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Evaluation of host biomarkers to support the development of a point-of-care diagnostic test to guide antibiotic use in bacterial/non-bacterial acute febrile illness cases

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1 1 **Evaluation of host biomarkers to support the development of a point-of-care diagnostic**
2 2 **test to guide antibiotic use in bacterial/non-bacterial acute febrile illness cases**

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1
2 45 **ABSTRACT**

3
4 46 **Background**

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6 47 Globally, acute febrile illness (AFI) is one of the main reasons individuals present to primary
7 healthcare facilities, particularly children. Differentiating bacterial from non-bacterial AFI is
8 often difficult, in case of doubt, it is unsurprising that healthcare providers prescribe antibiotics
9 to avoid negative outcomes in their patients which leads to an increase in the spread of
10 antimicrobial resistance. Host biomarkers have the potential to inform the aetiology of AFI,
11 but which biomarkers are most appropriate in resource-limited settings remains unclear, and
12 also if its possible to utilize markers in the same way in different global settings.

13
14 54 **Methods**

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16 55 We conducted the Biomarker for Fever Diagnostic (BFF-Dx) study to evaluate 18 different
17 host biomarkers, in a prospective study of 1915 patients with non-severe AFI in Brazil (n=500),
18 Malawi (n=1000), and Gabon (n=415) using a standardized approach. Bacterial and non-
19 bacterial classifications were made based on a 2-step process using laboratory testing and a
20 clinical panel.

21
22 60 **Findings**

23
24 61 The most widely known biomarkers, hematology biomarkers and C-reactive protein (CRP),
25 remain the best-performing in this non-severely ill population with area under the receiver
26 operating characteristic (AUROCs) of 0·8 (white blood cell count) or 0·71 (CRP) in the best
27 cases. None of the evaluated novel host biomarkers exhibited high performances in
28 distinguishing bacterial from non-bacterial infections in any of the settings (AUROC<0·70 in
29 most cases) and variation across locations was observed.

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31 67 **Interpretation**

32
33 68 There is a continued need for innovation in the host-biomarker space as the available markers
34 do not meet the needs of diverse populations around the globe. This highlights the importance

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3 70 of targeted evaluations in non-severe patients in multiple settings to understand true potentials
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5 71 for real-life use. The findings highlight that not one-marker fits all settings and novel
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7 72 innovations remain urgently needed.
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74 Keywords

75 Antimicrobial Resistance, AMR, CRP, Host Biomarkers, Prospective study, biomarker, non-
76 malaria fever, primary health care, Malawi, Brazil, Gabon

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1 2 3 77 INTRODUCTION 4 5

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7 78 Globally, acute febrile illness (AFI) is one of the leading reasons individuals, particularly
8 children aged less than 5 years, present to primary healthcare facilities [1]. AFI has various
9 causes, both infectious and non-infectious, that vary according to geography, age group, and
10 season [1]. In malaria-endemic settings, malaria was long considered the primary cause of all
11 fevers; however, the introduction of rapid diagnostic tests (RDTs) for malaria in the past decade
12 has disproved this. Modelling estimates suggest that approximately 70% of all fevers can be
13 attributed to non-malarial causes, even in malaria-endemic settings [2]. In the Integrated
14 Management of Childhood Illness (IMCI), introduced by the World Health Organization
15 (WHO) and UNICEF in the mid-1990s and subsequently implemented in more than 100
16 countries, the standard “fever” algorithm currently includes a malaria RDT but no diagnostic
17 test for other infections [3]. Hence, at primary care level, the only evidence-based treatment
18 decision that can be made relies on the malaria RDT, resulting in extremely high levels of
19 antibiotic use in malaria-negative patients [4]. In this context of limited knowledge about the
20 causes of AFI and limited diagnostic and human capacity, it is unsurprising that healthcare
21 providers prescribe antibiotics to avoid negative outcomes in their patients.

22
23 93 To assist healthcare providers with clinical decision-making, a simple diagnostic tool is
24 required to differentiate patients with AFI of bacterial and non-bacterial aetiology and provide
25 appropriate care. In well-resourced settings, in both high-income countries (HICs) and low-
26 and middle-income countries (LMICs), some nonspecific host-biomarkers are used for this
27 purpose, most frequently C-reactive protein (CRP) and procalcitonin (PCT), although these
28 biomarkers are less useful in settings with a higher frequency of comorbidities [5]. Thus, in
29 2015, an international group of experts was convened to define the target product profile (TPP)
30 of such a tool, specifically for low-resource settings, to guide product development and
31 implementation as part of integrated treatment management guidelines [6]. Since then, the

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3 102 ongoing viral pandemic (SARS-CoV-2) has further highlighted the challenge of differential
4 diagnosis and shows yet again that better antimicrobial stewardship interventions are needed
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6 103 to counter the overprescribing of antibiotics in patients with viral infections [7].
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10 105 Host biomarkers other than CRP and PCT have been evaluated for distinguishing bacterial
11 from non-bacterial infections, including human neutrophil lipocalin (HNL), heparin-binding
12 protein (HBP), and chitinase 3-like protein 1 (CHI3L1) [8]. There are also some commercially
13 available tests. ImmunoXpert™, from MeMed, uses a biomarker combination comprising
14 CRP, interferon gamma-inducible protein 10 (IP-10), and TNF-related apoptosis-inducing
15 ligand (TRAIL), while FebriDx®, from Lumos Diagnostics, uses an MxA and CRP biomarker
16 combination. While these biomarker signatures show promise, they have only been evaluated
17 in limited settings. Any potential impact of co-infections or comorbidities, common in LMICs,
18 on their effectiveness is unknown. Other characteristics of host-biomarker studies that hamper
19 direct comparisons include: (i) just one/a few biomarkers in the study; (ii) small sample sizes,
20 increasing the probability of recruiting unrepresentative study populations; (iii) narrow
21 population subgroups (e.g. children only, hospitalised only, respiratory infections only, etc),
22 limiting the generalisability of study results to the broader AFI population; (iv) studies
23 conducted in one country, so co-infections/comorbidities may not be comparable with those of
24 other countries; (v) retrospective studies that used convenience sampling and case-control
25 study designs, increasing the risk of bias; and (vi) the lack of a standard definitions for
26 classifying bacterial versus non-bacterial infections [9].
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30 122 Here, we describe the Biomarker for Fever Diagnostic (BFF-Dx) study, specifically designed
31 to evaluate host biomarkers to distinguish bacterial from non-bacterial infections in line with
32 the published TPP and the final use case of such diagnostic tests. To our knowledge, this is the
33 largest study to have evaluated host biomarkers in the intended target population from the
34 intended use setting. We prospectively evaluated 18 host-biomarkers in three distinct settings,
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3 127 in Brazil, Gabon, and Malawi with the main objective to provide a performance comparison of
4 host biomarkers in the non-severe AFI population from resource-limited settings [10]. The
5 described comparison was conducted within the pragmatic context of diagnostic product
6 development and aimed to identify host biomarkers or biomarker combinations for utilization
7 in next-generation rapid diagnostic tests.
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132 METHODOLOGY

133 Study settings

134 This multinational, cross-sectional study was conducted in Brazil, Gabon, and Malawi; Gabon
135 and Malawi were selected as high-malaria endemicity settings, while Brazil was selected as a
136 low-malaria endemic setting. The study sites were UPA Manguinhos and Family Health Clinics
137 Armando Palhares in Rio de Janeiro, Brazil; the Clinical Trials Unit Center of Medical
138 Research Lambaréné (CERMEL), Lambaréné, Gabon; and Malawi Epidemiology and
139 Intervention Research Unit (MEIRU), Chilumba campus, Malawi. The enrolment sites were
140 an urban primary healthcare facility, a hospital in a semi-rural setting, and a rural primary
141 healthcare facility in Brazil, Gabon, and Malawi, respectively. Participants were recruited from
142 October 2018 to July 2019, May to November 2019, and April 2017 to April 2018, in Brazil,
143 Gabon, and Malawi, respectively. The study protocol was submitted to clinicaltrial.gov
144 (NCT03047642) and ethical approval was obtained from all relevant institutional committees
145 in Brazil, Gabon and Malawi and all details of the design have been previously published [10].
146 Reporting complies with the STARD-15 checklist.

147

148 Study population and study procedure

149 Participants were obtained through convenience sampling and included both children and
150 adults, aged between 1 and 65 years, who presented at the outpatient clinics with a history of
151 fever of ≤ 7 days duration (Brazil and Gabon) or fever at presentation (Malawi). Patients with
152 signs of severe illness were not included in the study. The overarching study protocol was
153 slightly adapted to each site due to local requirements (logistical or ethical). Detailed criteria
154 for inclusion by study sites have been published previously [10]. Outcomes were based on the
155 TPP criteria and while no patient input was used, external expert input was used to define target
156 population and criteria. Only patients who met the eligibility criteria and who provided written

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3 157 consent (patient or guardian for children) were enrolled in the study. Data and samples were
4 systematically collected and analysed as previously described. To ensure consistent quality and
5 comparability of data testing was performed using the same standard operating procedures at
6 all sites or were performed after shipment to one reference lab [10].
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10 162 **Bacterial/non-bacterial classification and biomarker selection and testing**

163 A two-step process was used to classify the patients into “bacterial” and “non-bacterial”
164 groups. Briefly, the cause of fever (bacterial/non-bacterial) was first classified according to
165 laboratory-determined parameters (“electronic group”). Next, cases that could not be classified
166 by laboratory-determined parameters were assessed by a panel of three independent clinical
167 experts. These assessments, which were based on a patient’s history and clinical and laboratory
168 data, were then compared. If the three panel members unanimously assigned a diagnostic label,
169 patients were considered to have “bacterial” or “non-bacterial” infections; if two out of three
170 panel members reported a classification of “bacterial” or “non-bacterial”, these patients were
171 considered to have “probable bacterial infection” or “probable non-bacterial infection”,
172 respectively.

173 Data were analysed based on three groups of patients: 1) the “electronic group”, i.e. subjects
174 with a cause of fever defined based on laboratory parameters; 2) the “strict group”, which
175 comprised the electronic group and the patients that were unanimously classified by the clinical
176 panel of three experts; and 3) the “loose group”, which comprised the electronic and strict
177 groups as well as those patients for whom two of the clinical experts agreed they had either
178 probable bacterial or probable non-bacterial infection. Subjects with undetermined cause of
179 fever according to the three classification criteria considered (“electronic group”, “strict
180 group”, “loose group”) were excluded from the statistical analysis. This outcome-oriented

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3 181 approach, based on methods previously developed for host-biomarker studies and described
4 previously, was used to ensure the total intended-use population of any future test was
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6 182 represented in the final analysis [10, 11].
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10 184 The evaluated biomarkers were selected based on previously reported performances, and
11 haematological markers as well as CRP were included as comparators (Table 1 and
12
13 185 Supplementary Table 1) [8, 12].
14
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16 187 At the end of data collection, all biomarker data were analysed to assess the percentage of
17 missing values and the percentage of values below the lower limit or above the upper limit of
18 detection of the used tests. Biomarkers with more than 50% of missing data or more than 95%
19 of saturated values below the lower limit of quantification of the used test, were excluded from
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21 190 the following statistical analysis.
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3 192 **Table 1.** Novel biomarkers identified in the literature and evaluated in the BFF-Dx study,
4 including sample type used, evaluation method, and sample origin.
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Abbreviation	Biomarker name	Sample type	Evaluation method	Sample origin
AGP	A-1-acid glycoprotein	EDTA-plasma	Luminex	B, G, M
C2	Complement 2	EDTA-plasma	Luminex	B, G, M
C4b	Complement C4b	EDTA-plasma	Luminex	B, G, M
CHI3L1	Chitinase-3-like protein 1	EDTA-plasma	Luminex	B, G, M
CRP	C-reactive protein	EDTA-plasma	CRP Nycocard/ NycoCardReader II, ELISA	B, G, M
Gal-9	Galectin-9	EDTA-plasma	Luminex	B, G, M
HBP	Heparin-binding protein	EDTA-plasma	ELISA	B, M
HNL	Human neutrophil lipocalin	Heparin-activated plasma time-controlled activation#	ELISA	M
		EDTA-plasma	ELISA	B, G, M
HP	Haptoglobin	EDTA-plasma	Luminex	B, G, M
IFN-gamma	Interferon gamma	EDTA-plasma	Luminex	B, G, M
IL-4	Interleukin-4	EDTA-plasma	Luminex	B, G, M
IL-6	Interleukin-6	EDTA-plasma	Luminex	B, G, M
IP-10	Gamma-induced protein 10	EDTA-plasma	Luminex	B, G, M
LBP	Lipopolysaccharide binding protein	EDTA-plasma	Luminex	B, G, M
NGAL	Neutrophil gelatinase-associated lipocalin	Frozen heparin-activated plasma	Luminex	M
		EDTA-plasma	Luminex	B, G, M
PCT	Procalcitonin	EDTA-plasma	Luminex; ELISA	B, G, M
sPLA2	Secretory phospholipase 2	EDTA-plasma	Luminex	B, G, M
sTREM-1	Soluble triggering receptor expressed on myeloid cells 1	EDTA-plasma	Luminex	B, G, M
TRAIL	TNF-related apoptosis-inducing ligand	EDTA-plasma	Luminex	B, G, M

194 B, Brazil; G, Gabon; M, Malawi

195 # Whole blood samples were collected in lithium heparin tubes and activation was performed within 60 min prior to freezing
196 and subsequent ELISA testing [13]. All biomarkers were tested using the same standard operating procedures (SOPs) and all
197 sites were trained on the SOPs. For CRP and PCT different devices were used at different sites, repeat testing was performed
198 at the central facility (NMI).

