

NEWS AND VIEWS

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Infectious Diseases

Lecture - 03

23rd August, 2021



Dr Manu Prakash

Associate Professor
Department of Bioengineering
Stanford University, USA

Issue 10, August 2021

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Dr Sanjib Mohanty

&

Dr Praveen Kishore Sahu

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Dr Chanaki Amaratunga



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MERA-INDIA Newsletter 'News & Views' August 2021

Editorial

Dear readers,

MERA-India team presents to you the tenth issue of our newsletter.

In July 2021, the NIMR COVID-19 lab completed 50,000 RT-PCR tests. This accomplishment was celebrated at an event in NIMR where the Director NIMR congratulated the team members and presented them with certificates of appreciation.

We organized the second lecture of the Lecture Series on Infectious Diseases on 23rd July 2021. In this lecture, Dr Gyanu Lamichhane, Johns Hopkins University, spoke on the topic 'Atypical cell wall of mycobacteria: Its relevance to TB, treatment and drug-resistance'.

In the 'Guest Commentary' segment, Dr Sanjib Mohanty and Dr Pravin Kishore Sahu talk about the emerging concepts in the pathogenesis of cerebral malaria. For the 'Malaria Scientist to Watch' section, we interviewed Dr Chanaki Amaratunga (DeTACT Coordinator, MORU, Mahidol University, Thailand).

In the 'Research in Spotlight' section, we have highlighted four recent publications with significance in the field of malaria research. In the study by Mohan I *et al.*, published in Malaria Journal, the authors have analysed socio-economic determinants of malaria in India using data from longitudinal ageing survey. In the study by Zhang M *et al.*, published in Nature Communications, the common link through apicoplast derived genes in fever survival response as well as artemisinin resistance in *P. falciparum* through is shown. In the study by Giacometti M *et al.*, published in Adv Sci, the authors have described a new rapid lab-on-chip malaria diagnostic tool to detect and quantify the infected RBCs and the distinct stages of malaria parasite. In the study by Van Heerden A. *et al.*, published in Front Cell Infect Microbiol., the authors have used machine learning to stratify antimalarial compounds based on their modes on action using chemo-transcriptomic profiles. We congratulate the authors of these studies on their novel findings, and hope our readers will find these articles informative to read.

In the 'Upcoming Events' section, we have provided the details of the third lecture of the

'Lecture Series on Infectious Diseases' being organized by NIMR & MERA-India. This lecture will be delivered by Dr Manu Prakash, Stanford University, on 23rd August 2021 on the topic 'Frugal Science: Reimagining the role of technology in global health, science education and disease monitoring' (<http://lectureseries.meraindia.org.in/>).

We hope you will enjoy reading this issue.

For any feedback or suggestions towards the content of the newsletter, please write to us at meranewsletter@gmail.com.

With best wishes
MERA-India team

NIMR Activities: Celebration of 50,000 RT-PCR tests at NIMR-COVID-19 lab



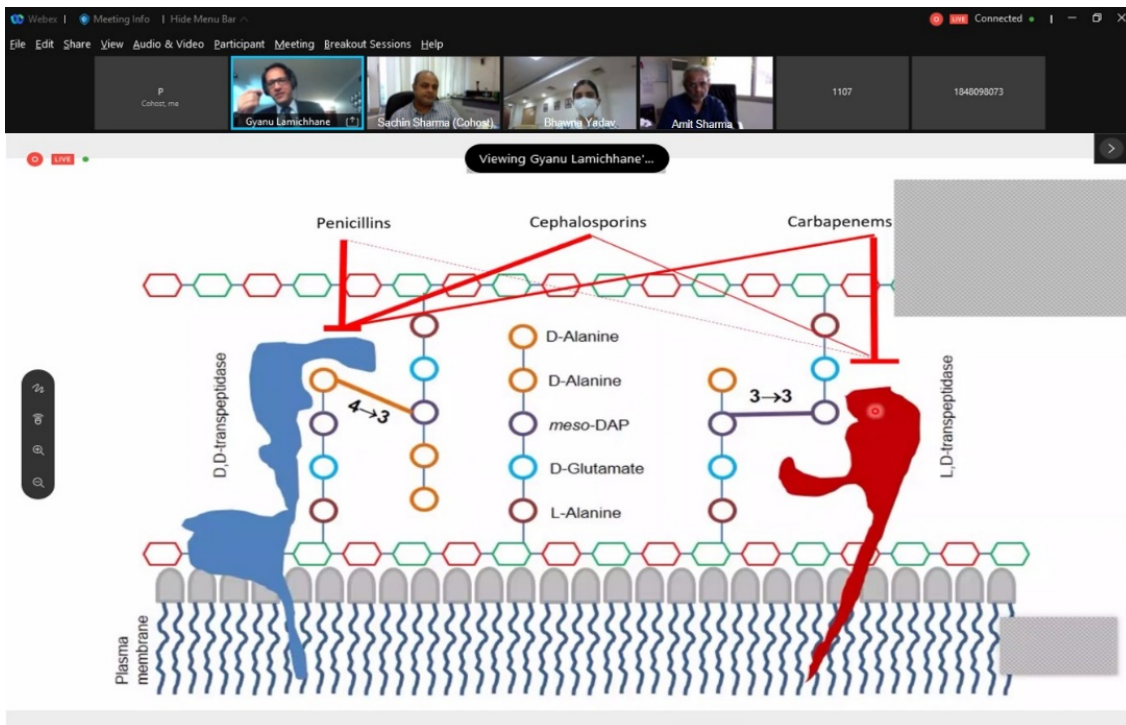
In March 2020, NIMR was assigned the responsibility to act as a regional as well as a central depot to check the integrity and quality of the COVID-19 kits (VTM, RNA-extraction and RT-PCR), and to distribute these kits. Approximately 12 million COVID-19 kits were distributed across India by NIMR managed depots.

