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MAJOR ARTICLE

Comparative Analysis of Initial Full Blood Count Parameters in Adults Infected With *Plasmodium falciparum* for Classification of Disease Severity and Previous Exposure Across Endemic (Gabon) and Nonendemic (Germany) Settings

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Background. The clinical presentation of individuals infected with *Plasmodium falciparum* is exceptionally diverse, ranging from asymptomatic parasitemia to life-threatening disease. Frequent previous exposure to *Plasmodium* spp results in partial protection from severe disease; however, this protection wanes in individuals emigrating from holoendemic regions, and there are currently no reliable biomarkers that accurately indicate this semi-immunity.

Methods. Data were analyzed from 1392 adults infected with *P falciparum* in Gabon and Germany. Full blood count parameters and ratios were evaluated individually and as a combined ensemble-based machine learning classifier to predict disease severity, ranging from asymptomatic infection to severe malaria. As a secondary objective, the influence of previous exposure to *Plasmodium* spp was assessed.

Results. Comparing asymptomatic parasitemia with uncomplicated malaria in Gabonese and comparing uncomplicated with severe malaria in German patients revealed significantly lower platelet counts (218 vs 150 $\times 10^3$/μL, $P < .0001$; 85 vs 40 $\times 10^3$/μL, $P < .0001$, respectively) and higher neutrophil counts (2.32 vs 2.57 $\times 10^3$/μL, $P = .0037$; 3.08 vs 4.49 $\times 10^3$/μL, $P < .0001$) in those with greater infection severity. The machine learning classifier outperformed single parameters in differentiating infection severity in both comparisons (area under the receiver operating characteristic curve, 0.94 and 0.84). Lymphocyte and monocyte counts showed a pattern that follows the level of previous malaria exposure, with lower cell counts in naive vs previously exposed patients, regardless of infection severity.

Conclusions. The value of simple full blood count parameters for classification of *P falciparum* infection severity and previous exposure is considerable. The accuracy can be increased by integrating individual parameters into a joint machine learning model.

Keywords. asymptomatic parasitemia; full blood count; machine learning; *Plasmodium falciparum*; severe malaria.

Malaria causes considerable morbidity and mortality, especially in sub-Saharan Africa [1]. Its clinical presentation is exceptionally diverse, ranging from asymptomatic to life-threatening infection. Previous exposure to *Plasmodium* spp is strongly associated with

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prognosis and outcome in malaria, with repeated infections leading to the development of semi-immunity and partial protection from severe disease [2]. However, semi-immunity wanes in individuals who emigrate from endemic regions, a patient group frequently traveling back to visit friends and relatives (VFR), thereby carrying a high risk of *Plasmodium* infection [3, 4]. As the epidemiology of malaria changes (eg, in light of current elimination efforts), persons in previously holoendemic regions might also be affected by waning semi-immunity and consequently an increased risk of severe disease [5].

To date, no consensus exists concerning the duration of semi-immunity or its decline. Moreover, no robust biomarkers exist that properly reflect antiparasite or clinical semi-immunity, which could help clinicians anticipate the risk profiles of patients with malaria. Severe malaria, characterized by organ dysfunction that often requires intensive care treatment, is commonly defined by standardized criteria [6]. However, treatment decisions are far less clear for patients at medium or low risk (eg, with regard to the possibility of outpatient treatment in VFR patients) [7, 8]. Similarly, there is ongoing debate about the definition and necessity of treatment of asymptomatic parasitemia [9, 10].

The differential blood count from routine hematologic analysis, readily available in most clinical settings, can provide information about the severity of a range of diseases. Changes in neutrophil to lymphocyte count ratio (NLCR) and monocyte to lymphocyte count ratio (MLCR) have especially been described as nonspecific markers of inflammation in the context of various infections [11–14]. In malariology, higher NLCR and MLCR were observed in patients with more severe disease as compared with controls in a nonendemic setting [15, 16]. Machine learning–based tools for laboratory and clinical markers have recently been tested to assist clinical decision making for treating malaria in returning travelers and in children in endemic areas [17, 18]. However, they primarily focus on detecting infections rather than differentiating various severity levels. Moreover, comparison and integration of data from nonendemic and endemic settings into a single analysis are lacking.

The aim of this multicenter cross-sectional study is to analyze the value of full blood count (FBC) parameters upon initial presentation for assessing disease severity in individuals infected with *Plasmodium falciparum* (*Pf*) as a potential aid for early clinical decision making. Furthermore, the influence of previous malaria exposure on these parameters is assessed.

METHODS

A pooled secondary analysis was performed with clinical and laboratory data from 9 prospective cohort studies conducted at Centre de Recherches Médicales de Lambaréné (CERMEL) in Gabon and Charité University Hospital in Berlin, Germany, and 1 retrospective study at Bernhard-Nocht-Institute for Tropical Medicine (BNITM), in Hamburg, Germany, between 2013 and 2023. All studies were approved by their ethics committees.

The primary objective was to assess the potential of FBC parameters for classification of disease severity in individuals infected with *Pf* in endemic and nonendemic settings. Initial routine FBCs were evaluated as single parameters and as a combined classifier to predict disease severity at first clinical presentation, ranging from asymptomatic infection to severe malaria. As a secondary objective, the influence of preexisting semi-immunity on these parameters was assessed.

Study Participants

Participants at CERMEL were recruited in the Moyen-Ogooué province of Gabon, a hyperendemic region with perennial *Pf* transmission [19]. Patients in Berlin were recruited at Charité University Hospital and patients in Hamburg from the database of the Center of Tropical Medicine at BNITM.

