Plasmodium knowlesi infection

is associated with elevated circulating biomarkers of brain injury and endothelial activation

Cesc Bertran-Cobo, Elin Dumont, Naqib Rafieqin Noordin, Meng-Yee Lai,
William Stone, Kevin KA Tetteh, Chris Drakeley, Sanjeev Krishna, Yee-Ling Lau, Samuel C Wassmer

Young Investigator Award

Attestation of eligibility

I confirm my eligibility
to present at the
Young Investigator Award
ASTMH Annual Meeting 2024

Training status

PhD student
(UCT, SA)
Expected degree completion:
September 2027

Affiliations

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

University of Cape Town · Universiteit van Kaapstad · Yunivesithi yaseKapa

ISGlobal

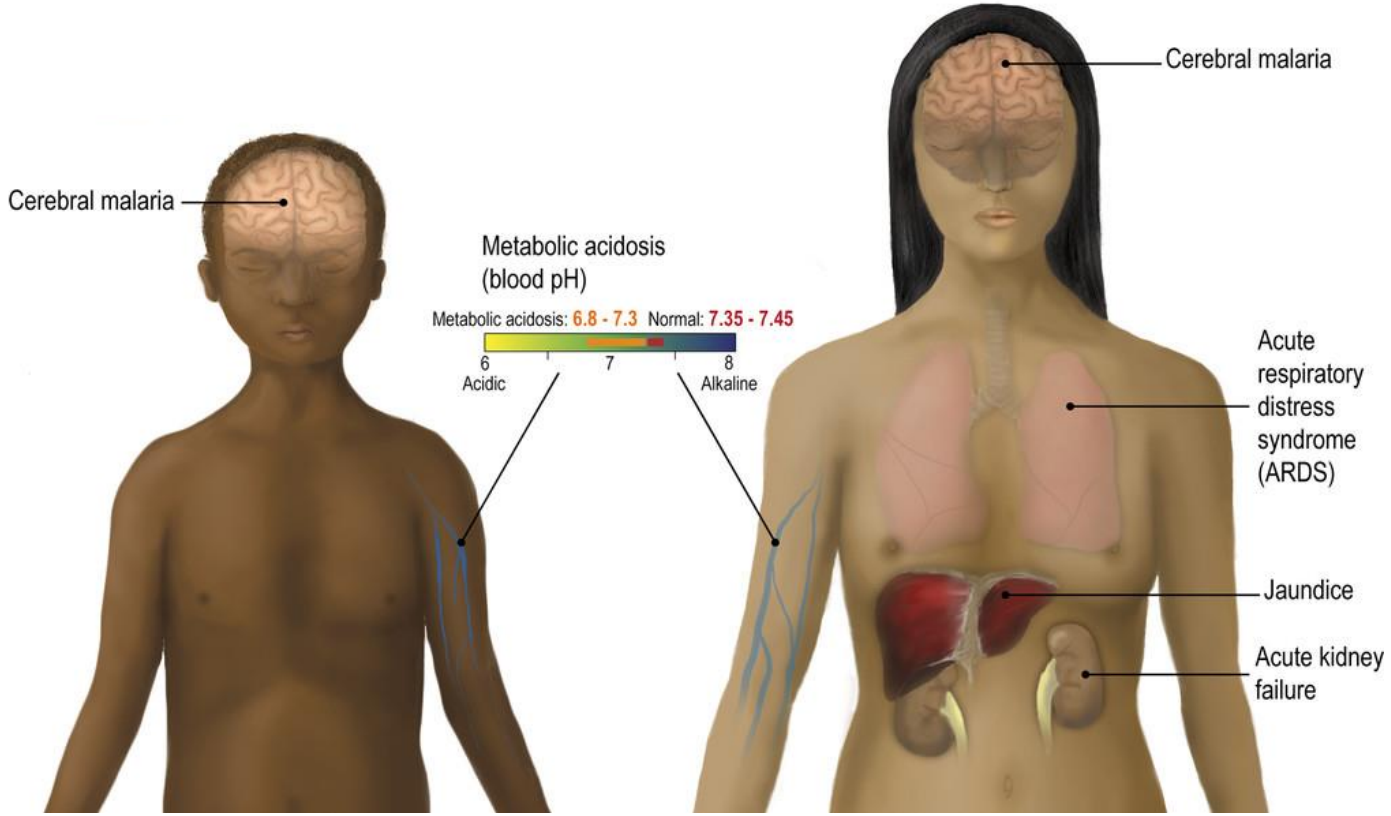
I **do not** hold a faculty position

Background

Severe malaria (SM) and cerebral malaria (CM)

CM diagnosis

- **WHO guidelines**
  - Confirmed infection (RDT / microscopy)
  - Unarousable coma
  - Unspecific
- **Malarial retinopathy**
  - Increases specificity in children
  - Lack of retinopathy does not rule out CM
  - Non-comatose severe malaria adults can present with CM

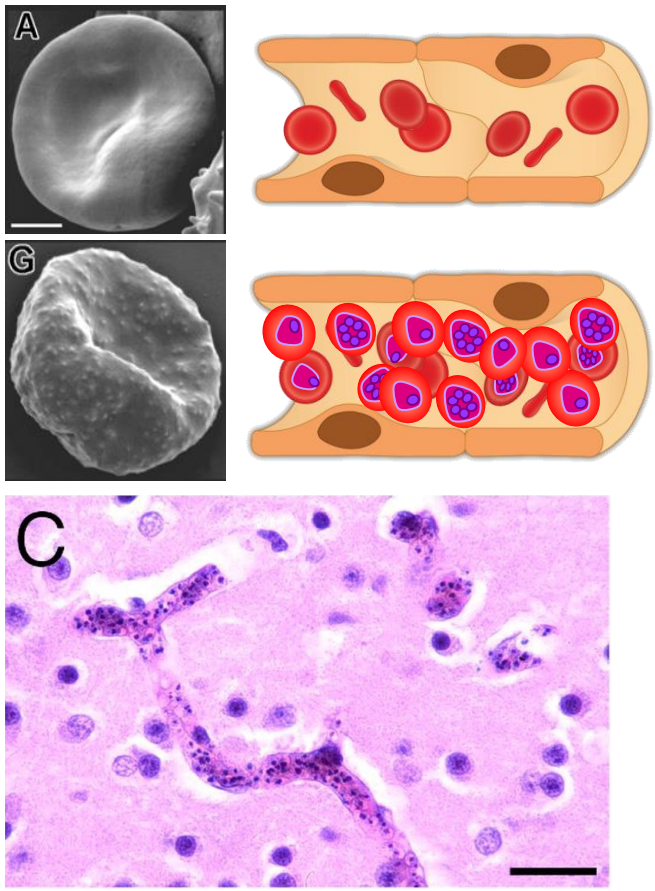


Gachot & Ringwald, WHO Tropical Medicine & International Health (2014); Wassmer *et al.*, AJTMH (2015); Hadjilaou *et al.*, Nature Neurology Reviews (2024)

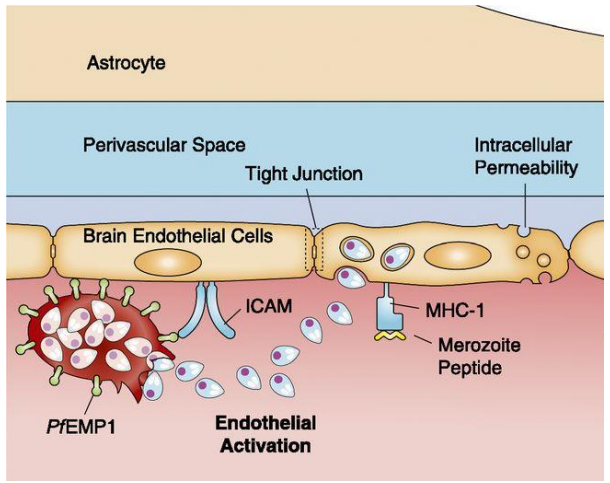
CM pathogenesis

Biological bases of CM in the brain vasculature (experimental CM and post-mortem findings)

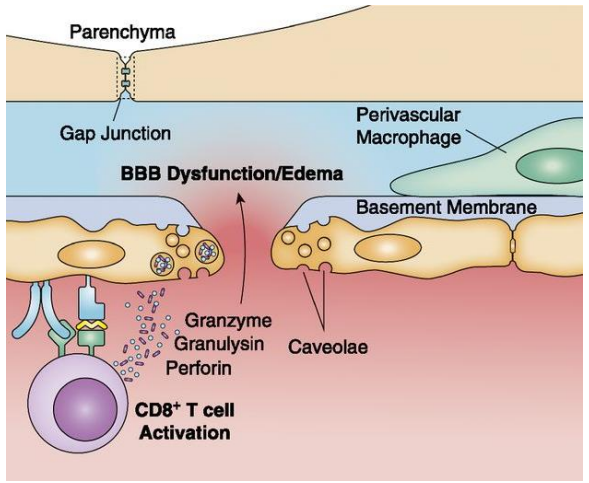
Sequestration (hallmark)