In October 2020, a COVID-19 diagnostic lab was set up at NIMR. Teams comprising of NIMR staff and additional staff recruited for this facility were formed. The team members were trained to use the PPE kit; to collect, handle and dispose the patient samples with complete safety and precautions, as well as the protocol for sample testing. The team members worked day and night in the COVID-19 lab to provide timely reports for the collected samples. The facility is also responsible to test the samples collected from the Parliament and the Ministries of Government of India. The average reporting time for the

results was within 6 hours of sample collection. While initially the lab was handling 100 samples/day, the capacity was increased to close to 600 samples/day during the pandemic peak period. No backlog for the sample reporting was observed at any point of time.

It was a proud moment for NIMR in July 2021, when our COVID-19 testing laboratory completed 50,000 RT-PCR tests. This could be achieved only due to the tireless efforts made by the teams. To celebrate this achievement, an event was organized at NIMR on 05th July 2021. The Director NIMR, Dr Amit Sharma, congratulated and praised the team members for their dedication and hard work; and presented them with certificates of appreciation.

Lecture 02 of Lecture Series on Infectious Diseases



The second lecture of the NIMR & MERA-India virtual lecture series on infectious diseases (June 2021-May, 2022), was scheduled on 19th July 2021. However, due to unavoidable circumstances, the lecture had to rescheduled for 23rd July 2021. The lecture was delivered by Dr Gyanu Lamichhane, Associate Professor, Johns Hopkins University on the topic 'Atypical cell wall of Mycobacteria: Its relevance to TB, treatment and drug-resistance'.

Dr Sachin Sharma, Chief Consultant, MERA-India welcomed everyone and introduced the speaker. In his lecture, Dr Gyanu described how according to the historical generalized cell wall model, it was thought that there was only one enzyme class D,D-transpeptidase (D,D-T) or penicillin-binding-protein, catalyzing the 4→3 interpeptide linkages to polymerize the peptidoglycan monomers in the bacterial cell wall. This guided the use of β -lactam drugs to inhibit cell wall synthesis by targeting this specific enzyme, leading to the inhibition of peptidoglycan synthesis and thus cell rupture and death. However, the discovery of L,D-

transpeptidase (L,D-Ts), which catalyzed the 3→3 linkages in the peptidoglycan polymers, led to the revision of the mycobacterium cell wall model. This also resulted in an understanding of the resistance to the old classes of β-lactam drugs, which were designed to inhibit D,D-T but missed L,D-T. He also talked about the current mouse models to study the course of mycobacterium infections, and the synergistic efficacy of β-lactam combinations against the mycobacterium infections by targeting both, the D,D-T as well as L,D-T.

The lecture was followed by answers to the audience questions. The session ended with a note of thanks from Dr Sachin Sharma.

The recording of this lecture is now available on MERA-India website (<https://www.meraindia.org.in/lecture-series>).