Individuals were eligible for inclusion if they fulfilled the following criteria: age ≥18 years, microscopically confirmed asexual *Pf* infection, and blood draw with FBC ≤24 hours after initiation of antimalarial treatment. In addition, there was a subset of symptomatic patients in Gabon (n = 508) whose diagnosis of uncomplicated malaria was established by a rapid diagnostic test based on PfHRP2 (*Pf* histidine-rich protein 2). All these patients denied previous malaria episodes in the last 28 days. Individuals were excluded in case of known liver disease, hematologic disease, immunodeficiencies, pregnancy, or breastfeeding. Asymptomatic infection was defined as asexual parasitemia without symptoms 7 days before and after diagnosis. Uncomplicated and severe malaria was defined according to World Health Organization (WHO) criteria [6]. All patients were treated according to the respective study protocols and Gabonese or German guidelines.

Infection severity was analyzed by 2 comparative analyses: asymptomatic parasitemia vs uncomplicated malaria in Gabon and uncomplicated vs severe malaria in Germany. For analyzing the influence of semi-immunity, previous malaria exposure was used as a proxy, as determined from participants' medical histories and current residences. Patients were classified as "endemic" with ongoing exposure to *Pf* in Gabon; "VFR," when born and raised in endemic regions but now living in nonendemic regions for >12 months; or "naive," with first-time *Pf* infection. VFR patients with migration ≤12 months prior were excluded to ensure clear differentiation from endemic patients. We then selected 2 patient subgroups with comparable infection severity: uncomplicated and severe malaria. Within each subgroup we compared patients with different degrees of previous exposure: endemic, VFR, and naive for uncomplicated malaria; VFR and naive for severe malaria.

Data and Statistical Analysis

Data were collected from the earliest time point at or after diagnosis and included demographic data, medical history, asexual parasitemia, and routine FBC parameters (platelet, total white blood cell, lymphocyte, monocyte, and neutrophil counts). Red blood cells (RBCs), hemoglobin, and hematocrit were excluded to avoid confounding with WHO criteria of severe malaria (circular reasoning). Eosinophils and basophils were excluded to avoid confounding by helminth coinfections. NLCR, MLCR, and platelet to lymphocyte count ratio (PLCR) were calculated by the absolute counts. Parasitologic examinations were performed with thick and thin blood smears, and parasitemia was calculated as follows: % RBC × RBC/μL. All parasitologic and hematologic examinations were performed by accredited laboratories at CERMEL, Charité, or BNITM.

Statistical analysis and machine learning were conducted in Python version 3.10. As most variables were not normally distributed, nonparametric tests were performed with *scipy*, including Mann-Whitney *U* tests for pairwise comparisons and Kruskal-Wallis *H* tests for comparisons with >2 groups. Post hoc pairwise tests were conducted with Dunn test implementation from *scikit-posthoc*. A significance level of $\alpha = .05$ was adopted. Random forest models were trained on an age-, sex-, and severity-balanced dataset through a leave-one-out cross-validation approach (Supplementary Methods).

RESULTS

Participant Characteristics

Overall, 3648 individuals were screened from the included studies and databases. Patients were excluded if any of the following applied: age <18 years (n = 1783), missing FBC (n = 264), non-*Pf* monoinfection (n = 100), coinfection or concomitant disease with known effect on FBC (n= 71), pretreatment (n = 7), recrudescence (n = 7) or gametocyte only (n = 1), double inclusion (n = 11), and pregnant or breastfeeding women (n = 12), resulting in 1392 evaluable cases. Of these, 723 were recruited in Gabon and 669 in Germany. No study in Gabon included patients with severe malaria and, conversely, no asymptomatic cases were identified in Germany.

Baseline characteristics are presented in Table 1. There was a predominance of male participants overall and in all subgroups. The median age ranged from 26 years in asymptomatic individuals to 52 years in naive travelers with severe malaria, and initial parasitemia ranged from a median 483 to 475 500 parasites/μL, respectively.

FBC Parameters as Markers for Infection Severity

Analysis of FBC parameters according to infection severity revealed notable differences in the endemic setting (asymptomatic parasitemia vs uncomplicated malaria) and nonendemic setting (uncomplicated vs severe malaria; Figure 1, Supplementary Table 1).

Table 1. Baseline Characteristics of Study Participants, Categorized According to Level of Endemicity, Disease Severity, and Previous Malaria Exposure

	Endemic Setting[a]		Nonendemic Setting[b]					
	Asymptomatic Infection	Uncomplicated Infection		Severe Infection			Total
		VFR	Naive	VFR	Naive		
No.	117	425	138	50	56		1392
Male	75 (64)	336 (78)	77 (56)	40 (80)	41 (71)		871 (63)
Age, y	26 (20–41)	41 (34–49)	31 (24–42)	48 (42–53)	52 (41–64)		36 (25–49)
Initial parasitemia, parasites/μL	483 (255–1025)	26 000 (21 500–77 085)	23 950 (14 025–46 550)	340 500 (134 250–479 125)	475 500 (207 000–747 450)		23 750 (4325–74 800)

Data are presented as No. (%) or median (IQR).

Abbreviation: VFR, visit friends and relatives.

[a]Previous exposure based on living in an endemic area.

[b]Previous exposure based on VFR (visiting patients of African descent living in nonendemic regions outside of Africa) or naive status (first-time *Plasmodium falciparum* infection).

