# Comparative analysis of initial Full Blood Count parameters in *Plasmodium falciparum* infected adults for classification of disease severity and previous exposure across endemic (Gabon) and non-endemic (Germany) settings

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# Supplementary Methods

## Statistical analysis and Machine Learning

To evaluate the diagnostic performance of FBC parameters in distinguishing severity levels, sensitivity, specificity, the positive likelihood ratio (LR+) and the negative likelihood ratio (LR-) were computed for continuous parameters with significant differences between severity groups. For optimal cut-off determination, Euclidean distance was computed based on sensitivity and specificity of the respective parameter. Patient data used for machine learning was balanced for age and sex, by fitting a k-nearest neighbour algorithm on the majority class. Models were trained using leave-one-out cross validation and predictions and predictive probability of test cases were used for subsequent performance analysis. Random Forest models trained to predict severity based on FBC parameters only, were limited to a maximum node depth of 3 and 38 and 40 estimators for the endemic and nonendemic setting, respectively. Models trained on Full blood count (FBC) and parasitic count were limited to 41 estimators in both settings. Random forest models with the same hyperparameters were trained on all samples used in the cross-validation before. SHAP (**SH**apley **A**dditive ex**P**lanations) [1] values were extracted to enhance model interpretability. Calculation and visualisation were performed using the *shap* package. Receiver Operating Characteristic (ROC) curves and Area Under the ROC curve (AUROC) values were obtained by using *scipy*'s trapezoidal integration. To assess model performance across multiple cross-validation splits, false positive rates (FPR) from all test sets were aggregated into a unified, sorted set of unique FPR values. True positive rates (TPR) were interpolated for these FPR values from each test set, averaged across splits, and used to generate the mean ROC curve. When calculating ROC curves for individual features (i.e., FBCs and their respective ratios), values were sorted depending on the positive outcome of the non-endemic setting. For instance, as the positive class was defined to be severe malaria, platelets were sorted in descending order. To maintain consistency across the entire analysis and facilitate direct comparison of the ROC curves between the non-endemic and endemic setting, we used the same sorting order for the endemic setting. This might result in AUROC values below 0.5, indicating a higher predictive performance for the negative class. This sorting choice did not affect any calculations and was done solely for visualisation purposes. The positive and negative likelihood ratios of the machine learning models were calculated using TPR and FPR obtained from the predicted probabilities from the random forests.

## Sensitivity analysis with external normalisation of FBC parameters according to genetic background

Several studies have described differences in the distribution of WBC subpopulations according to the genetic background [4,5]. To address potential underlying differences in the distribution of WBC subpopulations due to different genetic background between the African and European study population we performed a sensitivity analysis. For this we normalised absolute cell count values of WBCs, neutrophils, lymphocytes and monocytes for the non-African study population by mathematically applying an external harmonization factor. As no standardised correction factor exists and due to limited availability of extensive datasets on healthy persons in Gabon or Sub-Saharan Africa, we utilised the US-American National Health and Nutrition Examination Survey (NHANES) 2011-2012 dataset [6], despite its limitations in fully representing the African diversity of blood parameters. From this dataset, we extracted FBC profiles of all 809 "Non-Hispanic White" and all 433 "Non-Hispanic Black" adults with "excellent" and "very good" health statuses and calculated the correction factors as: (median cell $count_{[Non\text{-}Hispanic\ Black]}$)/(median cell $count_{[Non\text{-}Hispanic\ White]}$). The correction factors were found to be 1.11 for lymphocytes, 0.79 for neutrophils, and 0.80 for monocytes (**Supp. Table 8**). These factors we applied to adjust the respective FBC counts of the non-African population in all analyses.

This correction, affecting only the non-endemic groups, resulted in lower median WBC, monocyte, and neutrophils, and higher median lymphocytes compared to the non-harmonised cell counts in both severity groups. Correspondingly, harmonised median NLCR and PLCR were lower, while median MLCR higher than the non-harmonised. The respective blood count values after harmonisation are presented in **Supp. Table 9**. However, as the differences between the harmonised and unharmonised values were negligible, we solely present the findings from non-harmonised data in the subsequent analyses.