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3 201 **Statistical analysis**

4
5 202 a. Kruskal-Wallis Analysis and Definition of Covariates Influence on Biomarkers

6
7 203 A Kruskal-Wallis test, adjusted by Benjamini-Hochberg, was performed for each biomarker to
8 identify which covariates significantly affect the biomarker value. The covariates studied were
9 country (i.e., the country of origin of the patients), age, sex, malaria status, comorbidities (i.e.,
10 presence of one or more diseases among cardiovascular, neurological, respiratory, renal,
11 genitourinary, connective tissue, cancer, or infectious diseases), malnutrition status calculated
12 based on WHO body mass index criteria, self-reported use of antibiotics prior to visiting the
13 health facility, axillary temperature $\geq 38^{\circ}\text{C}$, and positive result to Chikungunya test. The
14 Kruskal-Wallis test was performed for each of the three patient groups defined in the previous
15 section (“electronic”, “strict”, “loose”). The results of the Kruskal-Wallis test allowed the
16 identification of covariates that most significantly impacted the biomarker distribution
17 ($p < 0.001$, adjusted by Benjamini-Hochberg). The most significant covariates were considered
18 for defining subgroups of patients in which the following univariate analyses were performed,
19 or included as covariates in the multivariate analyses.

20
21 216 a. Univariate analysis

22 As exploratory step, it was studied the ability of each biomarker to discriminate between
23 bacterial and non-bacterial infections was assessed by the area under the receiver operating
24 characteristic curve (AUROC). In particular, subjects were ranked based on the values of the
25 single variable of interest (i.e. based on ordered values) and, using this as score, calculated the
26 ROC curve and the corresponding area under the curve. Such univariate analysis was
27 conducted for each patient group (“electronic”, “strict”, “loose”) and specific patient subgroup
28 (Malaria status, Country and Age).

29
30 224 b. Multivariate analysis

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2
3 225 Multivariate classification models were developed to assess the discrimination ability of
4 combinations of biomarkers and covariates. For the multivariate analysis, both linear (logistic
5 regression) and non-linear classification models (RuleFit) were explored [14]. The candidate
6 features for each model included a group of host-biomarkers and some additional covariates
7 (age, temperature, fever duration, diastolic blood pressure, respiration rate, and pulse rate).
8
9 229 Regarding host-biomarkers, three different groups of biomarkers were considered:
10 haematology biomarkers only (i.e. white blood cell, neutrophil, red blood cell, lymphocyte
11 counts), protein biomarkers only (i.e. novel biomarkers + CRP), and haematology plus protein
12 biomarkers (i.e. all biomarkers).
13
14 234 For each patient subgroup and each candidate feature set, three multivariate models were
15 developed: i) a logistic regression model with stepwise (SW) feature selection; ii) a logistic
16 regression model with features selected based on recursive feature addition (RFA; a variant of
17 the method proposed in [15]); iii) RuleFit, a non-linear model in which a set of rules from an
18 ensemble of decision trees (typically from a tree-based model like a Random Forest or Gradient
19 Boosted Trees) is generated and then fit a sparse linear regression model (regularized with
20 LASSO), where the features are the rules generated from the trees [14, 15].
21 To further tackle the number of biomarkers and variables included in the best models, we
22 introduced an additional selection step, employing a plateau seeking approach. The primary
23 objective of this approach was to pinpoint a concise set of variables capable of attaining an
24 AUROC score similar to that of our comprehensive model, which already incorporated the
25 most impactful and previously selected variables. This was to ensure that our model is not only
26 effective in terms of performance but also efficient in its variable inclusion.
27
28 247 Each model was trained and tested using the following pipeline. The data were randomly split
29 into training and test sets (80% and 20% of the data, respectively) stratifying by the outcome
30 variable. Missing data in the training and test sets were imputed using the MICE (multiple
31 imputation by chained equations) package in R [16]. The first step in the pipeline was to
32 identify the variables that were significantly associated with the outcome. This was done by
33 fitting a logistic regression model with stepwise (SW) feature selection. The variables that
34 were selected in this step were then used to fit a logistic regression model with recursive feature
35 addition (RFA). The variables that were selected in this step were then used to fit a logistic
36 regression model with stepwise (SW) feature selection. The variables that were selected in this
37 step were then used to fit a logistic regression model with recursive feature addition (RFA). The
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51 recursive feature addition (RFA). The variables that were selected in this step were then used to fit
52 a logistic regression model with stepwise (SW) feature selection. The variables that were selected
53 in this step were then used to fit a logistic regression model with recursive feature addition (RFA).
54 The variables that were selected in this step were then used to fit a logistic regression model with
55 stepwise (SW) feature selection. The variables that were selected in this step were then used to fit
56 a logistic regression model with recursive feature addition (RFA). The variables that were selected
57 in this step were then used to fit a logistic regression model with stepwise (SW) feature selection.
58 The variables that were selected in this step were then used to fit a logistic regression model with
59 recursive feature addition (RFA). The variables that were selected in this step were then used to fit
60 a logistic regression model with stepwise (SW) feature selection.

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3 250 imputation by chained equation) algorithm. The `n_imp` parameter for MICE imputation was
4 set to 1, resulting in a single imputed dataset; however, the imputation process was integrated
5 in a robust bootstrapping pipeline, generating ten independent datasets. This approach ensured
6 variability in our results, stemming not only from the MICE imputation but also from the
7 bootstrapping process. This dual approach guarantees that each imputed dataset is distinct [16].
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9 255 All quantitative variables were scaled into the range [0,1] by subtracting their minimum value
10 and dividing by the difference between the maximum and minimum values in the training set.
11
12 257 The categorical variables with n categories were encoded using n-1 binary “dummy” variables.
13
14 258 The model was then trained on the imputed and scaled training set, and its performance was
15 assessed on the imputed and scaled test set by computing the AUROC. The AUROC on the
16 test set was also calculated for single host biomarkers, to allow a fair comparison of the
17 performance of the multivariate classification models vs. single host biomarkers.
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20 262 To assess the robustness and variability in the results of the developed models, the entire
21 pipeline were bootstrapped, i.e. it was run ten times with different random training-test set
22 splits. Finally, the mean and the standard deviation (SD) or the minimum and maximum
23 reached of the AUROC across the ten training-test splits were calculated for each multivariate
24 model and each single host biomarker.
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c. Software

267 All statistical analyses and model development were performed using the R programming
268 language (version 4.1.2). Specifically, the *mice* package was used for data imputation, while
269 the *pre* and *stats* packages were used for RuleFit and logistic regression model development,
270 respectively.

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273 Role of the funding source

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3 274 The funding organisations had no role in the study design, data collection, analysis and
4
5 275 interpretation of data. Further they had no role in writing of the report or decision to submit for
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7 276 publication.
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3 277 **RESULTS**
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8 278 **Study population**
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13 279 In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415;
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15 280 Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male.
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18 281 Children aged <5 years comprised 45/500 (9%), 182/415 (43.9%), and 367/1000 (36.7%)
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20 282 participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4)
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In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415; Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male. Children aged <5 years comprised 45/500 (9%), 182/415 (43.9%), and 367/1000 (36.7%) participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4) years (Table 2). Detailed baseline characteristics of patients and analyses of differences will be described in a separate manuscript (Alabi et al in preparation).

285 **Table 2: Baseline characteristics of patients.**

	Brazil	Gabon	Malawi	All
0–5 years (median, IQR, n)	3, [2-4], 45	3, [2-5], 182	3, [2-4], 137	3, [2-4], 594
5–15 years (median, IQR, n)	11, [8-14], 85	9, [7-12], 214	9 [7-12], 26	9, [7-12], 575
>15 years (median, IQR, n)	34, [24-45], 370	16, [16-16.5], 19	28, [21-35], 357	30, [21-42], 746
Male (%), n	49.6%, 248	45.1%, 187	42.7%, 217	45.0%, 862
Temperature, °C (median, IQR, n)	37.7, [36.7-38.4], 500	36.8, [36.4-37.4], 415	38.1, [37.7-38.8], 999	37.8, [37.3-38.5], 1914
WBC count, 10 ⁹ /L (median, IQR, n)	7.28, [5.47-10.39], 494	7.7, [5.7-10], 411	6.7, [5.1-9.8], 985	7.1, [5.3-9.8], 1890
Neutrophil count, 10 ⁹ /L (median, IQR, n)	4.97, [3.63-7.4], 494	2.77, [1.96-3.9], 408	4.3, [3.6-18.1], 906	4.1, [2.8-6], 1812
RBC count, 10 ⁹ /L (median, IQR, n)	40.1, [36.5-43.2], 494	33.2, [29.4-35.8], 412	36.2, [33.3-39.5], 984	36.3, [33-40.2], 1892
Lymphocyte count, 10 ⁹ /L (median, IQR, n)	1.15, [0.7-1.99], 493	2.73, [1.8-4.16], 411	1.5, [1.2-2.2], 982	1.63, [1-2.6], 1883
CRP NycoCard# – mg/L (median, IQR, n)	70.5, [35-98.75], 498	28, [5-73], 415	47, [12-66.6], 987	49, [13-98], 1900
Malaria-positive by RDT on-site (% all, n)	0.2%, 1	56.4%, 234	45.9%, 458	36.2%, 693
Malaria-positive by qPCR or microscopy (% all, n)	-	-	50.5%, 555	-
HIV-positive by RDT (% all, n)	1.4%, 7	1.2%, 5	4.2%, 44	2.8%, 54
History of antibiotic-use pre-presentation (% all, n)	8.8%, 44	2.41%, 10	7.2%, 74	6.5%, 124
History of antipyretic-use pre-presentation (% all, n)	83.2%, 416	79.76%, 331	55.1%, 511	62.2%, 1298

NycoCard was found to be equivalent to reference testing in the relevant range (Supplementary Figure 1). CRP, C-reactive protein; IQR, interquartile range; qPCR, quantitative PCR; RBC, red blood cell; RDT, rapid diagnostic test; WBC, white blood cell; - data not available.

288 Bacterial and non-bacterial outcomes by classification groups

289 Using the electronic classification grouping, 15·1% (290/1915) of cases were bacterial
290 infections, 20·2% (387/1915) were non-bacterial infections, and 64·5% (1238/1915) had an
291 undetermined cause of fever (Figure 1). Under the strict classification grouping, 24·3%
292 (366/1509), 66·9% (1010/1509), and 9·0% (133/1509) were classified as bacterial, non-
293 bacterial, and undetermined infections, respectively, while using the loose classification
294 grouping 25·7% (491/1915), 67·3% (1286/1915), and 7·0% (133/1915) were classified as
295 bacterial, non-bacterial, and undetermined infections, respectively (Figure 1). Subjects with
296 undetermined cause of fever/infections were excluded from the following univariate and
297 multivariate analyses.