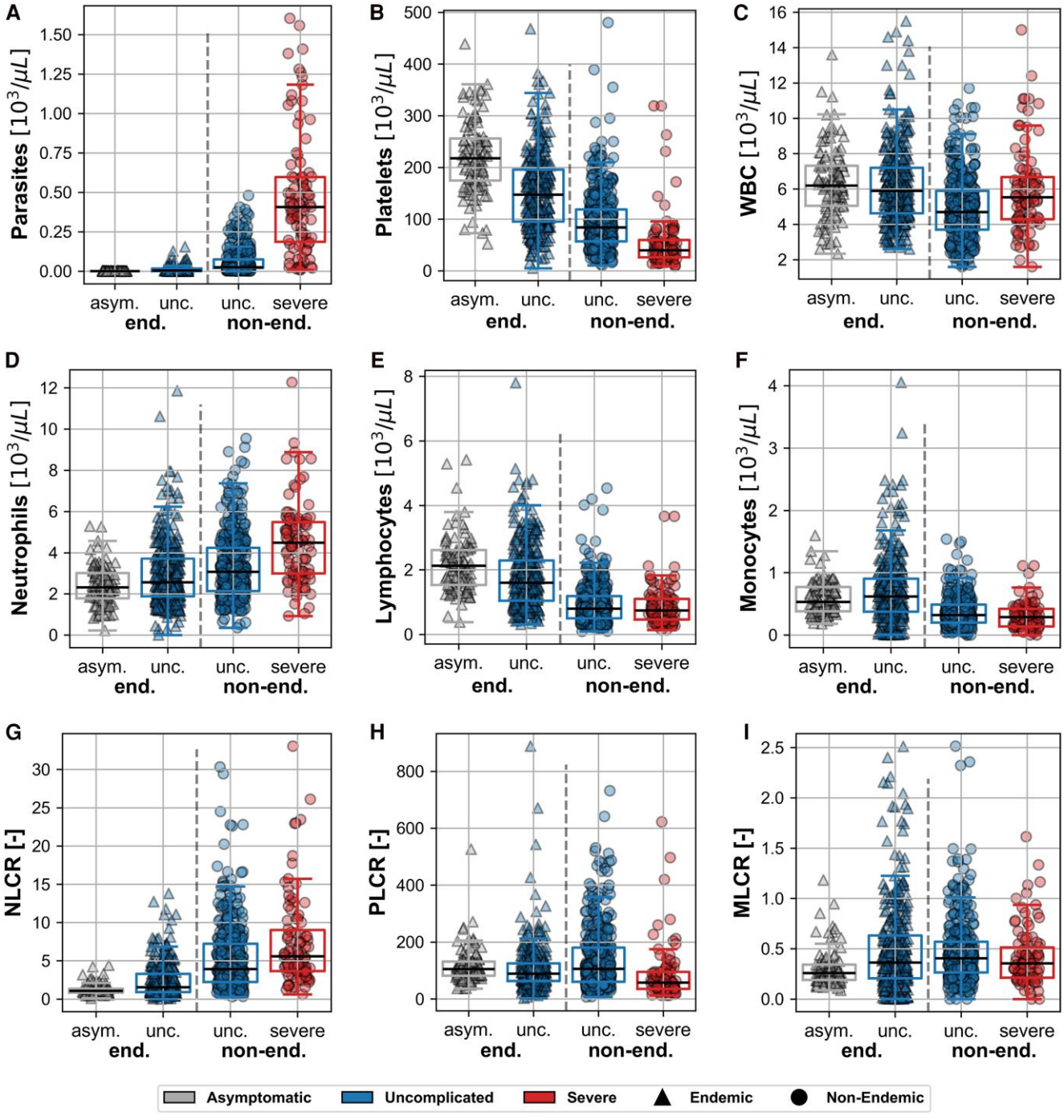

Figure 1. Distribution of parasitemia (*A*), full blood cell counts (*B–F*), and blood cell ratios (*G–I*) in individuals with different severity of *Plasmodium falciparum* infection: asymptomatic parasitemia (asym) and uncomplicated malaria (unc) in the endemic setting (end) and uncomplicated malaria and severe malaria in the nonendemic setting (non-end). Scatter points indicate individual patient values; line, box, and whiskers reflect the median, IQR, and 1.5 times the IQR, respectively. MLCR, monocyte to lymphocyte count ratio; NLCR, neutrophil to lymphocyte count ratio; PLCR, platelet to lymphocyte count ratio; WBC, white blood cells.

Platelet counts differed markedly between groups, with lower counts correlating with higher severity (endemic: 218 vs 150 × 10^3/μL for asymptomatic parasitemia vs uncomplicated malaria; nonendemic: 85 vs 40 × 10^3/μL for uncomplicated vs severe malaria, respectively). Neutrophils showed an opposite correlation with infection severity: higher counts in more severe cases in both settings (endemic: 2.32 vs 2.57 × 10^3/μL; nonendemic: 3.08 vs 4.49 × 10^3/μL). Conversely, lymphocyte counts were lower in endemic patients with uncomplicated malaria as compared with individuals with asymptomatic parasitemia (1.62 vs 2.16 × 10^3/μL) but were

similar between nonendemic patients with uncomplicated and severe malaria. The cell count ratios followed these trends, with lower PLCR and higher NLCR values in patients with more severe infection in both endemicity settings. In contrast to these clearly severity-associated markers, monocyte counts and the MLCR showed no clear trend among the severity subgroups. Overall, white blood cell counts did not correlate with infection severity in the endemic setting but were lower in uncomplicated vs severe malaria cases in the nonendemic setting (4.8 vs 5.5 $\times$ 10^3/μL; Figure 1, Supplementary Table 1).

Notably, FBC parameters could be leveraged to indicate disease severity: a platelet count $\leq 189 \times 10^3$/μL was the best criterion for uncomplicated malaria in the endemic setting (positive likelihood ratio [LR+], 2.34; LR–, 0.41; Supplementary Table 2). The area under the receiver operating characteristic curve (AUROC) for features ranged from 0.76 for platelets to 0.38 for MLCR (Figure 2*A*). In the nonendemic setting, platelets $\leq 61 \times 10^3$/μL most accurately discriminated patients with severe vs uncomplicated disease (LR+, 2.82; LR–, 0.29; Supplementary Table 3). AUROC values ranged from 0.79 for platelets to 0.54 for lymphocytes (Figure 2*B*).