# Online calculator to estimate severity of *Plasmodium* infection based on FBC values

To make the developed prediction models accessible to users, we created an online tool for predicting malaria disease severity in both endemic and non-endemic settings. The user interface requires the input of five parameters: platelets, WBC, lymphocytes, neutrophils and monocytes. Ratios for NLCR, MLCR, and PLCR are calculated automatically. Users can select from three different configurations A) highly sensitive, B) balanced, or C) highly specific for endemic settings and for non-endemic settings. These configurations were computed by selecting three thresholds of the predicted probability of the model (see below **Supp. Tables 6 and 7**).

In endemic settings, when choosing a predicted probability cut off which equally favours sensitivity and specificity the best values for positive and negative likelihood ratios were 0.19 and 0.20, respectively. In non-endemic settings, when choosing a similarly balanced scenario the best values for positive and negative likelihood ratios were 4.09 and 0.25, respectively. However, when choosing predicted probability cut offs favouring either specificity at the expense of sensitivity and vice versa indeed clinically favourable likelihood ratio values were achieved. Of highest interest are a 'high specificity & low sensitivity' scenario in endemic settings and a 'high sensitivity & low specificity' scenario in non-endemic settings. The former scenario yielded a positive likelihood ratio of more than 60 (LR+ of 61.00 corresponding with a LR- of 0.43) while the latter scenario had a negative likelihood ratio of below 0.1 (LR- of 0.06 corresponding with a LR+ of 2.64).

Upon choosing a given configuration the user can then enter the FBC values of the given *Plasmodium falciparum* infected individual. This will yield a prediction on whether this is likely a severe or non-severe infection for the chosen configuration. Additionally, users can input disease prevalence to optionally calculate negative and positive predictive values (NPV and PPV) for the chosen setting.
The website frontend was developed using HTML and JavaScript. The backend, deployed Python's Flask package [2] handling interaction with the machine learning models, database operations and I/O operations manages interactions with the machine learning models, database operations, and I/O operations. A SQLite database is used to collect user input data for future validation of the models. Both the website and database are hosted on the internal server infrastructure of the BNITM, and the collected data will be used exclusively for directly related research purposes. The website can be accessed under https://malaria-severity-prediction.bnitm.de/ and programming code is available under https://github.com/ClemensDie/malaria_severity_predictor.

# Supplementary Note

## Test-test-treat scenarios and potential use cases for malaria severity prediction using a rapid diagnostic test and a handheld haematology analyser

**Severity estimation in resource-limited, malaria-endemic settings**

To date, malaria light microscopy constitutes the diagnostic gold standard for malaria detection and *Plasmodium* parasite quantification in the clinical routine. However, it is a well described phenomenon that malaria-endemic regions suffer from a shortage of light-microscopy-based malaria diagnostic services, particularly owing to a lack of trained microscopists. Therefore, RDTs have been widely deployed and constitute the main malaria diagnostic method in many, particularly low-resource settings [3]. They are simple and can be performed using a small quantity of blood, which is often sampled via a finger prick. However, to date they can only provide qualitative parasitological diagnostic test results and not on disease severity. Moreover, field testing in many resource-limited, malaria endemic settings is mostly operated by field workers who follow simple test-and-treat algorithms. Such field-worker-operated RDT programmes could potentially be complemented by handheld haematology analysers. These are simple, yield valid results from finger prick blood and can be performed both at the bedside, as in the field and would yield sufficient diagnostic information on all FBC values necessary for our machine learning online calculator. Thereby, the qualitative parasitological RDT-based result could be complemented by information on disease severity. Such a test-test-treat algorithm could pose a strategic advantage for malaria control programmes and malaria case management programmes, which shall be further illuminated in the following paragraphs.

**A) Treating asymptomatic *Plasmodium* infections in endemic settings**

At present there is no consensus in the scientific community on whether asymptomatic *Plasmodium* infections should be treated. While from a disease elimination perspective each infection should indeed be treated, case management programmes ponder on whether the clearance of asymptomatic *Plasmodium* infections might eventually result in the decline of population level immunity and subsequently lead to higher rates of complicated disease episodes not only among children, but also among adults. In malaria-endemic settings, where elimination seems unrealistic and in the event that clearance of asymptomatic *Plasmodium* infections might indeed be proven to be unfavourable due to above-described concerns, our machine learning model might provide decisive clarity on whom to treat among all individuals with a *Plasmodium* infection. Choosing our 'high specificity & low sensitivity' configuration in our online calculator would ensure treatment delivery to only individuals whose asymptomatic *Plasmodium* infection, from a laboratory standpoint, already resembles uncomplicated malaria. This approach would seem less likely to significantly decrease population level immunity compared with treating virtually all *Plasmodium* infections and would potentially correctly treat those individuals whose *Plasmodium* infection might have soon progressed to being symptomatic due to having close laboratory value resemblance with uncomplicated malaria patients.

