



NON-MALARIA RELATED ACUTE FEVER, A PRELIMINARY STUDY FROM KINSHASA THE DEMOCRATIC REPUBLIC OF THE CONGO

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ABSTRACT

Background: Fever is a key symptom that motivates medical consultation mainly in tropical areas. This study aimed to assess non-malaria cases among those attending a primary health care facility with acute fever in Kinshasa suburb.

Methods: Participants were screened independently with rapid diagnostic test (RDT) and microscopy. Dried blood spots were analyzed by polymerase chain reaction (PCR), which was considered as the golden standard for malaria diagnosis. A subpopulation benefited from blood culture to screen nonspecific bacterial infections.

Results: A total of 296 patients were enrolled. Malaria was detected in 44.6%, 38.5%, and 37.2%, respectively, by RDT, microscopy, and PCR. Blood culture was positive for 5/151 patients (3.3%), essentially those aged under 5 years (4/5). Sleeping under mosquito net was protective against malaria ($P = 0.026$). In the subgroup assessed for blood culture, 94/151 patients (62.3%) had neither unspecific bacterial infection nor malaria.

Conclusion: Neither malaria nor unspecific bacterial infection was responsible of the acute fever in more than half of the patients. These findings suggest that DTs (including viral agents) and diagnostic algorithms are paramount for the management of non-malaria acute fever.

Keywords: Acute fever, blood culture, Democratic Republic of the Congo, Kinshasa, non-malaria.

INTRODUCTION

Fever or history of fever is a key symptom that motivates medical consultation worldwide. In tropical malaria endemic areas, till recently, every case of fever used to be considered foremost as malaria and treated accordingly. Consequences were overtreatment, mistreatment, and spread of antimalarial drugs resistance. These consequences as well as the (relatively) high cost of the new artemisinin-based combination therapies motivated to adapt the guidelines by restricting antimalarial drugs to patients with a positive test.^[1] Meanwhile, malaria burden has declined in several endemic areas. Since early 2000 to now, the World Health Organization recorded an overall reduction of 37% in the malaria incidence and 60% in the malaria mortality.^[2] Consequently, clinicians practicing in a context of limited-laboratory infrastructure have to take into the account the increasing proportion cases of fever which are not attributable to a malaria infection. We assessed non-malaria cases among patients attending a primary health care (PHC) facility with acute fever in Kinshasa suburb.

MATERIALS AND METHODS

This was a cross-sectional study embedded into the Quinact clinical trial conducted at Lisungi Health Center in Kinshasa.^[3] All patients attending the study site from 25th February to 26th March 2014, with fever or history of fever were eligible. Patients (or parents/guardians if a patient was under 18 years old) were asked to sign (or thumb-print if they were illiterate) an informed consent form prior enrolment.

A questionnaire was filled with demographic and clinical (symptoms) data before blood sampling. Finger prick allowed collecting blood and performing rapid diagnostic test (RDT) for malaria (SD Bioline® Malaria Ag Pf/pan) according to manufacturer's instructions. At the same time, a thick and thin blood smears were prepared. After air-drying, the smears were stained with 10% Giemsa during 10 min. The thin smears were fixed with methanol before staining. The reading was done at 1,000X magnification, by two independent microscopists not aware of RDT results. Parasites were counted over 200 white blood cells (WBC), and density per μL estimated assuming 8,000 WBC/ μL . RDT and microscopy results were used for patients' management, according to national guidelines.

Finger-prick blood was spotted on filter paper (Whatman, Maidstone, UK), kept at room temperature, and used thereafter as a source of DNA. Parasite genomic DNA was extracted using QIAamp mini DNA kit (Qiagen, Germany) as described by the manufacturer and eluted in a final volume of 150 μL .

Analysis of the Plasmodium species was performed using polymerase chain reaction (PCR) assay targeting small subunit ribosomal ribonucleic acid (18S SSU rRNA) genes as described by Snounou *et al.*^[4] Briefly, the extracted parasite DNA was amplified by outer and nested species diagnostic PCR approach and the amplified product were run in 1.5% agarose (SeaKem™) ethidium bromide stained and 50 bp DNA was used as the ladder marker. Visualization of the bands was done

Table 1: Malaria tests positivity

Diagnostic test	Positive test in all age group		Positive test in <5 years		Positive test in ≥5 years	
	n/N	% (95% CI)	n	% (95% CI)	n	% (95% CI)
RDT	132/296	44.6 (39.0-50.2)	59/127	43.2 (37.7-55.2)	73/169	43.2 (35.7-50.7)
Microscopy	114/296	38.5 (33.0-44.0)	51/127	40.2 (31.6-48.8)	63/169	37.4 (29.9-44.6)
Malaria PCR	110/296	37.2 (31.6-42.7)	48/127	37.8 (29.3-46.3)	62/169	36.7 (29.4-44.0)

RDT: Rapid diagnostic test, PCR: Polymerase chain reaction, CI: Confidence interval

under ultraviolet trans-illuminator. PCR analysis was performed at the National Institute for Medical Research in Tanga, United Republic of Tanzania and the result was considered as the gold standard for malaria diagnosis.