Severity Classification by Machine Learning

To investigate whether the diagnostic capability of FBC values can be improved by combining the parameters, we developed a random forest model for severity classification, with an age-, sex-, and severity-balanced subset of patients in both settings (Supplementary Table 4). Indeed, the classifier could distinguish between asymptomatic parasitemia and uncomplicated malaria in the endemic setting with an AUROC of 0.94 (Figure 2*A* and 2*C*) and between uncomplicated and severe malaria in the nonendemic setting with an AUROC of 0.84 (Figure 2*B* and 2*D*), clearly outperforming individual parameters. In both settings, platelets were identified as the key model feature (Figure 2*E* and 2*F*), with higher values associated with lower severity. Lower MLCR and NLCR values and higher lymphocyte counts were significant for the model classifying asymptomatic infection (Figure 2*E*), whereas higher PLCR and lower neutrophil counts were important factors for classifying uncomplicated malaria (Figure 2*F*). The endemic classifier markedly outperforms parasitemia as a single parameter (AUROC, 0.94 vs 0.75), whereas parasitemia slightly outperforms the FBC-based classifier in the nonendemic setting (AUROC, 0.9 vs 0.84). Consequently, training a machine learning classifier on FBC and parasitemia significantly improved the model performance up to an AUROC of 0.95 in both settings (Figure 2*A* and 2*B*), while addition of RBCs did not result in further improvement (Supplementary Figure 1).

FBC Parameters as Markers of Previous Malaria Exposure

Even among individuals with similar severity (uncomplicated malaria; blue patient subgroups in Figure 1) some parameters, such as platelets, differed considerably between those in the endemic and nonendemic settings. We therefore systematically analyzed the influence of previous malaria exposure (endemic, VFR, naive) on FBC parameters within subgroups of patients with similar severity (uncomplicated or severe malaria).

The distribution of the strongest severity markers, platelets and neutrophils, generally followed the median parasite counts in the respective patient subgroups, independent of the degree of previous exposure (Figure 3, Supplementary Table 5). Accordingly, in patients with uncomplicated malaria, platelet counts were lowest in VFR patients, corresponding to the highest median parasitemia in this group, lower than in naive patients. Similarly, neutrophil counts were highest in VFR patients. The same trend was observed in patients with severe malaria. In contrast, lymphocyte and monocyte counts, which did not clearly correlate with infection severity, showed a distinct pattern that followed the degree of previous malaria exposure, with lower cell counts being associated with less preexposure in both severity groups. In uncomplicated malaria, patients from endemic areas had threefold higher monocyte and lymphocyte counts than naive patients, with VFR patients falling between the groups (monocytes: 0.62 vs 0.36 vs 0.23 $\times$ 10^3/μL for endemic, VFR, and naive patients, respectively; lymphocytes: 1.61 vs 0.89 vs 0.54 $\times$ 10^3/μL). A similar pattern, though inverse, was observed for the NLCR (1.55 vs 3.54 vs 5.6 $\times$ 10^3/μL), with the same trend in severe malaria.

DISCUSSION

Routine hematologic parameters have been shown to indicate disease severity in various conditions [11–13]. Here, we leveraged routine FBC measurements, usually one of the first laboratory tests performed upon presentation, to analyze differences in *Pf* infection severity as a potential aid for early treatment decisions. Examining 8 parameters not included in the WHO definition of severe malaria (ie, excluding hemoglobin and erythrocyte counts), we observed that platelets, neutrophils, PLCR, and NLCR can be used to discriminate between patients with varying disease severity in endemic and nonendemic settings. In both, platelets emerged as the most potent single discriminatory parameter, performing similarly well to peripheral parasitemia for discrimination of asymptomatic vs symptomatic malaria in endemic individuals. In contrast, monocytes and lymphocytes were clearly associated with previous exposure, not severity.

Thrombocytopenia is common in malaria and has been identified as a risk factor for adverse outcomes [20]. Underlying mechanisms include peripheral destruction, bone marrow alterations, and removal of platelets in the spleen [21]. While exerting direct antiplasmodial action through platelet factor 4 [22], platelets contribute to systemic and vascular inflammation and micro-occlusion [23]. The role of neutrophils in malaria