**B) Clinical research in malaria: Corroboration of inclusion criteria**

Furthermore, dedicated malaria research projects may use this haematology-based severity estimation to recruit a study population of specific interest. Using the 'high specificity & low sensitivity' configuration of our online calculator in malaria treatment studies would help corroborate the common inclusion criteria of symptomatic, clinical malaria. This is often defined as the presence of *Plasmodium* parasites in combination with the presence of symptoms. The latter of these two criteria is rather subjective due to it being a self-reported patient outcome and therefore, a haematology-based severity estimation could provide objective and useful supplementary information to yield additional validity of case classification.

**C) Facilitating prompt diagnosis of severe disease in non-endemic settings**

Also, non-endemic malaria regions, which are often high-resource settings, might benefit from FBC-based disease severity prediction using our online calculator. Contrary to malaria-endemic regions in non-endemic settings asymptomatically *Plasmodium* infected individuals, if existing at all, are very rare. Individuals residing in non-endemic regions have no immunity against malaria, and for semi-immune individuals immunity is described to wane over time. Therefore, disease manifestation tends to be on average more severe than in endemic settings, making prompt diagnosis and therapeutic management key. Most commonly, malaria in non-endemic settings develops with non-specific signs and symptoms prompting many diseased individuals to first seek contact with the healthcare system via their general practitioner. Malaria RDTs are commercially well available, rather inexpensive and therefore easily procurable for any medical setting in non-endemic countries. Furthermore, haematological analysis tends to be well available. As our data show, the combination of a positive RDT test-result with rapid initial FBC-based disease severity assessment, which could even be implemented automatically in a laboratory data information system, could lead to automatic labelling of patients with high risk of severe malaria in the hospital management system. For optimal performance we recommend the 'high sensitivity & low specificity' configuration, whose favourable LR- value of 0.06 will contribute a large if not conclusive decrease of severe disease likelihood. In such cases prompt treatment should ensue still, however, uncomplicated malaria management would probably suffice for a large part of such cases.

**D) Future research: Improving field-worker operated management guidelines on the treatment of febrile children in malaria-endemic regions by utilising FBC parameters and machine learning technology**

Furthermore, settings of high malaria transmission struggle not only to accurately distinguish between asymptomatic *Plasmodium* infection from clinical malaria, but also to correctly identify the root cause in febrile patients. According to guidelines of integrated community case management of malaria, febrile children in malaria-endemic areas should receive a malaria rapid diagnostic test, which - if positive - is followed by administration of an effective anti-malarial treatment course. Such practice is, of course, essentially good, however, has led to some case management algorithms, in which alternative fever causes were not adequately considered anymore once a positive malaria RDT result was present. Therefore, studies have suggested that a substantial proportion of African febrile children in malaria-endemic areas could receive inadequate causal fever management [3]. This phenomenon is explained by children who have developed a sufficient amount of anti-disease immunity against malaria so that they carry an asymptomatic concomitant *Plasmodium* parasitemia, which at point-of-care-RDT-testing might indeed be correctly detected from a parasitological perspective, however, incorrectly managed if the child's fever is of different etiology (e.g. bacterial pneumonia). In such scenarios, machine learning technology could help to distinguish between true malaria episodes and febrile episodes of other origin, ultimately enhancing the proportion of adequately managed febrile children, particularly in settings where most febrile episodes are not attributable to malaria. To facilitate this accordingly, studies should be conducted on febrile children in malaria-endemic areas to help identify true malaria episodes. To better understand this, further research is important in which machine learning models assess the discriminatory potential of blood parameters to distinguish between important differential diagnoses of a given target population. In the context of sub-Saharan African children bacterial sepsis, bacterial pneumonia and malaria are of special importance. Our current online calculator is only informed by individuals with *Plasmodium* infections and might therefore be of limited use in discriminating among different febrile infectious diseases. Should our calculator still be used in such a regard then the 'high specificity & low sensitivity' would in theory best facilitate the identification of patients with true malaria and separate them from those with a concomitant, asymptomatic *Plasmodium* infection.

