3 - 4 April 2025

Conference Venue

Institute of Tropical Medicine
University of Tübingen
Wilhelmstraße 27, 72074 Tübingen

16th Malaria Meeting
by DTG, DZIF and PEG

Program And Abstract Book

cermel

DZIF
German Center for Infection Research

Universitätsklinikum Tübingen

PEG
Paul-Ehrlich-Gesellschaft für Infektionstherapie e.V.

DTG

Eberhard Karls Universität Tübingen

Content

PROGRAM

Thursday, April 3

9:00 am **Registration**
Main lobby - with welcome coffee

Session I: Opening

Chairs: Peter G. Kremsner & Carsten Köhler

10:00 am **Peter G. Kremsner & Carsten Köhler**, Institute of Tropical Medicine Tübingen, Germany
Welcome address

10:05 am **Peter G. Kremsner**, Institute of Tropical Medicine Tübingen, Germany
Current innovations in Malaria out of Tübingen und Lambaréné

10:25 am **Artur Scherf**, Institute Pasteur Paris, France
Metabolomics reveals a natural inhibitor of host cytoadhesion in Malaria

10:45 am **Martin Grobusch**, Amsterdam Medical Center, The Netherlands
Antimalarial drug resistance from an European perspective

11:05 am **Samuel Wagner**
Rectorate of the Eberhard Karls University of Tübingen
Message from the Vice President of the University of Tübingen

11:15 am - 11:45 am **Coffee break**
Main lobby

Session II: Clinical Research and Translation

Chairs: Michael Ramharter & Sabine Belard

11:45 am **Jana Held**, Institute of Tropical Medicine Tübingen, Germany
Developing a non-artemesinin-based Malaria treatment: SINDOFO Project

11:55 am **Matthew McCall**, Radboud University Medical Center, The Netherlands
Pre-erythrocytic and transmission-blocking efficacy of ProC6C-AlOH/Matrix-M vaccine against controlled human malaria infection in Malian adults

12:05 pm **Florian Kurth**, Charité Berlin, Germany
Severe imported Plasmodium falciparum malaria with hyperparasitaemia: evaluation of predictors for critical disease in adult returning travelers

12:15 pm **Pinkus Tober-Lau**, Charité Berlin, Germany
Comparative analysis of initial Full Blood Counts in adults with P. falciparum malaria in endemic (Gabon) and non-endemic (Germany) settings: insights into disease severity and previous exposure

12:25 pm **Jeannot Zinsou**, Centre de Recherches Médicales de Lambaréné, Gabon
Safety and reactogenicity of the Malaria Vaccine candidate AnAPN1 in healthy adults in Gabon: a randomized, controlled, phase 1, dose-escalation clinical trial

12:35 pm **Jean Claude Dejon Agobe**, Centre de Recherches Médicales de Lambaréné, Gabon
Clinical assessment of the safety and efficacy of Artemisia annua infusion preparation for the treatment of asymptomatic Plasmodium falciparum infection in semi-immune adults in Gabon

12:45 pm **Jean Claude Dejon Agobe**, Centre de Recherches Médicales de Lambaréné, Gabon
Phase I trial for the establishment of controlled human malaria infection by bites of Plasmodium falciparum-infected Anopheles gambiae in Lambaréné

12:55 pm **Christian Chassem Lapue**, Centre de Recherches Médicales de Lambaréné, Gabon
Haemoglobin levels of patients infected with Plasmodium spp. compared with those of patients coinfected with Plasmodium spp. and helminths in Moyen-Ogooué, Gabon

1:05 pm **Christian Chassem Lapue**, Centre de Recherches Médicales de Lambaréné, Gabon
Characteristics of submicroscopic Plasmodium falciparum infection in a rural and semiurban area of Gabon, Central Africa

1:15 pm - 2:15 pm **Lunch**
Main lobby

Session III: Diagnostics and Immunology

Chairs: Andrea Kreidenweiss & Rolf Fendel

2:15 pm **Ghyslain MOMBO-NGOMA**, Bernhard-Nocht-Institute of Tropical Medicine Hamburg, Germany
Point of care diagnosis of Malaria in Saliva samples (PROMISE)

2:25 pm **Andrea Kreidenweiss**, Institute of Tropical Medicine Tübingen, Germany
Molecular detection of malaria diagnostic-resistant pfhrp2/pfhrp3 deleted Plasmodium falciparum strains

2:35 pm **Constanze Heinzel**, Institute of Tropical Medicine Tübingen, Germany
Establishment of a saliva-based Plasmodium falciparum histidine-rich

protein 2 (pfHRP2) ELISA for the non-invasive detection of malaria

2:45 pm **Albert Lalremruata**, Institute of Tropical Medicine Tübingen, Germany
Mobile laboratory for Malaria: field evaluation of a simplified PCR-Free detection assay (Phase 1)

2:55 pm **Nouhoun Barry**, Institute of Tropical Medicine Tübingen, Germany
Evolution of Malaria antigen-specific immune responses and antibody-dependent respiratory burst activity in longitudinal Controlled Human Malaria Infection model

3:05 pm **Rajagopal Murugan**, Leiden University Medical Center, The Netherlands
Immunity against protective late-liver stage arresting GA2 parasites

3:15 pm **Galal Metwally, Nahla**, Bernhard-Nocht-Institute of Tropical Medicine Hamburg, Germany
Role of human miRNAs in the pathogenesis of human malaria caused by Plasmodium falciparum infection

3:25 pm **Karolina Krämer**, MVZ Dr. Stein + Kollegen GbR, Germany
Diagnosing malaria with a fluorescence flow cytometer (XN-31, Sysmex Deutschland GmbH): A 4 years prospective study of diagnostic accuracy of XN-31 in comparison to microscopy or PCR in a private laboratory

3:35 pm **Lara Bardtke**, Charité Berlin, Germany
The role of the innate immune system in semi-immunity to malaria

3:45 pm - 4:15 pm **Coffee break**
Main lobby

Session IV: Epidemiology

Chairs: Jürgen May & Steffen Borrmann

4:15 pm **Bryan Nyawanda**, Swiss Tropical and Public Health Institute, Switzerland
Bayesian spatio-temporal modelling to assess the impact of climate variability and control interventions on the burden of malaria in Kenya

4:25 pm **Anton Beloconi**, Swiss Tropical and Public Health Institute, Switzerland
Age-structured malaria transmission modelling incorporating the effects of climate and control interventions

4:35 pm **Ayten Sultanli**, Institute of Tropical Medicine Tübingen, Germany
Management of Malaria in Neonates: a Systematic Review

4:45 pm **Hounmenou Zinsou Alex**, Centre de Recherches Médicales de Lambaréné, Gabon
Malaria and hypertension among volunteers attending the health services of Lambaréné in Gabon

4:55 pm **Dollon Jacques Mbama Ntabi**, Congolese Foundation for Medical Research, Congo
Contribution of Anopheles gambiae s.l. to malaria transmission during the dry season in Djoumouna and Ntoula villages in the Republic of the Congo

5:05 pm **Ikegbunam Moses**, Institute of Tropical Medicine Tübingen, Germany
NO evidence of piperaquine resistance in Southeast Nigeria

5:15 pm **Miriam Rodi**, Institute of Tropical Medicine Tübingen, Germany
Genetic diversity of Plasmodium malariae in sub-Saharan Africa: a two-marker genotyping approach for molecular epidemiological studies

5:25 pm **Francis Bohissou**, Institute of Tropical Medicine Tübingen, Germany
Molecular markers of resistance to sulfadoxine-pyrimethamine in Plasmodium falciparum isolates from children living in two rural areas in Southern Benin

6:45 pm **Scientific Networking Dinner**
Neckawa

Friday, April 4

08:45 am **Registration**
Main lobby - with welcome coffee

Session IV: Epidemiology (continued)

Chairs: Thirumalaisamy P. Velavan & Ayola Akim Adegnika

9:00 am **Ralf Krumkamp**, Bernhard-Nocht-Institute of Tropical Medicine Hamburg, Germany
Recurrent events in clinical cohorts: modeling the occurrence of malaria infections

9:10 am **Mandeep Kaur**, European Vaccine Initiative Heidelberg, Germany
Exploring cultural beliefs, practices, and perceptions of pregnancy and vaccination: a focus on placental Malaria and vaccine acceptability among pregnant women in Malawi

9:20 am **Jordi Landier**, French National Research Institute for Sustainable Development, France
Plasmodium falciparum carriage in a population under long-term, intensive malaria control: a one-year cohort study in Kedougou region, Senegal

9:30 am **Juliana Inoue**, Institute of Tropical Medicine Tübingen, Germany
High-resolution dynamics of Plasmodium falciparum genotype and Plasmodium species diversity in asymptomatic adults in Gabon

9:40 am **Alabi Ayodele**, Centre de Recherches Médicales de Lambaréné, Gabon
Trends in malaria incidence in children living in Lambaréné, Gabon, pre and post Covid-19 pandemic (2014-2024)

9:50 am **Rella Zoleko-Manego**, Bernhard-Nocht-Institute of Tropical Medicine Hamburg, Germany
Plasmodium falciparum infection and malaria prevalence: community and hospital survey in central region of Gabon

10:00 am **Josiane Honkpehedji**, Centre de Recherches Médicales de Lambaréné, Gabon
Prevalence and risk factors for Plasmodium spp. infection during pregnancy: evidence from antenatal care-based diagnosis

10:10 am **Olusola Ojurongbe**, Ladoke Akintola University of Technology, Nigeria
Genomic surveillance of Plasmodium falciparum resistance to Sulfadoxine-Pyrimethamine in pregnant women in Nigeria: insights from in-country nanopore sequencing

10:20 am - 10:50 am **Coffee break**
Main lobby

Session V: Parasitology

Chairs: Gabriele Pradel & Friedrich Frischknecht

10:50 am **Friedrich Frischknecht**, Heidelberg University, Germany
Experimental vaccination with a single dose injection of Plasmodium sporozoites

11:00 am **Gabriele Pradel**, RWTH Aachen University, Germany
What makes a man a man: Zinc finger proteins in male sexual development of Plasmodium falciparum

11:10 am **Sandra Bennink**, RWTH Aachen University, Germany
The regulation of translational control during human-to-mosquito transmission of Plasmodium falciparum gametocytes

11:20 am **Timo Reiß**, RWTH Aachen University, Germany
The complement protein C3b plays an essential role during merozoite invasion in red blood cells

11:30 am **Pablo Cortes**, Max Planck Institute for Infection Biology Berlin, Germany
PfDPAP2 is a crystalloid-associated protein essential for mosquito transmission of Plasmodium falciparum

11:40 am **Lina Maacke**, Interfaculty Institute of Biochemistry - University of Tübingen, Germany
Investigating the role of gdv1 as RNA during sexual commitment of Plasmodium falciparum

11:50 am - 12:50 pm **Lunch**
Main lobby

Session V: Parasitology (continued)

Chairs: Selidji Agnandji & Ghyslain Mombo-Ngoma

12:50 pm **Markus Ganter**, University Hospital Heidelberg, Germany
Discovery of small-molecule inhibitors of the Plasmodium falciparum protein kinase CRK4

1:00 pm **Anna Karolina Kneller**, Charité Berlin, Germany
Genotyping of Plasmodium falciparum sickle associated single nucleotide polymorphisms (Pfsa SNPs) in Africa

1:10 pm **Umit Y. Kina**, Humboldt University Berlin, Germany
Cytolysin secreting genetically attenuated Plasmodium yoelii parasites as whole sporozoite vaccines

1:20 pm **Oskar Werb**, Humboldt University Berlin, Germany
Uncovering diversity: complete mitochondrial genomes of African bat hepatocystis reveal conserved structure but unusual size

1:30 pm **Dearie Glory Okwu**, Centre de Recherches Médicales de Lambaréné, Gabon
Safety and tolerability of Artemether-Lumefantrine + Atovaquone-Proguanil tri-therapy for treatment of uncomplicated Malaria in adults and adolescents in Gabon- ASAAP Project - Pilot Study

1:40 pm **Lars Sippmann**, University Hospital Heidelberg
Determination of the food vacuole proteome of Plasmodium falciparum via proximitybiotinylation

1:50 pm **Christoph Wenz**, Heidelberg University, Germany
Assessing the impact of artemisinin resistance on P. falciparum transmission in insecticide resistant Anopheles gambiae.

2:00 pm - 2:30 pm **Coffee break**
Main lobby

Session V: Parasitology (continued)

Chairs: Francine Ntoumi, Jana Held & Meral Esen

2:30 pm **Lais Pessanha de Carvalho**, Institute of Tropical Medicine Tübingen, Germany
Boromycin: an antimalarial candidate with potent transmission-blocking activity

2:40 pm **Thomas Schäfer**, Institute of Tropical Medicine Tübingen, Germany
Dehalogenil: A novel compound with potent, multi-stage antimalarial activity and a high resistance barrier

2:50 pm **Victor Asua**, Institute of Tropical Medicine Tübingen, Germany
Evolution of Plasmodium falciparum resistance to antifolate antimalarial drugs in Uganda

3:00 pm **Mia Heuser**, Interfaculty Institute of Biochemistry - University of Tübingen, Germany
Investigation of the molecular functions of chromatin remodeling proteins during sexual commitment in the malaria parasite Plasmodium falciparum

3:10 pm **Dollon Jacques Mbama Ntabi**, Congolese Foundation for Medical Research, Congo
Assessment of molecular markers of knockdown resistance in the Anopheles population in Madibou and Goma Tse-Tse, Republic of Congo

3:20 pm **Emma Gladis Malinga**, Institute of Tropical Medicine Tübingen, Germany
Haematological recovery after antimalarial treatment of uncomplicated malaria in a subset of population of the 1-D-CURE study

3:30 pm **End of 16th Malaria Meeting**
Informal discussions with coffee

Arrival

By public transport from Tübingen main station:

- Bus lines 1, 2 and 6 to Lothar-Meier-Bau
- Bus lines 3 and 4 to Uni/Neue Aula

Public parking spaces available (6 - 10€ per day)

Scientific Networking Dinner

Neckawa
Wöhrdstrasse 25
72072 Tübingen

20 min walk from the ITM Tübingen.

Contact during the meeting

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Sponsors

This event is supported by BioMérieux Germany and Xpedite Diagnostics GmbH, each generously contributing €600. We sincerely appreciate their support in making this conference possible.