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299 Exclusion of biomarkers with too many missing or saturated values

300 The biomarkers C4b, HNL and PCT had more than 50% missing values and were therefore
301 excluded. The high number of missing values is due to fact that biomarkers were analysed in
302 groups based on the required dilution using Luminex platform. For some biomarkers the
303 dilution was not optimal, and it was only possible to re-measure biomarkers with a different
304 dilution a limited number of times. IFN-gamma and sTREM-1 were excluded due to more than
305 95% of values saturated to the minimum/maximum level detectable by the measurement
306 instrument. All the biomarkers retained in the analysis had less than 12% missing values
307 (Supplementary Table 3).

308 Identification of relevant subgroups for analyses

309 According to the Kruskal-Wallis analysis on the “electronic group”, the variables “country”,
310 “malaria status” and “age” had a strong ($p<0.001$) or high ($0.001< p<0.01$) effect on many of
311 the host biomarkers (Supplementary Table 4). The variables “sex”, “comorbidities”, “history
312 of antibiotic use” showed no ($p>0.05$) or slight ($p<0.05$) associations with all the host
313 biomarkers. The effects of “chikungunya status” and “fever above 38°C” were generally
314 significant ($p<0.01$), but the sample sizes for these groups were either too small or exhibited
315 an imbalance. Primarily centered on populations grouped by study country and malaria status
316 variables - both of which were strongly associated with the biomarker value in the “strict”
317 and “loose” groups (Supplementary Table 5, 6) - other significant covariates were also
318 included in the multivariate analysis. This inclusion was due to their influence, and factors
319 like the study country were considered as variables in the overall scenario.

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321 Individual host-biomarker performance – univariate analysis

322 The performance of 18 host biomarkers was consistent across the three patient classification
323 groups in each of the settings (Table 3). White blood cell (WBC) and neutrophil counts were
324 the most effective biomarkers for differentiating bacterial and non-bacterial infections. For the
325 malaria-negative population, the mean (95% confidence interval) of AUROC for WBCs was
326 between 0.60 (0.48–0.72) and 0.83 (0.77–0.88) and for neutrophils it was between 0.67 (0.57–
327 0.77) and 0.80 (0.74–0.86) across the three countries and the three groups (“electronic”,
328 “strict”, “loose”). Neutrophil and WBC counts showed the highest AUROCs in the Brazilian
329 population, between 0.80 (0.74–0.86) and 0.83 (0.77–0.88), respectively. All protein
330 biomarkers showed relatively poor performances (<0.7 in most cases, Table 4) in all three
331 settings. Galactin-9, CRP, IP-10, and NGAL were the best-performing protein biomarkers
332 across the three settings and criteria. Protein biomarkers showed better performances in Malawi

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3 333 and Gabon, as in Brazil most protein biomarkers showed performances of <0·6. When the
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5 334 biomarker results were stratified by age, the AUROCs were slightly higher for children (≤ 15
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7 years) compared with those seen for adults in the malaria-negative population (Supplementary
8
9 Tables 9-11). Among the malaria-positive population, WBC, lymphocyte, and neutrophil
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11 counts were the best-performing biomarkers in both Gabon and Malawi (in most cases between
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13 337 0·6 and 0·7).
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Table 3: Univariate analysis of 18 individual biomarkers[#] among malaria-negative patients. Common biomarkers such as CRP and haematological biomarkers were included for reference. In this context we defined performance as follows: green (AUROC ≥ 0.7), yellow (AUROC > 0.65 and < 0.7), orange (AUROC 0.55 – 0.65), and red (AUROC < 0.6).

	Brazil			Gabon			Malawi		
	AUROC** (CI), N			AUROC** (CI), N			AUROC** (CI), N		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
Haematological biomarkers									
Lymphocyte count	0.67 (0.59-0.74), 257	0.66 (0.59-0.72), 408	0.66 (0.6-0.72), 442	0.58 (0.45-0.71), 81	0.52 (0.4-0.63), 167	0.55 (0.45-0.65), 222	0.66 (0.47-0.64), 254	0.51 (0.45-0.58), 303	0.52 (0.47-0.58), 461
Neutrophil count	0.77 (0.7-0.84), 257	0.8 (0.74-0.86), 408	0.79 (0.73-0.84), 442	0.78 (0.66-0.89), 80	0.72 (0.62-0.83), 165	0.67 (0.57-0.77), 219	0.78 (0.58-0.74), 243	0.73 (0.67-0.79), 273	0.7 (0.65-0.76), 414
RBC count	0.61 (0.52-0.69), 258	0.58 (0.51-0.65), 408	0.58 (0.51-0.64), 442	0.55 (0.41-0.68), 81	0.52 (0.41-0.63), 167	0.53 (0.43-0.63), 222	0.66 (0.46-0.55), 255	0.53 (0.46-0.59), 305	0.56 (0.5-0.61), 463
WBC count	0.81 (0.75-0.87), 257	0.83 (0.77-0.88), 408	0.82 (0.77-0.87), 442	0.67 (0.54-0.79), 81	0.6 (0.48-0.72), 167	0.61 (0.5-0.71), 222	0.66 (0.56-0.78), 255	0.72 (0.66-0.78), 304	0.68 (0.63-0.73), 461
Protein biomarkers									
AGP	0.59 (0.51-0.68), 252	0.54 (0.47-0.61), 402	0.52 (0.46-0.59), 434	0.77 (0.65-0.9), 80	0.7 (0.59-0.82), 163	0.65 (0.55-0.75), 220	0.66 (0.46-0.66), 258	0.54 (0.48-0.6), 309	0.54 (0.49-0.59), 466
Chitinase 3-like 1	0.58 (0.5-0.66), 246	0.54 (0.47-0.6), 394	0.55 (0.49-0.61), 424	0.6 (0.46-0.74), 79	0.6 (0.48-0.72), 162	0.62 (0.52-0.72), 217	0.69 (0.39-0.69), 255	0.5 (0.43-0.56), 304	0.5 (0.44-0.55), 462
CRP*	0.61 (0.52-0.69), 259	0.61 (0.54-0.68), 412	0.62 (0.55-0.68), 446	0.71 (0.59-0.82), 81	0.65 (0.55-0.75), 167	0.63 (0.53-0.72), 224	0.65 (0.45-0.65), 256	0.6 (0.54-0.67), 305	0.58 (0.53-0.63), 462
IP-10/IP-10/CRG-2	0.6 (0.52-0.68), 252	0.53 (0.46-0.59), 402	0.53 (0.47-0.59), 434	0.6 (0.48-0.73), 80	0.51 (0.4-0.62), 164	0.52 (0.43-0.62), 221	0.66 (0.56-0.66), 258	0.6 (0.53-0.66), 309	0.61 (0.56-0.66), 466
Galectin-9	0.63 (0.55-0.71), 252	0.56 (0.49-0.63), 401	0.57 (0.5-0.63), 433	0.7 (0.58-0.83), 80	0.6 (0.48-0.71), 163	0.54 (0.43-0.64), 219	0.69 (0.52-0.68), 258	0.61 (0.55-0.67), 309	0.63 (0.57-0.68), 466
hCC2	0.51 (0.43-0.6), 244	0.51 (0.44-0.58), 392	0.52 (0.46-0.59), 424	0.55 (0.41-0.69), 77	0.52 (0.4-0.64), 159	0.51 (0.41-0.61), 216	0.59 (0.49-0.69), 258	0.55 (0.49-0.62), 309	0.55 (0.5-0.6), 466
HBP***	0.67 (0.52-0.81), 113	0.68 (0.55-0.8), 144	0.64 (0.51-0.76), 151	0.53 (0.39-0.68), 153	0.55 (0.44-0.66), 106	0.52 (0.41-0.63), 124

HPTGN	0·48 (0·4-0·57), 248	0·51 (0·44-0·58), 398	0·51 (0·45-0·58), 430	0·64 (0·5-0·78), 77	0·62 (0·51-0·74), 159	0·55 (0·45-0·66), 214	0·54 (0·45-0·57), 157	0·51 (0·45-0·58), 307	0·51 (0·46-0·57), 464
IL-4	0·58 (0·5-0·65), 249	0·53 (0·47-0·59), 398	0·54 (0·48-0·59), 429	0·46 (0·4-0·52), 79	0·49 (0·45-0·53), 163	0·51 (0·47-0·55), 220	0·48 (0·44-0·57), 157	0·48 (0·42-0·53), 306	0·47 (0·42-0·51), 463
IL-6	0·49 (0·43-0·54), 247	0·49 (0·44-0·54), 395	0·48 (0·43-0·52), 426	0·51 (0·47-0·55), 80	0·51 (0·48-0·55), 164	0·51 (0·47-0·55), 221	0·50 (0·47-0·58), 158	0·61 (0·55-0·67), 307	0·59 (0·54-0·64), 465
LBP	0·58 (0·5-0·66), 248	0·54 (0·48-0·61), 397	0·52 (0·46-0·58), 429	0·69 (0·56-0·83), 78	0·67 (0·55-0·78), 160	0·6 (0·5-0·71), 217	0·56 (0·42-0·57), 157	0·54 (0·47-0·61), 267	0·53 (0·47-0·59), 394
Lipocalin-2/NGAL	0·49 (0·41-0·57), 249	0·51 (0·44-0·57), 396	0·51 (0·44-0·57), 428	0·67 (0·54-0·8), 79	0·6 (0·49-0·72), 163	0·58 (0·48-0·68), 219	0·56 (0·46-0·56), 156	0·65 (0·59-0·72), 265	0·61 (0·56-0·67), 392
sPLA/Lp-PLA2	0·54 (0·46-0·62), 252	0·53 (0·46-0·59), 402	0·52 (0·45-0·58), 434	0·58 (0·44-0·71), 80	0·54 (0·43-0·65), 164	0·58 (0·48-0·68), 221	0·53 (0·47-0·58), 158	0·55 (0·49-0·61), 308	0·56 (0·51-0·61), 466
TRAIL	0·56 (0·49-0·64), 252	0·53 (0·47-0·59), 402	0·53 (0·48-0·59), 434	0·5 (0·5-0·5), 74	0·5 (0·49-0·5), 156	0·49 (0·48-0·5), 212	0·51 (0·51-0·51), 157	0·62 (0·56-0·68), 306	0·62 (0·57-0·67), 463

*CRP was measured with a NycoCard device. **AUROC has a value between 0 and 1, where 1 corresponds to an effect classifier, 0·5 to one that assigns classes randomly. #Freeze-thaw experiments to evaluate the stability of the biomarkers after five cycles (referred to as “treated”) were performed with Luminex 9- and 2-plexes. Three samples each were freeze-thawed up to six times and compared with samples after the first thawing (referred to as “untreated”; biomarkers were considered stable with 80–120% recovery). Samples were analysed in triplicate and showed good stability up to five freeze-thaw cycles for all analytes showing acceptable results, except for the C2 and C4b biomarkers (C2: 2/3 [66·7%] samples were stable; C4b: two samples failed the sixth freeze-thaw cycle). As a result, these biomarkers were excluded as they would never be suitable as the basis of a diagnostic test. ***HBP was evaluated in a small group of patients in Malawi and Brazil; however, HBP did not show promise and was not evaluated further.

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3 **349 Combinations of host-biomarkers and additional covariates – multivariate analysis**

4
5 350 The best-performing biomarkers in the univariate analysis were compared with the best
6 performances from the multivariate analyses, which several feature-selected biomarkers and
7 covariates (Table 4 and Supplementary Tables 15-20). In most cases the best combination of
8 biomarkers showed higher AUROCs than the top-performing individual biomarkers, with a
9 low/moderate “gain” (range 1–13%). The best-performing AUROCs were very similar,
10 irrespective of the multivariate model used, especially for the “strict” and “loose” groups
11 (difference in AUROC range 0·02–0·03 for Malawi and Brazil). Biomarkers identified as top
12 performing by the multivariate analyses differed depending on the model used. While SW and
13 RFA selected three to five biomarkers or combinations, RuleFit selected more biomarkers (ten
14 variables on average) to be part of the signature. The relatively low increase in AUROC when
15 comparing the top-performing single biomarker with multivariate models indicates that
16 biomarkers in addition to the single best-performing biomarker do not make a major
17 contribution.

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Table 4: Multivariate analysis of biomarkers among malaria-negative patients, including the gain/loss of performance when comparing multivariate analysis and single host-biomarkers comprising both haematological and protein host-biomarkers.