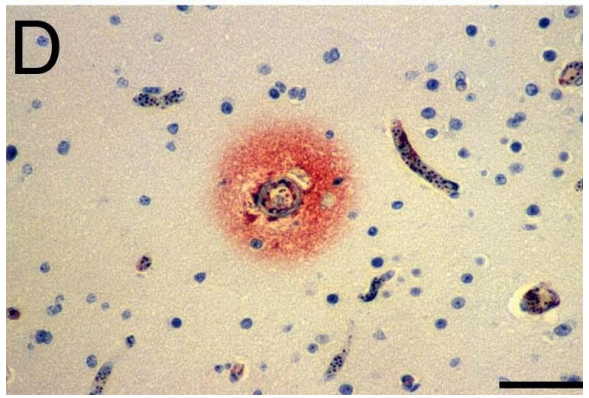


Endothelial activation



Loss of vascular integrity

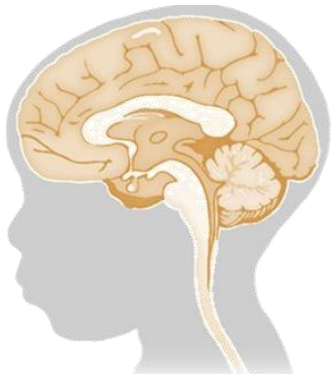




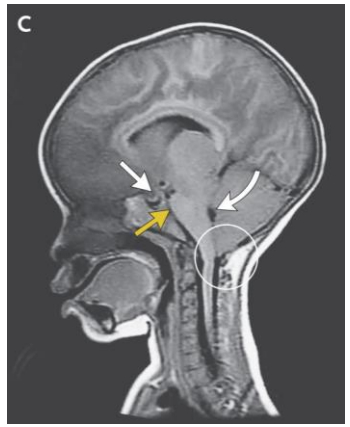
Dorovini-Zis *et al.*, American Journal of Pathology (2011); Riggle *et al.*, Journal of Immunology (2020); Wassmer *et al.*, Trends in Parasitology (2024); Hadjilaou *et al.*, Nature Neurology Reviews (2024)

CM and *in-vivo* brain imaging, a game-changer

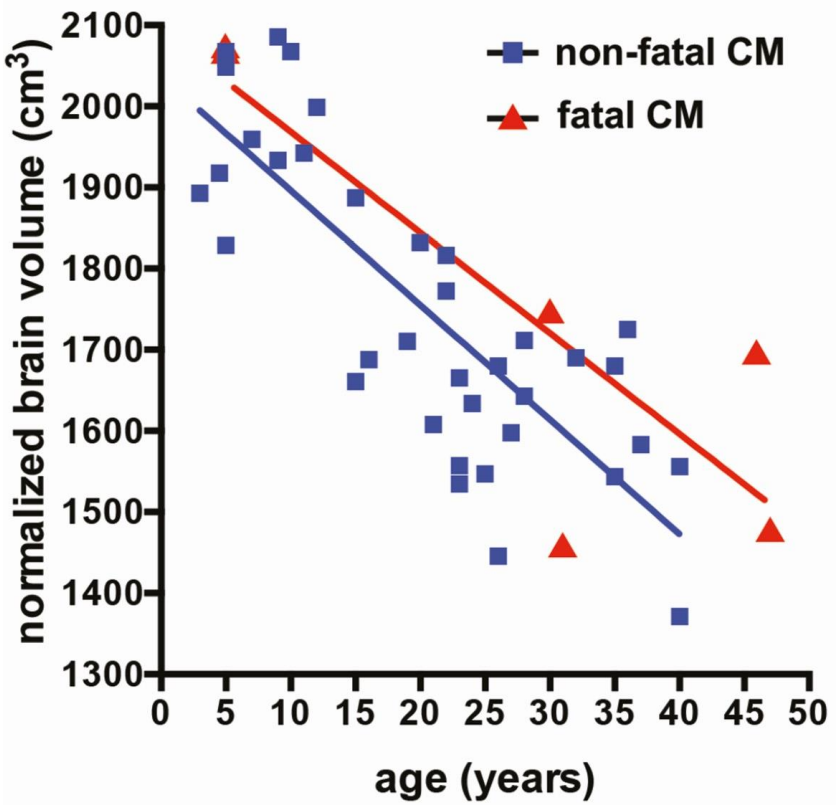
Neuroimaging findings include brain swelling in children, versus with severe hypoxia in adults, with or without coma

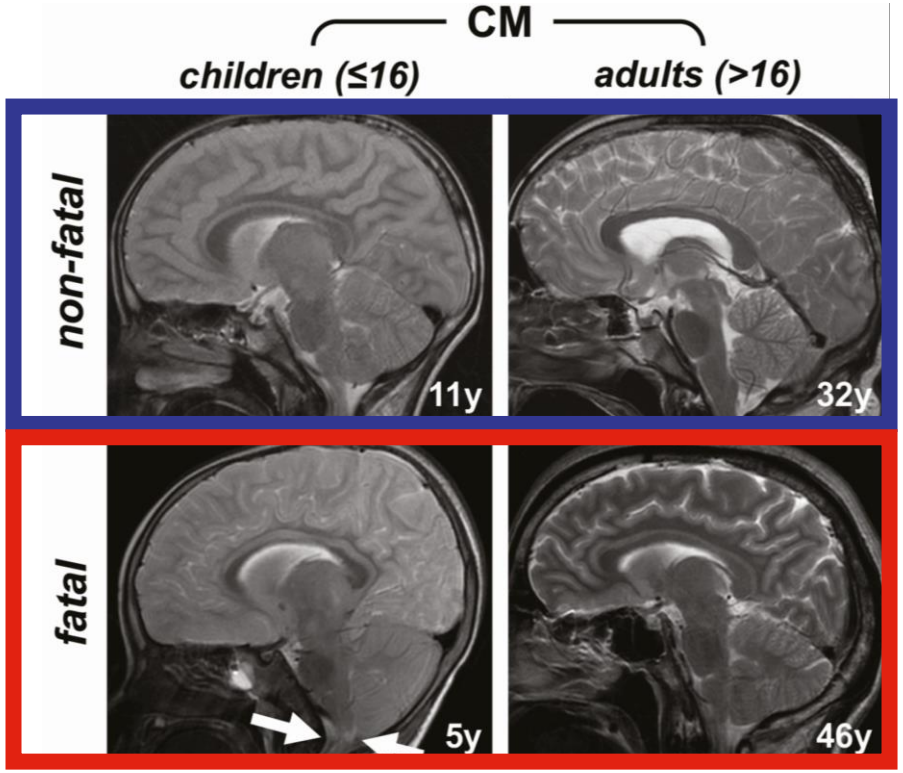


Healthy child



Child with CM





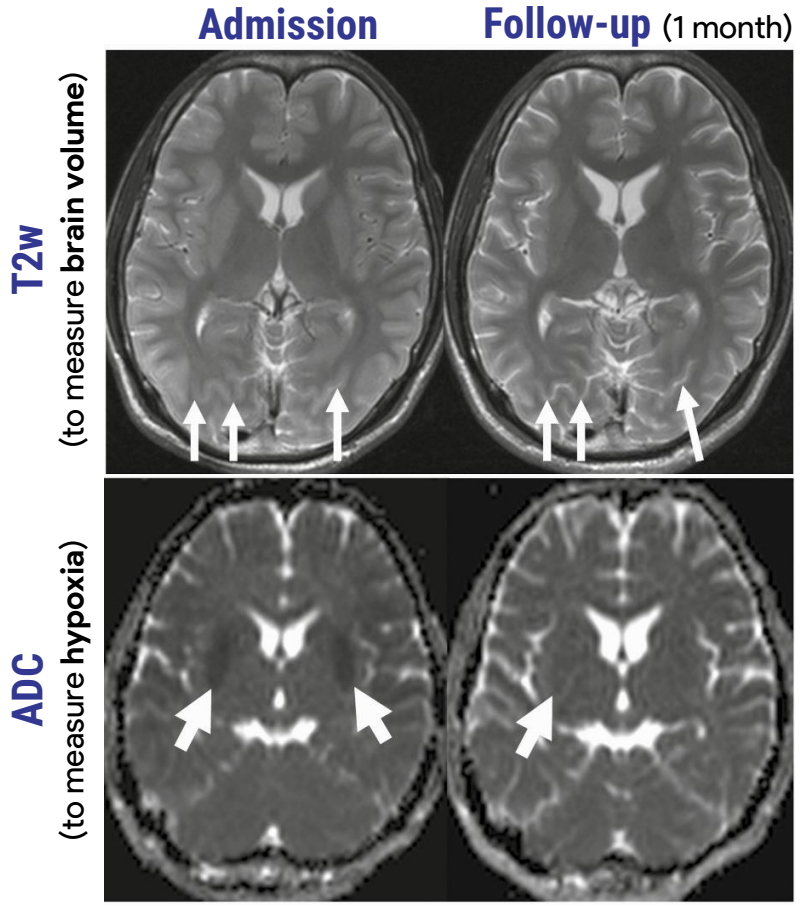
Seydel *et al.*, New England Journal of Medicine (2015); Sahu *et al.*, Clinical Infectious Diseases (2021); Riggle *et al.*, Journal of Immunology (2020)

