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(RESEARCH ARTICLE)

Malaria parasite density estimation using actual and assumed white blood cell counts in *Plasmodium falciparum*-infected patients in Dak Lak province, Vietnam

Quang Hong Huynh [1,*], Khanh Van Chau [1], Hien Thanh Pham[1], Hien Tinh Tran [2], Nhien Thuy Thanh Nguyen [2] and Hong Duc Nguyen [1]

[1] *Institute of Malariology, Parasitology and Entomology Quy Nhon, Binh Dinh, Vietnam.*
[2] *Oxford University Clinical Research Unit (OUCRU), Vietnam.*

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Abstract

Estimating malaria parasite density is necessary for disease management, clinical trials and drug efficacy studies. This study was conducted to compare the malaria parasite density among patients using actual white blood cell (WBC) and the assumed WBC counts (8.0 ×109/l). A cross-sectional study was conducted in Dak Lak, Vietnam. WBC and asexual malaria parasite counts were performed on blood films. Eighty patients were enrolled. The mean (±SD) of WBCs was 5.84±1.63 ×109/l. The median (IQR) of parasite density using the assumed WBCs (8000 cells/µl) (11057.50 [2388.50-34210.75] rings/µl) was significantly higher than that estimation based on the actual WBC count (6898.61 [1892.12-24623.10] rings/µl) (p<0.001). Therefore, this study recommended the use of actual WBC count to estimate malaria parasite density in *P. falciparum* – infected patients in Dak Lak, Vietnam.

Keywords: Malaria; Parasite density; Assumed white blood cell; Actual white blood cell

1. Introduction

Malaria continues to be the leading cause of morbidity and mortality worldwide. In 2018, an estimated 228 million cases of malaria occurred worldwide. World Health Organization (WHO) South-East Asia Region accounted for 3.4% of the cases. Moreover, there were an estimated 405.000 deaths from malaria globally [1]. In the Greater Mekong Subregion (GMS) of Southeast Asia, the tropical and subtropical climate is highly conducive to malaria transmission [2]. Despite that several countries in this region are moving toward malaria elimination, malaria remains highly prevalent along international borders, where hill tribes and ethnic minorities reside.

Estimating malaria parasite density (number of parasites per µl of blood) is necessary for disease management, clinical trials and drug efficacy studies [3]. It is the only method that is widely and practically available for quantifying malaria parasite density by comparing the ratio of counted parasites within a given number of microscopic fields, against either counted white blood cells (WBCs) or counted red blood cells (RBCs) within those same fields, and then multiplying that ratio by either the measured or estimated/assumed density of the patient's WBCs or RBCs [4, 5].

Due to the lack of laboratory facilities in most malaria-endemic countries to quantify patient's WBCs, WHO recommended the use of an assumed WBC value of 8,000/µl [3], which was based on a study with a Nigerian population with a large proportion of children younger than five years [6]. The use of assumed WBCs rather than actual WBCs might lead to over-estimation or under-estimation of the parasite density in the case of other infections, or the contrary, depending on several factors, such as severity of malaria and other infections and the acceptable reference value for WBCs in the specific settings [7, 8]. Recent studies found that estimating malaria parasite densities using this WBC value

* Corresponding author: Quang Hong Huynh
Institute of Malariology, Parasitology and Entomology Quy Nhon, Binh Dinh, Vietnam.

yielded results that were inconsistent with the actual observed malaria parasite density of patients in some areas [1, 9, 10, 11]

Therefore, this study was conducted to compare the parasite densities calculated with the actual and assumed WBCs of uncomplicated malaria patients in Dak Lak, Vietnam to access the impact of using assumed WBCs to estimate parasitemia of *P. falciparum* infection.