Classification group	Best multivariate model/models: mean (min-max) AUROC	Best host-biomarker: mean (min-max) AUROC	Multivariate AUROC gain/loss (%) *** multivariate and single host-biomarkers ratio
Overall (Brazil + Gabon + Malawi)*			
L	SW/RFA/RF:0·75 (0.69-0.81)	WBC count: 0·7 (0.64, 0.76)	+7%
S	SW:0.83 (0.75 - 0.91)	WBC count: 0·78 (0.72 - 0.84)	+6%
E	SW/RFA:0.83 (0.77 - 0.89)	WBC count: 0·77 (0.69 - 0.85)	+8%
Brazil			
L	SW: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
S	RFA: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
E	SW: 0·85 (0.73 - 0.97)	WBC count: 0·83 (0.69 - 0.97)	+2%
Gabon**			
L	SW/RFA: 0·7 (0.46 - 0.94)	WBC count: 0·7 (0.64 - 0.76)	..
S	SW/RFA: 0·76 (0.52 - 0.96)	WBC count: 0·78 (0.72 - 0.84)	-3%
E	RFA: 0·77 (0.63 - 0.91)	WBC count: 0·77 (0.69 - 0.85)	..
Malawi			
L	SW/RFA: 0·74 (0.62 - 0.86)	neutrophil count: 0·72 (0.66 - 0.78)	+3%
S	SW: 0·73 (0.61 - 0.85)	neutrophil count: 0·72 (0.58 - 0.86)	+ 1%
E	RFA: 0·72 (0.60 - 0.84)	WBC count: 0·7 (0.56, 0.84)	+ 2%

366 E, electronic classification group; S, strict classification group; L, loose classification group; RF, RuleFit; RFA, logistic
 367 recursive feature addition; SW, stepwise logistic regression.
 368

369 * In the “Overall” scenario, the model was developed using the data of all countries and the variable indicating the country
 370 was used as a covariate in the model.

371 **Multivariate performances for Gabon were computed using as a predictor model the model trained in the “Overall” scenario
 372 (all participants from the three analysed countries) then evaluated using Gabon data only. Indeed, the sample size of Gabon
 373 data was not sufficient to allow the development of a reliable model specific for this country.

374 *** Performance comparison was computed as: [(multivariate AUROC – univariate AUROC) / univariate AUROC] * 100
 375 Green (gain, i.e. the multivariate models show better performances than univariate models); red (loss, i.e. the univariate models
 376 show better performances than multivariate models).

377 DISCUSSION

378 We present the most extensive and diverse host-biomarker evaluation study to differentiate
379 bacterial from non-bacterial infections in LMICs. The study aimed to identify if next-
380 generation host-biomarkers for distinguishing bacterial from non-bacterial cases of AFI, which
381 could replace existing biomarkers such as CRP, PCT, and WBC/neutrophil assessments. The
382 data show that none of the promising host-biomarkers exhibited high AUROCs in our non-
383 severe AFI population in either low malaria prevalence (Brazil) or high malaria prevalence
384 (Gabon, Malawi) settings. Haematology biomarkers and CRP were included a baseline to
385 identify better-performing markers; however, they remain those with the highest AUROC
386 values (approximately 0·60–0·70 AUROC) in our population.

387 Overall, the performance of all markers was underwhelming, yet not surprising. It aligns with
388 previous data where a marked reduction in performance was observed when shifting the
389 population from in- to outpatients [17-19]. Previously, it was hypothesised that the decrease in
390 performance in host biomarkers between HIC and LMIC settings, or even between Africa and
391 Asia, was due to the untreated comorbidities (e.g. diabetes, malaria, neglected tropical diseases)
392 which contribute to inflammation and the nonspecific triggering of host biomarkers, unrelated
393 to the current acute presentation [19, 20]. In our data the performance was indeed poorer in
394 malaria-positive patients (AUROC <0·6); however, even in the malaria-negative population,
395 biomarkers showed low performances (~0·6–0·7) in our cohort. Similarly, sex and arboviral
396 status appeared to have no major effect on biomarker performance. Notably, Our data notably
397 indicated that combining biomarkers can enhance performance. However, this improvement
398 was not consistently observed. When combining several biomarkers and additional covariates,
399 the “gain” in AUROC values was low/moderate (range 1–13%) compared to the top-
400 performing individual biomarkers. From a diagnostic development perspective, a low gain in

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3 401 performance would not justify the additional complexity and cost of developing a simple
4 402 multiplex test.

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7 403 Adding to the challenges of host-biomarker studies is the lack of consistent reference standards
8 404 and that most studies have focused their analyses solely on the subpopulation of patients with
9 405 a microbiologically confirmed diagnosis. This approach ignores the largest group (>70%) of
10 406 patients and intended-use population of any future test [21]. The group with laboratory
11 407 confirmed diagnosis will decrease further in the non-severe AFI population; presenting at
12 408 primary care level. Going forward more clarity will likely follow as a recent host-biomarker
13 409 test (BVtest, MeMed, Israel) was approved by the FDA and subsequent guidance will prescribe
14 410 more clearly how studies have to be designed to standardize the classification of “bacterial”
15 411 vs “non-bacterial” evaluated to guide prescribing for bacterial or non-bacterial infections [9,
16 412 22]. Our protocol is aligned with the FDA approved classification hence we are confident our
17 413 methodology is robust.

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19
20 414 While our study aimed to mitigate the challenges described, it still had several limitations. The
21 415 study did not include a control group, so no baseline information was available for biomarker
22 416 performance or asymptomatic carrier populations. The enrolment period in Brazil and Gabon
23 417 lasted for less than one year and given the heterogeneity of causes of AFI across time a the
24 418 performance of the biomarkers may not be generalisable to different times of the year and
25 419 geographical settings, particularly in Asia. The study utilised a two-step process to classify
26 420 outcomes, and the clinical classification based on recorded clinical information may have
27 421 introduced subjectivity. Notably, clinicians had access to the haematology biomarker results
28 422 (WBCs, neutrophils) during outcome classification, which might have introduced a bias in
29 423 favour of these biomarkers. However, comparing AUROCs between all classification groups
30 424 (E, L, S) suggests this potential bias had no major impact as the results are similar across
31 425 groups. There were some heterogeneities in the inclusion criteria across the various study sites,

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3 426 including age groups and fever criteria. In Brazil and Gabon, the inclusion criterion was a
4 427 history of fever in the past 7 days, while it was fever at presentation in Malawi. Studies have
5 428 found that acute fever at presentation has implications for the interpretation of host biomarkers
6 429 [23]; however, our sub-analysis by acute fever showed no differences, so we do not consider
7 430 that these different inclusion criteria impacted interpretation. Despite best efforts to standardise
8 431 procedures, there was a level of adaptability required in the choice of testing methods by the
9 432 clinical teams in each country, in particular for arbovirus and respiratory pathogen detection.
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20 433 Overall, the results of this diverse study highlight the difficulties in identifying single host-
21 434 biomarkers or simple host-biomarker combinations that can help solve the problem of
22 435 undifferentiated prescribing at primary healthcare, particularly to be used across diverse global
23 436 settings. On the seventh birthday of the original TPP for a diagnostic assay to distinguish
24 437 bacterial and non-bacterial infections in resource-limited settings, a more recent consultation
25 438 confirmed that the need for such an assay remains and is in fact increasingly urgent [6, 24]. Yet
26 439 again, the consultation concluded primary healthcare clinics and their equivalents must have
27 440 the ability to perform tests other than just malaria RDTs [24]. The lack of diagnostics
28 441 infrastructure at the lower levels of health systems is well documented and requires urgent
29 442 improvement to support medical staff in their decision making. While no novel host-biomarker
30 443 assay meets these needs, evidence for existing biomarkers, e.g. CRP, and various haematology
31 444 biomarkers, should be utilised to drive such improvements, albeit utilizing slightly different
32 445 approaches and cut-offs across settings. Recent studies have shown that even simple host-
33 446 biomarkers, such as CRP, can have a major impact on how clinical staff use antibiotics [25,
34 447 26]. The current study confirms that the existing biomarkers are imperfect and hence should
35 448 only be used as guidance, in conjunction with expanded clinical algorithms [27, 28]. Such
36 449 guidelines, alongside adopted policies and accessible haematology/biochemistry data could
37 450 enable healthcare workers to use simple tools to gain additional data points to help form a more

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3 451 evidence-based diagnosis that has to be guided by the local epidemiology. Optimising existing
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5 452 haematology or biochemistry tools and their maintenance requirements to meet the needs of
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7 453 low resourced settings could be one step towards more expanded use of these well-known
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9 454 markers. In conclusion, our study reinforces the continued need for innovation in the host-
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11 455 biomarker space and highlights the importance of targeted evaluations of such innovations, in
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13 456 diverse intended-use settings, to fully understand their true value.
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For peer review only

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4

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16 467 **Declaration of Interest**
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19 diagnostic during the study period.
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21 470
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23 471 **Author contribution**
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25 472 SD, CE, SO, AM, AMS, SG, STA, MML, ATA conceptualised the study and study design;
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27 473 CE, AS, SG, STA, AMS, JKM, VH, JM, ALK, AA, JCBO, MML, PNE, JAM, PB, LB, AdRM,
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29 474 BCC, MAMS, AMBdF, EAdS, RdS, MCSL, JH, AG, MJ, NSM, CH, SJL, implemented the
30 study and data collection; MA, MV, SL, SO, BDC, BLFC, SD, SP, SG, AMS, STA conducted
31 data analysis and interpretation. BLFC, SD wrote the first draft of the manuscript and all
32 authors contributed to the final version of the manuscript.
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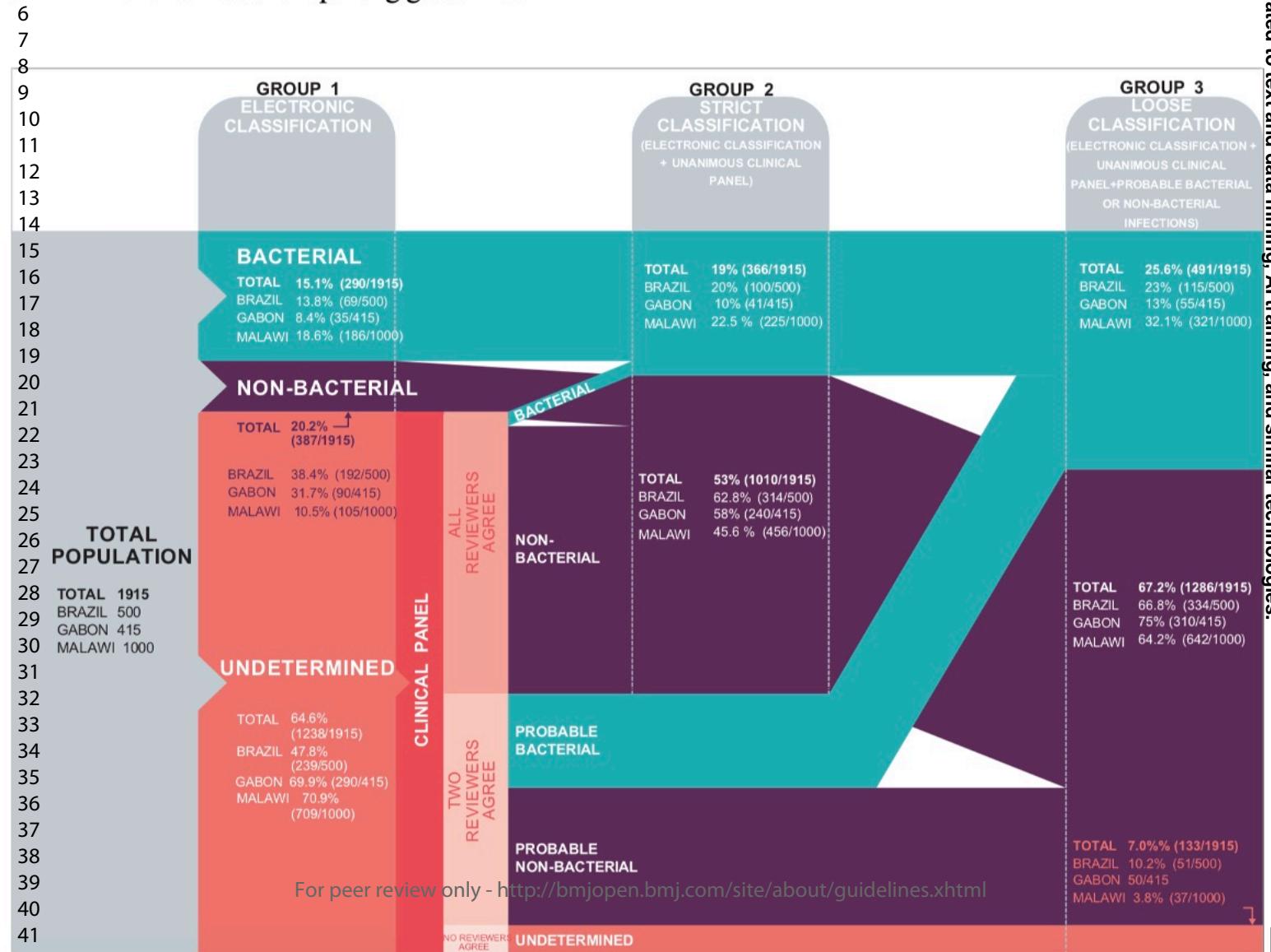
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Figure 1: Classification criteria to assign bacterial versus non-bacterial infection categories for the analysis.