CM and *in-vivo* brain imaging, a game-changer

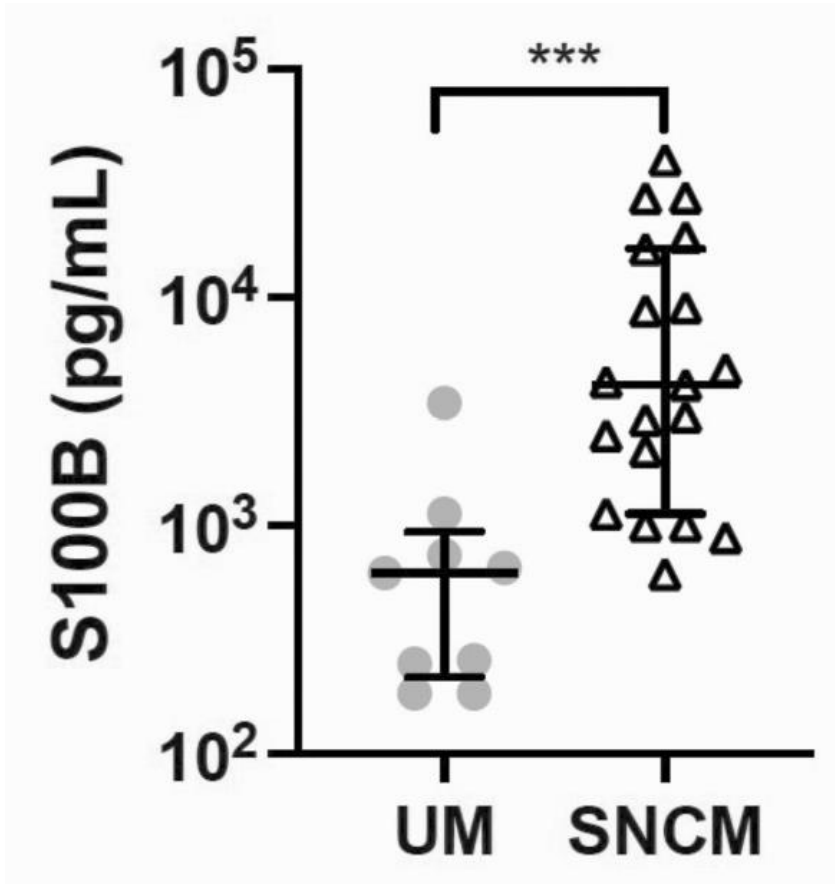
Neuroimaging signs of severe hypoxia are associated with blood biomarkers of brain injury in non-comatose patients

Non-comatose patient diagnosed with severe non-cerebral malaria

↓ **ADC values** on brain imaging



↑ Plasma **S100B** levels



Sahu *et al.*, Clinical Infectious Diseases (2021)

CM in other *Plasmodium spp.*

Rev Peru Med Exp Salud Publica. 2022;39(2):241-4.

REPORTE DE CASO

MALARIA CEREBRAL CON PANCITOPENIA POR *Plasmodium vivax* EN LA AMAZONÍA PERUANA: REPORTE DE CASO

Marco Paredes-Obando[1,a], Alfrando Moreno[1,a], Eduardo Panduro-García[1,a], André Ferreyra[1,a], Diego Chuquipiondo-Galdos[1,a], Jhosephi J. Vásquez Ascate[1,a], Jorge Sibina-Vela[1,2,b], Edgar A. Ramírez-García[1,2,c], Juan C. Celis-Salinas[1,2,c], Martín Casapía-Morales[1,2,c]

[1] Facultad de Medicina Humana de la Universidad Nacional de la Amazonía Peruana, Iquitos, Perú.
[2] Hospital Regional de Loreto, Iquitos, Perú.
[a] Estudiante de Medicina; [b] médico radiólogo; [c] médico infectólogo.

RESUMEN

Plasmodium vivax es la especie más común en la Amazonía peruana y ocasiona el 81% del total de casos de malaria. Presentamos el caso de un paciente adulto varón con malaria cerebral por *Plasmodium vivax*, que inicia con malestar general y fiebre, luego presenta convulsiones más de dos veces al día con pérdida de consciencia y limitación funcional motora. Se le realiza gota gruesa donc
depresión de las tres series sanguíneas. Se inicia tratamiento c
transfunde un paquete globular, y continua con primaquina p
con secuela neurológica en extremidad inferior izquierda.

Palabras clave: Malaria Cerebral; *Plasmodium vivax*; Convuls

Plasmodium vivax CEREBRAL MAL
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REPORT

ABSTRACT

Plasmodium vivax is the most common species in the Per
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who started with general malaise and fever, then presente
of consciousness and motor functional limitation. *Plasmod*
blood smear; we also found low counts of all three bloo
and clindamycin for five days, then one unit of packed r
continued with primaquine for seven days. The patient sh
sequelae in one lower limb.

Keywords: Cerebral Malaria; *Plasmodium vivax*; Seizures;

Citar como: Paredes-Obando M, Moreno A, Panduro-García E, Ferreyra A, Chuquipiondo-Galdos D, Vásquez Ascate JJ, *et al.* Malaria cerebral con pancitopenia por *Plasmodium vivax* en la Amazonía peruana: reporte de caso. Rev Peru Med Exp Salud Publica. 2021;39(2):241-5. doi: https://doi.org/10.17843/rpmesp.2022.392.10739.

Correspondencia: Marco Paredes Obando; marcofabrizio26@gmail.com

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En Línea: 30/06/2022

INTRODUCCIÓN

La malaria cerebral (MC) es una complicación
encefalopatía difusa asociada a coma y convulsione
por infección de *Plasmodium falciparum* en niños
causa paludismo no complicado; sin embargo, se l
complicada, pero es poco usual la afectación cerebra
vivax representan el 81% de todos los casos diagn
de 42,68 por cada 100 mil habitantes y mayor frecuencia en hombres (54%) [3,4]. Un estudio realizado en la zona tropical de Piura en Perú, en el 2008, reportó 0,4% de pacientes críticos de

Neurological involvement associated with *Plasmodium vivax* malaria from Pakistan

Tropical Doctor
2018, Vol. 48(1) 52–54
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DOI: 10.1177/0049475517722877
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SAGE

Yousaf Abdullah Khan[1,*], Usman Hameed Mian[1,*], Najia Karim Ghanchi[2], Ali Bin Sarwar Zubairi[3] and Mohammad Asim Beg[4]

Abstract

Plasmodium vivax is the most common specie causing malaria outside Africa with approximately 13.8 million reported cases worldwide. We report case of *P. vivax* infection with cerebral involvement. A nine year old boy presented with high grade fever accompanied by projectile vomiting and abnormal behavior later he developed seizures, shock, and

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[4]Professor, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan
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Email: masim.beg@aku.edu

Mukhtar *et al. Malar J* (2019) 18:316
https://doi.org/10.1186/s12936-019-2961-1

Malaria Journal

CASE REPORT Open Access

Plasmodium vivax cerebral malaria in an adult patient in Sudan

Maowia M. Mukhtar[1*], Omer A. Eisawi[2], Seth A. Amanfo[3], Elwaleed M. Elamin[1], Zeinab S. Imam[1], Faiza M. Osman[4] and Manasik E. Hamed[1]

Abstract

Background: *Plasmodium vivax* infection is rising in sub-Saharan Africa, where *Plasmodium falciparum* is responsible for more than 90% of malaria cases. While *P. vivax* is identified as a major cause of severe and cerebral malaria in South east Asia, the Pacific and South America, most of the severe and cerebral cases in Africa were attributed to *P. falciparum*. Cases of severe malaria due to *P. vivax* are emerging in Africa. A few severe *P. vivax* cases were reported in Eastern
of accurate diagnosis, low parasitaemia and seldom use of rapid

sented to the Al Kuwaiti hospital in the Sudan capital Khar-
ever (measured temperature was 38 °C), sweating, chills,
admission. He rapidly deteriorated into a coma state within
e was admitted to the intensive care unit and was suspected of
the patient was suffering from spinal cord disc. Brain CT scan
al tests, and blood film for malaria were performed. The results
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sites were seen in the blood film with high parasitaemia (quan-
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Plasmodium multi-species multiplex Polymerase Chain Reac-
/kg body weight for 10 days followed by primaquine 15 mg/
h and the patients was cured and released from the hospital.
of cerebral malaria in adults in Sudan and should be consid-
proper management of patients.

