDC	-0.006 (-0.015, 0.002)	0.127	-0.009 (-0.017, -0.001)	0.021	-0.013 (-0.023, -0.004)	**0.005**	0.001 (-0.003, 0.004)	0.760	-0.002 (-0.005, 0.002)	0.342	0.004 (-0.001, 0.002)	0.123
IL-12p70	-0.003 (-0.029, 0.022)	0.791	-0.010 (-0.034, 0.014)	0.407	-0.012 (-0.040, 0.017)	0.407	-0.001 (-0.012, 0.009)	0.774	0.001 (-0.012, 0.010)	0.886	0.005 (-0.009, 0.019)	0.502
IL-13	-0.012 (-0.053, 0.029)	0.581	-0.021 (-0.061, 0.018)	0.276	-0.031 (-0.078, 0.016)	0.192	0.001 (-0.016, 0.018)	0.897	0.001 (-0.018, 0.018)	0.994	0.015 (-0.007, 0.038)	0.186
IL-15	-0.003 (-0.024, 0.018)	0.780	-0.001 (-0.021, 0.018)	0.883	-0.003 (-0.027, 0.020)	0.797	0.003 (-0.005, 0.011)	0.475	-0.001 (-0.010 0.009)	0.900	0.006 (-0.005, 0.018)	0.284
sCD40L	-0.014 (-0.038, 0.010)	0.256	-0.021 (0.044, 0.002)	0.075	-0.032 (-0.059, -0.005)	**0.020**	-0.002 (-0.012, 0.007)	0.613	-0.009 (-0.020, 0.001)	0.083	0.003 (-0.010, 0.016)	0.652

IL-17A	-.0101707 .0480885	0.201	0.008 (-0.020, 0.035)	0.589	0.022 (-0.010, 0.056)	0.178	0.007 (-0.005, 0.018)	0.257	0.007 (-0.005, 0.020)	0.262	0.008 (-0.008, 0.024)	0.339
IL-1RA	-0.011 (-0.033, 0.011)	0.312	-0.012 (-0.033, 0.009)	0.257	-0.017 (-0.042, 0.007)	0.168	-0.003 (-0.012, 0.006)	0.471	-0.006 (-0.016, 0.003)	0.204	0.006 (-0.006, 0.019)	0.292
IL-1α	0.002 (-0.092, 0.096)	0.966	0.005 (-0.084, 0.095)	0.904	0.013 (-0.093, 0.120)	0.806	0.028 (-0.009, 0.065)	0.143	-0.020 (-0.043, 0.039)	0.925	0.039 (-0.012, 0.091)	0.133
IL-9	001 (-0.033, 0.036)	0.936	0.001 (-0.031, 0.034)	0.934	-0.003 (-0.042, 0.035)	0.868	0.005 (-0.009, 0.019)	0.470	0.001 (-0.014, 0.016)	0.862	0.017 (-0.002, 0.036)	0.076
IL-1β	-0.005 (-0.035, 0.025)	0.740	-0.003 (-0.032, 0.025)	0.826	-0.014 (-0.048, 0.020)	0.405	0.002 (-0.010) 0.014)	0.711	0.001 (-0.012, 0.015)	0.835	0.011 (-0.005, 0.027)	0.189
IL-2	-0.006 (-0.036, 0.024)	0.697	-0.015 (-0.043, 0.014)	0.305	0.012 (-0.046, 0.022)	0.499	0.005 (-0.007, 0.017)	0.386	-0.001 (-0.013, 0.013)	0.969	0.009 (-0.008, 0.025)	0.301
IL-3	0.031(0.008, 0.054)	**0.008**	0.027 (0.005, 0.049)	**0.017**	0.029 (0.003, 0.055)	0.027	0.013 (0.003, 0.022)	**0.007**	0.007 (-0.003, 0.017)	0.183	0.009 (-0.004, 0.022)	0.166
IL-4	0.007 (-0.011, 0.025)	0.458	0.005 (-0.012, 0.023)	0.550	0.001 (-0.020, 0.022)	0.934	0.007 (-0.001, 0.014)	0.056	-0.001 (-0.008, 0.008)	0.933	0.008 (-0.002, 0.018)	0.115
IL-5	0.012 (-0.009, 0.034)	0.264	0.009 (-0.012, 0.030)	0.385	0.001 (-0.024, 0.025)	0.964	0.005 (-0.004, 0.014)	0.270	0.008 (-0.009, 0.010)	0.871	0.014 (0.002, 0.026)	**0.020**
IL-6	-0.016 (-0.050, 0.019)	0.382	-0.020 (-0.053, 0.013)	0.229	(-0.074, 0.004)	0.081	-0.006 (-0.020, 0.008)	0.427	-0.007 (-0.022, 0.008)	0.384	.0014114 .039491	**0.035**
IL-7	0.005 (-0.012, 0.021)	0.564	0.001 (-0.014, 0.017)	0.873	0.001 (-0.018, 0.020)	0.899	0.002 (-0.005, 0.008)	0.606	0.001 (-0.006, 0.008)	0.781	0.004 (-0.004, 0.014)	0.325
IL-8	-0.007 (-0.046, 0.032)	0.734	-0.021 (-0.058, 0.015)	0.255	-0.024 (-0.068, 0.020)	0.279	-0.004 (-0.019, 0.012)	0.645	-0.001 (-0.017, 0.017)	0.957	0.024 (0.003, 0.046)	**0.024**
IP-10	-0.009 (-0.033, 0.016)	0.490	-0.005 (-0.029, 0.018)	0.656	0.011 (-0.039, 0.017)	0.444	-0.001 (0.011, 0.009)	0.808	-0.005 (-0.016, 0.006)	0.352	0.008 (-0.005, 0.022)	0.241
MCP-1	-0.029 (-0.050, -0.009)	**0.006**	-0.025 (-0.045, -0.005)	**0.014**	-0.048 (-0.071, -0.025)	< **0.001**	-0.007 (-0.016, 0.001)	0.090	-0.008 (-0.018, 0.001)	0.062	0.012 (0.001, 0.023)	**0.048**
MIP-1α	0.009 (-0.048, 0.066)	0.760	0.009 (-0.045, 0.064)	0.732	0.030 (-0.034, 0.095)	0.356	-0.13 (-0.036, 0.010)	0.270	0.008 (-0.017, 0.033)	0.531	0.008 (-0.023, 0.040)	0.607
MIP-1β	-0.015 (-0.043, 0.014)	0.308	-0.015 (-0.042, 0.012)	0.273	-0.028 (-0.060, 0.005)	0.092	-0.003 (-0.014, 0.009)	0.641	-0.008 (-0.020, 0.004)	0.208	0.023 (0.008, 0.039)	**0.003**
TNF-α	-0.044 (-0.068, -0.020)	< **0.001**	-0.049 (-0.071, -0.027)	< **0.001**	-0.077 (-0.102, -0.051)	< **0.001**	-0.006 (-0.015, 0.004)	0.258	0.017 (-0.028, -0.007)	**0.001**	0.026 (0.013, 0.039)	< **0.001**
TNF-α	0.007 (-0.016, 0.030)	0.564	0.003 (-0.019, 0.025)	0.805	-0.007 (-0.034, 0.019)	0.573	0.006 (-0.006, 0.015)	0.228	0.001 (-0.009, 0.012)	0.899	0.005 (-0.008, 0.018)	0.454
VEGF	0.011 (-0.010, 0.032)	0.310	0.002 (-0.019, 0.022)	0.878	0.003 (-0.021, 0.028)	0.779	0.006 (-0.003, 0.014)	0.199	-0.002 (-0.012, 0.007)	0.643	0.015 (0.003, 0.026)	**0.015**