# Supplementary Tables

**Supplementary Table 1:** Statistical analysis of the comparison between severity groups

| **Endemicity** | | **endemic** | | | **non-endemic** | |
|---|---|---|---|---|---|---|
| **Infection severity** | asymptomatic | uncomplicated | | uncomplicated | severe | |
| N | 117 | 606 | | 565 | 105 | |
| $10^3/\mu l$ | Median<br>IQR | Median<br>IQR | p | Median<br>IQR | Median<br>IQR | p |
| Platelets | 218<br>174-256 | 150<br>96-197 | <.0001* | 85<br>58-122 | 40<br>26-57 | <.0001* |
| WBC | 6.3<br>5.1-7.5 | 5.9<br>4.7-7.3 | 0.16 | 4.8<br>3.7-6.0 | 5.5<br>4.3-6.8 | 0.0006* |
| Neutrophils | 2.32<br>1.78-3.00 | 2.57<br>1.90-3.72 | 0.0037* | 3.08<br>2.19-4.24 | 4.49<br>2.98-5.53 | <.0001* |
| Lymphocytes | 2.16<br>1.55-2.63 | 1.62<br>1.05-2.33 | <.0001* | 0.79<br>0.48-1.20 | 0.75<br>0.46-1.10 | 0.1685 |
| Monocytes | 0.55<br>0.38-0.79 | 0.62<br>0.38-0.90 | 0.28 | 0.32<br>0.20-0.49 | 0.29<br>0.14-0.42 | 0.0083* |
| NLCR | 1.08<br>0.84-1.38 | 1.55<br>0.91-3.32 | <.0001* | 3.92<br>2.26-7.32 | 5.71<br>3.72-10.07 | 0.0001* |
| PLCR | 103.55<br>80.38-131.21 | 88.35<br>62.29-123.73 | 0.003* | 106.78<br>60.81-182.01 | 57.71<br>35.55-95.26 | <.0001* |
| MLCR | 0.26<br>0.20-0.35 | 0.36<br>0.21-0.63 | 0.0001* | 0.41<br>0.27-0.58 | 0.35<br>0.21-0.52 | 0.0812 |
| RBC [$10^6/\mu l$] | 4.59<br>4.12-5.00 | 4.35<br>4.02-4.80 | 0.0095* | 4.70<br>4.30-5.08 | 4.23<br>3.52-4.81 | <.0001* |
| Eosinophils | 0.88<br>0.33-1.63 | 0.25<br>0.07-0.82 | <.0001* | 0.01<br>0.00-0.03 | 0.01<br>0.00-0.01 | 0.5816 |
| Basophils | 0.06<br>0.04-0.08 | 0.09<br>0.06-0.16 | <.0001* | 0.02<br>0.01-0.03 | 0.02<br>0.00-0.04 | 0.8744 |

Data for cell counts are presented in $10^3/\mu l$ (unless otherwise indicated). P-values obtained using the Mann-Whitney U tests for pairwise comparisons. Significant p-values ($p < 0.05$) are indicated by *.

**Supplementary Table 2:** Diagnostic performance characteristics of single parameters for distinguishing between asymptomatic Plasmodium infections and uncomplicated malaria in patients from an endemic region (Gabon).

| Characteristic | Normal ranges | Cut off** | Sensitivity | Specificity | Positive likelihood ratio | Negative likelihood ratio |
|---|---|---|---|---|---|---|
| Parasites per µL | N/A | + 1,586 | 60% | 87% | 4.69 | 0.46 |
| Platelets, $10^3$/µl | 140 - 440 | - 189 | 71% | 70% | 2.34 | 0.41 |
| WBC, $10^3$/µl | 4.0 - 10.0 | NA* | NA* | NA* | NA* | NA* |
| Neutrophils, $10^3$/µl | 1.5 - 8.5 | + 2.39 | 57% | 55% | 1.26 | 0.78 |
| Lymphocytes, $10^3$/µl | 0.8 - 4.0 | - 1.85 | 61% | 63% | 1.62 | 0.63 |
| Monocytes, $10^3$/µl | 0.0 - 1.2 | NA* | NA* | NA* | NA* | NA* |
| NLCR | N/A | + 1.37 | 55% | 75% | 2.22 | 0.59 |
| PLCR | N/A | - 90.14 | 52% | 65% | 1.51 | 0.73 |
| MLCR | N/A | - 0.25 | 33% | 59% | 0.80 | 1.14 |

* No significant difference between the two groups; therefore, no performance characteristics were computed for this parameter

** '+' indicates that any value above the cut-off is associated with uncomplicated malaria; '-' indicates that any value below the cut-off is associated with uncomplicated malaria.