Abstracts

Session I - Opening

Date / Time: Thursday, April 03, 10:00 AM - 11:15 AM

Current innovations in Malaria out of Tübingen und Lambaréné

Author: Peter G. Kremsner

Metabolomics reveals a natural inhibitor of host cytoadhesion in Malaria

Author: Arthur Scherf

Antimalarial drug resistance from an European perspective

Author: Martin Grobusch

Developing a non-artemisinin-based Malaria treatment: SINDOFO Project

Dearie Glory Okwu[1,2], Ghyslain Mombo-Ngoma[1,2], Quique Bassat[3,4], Wenceslas Lendamba[1,2], Halidou Tinto[5], Innocent Valea[5], Moussa Lingani[5], Jessica Dalsuco[3,4], Carole Amroune[4], Bernhards Ogutu[6], Consolata Nganga[6], Etienne Guirou[7], Helen Demarest[7], Anne Claire Marrast[7], Conor Cahill[7], Hardik Patel[8], Siddharth Brahmbhatt[8], Vanesa Nkwouano[9], & Jana Held[9].

[1] Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon
[2] Bernhards Notch Institute of Tropical Medicine (BNITM), Hamburg, Germany
[3] Barcelona Institute for Global Health (ISGlobal), Spain
[4] Manhiça Health Research Center - Fundaçao Manhiça (CISM-FM)
[5] Centre National de la Recherche Scientifique et Technologique - Institut de Recherches en Sciences de la Santé (CNRST-IRSS), Burkina Faso
[6] Center For Research Therapeutic Sciences - Strathmore University (CREATES-SU), Kenya
[7] Medicines for Malaria Venture (MMV), Switzerland
[8] Zydus Lifesciences Limited, India
[9] Institute of Tropical Medicine, Travel Medicine and Human Parasitology, University Hospital Tuebingen (ITM-EKUT), Germany

Malaria remains a life-threatening disease, with an estimated 263 million cases and 597,000 deaths in 2023. Artemisinin-based Combination Therapies (ACTs) are the current first-line treatment, administered over three days. However, their effectiveness is increasingly compromised by poor patient adherence and rising resistance to artemisinins and partner drugs. While resistance first emerged in Southeast Asia, growing evidence now indicates its spread to sub-Saharan Africa, threatening malaria control efforts.

To counteract these challenges, a novel, non-artemisinin-based regimen with a shorter treatment duration is urgently needed. The SINDOFO project evaluates a promising combination therapy—ZY19489, a triaminopyrimidine, and ferroquine (FQ), a next-generation 4-aminoquinoline—administered either as a single-day treatment or over two consecutive days. Sponsored by Zydus Lifesciences Limited, the project consists of two clinical trials assessing the regimen's safety, efficacy, tolerability, and pharmacokinetics.

The first trial, a Phase Ib study in Lambaréné, Gabon, is a single-center, randomized, double-blind, placebo-controlled dose-escalation study in asymptomatic adults. It establishes safety, tolerability and pharmacokinetics across three cohorts of 12 participants each, monitored over 64 days. In each cohort, participants are randomized 2:1 to receive either ZY19489-FQ or a placebo. The second trial, a Phase II multicenter, randomized, controlled study, assesses the regimen's efficacy over 64 days. Conducted in Gabon, Burkina Faso, Mozambique, and Kenya, it involves 135 symptomatic adults, adolescents, and children, divided into three parallel cohorts. Participants are randomized 2:1 to receive either ZY19489-FQ or the standard of care.

Beyond advancing treatment options, SINDOFO prioritizes capacity building in Africa by decentralizing research responsibilities. Unlike conventional approaches that rely on Northern laboratories, this initiative strengthens regional expertise: Kenya leads informed consent formulation, Burkina Faso manages data systems, Mozambique ensures microscopy quality control, and Gabon conducts PCR-based molecular analysis. The project also supports PhD research, emphasizing gender balance in scientific leadership.

By fostering locally driven malaria research and innovation, SINDOFO not only contributes to the fight against drug-resistant malaria but also establishes a sustainable framework for future scientific advancements.

The SINDOFO project is part of the EDCTP2 Program supported by the European Union.

Keywords: ZY19489, Ferroquine, Safety, Tolerability, Pharmacokinetics, capacity-strengthening.

Pre-erythrocytic and transmission-blocking efficacy of ProC6C-AlOH/Matrix-M vaccine against controlled human malaria infection in Malian adults

Mamady Kone[1], Adama Sacko[1], Moussa Thienta[1], Daman Sylla[1], Bourama Kamate[1], Jordan Plieskatt[2], Markus Gmeiner[3], Bayaya Haidara[1], Abdoulaye Katile[1], Moussa B. Kanoute[1], Moussa Diallo[1], Bakary Traore[1], Abdrahamane Fofana[1], Ousmane A Poudiouguo[1], Boubacar Coulibaly[1], Hawa S Bayogo[1], Yacouba Dembele[1], Ibrahim Diarra[1], Salifou M Kone[1], Issa Diallo [1], Sekou Goita[1], Karim Bengaly[1], Moridie Sidibe[1], Melissa Kapulu[4], Amagana Dolo[1], Allaye Tolo1[1] , M'Bouye Doucoure[1], Souleymane Traore[1], Mahamadou L Diarra[1], Amatigue Zeguime[1], Teun Bousema[3], Ebenezer Ofori[2], Sale Sidibe[1], Yacouba Diarra[1], Mohammad Naghizadeh[1], Katharine Collins[3], Mahamadou S Sissoko[1], Matthew BB McCall[3], Michael Theisen[2], Issaka Sagara[1].

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Multi-stage malaria vaccines against offer distinct potential by compensating for sub-optimal stage-specific protection and inhibiting the spread of escape mutants. ProC6C is a recombinant pre-erythrocytic and transmission-blocking vaccine against *Plasmodium falciparum* (*Pf*), consisting of the Pro domain of Pfs230 (Pro) coupled to the 6-Cysteine C-terminal fragment of Pfs48/45 (6C) through a spacer sequence containing minor and major repeat regions of PfCSP. The transmission-blocking efficacy of ProC6C was demonstrated in recent field studies in Burkina Faso and Mali, but its pre-erythrocytic efficacy in humans has not yet been assessed. We previously developed a Controlled Human Malaria Infection model in African adults to include assessment of transmission-blocking interventions (CHMI-Trans). Here we assessed the safety, tolerability and immunogenicity of ProC6C-AlOH/Matrix-M vs. rabies vaccine control, as well as its pre-erythrocytic and transmission-blocking efficacy against CHMI-Trans, in healthy Malian adults in a randomized, double-blind trial.

Participants were enrolled following serological stratification for prior malaria exposure and randomised 1:1 to receive 3 doses of ProC6C or rabies vaccine at monthly intervals. Three months post-3rd dose, n=32 participants were inoculated intradermally with 22,500 PfSPZ Challenge (NF54) and followed daily for assessment of safety parameters and parasitemia by blood smear and (retrospectively) 18S qPCR. Upon parasitemia >1000 *Pf*/µL, or any parasite density with ≥1 malaria symptoms, participants were treated with a sub-curative dose (480mg) of piperaquine, in order to suppress asexual parasites but allow gametocyte development to continue. All participants underwent direct skin feeding (DSF) assays with 2x 30 *Anopheles coluzzii* on their forearms on 1-3 occasions, with timing based, where applicable, on the detection of gametocytes by thick blood smear. At end of study, all participants were treated with a standard curative regimen of artemether/lumefantrine plus a stat dose of primaquine (0.25 mg/kg) to kill any residual gametocytes. The primary efficacy endpoint was time to *Pf* parasitaemia by qPCR; exploratory efficacy endpoints included *Pf*-oocyst positivity and density in mosquitoes following DSF on gametocytaemic participants.

Severe imported *Plasmodium falciparum* malaria with hyperparasitaemia: evaluation of predictors for critical disease in adult returning travelers

Tilman Lingscheid[1], Johannes Jochum[2], Pinkus Tober-Lau[1], Johanna Schöllgen[1], Regina Stegherr[3], Juliane Dörfler[1], Frieder Pfäfflin[1], Leif Erik Sander[1,4,5], Thomas Zoller[1], Michael Ramharter[2,6,7], Florian Kurth[1,4,6].

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[4] German Center for Lung Research, DZL, Berlin, Germany
[5] Berlin Institute of Health, Berlin, Germany
[6] Centre de Recherches Médicale de Lambaréné, Gabon
[7] DZIF Hamburg Borstel

Background:
Severe *Plasmodium falciparum* (P.f.) malaria remains a major health threat for travellers. WHO has defined criteria for severe malaria, including hyperparasitaemia ≥10%, as major risk factors for adverse outcome. Additionally, WHO recognizes "uncomplicated hyperparasitaemia" (4-10%), a parasite density usually defining severe malaria outside endemic areas. Overall, the role of hyperparasitaemia as risk factor in imported severe malaria is unclear, with most data predating the artemisinin era.

Methods:
We retrospectively analysed adult in-patients with hyperparasitaemia (≥4%) and/or severe P.f. malaria according to WHO criteria who received artemisinin-based treatment at two German university hospitals 2013-2023, to assess the risk for critical disease with need for organ replacement therapy or vasopressors. Based on multivariable nominal logistic regression results, we developed a scoring system to predict critical disease, which was validated on an independent patient cohort.

Results:
Of 168 included patients, 33 (20%) developed critical disease. All critical patients presented with at least one WHO criterion other than hyperparasitaemia, while none of the 72 patients with isolated hyperparasitaemia developed critical disease. Logistic regression confirmed that hyperparasitaemia on admission was not an independent indicator of critical disease (aOR 0.85 95%CI 0.23-3.12) in contrast to creatinine >3mg/dL (aOR 6.74 95%CI 1.06-42.75), oligo-/anuria (aOR 5.94 95%CI 1.27-27.82), lactate >5mmol/L (aOR 8.16 95%CI 8.16-35.03), confusion (aOR 4.07 95%CI 1.39-11.94), and circulatory shock and respiratory failure, which are inherently critical conditions. The risk score accurately identified patients at risk for critical disease (AUC=0.99) and correctly identified all 33 critical patients with, and 131/135 (97.0%) without critical disease. In an independent cohort, the score and threshold correctly identified all 8 patients with critical and 39/44 (88.6%) patients without critical disease.

Conclusion:
A weighted scoring system based on the WHO criteria for severe malaria can improve identification of patients with highest individual risk for critical disease, whereas isolated hyperparasitaemia is no independent risk factor in patients treated with artemisinins.

Comparative analysis of initial Full Blood Counts in adults with *P. falciparum* malaria in endemic (Gabon) and non-endemic (Germany) settings: insights into disease severity and previous exposure

Pinkus Tober-Lau[1], Cäcilie von Wedel[1,2], Lars C. Matthies[2,3], Clemens Dierks[1,4], Michael Ramharter[2,3,5,] Ghyslain Mombo-Ngoma[2,6,7], Johannes Mischlinger[2,5,] Florian Kurth[1,2].

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[2] Centre de Recherches Médicales de Lambaréné, Gabon
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[7] Section of Implementation Research, Bernhard Nocht Institute for Tropical Medicine & I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Germany

Background:
The clinical presentation of individuals infected with *Plasmodium falciparum* is exceptionally diverse, ranging from asymptomatic parasitemia to life-threatening disease. Frequent previous exposure to *Plasmodium spp.* results in partial protection from severe disease. However, this protection wanes in individuals emigrating from holo-endemic regions and there are currently no reliable biomarkers that accurately indicate this acquired semi-immunity.

Methods:
Data were analyzed from 1392 *P. falciparum* infected adults with varying disease severity in Gabon and Germany. Full blood count (FBC) parameters and ratios were evaluated individually and as a combined ensemble-based machine learning (ML) classifier to predict disease severity, ranging from asymptomatic infection to severe malaria. As a secondary objective, the influence of previous exposure to *Plasmodium spp.* on FBC parameters was assessed.

Results:
Comparing asymptomatic parasitemia with uncomplicated malaria in Gabonese and uncomplicated with severe malaria in German patients revealed significantly lower platelet (218 vs 150 x103/µl, $p<0.0001$, and 85 vs 40 x103/µl, $p<0.0001$, respectively) and higher neutrophil counts (2.32 vs 2.57 x103/µl, $p=0.0037$, and 3.08 vs 4.49 x103/µl, $p<0.0001$, respectively) in those with greater infection severity. The ML classifier outperformed single parameters in differentiating infection severity in both comparisons (AUROC 0.94 and 0.84, respectively). Conversely, lymphocyte and monocyte counts showed a pattern that follows the level of previous malaria exposure, with lower cell counts in naive compared to previously exposed patients, regardless of infection severity.

Conclusions:
The value of simple FBC parameters for classification of *P. falciparum* infection severity and previous exposure is considerable. The accuracy can be increased by integrating individual parameters into a joint ML model.

Safety and reactogenicity of the Malaria Vaccine candidate AnAPN1 in healthy adults in Gabon: a randomized, controlled, phase 1, dose-escalation clinical trial

JF Zinsou[1,2], GC Ongouta[1], JR Edoa[1], Judith Barth[1], E Dansou[1], L Dahmash[3], R Bikangui[1], R Adamou[1], Benjamin Mordmüller[5], RR Dinglasan[3] and AA Adegnika[1,2,4].

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[2] Institut für Tropenmedizin (ITM), Universitätsklinikum Tübingen, Tübingen, Germany

[3] University of Florida, Department of Infectious Diseases & Immunology and Emerging Pathogens Institute, College of Veterinary Medicine, Gainesville, FL USA

[4] Deutsches Zentrum für Infektionsforschung (DZIF), Tübingen, Germany

[5] Department of Medical Microbiology, Radboud UMC, Nijmegen, The Netherlands

Background:

Malaria remains a major global public health issue in endemic regions. In preclinical studies, the recombinant AnAPN1 vaccine candidate has shown promising activity. Here, we assessed the safety, tolerability, and reactogenicity of three doses of the AnAPN1 vaccine, formulated with and without the GLA-LSQ (glucopyranosyl lipid adjuvant containing QS21/saponin) adjuvant, administered to healthy adults.

Method:

A randomized, double-blind, controlled, dose-escalation phase1 clinical trial was conducted in Lambaréné, Gabon among 33 healthy adult volunteers aged 18-45 years. Participants were randomly assigned to one of 3 ascending dose groups. In each cohort, 9 participants received AnAPN1 with GLA-LSQ and 2 received the vaccine without adjuvant. The 20µg, 50µg, and 100µg vaccine doses were administered intramuscularly at days 0, 28, and 56-day in the first, second, and third cohorts, respectively. The vaccine's safety and reactogenicity were evaluated by means of close participant monitoring for any adverse effects occurring during and after vaccinations.

