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Research Article

Appropriateness of Assumed Versus Absolute White Blood Cell Counts for Estimation of Malaria Parasite Density in Children Population in Ibadan Southwest Nigeria

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Abstract

Estimation of malaria parasite density is important in diagnosis and assessment of individuals on antimalarial drugs. The use of either patient's actual white blood cell (WBC) counts or an assumed value of 8000/mm^3 to calculate malaria parasite density is still controversially discussed in literature. This study was carried out to investigate the agreement between the two methods of calculating malaria parasite density in children. Data on parasite and WBC counts were extracted from 796 case record forms of children aged 3 to 120 months who participated in four antimalarial clinical trials conducted between 1998 and 2014. Criteria for enrolment into the clinical trials included symptoms compatible with acute uncomplicated malaria, microscopically confirmed malaria parasitaemia of at least 1000/µL and absence of danger signs of severe malaria. All the studies received relevant ethical approval. A Bland Altman plots was used to show level of agreement or bias between the two methods. Male participants constituted 54.9%. Overall mean age was 47±31 months and mean WBC was 7807±4888/mm^3. Geometric mean parasite density using assumed and actual WBC were 15,870 parasite/µL and 14,139 parasite/µL (ρ<0.001) respectively. Bland Altman plots showed that mean differences between parasite densities calculated from assumed and actual WBC densities were close to zero suggesting no remarkable systematic bias. Using an assumed white blood cell counts for calculating parasite density appears appropriate in children aged 3 to 12months for in Southwest Nigeria.
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Key Words: Parasite Density, Assumed White Blood Cell Count, Actual White Blood Cell Count and Antimalarial Drug.

INTRODUCTION

Despite the huge amount of money invested in malaria control and elimination and the success recorded evidenced by the decline in the incidence and death from malaria globally (76 to 63 per 1,000 population at risk from 2010 to 2016 representing an 18% decline) (WHO, 2017), malaria remains a public health infection of importance. Malaria accounts for 43% of global death among under 5 African children (WHO, 2017). An important strategy for reducing the morbidity and mortality due to malaria is early diagnosis and prompt treatment with efficacious drugs (Pagnoni *et al.*, 1997). Microscopic examination of blood smear for detection of presence of malaria parasite and the magnitude of the parasitization known as parasite density are essential for accurate diagnosis. Consequently, WHO insists on this method or other methods of parasitological diagnosis such as Rapid Diagnosis Test (RDT) before instituting treatment(Organization, 2015).

In addition, estimating malaria parasite density is necessary for monitoring response to treatment, as an indicator of outcome in clinical trials and drug efficacy studies (WHO, 2010a, WHO, 2010b). In quantifying the malaria parasite density asexual parasites are counted against WBC within the same fields, and then multiplied by the assumed WBC or the patient's WBC (O'Meara *et al.*, 2006). Complete blood counts, particularly WBC counts, can be performed with automated haematological analyser, or manually using stains, a microscope and a cell counting chamber and counters (Liu, 2016). However, in most field settings, it is not practicable to obtain WBC value for malaria patients by automated blood cell counters, due to the inadequate availability of laboratory facilities, lack of constant electricity and cost. In addition, venipuncture is culturally not acceptable in the community and is viewed with suspicion. Conventionally, an assumed WBC count of 8,000/mm^3, which was recommended by WHO in 2010, is used in estimating malaria parasite density. However, using the conventional method of assumed WBCs of 8,000/mm^3 to quantify parasite densities may generate errors which could influence decisions and conclusions (McKenzie *et al.*, 2005, O'Meara *et al.*, 2007) considering the racial/ethnic differences in WBC count. Studies have shown that individuals of African ancestry have lower WBC count than their counterparts from other continents (Bain, 1996, Beutler and West, 2005, Urquhart *et al.*, 2008). Thus, there might be over-estimation or under estimation of the parasite density with the use of assumed WBC count rather than actual WBC count (Jeremiah and Uko, 2007, O'Meara *et al.*, 2007, Laman *et al.*, 2014, Haggaz *et al.*, 2014, Alves-Junior *et al.*, 2014, Bilal *et al.*, 2015, Adu-Gyasi *et al.*, 2012, Adu-Gyasi *et al.*, 2015) Furthermore, WBC count, particularly in malaria-infected individuals, can vary by as much as ten-fold between