The effect of antibody responses on cytokine, chemokine and growth factors concentrations was assessed through univariable linear regressions for each analyte, with analyte concentration as outcome (pg/mL) and antibody as the predictor variables. CI: confident interval. P< 0.05 are in bold.

CHAPTER IV: DISCUSSION

Despite the ongoing efforts of various programs to control the incidence of malaria, Cameroon remains one of the major contributors to the high incidence of malaria in sub-Saharan Africa (World Health Organization, 2019). This may be due to the high prevalence of asymptomatic infections (Nyasa *et al.*, 2015; Tientche *et al.*, 2016; Roman *et al.*, 2018), which has been shown to play an important role in the maintenance of malaria transmission and thus considered as an obstacle to malaria control and elimination worldwide (Lin *et al.*, 2014; Adhikari *et al.*, 2018). Eliminating malaria in sub-Saharan Africa will require a clear understanding of the mechanisms involved in asymptomatic parasite carriage and the impact of asymptomatic parasitaemia on individual health. Therefore this study aimed to define the immunological correlates of asymptomatic malarial parasitaemia in a *P. falciparum* highly exposed individuals in Cameroon.

Infection with malaria parasites was determined based on sample positivity by multiplex PCR. Participants identified as asymptomatic for the infection were followed-up weekly over a period of ten weeks to identify associated epidemiological and immunological factors of persistent asymptomatic infection. High prevalence (75.4%) of asymptomatic *Plasmodium* infections were observed in the study area, consistent with observations in school children in other areas of high transmission of *P. falciparum* in Cameroon (Tientche *et al.*, 2016). This high prevalence of asymptomatic carriage of *Plasmodium* parasitaemia could be explained in the context of frequent exposure to parasites leading to a state of premunition by most residents (Doolan *et al.*, 2009; Pinkevych *et al.*, 2012; Lindblade *et al.*, 2013; Antonio-Nkondjio *et al.*, 2019). However, this prevalence was twice as high compared to that reported in a community study in the southwest region of Cameroon using microscopy for the detection of malaria parasites (35.2 % in the rainy season and 17.8 % in the dry season) (Nyasa *et al.*, 2015). These data highlights the need for a massive deployment of molecular diagnostic tools to target low-density sub-microscopic infections (Mwingira *et al.*, 2014; Cook *et al.*, 2015), which serve as the infectious reservoirs (Okell *et al.*, 2009; Slater *et al.*, 2019). Additionally, 31.7% of the infections were under the detection limit of light microscopy. Conventional routine malaria diagnostic tools (microscopy and RDT) with a detection limit of 50-200 parasites/μL have been shown to greatly underestimate the burden of asymptomatic infections (Bousema *et al.*, 2014; Ayong *et al.*, 2019) as these infections generally correlate with low parasite densities (Okell *et al.*, 2012; Bousema *et al.*, 2014; Björkman, 2018).

Asymptomatic parasitaemia risk factors analysis showed that, submicroscopic or low parasitaemia levels increased more than twice the odds of being asymptomatic for malaria parasitaemia. Additionally, median blood parasitaemia was lower, albeit not significant, in the asymptomatic participants when compared to those with symptoms. These data support the hypothesis

that, asymptomatic *Plasmodium* infections are usually correlated with low parasite densities (Okell *et al.*, 2012; Bousema *et al.*, 2014; Björkman, 2018). Indeed, all clinical symptoms associated with malaria parasitaemia are caused by asexual erythrocytic or blood-stage parasites. Lysis of infected red blood cells releases into the bloodstream not only merozoites but also parasite byproducts such as haemozoin, glycosylphosphatidylinositol (GPI), and other toxic factors that trigger the production of pyrogenic inflammatory mediators by macrophages and other innate immune cells (Oakley *et al.*, 2011). These mediators in turn stimulate thermoregulatory regions of the brain to increase body temperature. The increase in parasitaemia is associated with an increase in the amount of toxins and pyrogenic mediators, thereby increasing body temperature and the risk of fever. The parasite density threshold associated with the onset of fever in human hosts was considered a potential indicator of the risk of symptomatic malaria in a parasite carrier (Smith *et al.*, 2006). Furthermore, parasitaemia was negatively correlated with age in both asymptomatic and symptomatic individuals and the median age was significantly higher in asymptomatic individuals than in those with symptomatic infections. Age is considered to be one of the main factors associated with acquired immunity in malaria endemic areas and previous studies showed that adults are more susceptible to asymptomatic infections owng to acquired immunity from repeated exposures to malaria parasites (Doolan *et al.*, 2009; Pinkevych *et al.*, 2012; Lindblade *et al.*, 2013).