2. Material and methods

2.1. Study population

A cross-sectional study was conducted in Dak Lak, Vietnam from August 2019 to April 2020. After signing an informed consent, febrile (temperature ≥37.5 °C) patient was screened and clinically examined for malaria. Malaria screening was performed using microscopy. Patients with confirmed diagnosis of malaria were treated with Artesunate/Mefloquin. Demographic and clinical characteristics of patients were recorded. WBCs count was determined by an automated cell counter (Abacus 380, Hungary).

The standard procedure used for the diagnosis of malaria was the examination of thick and thin blood smears for malaria parasite by with Wright and Giemsa staining and finding organism under light microscopy by laboratorists. After the detection of malarial parasites, thin smears were used to identify the parasite species and thick smears were used to identify parasite density.

Parasite density, expressed as the number of asexual parasites per µl of blood, will be calculated by dividing the number of parasites by the number of white blood cells counted and then multiplying by an assumed white blood cell density (8000 per µl) or the actual white blood cells count.

$$\text{Parasite density (per µl)} = \frac{\text{Number of parasites counted} \times (\text{WBCs/µl})}{\text{Number of leukocytes counted}}$$

2.2. Statistical analysis

Parasite densities estimated with assumed and actual white blood cell counts followed a skewed distribution. Therefore, the differences in these two figures were analyzed by using Wilcoxon signed rank test. Data analysis was performed using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Between August 2019 and April 2020, 80 patients who were infected with *P. falciparum*, were recruited to this study. The basic characteristics of patients are shown in Table 1. The age of patients ranged from 14 to 57 years. There was a male predominance, with a male to female ratio of 77:3. Most patients represented at the hospital with fever, the mean temperature was 39 (±0.6) degrees Celsius. The number of WBCs ranged from 2120 to 10200 cells/µl while the mean WBCs was 5840 cells/µl.

Table 1 Characteristics of patients

Characteristics	Mean (SD)	Range
Age (year)	33 (±10)	14-57
Sex (male:female ratio)	77:3	
Height (cm)	163 (±5)	153-172
Weight (kg)	58 (±5)	45-68
Temperature (^oC)	39 (±0.6)	37-41
Hemoglobin (g/dL)	128.65 (±15.40)	90-166
WBC count ($\times 10^3$/µl)	5.84 (±1.63)	2.12-10.20
Platelet count ($\times 10^3$/µl)	132.59 (±64.19)	28-320

The median (IQR) of parasite density using the assumed WBCs (8000 cells/µl) was significantly higher than that estimation based on the actual WBC count (11057.50 [2388.50-34210.75] vs. 6898.61 [1892.12-24623.10] rings/µl, p<0.001); figure 1. The two parasitemias were positively correlated (r=0.938, p<0.001); figure 2.

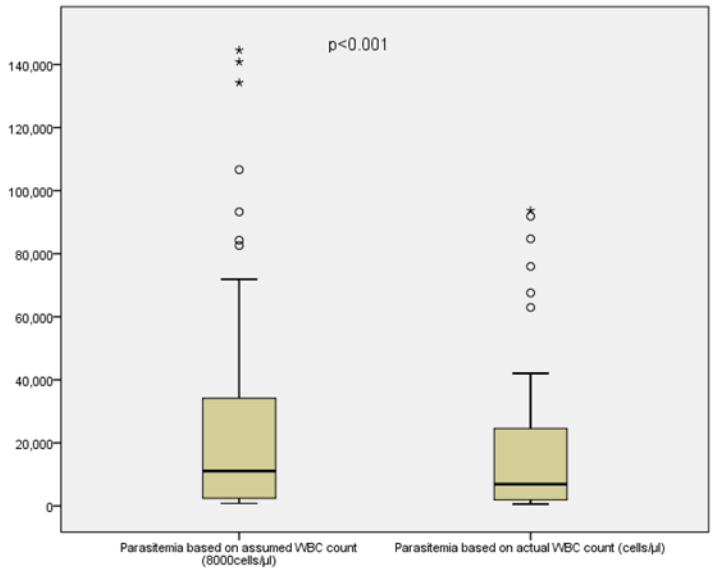

Figure 1 Comparison of parasite density using actual and assumed WBC counts of 8000 cells/µl