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individuals (McKenzie *et al.,* 2005). Previous studies show discrepancies using assumed versus actual WBC in calculating malaria parasite density (Jeremiah and Uko, 2007, Adu-Gyasi *et al.,* 2012, Laman *et al.,* 2014, Haggaz *et al.,* 2014, Alves-Junior *et al.,* 2014, Adu-Gyasi *et al.,* 2015, Bilal *et al.,* 2015). Studies conducted by Jeremiah and Uko in 2007, Haggaz *et al.,* in 2014, Alves-Junior *et al.,* in 2014, Adu-Gyasi *et al.,* in 2015 and Bilal *et al.,* in 2015 all showed that malaria parasite density calculated using assumed WBC was significantly higher than using actual WBC (Jeremiah and Uko, 2007, Haggaz *et al.,* 2014, Alves-Junior *et al.,* 2014, Adu-Gyasi *et al.,* 2015, Bilal *et al.,* 2015). Adu-Gyasi *et al.,* in 2012 showed that malaria parasite density calculated using assumed WBC was significantly lower than using actual WBC (Adu-Gyasi *et al.,* 2012) while Laman *et al.,* in 2014 showed that there was no statistically significant difference using either assumed or actual WBC in calculation malaria parasite density (Laman *et al.,* 2014). White blood cell (WBC) counts during malaria infection are generally characterized as being low, normal or high,*(*Sowunmi *et al.,* 1995) a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis (McKenzie *et al.,* 2005). Leucocytosis is typically reported in a fraction of cases and may be associated with concurrent infections and/or poor prognosis (McKenzie *et al.,* 2005).

When clinical trials are done to test the efficacy of new antimalarial drugs, an important measure of therapeutic response is the parasite density which may be overestimated or underestimated when assumed WBC count is used. When assessing enrolees for clinical trials, using estimated WBC count could label an enrolee hyperparasitaemic when the parasite density is overestimated and vice-versa which would determine the intervention the enrolee receives. It is however preferable to use actual WBC count when calculating parasite density. This might be unachievable in most malaria endemic regions because of paucity of funds to procure automated haematological analyser and none availability of constant electricity to run the machines even when one exists. This underscores the need for an acceptable reference value for WBCs count in children in different regions of the world. There are very few published studies that have compared parasite densities using both actual and assumed WBC count when assessing the efficacy of new antimalarial drugs.

This study was carried out to determine the agreement between the parasite densities obtained using the assumed 8,000/mm^3 of blood and the actual WBC count of the patient in Ibadan, Southwest Nigeria

MATERIALS AND METHODS

Study design, site and population: This study presents findings from data extracted from data of four previous clinical trials of some antimalarial drugs conducted between July 1998 and November 2014. All the four clinical trials were carried out in Ibadan. Blood film for microscopy and white blood cell count were carried out at the hematology laboratory of the University College Hospital Ibadan, Oyo State, Nigeria. The study participants were mainly Yoruba and were all resident of Ibadan an area of intense malaria transmission(Salako *et al.,* 1990) who lived within 15-20km distance from the study center.

Sample size and sampling: The minimum sample size required to detect a difference in parasite count between the two methods, actual WBC and assumed 8,000/mm^3 was calculated using a sample size for paired mean values. Assuming that the standard deviation for the WHO recommended 8,000/mm^3 is 19, 168.8 as reported by Haggaz *et al.,* and the present hopes to detect a difference that is 25% of SD (4792.2). At 80% power and 95% level of confidence, an estimated 502 participants were required.