N.B.: As comparison the discriminatory potential of parasitaemia and red blood cells is displayed

**Supplementary Table 3:** Diagnostic performance characteristics of single parameters for distinguishing between uncomplicated and severe malaria patients from a non-endemic region (Germany).

| Characteristic | Normal ranges | Cut off** | Sensitivity | Specificity | Positive likelihood ratio | Negative likelihood ratio |
|---|---|---|---|---|---|---|
| Parasites per µL | N/A | + 129,000 | 83% | 88% | 6.67 | 0.19 |
| Platelets, $10^3$/µl | 150 - 400 | - 61 | 79% | 72% | 2.82 | 0.29 |
| WBC, $10^3$/µl | 3.80 - 11.0 | + 5.25 | 58% | 60% | 1.45 | 0.70 |
| Neutrophils, $10^3$/µl | 1.50 - 7.70 | + 3.99 | 60% | 71% | 2.06 | 0.57 |
| Lymphocytes, $10^3$/µl | 1.10 - 3.40 | NA* | NA* | NA* | NA* | NA* |
| Monocytes, $10^3$/µl | 0.20 - 0.90 | - 0.29 | 53% | 56% | 1.20 | 0.84 |
| NLCR | N/A | + 4.17 | 70% | 53% | 1.48 | 0.57 |
| PLCR | N/A | - 68.67 | 65% | 70% | 2.14 | 0.50 |
| MLCR | N/A | NA* | NA* | NA* | NA* | NA* |

* No significant difference between the two groups; therefore, no performance characteristics were computed for this parameter

** '+' indicates that any value above the cut-off is associated with severe malaria; '-' indicates that any value below the cut-off is associated with severe malaria

N.B.: As comparison the discriminatory potential of parasitaemia and red blood cells is displayed

**Supplementary Table 4:** Demographic characteristics of the subset of participants used to train and test machine learning models for severity classification in an endemic and non-endemic setting.

| **Classifier** | **endemic** | | **non-endemic** | |
|---|---|---|---|---|
| **Groups** | asymptomatic | uncomplicated | uncomplicated | severe |
| N | 107 | 107 | 93 | 92 |
| Male, n % | 0.63 | 0.65 | 0.7 | 0.73 |
| Age in y, median (IQR) | 26 (21.0 – 41.5) | 25 (21.0 – 41.0) | 50 (42.5 – 59.0) | 50 (42.3-59.0) |
| parasitemia in p*$10^3$/µl, median (IQR) | 0.5 (0.3 – 1.0) | 4.6 (0.6-16.8) | 27.5 (17.7 – 74.6) | 407 (188.3 – 592.4) |

**Supplementary Table 5**: Statistical analysis of the comparison between subgroups of different previous exposures within severity groups

