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Assessment of the performance of malaria rapid diagnostic test in acutely malnourished children under five years of age in Nanoro - Burkina Faso

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The interaction of malaria with malnutrition is complex. In areas where malnutrition among children is prevalent, management of malaria is not standardized. In Burkina Faso, malaria treatment is prescribed after positive malaria rapid diagnostic test (RDT) or thick blood smears confirmation regardless of the nutritional status of the child. The study aims to assess the performance of malaria RDT in acute malnourished children under five years of age. A descriptive cross-sectional study was carried out from June 1st to August 31th 2014 in the health district of Nanoro in Burkina Faso. The study involved the children less than 5 years of age who were admitted for acute malnutrition and tested for malaria using RDT. The diagnostic values were then assessed for their agreement with the gold standard of the World Health Organization (thick blood smears) using Cohen-Kappa coefficient. In total, RDT and thick blood smear results were obtained from 131 children (aged 1-59 months). RDT was positive in 87 tested children (66.4%), while the thick smear indicated that only 47 were infected by malaria (35.9%) and Cohen kappa coefficient was 0.44. The sensitivity, specificity, positive predictive value and negative predictive value of RDT for malaria compared to microscopy were respectively 100% (95% CI: 92.5 - 100), 52.4% (95% CI: 51.1 - 52.9), 54% (95% CI: 43 - 64.8), 100% (95% CI: 92.5 - 100). Their timeliness was 8 min (± 3.47 min). Using malaria RDT in acutely malnourished children results in high number of false positive.

Key words: Performance, sensitivity, specificity, acute malnutrition, rapid diagnostic test, thick blood smear, Burkina Faso.

INTRODUCTION

Malnutrition is a pathological condition resulting from the deficiency, excess or imbalance in intake of one or more macronutrients and/or micronutrients. It compromises natural immunity leading to increased susceptibility to infections such as malaria (Caulfield et al., 2004). Malnutrition is a major public health problem (Food and Agricultural Organization, 2012).

Malaria is a public health problem (World Health

Organization, 2017) and it is a major cause of mortality in countries where it remains endemic. According to the WHO report data in 2017, an estimated 216 million cases of malaria occurred worldwide with 445,000 malaria deaths (WHO, 2017). Africa region is the most affected, with children less than five years of age and pregnant women being the most affected (Burki, 2013; WHO, 2017). The problem of the malaria-malnutrition interaction is complex (Lapidus et al., 2009; Müller et al., 2003; Oldenburg et al., 2018). But as reported previously, this association seems to be common in endemic countries (Lapidus et al., 2009; Müller et al., 2003; Nyakeriga et al., 2004; Osterbauer et al., 2012; Shankar, 2000).

In Burkina Faso, despite the public health importance of both malaria and malnutrition, there are no national data on their association and co-occurrence. A hospital-based study carried out in Kaya (Center North of Burkina Faso) in 2013 estimated that one fifth of the acutely malnourished children were affected by malaria (Ouedraogo et al., 2013). Since 2013, with the WHO recommendation on the use of RDT for malaria, new guidelines on the implementation of histidine-rich protein 2 (HRP2)-based RDT were adopted for all patients with fever living in localities where microscopy is not available regardless of their nutritional status (Ministère de la Sanè du Burkina Faso, Programme National de Lutte Contre le Paludisme, 2014). Results obtained by both tests, RDT and microscopy are not always consistent. RDT antigen-based tests can be positive for a certain period post-infection even if the infection has been treated and the parasite has been cleared (WHO/Roll Back Malaria, 2005). The antigen HRP2 remains in the bloodstream for up to five weeks, time by which the parasite has been cleared probably by treatment, that is, the microscopy would be negative. The difference could be due to the cross-reactions with heterophile antibodies in the patient's plasma when using OptiMAL-IT (DiaMed Basel, Switzerland) as the RDT (Valea et al., 2009). From another hand, it has been shown that malaria infection can be detected by polymerase chain reaction (PCR) in samples negative for microscopy but positive by RDT (Berzosa et al., 2018; Fançony et al., 2013; Moody, 2002; Okell et al., 2009; Tham et al., 1999). The WHO strategy on the diagnosis of malaria in endemic areas in the community using RDT has been implemented with no evidence on its sensitivity and specificity in malnourished patients. We hypothesize that differences between the two tests RDT and microscopy will be exacerbated in these children. In the present analysis, we aimed at testing the diagnostic performance of HRP2 -RDT for malaria compared to microscopy in malnourished children.

