Acute febrile illness surveillance in a malaria endemic region of Madagascar: biomarker discovery for differentiation of bacterial and viral illnesses

Laetitia Cortes[1]; Yiyong Zhou[1]; Melina Messaoudi[5]; Muriel Maeder[2]; Rudolf Guilbaud[1]; Michael Schirm[1]; Carole Chedid[4,5]; Jonathan Hoffmann[5]; Bénédicte Contamin[2]; Martin Randriamarotia[3]; Florence Pradel[5]; Juliet Bryant[5]; Valentina Picot[5]; Eustache Paramithiotis[1] ; Glaucia Paranhos-Baccalà[5]

[1]*Caprion, Montreal, CANADA;* [2]*Centre d'Infectiologie Charles Mérieux (CICM), Antananarivo, Madagascar;* [3] *Fondation Médicale d'Ampasimanjeva (FMA), Ampasimanjeva, Madagascar;* [4]*Département de Biologie, Ecole Normale Supérieure de Lyon, Lyon, France;* [5]*Emerging Pathogens Laboratory, Fondation Mérieux, Lyon, France.*

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BACKGROUND

More than half of deaths in children under the age of five in low-income countries can be attributed to only five infectious diseases, or a combination of them: acute respiratory infections (for most, pneumonia), malaria, diarrhea, measles and HIV/AIDS.

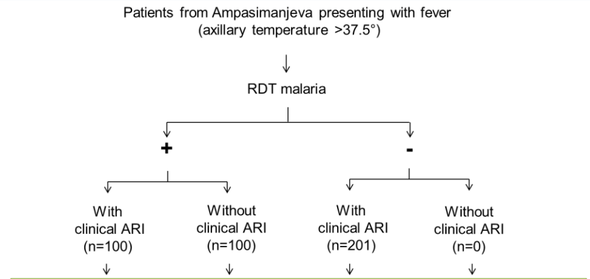
In Madagascar, 20% of child deaths under 5 are attributed to malaria, but from several studies undertaken separately in 15 countries reported that on average there is a 61% overestimation of malaria cases; this highlights one of the major problems regarding the clinical diagnosis overlap between malaria and ALRI - pneumonia and its important implication in the case management strategies and evaluation of disease-specific interventions in regions in which these infections are prevalent.

OBJECTIVES

1. To identify (discover, qualify and verify) candidate diagnostic biomarkers in sera of children under five of age with malaria and clinical respiratory infections living in a malaria endemic region of Madagascar.

2. To establish a correlation between the type of pathogens identified and C-reactive protein (CRP), Procalcitonin (PCT) and Interferon-gamma induced protein 10 (IP-10).

METHOD



Whole-blood samples:
- Real-time PCR amplification for *S. pneumoniae* , *H. influenzae*, *S. aureus*
- Multiple Reaction Monitoring- Mass spectometry (MRM-MS) assay
- CRP, PCT and IP-10 quantification

Nasal swabs:
- Multiplex real-time PCR assay allowing the identification of 22 respiratory pathogens (Fast-track Diagnostics respiratory pathogens kit)

MAIN OBSERVATIONS

Fever etiologies

1. A total of 200 individuals (49.9%) were malaria positive. Clinical respiratory infections were recorded in 75.1% of the patients. Prevalence of malaria and clinical respiratory co-infection reached 25.9%.

2. Individuals with clinical respiratory infection were significantly more at risk to be infected by a strict pathogen (n=71/101, 70.3%, P<0.0001, OR=3.0, 95% IC [1.9; 5.2]).

3. Absence of PCR detected pathogens (NoRP) was mainly observed among children infected with malaria (n=49/98, 50%).

Host blood-based biomarkers investigation

4. Blood PCT levels were significantly induced in malaria (mean=15.3 µg/ml) compared to clinical respiratory infection (mean=2.0 µg/ml, P<0.001) but not compared to co-infection of malaria and respiratory infection (mean=10.8 µg/ml, NS).