| **Severity** | **uncomplicated** | | | | | | | **severe** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Previous exposure** | endemic | VFR | naive | p | | | | VFR | naive | p |
| N | 606 | 428 | 137 | | | | | 48 | 57 | |
| $10^3$/µl | Median IQR | Median IQR | Median IQR | Over all | endemic vs VFR | VFR vs naive | endemic vs naive | Median IQR | Median IQR | |
| Platelets | 150 | 82 | 99 | <0.0001* | <0.0001* | 0.0034* | 0.001* | 50 | 34 | 0.0064* |
| | 96-197 | 57-113 | 61-166 | | | | | 33.3-67.5 | 22.3-50.3 | |
| WBC | 5.90 | 5.00 | 4.35 | <0.0001* | 0.0021* | 0.26 | <0.0001* | 5.55 | 5.34 | 0.7879 |
| | 4.70-7.30 | 3.90-6.19 | 3.15- 5.58 | | | | | 4.22-6.65 | 4.38-7.10 | |
| Lymphocytes | 1.61 | 0.89 | 0.54 | <0.0001* | <0.0001* | 0.0009* | <0.0001* | 0.92 | 0.70 | 0.0087* |
| | 1.05- 2.33 | 0.60- 1.30 | 0.35- 0.84 | | | | | 0.53-1.28 | 0.36-0.87 | |
| Monocytes | 0.62 | 0.36 | 0.23 | <0.0001* | <0.0001* | 0.012* | <0.0001* | 0.32 | 0.24 | 0.0619 |
| | 0.38- 0.90 | 0.22- 0.52 | 0.14- 0.34 | | | | | 0.19-0.46 | 0.14-0.38 | |
| Neutrophils | 2.57 | 3.08 | 3.06 | 0.0005* | 0.0025* | 0.1615 | 0.0410* | 4.17 | 4.97 | 0.1231 |
| | 1.90-3.72 | 2.22-4.2 | 2.21-4.36 | | | | | 3.00-5.05 | 2.98-5.79 | |
| MLCR | 0.36 | 0.40 | 0.42 | 0.1778 | NA | NA | NA | 0.34 | 0.37 | 0.3060 |
| | 0.21-0.63 | 0.27-0.57 | 0.26-0.63 | | | | | 0.21-0.44 | 0.21-0.58 | |
| NLCR | 1.55 | 3.54 | 5.60 | <0.0001* | <0.0001* | 0.0006* | <0.0001* | 4.73 | 7.12 | 0.0059* |
| | 0.91-3.32 | 2.00-6.25 | 3.32-9.24 | | | | | 3.34-7.47 | 4.25-13.26 | |
| PLCR | 88.35 | 89.74 | 174.07 | <0.0001* | 0.1249 | <0.0001* | <0.0001* | 57.78 | 48.61 | 0.9123 |
| | 62.29-123.73 | 58.68-142.91 | 105.81-287.64 | | | | | 35.76-91.30 | 34.81-100.18 | |

Data for cell counts are presented in $10^3$/µl. P-values obtained using the Mann-Whitney U tests for pairwise comparisons and Kruskal Wallis H tests for comparisons with >2 groups. Significant p-values ($p < 0.05$) are indicated by *.

**Supplementary Table 6:** Diagnostic performance characteristics of machine learning model for distinguishing between asymptomatic parasitaemia and uncomplicated malaria in patients in endemic settings

| Scenario | Cut off* | Sensitivity | Specificity | Positive likelihood ratio | Negative likelihood ratio |
|---|---|---|---|---|---|
| High specificity & low sensitivity | 79% | 57% | 99% | 61.00 | 0.43 |
| Balanced sensitivity & specificity | 52% | 83% | 90% | 0.19 | 0.20 |
| High sensitivity & low specificity | 30% | 93% | 72% | 3.30 | 0.10 |

* Cut off on the predicted probability of the machine learning model to identify an uncomplicated malaria case among all endemic *Plasmodium* infected individuals

**Supplementary Table 7:** Diagnostic performance characteristics of machine learning model for distinguishing between uncomplicated and severe malaria in patients in non-endemic settings

| Scenario | Cut off* | Sensitivity | Specificity | Positive likelihood ratio | Negative likelihood ratio |
|---|---|---|---|---|---|
| High specificity & low sensitivity | 68% | 67% | 94% | 12.00 | 0.35 |
| Balanced sensitivity & specificity | 40% | 88% | 79% | 4.09 | 0.25 |
| High sensitivity & low specificity | 25% | 96% | 64% | 2.64 | 0.06 |

* Cut off on the predicted probability of the machine learning model to identify a severe malaria case among all non-endemic malaria cases

**Supplementary Table 8**: FBC profiles of the National Health and Nutrition Examination Survey (NHANES) 2011-2012 Data set in the United States

| Blood cell count in $10^3/\mu l$ median (IQR) | Non-Hispanic Black | Non-Hispanic White | Correction factor |
|---|---|---|---|
| N | 809 | 433 | |
| WBC | 5.9 (4.9-7.3) | 6.4 (5.5-7.9) | 0.92 |
| Lymphocytes | 2.0 (1.6-2.5) | 1.8 (1.5-2.3) | 1.11 |
| Monocytes | 0.4 (0.4-0.6) | 0.5 (0.4-0.6) | 0.80 |
| Segmented Neutrophils | 3.1 (2.3-4.2) | 3.9 (3.0-4.9) | 0.79 |

White blood cells, Lymphocytes, Monocytes and segmented neutrophils of all "Non-Hispanic White" and all "Non-Hispanic Black" individuals in "excellent" and "very good" health statuses. Correction factor was calculated by (median cell count$_{[\text{Non-Hispanic Black}]}$)/(median cell count$_{[\text{Non-Hispanic White}]}$).