The flows in different colours (turquoise=bacteria, purple=non-bacterial, red=undetermined) represent the proportion of patients that were assigned into the respective group (bacteria/non-bacteria/undetermined) after each classification step. Group 1 representing only patients assigned using laboratory data; group 2 representing patients with a unanimous decision after review by the clinical panel; group 3 including all patients, even if only 2 panel members agreed on the probable cause. The study follows the STARD-15 checklist and reporting guidelines.



Supplementary Material

Evaluation of host biomarkers to support the development of a point-of-care diagnostic test to guide antibiotic use in bacterial/non-bacterial acute febrile illness cases

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Biomarker selection

Biomarkers evaluated were selected based on reported performances for distinguishing bacterial versus non-bacterial infections in prior publications, which were systematically reviewed in 2016 by Kapasi et al.¹ and other key publications (Supplementary Table 1). Biomarker performances reported in the 2016 systematic review were compared with reported performances in a later systematic review conducted in 2020.²

Supplementary Table 1. Biomarkers included based on Kapasi et al.'s (2016) systematic review and other key publications.

Biomarker	Performance, 2016 systematic review
C-reactive protein (CRP)	1
FebriDx (MxA+CRP)	2
Galectin-9	2
Gamma-induced protein 10 (IP-10)	2*
Haptoglobin	2 [#]
Heparin-binding protein (HBP)	3
Human neutrophil lipocalin (HNL)	2
Interferon gamma (IFN-gamma)	3
Interleukin-4 (IL-4)	2
Interleukin-6 (IL-6)	3
Lipopolysaccharide binding protein (LBP)	3 ^{\$}
Procalcitonin (PCT)	1
Secretory phospholipase 2 (sPLA2)	2
Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1)	3 ^{\$}
TNF-related apoptosis-inducing ligand (TRAIL)	2*
<i>Included based on key publications in the field</i>	
Biomarker	Publication
A-1-acid glycoprotein	Struck et al. ³
Chitinase-3-like protein 1 (CHI3L1)	Erdman et al. ⁴
Complement 2	Struck et al. ³
Complement C4b	Struck et al. ³
Neutrophil gelatinase-associated lipocalin (NGAL)	Huang et al. ⁵

Performances were scored as: 1, high-performing biomarker (meets the current TPP minimum diagnostic performance criteria, i.e. ≥ 0.90 and 0.80 sensitivity/specificity); 2, moderately performing biomarker (≥ 0.65 and 0.65 and < 0.90 and 0.80 sensitivity/specificity); 3, AUROC > 0.8 ; 4, low-performing biomarker; 5, not evaluated. *As part of the signature CRP+IP-10+TRAIL; # as part of the signature Haptoglobin+IL-10+TIMP1; \$ in respiratory tract infections as part of the signature CRP+LBP; § as part of the signature sTREM+CRP; 1 only in the context of meningitis, otherwise low performance.

Reference laboratory methodology

Materials, equipment, and software

All assay reagents used were delivered with the commercial kits and were used as described in the corresponding kit manuals. Supplementary Table 2 shows the commercial human multi-analyte kits and ELISA kits used.

Supplementary Table 2: Commercial human multi-analyte kits and ELISA kits used.

Analytes	Assay type	Provider	Reference laboratory that performed the analysis
CHI3L1, Gal-9, IL-4, IL-6, IP-10, IFN-gamma, sPLA2, sTREM-1, TRAIL	Luminex, 9-plex	Biotechne/ Systems R&D	NMI
NGAL, LBP	Luminex, 2-plex	Biotechne/ Systems R&D	NMI
C2, C4b	Luminex, 2-plex	Merck	NMI
HP, AGP	Luminex, 2-plex	Merck	NMI
PCT	Luminex, 1-plex	Biotechne/ Systems R&D	NMI
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HNL	ELISA	Diagnostics Development	NMI

CRP	ELISA	Biotechne/ Systems	R&D
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HBP	ELISA	Axis-Shield	on-site

NMI, The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany; MVZ Labor, Dr. Limbach & Kollegen, Heidelberg, Germany

For data generation, the Luminex FLEXMAP 3D instrument, operated with xPONENT Software V4.2, was used for the bead-based Luminex assays. The data evaluation was performed using Bio-Rad Bio-Plex Manager Software 6.1.1. To generate the data for the ELISAs at NMI a BioTek ELx 808 absorption reader was used. The embedded software Gen5 (BioTek) was used for data evaluation. At MVZ Limbach, a Cobas 8000 immunoanalyzer (Roche Diagnostics) was used for data generation.

Methods

All assays were processed according to the manufacturer's protocol. Standard curves, quality control (QC) samples, and blanks were analysed in duplicate; samples were assayed singly. Two or three QC samples were measured on each assay plate. QC samples were taken to cover the range of the standard curve (low, mid, and high level). All QC samples were prepared and aliquoted in larger quantities at the beginning of sample screening so that a fresh aliquot could be used for each measurement, and all QC samples underwent the same freeze–thaw cycle. The performance of the standard curves was controlled over the entire measurement period based on %CVs of the standard point duplicates (<20% and <25% for the last standard point) and percentage recovery on the basis of the nominal concentrations. If permitted by the dilution factor, samples out of the dynamic range were re-analysed with a lower or higher dilution factor.

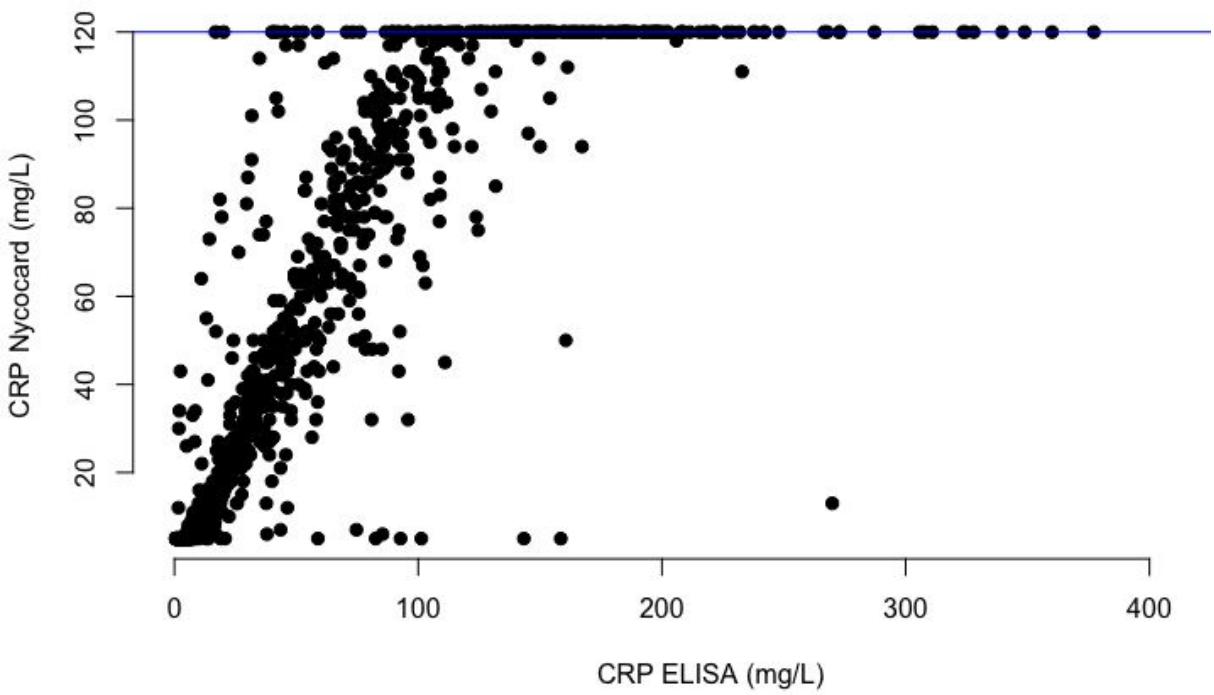
Heparin-binding protein (HBP) assay

The commercially available Axis-Shield heparin-binding protein ELISA for citrated plasma was validated for human EDTA plasma. Calibration curve, limit of detection (LOD), assay range, precision, parallelism, and spike-in recovery experiments were performed.

The ELISA was processed according to the assay protocol provided with the kit. Validation was performed using a fit-for-purpose approach and under consideration of the recommendations for assay validation given in guidelines from health authorities (European Medicine Agency (2011); Food and Drug Administration (2018)). This was a short validation with a limited number of samples.

Except for the percentage recovery, all analysed parameters met the criteria during the validation of the HBP ELISA using human EDTA plasma instead of the recommended citrated plasma matrix. The assay performance seemed to be stable for the sample evaluation using the kit.

Supplementary Figure 1: Analytical assessment of CRP Nycocard vs CRP ELISA



Statistical analysis

This section contains additional figures and tables related to the statistical analysis.

Supplementary Table 3: Number and percentage of missing values for the biomarkers included in the statistical analysis

	Electronic group [¶] [n (%)]	Strict group [§] [n (%)]	Loose group [#] [n (%)]
White blood cells	6 (0.8%)	11 (0.8%)	15 (0.8%)
HAEMATO COUNT	6 (0.8%)	11 (0.8%)	15 (0.8%)
Lymphocytes	6 (0.8%)	12 (0.9%)	17 (1%)
Neutrophils	22 (3%)	64 (5%)	90 (5%)
CRP NYCOCARD	5 (0.7%)	10 (0.7%)	14 (0.8%)
IL-6	10 (1.5%)	20 (1%)	24 (1%)
Gal-9	10 (1.5%)	20 (1%)	24 (1%)
CHI3L1	10 (1.5%)	20 (1%)	25 (1%)
IP-10	10 (1.5%)	20 (1%)	24 (1%)
TRAIL	10 (1.5%)	20 (1%)	24 (1%)
IL-4	13 (2%)	24 (2%)	29 (2%)
sPLA2	10 (1.5%)	20 (1%)	24 (1%)
NGAL	29 (4%)	138 (10%)	197 (11%)
LBP	30 (4%)	139 (10%)	198 (11%)
C2	10 (1.5%)	21 (1.5%)	25 (1%)
AGP	10 (1.5%)	21 (1.5%)	25 (1%)
HP	11 (1.6%)	24 (2%)	29 (2%)