MATERIALS AND METHODS

Study site and period

This study was carried out between June 1st and August 31th 2014, which corresponded to the rainy season in the region by the Clinical Research Unit of Nanoro (CRUN). It included six health centers of the Nanoro Health District: four peripheral health centers (Godo, Séguédin, Soum and Nazoanga) and two health facilities in the city of Nanoro. Nanoro is located at 85 km from Ouagadougou, the capital city of Burkina Faso. It is an agricultural area which depends entirely on rainfall. Rainfall is capricious and insufficient. It varies between 450 and 700 mm per year (District Sanitaire de Nanoro). Malaria is hyper endemic in the area (Derra et al., 2012). The district has two centers for education and raising awareness regarding nutrition and hygiene in the community located in Nanoro and Nazoanga. The district has another center of rehabilitation and nutritional education (CRNE) for treatment of children with severe acute malnutrition.

Study population

In this descriptive cross-sectional study, 0-5 year old children of both sex living in Nanoro health district area who attended, during the study period, the outpatient clinic at one of the collection sites were included. Other health conditions were not considered in the inclusion or exclusion criteria (that is, children with reported or diagnosed infectious diseases such as HIV or tuberculosis were included). The outpatient clinic is a community health center that screens children for acute malnutrition; treats children with moderate acute malnutrition while refers children with severe acute malnutrition to the CRNE. Data were collected in children suffering from acute malnutrition indicated by a weight-for-length/height Z score ≤ -2 Standard Deviations (SD) of the median of the WHO (2006) and followed up by “Emergency Against Acute Malnutrition in the North Center” for treatment.

Laboratory procedures

Malaria RDT, SD Bioline Ag Pf / Pan (Standard Diagnosis, Korea) was used throughout this study in all sites. The test was performed according to the manufacturer instructions as follow. Trained nurses held the finger of the patient and cleaned it using the alcohol swab provided in the test kit. The fingertip is let to dry and then it is slightly pressed and pricked with a sterile lancet. A blood drop (5 µl) is collected using a capillary tube (included in the test kit) and deposited in the round hole (hole "S" of test cassette). Four drops of the buffer are added carefully in the square hole of the test cassette. The test is read after a waiting period of 5 to 10 min. The appearance of the band C only means the test is negative. When both the control band C and Pf line or the band C and the Pan line are visible, the test is positive. The test is invalid if the band C is not visible and is performed again following the same steps described above (WHO, 2018).

At the same time, in every eligible participant, a thick blood smear was also performed by the trained nurses who carried out the RDT test. The thick blood smear was dried immediately and then transported to the laboratory of the Clinical Research Unit of Nanoro for processing and microscopy. At the laboratory, the slides were dried, stained with Giemsa 10% in water (V/V) (Gupta and

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Table 1. Selected population characteristics.

Designation	Value
Female n (%)	69 (52.3)
Median age (months [min-max])	15 [1-59]
Mean temperature (°C ± SD)	37.9 ± 1.08)
Fever[1] or history of fever within 24 h n (%)	107 (81.7)
Severe acute malnutrition[2] n (%)	76 (58.0)
Rural residence n (%)	131 (100)
Mother with no education n (%)	124 (94.7)

[1] Fever is defined as axillary temperature higher than 37.5°C. [2]Weight-for-length/height Z-score <-3 SD or Weight-for-length/height Z-score <-2 SD + oedema

Singla, 2012) and read after 5 to 10 min by two experienced microscopists. In case of discrepancy of the two results, a third reading was done. The microscopists were not involved in performing the RDT, and were masked to the results of the RDT as well as to the clinical status (febrile or non-febrile) of the children. A number of microscopic fields corresponding to 200 White Blood Cells were read in the thick film. The parasite density was calculated (for *Plasmodium falciparum* only) in the conventional way according to the WHO criteria.

Sample size calculation

To our knowledge, there were no previous studies that assessed the performance of RDT compared to the gold standard "microscopy" in moderate and severe malnourished children. The sample size calculation was based on "exhaustive inclusion", that is, we included all the eligible participants during the selected period of high malaria transmission (1st June – 31st August).

Ethical considerations

Local authorities (physician responsible of the district, director of the Medical Center with Surgical Antenna Saint Camille of Nanoro, Clinical Research Unit of Nanoro, center of rehabilitation and nutritional education' Chief, the physician in charge of the Medical Center with Surgical Antenna Saint Camille of Nanoro) were informed of the investigation and their authorization was acquired before starting the study activities. Study protocol was also approved by the local ethical committee of "Institut de Recherche en Sciences de la Santé".

The field team has been trained to follow good clinical practices and study survey procedures. Before collecting patient's data, informed consent was obtained from the legal guardian accompanying the child participating in the study. Legal guardians were informed that their participation in the study is not compulsory nor a condition to benefit from the medical care of their child. In addition, it was agreed that the information collected in this study are and will remain confidential.