**Supplementary Table 9**: Parameters in non-endemic setting harmonised by applying the calculated correction factor to adjust the respective FBC counts of the non-African population

| | harmonised uncomplicated | harmonised severe | |
|---|---|---|---|
| $10^3/\mu l$ | Median<br>IQR | Median<br>IQR | p |
| WBC | 4,75<br>3.62-5.96 | 5,48<br>4.09-6.56 | **0.0021** |
| Lymphocytes | 0,82<br>0.51-1.23 | 0,82<br>0.46-1.16 | 0.4690 |
| Monocytes | 0,31<br>0.18-0.48 | 0,25<br>0.13-0.38 | **0.0026** |
| Neutrophils | 2,97<br>1.98-3.91 | 4,04<br>2.69-4.92 | **<.0001** |

P-values obtained using the Mann-Whitney U tests for pairwise comparisons. Significant p-values ($p < 0.05$) are indicated in bold.

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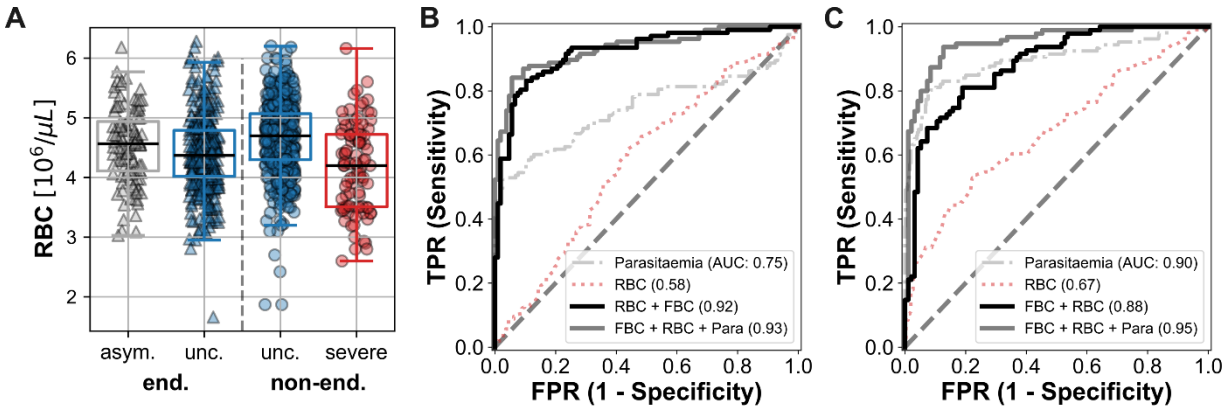
# Supplementary Figure 1




## Legend

Distribution of Red blood cells (RBCs) in individuals with different severity of *P. falciparum* infection, i.e., asymptomatic parasitaemia (asym.) and uncomplicated malaria (unc.) in the endemic setting (end.), and uncomplicated malaria and severe malaria in the non-endemic setting (non-end.). Scatter points indicate individual patient values, boxes and whiskers reflect the median, the interquartile range and 1.5 times the interquartile range, respectively (A). Prediction performance of a random forest-based classifier to distinguish asymptomatic parasitaemia from uncomplicated malaria in the endemic setting (B) and uncomplicated from severe malaria in the non-endemic setting (C) as shown in Fig. 2, including RBCs as additional markers to Full blood count parameters (FBC) with or without parasitaemia (Para).

## Alt Text

Three panels labelled from A to C with one boxplot graph A displaying the distribution of Red blood cells with different severity of *P. falciparum* infection in the endemic setting and non-endemic setting. Graph A and B displaying each 4 ROC curves of Full blood count parameters including Red blood cells indicating the prediction performance of a random forest-based classifier to distinguish between different severity groups of malaria infection in the endemic setting and non-endemic setting.