Guest Commentary

Emerging concepts in the pathogenesis of Cerebral Malaria

[Dr Sanjib Mohanty](#)^{1*} (MD) and [Dr Praveen Kishore Sahu](#)² (PhD)

¹ Senior Consultant, Infectious Disease Biology Unit, Ispat General Hospital Rourkela and Dept. of Molecular Biology & Infectious Diseases, Community Welfare Society Hospital Rourkela, Odisha, India

Email: sanjib.mohanty54@gmail.com

*Corresponding author

² Senior Scientist, Molecular Biology & Immunology Lab, Ispat General Hospital Rourkela and

Dept. of Molecular Biology & Infectious Diseases, Community Welfare Society Hospital Rourkela, Odisha, India

Email: praveenkishore.sahu@gmail.com

Cerebral malaria (CM) is a dreaded neurological complication of *Plasmodium falciparum* malaria infection in humans, though it can manifest during *Plasmodium vivax* infection as per few case reports. The pathogenesis of CM is multifactorial, and is yet to be unraveled completely. Previous understandings were solely limited to autopsy studies, while a robust animal model is still elusive, despite significant progress. Central to the pathogenesis of CM is the cytoadherence of parasitized erythrocytes (pRBCs) to the host endothelial receptors causing hypoxia and energy deprivation in brain tissues, which are highly sensitive to oxygen and energy depletion. The process leads to a release of several inflammatory mediators like TNF-α, interleukins and free-radicals thereby upregulating a multitude of host vascular receptors, prominent being EPCR, ICAM, CD-36 etc. The pRBCs express a family of proteins called PfEMP1 (*Plasmodium falciparum* erythrocyte membrane protein 1), forming ligands with these for sequestration. A landmark autopsy case-series on 23 fatal CM patients from Rourkela, India in 1994, had shown evidence of

clogged-capillaries of various degrees in different parts of brain, leukocytes margination to endothelial walls and brain parenchyma, pointing towards a breach in the blood-brain-barrier (BBB) [1]. The outcome of these processes is known as 'brain edema', however brain swelling for a long time, was believed as an agonal event and not a major mechanism causing CM. Previous autopsy studies from Vietnam [2] and Africa [3] had also demonstrated gross swelling of brain which can be fatal, however could not explain how. Also, due to possible biasness in autopsy data in CM and live brain-biopsy being infeasible, malariologists in order to understand the pathophysiology of CM, took resort to 'neuroimaging'.

Computerized Tomography (CT) of the brain in CM patients were undertaken in small cohorts in different parts of the world with varying findings [4,5], but brain swelling was not consistently demonstrated. Mannitol, an osmotic diuretic, which helps in reducing the brain edema had inconsistent effects. Our institute at Rourkela, Odisha– a hotspot in the malaria endemic region, took up a Mannitol clinical trial, the largest series of 126 adult CM patients till date whose brain CT scans revealed majority with mild to severe brain swelling, consistent with high opening cerebrospinal fluid (CSF) pressure [6]. Nonetheless, since Mannitol arm presented higher mortality and prolonged coma recovery time than placebo, the question arose that what led to this adverse effect, and what could be the possible mechanism(s) of brain swelling?

Meanwhile, to understand the landscape of the CM pathogenesis, colossal progress was being made (and still is), to enhance the diagnostic and prognostic capacities and to evaluate this neurological syndrome (CM) through an array of tools and biomarkers [7]. At the same time, high-resolution brain imaging of CM patients 'in-life', using Magnetic Resonance Imaging (MRI) became more relevant. Among several small case series and reports published, the pioneering study was on 24 patients from Thailand in 1995, that opened the gateway for malariologists to investigate CM using MRI [8]. And the largest systematic MRI study on CM was from Malawi, east Africa- which showed varying degrees of brain swelling and established the fact that herniation of brain leads to respiratory failure and deaths in children with CM [9]. However, even more complex questions arose, like what are the underlying mechanisms of the brain swelling leading to death, and whether pathogenesis of CM in children differ than adults?

Classically, we knew that brain edema is either 'cytotoxic' i.e, brain tissue deprived of oxygen and energy could swell due to dysfunction of ATPase pumps; or, vasogenic, i.e. due to a breach in the BBB. Our Subsequent MRI studies then revealed the occurrence of reversible vasogenic oedema (within 48-72 hours) in both adults and children from Rourkela. This phenomenon was predominant in the posterior part of the brain, the primary site of inflammation and parasite cyto-adherence with weakened blood vessel walls, suggesting a consequence of endothelial dysfunction, defined as posterior reversible encephalopathy syndrome (PRES) or PRES-like symptoms earlier only known to occur in hypertension and some drug effects. Strikingly, there were changes in the basal nuclei area in a subset of patients, suggestive of venous congestion, presumably owing to pRBC sequestration leading to cytotoxic edema [10]. Despite the small sample size, this study was among the very first of its kind, and the experimental evidence corroborated the hypotheses that an impaired BBB and vascular engorgement are responsible for the increased brain volume in CM. Consistent with the Rourkela study, very soon a case series from Zambia corroborated that PRES is a common syndrome of CM in rapidly reversible

malarial coma, regardless of any association with age or multiorgan complications of malaria [11].