The length of stay in the study area > 10 years and living in houses with less than 5 inhabitants increased the odds of asymptomatic infections. This could be explained by the fact that participants who have stayed within the locality for more than 10 years, consisted mainly of adults. Likewise, a higher proportion of adults (72.4 %) in the households with fewer inhabitants (<5 inhabitants) compared to that (43.5%) with more occupants (>9 inhabitants) was observed in the study population. This explain the observed trends, given that, adults are more likely to develop asymptomatic malaria due to acquired immunity to malaria parasites (Doolan *et al.*, 2009; Pinkevych *et al.*, 2012; Lindblade *et al.*, 2013). Consistent with these findings, recent data showed that having young children in the household was an independent risk factor of symptomatic malaria (Carlucci *et al.*, 2017). No history of fever in the last three months was also associated with asymptomatic malarial parasitaemia. This is consistent with previous data reporting that four or more malaria-related fever episodes in the past 6 months, was significantly associated with an elevated risk of *P. falciparum* infection (Maziarz *et al.*, 2018). A history of fever in the last three months indicate that these participants have recently suffered from clinical malaria, suggesting the inadequate acquired malaria immunity in these individuals, the potential reason of which they are susceptible to symptomatic malaria.

In contrast to the findings that demonstrated the association between asymptomatic malaria and anemia (Matangila *et al.*, 2014; Nyasa *et al.*, 2015), this study, showed that, haemoglobin levels were significantly higher in asymptomatic individuals compared to those with symptomatic infections. This

could be due to the higher parasite density observed in symptomatic individuals compared to asymptomatic subjects, as haemoglobin levels were negatively correlated with parasite densities in this population. Indeed, malaria parasites destroy red blood cells during their multiplication, which can lead to anaemia. Moreover, it has been shown that the severity of anaemia increases with the increasing intensity of malaria infection (Abdulkareem *et al.*, 2017). In fact, parasite byproducts (haemozoin and GPI, known to be correlated with parasitaemia), released by parasitized erythrocytes following schizont rupture may activate multiple immune cells to produce inflammatory markers, including tumor necrosis factor (TNF)-α, interferon (IFN)-γ and nitric oxide (NO) known to be involved in suppression of erythropoiesis (Pathak and Ghosh, 2016; Dufour *et al.*, 2003; Keller *et al.*, 2004), thus resulting to anaemia (clinical disease).

Similar to the studies undertaken in other endemic areas, a high rate of persistent asymptomatic infections (55.4%) based on a 10-week follow-up duration was observed in this study area, providing for the first time evidence of long-term asymptomatic *Plasmodium* parasite carriage in Cameroon. Indeed, in Ghana, a longitudinal study showed that naturally occurring *Plasmodium* infections last on average 5–6 months (Felger *et al.*, 2012). In Myanmar, *P. falciparum* carriage of at least 6–9 months was observed (Landier *et al.*, 2017). A study in Cambodia, which monitored 24 adults with asymptomatic *falciparum* infections monthly, found that 13% carried parasites for 2–4 months (Tripura *et al.*, 2016). Additionally, a recent cohort study in Vietnam found the median duration of *P. falciparum* infection and *P. vivax* of 6 and 2 months, respectively (Nguyen *et al.*, 2018). Together, our findings and other data indicate that chronic carriage of malaria parasites occurs in a wide range of malaria endemicities, although its frequency and duration is likely context-specific which could be due to the difference in study design and/or the malaria endemicity (Drakeley *et al.*, 2018). During the follow-up study, we observed an important fluctuation in parasite densities over time persistent infection in these highly exposed individuals in Cameroon. These results are in line with those reported in Vietnamiam population by Nguyen et al. (Nguyen *et al.*, 2018), where the parasite densities of persistent infections oscillated; from ultra-low-density infections (submicroscopic) to high-density infections. Taken together, these findings suggest a dynamic interaction between parasites and host defence mechanisms during persistent asymptomatic infections. As expected, in long-term asymptomatic participants, the median Hb level was significantly higher in the first week than the fourth and eleventh weeks before treatment, then increased after treatment. These results show one of the silent and long-term health consequences of asymptomatic infections (Matangila *et al.*, 2014; Akiyama *et al.*, 2016), and underscore the need to tackle asymptomatic malaria parasitaemia as a public health problem in malaria endemic communities, particularly those with high stable transmission patterns of *P. falciparum*. Additionally, these findings support recent arguments that the term "asymptomatic" during *Plasmodium* infection is a misnomer. Interestingly, despite the decrease in

median Hb during persistent asymptomatic infections, median Hb levels remained above the anaemia threshold (Hb> 11 g/dl) throughout the follow-up period, indicating a possible tight balancing of erythropoiesis processes during in asymptomatic parasite carriers. None of the potential epidemiological risk factors analysed predicted the persistent asymptomatic parasite carriage in this study area, indicating the need to study other host factors such as immune or genetic factors, as well as other parasite factors that may explain the persistent parasitaemia.

Similar to a study undertaken in a low malaria transmission setting in Columbia, wherein only 18.4% of asymptomatic carriers developed a malarial fever after two weeks of follow-up (Cucunubá *et al.*, 2008), an early conversion rate from asymptomatic to symptomatic infection of 15.4% (24) was obtained in the present study, indicating that early development of febrile disease in asymptomatic carriers is independent of transmission intensity. Risk analysis in this study identified gender (female) and anaemia as predictors of early fever occurrence. Indeed, malarial anaemia is known to correlate with increased release of parasite-derived pathogen-associated molecular patterns such as haemozoin and malaria GPI, which bind toll-like receptors stimulating an inflammatory response leading to a febrile disease (Pichyangkul *et al.*, 2004; Nebl *et al.*, 2005; Coban *et al.*, 2005). The underlying reasons why females have a higher risk of anaemia during asymptomatic *Plasmodium* infection are unclear, but this could be due to natural physiological differences such as sex hormone-dependent stimulation of erythropoiesis, known to be driven by testosterone which is abundant in males compared to females (Murphy, 2014). Furthermore, a previous study showed that, females were 3 time more prone to anaemia than males (Sumbele *et al.*, 2016). This indicates that the association between female gender and early clinical conversion may be indirectly related to the observed anaemia in women. Together these findings suggest a strong association between blood haemoglobin levels and febrile disease during asymptomatic *Plasmodium* infection.