¶ Total number of subjects in the Electronic group: 677

§ Total number of subjects in the Strict group: 1376

Total number of subjects in the Loose group: 1777

Kruskal-Wallis tables

Supplementary Table 4: Kruskal-Wallis table results for the electronic classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	1.214 5E-13	1.980 8E-01	1.098 5E-02	3.440 8E-01	8.4018E-01	2.7154E-01	4.3535E-01	3.4408E-01	5.4183E-09
HAEMA TO COUNT	2.804 0E-45	1.044 6E-09	4.346 1E-28	1.318 5E-36	6.8045E-02	9.1321E-01	6.9000E-01	9.9455E-01	3.6951E-08
Lymphocytes	1.385 0E-45	8.068 0E-03	3.156 2E-29	4.541 4E-32	1.0022E-05	4.4874E-01	4.5900E-01	5.4198E-08	1.9910E-11
Neutrophils	5.649 5E-03	3.914 7E-01	1.133 7E-04	1.867 4E-17	1.5980E-02	4.2719E-01	4.3608E-01	3.0003E-08	6.5439E-04
CRP NYCOCARD	1.448 5E-03	4.229 7E-01	1.386 1E-15	3.033 2E-07	2.1171E-01	4.6667E-01	8.4615E-01	3.0231E-03	2.1171E-01
IL-6	9.262 6E-06	2.527 7E-01	4.668 6E-34	4.281 0E-21	6.1106E-03	7.1615E-01	5.8674E-02	2.0177E-10	9.2626E-06
Gal-9	7.808 4E-11	3.329 6E-01	1.273 1E-07	2.247 1E-07	4.3173E-01	5.3845E-01	9.9020E-02	3.6659E-01	8.5282E-04
CHI3L1	3.687 4E-01	1.542 7E-01	2.259 3E-04	3.594 2E-05	9.0961E-01	8.0977E-01	7.9973E-01	2.5264E-02	2.5264E-02
IP-10	7.023 5E-01	7.023 5E-01	4.042 9E-09	7.048 6E-10	4.9729E-01	7.0235E-01	4.0169E-01	3.6086E-08	3.3476E-01
TRAIL	1.410 8E-03	1.542 9E-02	6.771 0E-19	6.947 3E-56	9.2177E-01	2.2485E-02	9.5591E-01	9.7926E-04	1.8702E-06
IL-4	1.419 0E-03	8.956 6E-02	1.789 6E-25	1.117 9E-73	4.2256E-01	8.9341E-03	8.9692E-01	3.0403E-03	2.2958E-09
sPLA2	9.599 3E-05	9.212 7E-01	2.847 7E-20	5.681 0E-03	1.5011E-01	9.2127E-01	6.1633E-01	7.4323E-03	7.4323E-03
NGAL	2.684 1E-02	7.192 4E-01	1.249 8E-05	6.460 4E-21	7.1924E-01	2.6841E-02	5.1387E-01	1.2498E-05	9.6273E-03
LBP	2.265 8E-11	5.148 1E-02	1.852 7E-54	2.154 4E-101	8.2974E-02	5.3837E-03	1.1745E-01	3.5938E-09	6.0583E-19
C2	1.721 9E-02	3.006 3E-01	6.862 8E-13	6.862 8E-13	6.2951E-02	8.5874E-01	5.6324E-01	4.4637E-01	6.2045E-03
AGP	5.188 8E-03	2.027 4E-01	3.674 7E-16	1.344 5E-16	1.5176E-01	9.8963E-01	6.3154E-01	2.3325E-01	3.1922E-05
HP	2.942 0E-07	2.739 0E-01	1.839 3E-25	2.499 7E-25	2.7390E-01	2.7390E-01	4.0178E-01	7.2077E-01	2.9140E-03
C4b	5.615 9E-19	6.701 0E-02	4.504 1E-81	1.949 1E-84	6.7179E-03	6.7179E-03	3.3168E-01	1.8052E-01	8.0363E-18

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 5: Kruskal-Wallis table results for the strict classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	3.114 9E-20	2.409 1E-01	3.674 9E-09	9.399 7E-03	3.1632E-01	6.3502E-02	6.3502E-02	9.1443E-01	1.7973E-08
HAEMA TO COUNT	6.183 5E-100	1.999 4E-04	5.630 4E-55	3.785 2E-68	1.6199E-04	8.0189E-01	7.1282E-01	2.9137E-01	1.7149E-10
Lymphocytes	8.477 8E-84	1.529 1E-01	2.677 9E-44	2.740 4E-58	6.3047E-07	6.1980E-03	4.5554E-01	7.1024E-22	8.6226E-15
Neutrophils	8.951 3E-04	1.715 2E-01	7.983 8E-14	1.913 4E-37	4.5549E-02	5.2789E-01	4.5549E-02	3.0001E-19	4.1217E-02
CRP NYCOCA RD	1.654 7E-02	5.765 6E-02	2.457 0E-38	6.299 1E-11	7.4370E-01	3.0220E-01	7.4370E-01	9.7289E-15	3.0220E-01
IL-6	2.570 4E-02	1.288 8E-01	2.513 1E-68	3.475 8E-27	1.4641E-01	8.1220E-01	6.6933E-02	4.3924E-26	2.5371E-04
Gal-9	7.442 4E-19	3.545 5E-03	1.343 2E-11	1.375 7E-08	1.1615E-01	3.9116E-01	1.3397E-01	2.2573E-01	2.4249E-03
CHI3L1	2.833 5E-01	1.543 3E-01	3.678 7E-11	7.431 9E-16	2.8335E-01	2.8335E-01	2.8335E-01	8.7744E-06	1.5017E-03
IP-10	2.452 1E-01	6.871 6E-01	8.565 6E-31	1.550 3E-36	2.1157E-01	3.0336E-01	3.2906E-01	4.1236E-22	3.2906E-01
TRAIL	6.435 8E-04	2.420 6E-01	3.746 7E-46	4.580 6E-127	7.7652E-01	8.3869E-04	7.7652E-01	2.8337E-17	1.7642E-08
IL-4	4.210 8E-04	5.985 8E-01	2.594 9E-55	2.708 3E-159	3.3368E-01	8.0705E-05	6.5563E-01	2.2888E-11	2.2888E-11
sPLA2	3.000 5E-14	1.126 4E-01	4.135 5E-60	4.705 5E-09	6.7473E-04	2.2676E-01	3.6531E-01	1.0844E-09	4.7059E-05
NGAL	7.746 2E-02	1.130 0E-01	6.092 7E-16	1.372 0E-35	5.9955E-01	4.9221E-02	4.4419E-01	1.4382E-19	8.8808E-03
LBP	1.350 9E-14	3.412 3E-01	6.066 0E-94	1.936 0E-197	2.1248E-02	3.6673E-05	3.0644E-01	2.3473E-28	7.4289E-21
C2	7.267 4E-07	4.315 7E-01	2.314 5E-26	4.532 4E-25	6.8236E-03	4.3157E-01	4.3157E-01	8.8206E-03	2.1062E-03
AGP	4.851 3E-04	1.737 9E-01	5.058 7E-21	7.149 6E-23	1.5900E-01	7.9521E-01	9.7767E-01	1.1305E-01	1.4880E-05
HP	1.212 7E-13	6.331 1E-01	1.636 6E-46	3.005 3E-46	2.9299E-03	5.6523E-01	5.6523E-01	9.0316E-01	4.8596E-04

C4b	6.319 3E-21	1.923 1E-02	1.666 4E-139	3.199 9E-147	1.9749E-04	2.6638E-04	9.3349 E-01	8.0678E-03	3.0903E-25
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Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 6: Kruskal-Wallis table results for the loose classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^\circ\text{C}$	Chikungunya
White blood cells	2.057 4E-28	9.875 9E-01	1.848 4E-08	4.526 0E-03	9.0171E-02	4.8259E-02	1.0890 E-01	7.4007E-01	1.8484E-08
HAEMA TO COUNT	1.308 3E-126	1.861 9E-04	6.283 5E-56	7.796 2E-76	1.1102E-06	7.8862E-01	7.9391 E-01	2.9434E-01	1.2853E-10
Lymphocytes	4.965 1E-101	2.946 1E-01	4.679 6E-45	1.637 2E-67	4.8743E-07	6.6823E-04	2.9461 E-01	2.4236E-29	4.3110E-15
Neutrophils	1.131 0E-04	7.267 7E-01	7.274 2E-15	1.612 7E-46	2.0313E-01	4.6743E-01	2.0038 E-01	1.2920E-24	2.9723E-02
CRP NYCOCARD	1.361 4E-01	4.412 3E-03	1.034 7E-57	2.470 3E-15	4.0226E-01	5.2068E-01	5.9738 E-01	6.7648E-18	1.3614E-01
IL-6	9.525 0E-02	4.873 6E-02	8.630 3E-95	1.968 8E-31	1.5356E-01	8.2374E-01	9.3076 E-02	6.1774E-34	2.1766E-05
Gal-9	2.046 3E-27	1.443 1E-03	1.931 8E-13	6.827 3E-10	2.3586E-01	2.3586E-01	3.6447 E-02	2.3586E-01	3.0166E-03
CHI3L1	2.748 3E-01	5.354 1E-02	3.612 8E-14	3.612 8E-14	2.8535E-01	7.9359E-01	3.0946 E-01	1.4718E-04	7.1655E-04
IP-10	4.138 4E-01	7.867 4E-01	6.519 3E-43	4.220 2E-47	7.9605E-02	3.6101E-01	4.1384 E-01	1.4436E-34	4.1902E-01
TRAIL	2.472 2E-02	1.391 8E-01	6.282 8E-56	2.918 5E-156	8.2684E-01	6.2797E-05	8.2684 E-01	2.4486E-17	1.1148E-09
IL-4	1.144 8E-02	3.191 1E-01	3.084 4E-69	4E-206	1.748 0E-01	3.9276E-01	4.7672E-08	5.7785 E-01	2.1611E-12
sPLA2	8.375 3E-18	2.731 7E-01	1.589 0E-82	1.270 2E-09	1.2356E-04	3.7225E-01	4.1002 E-01	8.1232E-15	4.0213E-05

NGAL	1.570 6E-01	2.065 0E-02	3.748 6E-27	2.284 8E-43	3.7129E- 01	1.4239E- 01	3.9957 E-01	1.3734E- -24	5.3057E- 03
LBP	1.656 7E-10	4.386 5E-01	2.110 116	2.427 254	8.2765E- 03	5.4993E- 07	6.1624 E-01	1.4861E- -39	1.4254E- 24
C2	2.103 5E-04	1.459 3E-01	7.600 5E-28	2.186 5E-27	4.8543E- 02	2.9326E- 01	3.8932 E-01	9.8425E- -03	1.2901E- 03
AGP	2.507 6E-03	9.527 3E-02	1.987 0E-26	3.272 6E-28	9.3140E- 02	8.9492E- 01	9.5756 E-01	9.5273E- -02	3.2225E- 06
HP	5.764 0E-15	7.268 5E-01	2.837 6E-51	7.966 7E-51	7.2760E- 03	6.9555E- 01	6.9555 E-01	9.7145E- -01	1.7228E- 04
C4b	3.907 7E-15	9.303 7E-03	9.356 160	3.444 171	6.9926E- 04	2.2357E- 03	8.6228 E-01	2.2357E- -03	1.0351E- 29

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Supplementary Table 7: Univariate analysis – Overall (malaria-positive and malaria-negative) population