Following the discovery of PRES and PRES-like syndromes in CM, a case report [12] showed multiple vasoconstrictions in the brain of a CM patient upon MR angiography, which is known as RCVS (Reversible cerebral vasoconstriction syndrome). The mechanism of RCVS in CM needs further evaluation through larger sample sizes. The major difference between RCVS from PRES could be due to sympathetic hyperactivity caused by dysregulated vascular tone, endothelial dysfunction through pRBC adherence, and oxidative stress leading to vasoconstriction of multiple cerebral arteries without evident aneurysm. RCVS could be uniphasic and reversible, meaning it may structurally mimic PRES-induced CM but not the same, and that, RCVS could be triggered by several other causes as described earlier [13,14].

Nevertheless, the advances from neuroimaging studies couldn't explain the fatality in CM or the age factor in the pathogenesis in CM. The most recent MRI study has shown that profound brain hypoxia measurable by low apparent diffusion coefficient (ADC) values, elevated plasma Lipocalin-2 and high PfHRP2 can predict negative outcomes (deaths) in adult CM compared to children, in the Rourkela cohort [15]. The findings were promising and supported the hypothesis that adults may have multiple localization of restricted diffusion, and fatal CM was perhaps associated with decreased ADC, suggesting cytotoxic edema in adults. Interestingly, in the children group, only one had brainstem herniation and low ADC values and rest two had high ADC values, indicating vasogenic edema. Although a very small number of fatalities occurred in this study, yet age of the patients was a significant factor. Hence disease courses may be differentially targeted by specific adjunctive therapies according to age-group.

Despite the recent advancements, many pieces are still missing from the complex puzzle. First, mechanistic basis of endothelial dysfunction and sequestered pRBCs, which indeed causes vasogenic edema in PRES or the breach in the integrity of BBB, is yet to be elucidated perfectly. Second, it is also unclear whether the differential PfEMP1 expression and degree of sequestration in the endothelium leads to the impaired venular circulation and segmental constriction of cerebral arteries. Nevertheless, more specific and underlying pathogenetic mechanisms of CM and features associated with brain swelling can be deduced using multidisciplinary clinical (neuro-radioimaging) and laboratory (*var* gene transcriptomics, NextGen sequencing, and multiplexed sero-profiling) investigations, clubbed with advanced machine-learning models, which are currently underway. All these emerging approaches will reveal more novel concepts and trends, paving way to discover adjunctive therapies for CM patients, which are unfortunately scarce at present.

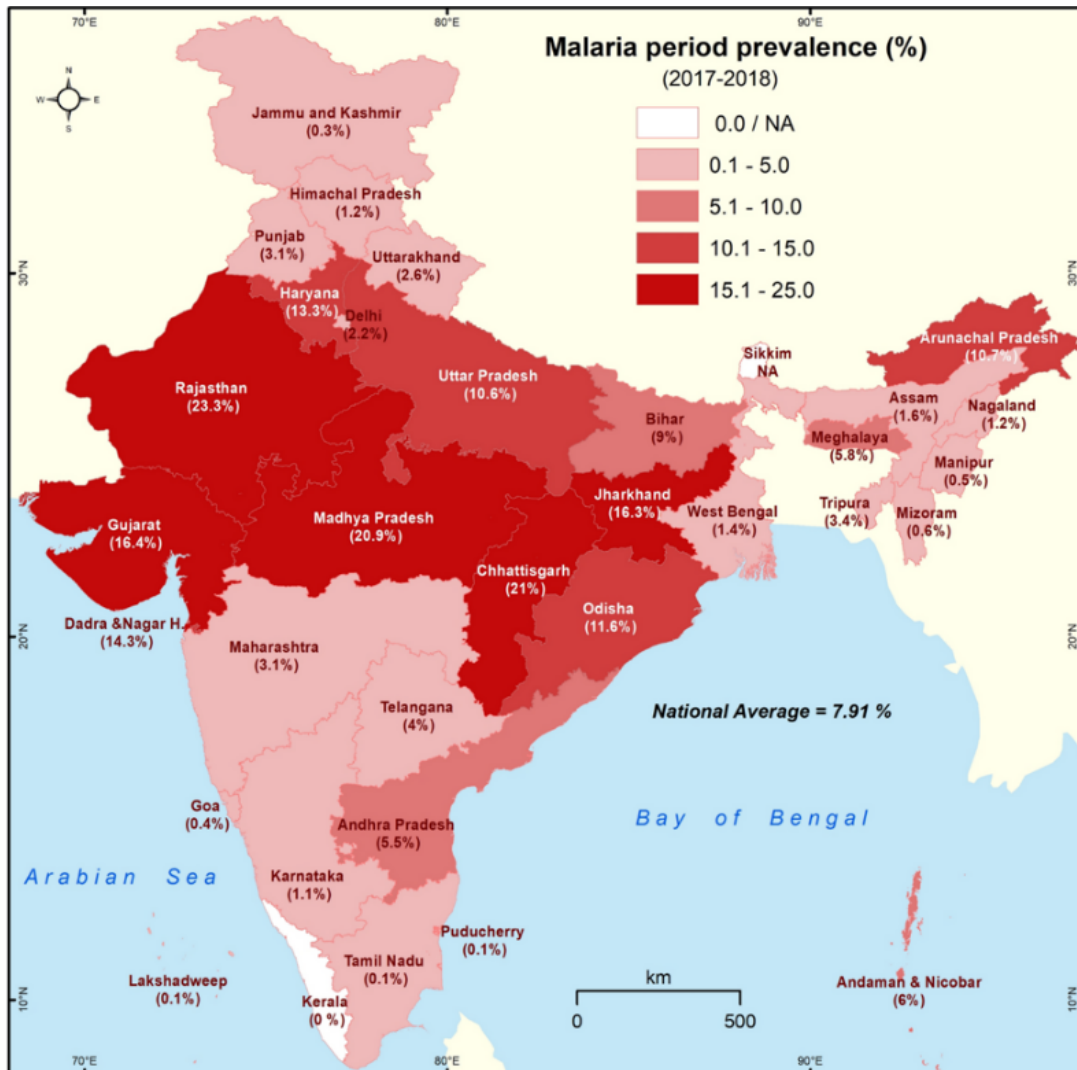
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Research in Spotlight