Results:

Between October and December 2023, 55 participants were screened, of whom 33 eligible participants were recruited and allocated to either the 20µg, 50 µg, or 100µg dose in 3 sequential cohorts of 11 individuals each. The median age is 29 years (range: 18-43 y). Participants in cohort 1 (n=10), cohort 2 (n=10) and cohort 3 (n=11) received the complete 3-dose vaccination schedule. The most frequently solicited local adverse events were mild to moderate injection site pain, representing 92% (44/48) of local events, and mild headache 36% (29/81) and nausea 19% (15/81) at a systemic level. No vaccine related serious adverse events was reported.

Conclusion:

Overall, these results suggest that the administration of the AnAPN1 malaria candidate vaccine is safe and well tolerated in healthy Gabonese adults.

Clinical assessment of the safety and efficacy of *Artemisia annua* infusion preparation for the treatment of asymptomatic *Plasmodium falciparum* infection in semi-immune adults in Gabon

Jean Claude Dejon Agobé[1,2], Christian Chassem Lapue[1], Peggy Gandia[3], Marc Litaudon[4]. Ayola Akim Adegnika[1,2], Steffen Borrmann[1,2].

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Background:
The use of *Artemisia annua* dried leaf infusions for the treatment of malaria is increasing in sub-Saharan countries. The increase in the availability of *A. annua* plants combined with the recurrence of malaria attacks in endemic communities where ACTs, although effective for the treatment of malaria, are not always available or available at high prices, could explain the situation. However, because of the lack of robust evidence on the clinical efficacy of *A. annua* infusion, mainly due to the quality of existing trials, WHO currently discourages the use of *Artemisia* herbal preparations. To address this research gap and highlight the potential risk or benefit of the use of *Artemisia* preparations, the present study aims to assess the antiparasitic action of *A. annua* infusion, establish the pharmacokinetics of artemisinin and associated compounds in plasma, and assess its potential role in malaria transmission.

Methods:
We will conduct a randomised, open-label, superiority trial to compare *Artemisia annua* infusion against placebo (*Centaurium erythrea* infusion) for the clearance of asymptomatic *P. falciparum* infections in adult volunteers. The participants will be treated with an infusion of 5 g of *A. annua* dried leaves in 1 L of water given in three doses per day for 7 consecutive days. Follow-up will be for 28 days in total to capture recrudescent infections. The detection of *Plasmodium* parasites will be performed via Giemsa-stained thick blood smears and qPCR. We will randomise 20 volunteers to receive either *Artemisia annua* infusion (group A) or placebo infusion (group B). Should 10 out of 10 volunteers in group A be asexual parasite free by day 7 (the pre-defined criterion for efficacy) but also more than 6 volunteers in group B (spontaneous clearance), we will randomise 20 additional volunteers in group A and B. The primary endpoint is clearance of asymptomatic, asexual parasitaemia by microscopy.

Outlook:
The study is designed to demonstrate the capacity of *Artemisia annua* infusion to clear 100% of asymptomatic *P. falciparum* infections in semi-immune adult volunteers by day 7, our criterion for efficacy. We will present the design of the study and discuss the potential role for *Artemisia annua* infusion in the treatment of malaria.

Phase I trial for the establishment of controlled human malaria infection by bites of *Plasmodium falciparum*-infected *Anopheles gambiae* in Lambaréné

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Background: Controlled human malaria infection (CHMI) is a model for investigating immunity to malaria and antimalarial interventions. The use of laboratory-adapted *P. falciparum* (Pf) strains in such a model to infect volunteers does not take into account the genetic heterogeneity of *Plasmodium* infections typically present in malaria-endemic regions, and might not therefore predict the efficacy of vaccine candidates or antimalarial drugs in endemic settings. Our objective is to establish a safe protocol for the controlled infection of healthy semi-immune adult volunteers in Gabon with local strains of Pf via bites of infected *A. gambiae s.s.*

Methods: *P. falciparum*-infected *A. gambiae* Kisumu (Pf-iAgK) strain mosquitoes will be produced via membrane feeding of a gametocyte-containing Pf isolate obtained from a suitable local donor. First, 10 healthy semi-immune adults will be exposed to the bite of five Pf-iAgK mosquitoes. If at least 50% of exposed participants develop qPCR-positive blood stage infections during daily follow-up until day 28, 10 additional participants will be included to reach the 20-target sample size. Otherwise, the same procedure will be applied, using 10 Pf-iAgK mosquitoes per participant. The overall aim is to determine the number of Pf-iAgK needed to infect at least 80% of semi-immune adult volunteers. We will also address secondary parasite genetic and immunological research questions.

Outlook: We will present the design of the study and discuss potential future research avenues using this new platform.

Haemoglobin levels of patients infected with *Plasmodium* spp. compared with those of patients coinfected with *Plasmodium* spp. and helminths in Moyen-Ogooué, Gabon.

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Background:

Malaria, caused by Plasmodium protozoa, is the deadliest parasitic disease worldwide; it affects red blood cells and leads to significant morbidity and mortality. This study aimed to compare hemoglobin levels between malaria-infected individuals and those coinfected with Plasmodium and helminths in Moyen-Ogooué, Gabon.

Methods:

This was a cross-sectional study conducted from September 2022 to September 2023 in which participants were randomly included. Soil-transmitted helminths (STHs) were diagnosed via the Kato–Katz, coproculture, and Harada–Mori techniques, whereas Plasmodium and filaria infections were identified via thick blood smears and leucoconcentration. Hematological parameters were analysed via a Pentra ABX60 analyser.

Results:

A total of 142 participants infected with *Plasmodium spp* were included in the present analysis. Among them, 54 (38%) were co-infected with helminths, which included STHs (22%), *Schistosoma haematobium* (12%) and filariae; *Loa-loa* and *Mansonela perstans* (11%). The mean haemoglobin (±SD) was 10.6 (±2) among malaria mono-infected individuals and 11.4 (±1.6) among those co-infected. A linear regression analysis revealed a statistically significant association between haemoglobin level and co-infection (β=0.57, 95% CI: -0.01 – 1.15, p=0.05), and age (β=0.05, 95% CI: 0.04 – 0.07, p=<0.001). Stratified analysis on sex shown a stronger association between haemoglobin level and co-infection in women (β=0.74, 95% CI: 0.02 – 1.46, p=<0.04), while this association disappeared in men (β=0.55, 95% CI: -0.03 – 1.13, p=0.07).

Conclusion:

In summary, the presence of helminths in individuals infected with *Plasmodium* spp. appears to have a positive effect on haemoglobin, particularly in women. Further investigations are needed to better understand these interactions.

Funding source: DZIF

Characteristics of submicroscopic *Plasmodium falciparum* infection in a rural and semiurban area of Gabon, Central Africa

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Background:
Submicroscopic *Plasmodium falciparum* infection (SmPfI) is a low-density parasitemia that cannot be identified by standard microscopy; instead, it can be detected only by molecular diagnostic methods. It is often asymptomatic and promotes the transmission of the parasite, particularly in sub-Saharan regions. This analysis aims to describe the characteristics of patients with SmPfI and investigate associated factors.

Methods:
This is an ongoing cross-sectional hospital- and community-based study that started in April 2024. A questionnaire was administered to each participant to collect signs or symptoms at inclusion, and vital parameters were recorded. Blood samples were collected and tested for the detection of malaria parasites via microscopy and RT–qPCR. Cell blood counts were performed via a Pentra ABX60 analyser.
Results: Out of the 309 participants included in the present analysis, 195 (63%) were positive for *P. falciparum* infection and constituted our study population. Among them, 125 (64%) were positive by RT-qPCR only and constituted the SmPfI group, whereas their positive counterparts by microscopy constituted the microscopic *P. falciparum* infection (mPfI) group. Compared with those in the mPfI group, participants in the SmPfI group were older (19.6 *vs* 31.0 years old, $p<0.001$) and presented significantly higher platelet levels (201800 *vs* 236500 platelets/l, $p=0.02$), whereas only a trend toward an increased haemoglobin level (11.3 *vs* 12.1 g/l, $p=0.05$) was observed. No significant differences were observed for location ($p=0.20$) or sex ($p=0.73$) between the groups, but a trend toward a lower proportion of participants with symptoms (60% *vs* 44%, $p=0.05$) was observed in the SmPfI group. In the linear multivariate analysis adjusted for age and sex, the higher platelet count in the submicroscopic group remained statistically significant (β=49280, 95% CI: 20800 – 77760, *p=0.001*), whereas the difference in the hemoglobin level disappeared (β=0.36, 95% CI: -0.44 – 1.17, *p=0.38*).

Conclusion:
SmPfI is highly prevalent in our population, positively associated with age, with no additional effect on haemoglobin levels compared with microscopic infection.

Funding source: DZIF

Point of care diagnosis of Malaria in Saliva samples (PROMISE)

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Background:
Using blood samples for malaria diagnostic tests has limitations due to blood taboos, patient inconvenience, needle-sticks injuries with potential disease transmission, and complications after repeated sampling in anaemic patients or children. A rapid point-of-care test suited for home and field station testing, based on easily assessable body fluids, with a performance on par with the golden standard in clinical routine (microscopy), is therefore needed for diagnosis of suspected cases.

Objectives: The main goal is to develop a prototype of a qualitative, binary Lateral Flow (LF) malaria test and to document the specifications of the test in validating clinical trials primarily in sub-Saharan African countries, and South Korea.

Methods:
In a disruptive approach, we use the activity of Plasmodium enzyme Topoisomerase 1 (pTOP1) as biomarker, as this allows for much improved sensitivity compared to current rapid tests, while maintaining the simplicity of a lateral flow home-test. Part of our concept is also a point-of-care device for saliva sample management. The PROMISE LF device is a binary (yes-no) test targeted for use as home test and low-resource field settings. The test is done in two steps. First, a saliva sample is collected with a simple device before being transferred to a lateral flow stick. There are two readouts: Yes (red coloured test line) for positive infection and No (blank test line) for malaria negative.

Expected outcome:
The novel LF device will ensure sensitive and rapid malaria diagnosis of individuals who do not accept blood withdrawal and ameliorate complications of repeated blood sampling. The current gold standard of malaria diagnosis in clinical routine is light microscopy which often is unavailable in malaria-endemic regions. Here, antigen rapid diagnostic tests (RDT) are usually used. Compared to RDT, the LF provides a higher sensitivity owing to the choice of biomarker, also allowing use of saliva instead of blood.

Molecular detection of malaria diagnostic-resistant *pfhrp2/pfhrp3* deleted Plasmodium falciparum strains

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Plasmodium falciparum parasites with deletions in the histidine-rich protein 2 and 3 genes, pfhrp2 and pfhrp3, pose a significant challenge to malaria diagnostics and control efforts by evading detection through HRP2-based rapid diagnostic tests (RDTs). This diagnostic resistance jeopardizes effective treatment and surveillance strategies. First identified in South America in 2007, pfhrp2/pfhrp3 gene deletions have now been documented across Africa, including East, Central, and West Africa. Their emergence may stem not only from increased use of PfHRP2-based RDTs but also from pre-existing genetic diversity in P. falciparum populations.

We investigated the prevalence of pfhrp2- and pfhrp3-deleted strains across multiple sites in Africa, including Ethiopia, Central, and West Africa. The analysis, using a highly sensitive multiplex qPCR, revealed varying prevalence of gene deletions, with East Africa showing higher rates compared to other regions. Despite generally low prevalence in Central and West Africa, double-deleted strains were identified in some areas. These findings highlight the potential for false-negative RDT results in specific regions, particularly where gene deletions are more prevalent. Continuous monitoring is essential to detect shifts in prevalence and to safeguard the effectiveness of RDTs in malaria control programs.

Establishment of a saliva-based *Plasmodium falciparum* histidine-rich protein 2 (*pf*HRP2) ELISA for the non-invasive detection of malaria

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To this day, malaria caused by *Plasmodium falciparum* remains a leading cause of death in young children in Africa. Rapid diagnosis and treatment are crucial to reduce the disease burden. For this project, we explored a non-invasive diagnostic option, which could be particularly beneficial for infants. We established a highly sensitive ELISA detecting *pf*HRP2 in saliva, with additional reactivity to *pf*HRP3. During ELISA optimisation, multiple capture and detection antibody pairs, various buffers and incubation conditions were compared. The assay detection limit for HRP2 was low at approximately 5 pg/ml. A limited number of malaria-positive children from Gabon (n=4) and spiked saliva samples served as positive controls. The background signal of the extensive negative cohort (n=76) was minimal, showing a favourable positive-to-negative ratio. In summary, a sensitive, salivary *pf*HRP2 ELISA was developed, which can be extensively tested in the next phase with field samples.

Additionally, a second protocol for *pf*HRP2-specific detection was established without cross-reactivity to *pf*HRP3. This tool could help discover HRP2 deletions in *Plasmodium falciparum*.

Mobile Laboratory for Malaria: Field Evaluation of a Simplified PCR-Free Detection Assay (Phase 1)

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Background:
Accurate diagnosis is essential for effective case management of malaria infection. Microscopy and antigen-detecting rapid diagnostic tests (RDTs) are the most widely used malaria diagnostic tools for clinical management and surveillance; however, these diagnostic tests have limited sensitivity in detecting low-density infections. Accurate identification of submicroscopic parasitemia is particularly important for malaria elimination campaigns and research, such as controlled human malaria infection studies. Ultra-sensitive nucleic acid amplification technology (NAAT) assays, such as reverse transcription quantitative PCR (RT-qPCR), achieve detection levels of 6 parasites/mL (0.06 parasites per µL); however, the complexity of PCR workflows and reliance on sophisticated laboratory equipment limit their use to centralized facilities and result in long diagnostic turnaround times. To address these limitations, our DZIF-funded malaria diagnostics project (TTU 03.811, MALDx) aims to simplify NAAT-based malaria diagnostics for rapid field use.

Methods:
First, we simplified nucleic acid extraction by replacing traditional silica spin column purification with a magnetic bead suspension method that eliminates the need for high-speed centrifugation. Next, we developed a novel isothermal amplification technique based on reverse transcription-recombinase-polymerase-aided amplification (RT-RAA) as an alternative to PCR. We then streamlined workflows to enable molecular diagnosis of malaria directly in the field using a mobile suitcase laboratory powered by a solar panel battery. Finally, we evaluated the performance of the RT-RAA assay compared to microscopy, RDT, and ultrasensitive RT-qPCR in a prospective field trial in Lambaréné and surrounding villages in Gabon. The sensitivity of RT-RAA was assessed using two nucleic acid extraction methods.