Khartoum, Sudan
Full list of author information is available at the end of the article

could be attributed to *P. vivax* ranging between 1900 and 10,000 globally.

Severe cases and deaths due to *P. vivax* malaria were reported from all endemic regions. In 2015, severe vivax malaria was attributed to cause 16% of all malaria related mortality outside sub-Saharan Africa [2]. The risk of death from *P. vivax* malaria was estimated ranging between 0.012 and 0.063%, while the risk of severe disease was estimated between 0.29 and 0.82% [2].

Plasmodium vivax infection is an emerging public health problem in Sudan with an overall prevalence of 26.6% among malaria cases in different regions of the

CM in other *Plasmodium spp.*

Rev Peru Med Exp Salud Publica. 2022;39(2):241-4.

REPORTE DE CASO

MALARIA CEREBRAL *Plasmodium vivax* EN L REPORTE DE CASO

Marco Paredes-Obando[1,a], Alfrand
André Ferreyra[1,a], Diego Chuquipic
Jorge Sibina-Vela[1,2,b], Edgar A. Ra
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Plasmodium vivax CEREB PANCYTHOPENIA IN T REPORT

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INTRODUCCIÓN

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Malaria Journal

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malaria

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Cox-Singh *et al. Malaria Journal* 2010, **9**:10
http://www.malariajournal.com/content/9/1/10

MALARIA JOURNAL

CASE REPORT Open Access

Severe malaria - a case of fatal *Plasmodium knowlesi* infection with *post-mortem* findings: a case report

Janet Cox-Singh[1,2*†], Jessie Hiu[3†], Sebastian B Lucas[4], Paul C Divis[2], Mohammad Zulkarnaen[2], Patricia Chandran[5], Kum T Wong[5], Patricia Adem[6], Sherif R Zaki[6], Balbir Singh[2], Sanjeev Krishna[1,2]

Abstract

Background: Zoonotic malaria caused by *Plasmodium knowlesi* is an important, but newly recognized, human pathogen. For the first time, post-mortem findings from a fatal case of knowlesi malaria are reported here.

Case presentation: A formerly healthy 40 year-old male became symptomatic 10 days after spending time in the jungle of North Borneo. Four days later, he presented to hospital in a state of collapse and died within two hours. He was hyponatraemic and had elevated blood urea, potassium, lactate dehydrogenase and amino transferase values; he was also thrombocytopenic and eosinophilic. Dengue haemorrhagic shock was suspected and a post-mortem examination performed. Investigations for dengue virus were negative. Blood for malaria parasites indicated hyperparasitaemia and single species *P. knowlesi* infection was confirmed by nested-PCR. Macroscopic pathology of the brain and endocardium showed multiple petechial haemorrhages, the liver and spleen were enlarged and lungs had features consistent with ARDS. Microscopic pathology showed sequestration of pigmented parasitized red blood cells in the vessels of the cerebrum, cerebellum, heart and kidney without evidence of chronic inflammatory reaction in the brain or any other organ examined. Brain sections were negative for intracellular adhesion molecule-1. The spleen and liver had abundant pigment containing macrophages and parasitized red blood cells. The kidney had evidence of acute tubular necrosis and endothelial cells in heart sections were prominent.

Conclusions: The overall picture in this case was one of systemic malaria infection that fit the WHO classification for severe malaria. Post-mortem findings in this case were unexpectedly similar to those that define fatal falciparum malaria, including cerebral pathology. There were important differences including the absence of coma despite petechial haemorrhages and parasite sequestration in the brain. These results suggest that further study of knowlesi malaria will aid the interpretation of, often conflicting, information on malaria pathophysiology in humans.

CM in other *Plasmodium spp.*

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REPORTE DE CASO

MALARIA CEREB
Plasmodium vivax
REPORTE DE CA

Marco Paredes-Obando[1,a], André Ferreyra[1,a], Diego C
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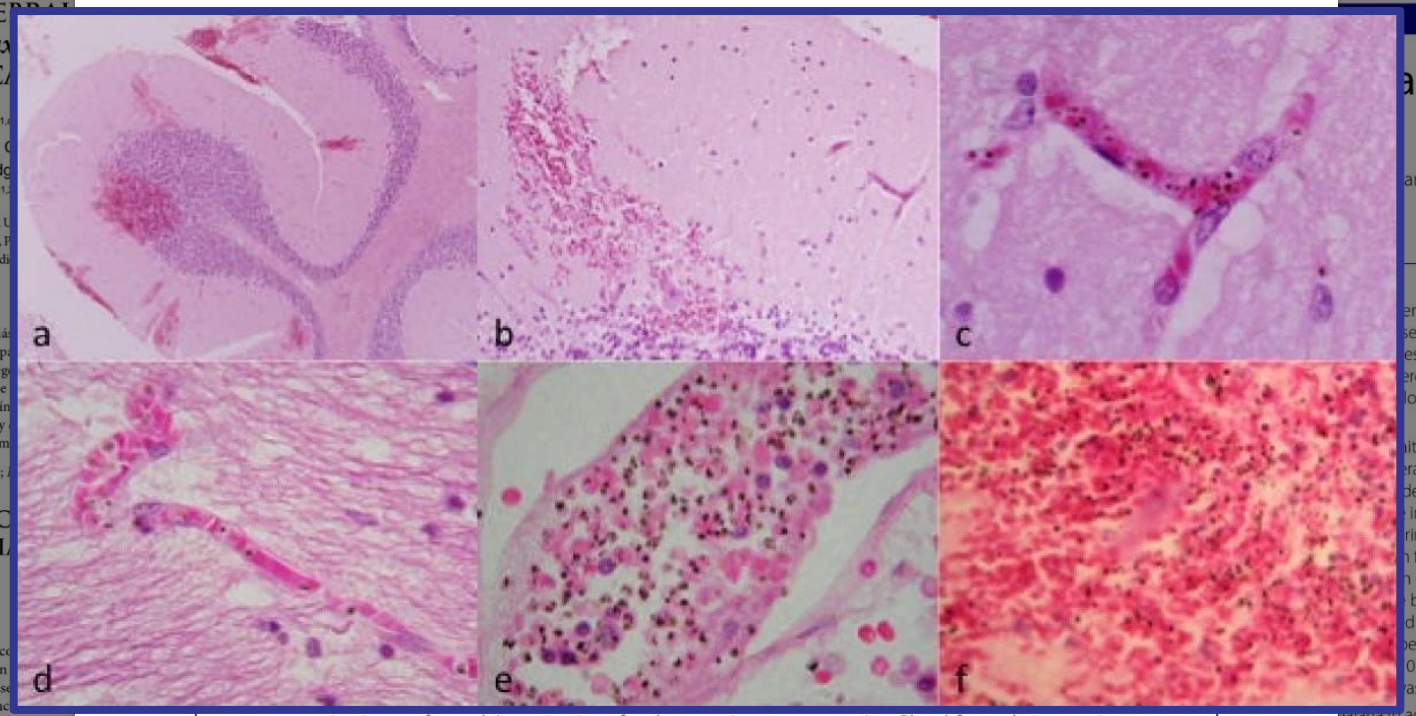

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problem in Sudan with an overall prevalence of
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Research question

Proof-of-concept study

Do patients with ***Plasmodium knowlesi*** (*Pk*) malaria present with increased levels of **brain injury biomarkers** when compared to uninfected controls?