Significant gaps exist in the understanding of anti-parasite and anti-disease immunity dynamics during persistent asymptomatic parasite carriage in areas with high transmission of *P. falciparum*. This study took advantage of longitudinal follow-up of asymptomatic *Plasmodium*-infected individuals to define the contribution of antibody and cytokine responses in the maintenance of asymptomatically long-term parasitaemia amongst highly exposed persons in Cameroon. Antibody levels or avidity were comparable between infected groups (LT vs ST vs SY or AS vs SY) or between infected and non-infected groups, indicating that antibody levels or qualities may not predict the protection against clinical infection or absence of an infection. These results are consistent with data previously reported by Bereczky et al (Bereczky *et al.*, 2004), who found no association between total antiplasmodial IgG antibody levels and protection from clinical malaria based on a longitudinal study in individuals aged 1-84 years old from a holoendemic area in Tanzania. However, the growth inhibition index of *P.*

falciparum was significantly higher in the long-term compared to the short-tern asymptomatic and symptomatic individuals, suggesting that antibody activity may play a role in protection against the development of the clinical disease. Antibodies are known to suppress parasite growth via a number of mechanisms, including inhibition of merozoite egress and invasion, antibody-dependent cellular inhibition, complement-mediated lysis or opsonic phagocytosis, and antibody-dependent respiratory burst (Bouharoun-Tayoun *et al.*, 1990; Thuilliez *et al.*, 2010; Duncan *et al.*, 2012; Osier *et al.*, 2014; Boyle *et al.*, 2015a). Otherwise, antibody levels against TE, ME, SE and MSP-1 antigens were positively correlated with age and negatively correlated with parasitaemia, thus indicating that antibody responses are acquired with age or cumulative exposure and play an important role in controlling the parasite load during *Plasmodium* infection (Doolan *et al.*, 2009; Dobaño and Moncunill, 2018). However, the increased levels of anti-MSP-4 with parasitaemia indicate that the MSP-4 antigen could be considered as a marker of exposure and not as a marker of protection (van den Hoogen *et al.*, 2019).

Consistent with no significant difference in median antibody between study groups, we found a stable median antibody level over time in the persistent asymptomatic parasite carriers, suggesting a limited boosting in antibody responses by persistent parasitaemia. Antimalarial treatment of persistent asymptomatic infections resulted in declines in antiplasmodial plasma antibody levels, indicating that persistent parasitaemia is required for sustained antibody responses within endemic populations. However, the data show an increased pattern of antibody avidity to some plasmodial antigen (TE and EBA-175) over time, which significantly decreased after antimalarial treatment. It has been speculated that in the presence of continuous low antigen levels, matured affinity plasma cells that secrete high-affinity antibodies (at low levels) are favoured at the expense of low-affinity plasma cells (Ssewanyana *et al.*, 2017), thereby, keeping parasitaemia below the pyrogenic threshold. In contrast, to our findings, antibody responses to several *Plasmodium* antigens have been shown to increased over time in infected individuals living in low-seasonal malaria transmission setting in Thailand (Baum *et al.*, 2016). This increasing trend coincided with before, during and, after the long rainy season, which was associated with low, peak, and decline of parasite transmission season in the region. The stability in antibody responses found in this study could also be explained by the stability in malaria transmission in this study area. A study has shown that convalescence state is associated with decreased levels of antibody (Partey *et al.*, 2018). Furthermore, studies have found a decline of the breadth and magnitude of immunoglobulin G (IgG) specific for 862 *P. falciparum* proteins/polypeptides in asymptomatic *P. falciparum* infection from the wet to the dry season. Overall, these data show that antibody profiles in persistent infection could vary according to malaria transmission context.

Consistent with the role of balance between pro-and anti-inflammatory cytokine responses in the disease outcome, this study showed that clinical conversion from asymptomatic to symptomatic

malaria was linked to increased baseline levels of IL-10/pro-inflammatory cytokine ratios in the asymptomatic subjects. IL-10 is an anti-inflammatory cytokine with controversial roles in the protection and malaria pathogenesis, according on the timing of its production (Kumar *et al.*, 2019). In line with our findings, increased IL-10 levels have been found as the primary predictor of clinical conversion from asymptomatic to symptomatic visceral leishmaniasis in some endemic areas (Topno *et al.*, 2019). On the other hand, IL-10 levels, as well as IL-10/pro-inflammatory cytokine ratios, were significantly higher in the symptomatic individuals compared to the asymptomatic subjects, indicating the role of IL-10 in the development of clinical malaria. These data are supported by findings from other studies which have associated increased levels and activation of Treg cells, the main source of the IL-10 with symptomatic malaria instead of asymptomatic infections (Boyle *et al.*, 2015b; Frimpong *et al.*, 2018). IL-10 is an anti-inflammatory cytokine that acts mainly by inhibiting monocytes and macrophages in the production of pro-inflammatory cytokines such as IL-6, TNF, and IL-l (de Waal Malefyt *et al.*, 1991). High levels of IL-10 are thought to be a consequence of increased inflammation and may be beneficial by reducing the inflammatory response but suggesting that the immune system had poorer control of peripheral parasites. In this study, higher levels of IL-6, IL-1RA and MCP-1 were also associated with symptomatic malaria. Higher levels of IL-1RA and MCP-1 have been associated with a gradual increase in disease severity from healthy controls to uncomplicated malaria to cerebral malaria, and very high levels in children who die of cerebral malaria (Jakobsen *et al.*, 1994; John *et al.*, 2008). Like IL-10, IL-1RA is a potent anti-inflammatory cytokine that can also play a critical role in controlling inflammation by regulating the function of the pro-inflammatory cytokines IL-1α/IL-1β (Arend *et al.*, 1998). Notably, IL-10 is a potent inducer of the anti-inflammatory cytokine IL-1RA, which can be described as one of the mechanisms by which IL-10 exerts its anti-inflammatory effects (Tamassia *et al.*, 2010). These symptomatic malaria associated-cytokines and IL-10/pro-inflammatory cytokine ratios were positively correlated with parasite densities, indicating that their responses are dependent on the parasite load. This is supported by the observed high parasite densities in symptomatic compared to asymptomatic individuals, despite the non-significance. Although little is known concerning the potential role of IL-8 in the protection against clinical malaria, this study revealed significantly higher levels of IL-8 in long-term asymptomatic compared to short-term asymptomatic individuals. IL-8 is a chemoattractant cytokine known to be involved in neutrophil recruitment and activation, and previously associated with severe malaria pathogenesis (Lyke *et al.*, 2004; Mandala *et al.*, 2017). A recent study reported higher levels of IL-8 production by PBMCs from asymptomatic individuals compared to symptomatic carriers (Lehmann *et al.*, 2017). Furthermore, the Malian Fulani ethnic group known to be less susceptible to *P. falciparum* malaria as reflected by lower parasitaemia and fewer clinical symptoms exhibited a higher level of IL-8 and IL-12p70 than the Sympatric ethnic groups (Boström *et al.*, 2012). Together, these findings suggest a potential dual role