Results:
Between April and June 2024, we evaluated 165 individuals. The sensitivity of RT-RAA reached 64.4% (extraction method 1) and 98.7% (extraction method 2) compared to ultrasensitive RT-qPCR and was superior to the sensitivities of microscopy and RDT. Cohen's kappa agreement between RT-RAA and reference was good for method 1 ($\kappa = 0.61$) and excellent for method 2 ($\kappa = 0.98$); in contrast, agreement was poor for microscopy ($\kappa = 0.27$) and fair for RDTs ($\kappa = 0.41$).

Conclusion:
This innovative and simplified molecular assay shows significant potential as a point-of-need screening or diagnostic test, offering high sensitivity comparable to ultrasensitive RT-qPCR. It can be used in settings where such diagnostic accuracy is critical, including drug/vaccine trial screening in endemic areas, mass drug administration test-and-treat campaigns, and intermittent screening and treatment (ISTp) for pregnant women.

Evolution of Malaria Antigen-specific immune responses and Antibody-Dependent Respiratory Burst activity in longitudinal Controlled Human Malaria Infection model

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Background:
Malaria remains a significant global health burden, particularly in endemic regions such as Gabon. Understanding the immune response to *Plasmodium falciparum* infections is crucial for understanding the disease and vaccine development. This study evaluates the immune response profile to 13 *P. falciparum* antigens covering pre-erythrocytic stage as well as blood stage and explores the correlation between immune response magnitude and Antibody-Dependent Respiratory Burst (ADRB) activity using freshly isolated neutrophils in a Controlled Human Malaria Infection (CHMI) study.

Methods:
Participants from a malaria-endemic area in Gabon received six infections of Pf sporozoites (*PfSPZ)* Challenge at a dose of 3,200 parasites at eight-week intervals. Plasma samples were collected at multiple time points to quantify antigen-specific antibody responses using Bio-Plex assay. ADRB activity was assessed through a luminol-based chemiluminescence assay. Correlations between antibody levels and ADRB activity were analyzed using Spearman's rank correlation.

Results:
Antibody responses varied across the 13 *P. falciparum* antigens. AMA-1 antigen has the higher response ranging from 1,000 to 10,000 MFI and eTRAMP-5 the lowest response from 25 to 100 MFI. A significant correlation was observed between blood stage antigen-specific antibody levels and ADRB activity ($P<0.005$), suggesting a potential functional role of these antibodies in antiparasitic neutrophil activity.

Conclusions:
This study provides insight into the immune response against multiple *P. falciparum* antigens in a malaria-endemic setting. The observed correlation between antibody responses and ADRB activity highlights the importance of functional immunity in malaria protection. These findings contribute to the selection of promising antigenic targets for malaria vaccine development.

Immunity against protective late-liver stage arresting GA2 parasites

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Whole sporozoite based immunization remains a valuable strategy to induce protective immune responses against *Plasmodium falciparum* (Pf) malaria in humans. Previous studies indicate that parasite attenuation at distinct life stages impacts the induction of protective immunity. The most promising approach had been chemo-attenuating the blood stage parasites upon immunizing with live metabolically active sporozoites.

Here we explored genetic attenuation (GA) strategies that arrest the parasite at key intra-hepatocytic development stages in the liver. Our clinical results illustrate the superior immunogenicity and protective capacity of the parasites arresting late (GA2) compared to early (GA1) during liver stage development. Our results highlight the potential of late liver stage antigens in inducing protective immunity and merit further exploration for future malaria vaccine programs.

Role of human miRNAs in the pathogenesis of human malaria caused by *Plasmodium falciparum* infection

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Malaria infection is known to severely compromise endothelial cells (EC). The pathogenic consequences of ECs activation might lead to organ failure and death. The organ-specific response of the endothelium has not been described in detail. It is not known whether microRNAs play a specific role in the complications of severe malaria, although microRNAs control 60% of the genes expressed in the human body.

In this study, we report microRNA candidates that are specifically expressed in both ECs types and their secreted extracellular vesicles (EVs). We were able to show that shear stress plays a role in the switching on of variable signaling pathways in brain ECs, such as IL-8 signaling and tight junctions. Specific miRNAs that we found to be differentially expressed in the ECs seem to control these pathways. Incubation with ring stage infected red blood cells (iRBCs) results in activation of endocytic pathways in brain ECs. In contrast, in lung cells, the most prominent activated pathway was the electron transport pathway which was found to be activated in immune cells during inflammation. Data analysis showed that endocytosis and electron transport pathways were targeted by some miRNA candidates. These miRNAs were significantly altered after 8 hours of coincubation with ring stage iRBCs at a shear stress of 1.5 dyne/cm2.

We hypothesize that EC dysfunction is a precursor to severe malaria complications. This disrupts the balance between vasoconstriction and vasodilation, predisposing to cytoadhesion of iRBCs, endothelial proliferation and blood brain barrier leakage. Certain miRNA candidates may be involved in the control of these events within ECs. Through cell-to-cell communication between iRBCs and ECs, these miRNAs can sense the presence of iRBCs and thus stimulate the initiating signaling pathways to respond to infection.

Diagnosing malaria with a fluorescence flow cytometer (XN-31, Sysmex Deutschland GmbH): A 4 years prospective study of diagnostic accuracy of XN-31 in comparison to microscopy or PCR in a private laboratory

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Question: Malaria is a medical emergency that requires rapid and accurate diagnosis. Fluorescence flow cytometry using XN-31 offers the possibility of fast and easy-to-perform diagnostics for the detection of malaria, including the quantification and differentiation of *Plasmodium falciparum* from the other species, fully automated with a limit of detection (LoQ) of 20 parasites/µl.

Methods: 891 samples were measured using XN-31. Thick and thin film were subsequently performed. All samples that could not be clearly evaluated in the thin film were confirmed by PCR. In addition, an intralaboratory comparison with 10 measurements was performed.

Results: 823 negative and 45 malaria positive blood samples were examined. Thick film showed a high sensitivity of 91%, thin film of 80% and XN-31 an excellent sensitivity of 100% in comparison to the event "malaria". Specificity and PPV was 100% for all three methods. NPV was 99.5% for the thick film, 98.9% for thin film and 100% for XN-31. Agreement was high with Cohen's kappa of 0.92 for thick and 0.81 for thin film. In 23 cases, XN-31 result was not evaluable (2.6%). None of the samples were positive for malaria. XN-31 showed a correct differentiation between P. falciparum and P. Non-falciparum. In 8 out of 45 cases a positive result with species unknown (UNC) was indicated. The mean parasitaemia was 0.0014. In all these cases thin film was assessed as negative, the PCR was able to detect P. falciparum in all cases. Intralaboratory comparison showed the same evaluation of the result, with a mean density of 0.0208 (SD 0.0003).

Conclusions: XN-31 offers a reliable exclusion of malaria, as well as an equally reliable suspected diagnosis with the differentiation of P. falciparum and P. non-falciparum including determination of parasitaemia within a few minutes. Positive results should be confirmed by microscopy of the thin film or PCR. Microscopy of the thick film as an enrichment method can be dispensed with when using XN-31.

The role of the innate immune system in semi-immunity to malaria

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The outcome of malaria fundamentally depends on the patient's history of previous *Plasmodium* spp. infections. In general, infections are tolerated better by patients that have been exposed repeatedly when compared to naïve patients. People living in an area of high malaria transmission develop a state of "semi-immunity" eventually even leading to asymptomatic parasitaemia – the result of an interplay of anti-parasite and clinical immunity. While the role of B and T cells in the development of semi-immunity has been extensively studied, much less is known about the involvement of the innate immune system. The much newer concept of innate adaptations includes both trained immunity as well as innate tolerance in response to repeated stimuli. We hypothesise that innate adaptations play a role in the development of semi-immunity to malaria by acting more anti-inflammatory and/or anti-parasitic in repeated infections. To test this, patients with varying histories of exposure to malaria are recruited within the DEMIT study at Charité Universitätsmedizin Berlin (Germany) and CERMEL (Lambaréné, Gabon). By deep phenotyping of the innate compartment of matched symptomatic and asymptomatic patients, using CyTOF and scRNAseq, we aim to unravel anti-parasitic and anti-disease adaptations. To further investigate the role of innate "trained immunity" in a tractable animal model, we infection-treated wild type and RAG-/- mice lacking B and T cells to prime innate immune cells. We did not observe any significant difference in the innate compartment of pre-infected and uninfected-untreated mice after clearance. Upon rechallenge, previously infected RAG-/- mice also had similar parasitaemia and clinical score to naïve animals, while previously infected wild type mice were protected. These data suggest that innate immune cells do not contribute to protection, but do not preclude the possibility that they may modulate disease progression in concert with the adaptive response.

Bayesian spatio-temporal modelling to assess the impact of climate variability and control interventions on the burden of malaria in Kenya

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Malaria remains one of the most persistent and challenging infectious diseases globally, with sub-Saharan Africa (SSA) bearing the greatest burden, particularly among children under 5 years. In Kenya, malaria burden is heterogeneous, with western regions experiencing the highest incidence. While global malaria trends declined between 2000 and 2015, this progress has since plateaued, raising concerns about resurgence and expansion to new areas due to factors such as climate change, insecticide and drug resistance, and land use changes. This study investigated the effects of climatic, environmental, and non-climatic factors on malaria incidence and mortality in Kenya, utilizing data from the Kisumu Health and Demograpic Surveillance System - HDSS (2008–2022) and the Kenya Malaria Indicator Surveys - MIS (2015 and 2020). Advanced Bayesian spatio-temporal methods including geostatistical models, and Empirical Dynamic Modelling including Convergent Cross Mapping (CCM) for causal inference were employed. Climatic factors, such as temperature and rainfall, showed stronger effects on malaria incidence and mortality compared to interventions like bed net use. Higher SES and proximity to health facilities were associated with reduced mortality, while the incidence of malaria was higher in younger children. Between 2015 and 2020, parasitemia prevalence decreased overall, but this reduction was highly heterogeneous. Northern semi-arid regions experienced increased risk in 2020. Nightlights and ITN use were associated with reduced parasitemia risk. Additionally, CCM confirmed causal links between climatic suitability, interventions, and malaria incidence. The method identified optimal lag times and climatic ranges for malaria transmission. Empirical dynamic modelling provided effective short-term malaria incidence forecasting. These findings emphasize the importance of fine-scale data and highlight spatial heterogeneity in malaria risk in Kenya. The results support the National Malaria Control Program (NMCP) and county health departments in targeting interventions more effectively, offering valuable tools for malaria prediction and adaptation to changing climatic conditions.

Age-structured malaria transmission modelling incorporating the effects of climate and control interventions

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Climate significantly influences malaria incidence, particularly in regions with epidemic transmission. However, its role in moderate-to-high transmission areas with intensive malaria control interventions is less understood, partly due to limited availability of high-quality, long-term malaria data. In these regions, malaria transmission dynamics are primarily driven by seasonal variations in mosquito populations and densities, which are closely linked to local rainfall and temperature patterns. Additionally, immunity to malaria develops during childhood through repeated exposure, allowing adults to tolerate infections without experiencing symptoms. As a result, the impacts of climate variability and control interventions on malaria transmission dynamics may differ across age groups. Models able to explicitly quantify these effects on the disease transmission parameters are scarce. Here, we introduce a novel age-structured, process-based stochastic transmission model that disentangles the contributions of climatic factors and control interventions on malaria incidence across different age groups. The model is fitted using a sequential Monte Carlo method based on particle filtering for likelihood maximization by iterated filtering. Using population-based infectious disease surveillance data from Siaya County, western Kenya (2008–2022), we demonstrate the model's ability to assess the effects of climatic variability, immunity development, and malaria control intervention. This framework enhances the understanding of malaria dynamics across age groups and provides insights to guide targeted, sustainable control strategies under varying climatic and intervention scenarios.

Keywords: climate change, control interventions, bed nets, age-structured models, stochastic transmission models, western Kenya.

Management of Malaria in Neonates: a Systematic Review.

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Background:
Neonatal malaria, arising from vertical or vector-borne transmission, is life-threatening but often underdiagnosed due to symptom overlap with sepsis. Management strategies vary globally, reflecting a lack of specific guidelines for this age group. This review identifies approaches for managing malaria in neonates within the first month of life.

Methods:
We systematically searched PubMed and Web of Science (January 2003–December 2023) for original studies using terms related to 'management,' 'neonatal,' and 'malaria.' Inclusion criteria were age 0–28 days, parasitological malaria confirmation, and antimalarial treatment data. All study designs, including case reports, were eligible. Data extraction and risk of bias assessment used JBI tools. PROSPERO registration: CRD42023414278.

Results:
Among 4030 records, 38 studies were included, involving 42 neonates. Most studies (21/28, 75%) were from endemic regions, with India (39.3%) and Colombia (10.7%) contributing the most cases. Maternal malaria history was reported in 72.7% (16/22) of cases, and 80% of these mothers received preventive treatment during pregnancy. Microscopy diagnosed 96.4% (27/28) of cases. Plasmodium vivax (70.4%) was the predominant species, followed by Plasmodium falciparum (25.9%). Congenital malaria accounted for 86.4% (19/22) of cases. Fever was the most common symptom in P. vivax (84.2%) and P. falciparum (71.4%), while anemia was more frequent in P. vivax (79% vs. 28.5%). Initial misdiagnosis as sepsis occurred in 77.8% (14/18) of cases. Treatments included chloroquine for P. vivax (52.5%) and artemisinin-based or non-artemisinin-based therapies for P. falciparum. Oral treatments (14.3%) involved crushed tablets delivered via orogastric tube. Parasite clearance time averaged 5 days.

Discussion:
Neonatal malaria is frequently misdiagnosed, with variable management practices. Findings highlight the need for standardized guidelines, improved diagnostics, and tailored treatments to address the unique challenges in this population.

Malaria and Hypertension among Volunteers attending the health services of Lambaréné in Gabon

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Introduction:
Recent decades have been marked by an increase in the incidence of non-communicable diseases, among which are cardiovascular diseases especially high blood pressure. This growing phenomenon, which is evolving in parallel with the ongoing state of malaria endemic in sub-Saharan Africa, remains of concern due to its alarming number of deaths. Although the potential link between these two entities i.e. hypertension and malaria has been described in the literature, there are few studies on African populations where malaria is endemic. Our study aims to explore the association between malaria parasitemia and hypertension in patients and volunteers screened for malaria in Gabon.