Bertran-Cobo *et al.*, Journal of Infectious Diseases (in press)

Methodology: Participants and samples

UNIVERSITY OF MALAYA
The Leader in Research & Innovation

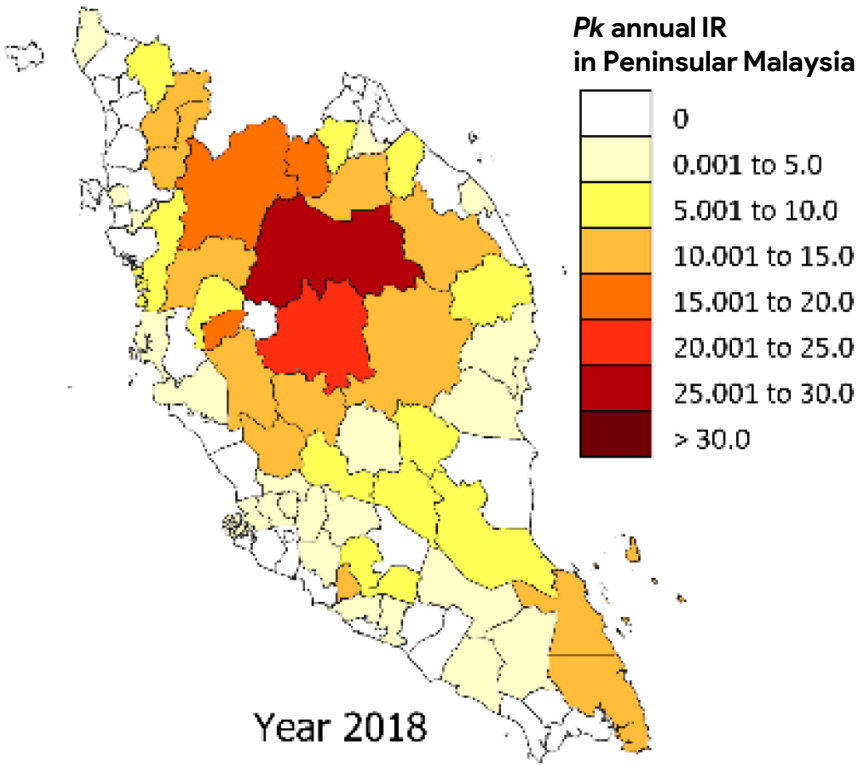
Collaboration with Prof Yee Ling Lau, University Malaya

In Malaysia:

- No reports of *P. falciparum* or *P. vivax* since 2018
- Emerging concern of ***Pk* infections** with **19,625 cases** and **57 deaths** since 2017

Prof Yee Ling's parent study:

- Aimed at determining prevalence of low-density malaria (all *Plasmodium spp.*)
- In previously reported malarious localities in **Peninsular Malaysia**
- Availability of **archived serum samples**



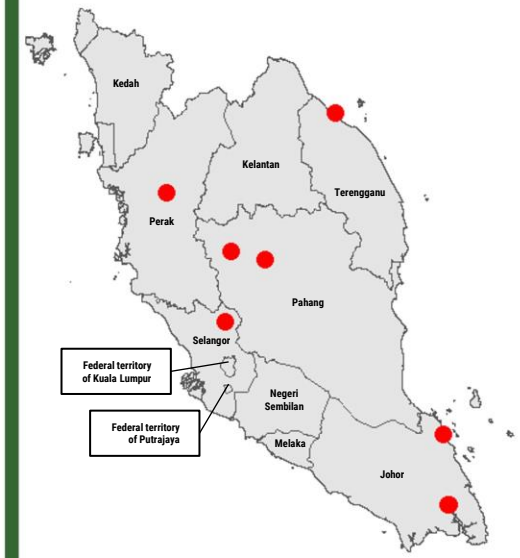
Noordin *et al.*, AJTMH (2020); Phang *et al.*, Int J Environ Res Public Health (2020)

Methodology: Participants and samples

Peninsular Malaysia, recruitment between December 2019 and January 2023

Pk-infected patients (N=19)

Inclusion criteria: Tested positive for *Pk* infection on **microscopy**, confirmation by **PCR**



Plasma samples obtained from patients with *Plasmodium knowlesi* infection		
State	**Hospital**	**No. samples**
Johor	Hospital Kota Tinggi	1
	Hospital Mersing	1
	Pejabat Kesihatan Daerah Kota Tinggi	1
Pahang	Hospital Kuala Lipis	4
	Klinik Kesihatan Betau	1
Perak	Hospital Sungai Siput	2
Selangor	Hospital Kuala Kubu Bharu	8
Trengganu	Hospital Setiu	1

Healthy controls (N=19)

Active sample screening of individuals from communities in Johor, Selangor, Negeri Sembilan, and Kedah

Inclusion criteria: **Asymptomatic**, ≥18yo, high-risk groups (i.e., working near to forest fringes, army, etc)

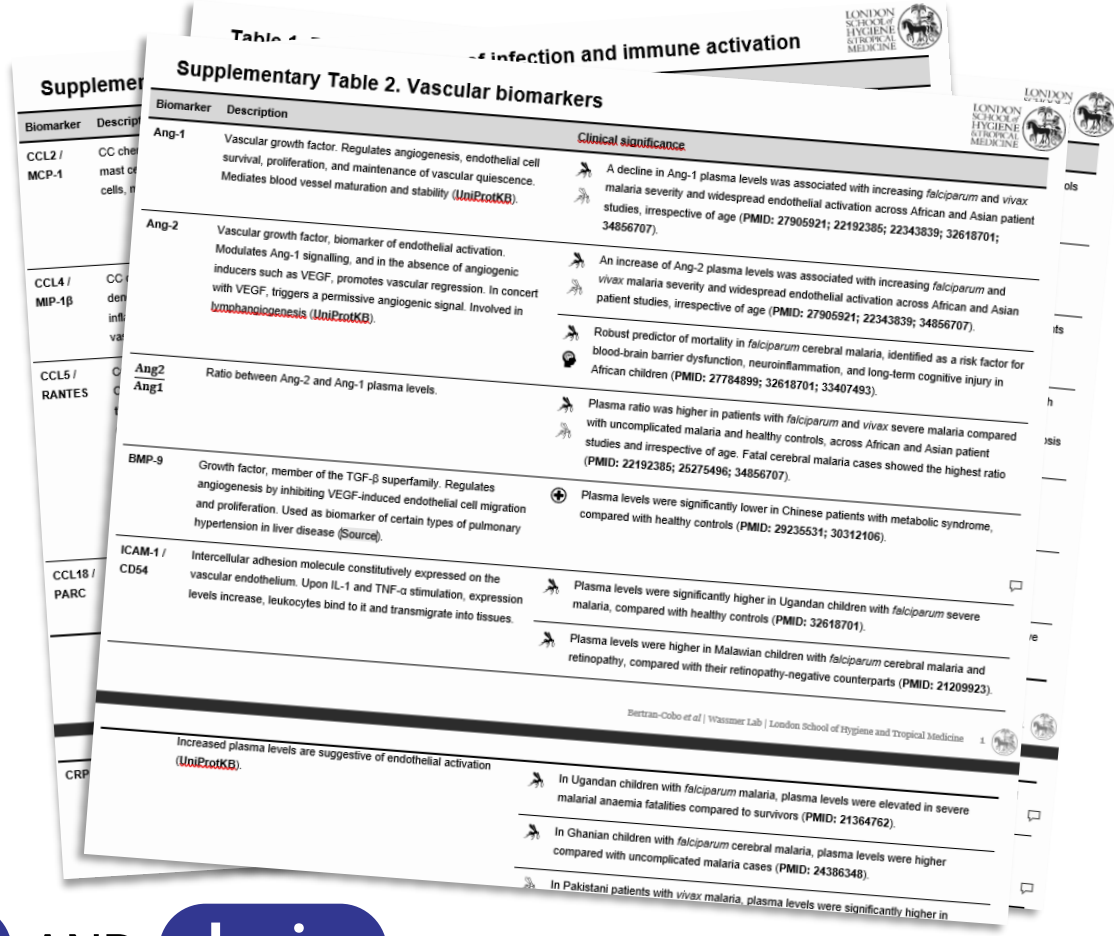
Methodology: Biomarker panel

Semi-systematic search

Search limited to PubMed

Filters:

- Research on **plasma levels** in **human patients**
- Publications from **2010** included
- From scientific journals ranked in **Q1** by **SJR**