Data of clinical trials participants included in this study were children aged 3 – 120 months who presented with fever (axillary $\geq 37.4^0$C) or a history of fever within 48 hours of presentation and had microscopically confirmed parasitaemia of at least 1,000 asexual parasites /µL were enrolled into the studies. Patients with severe anaemia (PCV <15%), clinical feature of severe and complicated malaria (WHO 2015), evidence of chronic disease (e.g. hemoglobinopathy, renal or liver impairment etc.) were excluded from the studies. Children who vomited the study drug recurrently, received other antimalarial drugs or failed to comply with the study protocol were withdrawn from the studies.

Data collection and variables: Case record forms of each patients were reviewed and data points extracted include: study identification number, date of enrolment, demographic data (gender and age in months), weight at enrolment in kilogram (kg), temperature at enrolment in degree Celsius (^{0}C), parasite count at enrolment, haematocrit at enrolment and white blood cell count (WBC) at enrolment. Fever was defined as temperature $\geq 37.4^0$C. Anaemia was defined as haematocrit less than 30% and sub categorised as mild anaemia (21-29%) and moderate anaemia (15-20%). We defined leucocytosis for ages 3-23 months, 2 – 9 years and >9 years as WBC count greater than 14,000 cells per mm^3, 12,000 cells per mm^3, and 10,500 cells per mm^3, respectively(Stanley, 2016). Leukopenia was defined for ages 3-23 months and >2 years as WBC count less than 6,000 cells per mm3 and 4,000 cells per mm3 respectively(Stanley, 2016). For all the clinical trials, diagnosis of malaria was based on microscopy performed following standard procedure recommended by World Health Organization (WHO, 2010a).

Ethical Consideration: All the clinical trials from which the data were extracted were conducted in line with the declaration of Helsinki. Ethical approval for all the clinical trials was obtained from the University of Ibadan/University College Hospital (UI/UCH) Institutional Review Committee. Written informed consent or witnessed verbal informed consent were obtained from the parents or guardians of prospective enrolees before enrolment into the clinical trial studies.

Data analysis: Data from 796 patients' case record files (CRF) were analysed. The focus of the analysis of this study was the parasite count and the white blood cell count. The actual and assumed parasite densities were calculated using the actual WBCc and assumed WBCc of 8,000/mm^3 (WHO, 2010a) respectively using the formula:

Conventional

$$\text{Parasite density (µL)} = \frac{\text{Number of parasite x 8,000/mm}^3}{\text{No of WBC}}$$

Ideal

$$\text{Parasite density (µL)} = \frac{\text{Number of parasite x Patients' WBC}}{\text{No of WBC}}$$

Study identification number, date of enrolment, demographic data (gender and age in months), weight at enrolment in kilogram (kg), parasite count at enrolment, white blood cell count (WBC) at enrolment, assumed and actual parasite densities for each enrolee were entered into a spread sheet designed using IBM Statistical Package for the Social Sciences (SPSS, version 23).

The data were then analysed using various statistical tools which includes mean, geometric mean, standard deviation of normally distributed data. Student paired t-test was used to analyse association between parasite density and haematological parameters. A Bland-Altman plot was used to display graphical representation of agreement, bias and 95% CI between the two methods.

RESULTS

Of the 796 study participants, 437 (54.9%) were males and the mean age of the patients was 47months ± 31 (range, 3-120 months). At enrolment, the overall mean weight was 13.9kg ± 5.4 (range, 5-31.5kg). Further clinical and demographic details are shown on Table 1. Anaemia was detected in 336 (42.2%) with 37.7% (n = 300) being mildly anaemic while 4.5% (n = 36) were moderately anaemic. We found 7.3% (n=58) had leucocytosis, 84.8% (n=675) had normal WBC count while 7.9% (n=63) had leukopenia at enrolment irrespective of their age.