Methods:
This study was conducted at the Centre de Recherches Médicales de Lambaréné. The data were collected retrospectively during field and screening activities in the dispensaries of Lambaréné from 2017 to 2020 and prospectively on consenting adult subjects seen in routine consultation in the clinical operations department of the said centre from 2020 to 2023. Recruited populations underwent a malaria screening test and a blood pressure measurement. Blood pressure diagnosis was made based on systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure, greater than 90 mm Hg. Malaria parasitemia was determined by a rapid diagnostic test.

Results:
A total of 913 participants were enrolled in this study with a male-to-female ratio of 0.86. The majority of participants lived in urban areas, 70.1% (640/913). In this study, the prevalence of malaria was estimated at 69% (630/913) Cases of hypertension were encountered with an estimated prevalence of 35.9% (n=226) and 35.9% (N=226) of participants with hypertension were positive for malaria screening. Our study revealed a significant association between malaria and diastolic blood pressure (p-value=0.005).

Conclusion:
Although the risk of non-communicable diseases such as cardiovascular disease is related to habits and lifestyle, although it is partly attributable to malaria infection in the literature by some authors high blood pressure is very little related to malaria in our study.

Key words: malaria, hypertension, Gabon.

Malaria and Hypertension among Volunteers attending the health services of Lambaréné in Gabon

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Background:
Mosquitoes belonging to the *Anopheles gambiae* sensu lato complex play a major role in malaria transmission across Africa. This study assessed the relative importance of members of *An. gambiae* s.l. in malaria transmission in two rural villages in the Republic of the Congo.

Methods:
Adult mosquitoes were collected using electric aspirators from June to September 2022 in Djoumouna and Ntoula villages and were sorted by taxa based on their morphological features. *Anopheles gambiae* s.l. females were also molecularly identified. A TaqMan-based assay and a nested polymerase chain reaction (PCR) were performed to determine *Plasmodium* spp. in the mosquitoes. Entomological indexes were estimated, including manbiting rate, entomological inoculation rate (EIR), and diversity index.

Results.
Among 176 mosquitoes collected, *An. gambiae* s.l. was predominant (85.8%), followed by *Culex* spp. (13.6%) and *Aedes* spp. (0.6%). Three members of the *An. gambiae* s.l. complex were collected in both villages, namely *An. gambiae* sensu stricto (74.3%), *Anopheles coluzzii* (22.9%) and *Anopheles arabiensis* (2.8%). Three *Plasmodium* species were detected in *An. gambiae* s.s. and *An. coluzzii* (*Plasmodium falciparum, P. malariae* and *P. ovale*), while only *P. falciparum* and *P. malariae* were found in *An. arabiensis*. In general, the *Plasmodium* infection rate was 35.1% (53/151) using the TaqMan-based assay, and nested PCR confirmed 77.4% (41/53) of those infections. The nightly EIR of *An. gambiae* s.l. was 0.125 infectious bites per person per night (ib/p/n) in Djoumouna and 0.08 ib/p/n in Ntoula. The EIR of *An. gambiae* s.s. in Djoumouna (0.11 ib/p/n) and Ntoula (0.04 ib/p/n) was higher than that of *An. coluzzii* (0.01 and 0.03 ib/p/n) and *An. arabiensis* (0.005 and 0.0 ib/p/n).

Conclusions.
This study provides baseline information on the dominant vectors and dynamics of malaria transmission in the rural areas of the Republic of the Congo during the dry season.
In the two considered villages, *An. gambiae* s.s. appears to play a predominant role in *Plasmodium* spp. transmission.

Keywords. *Anopheles gambiae* sensu lato, *Plasmodium species*, Malaria transmission, Rural areas, Republic of the Congo.

NO evidence of piperaquine resistance in Southeast Nigeria

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Artemisinin-based combination therapies (ACTs) are currently the first-line treatment for uncomplicated malaria. However, ACTs are being threatened by the emergence of *Plasmodium falciparum* strains resistant to artemisinins as well as their partner drugs. Periodical surveillance of drug efficacy and molecular markers associated with resistance is required to monitor this. Resistance to piperaquine (PPQ), the partner drug in the ACT dihydroartemisinin-piperaquine, is spreading in Asia, and mutations associated with PPQ resistance have been reported in some African countries. In Nigeria, this has never been reported. This study aimed to assess biomarkers of PPQ resistance—specifically, the T93S, H97Y, and F145I SNPs in exons 2 and 3 of the *P. falciparum* chloroquine resistance transporter (*pfcrt*) gene—and to examine amplification of the *plasmepsin* 2 and 3 genes (*pfpm2/3*) in 268 patient samples collected from Nnewi, Southeastern Nigeria, in 2019. SNPs were identified using PCR followed by sequencing, while *pfpm2/3* copy number was determined by qPCR. No *pfcrt* SNPs were detected, and no increase in copy number for *pfpm2* or *pfpm3* was observed in any sample. The absence of these PPQ resistance markers suggests that PPQ remains an effective partner drug in ACTs in this region. However, continuous monitoring of circulating *P. falciparum* parasites in Nigeria is crucial to promptly detect and respond to potential resistance development.

Keywords: Piperaquine; Resistance; *Plasmodium falciparum*, Southeastern Nigeria, *pfcrt*, Plasmepsin 2, Plasmepsin 3 and Nigeria.

Genetic diversity of *Plasmodium malariae* in sub-Saharan Africa: a two-marker genotyping approach for molecular epidemiological studies

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The prevalence of *Plasmodium malariae* has been underestimated in many endemic countries so far. Only recently, epidemiological studies using highly sensitive molecular methods revealed a high prevalence of the species. However, limited data exists on its genetic diversity. We aimed to develop a genotyping approach for *P. malariae* based on size polymorphic regions, facilitating its use in molecular epidemiological studies. Four potential markers – two microsatellites Pm02 and Pm02, as well as *P. malariae* thrombospondin-related anonymous protein (*pmtrap*), and *P. malariae* merozoite surface protein fragment 2 (*pmmsp1* F2) – were amplified using a nested PCR approach and analysed via automated capillary gel electrophoresis. The genetic diversity was assessed in a study population of 95 *P. malariae* samples from Lambaréné and surrounding villages in Gabon. Among the four markers, *pmtrap* showed the highest allelic diversity (mean MOI = 1.61), followed by *pmmsp1* F2 (He = 0.81). Applying these two markers on a separate sample set of 21 *P. malariae* positive individuals monitored over one week, demonstrated a consistent performance. Additionally, we saw a substantial complexity and dynamic changes in the different *P. malariae* genotypes in a group of asymptomatic Gabonese study participants.
We successfully developed a genotyping panel for *P. malariae* based on two markers, *pmtrap* and *pmmsp1* F2. Our approach is adaptable to other endemic settings. This will enable further detailed investigations into the genetic diversity of *P. malariae* and contribute valuable insights into its molecular epidemiology.

Molecular markers of resistance to sulfadoxine-pyrimethamine in *Plasmodium falciparum* isolates from children living in two rural areas in Southern Benin

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Introduction:
In Benin, sulphadoxine-pyrimethamine (SP) is used for intermittent preventive treatment during pregnancy (IPTp), seasonal malaria chemoprevention (SMC) and perennial malaria chemoprevention (PMC). However, the effectiveness of SP is threatened by *P. falciparum* resistance, linked to specific point mutations in the *Pfdhps* and *Pfdhfr* genes. These mutations compromise SP efficacy, necessitating ongoing surveillance. The study focused on analysing SNPs of *Pfdhfr* and *Pfdhps* in *P. falciparum* strains from children in Ketonou and Kpome in Southern Benin.

Methods:
Dried blood spots (n=92) were collected from children aged 6 months to 10 years during a cross-sectional study in 2017. The study was conducted in two rural areas: Ketonou, near the Nigerian border, and Kpome. DNA was extracted using the Qiagen kit, and *Pfdhps* and *Pfdhfr* genes were amplified using nested PCR. Mutations conferring resistance were identified by sequencing the resulting products.

Results:
The *Pfdhfr* N51I, C59R, S108N and *Pfdhps* A437G mutations were detected at high frequencies in both study areas, ranging from 95% to 100%. No mutations were identified at K540E in *Pfdhps*. The *Pfdhps* mutations I431V and A581G were observed at an overall prevalence of 8.3%, exclusively in Ketonou, whose estimated prevalence was 22.3%. The most frequently haplotype was the quadruple *Pfdhfr/Pfdhps* mutant CIRNI/ISGKAA, which accounted for 64.9% (37/57) in both areas, including 57.1% (12/21) in Ketonou and 69.4% (25/36) in Kpome. The octuple mutant CIRNI/VAGKGS was detected only in Ketonou, with prevalence of 23.8% (5/21).

Conclusions:
The study indicated that using SP in Benin remains effective. However, it highlighted the spread of the *Pfdhps* I431V mutation from Nigeria into Benin, though its role in SP resistance remains uncertain. Monitoring was recommended to track resistance patterns and evaluate the potential impact of the I431V mutation on SP efficacy.

Keywords: *Plasmodium falciparum*, *Pfdhfr*, *Pfdhps*, Benin.

Recurrent events in clinical cohorts: modeling the occurrence of malaria infections

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There is limited methodological information about recurrent events in longitudinal studies. However, in diseases like malaria, recurrent events are common and they are the subject of analyses in many clinical studies. This study mathematically describes recurrent malaria infections in a naïve cohort and highlights the necessary assumptions to inform study planning.

The risk to experience recurrent events is given by the incidence rate (IR), which represents the observed number of events in a population at risk over time. Mathematical models parameterized with an IR of 4 events per person year (representing a high transmission setting) were established to show the proportion of study individuals experiencing recurrent infections over a period of 2 years. The transition between these states is described by a system of ordinary differential equations.

At 12 months, 33% and at 24 months, 55% of the study population experienced at least one malaria infection. Over time, individuals who experienced their first infection could become reinfected, and as the number of recurrent infections increased, the rate of single infections decreased. At 24 months, 14% experienced two, 4% experienced three, and 1% experienced four or more infections. The number of patients who experienced recurrent infections was 19% at 12 months and 35% at 24 months. At month 12, an average of 4 and at month 24, an average of 8 infections per individual were observed, representing the IR used to calculate the model.

Recurrent infections in longitudinal studies cannot be directly estimated from disease frequency data. However, this study provides a simple set of equations to calculate the number of expected recurrent events. The presented formulas represent simple transmission dynamics and ignore factors such as heterogeneous infection risk or immunity development over time. However, the model can easily be adapted to represent additional transmission and infection dynamics.

Exploring Cultural Beliefs, Practices, and Perceptions of Pregnancy and Vaccination: A Focus on Placental Malaria and Vaccine Acceptability Among Pregnant Women in Malawi

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Placental malaria (PM) is a severe complication of malaria during pregnancy, particularly prevalent in Sub-Saharan Africa poses significant risks to maternal and neonatal health, leading to adverse outcomes such as maternal anaemia, premature delivery, low birth weight, and increased neonatal mortality. The existing prevention strategies are losing effectiveness due to drug resistance. Recent advances in promising vaccines candidates PRIMVAC and PAMVAC, could enhance malaria control efforts. However, the operational acceptability of these new interventions is crucial for their public health impact. Cultural beliefs, misconceptions and fears surrounding vaccines can influence its acceptance, making it a necessary prerequisite to understand the specific attitudes towards pregnancy and vaccination before developing and rolling out PM vaccines. In this study we aimed to identify the facilitators and barriers to the acceptability of the PM vaccines in Malawi.

We conducted a mixed method study of 6 focus group discussions, 300 surveys with women of reproductive age, and 62 semi structured interviews with women of reproductive age, older women who pass on cultural ideas concerning pregnancies; mothers and Traditional Birth Attendants (TBAs) their family members and traditional leaders. In preliminary analysis, the major barriers include limited awareness and understanding of PM as a fatal disease, along with the cultural beliefs of non-physical attacks during pregnancy, accessibility challenges and socioeconomic factors. Perception that vaccines are not cures and concerns about the increasing number of vaccines (vaccine fatigue) also contribute to hesitancy. Facilitators include involving community leaders, health surveillance assistants (HSAs) as well as TBAs. Ensuring local availability would further enhance accessibility by reducing travel costs. Tailored educational campaigns and community engagement strategies can help address doubts and enhance vaccine acceptance.

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Plasmodium falciparum carriage in a population under long-term, intensive malaria control: a one-year cohort study in Kedougou region, Senegal

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Background.
In Sahelian Africa, successful interventions against malaria include vector control, improved access to care and seasonal malaria chemoprevention (SMC) in children. Yet, malaria increased in recent years. Up-to-date evidence is necessary to design additional interventions and restore progress towards elimination. We aimed to capture current distribution and risk factors of *Plasmodium falciparum* infection in Kedougou, Senegal's most affected region.

Methods.
We included all individuals over 6 months from randomly selected households of four villages in a one-year open cohort. During four surveys spanning the dry and wet seasons, we collected sociodemographic and behavioural data, and detected *P. Falciparum* using qPCR on capillary dried blood samples. We analysed risk factors associated with *P. falciparum* carriage using multilevel logistic regression.

Findings.
We included 763 participants in 69 households. SMC-eligible children had the lowest *P. falciparum* infection prevalence (dry season: 4.3%; wet season: 9.1%). Older age groups had similar dry season prevalence (10%). During the wet season, prevalence increased in adults aged 15-24 (32%), and 35-49 years (24.7%). Participants aged 10-19 had the highest clinical burden (100 cases per 1000 person.month), ten-fold higher than SMC-eligible children. Outdoor nighttime activity was associated with *P. Falciparum* infection.

Interpretation.
Ongoing intensive control reduces malaria in SMC-eligible children. Older individuals bear an important clinical burden and harbour high prevalence during the wet season. Elimination-oriented interventions must tackle the parasite reservoir, involving whole communities and specifically young adults. Funding. Excellence Initiative of Aix-Marseille University - A*MIDEX, a French "Investissements d'Avenir" programme: the MARS project (A*Midex International 2018).