of IL-8 in protection against clinical malaria and the development of the disease that might also depend on the timing of its release.

In line with fluctuating parasite densities over time, significant changes were observed in several pro- and anti-inflammatory cytokine levels over the 10 week infections follow-up period, indicating a dynamic interaction between the parasite and host defence mechanisms during persistent asymptomatic infections (Drakeley *et al.*, 2018). However, no significant changes were observed in IL-10 anti-/pro-inflammatory cytokine ratios over the 10-week follow-up as well as in levels of the key regulatory cytokines TNF-α, IL-10, IL-1RA, IP-10, MCP-1 and Eotaxin. Together, these findings show the important role of these cytokines as well as a balanced anti-/pro-inflammatory cytokine response in the maintenance of asymptomatic parasite carriage over extended durations. Previous studies have shown that the clinical outcome of malaria infection is largely dictated by the balance between pro-inflammatory and anti-inflammatory markers, i.e. whether it leads to protection and/or immunopathology (Prakash *et al.*, 2006; Wilson *et al.*, 2010; Rovira-Vallbona *et al.*, 2012). Acute malaria infection is associated with an increase of inflammatory cytokine levels in the peripheral blood, which mediates cellular responses and contributes to parasite clearance, but could also be responsible for the symptoms and pathogenesis of severe malaria. Thus, dampened inflammatory cytokine responses by increased levels of regulatory cytokine response may protect individuals from symptomatic disease while at the same time interfer with their ability to prevent re-infection. The importance of balanced cytokine responses in the maintenance of the persistent asymptomatic infection was supported by the observed decline in IL-10 anti-/pro-inflammatory cytokine ratios as well as regulatory IL-10 and MCP-1 cytokine levels after antimalarial treatment. Other studies have shown that the malaria convalescence phase was associated with a significant decrease in IL-10, MCP-1, IFN-γ, TNF-α cytokine levels (Gonçalves *et al.*, 2012; Mandala *et al.*, 2017). IL-10 is known to down-regulate inflammatory responses during the acute phase of infection and its decrease correlated with recovery (Rodrigues-da-Silva *et al.*, 2014).

Different susceptibility to clinical malaria disease in children vs adults is associated with age-related differences in immunity due to cumulative exposure to *Plasmodium* infections (Griffin *et al.*, 2015; White and Watson, 2021). Interestingly, results from this study showed that age independently predicted the levels of cellular immune mediators with different patterns. Age was independently associated with increased antibody levels to TE and SE and decreased cytokine (TNF-α, IFN-γ, IL-9, IL-8, MDC and sCD40L) levels in infected individuals. These observations are consistent with the idea that repeated exposure to malaria parasites leads to acquired anti-parasite immunity and may drive the host towards a disease tolerance state in order to reduce the negative impacts of infection-related pathology (Ademolue and Awandare, 2018; Aguilar *et al.*, 2019). Similarly, recent studies have shown

that both inflammatory and regulatory cytokine responses to malaria decreased with increasing age in highly exposed individuals (Farrington *et al.*, 2017; Aguilar *et al.*, 2019). Repeated exposure to *P. falciparum* leads to the establishment of tolerance (Portugal *et al.*, 2014), which may be associated with the loss and/or altered function of both innate and adaptive immune cell types, indicating the role of both exhaustion and immunoregulatory pathways in the protection from disease (Boutlis *et al.*, 2006; Illingworth *et al.*, 2013; Horne-Debets *et al.*, 2013). Parasite densities independently predicted decreased levels of anti-plasmodial antibody and increased levels of cytokine such as IL-10, TNF-α, IL-1RA, IP-10 and MCP-1 in this study population. Indeed, high parasite density would lead to high levels of parasite associated antigens such as glycophosphatidylinositol (GPI) anchors (Schofield and Fiona, 1993; Mbengue *et al.*, 2015), and high levels of damage-associated molecular patterns (DAMPs) such as haem (Mendonça *et al.*, 2016), which, consequently, should induce corresponding high levels of pro-inflammatory response. This increase in inflammation is associated with an increase in the regulatory response mediated mainly by IL-10 which has a number of effects such as the inhibition of the secretion of IL-6, IFN-γ and TNF -α. These results are supported by the observation that parasitaemia was negatively correlated with age. Furthermore, other studies have also found a positive association between pro-/anti-inflammatory cytokine levels, including TNF-α, IL-2R, IL-12, MCP-1, TNFR1 and IL-10 and parasitaemia (Rodrigues-da-Silva *et al.*, 2014; Ademolue *et al.*, 2017).

CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

CONCLUSIONS

The aim of this study was to define the immunological determinants of persistent asymptomatic malarial parasitaemia in a *Plasmodium falciparum* hyper-endemic setting in Cameroon. The data from this study show:

1- High prevalence (75.4%) and evidence of persistent asymptomatic malarial parasitaemia in the health district of Esse, a *P. falciparum* hyper-endemic zone in Cameroon. Low parasite densities and no history of fever in the preceding three months were identified as independent risk factors of asymptomatic malarial parasitaemia, whereas female gender and anaemia were independently associated with early conversion from asymptomatic to symptomatic infections.

2- Specific anti-plasmodial antibody responses play important roles in controlling blood parasite densities. This effect of antiplasmodial antibodies are independent of plasma levels and avidity of antibodies against the *P. falciparum* proteins EBA-175, MSP-1 and Msp-4. Persistent parasitaemia is required to sustain antiplasmodial antibody levels and avidity in asymptomatic individuals.

3- High IL-10/pro-inflammatory cytokine ratios are associated with clinical disease, whereas high levels of IL-8 chemokine predicts persistent asymptomatic parasite carriage in highly exposed individuals. Additionally, balance in main anti-/pro-inflammatory cytokine ratios is crucial for the maintenance of asymptomatic parasitaemia in the study population.

RECOMMENDATIONS

Based on the findings obtained from this study, we recommend:

1- To the National Malaria Control Program (NMCP), the systematically diagnosis and treatment of malaria infections in rural communities using molecular diagnostic tools such as RT-LAMP to target low-density infection, as more than 31.7% of the infected individuals is likely to be submicroscopy.

2- To the Ministry of Public Health, with the aim of eliminating malaria, to consider taking asymptomatic infections into account in the malaria control policy because these infections represent more than 80% of total infections, thus constituting the reservoirs of malaria transmission.

PERSPECTIVES

As perspectives the following studies are worth pursuing:

1- Use methods a high-throughput method such as protein-arrays to screen more antigens to confirm non-association between antibody levels and persistent infection.

2- Determine the immune cells subsets (innate and adaptive) directly implicated in the establishment and maintenance of asymptomatic malaria parasitaemia.

3- Determine the host and parasite genetic factors that could predict the persistence of asymptomatic parasitaemia

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APPENDICES

Appendix 1: Ethical clearance

COMITE NATIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE

Arrêté N° 0977/A/MINSANTE/SESP/SG/DROS/ du 18 avril 2012 portant création, organisation et fonctionnement des comités d'éthique de la recherche pour la santé humaine au sein des structures relevant du Ministère en charge de la santé publique

N° 2018/09/1104/CE/CNERSH/SP

Yaoundé, le 17 septembre 2018

Cnethique_minsante@yahoo.fr

CLAIRANCE ETHIQUE

Le Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session extraordinaire du 04 mai 2018, a examiné le projet de recherche intitulé: **«Caractérisation immunologique du paludisme asymptomatique dans la localité d'Esse Centre Cameroun»** soumis par **Monsieur FOGANG Balotin,** Investigateur Principal, étudiant à la Faculté des Sciences ; Université de Yaoundé 1.

Le projet est d'un grand intérêt scientifique et social. L'objectif de cette étude est de définir les déterminants immunologiques du paludisme asymptomatique dans une localité du centre Cameroun. La procédure de l'étude est bien documentée et claire. Les risques liés au prélèvement de sang sont précisés ainsi que les mesures pour les éviter et les minimiser. La notice d'information et les formulaires de consentement éclairé, en français et en anglais, sont bien élaborés et simples à comprendre. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des Investigateurs les décrivent comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d'Ethique approuve pour une durée d'un an, la mise en œuvre de la présente version du protocole.

L'étudiant **FOGANG Balotin** est responsable du respect scrupuleux du protocole approuvé et ne devrait y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. Les investigateurs sont appelés à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations susmentionnées.

En foi de quoi, la présente clairance éthique est délivrée pour servir et valoir ce que de droit.

Ampliations

- MINSANTE

Le Président

Pr Lazare KAPTUE

N.B : cette clairance éthique ne vous dispense pas de l'autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.

Appendix 2: Study site authorisation

REPUBLIQUE DU CAMEROUN
Paix-Travail-Patrie
****
MINISTERE DE L'ADMINISTRATION TERRITORIALE ET DE LA DECENTRALISATION
****
REGION DU CENTRE
****
DEPARTEMENT DE LA MEFOU ET AFAMBA
****
ARRONDISSEMENT D'ESSE
****
SOUS-PREFECTURE D'ESSE
****
SECRETARIAT PARTICULIER
****

REPUBLIC OF CAMEROON
Peace-Work-Fatherland
****
MINISTRY OF TERRITORIAL ADMINISTRATION AND DECENTRALIZATION
****
CENTRE REGION
****
MEFOU AND AFAMBA DIVISION
****
ESSE SUB DIVISION
****
DIVISIONAL OFFICE ESSE
****
PRIVATE SECRETARIAT
****

Esse, le 12 JAN 2018

N°100 /ARA/JO5-02/SP

AUTORISATION DE RECHERCHE ACADEMIQUE

LE SOUS-PREFET DE L'ARRONDISSEMENT D'ESSE soussigné, accorde à Monsieur FOGANG Balotin, Etudiant en première année de Doctorat en Biologie des Organismes Animaux à la Faculté des Sciences de l'Université de Yaoundé I, une autorisation de recherche académique sur la caractérisation immunogénétique du paludisme asymptomatique au Cameroun, suite à sa demande introduite en date du 18 Janvier 2018 dans les services de la Sous-préfecture.

L'intéressé prendra toutes les dispositions nécessaires pour que lesdits travaux de recherche ne portent pas atteinte à l'ordre public et rendra compte aux autorités locales de toutes difficultés éventuellement rencontrées.

En foi de quoi, la présente autorisation de recherche académique est établie et délivrée à l'intéressé pour servir et valoir ce droit.

LE SOUS-PREFET

REPUBLIQUE DU CAMEROUN Département de la Mefou et Afamba Le Sous-Préfet Arrondissement d'ESSE REGION DU CENTRE

Muranjo Nsangue Elie
Diplômé de l'ENAM
ATPS

AMPLIATIONS
- Préfet/ MAF/Mfou
- Maire/Commune/Esse
- Responsables/FMO/Esse
- Directeur/Hôpital de District de Santé/Esse
- Chefs de village/Esse
- Intéressé
- Chrono-Archives

Appendix 3: Notice d'information

Titre du projet : Caractérisation immunogénétique du paludisme asymptomatique à *Plasmodium falciparum* dans une zone hyper-endémique au Cameroun.