5. Blood PCT levels were significantly higher in severe malaria cases (n=18/401, mean=24.5 µg/ml, P<0.001) than in non-severe malaria cases confirming its added value as a prognosis biomarker of severe malaria.

6. Combination of PCT/CRP/IP-10 was not as sensitive as single biomarkers or complex molecular signatures to identify strict-pathogens in different clinical fever etiologies

MAIN RESULTS

1. Socio-demographic characteristics

Variables	Categories	N (%) or *median (IQR)*
Gender	Male	190 (47.4)
	Female	211 (52.6)
Age (months)	-	*24 (12 - 39)*
	< 12	94 (23.4)
	12 - 24	111 (27.6)
	25 - 34	64 (16.0)
	35 - 44	61 (15.3)
	≥ 45	71 (17.7)
Nutritional status	Adequate nutrition	328 (81.8)
	Moderate malnutrition	44 (11.0)
	Severe malnutrition	19 (4.7)
	Excessive weight	10 (2.5)
Sickle cell disease	No disease (AA hemoglobin)	307 (76.6)
	Heterozygous (AS hemoglobin)	82 (20.4)
	Homozygous (SS hemoglobin)	12 (3.0)
Clinical observations	Pneumonia	117 (29.2)
	Malaria	100 (24.9)
	Acute respiratory infection (ARI)	84 (21.0)
	Malaria + ARI	90 (22.4)
	Malaria + pneumonia	10 (2.5)
Detection of respiratory pathogen	No pathogen detected	98 (24.5)
	Viral infection	193 (48.1)
	Viral co-infection	92 (23.0)
	Viral/bacterial co-infection	14 (3.5)
	Bacterial infection	4 (<1)

Table 1. IQR: interquartile range. Malaria was diagnosed using Rapid Diagnostic Test (RDT). Pathogens were detected and identified by real-time RT-qPCR.

2. Principle component analysis of plasma changes

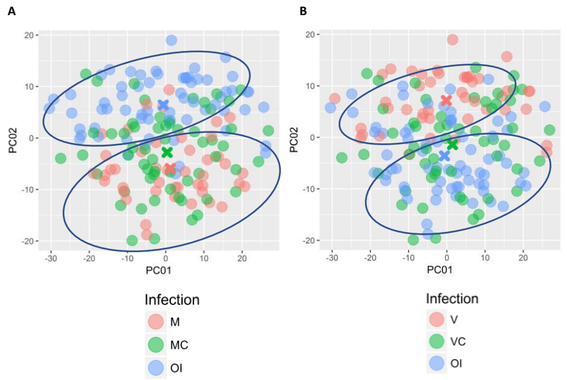


Figure 2. Principle component analysis of plasma changes in subjects infected with malaria (M), malaria plus other coinfections (MC), and all other infections (OI) (Figure 2.A), or in subjects infected with respiratory virus (V), virus plus other coinfections (MC), and all other infections (OI) (Figure 2B). Each dot represents an individual, color-coded according to group membership. Color coded X indicate the corresponding group mean.

3. Host plasma changes according to pathogen type

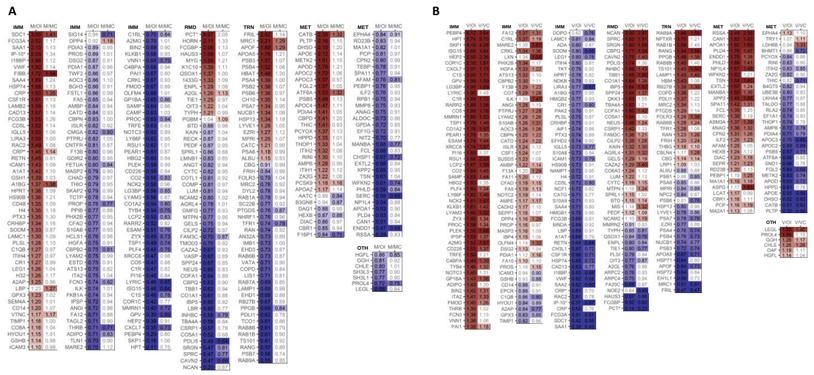


Figure 3. Cross-sectional comparison of host protein expression change ratios: A. in subjects infected with malaria (M) vs all other infections (OI), and subjects infected with malaria (M) vs malaria plus all other coinfections (MC); **B.** in subjects infected with respiratory viruses (V) vs all other infections (OI), and subjects infected with respiratory viruses (V) vs virus plus all other coinfections (MC). Significant changes ($p > 0.05$) are color-coded red for increased and blue for decreased expression ratios, respectively. Proteins linked to immune (IMM), tissue remodelling (RMD), transport (TRN), and metabolic (MET) functions are grouped together. OTH = other functions