High-resolution dynamics of *Plasmodium falciparum* genotype and *Plasmodium* species diversity in asymptomatic adults in Gabon

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In malaria-endemic regions, individuals often harbour infections caused by multiple *Plasmodium* species and genotypes, with asymptomatic cases showing dynamic changes in allele detection over time. This complexity hinders antimalarial drug clinical trials as proper differentiation of new infection and recrudescence is critical in this scenario. This study aimed to evaluate whether a baseline blood sample from 49 asymptomatic participants in a clinical trial reflects the diversity of *Plasmodium falciparum* genotypes and *Plasmodium* species observed throughout a seven-day follow-up period. Blood samples were serially collected at baseline, every 8 hours for the first 72 hours, and daily thereafter until day 7. *Plasmodium falciparum* diversity was assessed by analysing the length polymorphism of *Plasmodium falciparum* merozoite surface protein 1 (*pfmsp1*) gene through nested PCR and capillary electrophoresis. *Plasmodium* species were identified by qPCR targeting specific regions of the 18S rRNA gene for each species. Results showed that 73% of participants (35/48) had multiple *pfmsp1* genotypes over the study period, with some appearing only at specific time points. In 35% of participants (17/48), all genotypes found throughout follow-up were already present at baseline. However, 65% (31/48) had 85 new genotypes that were not detected at baseline. Notably, additional sampling at 24 hours revealed all genotypes found during follow-up in 50% of participants. Mixed *Plasmodium* species infections were detected at baseline in 57% (28/49) of individuals, with follow-up revealing variable detection of non-*falciparum* species. These findings highlight the complex intra-host dynamics of *Plasmodium* infections and underscore the importance of serial sampling in clinical trials. Such an approach ensures a comprehensive understanding of *Plasmodium* genotypic diversity, which is essential for evaluating the efficacy of antimalarial treatments and tracking the evolution of the infection.

Trends in malaria incidence in children living in Lambaréné, Gabon, pre and post Covid-19 pandemic (2014-2024)

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Background
Malaria remains a huge global public health problem. There was a global increase in malaria mortality post Covid-19 pandemic, with around 627,000 deaths registered, 80% of which were children aged 6-59 months. This was in contrast to the continuous decline in malaria cases observed pre-pandemic. In Gabon, malaria is listed as a priority health concern in the national health policy. The initial target of the 2017-2021 national malaria strategic plan was a 40% reduction in malaria-related morbidity and mortality by December 2021, when compared to 2015. In order to investigate the impact of Covid-19 on malaria incidence, and if the global trend was replicated in Lambaréné, Gabon, we are comparing incidence rates in research studies conducted pre-pandemic, during the pandemic, and after established covid-19 endemicity in Gabon.

Methods
We are conducting a retrospective analysis of malaria incidence in five cohort studies, defined at one year of follow-up, conducted between November 17th 2014 to July 23rd 2024 at the Centre de Recherche de Lambaréné (CERMEL) in children aged 1-12 years old living in Lambaréné, Gabon. A total of 905 participants are included in the analyses. The primary objective is analyzed using descriptive trend analysis. The manner of the active visits and frequent testing for malaria using malaria slides (gold standard) ensured that we captured of a high proportion of infections in the cohort.

Results
A total of 398 children were enrolled in the post-pandemic study conducted between August 2022 to February 2023. Median age was 6 years (interquartile range 4-9). Overall malaria Incidence rate at (based on first infection) and proportion at 6 months was 0.95 per person-year and 49%, respectively. Further analyses of the pandemic and pre-pandemic cohorts are ongoing.

Conclusions
Malaria incidence in Lambaréné, Gabon, remains high after establishment of covid-19 endemicity, exposing more children to the risk of malaria transmission.

Plasmodium falciparum infection and malaria prevalence: Community and hospital survey in central region of Gabon

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Introduction and objective
Malaria remains one of the commonest causes of death and serious morbidity, especially for children and pregnant women particularly in sub-Saharan Africa. The epidemiology of malaria has been changing over the past 10 years. Use of artemisinin-based combination therapies and increased coverage with insecticide-treated nets have contributed to change malaria epidemiology. There is still a need to evaluate the malaria prevalence in this context of malaria epidemiology change. We aimed to evaluate malaria prevalence in adult and children particularly in infant in order to better plan interventions for malaria control.

Methods
A facility-based and community-based cross-sectional study were conducted at the Centre de Recherches Médicales de Lambaréné (CERMEL) in the central region of Gabon from September 2022 to September 2023. Patients of all ages with malaria like symptom attending Sindara facility (CERMEL's satellite site) were screened for malaria. Patients were screened for plasmodium detection in the community of Lambaréné and Fougamou and their respective surrounding villages. Plasmodium infection or malaria were diagnosed either by rapid diagnostic test (RDT) or by microscopy. Descriptive analysis of data on parasite densities were presented.

Results
1500 and 2258 individuals screened in Sindara facility and in community were included in this analysis respectively. Median age was 13 years (IQR, 4-32), 425 (28,3 %) were children less than 5 years while 740 (49,3%) were adolescents and adults in Sindara facility. Infant represented 12,4% of the study population. In The community-based survey, median age was 10 years (IQR, 4-28). 617 (27,3%) were children less than 5 years, 924 (40,9%) were adolescent and adults and infant represent 9,7% (218) of population surveyed. Out of 1366 individuals assessed at Sindara facility, the malaria prevalence was 49,1 % and 37,9 % by RDT and microscopy respectively. Prevalence of malaria was 49,9% and 31,2 % by RDT and microscopy respectively in community-based survey. proportion of Plasmodium infection positive RDTs was similar (p-value=??) in children (30,6% and 5,9%) compared to adolescents and adults (36,7% and 33,5%) in Sindara facility and community respectively. Out of 760 and 1375 participants assessed for malaria by microscopy in Sindara hospital and community similar results were observed. Malaria prevalence among infant under two years was 9,6 and 6,4% in both survey, 36% of them had a parasite count above 10,000/µL.

Conclusions
A low prevalence of malaria was observed in infant compare to children and adolescent and adults in both, community and hospital survey. Infants under two years old had the highest rate of parasitaemia, above 10,000/µl, putting them at risk to develop severe malaria. Malaria control program should continue to focus on infant less than five year and malaria intervention should design their plan based on the current malaria epidemiology profile.

Prevalence and risk factors for *Plasmodium spp.* infection during pregnancy: evidence from antenatal care-based diagnosis

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Background:
Malaria during pregnancy remains a significant public health challenge in endemic regions, leading to maternal morbidity, adverse pregnancy outcomes and increased neonatal mortality. Despite the implementation of preventive strategies such as intermittent preventive treatment in pregnancy (IPTp) and the use of insecticide-treated bed nets (ITNs), *Plasmodium falciparum (Pf)* infections persist among pregnant women. Understanding the prevalence and risk factors associated with malaria in pregnancy is crucial for optimizing control strategies and improving maternal and infant health outcomes. This study aims to assess the prevalence of Plasmodium spp. infections among pregnant women attending antenatal care (ANC) in Lambaréné and to ascertain the association between prevalence and preventive measures.

Methods:
A cross-sectional study was conducted as part of the CENtre de SAnté MATernelle et Infantile de Lambaréné (CENSAMATIL) project (https://www.cermel.org/CENSAMATIL.php) in Lambaréné, Gabon. Pregnant women attending ANC visits at health facilities within CENSAMATIL during the first trimester from December 2022 to January 2024 were enrolled. Malaria was diagnosed using rapid diagnostic tests (RDTs), thick blood smear (TBS) and polymerase chain reaction (PCR) assays to differentiate between active infection and asymptomatic carriage and to determine its prevalence. Data on IPTp use, ITN use, and other malaria prevention practices were collected through structured questionnaires and ANC records.

Results:
A total of 827 pregnant women with a mean age of 26.2 years (SD: 6.77) were included in the study. The majority (60.3%) were aged 18-29 years, most lived in semi-urban areas (84.6%), while 15.4% were from rural areas. Multiparity was observed in 70.5% of women, while 28.3% were primiparous. The overall prevalence of malaria among pregnant women was 30.5% (252/827). During CPN1, 514 women were tested for malaria, resulting in a prevalence of 34.4% (177/514). Of the infected women, 14.4% were symptomatic, and 81.7% were asymptomatic. Many pregnant women did not adhere strictly to the recommended ANC attendance schedule. While CPN1 attendance was relatively high (514/827, 62.2%), The majority of pregnant women has their first antenatal visit at the first trimester, 60.1% (309/514) following in the second 31.1% (160/514) and few had their first visit 8.8% (45/514) in the third trimester. In addition, gaps in IPTp-SP administration were observed at CPN1, with 35.6% of women not receiving a first dose and 35.4% reporting to not use the ITNs. However, there is no evidence for pregnancy age to be associated with malaria.

Conclusion:
These findings suggest that some women-initiated ANC later than recommended, potentially delaying malaria screening and the start of preventive measures such as IPTp-SP. Despite the availability of ANC services, attendance delays and gaps in adherence to preventive measures increase the risk of malaria. These factors could contribute to the current persistent burden of malaria in pregnancy.

Genomic Surveillance of *Plasmodium falciparum* Resistance to Sulfadoxine-Pyrimethamine in Pregnant Women in Nigeria: Insights from In-Country Nanopore Sequencing

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Background:
Malaria in pregnancy threatens maternal and fetal health, especially with emerging Plasmodium falciparum resistance to IPTp-SP. This study uses Oxford Nanopore sequencing to analyze resistance dynamics, persistence, reinfection, and treatment effectiveness in Nigerian pregnant women.

Methods:
A cohort of 140 pregnant women in Ogbomoso, Nigeria, receiving IPTp-SP was followed during the second and third trimesters. Paired dry blood spots were collected and screened for *Plasmodium falciparum* using 18S rRNA PCR and VarATS qPCR. Multiplex PCR was performed using 9 primer pairs targeting key drug-resistance genes, polymorphic markers, and the vaccine target. The resulting PCR products were sequenced using ONT. Within sample allelic frequency, heterozygosity was analyzed between the positive samples' two-time points.

Results:
Among 140 pregnant women, *P. falciparum* was detected in 19% and 15% during the second and third trimesters, respectively. Ten (7%) remained positive across both trimesters, with a significant parasitemia decrease from 81,225 to 10,309 parasites/µL (P = 0.001). Mutational analysis identified *pfcrt* K76T, *pfmdr1* (N86Y, Y184F, N1042D), and a novel *pfmdr1* F1063Y mutation in all paired samples. Three *pfdhfr* (N51I, C59R, S108N) and six *pfdhps* mutations were consistently detected. Additionally, 38 *pfama1* and 25 *pfcsp* mutations remained unchanged, while *pfkelch13* mutations were absent. Allelic frequency analysis showed significant differences between trimesters (P = 0.05), indicating distinct parasite populations over time. However, low heterozygosity (*He*) suggests recrudescence as the predominant pattern, with a slight increase from 0.04 to 0.08 (P = 0.04), indicating some introduction of new variants.

Experimental vaccination with a single dose injection of *Plasmodium* sporozoites

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The recently approved first malaria vaccine based on a single antigen affords only partial protection for a limited time and necessitates multiple vaccinations. Hence, an effective vaccine against malaria remains elusive. Among other approaches, genetically modified *Plasmodium* lines that arrest their development in the liver are currently being explored as live attenuated vaccines against malaria. For these approaches high numbers of parasites need to be injected several times to achieve sterile protection and there is a chance for breakthrough infections during vaccinations. Here we present an attenuation strategy for experimental single-shot vaccination by sporozoites of genetically altered parasite lines that show reduced blood stage growth and cause self-limiting infections. Despite these characteristics the parasites retain capacity to be transmitted to and by mosquitoes. Infection of mice by a low number of mosquito bites or single injection of *Plasmodium* sporozoites leads to full protection from lethal malaria in highly susceptible mice. But mice are easy to vaccinate. Hence *P. falciparum* parasite lines lacking similar genes are currently being explored to inform on the establishment of human immunity.

What makes a man a man: Zinc finger proteins in male sexual development of *Plasmodium falciparum*

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Development and maturation of *Plasmodium falciparum* gametocytes require a well-coordinated programme of gene expression that involves a wide spectrum of regulatory proteins, ranging from histone modifiers to transcription factors to RNA-binding proteins. Various recent studies have demonstrated a crucial role of zinc finger proteins (ZFPs) during regulation of sexual development. These include the CCCH-ZFPs MD3 and ZNF4 as well as the RING finger E3 ligase RNF1. We show that parasites deficient of MD3 are impaired in gametocyte maturation, while ZNF4 deficiency impairs male gametocytogenesis by downregulation of male-enriched genes associated to the axoneme formation. Further, MD3 and RNF1 share an interactome particularly involving RNA-binding proteins like PABP1 and ALBA3, with translational initiators, regulators and repressors like eIF2, PUF1, NOT2 and CITH, and with further regulators of sexual development, including ZNF4 and GD1. We conclude that the three ZFPs are part of a regulator complex crucial for post-transcriptional fine-tuning of male gametocytogenesis.

The regulation of translational control during human-to-mosquito transmission of *Plasmodium falciparum* gametocytes

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The transmission of *Plasmodium falciparum* gametocytes from the human host to the mosquito is associated with a major change in environmental conditions that requires a rapid adaption of the parasite. To pro-actively prepare for the change of hosts, mRNA transcripts essential for development of the midgut stages are synthesized and stored in stress granules in female gametocytes. After transmission, the transcripts are released and translation occurs.

We have identified the protein 7-Helix-1 as a constituent of stress granules and a crucial component of the repressor complex in *P. falciparum* gametocytes. In order to investigate the stress granule composition and dynamics in *P. falciparum*, we have performed BioID experiments in immature and mature gametocytes. The results identified regulators of translation as well as RNA-binding proteins as interactors of 7-Helix-1. Surprisingly, the analysis also revealed components of the transcription machinery and nuclear proteins as being part of the interactome.

In silico analyses further showed that 7-Helix-1 is homologous to the human stress regulator hLanCL2, which acts as a regulator in the mTORC signaling pathway. We therefore hypothesize that translation following human-to-mosquito transmission is regulated by an mTORC-like signaling pathway, although the main components of the pathway have been lost during the evolution of the parasite.

Several proteins homologous to human mTORC-regulating kinases have been identified in *P. falciparum*. Among these are the kinases KIN, PI3K and PKB. Chemical inhibition of KIN by dorsomorphin disturbed the stress response and translational regulation of the parasite. Dorsomorphin-treatment impaired the parasite growth and led to an arrest of development in the schizont stage. Furthermore, dorsomorphin-treatment does not affect gametocyte formation but blocks maturation at stage IV, underscoring KIN's critical role sexual-stage development.

The complement protein C3b plays an essential role during merozoite invasion in red blood cells

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The obligate intracellular parasite *Plasmodium falciparum* must periodically exit its host cell, the erythrocyte, during its life cycle. Over 48 hours, the parasite forms up to 32 merozoites, which are then released into the bloodstream to rapidly reinvade new red blood cells. During this phase, the parasite faces two critical challenges: evading the immune system and successfully invading erythrocytes.

Our previous research demonstrated that *P. falciparum* utilizes the complement regulator factor H to evade complement-mediated lysis. Building on this, we investigated the role of the complement protein C3b, a key driver of the alternative complement pathway, in parasite invasion. We showed via merozoite invasion assays that the acquisition of C3b, but not inactivated C3b, in a complement-free system significantly enhances the invasion rate of merozoites into red blood cells. Growth assays further confirmed this effect, revealing increased parasitemia in the presence of C3b.