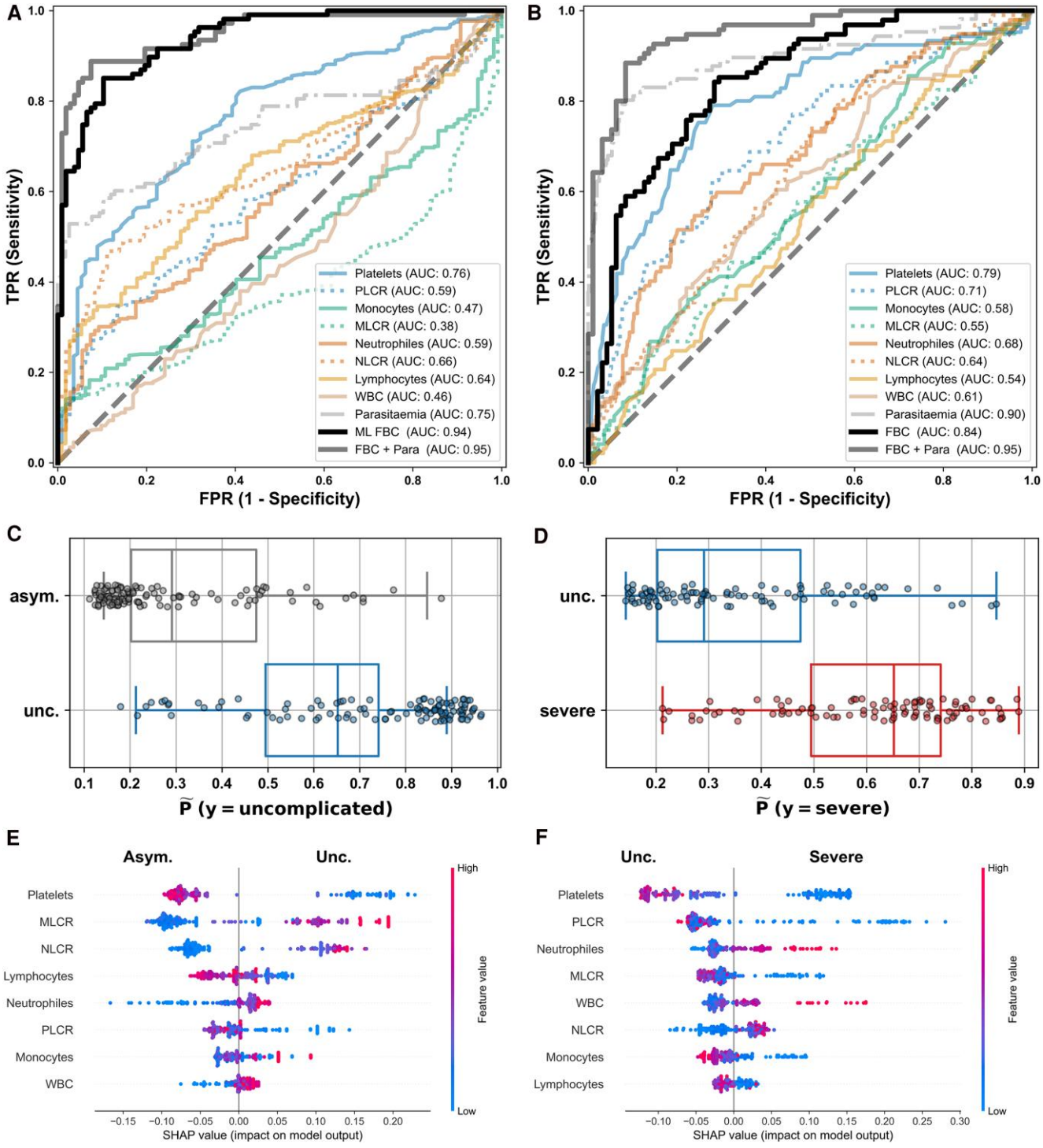

Figure 2. Classifier evaluation in distinguishing asymptomatic *Plasmodium falciparum* infection from uncomplicated malaria in endemic participants (*A*, *C*, *E*) and uncomplicated from severe malaria in nonendemic participants (*B*, *D*, *F*). Both classifier panels are structured in the same way. *A* and *B*, The prediction performance of random forest–based classifiers and individual parameters (FBCs, their ratios, and *Plasmodium* parasitemia in the blood) in both investigated classes. To ensure consistent ROC curve comparison between nonendemic and endemic settings, the same sorting order was used for both. Although this could produce AUROC values <0.5 (indicating better prediction for the negative class), the sorting choice was solely for visualization and did not affect the calculations. *C* and *D*, Predicted probabilities of machine learning models. Points indicate individual patient values; line, box, and whiskers reflect the median, IQR, and 1.5 times the IQR, respectively. *E* and *F*, SHAP values, indicating the impact of the features on the final model decision. ROC curves of machine learning classifiers were averaged over the performance in validation data of the cross validation. asym, asymptomatic; AUC, area under the curve; AUROC, area under the receiver operating characteristic curve; FBC, full blood count; ML, machine learning; MLCR, monocyte to lymphocyte count ratio; NLCR, neutrophil to lymphocyte count ratio; Para, parasitemia; PLCR, platelet to lymphocyte count ratio; ROC, receiver operating characteristic; SHAP, SHapley additive exPlanations; TPR, true positive rates; unc, uncomplicated; WBC, white blood cells.