Table 1.
Demographic and Clinical Parameters at Enrolment

Characteristics	N/%
Gender n=796	
Male	54.9 (437)
Female	45.1 (359)
Age in months	
Mean ± (SD)	47 ± 31
Range	3-120
Weight (kg)	
Mean ± (SD)	13.9 ± 5.4
Range	5-31.5
Temperature (^{0}C)	
≥37.4	74.0 (589)
Mean ± SD	38.2 ± 1.1
Range	35.6-41.0
White Blood Cells (/mm^3)	
Mean ± SD	7807 ± 4888
Range	2700-110300
Leucocytosis (%)	7.3 (58)
Leukopenia (%)	7.9 (63)
Haematocrit [PCV (%)]	
Mean ± SD	30 ± 5
Range	15-47
Anaemia (%)	
Mild Anaemia	37.7 (300)
Moderate Anaemia	4.5 (36)
Parasite density (/µL) using assumed WBC	
Mean ± SD	36217 ± 51432
Geometric mean	15870
Range	40-611,600
Parasite density (/µL) using actual WBC	
Mean ± SD	33409 ± 49217
Geometric mean	14138.79
Range	41-500,000

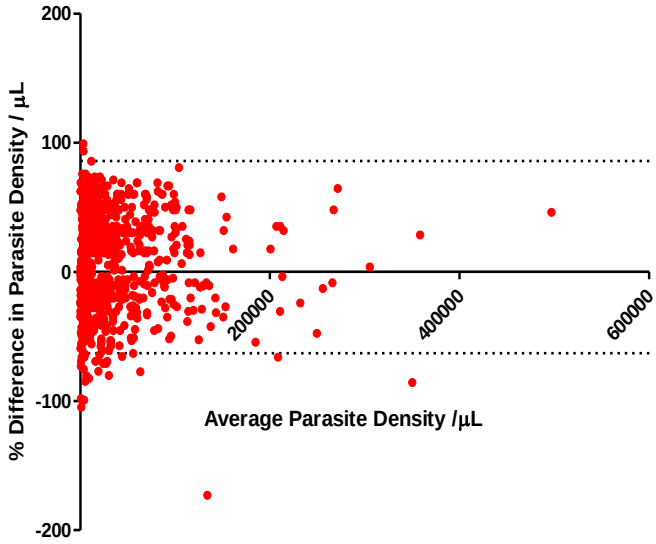

Figure 1:
Distribution of Parasite Density for All Enrollees

Table 2:
Demographics, Anthropometrics and Haematological Parameters of Participants

	Male	Female	All	P value
Age in months (mean ±SD)	47.4 ±32.1	46.7 ±31.1	47.1 ±31.6	0.761
Weight in kg (mean±SD)	14.2 ±5.4	13.5 ±5.4	13.9 ±5.4	0.076
Height in cm (mean±SD)	85.2 ±16.2	83.6 ±15.2	84.5 ±15.8	0.387
Haematocrit in % (mean±SD)	30.4 ±5.2	29.8 ±5.1	30.1 ±5.1	0.627
WBC in cell per mm^3 (mean±SD)	7731 ±5801	7900 ±3475	7807 ±4889	0.096

Figures 1 shows that Bland Altman plots and graphs for all the enrollee, the mean difference between parasite densities calculated from an assumed and actual WBC count were close to zero. The mean difference in parasite densities for all enrolee using both methods was within the 95% limits of agreement.

Table 2 shows the demographic, anthropometric and haematological parameters of all participants. There was no statistical difference in age, weight, height, haematocrit and WBC between both genders.

Figure 2 shows that enrollees that had leukopenia, there was an overestimation of parasite density when assumed WBC of 8,000/mm^3 was used in calculating the parasite density, for those that had leucocytosis there was an underestimation of the parasite density using the assumed while those with normal WBC count there was neither an overestimation nor an underestimation of the parasite density when the assumed WBC count was used