Mohan I. *et al.*, *Malar J*, 2021: Socio-economic and household determinants of malaria in adults aged 45 and above: analysis of longitudinal ageing survey in India

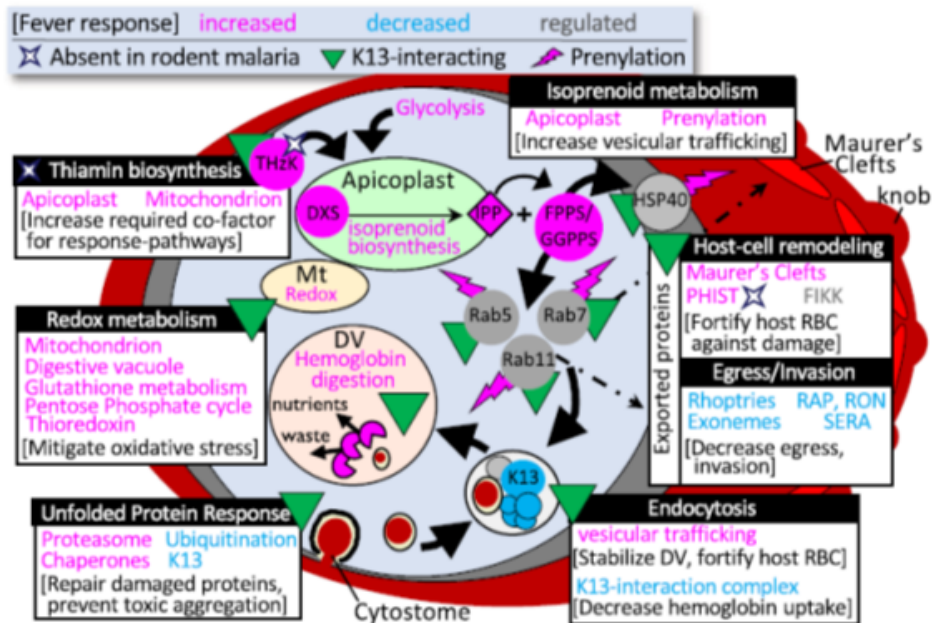


Source: <https://pubmed.ncbi.nlm.nih.gov/34233690/>

Authors of this [article](#) performed a pan-India (except Sikkim) study on the socio-economic and household determinants of malaria as a part of India's malaria elimination efforts. Analysis of dataset (≥ 45 years) from Longitudinal Ageing Survey of India (LASI) Wave 1 (2017-2018) indicated rural residence, illiteracy, ST population, working in agriculture occupation, household size with ≥ 6 , unimproved toilet facility, no water-source within a dwelling, using unclean fuel for cooking, and damp wall/ceiling are associated with increased risk of malaria. The self-reported period prevalence of malaria exhibited high prevalence in central and western India compared to the south, north and eastern regions with the average rate of 7.9 % in adults. The major limitation of this study is that it is restricted to age group ≥ 45 years and self-reported malaria prevalence which may affect

the accuracy. The study suggests that apart from parasite and vector control strategies, improving the socio-economic and living conditions may aid the malaria elimination efforts.