	Overall - Malaria negatives			Overall - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.74, (0.7- 0.79), 493	0.75, (0.71- 0.78), 880	0.72, (0.68- 0.75), 1127	0.65, (0.57- 0.73), 174	0.65, (0.58- 0.71), 481	0.64, (0.59- 0.7), 630
RBC count	0.58, (0.53- 0.63), 494	0.52, (0.48- 0.56), 880	0.51, (0.47- 0.54), 1127	0.58, (0.5- 0.67), 175	0.5, (0.44- 0.56), 481	0.51, (0.46- 0.57), 630
Lymphocyte count	0.66, (0.61- 0.71), 491	0.57, (0.53- 0.61), 877	0.55, (0.51- 0.58), 1123	0.63, (0.54- 0.71), 174	0.57, (0.5- 0.63), 480	0.54, (0.49- 0.6), 627
Neutrophil count	0.71, (0.66- 0.75), 480	0.75, (0.71- 0.79), 847	0.73, (0.69- 0.76), 1079	0.67, (0.59- 0.75), 172	0.65, (0.58- 0.71), 461	0.65, (0.59- 0.71), 603
IL-4	0.36, (0.31- 0.42), 486	0.4, (0.35- 0.44), 868	0.61, (0.57- 0.64), 1113	0.66, (0.58- 0.74), 175	0.59, (0.53- 0.65), 478	0.58, (0.53- 0.63), 624
TRAIL	0.36, (0.3- 0.41), 489	0.63, (0.59- 0.67), 871	0.63, (0.59- 0.67), 1117	0.68, (0.6- 0.76), 175	0.6, (0.54- 0.66), 478	0.58, (0.53- 0.64), 625
IL-6	0.61, (0.55- 0.66), 489	0.49, (0.45- 0.53), 873	0.49, (0.45- 0.53), 1120	0.42, (0.33- 0.5), 175	0.57, (0.5- 0.63), 478	0.53, (0.48- 0.59), 626
CRP NycoCard	0.52, (0.47- 0.57), 496	0.57, (0.53- 0.61), 884	0.57, (0.53- 0.6), 1132	0.52, (0.43- 0.6), 175	0.49, (0.43- 0.56), 481	0.5, (0.44- 0.55), 630
Gal-9	0.52, (0.47- 0.57), 490	0.54, (0.5- 0.58), 875	0.56, (0.52- 0.59), 1122	0.57, (0.48- 0.65), 176	0.54, (0.48- 0.6), 480	0.53, (0.48- 0.59), 629
CHI3L1	0.56, (0.51- 0.62), 489	0.55, (0.51- 0.59), 873	0.55, (0.51- 0.59), 1119	0.5, (0.41- 0.59), 176	0.52, (0.45- 0.58), 480	0.5, (0.44- 0.55), 627
IP-10	0.53, (0.48- 0.58), 489	0.52, (0.48- 0.56), 874	0.52, (0.49- 0.56), 1120	0.56, (0.47- 0.64), 176	0.53, (0.47- 0.59), 478	0.51, (0.45- 0.56), 627
sPLA2	0.52, (0.47- 0.57), 490	0.52, (0.48- 0.56), 874	0.52, (0.49- 0.56), 1121	0.49, (0.4- 0.58), 176	0.54, (0.48- 0.61), 479	0.54, (0.49- 0.6), 628

NGAL	0.61, (0.56-0.66), 489	0.62, (0.57-0.66), 833	0.6, (0.57-0.64), 1049	0.61, (0.52-0.7), 157	0.56, (0.49-0.62), 403	0.56, (0.51-0.62), 527
LBP	0.74, (0.69-0.78), 488	0.69, (0.65-0.73), 832	0.67, (0.64-0.71), 1048	0.67, (0.58-0.76), 158	0.58, (0.52-0.64), 404	0.57, (0.51-0.62), 529
C2	0.59, (0.54-0.64), 483	0.56, (0.52-0.6), 866	0.56, (0.52-0.59), 1113	0.63, (0.55-0.72), 176	0.59, (0.53-0.66), 480	0.56, (0.5-0.61), 629
AGP	0.67, (0.62-0.72), 490	0.6, (0.56-0.64), 874	0.58, (0.55-0.62), 1120	0.52, (0.43-0.6), 176	0.52, (0.45-0.59), 480	0.53, (0.47-0.59), 629
HBP	0.67, (0.57-0.76), 179	0.64, (0.56-0.72), 254	0.61, (0.53-0.68), 280	0.55, (0.37-0.72), 57	0.52, (0.42-0.63), 141	0.53, (0.43-0.64), 149
HP	0.55, (0.49-0.6), 489	0.5, (0.46-0.54), 871	0.52, (0.48-0.56), 1116	0.58, (0.49-0.66), 175	0.55, (0.48-0.61), 473	0.54, (0.48-0.59), 622

Supplementary Table 8: Univariate analysis – malaria-positive population

	Malawi - Malaria positives			Gabon - Malaria positives			
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict
WBC count	0.67 (0.58-0.76), 132	0.68 (0.61 – 0.75), 369	0.67 (0.61-0.72), 491	0.67 (0.44-0.91), 42	0.61 (0.38-0.83), 112	0.61 (0.44-0.78), 139	
RBC count	0.69 (0.6-0.79), 131	0.55 (0.48-0.61), 367	0.53 (0.47-0.59), 488	0.56 (0.31-0.81), 43	0.51 (0.3-0.71), 113	0.49 (0.33-0.65), 140	
Lymphocyte count	0.7 (0.61-0.79), 131	0.59 (0.53-0.66), 368	0.57 (0.51-0.62), 488	0.72 (0.51-0.93), 42	0.66 (0.47-0.85), 112	0.67 (0.52-0.82), 139	
Neutrophil count	0.62 (0.52-0.72), 129	0.65 (0.57-0.72), 348	0.66 (0.6-0.72), 463	0.53 (0.31-0.76), 43	0.59 (0.39-0.79), 113	0.59 (0.43-0.75), 140	
IL-4	0.46 (0.36-0.56), 132	0.47 (0.4-0.53), 369	0.48 (0.42-0.53), 488	0.44 (0.38-0.5), 40	0.46 (0.44-0.49), 103	0.5 (0.42-0.57), 127	
TRAIL	0.6 (0.51-0.7), 132	0.55 (0.49-0.62), 369	0.54 (0.48-0.59), 488	0.5 (0.5-0.5), 43	0.5 (0.5-0.5), 109	0.53 (0.47-0.6), 136	
IL-6	0.6 (0.5-0.7), 131	0.58 (0.51-0.65), 367	0.54 (0.48-0.6), 485	0.45 (0.32 - 0.57), 42	0.47 (0.37-0.57), 103	0.45 (0.37-0.53), 127	
CRP NycoCard	0.48 (0.38-0.58), 131	0.54 (0.47-0.61), 367	0.53 (0.47-0.59), 489	0.59 (0.32-0.86), 44	0.59 (0.36-0.82), 114	0.57 (0.4-0.75), 141	
Gal-9	0.58 (0.48-0.69), 132	0.56 (0.49-0.62), 369	0.54 (0.47-0.6), 491	0.57 (0.34-0.8), 43	0.5 (0.32-0.68), 109	0.56 (0.42-0.71), 136	
CHI3L1	0.56 (0.46-0.66), 132	0.55 (0.48-0.62), 367	0.55 (0.49-0.61), 487	0.52 (0.26-0.79), 43	0.53 (0.31-0.75), 106	0.63 (0.44-0.81), 131	
IP-10	0.67 (0.58-0.76), 132	0.56 (0.49-0.63), 363	0.52 (0.46-0.59), 484	0.51 (0.33-0.69), 40	0.49 (0.35-0.63), 104	0.48 (0.35-0.61), 129	
sPLA2	0.53 (0.43-0.64), 133	0.56 (0.48-0.63), 370	0.56 (0.5-0.62), 492	0.49 (0.24-0.74), 43	0.56 (0.34-0.77), 109	0.49 (0.32-0.67), 136	
NGAL	0.5 (0.39-0.61), 114	0.5 (0.43-0.58), 291	0.49 (0.42-0.55), 386	0.65 (0.44-0.91), 41	0.59 (0.41-0.77), 106	0.54 (0.38-0.7), 131	

LBP	0.47 (0.35-0.59), 115	0.54 (0.46-0.61), 295	0.54 (0.48-0.6), 393	0.6 (0.34 -0.85), 42	0.58 (0.37-0.8), 105	0.65 (0.48-0.81), 131
C2	0.62 (0.52-0.72), 133	0.57 (0.5-0.64), 369	0.54 (0.48-0.6), 491	0.72 (0.54-0.9), 43	0.72 (0.57-0.87), 105	0.64 (0.48-0.8), 131
AGP	0.54 (0.44 -0.64), 133	0.52 (0.44-0.59), 371	0.48 (0.42-0.54), 493	0.51 (0.27-0.75), 43	0.53 (0.33-0.74), 109	0.58 (0.41-0.76), 136
HBP	0.55, (0.37-0.72), 57	0.53, (0.43-0.64), 143	0.54, (0.44-0.64), 151
HP	0.58 (0.48-0.68), 133	0.54 (0.47-0.61), 365	0.51 (0.45-0.57), 487	0.57 (0.33-0.8), 42	0.56 (0.36-0.76), 107	0.61 (0.46-0.77), 134

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65,) red (AUROC < 0.6)

Univariate analysis – age subgroups

Supplementary Table 9: Univariate analysis - age less than 6 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.83, (0.73-0.94), 61	0.79, (0.71-0.87), 122	0.76, (0.69-0.84), 170	0.52, (0.25-0.78), 21	0.65, (0.46-0.85), 34	0.69, (0.51-0.86), 38	0.78, (0.62-0.94), 32	0.68, (0.52-0.83), 75	0.65, (0.52-0.79), 105
RBC count	0.65, (0.49-0.8), 62	0.58, (0.48-0.68), 123	0.58, (0.5-0.67), 172	0.6, (0.33-0.86), 21	0.56, (0.35-0.77), 33	0.59, (0.39-0.78), 37	0.6, (0.4-0.81), 32	0.56, (0.4-0.72), 75	0.53, (0.38-0.67), 105
Lymphocyte count	0.58, (0.43-0.72), 60	0.53, (0.42-0.64), 121	0.48, (0.38-0.57), 170	0.63, (0.36-0.89), 21	0.67, (0.44-0.91), 34	0.7, (0.5-0.9), 38	0.71, (0.53-0.89), 32	0.6, (0.44-0.76), 75	0.63, (0.49-0.76), 105
Neutrophil count	0.82, (0.7-0.93), 57	0.79, (0.7-0.88), 108	0.77, (0.69-0.86), 148	0.58, (0.32-0.85), 21	0.56, (0.36-0.77), 34	0.6, (0.41-0.79), 38	0.86, (0.72-0.99), 32	0.79, (0.67-0.92), 74	0.7, (0.58-0.83), 103
IL-4	0.54, (0.39-0.68), 63	0.5, (0.41-0.59), 125	0.48, (0.41-0.56), 174	0.63, (0.38-0.88), 20	0.66, (0.49-0.84), 31	0.62, (0.44-0.8), 33	0.43, (0.31-0.55), 30	0.49, (0.43-0.56), 72	0.51, (0.44-0.57), 103
TRAIL	0.57, (0.39-0.75), 63	0.6, (0.5-0.69), 125	0.59, (0.51-0.67), 174	0.5, (0.23-0.77), 20	0.63, (0.43-0.82), 31	0.59, (0.4-0.79), 33	0.5, (0.5-0.5), 28	0.5, (0.5-0.5), 69	0.49, (0.48-0.51), 99
IL-6	0.59, (0.44-0.73), 63	0.61, (0.52-0.7), 125	0.6, (0.52-0.68), 174	0.41, (0.29-0.53), 20	0.39, (0.29-0.49), 29	0.39, (0.3-0.49), 31	0.5, (0.5-0.5), 31	0.5, (0.5-0.5), 73	0.49, (0.47-0.5), 104
CRP NycoCard	0.56, (0.37-0.74), 61	0.61, (0.51-0.71), 121	0.59, (0.5-0.68), 169	0.49, (0.22-0.76), 21	0.59, (0.38-0.79), 34	0.6, (0.42-0.79), 38	0.76, (0.57-0.95), 32	0.62, (0.49-0.76), 75	0.57, (0.45-0.69), 106