Experiments conducted in a C5-deficient serum system, which allows C3b activation without subsequent formation of the terminal complement complex and thus cell lysis, confirmed that C3b facilitates parasite invasion. A concentration-dependent relationship between C3b level and invasion efficiency was also observed.

We hypothesize that *P. falciparum* exploits C3b to enhance its binding to the erythrocyte surface, potentially utilizing interactions between C3b, erythrocyte receptor CR1, and the merozoite surface protein PfRh4. Further investigations will focus on characterizing these interactions to uncover their precise role in parasite invasion.

PfDPAP2 is a crystalloid-associated protein essential for mosquito transmission of *Plasmodium falciparum*

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Successful transmission of *Plasmodium falciparum* between vertebrate and mosquito hosts depends on specialized parasite stages, including gametocytes, ookinetes, and sporozoites. Among proteins involved in these stages, we focused on *Pf*DPAP2, a putative protease with transcripts detected in gametocytes and ookinetes. We previously showed that *Pf*DPAP2 localizes to osmiophilic bodies in gametocytes, where it facilitates egress from erythrocytes, but its role in mosquito stages remained unexplored. Using genetically modified *P. falciparum* parasites capable of infecting *Anopheles gambiae*, we investigated *Pf*DPAP2's localization and function across transmission stages.

Our DPAP2-GFP parasite line revealed consistent localization to osmiophilic bodies in gametocytes. During mosquito-stage development, however, a single GFP-positive structure emerged ~8 hours post-infection, persisting through ookinete maturation and initial oocyst development, resembling the crystalloid, a very little understood transient organelle of the parasite. Colocalization with Lysotracker, a known marker of the organelle in other malaria species, confirmed DPAP2 as a crystalloid-associated protein. Functional studies using DPAP2 knockout (KO) parasites demonstrated impaired gamete egress as well as reduced ookinete formation, the later probably stemming from the defect in gamete egress. While DPAP2-KO ookinetes successfully traversed the mosquito midgut, they failed to progress beyond early oocysts, degenerating by 48 hpi and with no oocysts observed at 11 days post-infection. This defective oocyst development is evocative of defects observed with other crystalloid-associated proteins in murine models of malaria, emphasizing DPAP2's critical role in malaria transmission.

These findings identify DPAP2 as a critical component of the crystalloid and the first *P. falciparum* protein confirmed in this organelle. Notably, the essential role of DPAP2 in mosquito transmission contrasts with its reported non-essentiality in the murine malaria model *Plasmodium berghei*, underscoring the importance of studying *P. falciparum* directly. This work sheds light on crystalloid biology in *P. falciparum*, revealing its unique characteristics and the critical role of DPAP2 in parasite transmission, while highlighting intriguing questions about organelle function and protein targeting across parasite stages.

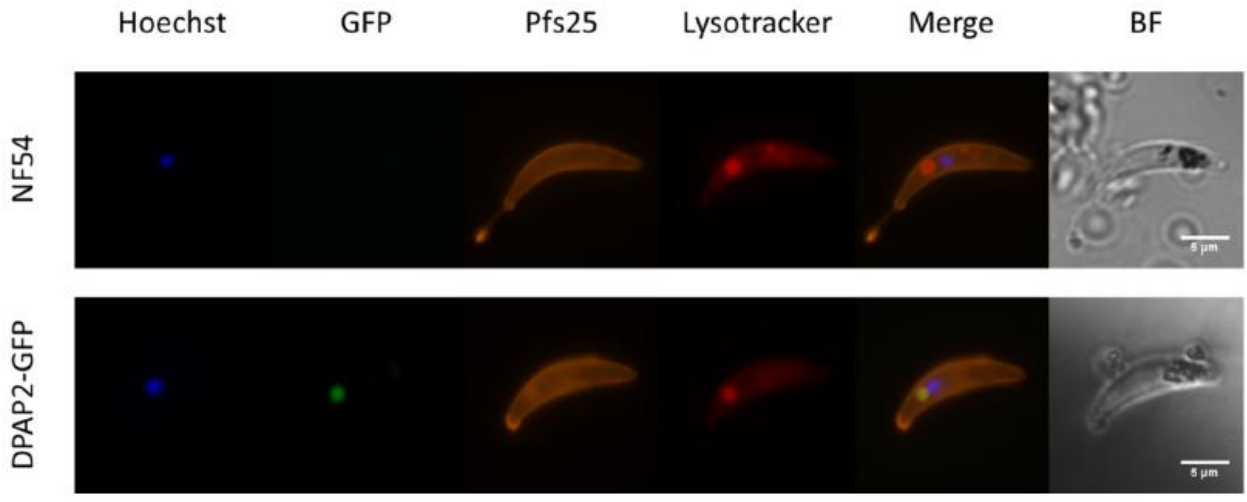

Fig 1: Live WT (NF54) and DPAP2-GFP ookinetes at 24 hours post infection.

Investigating the role of gdv1 asRNA during sexual commitment of *Plasmodium falciparu*m

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Plasmodium falciparum transmission depends on the parasite's ability to switch from asexual replication within the RBC to forming gametocytes. Only the gametocytes are transmissible to the mosquito. Therefore, understanding the mechanisms underlying sexual commitment is essential for unraveling the parasite's transmission dynamics. The transcription factor AP2-G is the master regulator of sexual commitment. In asexually growing parasites, AP2-G is silenced by heterochromatin formation through HP1. Expression of the upstream activator GDV1 leads to the removal of HP1 from *ap2-g* and the onset of sexual commitment. GDV1 is currently the most upstream factor described in the signaling cascade that initiates sexual commitment. However, the regulation of GDV1 expression is still not fully explained. Strikingly, in the *gdv1* locus, three long non-coding (lnc) RNAs are located. One of these lncRNA is complementary to *gdv1*, the *gdv1* asRNA. Previous studies showed that the *gdv1* asRNA is a potential antagonist of GDV1 expression since its knockout leads to a constant expression of GDV1.

With this work, we aim to gain a deeper understanding of the regulatory role of *gdv1* asRNA during sexual commitment and the underlying molecular mechanism. To investigate the potential functions of *gdv1* asRNA, we will establish parasite lines with *gdv1* asRNA overexpression. Commitment rates and the expression profiles of non-sexual commitment markers will be analyzed in these parasites. In addition, the effects on GDV1 expression will be monitored using western blot experiments. Finally, the localization of the *gdv1* asRNA will be determined by RNA FISH experiments to provide first insights into the underlying molecular mechanisms of a *gdv1* asRNA-based regulation.

In summary, these new findings will help us to better understand the molecular mechanisms leading to sexual commitment in *P. falciparum*, thus identifying potential weak points that can serve as targets to counteract malaria transmission.

Discovery of small-molecule inhibitors of the *Plasmodium falciparum* protein kinase CRK4

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Plasmodium falciparum proliferates through schizogony, where nuclei undergo several asynchronous rounds of DNA replication and nuclear division, producing multinucleated cells. The *Plasmodium*-specific kinase CRK4 is critical for the initial and the subsequent rounds of DNA replication. Thus, chemotherapeutic intervention targeting CRK4 would be efficacious throughout the >10-hour-long schizont stage. To identify potential inhibitors of CRK4, we used *in silico* molecular modelling and computational screening to compile a set of 33 small molecules, which may inhibit CRK4. We found that seven compounds perturb *P. falciparum* growth in culture with an EC_{50} < 10 µM. Next, we tested if these compounds phenocopy the CRK4 knockdown, which arrests at the trophozoite-to-schizont transition during intraerythrocytic development. While four compounds blocked parasite development either at the ring or the late schizont stage, we found that three compounds appeared to arrest parasite development consistent with an inhibition of CRK4. To further assess this, we combined genetic knockdown and small-molecule treatment, assuming that a partial knockdown of CRK4 would render the parasites more sensitive to a CRK4 inhibitor. Our data indicates that partial knockdown of CRK4 indeed sensitizes *P. falciparum* against low doses of one of the potential inhibitors. Together, our data suggest a chemical scaffold for further optimization. Ultimately, these small molecules might help us to better understand the biology of CRK4 and aid in the targeted design of novel chemotherapeutics to curb malaria.

Genotyping of Plasmodium falciparum sickle associated single nucleotide polymorphisms (Pfsa SNPs) in Africa

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Background:
Heterozygous carriers of the HbS allele (HbAS, sickle cell trait) are known to have relative protection against severe malaria and malaria-related death. Recent genome-wide association studies have identified four single nucleotide polymorphisms (SNPs) in the genome of *Plasmodium falciparum* that appear to decrease this protective effect. However, data on the distribution of these sickle-associated SNPs remains limited and are primarily derived from whole-genome sequencing.

Methods:
We developed a simple PCR protocol for genotyping *Plasmodium falciparum* sickle associated SNPs (Pfsa) using Sanger sequencing. Template parasite DNA was extracted from whole blood samples, including dried blood spots, collected from malaria patients at Centre de Recherches Médicales de Lambarene, Gabon, and Charité university hospital, Berlin, Germany.

Results:
In a proof-of-concept analysis of 60 patient samples from Gabon, we found three of the four Pfsa SNPs to be highly prevalent (50% Pfsa1+, 23% Pfsa2+, 29% Pfsa4+). Three samples exhibited a triple mutation, while 18 samples showed no mutations. The Pfsa1 SNP was also present in 26% of the isolates of imported malaria cases from Cameroon and Nigeria, whereas the Pfsa2 SNP was absent from all analysed isolates. Three additional SNPs appear frequently near the Pfsa loci.

Conclusion:
The protocol is robust and suitable for settings with limited laboratory equipment, even at low peripheral parasitaemia, and provides a feasible option for surveillance of the Pfsa SNPs distribution in Africa. The prevalence of Pfsa in the limited number of samples of our study follows the known prevalence of HbAS in Africa.

Cytolysin secreting genetically attenuated Plasmodium yoelii parasites as whole sporozoite vaccines

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Genetically attenuated live sporozoite vaccines have been shown to induce protective immunity in rodent malaria models and humans. Once inside the vertebrate host, *Plasmodium* parasites migrate into liver and invade hepatocytes. The main goal of genetic attenuation is complete developmental arrest during hepatocyte infection prior to blood infection. Single knockouts of essential liver stage genes are usually not sufficient; therefore, vaccine strategies typically include multiple gene deletions.

Here, we aim at generating late arresting genetically attenuated parasites (GAPs) by combining knock out of an essential liver stage gene with transgenic expression of a bacterial pore forming protein to disrupt the integrity of the parasite and/or parasitophorous vacuole membranes. The rationale is the assumption of improved release of *Plasmodium*-derived antigens into the hepatocyte cytoplasm and increased hepatocyte apoptosis, which might enhance MHC I peptide loading either directly or *via* cross-priming.

We generated two alternative *Plasmodium yoelii* parasite strains: *ΔLISP2::LLO* (*PyLISP2* replaced with secreted Listeriolysin O (LLO)) and *ΔLISP1::SLO* (*PyLISP1* replaced with secreted streptolysin O (SLO)). We show that infections with 5,000 sporozoites do not cause blood infections. Increasing sporozoite doses to 20,000 parasites resulted in breakthrough infections of the *ΔLISP2::LLO* parasite line, but not of *ΔLISP1::SLO* parasites, which remained blood film-negative even at 0.5 Mio. sporozoites. We next immunized BALB/c mice with three doses of sporozoites at 14 days intervals. Upon challenge with 10,000 *Py*WT sporozoites 30 days after the last immunization dose, all mice immunized with 5,000 *ΔLISP2::LLO* sporozoites remained free of blood stages, while *ΔLISP1::SLO* immunizations were less effective.

Our data support the notion of a delicate balance between safety and efficacy of GAP vaccine strains and signify development of improved GAPs for preclinical malaria vaccine testing.

Uncovering Diversity: Complete Mitochondrial Genomes of African Bat Hepatocystis Reveal Conserved Structure but Unusual Size

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The wide diversity of microorganisms harbored by bats includes seven distinct genera of malaria parasites. *Hepatocystis* parasites, most closely related to mammalian-infecting *Plasmodium* species, infect diverse mammalian hosts, including primates, bats, ungulates and rodents across the Old-World tropics of Africa, Asia, and Australia. Notably, African epauletted fruit bats feature exceptionally high prevalences of *Hepatocystis* infections of up to 100%. Phylogenetic studies of haemosporidian parasites have traditionally used partial mitochondrial gene sequences, however, more recently, complete mitochondrial genomes are used for improved resolution of taxonomic and evolutionary studies. Here we generated the first complete mitochondrial genomes of *Hepatocystis* parasites of African bats and an Australian bat host. Samples originate from *E. labiatus* fruit bats in northern Uganda and a *Pteropus* bat from southern Australia. We used a combination of published and newly designed primers for PCR amplification and Sanger sequencing. Complete mtDNA genomes were generated by individual sequence assemblies and annotated by GeSeq algorithm based on published haemosporidian mtDNA genomes. We successfully obtained six complete mitochondrial genomes of *Hepatocystis* parasites. Surprisingly the mtDNA genomes of African *Hepatocystis* feature a length of ~8.5 kb. These are the largest known mtDNA genomes malaria parasites. The size of mtDNA genomes of *Plasmodium* and other haemosporidians is usually between 6 kb and 7 kb. The overall structure is conserved, comprising the three protein-coding genes *cytb*, *cox1*, and *cox3* and fragmented rRNA genes. Our findings reveal unrecognized diversity in an otherwise assumed conserved genome of haemosporidian parasites.

Safety and Tolerability of Artemether-Lumefantrine + Atovaquone-Proguanil Tri-therapy for Treatment of uncomplicated Malaria in Adults and Adolescents in Gabon- ASAAP Project - Pilot Study

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Malaria is a lethal parasitic disease with an estimated 263 million cases occurring in 2023, resulting in 597000 deaths. Artemisinin-based Combination Therapies (ACTs), the current first-line treatment, are showing reduced efficacy in South-East Asia, with resistance spreading to Sub-Saharan Africa. A potential strategy to increase the lifespan of existing ACTs and preserve ACT efficacy in Africa is the introduction of a second partner drug, forming a triple-ACT (T-ACT) that further limits susceptibility to resistance. The ASAAP project consists of two clinical trials evaluating the efficacy, safety, tolerability, and pharmacokinetics of Artemether-lumefantrine (AL) combined with Atovaquone-Proguanil (AP) as a T-ACT. This abstract presents results from the first pilot clinical trial.