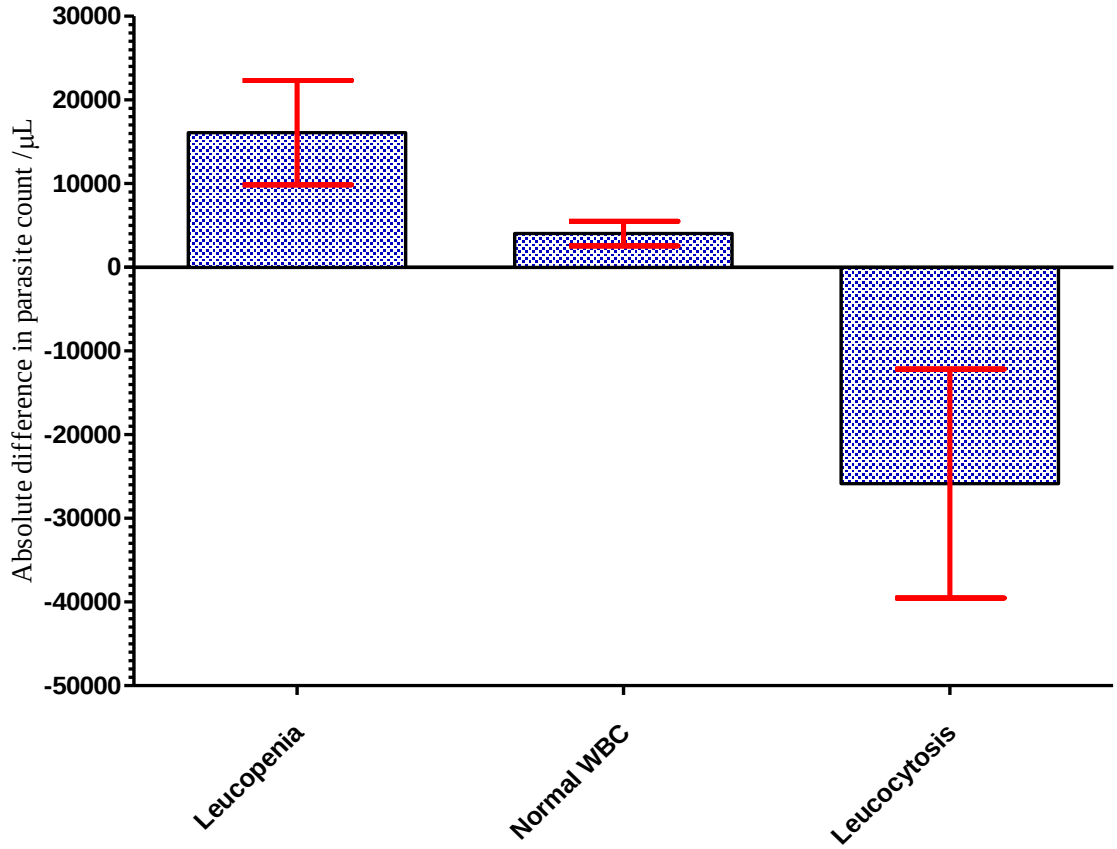

Figure 2:
Mean Values of Parasite Count by Level of WBC-for-Age

DISCUSSION

The importance of estimating malaria parasite density in patient management especially during clinical trials of antimalarial drug efficacy studies and epidemiological surveys cannot be over emphasized. In calculating this crucial parameter, ideally the patient's white blood cell count (WBCc) is used but because of its non-availability due to the need for specialised equipment, trained technologist, need for constant electricity involved, affordability and accessibility, prompt validation by constant servicing and maintenance this is usually not possible. For practical purposes, a reference assumed WBC count of 8,000/mm^3 has been arrived at (WHO, 2010a). During this retrospective study, we compared parasite densities obtained during antimalarial efficacy studies using the assumed WBC count and the study participant's WBC among children aged 3 – 120 months with a view to determining the appropriateness of the conventional WBC reference by evaluating it against individual WBC count. This was necessitated by previous reports that individuals of African ancestry have lower WBC count than their counterparts from other continents (Bain, 1996, Beutler and West, 2005, Urquhart *et al*., 2008).