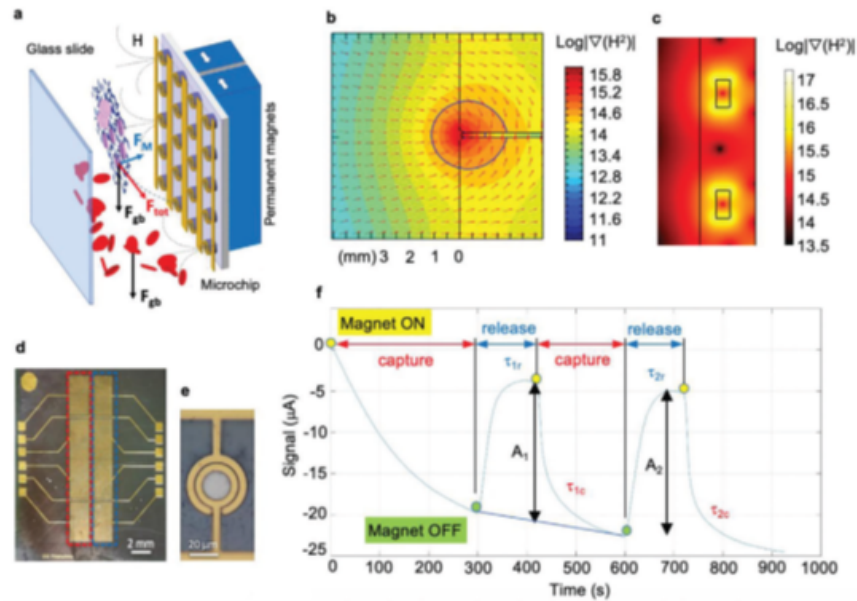
Zhang M. *et al.*, Nature Comm., 2021: The apicoplast link to fever-survival and artemisinin-resistance in the malaria parasite



Source: <https://www.nature.com/articles/s41467-021-24814-1>

In this study by [Zhang M. et al.](#), the authors conducted a large-scale genetic screen to identify the *P. falciparum* genes responsible for the survival at high temperatures during the febrile state of the host. In a parallel phenotyping, an overlap in responses was observed to the heat shock, and artemisinin resistance. The associated-pathways were identified, with most being involved in oxidative stress, unfolded protein response and host cell remodeling. However, surprisingly, apicoplast-derived genes, (isoprenoid metabolism genes), were also observed to be upregulated in the survival response to fever as well as artemisinin treatment, thus suggesting a link between the three.

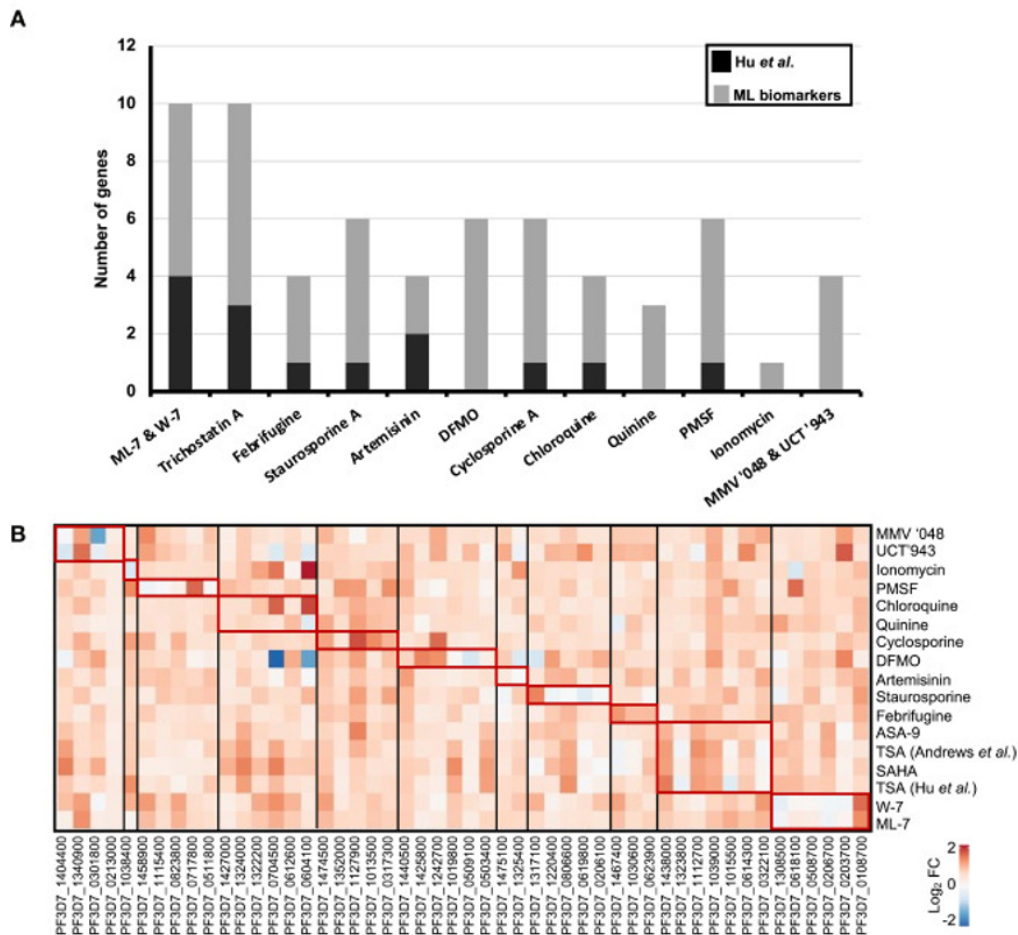
Giacometti M. *et al.*, *Adv Sci (Weinh)*, 2021: A Lab-On-chip Tool for Rapid, Quantitative, and Stage-selective Diagnosis of Malaria



Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8292881/>

In this study by [Giacometti M. *et al.*](#), the authors have described a new rapid lab-on-chip malaria diagnostic tool, TMek, to detect and quantify the infected RBCs and the distinct stages of malaria parasite. The infected RBCs display paramagnetic properties because of the hemozoin nanocrystals. This diagnostic tool is based on the differentiation and sorting of the malaria parasite infected RBCs on the basis of the gravity and magnetic forces, caused due to the presence of hemozoin crystals, and the detection of the ensuing electrical signal on a microchip. Further, the time and the amplitude for the electrical signal is distinct for the different parasite stages, and can thus distinguish between the ring, late trophozoite, and the gametocyte stages. The authors also validated this diagnostic method using patient samples.

Van Heerden A *et al.*, *Front Cell Infect Microbiol*, 2021: Machine Learning Uses Chemo-Transcriptomic Profiles to Stratify Antimalarial Compounds With Similar Mode of Action



Source: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8277430/>

The continued development of [antimalarial](#) resistance can impede the malaria elimination strategies. In phenotypic whole-cell screening, the exact target or mode of action (MoA) is determined only during hit-2-lead (H2L) or lead optimization (LO) phases of drug discovery process. In order to accelerate this process by providing data to H2L and LO strategies, authors of this study developed machine learning (ML) model that can stratify compounds with similar MoA using chemo-transcriptomic fingerprints. They developed rational gene approach to reduce the dimensionality of gene expression profiles and to identify predictive features for MoA. Among the three different ML algorithm categories, the multinomial logistic regression model successfully identified a set of 50 biomarkers with an accuracy of $76.6 \pm 6.4\%$ that define chemo-transcriptomic fingerprint of each compound. The ML model can be used to group novel compounds into MoAs associated with their predicted target and to extend to other life cycle stages like immature gametocytes.

Malaria Scientist to watch

An interview with Dr Chanaki Amaratunga



[Dr Chanaki Amaratunga](#)

DeTACT Coordinator

**Mahidol-Oxford Tropical Medicine Research Unit,
Faculty of Tropical Medicine, Mahidol University,
Bangkok, Thailand**

1. *What motivated you to work in the field of malaria research?*

I didn't want to work on malaria. I wanted to work on animal behavior, specifically elephant behaviour. After my undergraduate degree I met Prof Kamini Mendis who was head of the Malaria Research Unit in Sri Lanka at the time, to get a sense of how to do research. Prof Mendis, full of infectious energy, tried to connect me with scientists conducting research on elephant behavior, but there were no opportunities at the time. She convinced me to join the Malaria Research Unit, just to learn how to do research. I started working on vaccine trials for *Plasmodium vivax* malaria using a *Plasmodium cynomolgi-Macaca sinica* host-parasite system, which became my PhD research under the guidance of Dr Shiroma Handunnetti. I was drawn to the bigger picture of what working on malaria represented. Although I didn't enjoy conducting trials in animal models, I enjoyed being able to contribute towards understanding a disease of the developing world. Later I spent two years in the Malaria Group of the International Center for Genetic Engineering and Biotechnology in India and worked on protein expression. I wanted more connection with people working in the field, bringing research from bench to bedside. Unexpectedly this became possible after joining the National Institutes of Health in the United States where I had the opportunity to set up field sites in Cambodia for clinical studies of malaria. In Sri Lanka, India, the United States and Cambodia, I met colleagues working on different aspects of malaria, who have been inspiring and supportive. The dedication to their work, our continued friendship over the years, conversations about work, home and humanity with them also motivates me to continue working in the field of malaria research.