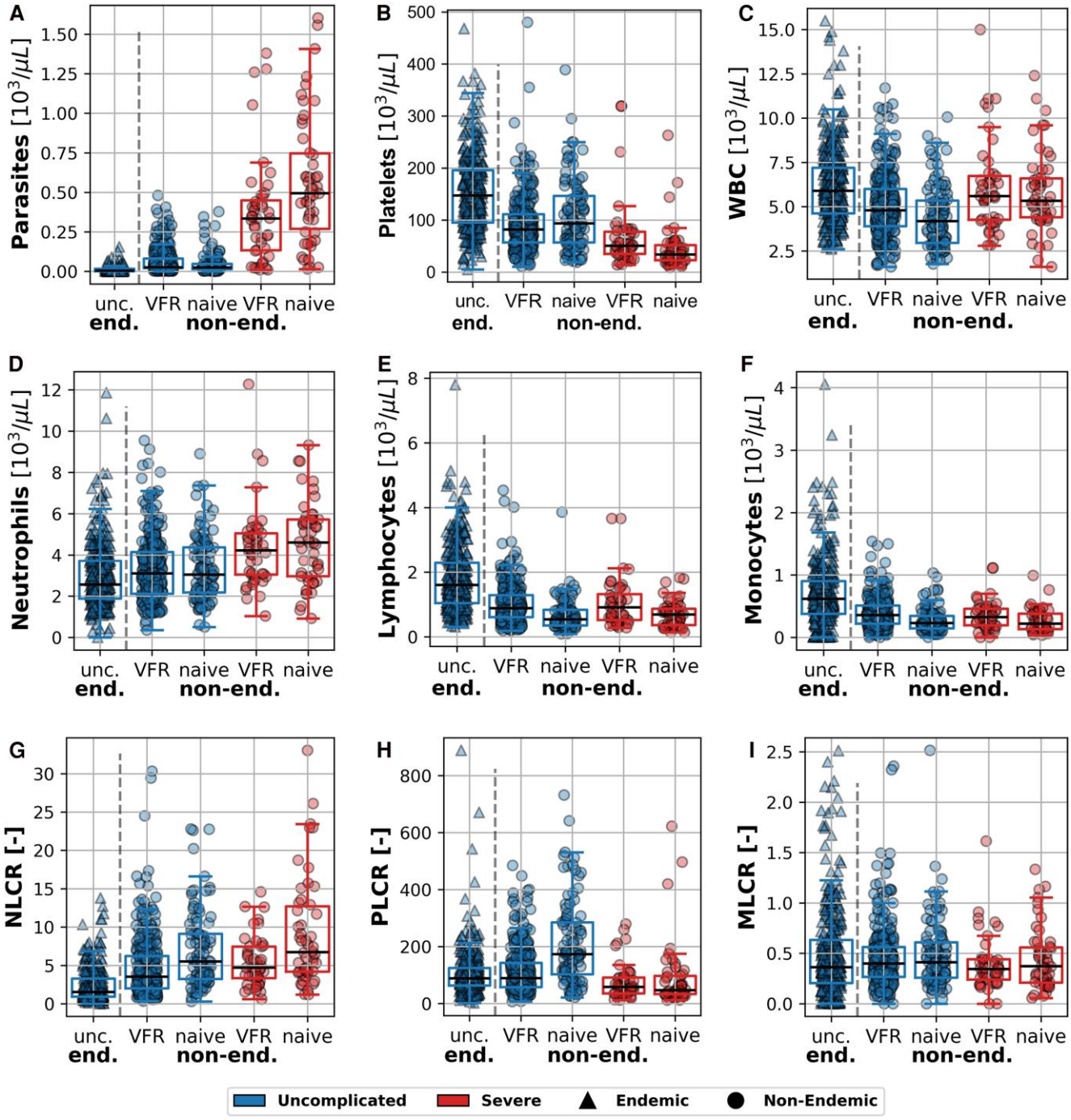

Figure 3. Distribution of parasitemia (*A*), full blood cell counts (*B–F*), and blood cell ratios (*G–I*) with various previous malaria exposure. Uncomplicated malaria cases are subdivided into endemic, VFR, and naive patients and severe malaria cases into VFR and naive patients. Scatter points indicate individual patient values; line, box, and whiskers reflect the median, IQR, and 1.5 times the IQR, respectively. end, endemic; MLCR, monocyte to lymphocyte count ratio; NLCR, neutrophil to lymphocyte count ratio; non-end, nonendemic; PLCR, platelet to lymphocyte count ratio; unc, uncomplicated; VFR, visit friends and relatives.

is less clear. Activated neutrophils support parasite elimination through phagocytosis and the production of reactive oxygen species and neutrophil extracellular traps, but they also contribute to damaging endothelium, increasing inflammation, and promoting parasite adhesion. Several studies have shown an association between neutrophilia and malaria severity [24, 25]. In our study, neutrophils were markedly higher in nonendemic patients with severe infection vs uncomplicated, with a much less pronounced difference in the endemic setting. Relating neutrophils to lymphocyte counts, the NLCR is considered a general

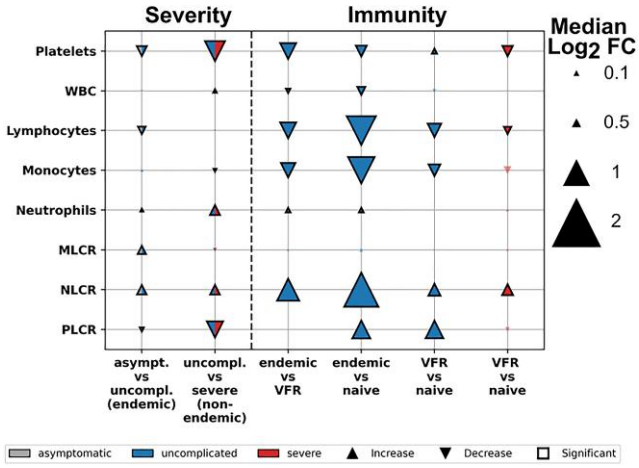

Figure 4. Trends of individual full blood count parameters in relation to *Plasmodium falciparum* infection severity and previous malaria exposure. Data are shown from one group with lower infection severity to the next group of higher infection severity or from a group with a higher degree of previous exposure to a group with a lower degree. The size of the triangles indicates the magnitude of the difference, with larger triangles representing greater differences. Comparisons were found significant if $P < .05$. FC, fold change; MLCR, monocyte to lymphocyte count ratio; NLCR, neutrophil to lymphocyte count ratio; PLCR, platelet to lymphocyte count ratio; VFR, visit friends and relatives; WBC, white blood cells.

marker of inflammation and stress [26]. In patients with imported malaria, the NLCR has been correlated with parasite density [15, 16]. In our study, the NLCR discriminated asymptomatic from uncomplicated cases in the endemic setting especially well, with the latter exhibiting higher neutrophil and lower lymphocyte counts. However, lymphocytes showed an even stronger association with previous malaria exposure in uncomplicated and severe malaria. Mechanisms of lymphopenia in malaria include lymphocyte sequestration in secondary lymphoid tissues and increased apoptosis [27]. As our data indicate, both might be dependent on the individual level of semi-immunity, which possibly explains the conflicting reports on lymphocyte levels in patients with malaria from different endemicity settings [15, 28].

While platelets, neutrophils, and NLCR displayed clear trends with disease severity, monocytes did not. In contrast, we observed a distinct correlation of monocyte counts with previous *Plasmodium* exposure, reflecting their potentially critical role in semi-immunity. Monocytes have recently been gaining interest in malaria, where they confer protection by clearance of parasites, as well as pathology, including systemic inflammation and vascular dysfunction [29]. Previous studies report ambiguous results for monocyte counts in malaria, depending on the study setting [30–32], possibly due to variations in the frequencies of different monocyte subsets [33]. Our findings underscore the complex role of monocytes in malaria, highlighting their distinct implication in immunity rather than disease severity. The precise mechanisms underlying these observations and their implications for malaria pathogenesis and immunity merit further research. Figure 4 shows an overview of the trends of FBC parameters in relation to infection severity and the degree of previous exposure found in our study.