In this study there was no significant difference in parasite density at enrolment between the two methods. In addition, Bland Altman plots showed that all enrollees irrespective of their ages, the mean difference between parasite densities calculated from an assumed and actual WBC count were close to zero in all cases. This is in agreement with what Laman *et al* reported in 2014 among Paupa New Guinea children with acute uncomplicated malaria (Laman *et al.,* 2014). However, Jeremiah and Uko, Haggaz *et al.,* Alves-Junior *et al.,* Adu-Gyasi *et al.,* and Bilal *et al.,* all showed that malaria parasite density calculated using assumed WBC was significantly higher than using actual WBC (Jeremiah and Uko, 2007, Haggaz *et al.,* 2014, Alves-Junior *et al.,* 2014, Adu-Gyasi *et al.,* 2015, Bilal *et al*., 2015). This may not be unrelated that the WBC count was calculated manually which was alluded by Jeremiah and Uko(Jeremiah and Uko, 2007) which may provide a wide margin of error unlike in our study which used automated haematology analyser. There is also a possibility that most of their study participants were leukopenic.

It is noteworthy, that there was no statistically significant difference when we compared the parasite densities obtained with the individual WBC count and the conventionally used assumed WBC count of 8,000/mm^3 (WHO, 2010a, WHO, 2010b), WBC for both age groups and total WBC. This is not surprising because Nigerian children constituted >50% of the study population used for arriving at the assumed WBC of 8,000/mm^3 by World Health Organization. Some earlier studies which reported significant difference between using assumed WBC count and actual WBC count in calculating parasite density might have looked at adult population rather than in children.(Haggaz *et al.,* 2014)

Haematological changes are a well-known feature of malarial infections (Adedapo *et al.,* 2007). These abnormalities are considered a hallmark of malaria and are reported to be most pronounced in *P. falciparum* infections (Kotepui *et al.,* 2015). These changes involve major cell lines including red blood cells (RBC), leukocytes and thrombocytes

(Erhart *et al.,* 2004). Haematological changes in the course of a malaria infection, such as anaemia, thrombocytopenia and leucocytosis or leucopoenia are well recognized. Anaemia has frequently been associated with malaria as result of haemolysis, decreased erythropoiesis and splenic sequestration (Trape, 1985). In this study, 42.2% of the patients presented with anaemia at enrolment compared with 44% recorded by Adedapo *et al* (Adedapo *et al.,* 2007). The difference in percentage is statistically insignificant.

Leucocytosis was less commonly observed in this study than leucopenia (7.3% vs 7.9%), as was reported by Sharma *et al* (1992) and Adedapo et (2007). Leucocytosis has been reported in acute Plasmodium infection (Stein, 1985, Ladhani *et al.,* 2002) and may suggest co-existing viral infection particularly with the presence of atypical lymphocytes (Eriksson *et al.,* 1989) and more so in children among whom viral infection may be common. Some cases of leukemoid reaction following malaria had also been documented in the past.(Riley and Robins, 1949, Soe *et al.,* 1991, Unnati *et al.,* 2009). However, some other studies had reported leucopenia among malarial patients (Arya and Prasad, 1989, Bhatnagar *et al.,* 2005, Liu, 2016).

Study participants with these outliers when calculating parasite densities using assumed WBC count of 8,000/mm^3 either underestimate or overestimate parasite densities respectively. In calculating parasite densities, the actual WBC count is most ideal, but it is far from reality. From this study, the outliers are in the minority hence, it can then be concluded that using an assumed WBC count of 8,000/mm^3 in calculating parasite densities is appropriate for this environment.

In conclusion, this study shows that there is no statistically significant difference in parasite density at enrolment between the two methods. Bland Altman plots also showed that for all study participants irrespective of their ages, the mean difference between parasite densities calculated from an assumed and actual WBC count were close to zero in all cases. In conclusion, using an assumed WBC count of 8,000/mm^3 in calculating parasite density should be considered appropriate in children aged 3 months to 120 months for in South western Nigeria.

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