2. *Please describe your current role in MORU DeTACT Project.*

DeTACT is the Development of Triple Artemisinin-based Combination Therapies project, funded with UK Aid from the UK government's Foreign, Commonwealth and Development Office, conducted by the Mahidol Oxford Tropical Medicine Research Unit (MORU), Thailand, with Prof Arjen Dondorp as principal investigator. The DeTACT project takes a holistic approach at addressing the problem of effectively treating multidrug-resistant *Plasmodium falciparum* malaria. We have liaised with pharmaceutical companies to produce triple artemisinin-based combination therapies (TACTs) and test these TACTs

for safety, tolerability and efficacy in 8 and 4 countries in Africa and Asia, respectively. Additionally, we are conducting mathematical modeling studies to predict the cost-effectiveness of TACTs in preventing or delaying artemisinin resistance, conducting market positioning studies to assess to what extent the antimalarial markets in Africa and Asia are ready to transition to TACTs, and conducting bioethical studies to understand ethical considerations of deploying TACTs in Africa in a paediatric population where ACTs are still effective. My role is to coordinate these different activities, which are led by experts in each field. This is made possible by a team of new inspirational colleagues that I have met at MORU and I must make special mention of Dr Mehul Dhorda, who is coordinating the DeTACT clinical trials in Africa.

3. If you were to pick one scientific discovery that has been crucial to our current understanding of malaria, which one would that be?

There are so many different aspects of malaria, and many important discoveries. Since I work on the treatment of malaria, I would pick the discovery of artemisinin by Chinese scientists, from *Artemisia annua*, a plant used in traditional Chinese medicine. A very potent drug that can very quickly kill Plasmodium parasites and has contributed towards saving millions of lives over the years since its discovery. Artemisinin-based combination therapy is now frontline treatment for *Plasmodium falciparum* malaria worldwide.

4. According to you, what is the biggest challenge to malaria eradication?

Malaria elimination is possible, as has been seen by several countries including Sri Lanka eliminating malaria. Malaria eradication is a bigger challenge and will require initially more countries achieving malaria elimination via multipronged strategies that are tailored carefully for each country. I think sustaining political will and funding are the biggest challenges for malaria eradication, which is now further strained due to the COVID-19 pandemic that has over-burdened health systems and services.

5. Apart from science and research, which other activities interest you?

These days while adhering to many rules due to the COVID-19 pandemic, I enjoy cooking and find it relaxing to break rules in the kitchen and see what happens when cooking experiments go wrong! I am enjoying discussions on Buddhist philosophy and spirituality. Writing stories for little children is something I have been doing for many years. I wanted to compile a book of bedtime stories when a friend had her first baby – that baby is now 18 years old and ‘Tales for a Moonbeam’ is yet to be completed!

Upcoming events

Lecture Series on Infectious Diseases: Lecture 03

NIMR NATIONAL INSTITUTE OF MALARIA RESEARCH
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NIMR & MERA-India present
Lecture Series on Infectious Diseases June 2021 - May 2022

Prof Adrian Hill, University of Oxford, UK
Dr Tavpritish Sethi, IIT Delhi, India
Prof Arjen Dondorp, Mahidol University, Thailand
Prof Anuradha Chowdhary, VPCI, University of Delhi, India
Dr Gyanu Lamichhane, Johns Hopkins University, USA
Dr N Regina Rabinovich, Harvard T.H. Chan, USA
Dr Saman Habib, CDRI, India
Prof Dominic Kwiatkowski, University of Oxford, UK
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Dr Manu Prakash, Stanford University, USA
Dr Shashank Tripathi, IISc, India
Dr Manju Rahi, ICMR Hgrs, India

@meraindia.org.in @MERAIndiaICMR @meraindiaicmr @meraindiaicmr meraindia.org.in meraindiaicmr@gmail.com

NIMR NATIONAL INSTITUTE OF MALARIA RESEARCH
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NIMR & MERA-India present Lecture: 03
Lecture Series on Infectious Diseases

“Frugal Science: Reimagining the role of technology in global health, science education and disease monitoring”

Dr Manu Prakash,
Department of Bioengineering,
Stanford University, USA

Registration link: <http://lectureseries.meraindia.org.in/>

Monday, 23rd August, 2021 | 10:00 IST

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The third lecture of the series will be by Dr Manu Prakash, Stanford University, on 23rd August 2021, who will be speaking on ‘Frugal Science: Reimagining the role of technology in global health, science education and disease monitoring’.

To register for this lecture, please visit: <http://lectureseries.meraindia.org.in/>

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Our mailing address is:

meraindiaicmr@gmail.com

Address: MERA India Secretariat, Room No. 344,
ICMR-National institute of Malaria Research,
Sector 8, Dwarka, New Delhi- 110 077

Telephone: 011-25307344

Website: <http://meraindia.org.in/>