Supplementary Table 2. Vascular biomarkers

Biomarker	Description	Clinical significance
Ang-1	Vascular growth factor. Regulates angiogenesis, endothelial cell survival, proliferation, and maintenance of vascular quiescence. Mediates blood vessel maturation and stability (UniProtKB).	A decline in Ang-1 plasma levels was associated with increasing *falciparum* and *vivax* malaria severity and widespread endothelial activation across African and Asian patient studies, irrespective of age (PMID: 27905921; 22192385; 22343839; 32618701; 34856707).
Ang-2	Vascular growth factor, biomarker of endothelial activation. Modulates Ang-1 signalling, and in the absence of angiogenic inducers such as VEGF, promotes vascular regression. In concert with VEGF, triggers a permissive angiogenic signal. Involved in lymphangiogenesis (UniProtKB).	An increase of Ang-2 plasma levels was associated with increasing *falciparum* and *vivax* malaria severity and widespread endothelial activation across African and Asian patient studies, irrespective of age (PMID: 27905921; 22343839; 34856707). Robust predictor of mortality in *falciparum* cerebral malaria, identified as a risk factor for blood-brain barrier dysfunction, neuroinflammation, and long-term cognitive injury in African children (PMID: 27784899; 32618701; 33407493).
Ang2 / Ang1	Ratio between Ang-2 and Ang-1 plasma levels.	Plasma ratio was higher in patients with *falciparum* and *vivax* severe malaria compared with uncomplicated malaria and healthy controls, across African and Asian patient studies and irrespective of age. Fatal cerebral malaria cases showed the highest ratio (PMID: 22192385; 25275496; 34856707).
BMP-9	Growth factor, member of the TGF-β superfamily. Regulates angiogenesis by inhibiting VEGF-induced endothelial cell migration and proliferation. Used as biomarker of certain types of pulmonary hypertension in liver disease (Source).	Plasma levels were significantly lower in Chinese patients with metabolic syndrome, compared with healthy controls (PMID: 29235531; 30312106).
ICAM-1 / CD54	Intercellular adhesion molecule constitutively expressed on the vascular endothelium. Upon IL-1 and TNF-α stimulation, expression levels increase, leukocytes bind to it and transmigrate into tissues.	Plasma levels were significantly higher in Ugandan children with *falciparum* severe malaria, compared with healthy controls (PMID: 32618701). Plasma levels were higher in Malawian children with *falciparum* cerebral malaria and retinopathy, compared with their retinopathy-negative counterparts (PMID: 21209923).
	Increased plasma levels are suggestive of endothelial activation (UniProtKB).	In Ugandan children with *falciparum* malaria, plasma levels were elevated in severe malarial anaemia fatalities compared to survivors (PMID: 21364762). In Ghanian children with *falciparum* cerebral malaria, plasma levels were higher compared with uncomplicated malaria cases (PMID: 24386348). In Pakistani patients with *vivax* malaria, plasma levels were significantly higher in

Plasma biomarkers of brain alterations or cerebral injury

Keywords:

[**NAME OF THE MARKER**] AND **plasma** AND **biomarker** AND **brain**

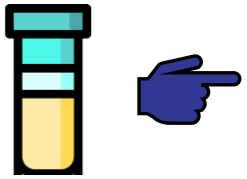
Plasma biomarkers of infection and immune activation, and vascular biomarkers

Keywords:

[**NAME OF THE MARKER**] AND **plasma** AND **biomarker** AND **malaria**

Bertran-Cobo *et al.*, Journal of Infectious Diseases (in press)

Methodology: Sample processing

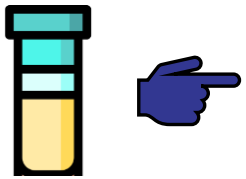


Luminex MAGPIX: 48 biomarkers (pg/mL)

Human Luminex® Discovery Assay (R&D Systems, #LXSAHM), 31-plex, 8-plex

Human ProcartaPlex™ Neuroscience (ThermoFisher, #EPX180-15837-901), 18-plex

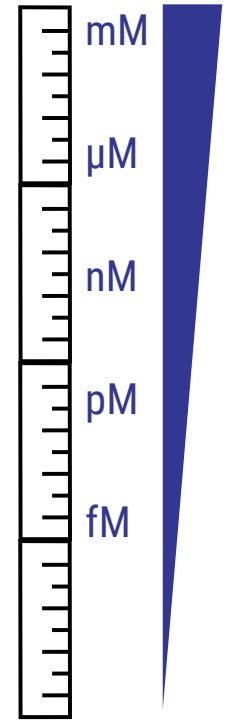
- **Multiplex** assay platform
- Simultaneous measurement of multiple biomarkers in one plasma sample
- Can detect molecule concentrations in the **picomolar** (pM) range
- High **sensitivity** and **reproducibility**



Single Molecule Array (Simoa) HD-X Analyzer™: 4 biomarkers (fg/mL)

Simoa HD-X Neurology (Quanterix, #102153), 4-plex

- **Ultrasensitive** digital immunoassay
- Detection and quantification of **low-abundance** plasma biomarkers
- Can detect molecule concentrations in the **sub-femtomolar** (sub-fM) range
- High **sensitivity**, **specificity**, and **reproducibility**



Results

Flowchart

Custom kits
48 biomarkers

Luminex QC
48 biomarkers

In-house assay for malaria **immunoseroprevalence**

Detect any recent or historical exposures to *Plasmodium spp.*

Custom kits
4 biomarkers

Simoa QC
4 biomarkers

R analysis
52 biomarkers

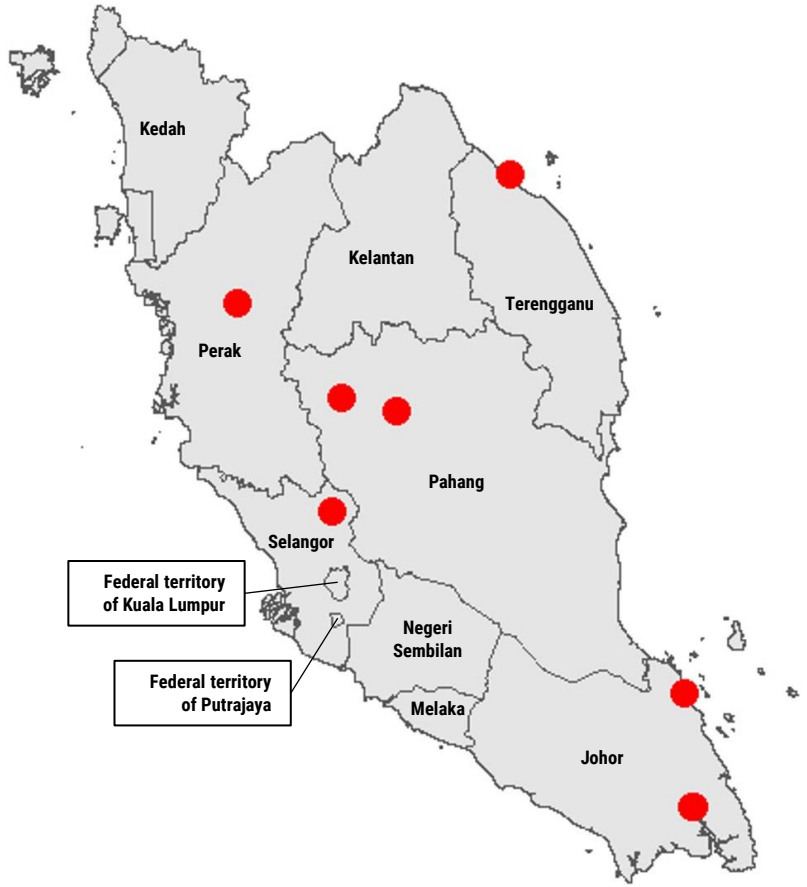
Statistical analysis

Data normality → **Baseline & seroimmune characteristics** → **Biomarker group comparisons** (T-test or Wilcoxon) → **Bonferroni correction** → **Correlation with parasitaemia & serum lactate** → **Hierarchical clustering**

Results

Cohort characteristics

	Pk-infected patients	Uninfected controls
Age	39 (±15)	39 (±15)
Gender	19/19 (100%) men	19/19 (100%) men
Parasitaemia	1.05 % (±2.15)	–
Low antibody reactivity vs any *Plasmodium spp*. antigens of recent or historical exposure	19/19 (100%) (**malaria naïve**)	19/19 (100%) (**malaria naïve**)



Results

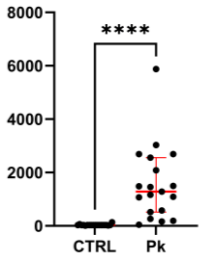
Brain injury biomarkers in *Pk*-infected patients and controls (Bonferroni-corrected results)

Biomarkers previously reported to be associated with **malaria infection** and/or **severity** in other *Plasmodium spp*.