Combining FBC parameters into ensemble-based machine learning models significantly improved the classification of disease severity. Again, platelets emerged as the most relevant contributors to both classifiers. Machine learning approaches have been gaining interest in life sciences lately, proving especially helpful for the combined analysis of multiple parameters. A particular challenge for their applicability is the lack of software solutions transforming the output of such models into easy-to-use tools for physicians [34]. We therefore provide an open access online calculator for both our machine learning models (https://malaria-severity-prediction.bnitm.de/). Upon entering FBC parameters of a patient infected with *Pf*, this calculator predicts the infection severity, allowing one to choose cutoffs for the predicted probability (ie, favoring specificity or sensitivity) in addition to the endemicity setting and baseline variables. When favoring specificity in an endemic setting, the model achieves a remarkable LR+ of 61 (sensitivity, 0.57; specificity, 0.99; Supplementary Table 6); in a nonendemic high-sensitivity configuration, it results in a favorably low LR– of 0.06 (sensitivity, 0.96; specificity, 0.64; Supplementary Table 7).

In practice, our calculator could serve as a tool for rapidly assessing disease severity to identify individuals with uncomplicated malaria vs asymptomatic infection in endemic settings and to support initial assessment of severe disease in nonendemic settings. Further validation of the suggested cutoff values for blood cell values and ratios is needed, and efforts should be made to address geographic discrepancies and other potential confounding factors in future studies. Configurations of the online calculator and potential use cases are described in the supplementary materials.

LIMITATIONS

Our study had several limitations. First, diagnosis of 508 patients with uncomplicated symptomatic malaria in Gabon relied solely on PfHRP2-based rapid diagnostic tests, which, while not gold standard, consistently detected only *Pf*. Given that the prevalence of *Pf* in the study region exceeds 95% [35], misclassification of non-*Pf* malaria is altogether highly unlikely. Even though PFHRP2-based assays may stay false positive up to approximately 1 month upon successful treatment, misclassification of false-positive test results due to remaining PfHRP2 is unlikely, since all 508 patients participated in an antimalarial treatment study that excluded individuals who took antimalarial drugs in the 28 days prior to malaria diagnosis [36]. Second, variations in FBC can be confounded by factors such as genetic and geographic background, age, concomitant medication, underlying comorbidities, and coinfections

[26, 37]. We excluded patients with known viral or bacterial coinfections and liver, blood, or immunologic diseases to minimize these effects. While isolated cases with undetected coinfections cannot entirely be ruled out, the large overall sample size likely renders such effects on group-level outcomes negligibly small. Third, all persons from endemic countries were retrospectively recruited for the analysis based on previous participation in a malaria study conducted at CERMEL. Since none of these studies focused on severe malaria, we could not include any case of severe malaria. Yet, in settings of hyperendemic malaria transmission, severe malaria is known to occur almost exclusively among children and usually not among semi-immune adults. Therefore, given that the generalizability of our results is limited to adults, we believe that this failure to include adults with severe malaria in Gabon constitutes only a minor limitation. Finally, the impact of genetic and geographic backgrounds remains insufficiently characterized. A sensitivity analysis with harmonization factors derived from a publicly available dataset (National Health and Nutrition Examination Survey Data, 2011–2012; Supplementary Methods [38]) revealed only negligible differences (Supplementary Tables 8 and 9). Nevertheless, population-specific reference ranges that account for genetic and environmental backgrounds are critical, and reliance on WHO global reference ranges or harmonization as in our sensitivity analysis may unintentionally perpetuate misconceptions about race in biomedical research. Developing locally adapted reference values would enhance clinical practice and facilitate multicentered or global comparative studies.

CONCLUSION

In summary, the present study provides extensive data on hematologic alterations across patients with *Pf* infection with widely varying levels of disease severity and previous malaria exposure in endemic and nonendemic settings. The results highlight the significance of platelets but also neutrophils for identifying patients with greater infection severity in both settings, whereas monocyte and lymphocyte levels mainly reflect the degree of previous exposure. The accuracy of identifying at-risk patients can be considerably increased by integrating parameters through a machine learning approach. For clinical translation of these findings, we provide a publicly accessible online calculator that offers a rapid prediction of a person's disease severity. The inherent diagnostic potential of this approach, based on simple FBC parameters that are readily available even in resource constraint settings, warrants prospective validation.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. World Health Organization. World malaria report 2023. Genève, Switzerland: World Health Organization, **2023**.
2. Mischlinger J, Rönnberg C, Álvarez-Martínez MJ, et al. Imported malaria in countries where malaria is not endemic: a comparison of semi-immune and non-immune travelers. Clin Microbiol Rev **2020**; 33:e00104-19.
3. Kurth F, Develoux M, Mechain M, et al. Intravenous artesunate reduces parasite clearance time, duration of intensive care, and hospital treatment in patients with severe malaria in Europe: the TropNet severe malaria study. Clin Infect Dis **2015**; 61:1441–4.
4. Kurth F, Develoux M, Mechain M, et al. Severe malaria in Europe: an 8-year multi-centre observational study. Malar J **2017**; 16:57.
5. Bittaye SO, Jagne A, Jaiteh LE, et al. Clinical manifestations and outcomes of severe malaria in adult patients admitted to a tertiary hospital in the Gambia. Malar J **2022**; 21:270.
6. World Health Organization. WHO guidelines for malaria. Genève, Switzerland: World Health Organization, **2023**.
7. Lingscheid T, Kurth F, Stegemann MS, et al. Outpatient treatment of imported uncomplicated *Plasmodium falciparum* malaria: results from a survey among TropNet and GeoSentinel experts for tropical medicine. J Travel Med **2020**; 27: taaa082.
8. Kurth F, Tober-Lau P, Lingscheid T, et al. Post-treatment haemolysis is common following oral artemisinin combination therapy of uncomplicated malaria in travellers. J Travel Med **2023**; 30:taad001.
9. Ogutu B, Tiono AB, Makanga M, et al. Treatment of asymptomatic carriers with artemether-lumefantrine: an opportunity to reduce the burden of malaria? Malar J **2010**; 9:30.
10. Chen I, Clarke SE, Gosling R, et al. "Asymptomatic" malaria: a chronic and debilitating infection that should be treated. PLoS Med **2016**; 13:e1001942.