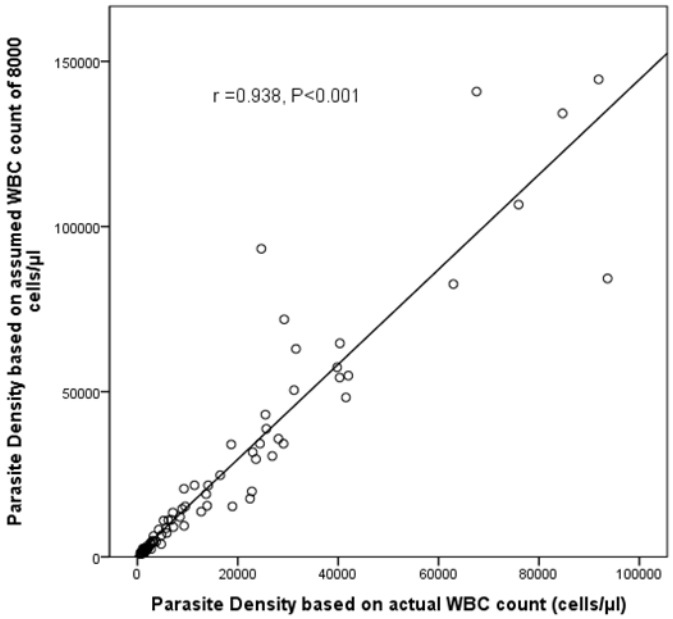

Figure 2 Correlation between parasite densities using actual and assumed WBC counts of 8000 cells/µl.

The main finding of this study was that the parasite density, which was calculated using the assumed WBC counts of 8000 cells/µl, was significantly higher than that calculated using the actual number of WBCs. This is related to a previous study in Nigerian children which showed that parasite density estimation using the assumed WBCs count of 8.0×10^9/L results in over-estimation of the parasite burden. The WBCs of individual patients should always be estimated when parasite density is required [1]. Another study conducted in pregnant women at central Sudan concluded that the estimated parasite density using actual WBC counts was significantly lower than the parasite density estimated using assumed WBC counts. Therefore, it is recommended to use the patient's actual WBC count in the estimation of the parasite density [12].

In addition, it was found that WBC counts were not significantly different between patients of different gender, axillary temperature, and body mass index levels, whereas they were significantly different between age groups of patients and the time points of measurement. [13].

4. Conclusion

The estimated parasite density using actual WBC counts was significantly lower than that estimated using assumed WBCs (8000 cells/µl) in patients in Dak Lak, Vietnam. Therefore, it is recommended that actual WBCs are used in the estimation of parasite density.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared that they have no conflicts of interest.

Statement of ethical approval

The research protocol was reviewed by the Institutional Review Board of the Institute of Malariology, Parasitology and Entomology Quy Nhon.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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Author's short biography

Quang Hong Huynh

Academic degree: MD, MSc, Mpharac, PhD, Professor
Research area: Tropical Diseases Research and Treatment
Years of experience: 22 years

Khanh Van Chau

Academic degree: Medical doctor.
Research area: Malariology & Tropical diseases.
Years of experience: 2 years

Hien Tinh Tran

Academic degree: Medical doctor, PhD, Professor.
Research area: Infectious Diseases and Global Health
Years of experience: 45 years

Nhien Thuy Thanh Nguyen

Academic degree: Doctor of molecular Biology, PhD, post-docs.
Research area: Medical Science & Tropical diseases.
Years of experience: 18 years

Hien Thanh Pham

Academic degree: Chemist and Pharmacist.
Research area: Malaria Pharmaceutical Unit
Years of experience: 17 years

Hong Duc Nguyen

Academic degree: Medical doctor, MSc in Parasitology
Research area: Malariology, Parasitology & Tropical diseases.
Years of experience: 24 years