Data analysis

Results obtained by RDT and microscopy were coded independently by two data clerks using the software Open Clinica Version 3.1.4. Descriptive statistics was performed using proportions, means or median. Additionally, the performance of RDT was evaluated by calculating the sensitivity, the specificity, positive predictive value, the negative predictive value, and the timeliness as follow:

Sensitivity: True Positive / (True Positive + False Negative)
Specificity: True Negative / (True Positive + True Negative)
Positive predictive value: True Positive / (True Positive + False Positive)
Negative predictive value: True Negative / (False Negative + True Negative)

Timeliness is the average of time required to get the RDT result (a sum of the time required for each RDT test divided by the number of tests). Additionally, the diagnosis accuracy (DA, a proportion of correctly classified subjects [True Positive + True Negative] among all subjects) and the Youden's index were also calculated. Youden's index is calculated by deducting 100 from the sum of test's sensitivity and specificity expressed as percentage (Ana-Maria, n.d.). Furthermore, the agreement between the microscopy and the RDT was computed using the Cohen-Kappa coefficient. Data were analyzed using the Stata10 -IC software (stata Corp LLC, TX).

RESULTS

A total of 131 malnourished children aged 1-59 months were enrolled in this study, two thirds were severely acute malnourished (defined as weight-for-length/height z-score less than -3 SD), and 52.3% were females. Measured fever or history of fever during the previous 24 h was reported in 81.7% of children. Patients' socio-demographic characteristics are summarized in Table 1.

RDT was positive in 66.4% of the tested children while the blood smear microscopy showed that only 35.9% were positive. There was a significant agreement between the microscopy and the RDT although moderate with a Cohen Kappa coefficient of 0.44 ($p < 0.0001$). Based on these data, we calculated the statistical measures of the RDT performance compared to microscopy which is the gold standard of the WHO (Table 2). The results showed that RDT was sensitive at 100% (95% Confidence Interval CI: 92.5 - 100) and specific at 52.4% (95% CI: 51.7 - 52.9). The RDT has positive predictive value of 54.0% (95% CI: 43 - 64.8), a negative predictive value of 100% (95% CI: 92.5 -100), a diagnostic accuracy of 69% and the Youndex Index of

Table 2. Diagnostic performance of RDT compared to microscopy (gold standard) for malaria detection.

Accuracy parameter	Value % (n/N)	Confidence intervals (95% CI)
Sensitivity	100(47/47)	92.5 – 100
Specificity	52.38(44/84)	41.19 – 63.40
Positive predictive value	54.02(47/87)	48.42 – 59.52
Negative predictive value	100(44/44)	93.5 – 100

52%.

The average time to obtain a result (timeliness, mean ± SD) was 8 ± 3.47 min. While, a thick blood smear requires in average 5 - 10 min for a microscopists to complete the reading. In this study, we have not recorded the timeliness of the microscopy test. Regarding the RDT results, 58.6% (51/87) of malaria cases were associated with assessed fever or a history of fever within the 24 h before outpatient facility. And according to the microscopy, 66.0% (31/47) of malaria cases were associated with fever or a history of fever within 24 h.

DISCUSSION

Malaria prevalence as tested by the RDT was almost two fold higher than malaria prevalence assessed by blood-smear microscopy. This difference between the two tests could mainly be explained by the RDT characteristics, the high number of false positive malaria cases in an endemic area, the compromised nutritional status and immune system in case of severe acute malnutrition and the high rate of malaria transmission during the rainy season when the study took place. Furthermore the RDT showed a high sensitivity (100%) with a low specificity (52.4%) for the detection of malaria infection compared to the reference blood smear microscopy.

The comparative studies we used are mostly from the general population. It has been systematically reported in other studies that RDT over estimates the prevalence of malaria infection at community level and at the health centers (Table 3) (Abba et al., 2011; Bisoffi et al., 2010; Dongmo, 2012; Ilombe et al., 2014; Ogouyèmi-Hounto et al., 2013; Samadoulougou et al., 2014; Valea et al., 2009). It is noteworthy to highlight that the prevalence found by the RDT and blood-smear microscopy were closer when malaria infection was not endemic and when the diagnosis was performed in symptoms suggestive of malaria cases vs. in community screening in malaria endemic areas.