11. Huang Y, Liu A, Liang L, et al. Diagnostic value of blood parameters for community-acquired pneumonia. Int Immunopharmacol **2018**; 64:10–5.
12. de Jager CPC, van Wijk PTL, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. Crit Care **2010**; 14:1–8.
13. McKenzie FE, Prudhomme WA, Magill AJ, et al. White blood cell counts and malaria. J Infect Dis **2005**; 192:323–30.
14. Sarkar S, Khanna P, Singh AK. The impact of neutrophil-lymphocyte count ratio in COVID-19: a systematic review and meta-analysis. J Intensive Care Med **2022**; 37:857–69.
15. van Wolfswinkel ME, Vliegenthart-Jongbloed K, de Mendonça Melo M, et al. Predictive value of lymphocytopenia and the neutrophil-lymphocyte count ratio for severe imported malaria. Malar J **2013**; 12:101.
16. Berens-Riha N, Kroidl I, Schunk M, et al. Evidence for significant influence of host immunity on changes in differential blood count during malaria. Malar J **2014**; 13: 155.
17. Balerdi-Sarasola L, Pedro F, Bottieau E, et al. MALrisk: a machine-learning–based tool to predict imported malaria in returned travellers with fever. J Travel Med **2024**; 31:taae054.
18. D'Abramo A, Rinaldi F, Vita S, et al. A machine learning approach for early identification of patients with severe imported malaria. Malar J **2024**; 23:46.
19. Ramharter M, Agnandji ST, Adegnika AA, et al. Development of sustainable research excellence with a global perspective on infectious diseases: Centre de Recherches Médicales de Lambaréné (CERMEL), Gabon. Wien Klin Wochenschr **2021**; 133:500–8.
20. Lacerda MVG, Mourão MPG, Coelho HCC, Santos JB. Thrombocytopenia in malaria: who cares? Mem Inst Oswaldo Cruz **2011**; 106(suppl 1):52–63.
21. Bayleyegn B, Asrie F, Yalew A, Woldu B. Role of platelet indices as a potential marker for malaria severity. J Parasitol Res **2021**; 2021:5531091.
22. McMorran BJ, Wieczorski L, Drysdale KE, et al. Platelet factor 4 and Duffy antigen required for platelet killing of *Plasmodium falciparum*. Science **2012**; 338: 1348–51.
23. McMorran BJ. Immune role of platelets in malaria. ISBT Sci Ser **2019**; 14:67–76.
24. Babatunde KA, Adenuga OF. Neutrophils in malaria: a double-edged sword role. Front Immunol **2022**; 13:922377.
25. Olliaro P, Djimdé A, Dorsey G, et al. Hematologic parameters in pediatric uncomplicated *Plasmodium falciparum* malaria in sub-Saharan Africa. Am J Trop Med Hyg **2011**; 85:619–25.
26. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy **2021**; 122:474–88.
27. Hviid L, Kemp K. What is the cause of lymphopenia in malaria? Infect Immun **2000**; 68:6087–9.
28. Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CRJC. Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. Br J Haematol **2002**; 119:839–47.
29. Chua CLL, Brown G, Hamilton JA, Rogerson S, Boeuf P. Monocytes and macrophages in malaria: protection or pathology? Trends Parasitol **2013**; 29:26–34.
30. Kotepui M, Phunphuech B, Phiwklam N, Chupeerach C, Duangmano S. Effect of malarial infection on haematological parameters in population near Thailand-Myanmar border. Malar J **2014**; 13:218.
31. Sakzabre D, Asiamah EA, Akorsu EE, et al. Haematological profile of adults with malaria parasitaemia visiting the Volta regional hospital, Ghana. Adv Hematol **2020**; 2020:9369758.
32. Maina RN, Walsh D, Gaddy C, et al. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. Malar J **2010**; 9(suppl 3):S4.
33. Dobbs KR, Embury P, Vulule J, et al. Monocyte dysregulation and systemic inflammation during pediatric falciparum malaria. JCI Insight **2017**; 2:e95352.
34. Hartl J, Kurth F, Kappert K, et al. Quantitative protein biomarker panels: a path to improved clinical practice through proteomics. EMBO Mol Med **2023**; 15:e16061.
35. Mischlinger J, Pitzinger P, Veletzky L, et al. Use of capillary blood samples leads to higher parasitemia estimates and higher diagnostic sensitivity of microscopic and molecular diagnostics of malaria than venous blood samples. J Infect Dis **2018**; 218:1296–305.
36. Lutete GT, Mombo-Ngoma G, Assi S-B, et al. Pyronaridine-artesunate real-world safety, tolerability, and effectiveness in malaria patients in 5 African countries: a single-arm, open-label, cohort event monitoring study. PLoS Med **2021**; 18: e1003669.
37. Naser RH, Rajaii T, Farash BRH, et al. Hematological changes due to malaria—an update. Mol Biochem Parasitol **2024**; 259:111635.
38. Centers for Disease Control and Prevention. National Center for Health Statistics: National Health and Nutrition Examination Survey data (NHANES) 2011–2012. Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/overview.aspx?BeginYear=2011. Accessed 4 August 2024.