The Phase IIb, double-blind, randomized, placebo-controlled trial was conducted in a semi-immune population in Lambaréné, Gabon. Adults and adolescents aged ≥15 years with uncomplicated *Plasmodium falciparum* malaria were enrolled. Sixty participants were randomized 2:1, with the intervention group (n=40) receiving AL twice daily + AP once daily over three consecutive days and the control group (n=20) receiving AL twice daily + placebo once daily over three days. Participants were followed for 42 days.

Of 85 screened participants (median age: 21 years, IQR:15-18), 60 were randomized; screening failures were mainly due to low parasite density and hyperbilirubinemia. Five participants vomited after drug administration, and one was lost to follow-up. Adverse events were reported in 45 participants (75%), all mild to moderate except one unrelated serious adverse event.

The results indicate good safety and tolerability in both arms, supporting further investigation in younger children (≤5 years), the group most vulnerable to malaria, to evaluate efficacy and safety.

Keywords: Malaria, Triple Therapy, Artemether-Lumefantrine, Atovaquone-Proguanil, Sub Saharan Africa, Semi-immune population, Efficacy, Safety

Determination of the food vacuole proteome of Plasmodium falciparum via proximity-biotinylation

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The injection of attenuated parasites at sporozoite stage is a previously used approach towards malaria vaccination. The (combined) knock-out of one or more genes can either induce an arrest at liver stage or a decreased growth rate in the blood stages to allow the host to confer immunity. Our previous screen and consecutive attenuations in the murine pathogen *P. berghei* revealed that some of the most promising targets were involved in hemoglobin digestion and located in the food vacuole. This highlighted the need to further improve our fundamental understanding of this compartment.

Here, we wanted to determine the proteome of the food vacuole by an unconventional application of proximity-biotinylation-based mass spectrometry. For this purpose, we used recombinantly expressed engineered ascorbate peroxidase (APEX2), which, after preloading into red blood cells, is taken up via the endocytotic pathway and will accumulate in the parasite's food vacuole. Subsequent biotinylation and pulldown with streptavidin-coated magnetic beads yields a food vacuolar proteome. We performed the same assay using a transgenic line with inducible vesicle fusion blockade to further examine vesicular contents. Accordant localization of a subset of enriched hits was confirmed via episomal tagging.

Our results suggest that the recombinant expression of a biotinylating enzyme and preloading of red blood cells can be a viable method to investigate the food vacuole and the endocytotic pathway. Those experiments provide us with a list of candidate proteins, which are currently under further investigation.

Assessing the impact of artemisinin resistance on *P. falciparum* transmission in insecticide resistant *Anopheles gambiae*

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Insecticide treated bed nets are the primary tool for malaria prevention, whilst anti-malarial drugs such as artemisinin derivates play a key role in treating malaria in sub-Saharan Africa; however, delayed clearance of infections associated with Kelch13 single nucleotide polymorphisms (SNPs) is increasingly reported. Whilst the fitness costs associated with drug resistance on the clinically relevant blood stages of *Plasmodium falciparum* are identified on a basic level, the effect on transmission through the mosquito stages remains poorly understood. Moreover, increasingly prevalent insecticide resistant (IR) *Anopheles* mosquitoes display metabolic differences which can affect the success in transmitting resistant parasites. Using CRISPR/Cas9 mediated mutagenesis, we first introduced field relevant Kelch13 point mutations into the *P. falciparum* strain NF54. To validate the conferred resistance *in vitro*, we perform ring stage survival assays. To investigate changes in transmission competence, we scored oocyst prevalence, intensity and ookinete abundance. We observe a significant increase in oocyst prevalence, indicating an advantage in mosquito stages for parasites harbouring K13 mutations, specifically Kelch13^{C469Y} and Kelch13^{R561H}. Next, we performed competition assays using NF54 wild type and determined the prevalence of mutant alleles in oocysts via droplet digital PCR, discovering no inherent fitness cost in either mutation. This study will help us gain insight into antimalarial resistance transmission in a field relevant setting. Future research will include introducing the mutations into field isolates, as well as targeting SNPs conferring resistance to Sulfadoxine and Pyrimethamine.

Boromycin: an antimalarial candidate with potent transmission-blocking activity

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Boromycin, a boron-containing antibiotic produced by *Streptomyces antibioticus,* has been described as a potent antivirus, anti Gram-positive bacteria and anti-protozoan agent. Antibiotics with antimalarial effect have been reported to target organelles of prokaryotic origin and to have a relatively slow onset of action due to their activity only on the second parasite´s life cycle. For these reasons, antibiotics have been used for malaria prophylaxis and for the treatment of malaria in combination with a fast-acting drug. Artemisinin combination therapies (ACTs) are the current treatment of malaria, and although still very efficacious, target only the asexual stages of the parasites, allowing the transmission of the disease after treatment. However, the emergence of artemisinin-partially resistant parasites in some countries in east Africa poses a threat to ACTs. Therefore, new alternatives are needed to replace ACTs in case of spread of resistance. Recently, boromycin was revealed as a potent, medium-acting, multi-stage antimalarial candidate. Boromycin´s activity against asexual stages of *P. falciparum* (sensitive and resistant strains) and *P. knowlesi* and mature gametocytes were in low nanomolar concentrations (IC_{50}: ~1 nM, and 8.5 nM, respectively). Further assays revealed that boromycin has a speed of action similar to chloroquine, eliminating all parasites after 72 h of drug incubation, and was also highly active against the artemisinin-resistant strain Dd2_R357T with parasite survival at 0.4% in comparison to 3.8% to dihydroartemisinin. Boromycin has also been shown to have a very low propensity for resistance development, and was similarly active against clinical isolates. So far, the apicoplast and ionophoric activity on potassium channels were excluded as the main target of boromycin, and further assays are planned to try to find out its MoA. Boromycin is a promising antimalarial candidate also with a great transmission blocking potential.

Dehalogenil: A novel compound with potent, multi-stage antimalarial activity and a high resistance barrier

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One of the important goals of current research on antimalarial compounds is the development of non-artemisinin combinations therapies targeting both asexual blood stages as well as the transmissive gametocytes to combat drug resistance and reduce malaria transmission. One especially promising compound in this regard is Dehalogenil, a semi-synthetic macrolide derived from the soil-dwelling myxobacterium *Sorangium cellulosum*. Compared to its precursor molecule Chlorotonil A, Dehalogenil possesses several improved characteristics, including higher antimalarial activity, increased solubility and a better safety margin. Dehalogenil is highly active against asexual blood stages of *Plasmodium falciparum* laboratory strains with different geographical origins and resistance backgrounds, clinical isolates from Gabon as well as against *P. knowlesi* (range of IC_{50} 13.5-32.5 nM). In addition, Dehalogenil also potently acts against mature *P. falciparum* gametocytes (IC_{50} 12.3 ± 10.2 nM). Experiments to determine the parasite reduction ratio revealed that Dehalogenil has a rapid onset of activity with no parasite growth after 24 hours and no parasites detectable after 48 hours. Dehalogenil is currently deemed "irresistible", with no parasite recrudescence in both one-step and step-wise resistance selection assays using *wt*-Dd2 and *pol*δ^{-}Dd2 parasites and culturing for 60 days and since 15 months, respectively. Taken together, these results show that Dehalogenil is a promising lead compound for the development of a novel antimalarial treatment targeting both symptom-causing and transmissive plasmodial stages.

Evolution of *Plasmodium falciparum* resistance to antifolate antimalarial drugs in Uganda

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Malaria, caused by *Plasmodium falciparum*, remains a major health threat in sub-Saharan Africa. Chemoprevention with sulfadoxine-pyrimethamine (SP) is recommended for vulnerable groups, but resistance threatens its effectiveness. Mutations in PfDHFR and PfDHPS drive SP resistance. Often, they are acquired sequentially, with the level of SP resistance dependent on the number of mutations. The evolution of resistance-associated mutations depends on the strength of selection pressure. For SP, it remains uncertain whether the current antifolate selection pressure drives further evolution. To address this question, we conducted spatio-temporal surveillance to track the temporal prevalence and assessed for signals of selection of key antifolate resistance-associated alleles at 16 sites across Uganda. Our analysis revealed a high prevalence (>90%) of five key mutations (PfDHFR N51I, C59R, S108N, and PfDHPS A437G, K540E). The PfDHFR I164L and PfDHPS A518G were more common in southwestern Uganda. Temporal analysis revealed an increase in the prevalence of PfDHFR I164L at multiple sites (Proportion mutant, 2016-2017: 0.19 [95% CI: 0.16 - 0.23], and 2022: 0.34 [95% CI: 0.31 - 0.37]). Analysis of IBD sharing patterns, parasite connectivity and signals of positive selection revealed (1) evidence of stable transmission of PfHDFR I164L mutants in southwestern Uganda, (2) potential local expansion of PfHDFR I164L mutants at Amolatar and Arua, and (3) recent expansion of PfHDFR I164L mutants plus positive selection around *pfdhfr* gene at Jinja. Although SP use has been limited for chemoprevention for nearly two decades, our comprehensive population-level assessment showed a continuing increase in PfDHFR I164L, with evidence of possible positive selection at one site. Whether additional drivers of SP resistance selection other than chemoprevention are at play remains unclear.

Investigation of the molecular functions of chromatin remodeling proteins during sexual commitment in the malaria parasite *Plasmodium falciparum*

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Plasmodium falciparum, the most lethal malaria parasite, causes severe morbidity and mortality, particularly in Africa. Its transmission depends on the ability to switch from asexual replication in red blood cells to gametocyte formation, the only stage transmissible to mosquitoes. This process is controlled by the transcription factor AP2-G, whose expression during asexual reproduction is suppressed by heterochromatin formation mediated by heterochromatin protein 1 (HP1). Activation of the upstream regulator GDV1 reverses this repression by removing HP1 from the *ap2-g* locus, thereby initiating sexual differentiation. However, the epigenetic mechanism controlling this process is poorly understood.

This project aims to define the epigenetic machinery regulating *ap2-g* expression and sexual commitment by focusing on three chromatin remodelers*: Pf_CHD1, Pf_ISWI*, and *Pf_SNF2L*. *Pf_CHD1*, a chromodomain DNA-binding helicase, has been identified as a potential GDV1 interaction partner, while *Pf_ISWI and Pf_SNF2L* are upregulated during sexual commitment, emphasizing their significance. CRISPR/Cas9 genome editing, including endogenous tagging and knockout via loxP-Cre recombinase, will enable a detailed analysis of the remodelers.

A combination of advanced techniques, including RT-qPCR, ChIP-qPCR, and RNA-seq, will be used to analyze *ap2-g*expression and trancriptomic changes to investigate the role of those chromatin remodelers in the epigenetic switch.

Anti-GFP Cut&Tag will provide a genome-wide binding profile of the remodelers, while immunofluorescence assays and live cell microscopy will explore subnuclear localization and interactions with GDV1.

These approaches will provide a comprehensive understanding of the chromatin remodeling machinery and its role in regulating *ap2-g* expression, contributing to insights into the parasite's sexual differentiation and to the development of strategies to control parasite transmission.

Assessment of molecular markers of knockdown resistance in the Anopheles population in Madibou and Goma Tse-Tse, Republic of Congo

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Background
Knock-down resistance caused by a single base pair mutation in the sodium channel gene is strongly associated with pyrethroid insecticide resistance in *Anopheles* mosquitoes in Central-West Africa. The aim of the study was to determine the molecular markers of the knock-down insecticide resistance in the *Anopheles* mosquitoes collected in southern Brazzaville, in the Republic of Congo.

Method. *Anopheles* mosquitoes were collected in household during a cross-sectional study conducted from March to September 2021 in Goma Tsé-Tsé health and Madibou districts. The *Anopheles* mosquitoes were identified using binocular magnifier and nested-PCR. The Knock-down resistances of Kdr-West and Kdr-East mutations were investigated using *TaqMan* assay (real time-PCR).

Results. A total of 585 *Anopheles mosquitoes* was analysis. *An. gambiae s.l*. was predominant (90.6%), followed by *An. funestus s.l*. (7%) and *An. moucheti* (2.4%). Among *An. gambiae s.l*., *An. gambiae s.s*. represented 82.9%, *An. coluzzi* 12.3%, and *An. arabiensis* 4.8%. The kdr-W (38%) and kdr-E (11%) mutations were detected, as well as the kdrW/kdrE mutation (5.8%). *An. gambiae s.s.* was the only species that carried all the mutations. The village of Djoumouna had the highest mosquito density and the presence of all kdr mutations. The kdrW mutation was slightly more frequent in the dry season (39%) than in the wet season (38%), while kdrE was higher in the wet season (14%) than in the dry season (8%). Double mutation was more common in the dry season (6.2%) than in the rainy season (5.5%).

Conclusion. This study reveals for the first time the presence of Kdr-E and Kdr-W mutation in *Anopheles* mosquitoes from the Republic of Congo, suggesting the necessity to integrate knock-down (Kdr) resistance surveillance to the national malaria control strategies.

Keywords: Malaria, Knock-down resistance, Insecticide, *Anopheles* mosquitoes, Republic of Congo

Haematological recovery after antimalarial treatment of uncomplicated malaria in a subset of population of the 1-D-CURE study

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Introduction

Malaria caused by protozoan parasites of the genus Plasmodium is according to the WHO responsible for 597 000 deaths in 2023 worldwide (WHO).

Haematological abnormalities are one of the most common complications in malaria.

The World Health Organization (WHO) protocol for the evaluation of an antimalarial drug or combination of drugs includes the restoration of haematological parameters as an efficacy criterion.

Data on the impact of antimalarial treatment on haematological recovery are scarce.

Thus, the aim of this study is to characterize the haematological profile of participants treated with antimalarial drugs for uncomplicated malaria.

Methodology - Study design and study population.

This study is a secondary analysis of participants included in the 1-D-CURE study.

Briefly, 1-D-CURE is a clinical trial assessing the efficacy and safety of a single-dose sulfadoxine-pyrimethamine plus artesunate-pyronaridine multidrug combination therapy for the treatment of uncomplicated malaria in adults and children in Gabon.

The study prospectively examines the results of peripheral blood counts of children, adolescents and adults with fever and who tested positive for uncomplicated malaria and treated at the Centre de Recherches Médicales de Lambaréné (CERMEL) and at its satellite sites in Four place and Tchibanga.

The time points for data analysis are D0 and D28.

Preliminary results.

In total, data from 80 participants are analysed in the study. The median age was 11 years.

Here we present preliminary results of haematological parameters.