The sensitivity found in this study was higher than that found by a meta-analysis combining over 70 studies in the general population [95.0%] (Abba et al., 2011). Predictably, the specificity found in this study was either lower or in the same range of specificity found in other studies in community or health settings in the general population. The characteristics of the RDT, the endemicity of malaria in the study region, and the high transmission during the rainy season all enhanced the risk of low specificity of the RDT (true positive). Additionally, it is hypothesized that the lower specificity of HRP2-based RDT among malnourished children is related to a weakened immunity which consequently causes a reduction or a lack of production of anti-HRP2 antibodies which neutralize HRP2 produced by malaria parasite. It is noteworthy to mention that the diagnosis of malaria infection based on microscopy might fail to detect submicroscopic infections because the detection limit of the thick film ranges between 10-20 parasites/µl (Haute Autorité de Santé, 2016). Furthermore, RDT is unstable in high temperatures (Valea et al., 2009). This could also apply to the local context where the temperatures are in average above 32°C during storage and in the field.

The low positive predictive value found in this study reflects also the low prevalence of malaria infection reported in this study. The negative predictive value was good and reflects conversely the low prevalence in this study population.

This study showed that in high malaria transmission-setting and where malnutrition is prevalent, the test should not only be conditioned by the presence of history of fever. Low specificity can result in treatment of fever as symptomatic malaria and may distract the clinician from correct care of the child. From another hand, our study results raise the potential risk of using blood-smear microscopy as the gold standard in high malaria transmission setting where malnutrition is prevalent because of the compromised immune system and the development of malaria disease in low density parasitemia (submicroscopic infections).

We recognize the limitations of our studies that are mainly the small sample size and the lack of tests of other morbidities that are either associated with- or risk factors for moderate and severe acute malnutrition such as HIV and tuberculosis. However, this study could be considered as a pilot study and more studies are needed in the context of infectious diseases (malaria) and malnutrition in order to build the evidence on the proper diagnosis and treatment of malaria. The prevalence of HIV/Tuberculosis are low in the study area (Nanoro Health District, 2014), this would limit their confounding of the findings in this study.

Table 3. Accuracy of RDT for malaria among children in few countries.

Study	Population characteristics	Malaria prevalence (Positive blood smear %)	Accuracy parameters (%)				Special comments
			Sensitivity	Specificity	Positive predictive value	Negative predictive value	
Ilombe et al. (2014), Democratic Republic of Congo	N: 872 (32% symptomatic) Age: 0.7-59.9 months enrolled at the community and health center(s)	34.4	Community 95.8 Health Center 98.0	79.4 66.9	60.0 68.8	98.3 96.5	Sensitivity of the RDT increased with parasite density.
Samadoulougou et al. (2014), Burkina Faso	N: 6102 Age: 6-59 months All 13 regions	66% (95% CI: 64.8-67.2)	89.9% (95% CI: 89.0-90.8)	50.4% (95% CI: 48.3-52.6)	77.9% (95% CI: 76.7-79.1)	72.1% (95% CI: 69.7-74.3)	Highest specificity before the rainy season 71.3% (95% CI: 65.6-77.0) and decreased to 44.3% (95% CI: 41.4-47.2) and 51.9% (95% CI: 48.1-55.7) during and after the rainy season
Dongmo (2012), Cameroon	N:336; Age: 1-79 years (16% were <5 years)	28.0	94.7	95.5	89.0	97.9	
Ogouyèmi-Hounto et al. (2012), Benin	N:354; Age: 6 months- 70 years (43% were <5 years)	22.8	96.3	95.6	86.7	98.9	All the participants were enrolled because of reported fever
Bisoffi et al. (2010), Burkina Faso	All (febrile and non-febrile) patients aged > 6 months N=5,236	Dry season: 17.9 Rainy season: 49.0	86% (95% CI: 78-92%) 94% (95% CI: 92-96)	90% (95% CI: 86-92) 78% (95% CI: 72-83%)	72% (95% CI: 63-79) 88% (95% CI: 85-91)	95% (95% CI: 92-97) 88% (95% CI: 83-92)	RDT performed only if patient had T° ≥ 37.5
Valéa et al. (2009), Burkina Faso	N: 464 Age: 6–59 months	82.8	98.7% (95% CI: 97.6–99.8)	96.2% (95% CI: 94.3–98.1)	99.2	93.9	All children reported for suspected malaria

Conclusion

RDT allows timely management of malnourished patients with febrile illness and restricts the use of antimalarial drugs to true positive malaria cases. In malnourished children the test has a low specificity compromising the treatment of malnourished children which have an already weakened immune system. Although RDT has low specificity in malnourished children, their performance was overall satisfactory in current conditions of use. Additionally, the study questions whether thick blood smear test and conditioning the malaria test only in febrile cases are appropriate in cases of severe acute malnutrition. More work needs to be carried out in the context of malnutrition and malaria before drawing an evidence-base recommendation on the best practices for malaria test and treatment in malnourished children. Until then, we recommend using RDT systematically in the case of acute malnutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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