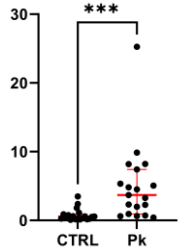
Significantly altered in our *Pk*-infected patients compared to controls

Increased

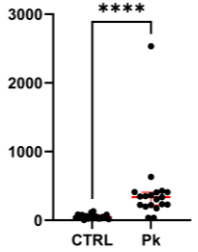
S100B



Tau (total)

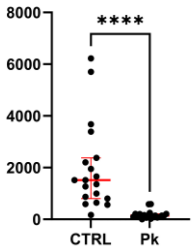


UCHL-1

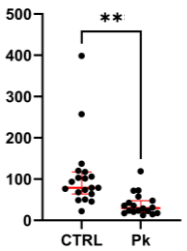


Decreased

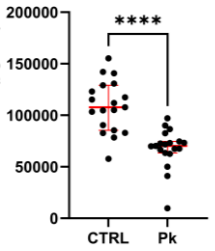
BDNF



GFAP

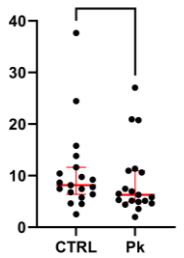


NCAM-1

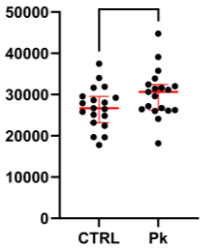


No significant differences

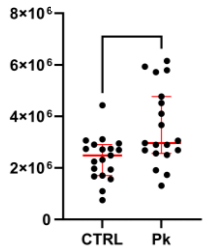
NfL



Lipocalin 2 / NGAL



Fetuin A



*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

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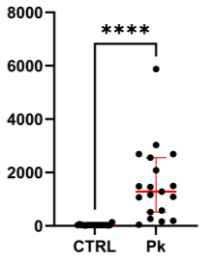
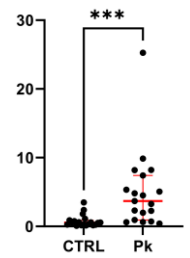
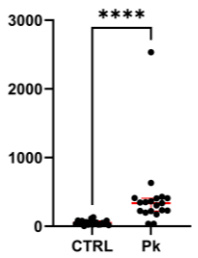


Tau (total)

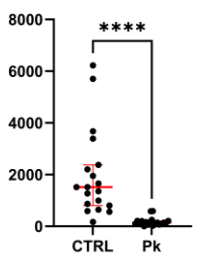


UCHL-1

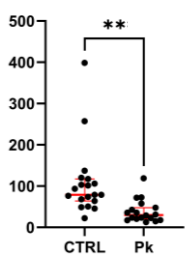


Decreased

BDNF



GFAP



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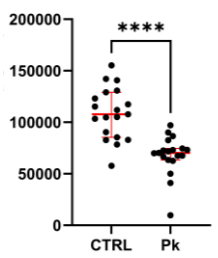
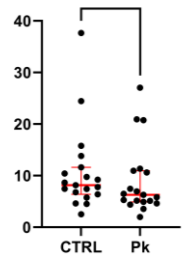
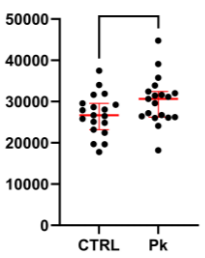


No significant differences

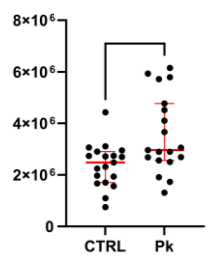
NfL



Lipocalin 2 / NGAL



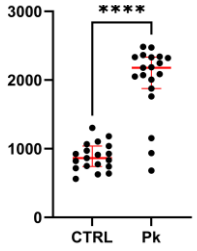
Fetuin A



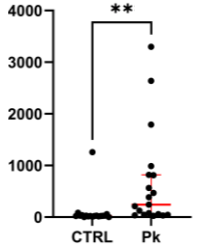
Biomarkers previously reported to be associated with **cognitive decline**, **brain injury**, and **neurodegeneration**

Increased

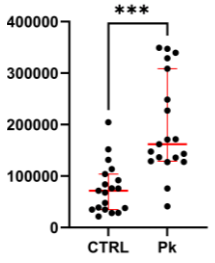
αSyn



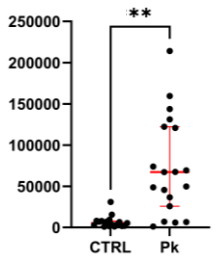
NRGN



Park7

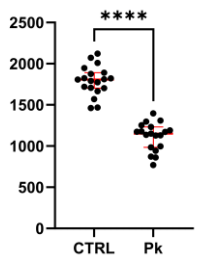


TDP-43

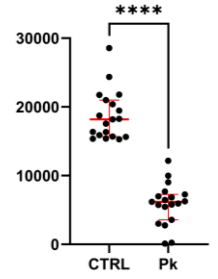


Decreased

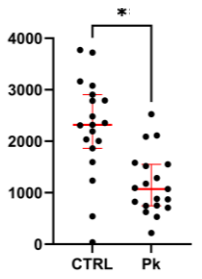
CaBD



CNTN1



KLK6



No differences

- APP
- $A\beta_{(1-42)}$
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- NGF- β
- YLK-40

*p<0.05 **p<0.01 ***p<0.001 ****p<0.0001

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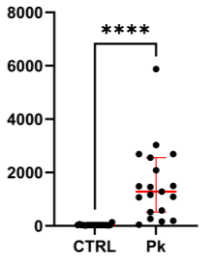
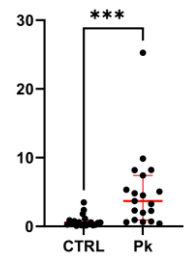
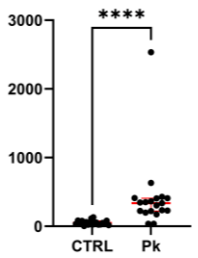
S100B



Tau (total)

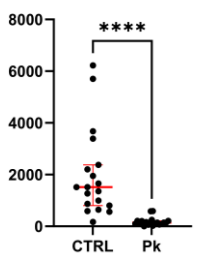


UCHL-1



Decreased

BDNF



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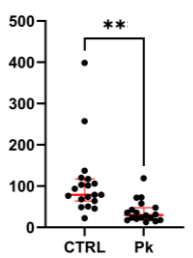
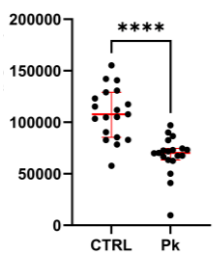
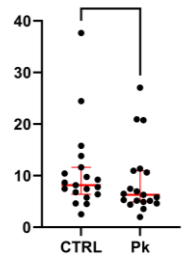


NCAM-1

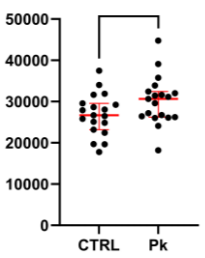


No significant differences

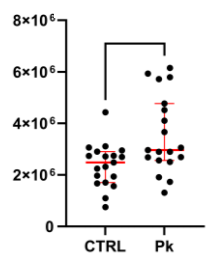
NfL



Lipocalin 2 / NGAL



Fetuin A



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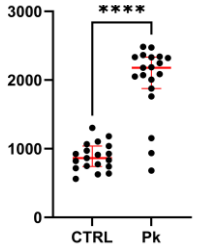
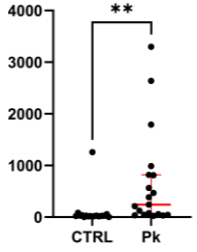


NRGN



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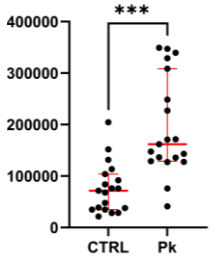
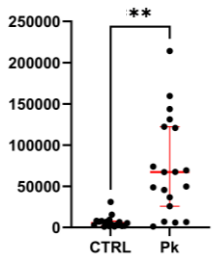
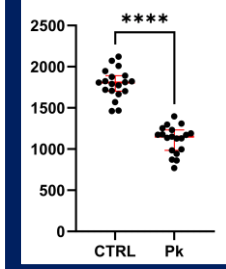