Blood culture was applied to screen nonspecific bacterial infections. Adults and children ≥15 years provided 20 mL venous blood, divided into two flasks (BacT/ALERT FA®, bioMérieux, France). Children younger than 15 years old provided 1 to 4 mL of venous blood (depending on the age) collected in one flask (BacT/ALERT FP®, bioMérieux, France). The flasks were transported to the laboratory of microbiology at Institut National de Recherche Biomédicale in Kinshasa. Thereafter, they were incubated at 35°C for a maximum of 7 days. Bacterial growth was checked daily. Blood culture was considered positive when bacterial growth could be observed by the naked eye. Gram-stain and bacteria identification were performed according to conventional methods. Only pathogenic bacteria were considered. Results were reported to the attending physician for consequent antibiotics treatment.

RESULTS

A total of 315 patients were invited to participate but 19 did not consent. 296 patients were included in this analysis. However, blood culture was realized only for 151 patients (for logistic reasons). The median age was 6 years (interquartile range: 2.5-25.3). Malaria was detected in 44.6%, 38.5%, and 37.2%, respectively, by RDT, microscopy, and PCR (Table 1). When stratified by age group, the proportion of malaria was similar in children below 5 years old compared to older patients group. Sleeping under mosquito net was a predictor of non-malaria acute fever (odds ratio = 0.57, 95% confidence interval = 0.35-0.93; $P = 0.026$).

Blood culture was positive for 5/151 patients (3.3%). Most of them (4/5) were aged under 5 years. The pathogens isolated were *Salmonella* sp. ($n = 3$), *Citrobacter freundii* ($n=1$), and *Streptococcus pneumoniae* ($n = 1$). All these five patients were malaria negative (Table 2).

In the subgroup assessed for blood culture, 94/151 (62.3%) had neither bacterial infection nor malaria. In the overall study population, 186/296 patients (62.8%) were malaria negative.

DISCUSSION

This study shows that majority of patients attending a health facility with acute fever in Kinshasa suburb have no malaria. In such circumstance, clinicians may be puzzled about the attitude to take, in particular when patients claim to know the diagnosis and have a prescription when laboratory results are negative.^[5,6] Both antimalarial drugs and antibiotics would be inappropriate to prevent over treatment and its consequences in terms of cost, adverse events and the selective pressure on resistant strains. And in some circumstances both may be needed. Clinicians are most challenged if non-malaria acute fever is not associated with other signs and symptoms of other conditions,^[6] i.e., antibiotics may be justified in some localized bacterial infections when blood culture is negative. In a larger study carried in Tanzania, acute fever was reported to be due to viral diseases in 70.5% of participants.^[7] Unfortunately, laboratory tests targeting bacterial or viral agents are challenging at PHC. Consequently, self-limiting viral infections may be unnecessarily treated with antimalarial drugs and/or antibiotics.^[7,8] Understanding the etiology of non-malaria acute fevers

Table 2: Bacterial infection and malaria

Malaria PCR	n (%)		
	Positive	Negative	Total
Blood culture			
Positive	0	5 (5.0)	5 (3.3)
Negative	52 (100)	94 (95.0)	146 (96.7)
Total	52 (100.0)	99 (100.0)	151

PCR: Polymerase chain reaction

in each particular context is believed to be the mainstay of improving outcomes of febrile illnesses.^[9]

RDT detected slightly more positive cases. It is known that RDTs may remain positive in the absence of viable malaria parasites because of persistent antigens that they detect.^[10,11] Nonetheless, they are more reliable at PHC for malaria diagnosis because of the poor quality of microscopy at that level.^[12]

CONCLUSION

Neither malaria nor unspecific bacterial infection was responsible of the acute fever in more than half of the patients. Consequently, both antimalarial drugs and antibiotics should be handled with care in such cases. We recommend to generate more data using DTs (including those detecting viral agents) to construct diagnostic algorithms adapted to the local context for the management of non-malaria acute fever at PHC.

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