4. Pathogen type distribution differs according to clinical fever etiologies

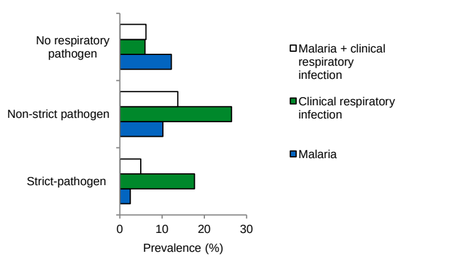


Figure 4. Each bar represents the number of PCR-positive detection among the different fever clinical etiologies. Strict pathogen includes: *S. pneumoniae* (blood), *Haemophilus influenzae* (blood), *S. aureus* (blood), HMPV (nasal swab), RSV (nasal swab) and Influenza virus (nasal swab). Non-strict pathogens group includes all remaining pathogens detected by FTD diagnostic kits (HRV, HBoV, HPIV, HCoV,…). No respiratory pathogen group includes all negative PCR-results.

5. Host blood-based biomarkers (CRP, PCT and IP-10) profiles vary according to clinical fever etiologies and pathogen type

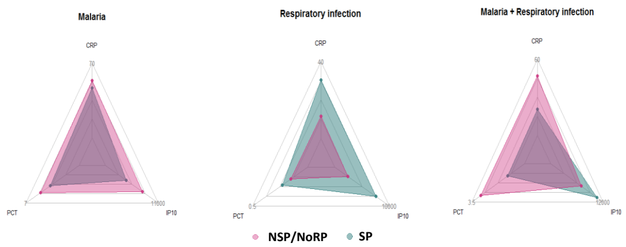


Figure 5. Each radar chart shows data from participants diagnosed with the same clinical condition. The three radial axes indicate the blood concentrations of C-reactive protein (CRP, mg/mL), procalcitonin (PCT, µg/L), and interferon-γ induced protein 10 (IP-10, pg/mL). Color coded triangles represent data from groups of participants infected by the same pathogen type. SP: strict pathogen; NSP: non-strict pathogen; NoRP: no detected pathogen; all pathogens identified by PCR. Dots represent the median blood concentration of CRP, PCT, and IP-10 in each participant group. No statistically significant difference between concentration medians was detected.

6. Rates of strict-pathogens correctly identified using biomarker combinations

Host blood-based biomarker caracteristics	MALARIA	CLINICAL RESPIRATORY INFECTION	MALARIA + CLINICAL RESPIRATORY INFECTION
Best concentration threshold for strict-pathogen identification	[**PCT**] < 5.09 µg/L	[**PCT**] > 0.15 µg/L	[**IP-10**]>7853.1 pg/ml
Rate of PS correctly identified	50%	66.20%	75.00%
P-value*	<0.01	<0.001	0.03
AUC	0.61 (0.44-0.77)	0.56(0.47-0.64)	0.57 (0.44-0.71)

*logistic regression

Table 2 First row: for each biomarker, concentration thresholds were obtained from Figure 4 to provide optimal discrimination between samples from participants with SP or with NSP/NoRP. Receiver operating curves (ROC) generated for each biomarker and each condition.

CONCLUSION

In our study, the combination of CRP, IP-10 and PCT does not appear to be adequate for early identification of strict pathogens infections among children presenting with fever.

Therefore a more complex biomarker signature emerging from MS analysis would be beneficial for discriminating patient who need preventive antibiotic therapy to prevent complication of respiratory infections.