TDP-43

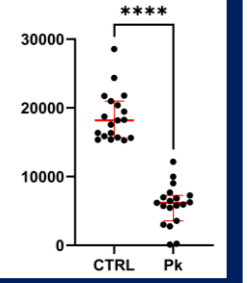


Decreased

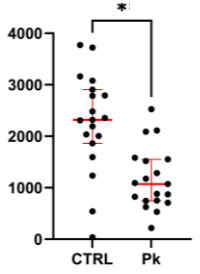
CaBD



CNTN1



KLK6



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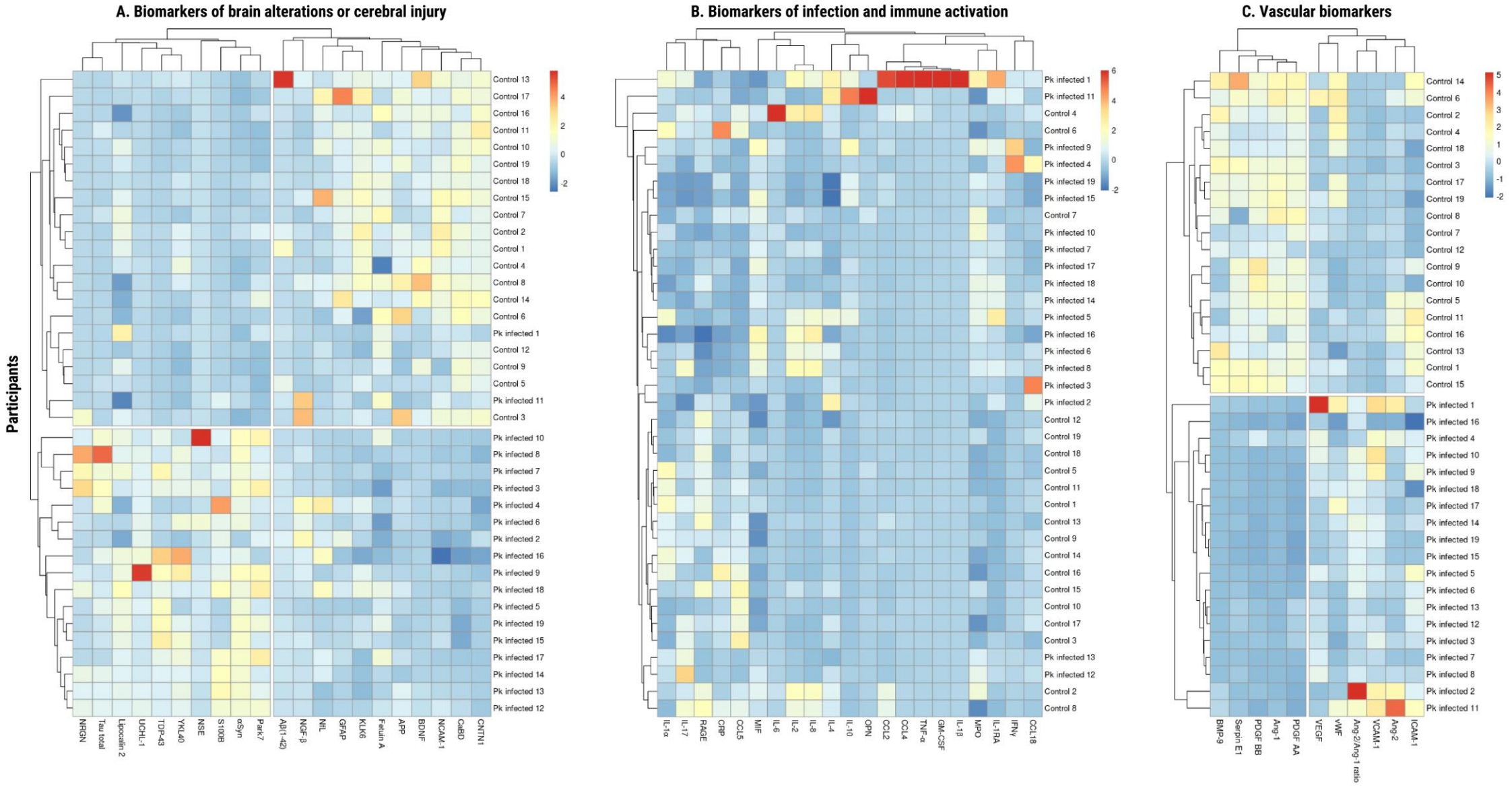
Results

Hierarchical clustering of subjects based on biomarker levels



Discussion

Children Adults

Similarities with malaria studies reporting brain injury biomarkers in other *Plasmodium spp.*

	Malaysia cohort		Other studies							
Biomarker	**Control group** pg/mL (IQR) (n=19)	***Pk*-infected** pg/mL (IQR) (n=19)	**Study**	**Country**	**Age**	***Plasmodium spp***	**Technique**	**Control group** pg/mL (IQR) (n)	**Case group 1** pg/mL (IQR) (n)	**Case group 2** pg/mL (IQR) (n)
BDNF	1,516.2 (1,454.1)	146.2 (115.8)	McDonald *et al*, 2017	Uganda		*falciparum*	ELISA	N/A	1.8 (2.5) † (n=100 SNCM)	1.1 (1.3) † (n=79 CM)
GFAP	78.1 (45.8)	29.9 (25.3)	Datta *et al*, 2023	Uganda		*falciparum*	SiMoA	100.6 (42.3) (n=20)	69. 6 (56.5) (n=30 SMA)	86.8 (57.7) (n=44 CM)
S100B	27.3 (0.0)	1,282.2 (1,777.2)	Mohanty *et al*, 2022	India		*falciparum*	Luminex	N/A	617.9 (490.9) (n=9 UM)	4,121.9 (11,006.9) (n=19 SNCM)
Tau total	0.6 (0.6)	3.7 (5.0)	Datta *et al*, 2021	Uganda		*falciparum*	SiMoA	2.6 (2.5) (n=118)	5.5 (5.4) (n=159 SMA)	7.1 (7.6) (n=182 CM)
UCHL1	49.3 (45.4)	336.6 (184.1)	Datta *et al*, 2023	Uganda		*falciparum*	SiMoA	9.7 (10.8) (n=20)	29.9 (27.9) (n=30 SMA)	52.9 (78.3) (n=44 CM)

† results reported in ng/mL (all other results are reported in pg/mL)

SNCM: severe non-cerebral malaria; **CM**: cerebral malaria; **SMA**: severe malarial anaemia

Discussion

Children Adults

Biomarker levels increase with malaria infection and/or severity

	Malaysia cohort		Other studies							
Biomarker	**Control group** pg/mL (IQR) (n=19)	***Pk*-infected** pg/mL (IQR) (n=19)	**Study**	**Country**	**Age**	***Plasmodium spp***	**Technique**	**Control group** pg/mL (IQR) (n)	**Case group 1** pg/mL (IQR) (n)	**Case group 2** pg/mL (IQR) (n)
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Discussion

Children Adults

Biomarker levels decrease with malaria infection and/or severity

	Malaysia cohort		Other studies							
Biomarker	**Control group** pg/mL (IQR) (n=19)	***Pk*-infected** pg/mL (IQR) (n=19)	**Study**	**Country**	**Age**	***Plasmodium spp***	**Technique**	**Control group** pg/mL (IQR) (n)	**Case group 1** pg/mL (IQR) (n)	**Case group 2** pg/mL (IQR) (n)
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Discussion

Limitations

- Clinical data on **neurological complications** among *Pk*-infected patients were **not collected** by the parent study, we were unable to
  - fully contextualize the observed alterations in brain injury biomarkers
  - investigate direct associations between altered biomarkers in *Pk* infection and **neurocognitive alterations**
- Tried to overcome this with **surrogate measurements of severity**
  - **parasitaemia** → showed no correlation with biomarker data
  - **serum lactate** → half our samples presented with insufficient volume to detect lactate concentrations
- **No follow-up** serum samples from the parent study, so we could not evaluate whether biomarker levels return to baseline or increase over time
- A **symptomatic**, **non-malaria control group** would have allowed stronger ascertainment of associations

Conclusions & future directions

Take home message

- Our study represents the first comprehensive assessment of **surrogate markers** of **cerebral involvement** in ***Pk*-infected patients** from Malaysia
- *Pk* infection may impact **brain** and **vascular health** through pathways similar to the ones described for *P. falciparum*, leading to elevated levels of brain injury and vascular biomarkers compared to healthy controls
- Our results provide a **proof of concept** that warrants further, more robust investigation

Ongoing research led by Lau & Wassmer labs

- Longitudinal study with infected patients, healthy controls, and symptomatic non-malaria controls, collecting
  - **Neuroimaging data**
  - **Neurocognitive evaluations**
  - **Blood biomarker data**
- In **well-characterized cohorts** of severe **knowlesi** (Malaysia) and **falciparum** patients (India)
- Comparative pathogenesis analyses during infection with *Pk* and other *Plasmodium spp*

Terima kasih! Thank you!

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