Investigateur Principal : Balotin Fogang, Faculté des sciences, Université de Yaoundé I, Cameroun. Tel: (237) 695374077. E-mail: b.fogang@yahoo.fr

Nous vous invitons à participer à cette étude sur la détermination des facteurs immunogénétiques associés au partage asymptomatique de *Plasmodium falciparum* dans une de transmission forte et stable.

Introduction. Le paludisme est une maladie causée un parasite du genre *Plasmodium* et transmit à l'homme par la piqure d'un moustique du genre anophèle. Généralement le paludisme se manifeste par des fièvres, les céphalées, les frissons, les douleurs abdominales, etc. Cependant, certaines personnes peuvent porter le parasite du paludisme pendant longtemps sans toutefois développé de symptômes et dont ne se font pas traité. Cette étude a pour but de tenté de mieux comprendre pourquoi parmi les personnes infectés vivants dans la même zone certaines font la maladie et d'autres pas.

Objectifs de l'étude :

Notre étude a pour objectifs de:

- Déterminer la prévalence et les facteurs immunologiques humoraux associés au paludisme asymptomatique *P. falciparum* dans une zone de transmission forte et stable de au Cameroun,
- Définir le rôle fonctionnel des anticorps antiplasmodiale dans le paludisme asymptomatique à *P. falciparum*,
- Investiguer le lien entre le paludisme asymptomatique *P. falciparum* et le polymorphisme de certains gènes de virulence du parasite ainsi que les traits de résistance aux antipaludiques.

Période de l'étude et population d'étude

Notre étude sera conduite entre le mois de Mars et le mois de juin 2018. Un infirmier effectuera des prélèvements de sang total chez certains résidents (enfants et adultes) de la communauté d'Esse ayant donné leur consentement par écrit.

Procédure

Si vous donner votre accord pour votre participation ou celle de votre enfant a cette étude, notre infirmier vous prélèvera environ 5 ml de votre sang ou de celui de votre enfant et nous vous poserons quelques questions sur vous ou votre enfant. Ensuite nous ferons un diagnostic rapide du paludisme et nous prendront aussi votre température corporelle. Si vous êtes positif au paludisme et avec une

température corporelle supérieure à 37,5, nous vous traiteront selon les recommandations du Programme Nationale de Lutte contre Paludisme. Si vous êtes positif mais ayant une température inférieure à 37,5 vous seriez suivit quotidiennement par un infirmier pendant une période de deux semaines et en cas de fièvre vous seriez immédiatement traité. A la fin des deux semaines tout le monde sera diagnostiqué de nouveau et les infectés seront traités.

Note : Après votre inclusion dans l'étude, vous pouvez suspendre à tout moment votre participation, ce qui n'entrainera aucune conséquence ou perte d'avantage auxquels vous avez droit.

Conservation des échantillons de sang

Dans le cadre de cette étude, les échantillons de sang prélevés seront utilisés pour des analyses immunologiques et génétiques. Ces échantillons seront traités aux Centre Pasteur du Cameroun Yaoundé et le reste sera conservé aussi longtemps que nécessaire. Toute recherche utilisant ces échantillons serait examinée par le Comité Nationale d'Ethique qui protège les droits et le bien-être des personnes qui participent à la recherche.

Considérations éthiques

Risques et Précaution à prendre

Au-delà d'une légère douleur que pouvez ressentir lors du prélèvement de sang et des effets secondaires liés à la prise des antipaludiques, il n'y aura pas de risque supplémentaire lié à la participation à cette étude. Dans le cas des effets secondaires après la prise du traitement antipaludique, vous devez aller voir le médecin de la communauté qui collabore avec l'enquête et dont recevoir un traitement approprié.

Bénéfices

Il n'y aura aucun avantage direct associé à votre participation ou à celui de votre enfant à cette étude dans la phase de collecte, stockage et analyse de laboratoire réalisés sur ces échantillons. Néanmoins, comme compensation de participation à l'étude, les personnes qui seront infectés par l'agent responsable du paludisme, bénéficieront d'une prise en charge gratuite. Nous vous délivrerons un antipaludique approprié selon la recommandation du Programme National de Lutte contre le Paludisme. Toutefois les résultats de cette étude pourront permettre de déterminer les biomarqueurs du paludisme asymptomatique; potentiel nouveaux candidat vaccin contre le paludisme et ainsi sauvé plusieurs vie dans les années à venir.

Confidentialité

Toute information relative à votre personne ou celle de votre enfant sera privée. Les informations spécifiques que nous apprenons sur vous ou votre enfant ne seront pas partager avec

n’importe qui sauf les chercheurs associés à cette étude. Tous rapports scientifiques liés à cette étude ne comporteront en aucun cas votre nom ou celui de votre enfant. Les échantillons de sang prélevés ne serviront en aucun cas à d’autres tests sans autorisation du Comité National d’Ethique; si les échantillons ne sont utilisés, ils sont détruits.

Pour des questions relatives au projet de recherche décrit ci-dessus, vous pouvez contacter :

Balotin Fogang, Faculté des Sciences, Université de Yaoundé I, Tel : (237) 695 37 40 77

Dr. Lawrence Ayong, Chef de l’Unité de Recherche sur le Paludisme, Centre Pasteur du Cameroun, Tel: (237) 651 11 76 00

Comité National d’Ethique de la Recherche pour la Santé Humaine, cnethique_minsante@yahoo.fr. Tel: 243 67 43 39

Appendix 4: Consentement éclairé (Adulte)

Je soussigne,_____(nom du participant) certifie avoir été invité à participer au travail de recherche intitulé «Caractérisation immunogénétique du paludisme asymptomatique à *Plasmodium falciparum* dans une zone hyper-endémique au Cameroun » dont l'investigateur principal se nomme Fogang Balotin, Université de Yaoundé I, BP812 Yaoundé Cameroun.

J'ai bien compris la notice d'information qui m'a été remise concernant cette étude. Je comprends que je pourrais ressentir un léger inconfort au moment de la piqûre et que, bien que les risques soient mineurs, des effets secondaires dus à la prise des antipaludiques peuvent survenir si je suis traité. Je suis conscient qu'il n'y a pas d'avantage de bénéfices que ceux cités dans la notice d'information.

J'ai lu la notice d'information, ou elle m'a été lue et expliquée. J'ai reçu des réponses satisfaisantes à toutes les questions que j'ai posées. Je peux joindre le coordonnateur du projet si j'ai des questions supplémentaires concernant le projet et mes droits de participant au projet.

J'accepte librement de participer à cette étude et je comprends que je peux me retirer du projet a tout moment sans que cela n'affecte en rien mes bénéfices en tant que participant au projet.

Nom du participant_____.

Date_____**(Jour/mois/année)**

Si le participant est incapable de lire ou d'écrire

J'ai été témoin d'une lecture précise du formulaire de consentement au participant et il a eu l'opportunité de poser des questions. Je confirme que la personne a donné librement son consentement.

Nom et Signature du témoin_____**Empreinte du participant**

Date_____**(Jour/Mois/Année)**

Nom de l'enquêteur _____.

Date_____**(Jour/Mois/Année)**

Une copie de la notice d'information et celui du formulaire de consentement vous sera fournie à votre demande.

Appendix 5: Consentement parental (mineur)

Je soussigne,_____(nom du parent/tuteur) représentant légal de (nom du participant) certifie avoir été invité à participer au travail de recherche intitulé «Caractérisation immunogénétique du paludisme asymptomatique à *Plasmodium falciparum* dans une zone hyper-endémique au Cameroun » dont l'investigateur principal se nomme Fogang Balotin, Université de Yaoundé I, BP812 Yaoundé Cameroun.

J'ai bien compris la notice d'information qui m'a été remise concernant cette étude. Je comprends que mon enfant pourra ressentir un léger inconfort au moment de la piqûre et que, bien que les risques soient mineurs, des effets secondaires dus à la prise des antipaludiques peuvent survenir si je suis traité. Je suis conscient qu'il n'y a pas d'avantage de bénéfices que ceux cités dans la notice d'information, ni pour moi, ni pour mon enfant.

J'ai lu la notice d'information, ou elle m'a été lue et expliquée. J'ai reçu des réponses satisfaisantes à toutes les questions que j'ai posées. Je peux joindre le coordonnateur du projet si j'ai des questions supplémentaires concernant le projet et mes droits de participant au projet.

J'accepte librement que mon enfant participe à cette étude et je comprends que je peux retirer mon enfant du projet a tout moment sans que cela n'affecte en rien ses bénéfices en tant que participant au projet.

Nom du participant et Signature du parent ou du tuteur _____.

Date_____(Jour/mois/année)

Si le parent/tuteur est incapable de lire ou d'écrire

J'ai été témoin d'une lecture précise du formulaire de consentement au parent/tuteur du participant et il a eu l'opportunité de poser des questions. Je confirme que la personne a donné librement son consentement.

Nom et Signature du témoin_____Empreinte du parent/tuteur

Date (Jour/Mois/Année)

Nom de l'enquêteur _____Signature_____.

Date_____(Jour/Mois/Année)

Une copie de la notice d'information et celui du formulaire de consentement vous sera fournie à votre demande.

Appendix 6: Questionnaire

Caractérisation immunogénétique du paludisme asymptomatique à *Plasmodium falciparum* **dans une zone hyper-endémique au Cameroun**

Date_____Site d'étude_____Resisdence_____code_____.

IDENTIFICATION DU PATIENT

Nom :

Prénom :

Age : Sexe : M__F Occupation_____.

INFORMATION SUR LE PALUDISME

Depuis combien de temps résidez-vous dans cette localité ?_____.

Disposez-vous d'une moustiquaire imprégnée ? Oui____Non

A quelle fréquence dormez-vous sous une moustiquaire ? Oui____Non

Utilisez-vous régulièrement des insecticides ? Oui____Non

Êtes-vous sur traitement antipaludique ? Oui____Non

Si oui lequel _____ .

Quand avez-vous eu votre dernière crise de paludisme ? _____.

A quand remonte votre dernière prise d'antipaludiques ? _____.

Avez-vous pris depuis ces trois derniers mois des:

Antiparasitaires Oui____Non

Des antibactériennes Oui____Non

Des antiviraux Oui____Non

RESULTATS PRELIMINAIRE

Température_____.

TDR control_____Pf _____Pan_____.

Goutte épaisse Négatif _____positif_____ Parasitemie (p/µl) _____.

Espèce *Plasmodium* _____.

Hémoglobine (g/dl) _____ Hématocrite (%)

PUBLICATIONS FROM THIS WORK

1- Fogang, B., Schoenhals, M., Maloba, F. M., Abite, M. F., Essangui, E., Donkeu, C., Cheteug, G., Kapen, M., Keumoe, R., Kemleu, S., Perraut, R., Megnekou, R., Lamb, T. J., & Ayong, L. S. (2022). Asymptomatic carriage of *Plasmodium falciparum* in children living in a hyperendemic area occurs independently of IgG responses but is associated with induction of IL-10 (p. 2022.05.04.22274662). medRxiv. https://doi.org/10.1101/2022.05.04.22274662. Submitted to the Journal of Infetious Disease.

2- Fogang, B., Biabi, M. F., Megnekou, R., Maloba, F. M., Essangui, E., Donkeu, C., Cheteug, G., Kapen, M., Keumoe, R., Kemleu, S., Nsango, S., Eboumbou, C., Lamb, T. J., & Ayong, L. (2021). High Prevalence of Asymptomatic Malarial Anemia and Association with Early Conversion from Asymptomatic to Symptomatic Infection in a *Plasmodium falciparum* Hyperendemic Setting in Cameroon. *The American Journal of Tropical Medicine and Hygiene*, 106(1), 293–302. https://doi.org/10.4269/ajtmh.21-0316.