Gal-9	0.79, (0.66- 0.92), 63	0.59, (0.49- 0.69), 125	0.57, (0.48- 0.66), 173	0.47, (0.2- 0.75), 20	0.5, (0.28- 0.72), 31	0.52, (0.3- 0.73), 33	0.66, (0.45- 0.87), 31	0.6, (0.43- 0.76), 72	0.54, (0.4- 0.69), 102
CHI3L1	0.56, (0.4- 0.72), 62	0.52, (0.42- 0.63), 124	0.54, (0.45- 0.63), 173	0.61, (0.35- 0.87), 20	0.66, (0.47- 0.86), 31	0.67, (0.49- 0.86), 33	0.68, (0.49- 0.88), 31	0.62, (0.45- 0.79), 73	0.61, (0.47- 0.75), 102
IP-10	0.67, (0.51- 0.83), 63	0.62, (0.52- 0.72), 125	0.6, (0.51- 0.68), 174	0.65, (0.39-0.9), 20	0.7, (0.51- 0.89), 31	0.64, (0.45- 0.84), 33	0.71, (0.53-0.9), 31	0.52, (0.38- 0.67), 73	0.51, (0.38- 0.63), 104
sPLA2	0.66, (0.5- 0.82), 63	0.55, (0.45- 0.66), 125	0.56, (0.47- 0.65), 174	0.65, (0.38- 0.91), 20	0.69, (0.48- 0.9), 31	0.68, (0.48- 0.88), 33	0.58, (0.37- 0.78), 31	0.57, (0.41- 0.72), 73	0.59, (0.45- 0.73), 104
NGAL	0.61, (0.44- 0.77), 63	0.68, (0.58- 0.78), 109	0.67, (0.59- 0.76), 144	0.67, (0.41- 0.93), 20	0.58, (0.38- 0.79), 31	0.52, (0.31- 0.72), 33	0.63, (0.43- 0.83), 31	0.6, (0.44- 0.77), 73	0.57, (0.43- 0.71), 103
LBP	0.47, (0.31- 0.63), 63	0.5, (0.39- 0.62), 109	0.53, (0.43- 0.63), 144	0.47, (0.2- 0.75), 20	0.46, (0.25- 0.68), 30	0.48, (0.27- 0.7), 32	0.73, (0.53- 0.93), 30	0.7, (0.53- 0.86), 70	0.59, (0.44- 0.75), 101
C2	0.51, (0.34- 0.69), 63	0.56, (0.45- 0.66), 125	0.52, (0.44- 0.61), 174	0.47, (0.18- 0.76), 19	0.64, (0.41- 0.87), 29	0.62, (0.4- 0.83), 31	0.51, (0.29- 0.73), 30	0.48, (0.32- 0.64), 71	0.5, (0.36- 0.64), 102
AGP	0.54, (0.38-0.7), 63	0.56, (0.45- 0.66), 125	0.57, (0.48- 0.66), 174	0.72, (0.48- 0.96), 20	0.57, (0.34- 0.81), 31	0.61, (0.39- 0.82), 33	0.8, (0.63- 0.98), 31	0.72, (0.56- 0.88), 72	0.62, (0.48- 0.76), 103
HBP	0.67, (0.45 -0.89), 26	0.55, (0 .37- 0.73), 4 5	0.54, (0 .37- 0.71), 4 8
HP	0.64, (0.49- 0.78), 62	0.57, (0.46- 0.67), 124	0.57, (0.48- 0.66), 173	0.68, (0.42- 0.93), 20	0.61, (0.38- 0.84), 31	0.62, (0.41- 0.84), 33	0.78, (0.59- 0.97), 28	0.72, (0.57- 0.88), 69	0.63, (0.49- 0.77), 100

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65), red (AUROC < 0.6)

Supplementary Table 10: Univariate analysis - aged between 7 and 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives				
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict
WBC count	0.49, (0.26- 0.73), 28	0.69, (0.54- 0.84), 50	0.75, (0.64- 0.86), 81	0.79, (0.61- 0.96), 34	0.83, (0.71- 0.95), 69	0.82, (0.71- 0.94), 75	0.46, (0.27- 0.65), 47	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112
RBC count	0.62, (0.41- 0.84), 28	0.54, (0.37- 0.7), 51	0.57, (0.44- 0.7), 82	0.7, (0.51- 0.88), 34	0.61, (0.45- 0.78), 69	0.6, (0.44- 0.75), 75	0.56, (0.38- 0.75), 47	0.55, (0.4- 0.7), 87	0.48, (0.35- 0.62), 112	0.48, (0.35- 0.62), 112	0.48, (0.35- 0.62), 112

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Lymphocyte count	0.76, (0.58-0.94), 28	0.67, (0.51-0.83), 51	0.62, (0.49-0.74), 82	0.6, (0.37-0.83), 34	0.69, (0.54-0.85), 69	0.71, (0.56-0.86), 75	0.59, (0.42-0.76), 47	0.61, (0.48-0.74), 87	0.55, (0.43-0.68), 112
Neutrophil count	0.46, (0.23-0.7), 26	0.7, (0.54-0.86), 45	0.76, (0.64-0.87), 73	0.73, (0.53-0.93), 34	0.82, (0.69-0.95), 69	0.8, (0.68-0.93), 75	0.66, (0.46-0.86), 46	0.61, (0.43-0.8), 86	0.61, (0.44-0.78), 111
IL-4	0.56, (0.34-0.78), 28	0.46, (0.31-0.6), 50	0.48, (0.37-0.6), 80	0.73, (0.53-0.92), 33	0.62, (0.47-0.77), 69	0.59, (0.45-0.74), 75	0.46, (0.41-0.5), 47	0.48, (0.46-0.5), 86	0.51, (0.45-0.57), 112
TRAIL	0.48, (0.23-0.73), 28	0.6, (0.45-0.76), 50	0.57, (0.45-0.7), 80	0.55, (0.34-0.77), 33	0.53, (0.38-0.68), 69	0.52, (0.38-0.66), 75	0.5, (0.5-0.5), 45	0.49, (0.48-0.51), 83	0.49, (0.47-0.5), 109
IL-6	0.45, (0.21-0.69), 28	0.56, (0.4-0.71), 51	0.55, (0.44-0.67), 82	0.46, (0.34-0.58), 33	0.44, (0.33-0.56), 69	0.43, (0.33-0.53), 75	0.53, (0.44-0.62), 47	0.53, (0.46-0.6), 86	0.54, (0.46-0.62), 112
CRP NycoCard	0.56, (0.34-0.78), 28	0.61, (0.46-0.77), 51	0.62, (0.5-0.74), 82	0.57, (0.33-0.81), 34	0.52, (0.35-0.68), 71	0.51, (0.35-0.68), 77	0.75, (0.59-0.92), 47	0.71, (0.55-0.87), 87	0.69, (0.56-0.83), 113
Gal-9	0.67, (0.43-0.9), 28	0.68, (0.53-0.84), 51	0.66, (0.54-0.78), 82	0.71, (0.52-0.9), 33	0.57, (0.41-0.73), 69	0.54, (0.39-0.7), 75	0.79, (0.62-0.95), 47	0.61, (0.44-0.77), 86	0.55, (0.39-0.71), 112
CHI3L1	0.53, (0.28-0.78), 28	0.6, (0.44-0.76), 51	0.61, (0.49-0.73), 82	0.69, (0.5-0.87), 32	0.66, (0.52-0.79), 67	0.59, (0.44-0.73), 71	0.53, (0.32-0.73), 46	0.58, (0.41-0.74), 84	0.62, (0.47-0.77), 110
IP-10	0.64, (0.42-0.86), 28	0.56, (0.39-0.72), 51	0.59, (0.46-0.72), 82	0.73, (0.53-0.92), 33	0.62, (0.46-0.78), 69	0.58, (0.42-0.73), 75	0.6, (0.41-0.78), 47	0.48, (0.31-0.66), 86	0.52, (0.37-0.67), 112
sPLA2	0.47, (0.21-0.72), 28	0.55, (0.39-0.72), 51	0.56, (0.43-0.68), 82	0.54, (0.33-0.76), 33	0.49, (0.35-0.64), 69	0.56, (0.43-0.7), 75	0.46, (0.28-0.64), 47	0.52, (0.36-0.67), 86	0.44, (0.29-0.59), 112
NGAL	0.56, (0.32-0.8), 28	0.68, (0.52-0.85), 46	0.73, (0.61-0.85), 73	0.71, (0.52-0.9), 33	0.68, (0.54-0.82), 69	0.64, (0.5-0.78), 75	0.7, (0.52-0.89), 46	0.6, (0.44-0.77), 85	0.59, (0.44-0.74), 111
LBP	0.54, (0.3-0.77), 28	0.59, (0.42-0.75), 46	0.58, (0.45-0.72), 73	0.68, (0.5-0.87), 33	0.66, (0.52-0.8), 69	0.67, (0.54-0.8), 75	0.71, (0.52-0.9), 46	0.66, (0.48-0.84), 85	0.63, (0.46-0.79), 111
C2	0.62, (0.34-0.9), 28	0.53, (0.36-0.7), 51	0.53, (0.41-0.66), 82	0.54, (0.31-0.76), 32	0.57, (0.4-0.74), 67	0.61, (0.45-0.77), 73	0.62, (0.42-0.81), 45	0.46, (0.27-0.65), 83	0.52, (0.36-0.68), 109
AGP	0.57, (0.3-0.83), 28	0.55, (0.39-0.71), 51	0.52, (0.39-0.65), 81	0.53, (0.3-0.76), 33	0.6, (0.44-0.75), 69	0.61, (0.46-0.75), 75	0.75, (0.56-0.94), 47	0.68, (0.5-0.86), 86	0.67, (0.52-0.83), 112
HBP	0.76, (0.28-1), 10	0.58, (0.29-0.87), 19	0.65, (0.39-0.91), 23	## Unbalance d classes	0.92, (0.69-1), 8	0.72, (0.28-1), 9

HP	0.5, (0.25-0.76), 28	0.51, (0.35-0.67), 51	0.5, (0.37-0.63), 82	0.52, (0.3-0.75), 32	0.62, (0.46-0.78), 68	0.6, (0.45-0.76), 74	0.53, (0.33-0.73), 47	0.54, (0.37-0.7), 85	0.53, (0.38-0.67), 109
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Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Supplementary Table 11: Univariate analysis - aged more than 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives					
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose
WBC count	0.67, (0.53-0.82), 66	0.71, (0.62-0.8), 132	0.68, (0.6-0.75), 210	0.84, (0.77-0.91), 202	0.84, (0.77-0.9), 305	0.83, (0.77-0.89), 329	2 patients in total	5 patients in total	5 patients in total	-	-	-
RBC count	0.59, (0.44-0.73), 65	0.53, (0.43-0.63), 131	0.51, (0.43-0.59), 209	0.56, (0.45-0.67), 203	0.56, (0.47-0.64), 306	0.55, (0.47-0.63), 330	-	-	-	-	-	-
Lymphocyte count	0.5, (0.34-0.66), 66	0.53, (0.43-0.63), 131	0.49, (0.41-0.57), 209	0.67, (0.58-0.76), 202	0.65, (0.57-0.72), 305	0.64, (0.57-0.71), 329	-	-	-	-	-	-
Neutrophil count	0.65, (0.49-0.81), 60	0.7, (0.6-0.8), 120	0.66, (0.59-0.74), 193	0.82, (0.74-0.9), 202	0.82, (0.76-0.89), 305	0.82, (0.75-0.88), 329	-	-	-	-	-	-
IL-4	0.4, (0.28-0.52), 66	0.47, (0.39-0.54), 131	0.45, (0.39-0.52), 209	0.56, (0.47-0.65), 196	0.53, (0.46-0.6), 298	0.54, (0.47-0.6), 321	-	-	-	-	-	-
TRAIL	0.68, (0.54-0.82), 66	0.65, (0.56-0.73), 131	0.66, (0.59-0.73), 209	0.57, (0.48-0.65), 199	0.54, (0.47-0.61), 302	0.54, (0.48-0.61), 326	-	-	-	-	-	-
IL-6	0.59, (0.46-0.72), 67	0.63, (0.54-0.72), 131	0.59, (0.52-0.66), 209	0.51, (0.44-0.58), 194	0.51, (0.45-0.58), 297	0.5, (0.44-0.56), 320	-	-	-	-	-	-
CRP NycoCard	0.53, (0.38-0.68), 67	0.6, (0.5-0.7), 133	0.57, (0.49-0.64), 211	0.66, (0.57-0.76), 204	0.65, (0.57-0.73), 307	0.66, (0.58-0.73), 331	-	-	-	-	-	-
Gal-9	0.72, (0.59-0.86), 67	0.6, (0.5-0.7), 133	0.63, (0.56-0.71), 211	0.61, (0.52-0.71), 199	0.56, (0.48-0.65), 301	0.57, (0.5-0.65), 325	-	-	-	-	-	-
CHI3L1	0.52, (0.36-0.67), 65	0.51, (0.41-0.61), 129	0.53, (0.45-0.61), 207	0.66, (0.58-0.75), 194	0.62, (0.54-0.69), 296	0.62, (0.55-0.69), 320	-	-	-	-	-	-
IP-10	0.64, (0.48-0.79), 67	0.59, (0.49-0.69), 133	0.61, (0.53-0.68), 210	0.59, (0.5-0.68), 199	0.52, (0.44-0.6), 302	0.53, (0.45-0.6), 326	-	-	-	-	-	-

Protected by copyright, including for uses related to text and data mining, AI training, and similar technologies.

1	sPLA2	0.53, (0.37-0.69), 67	0.54, (0.44-0.64), 132	0.54, (0.46-0.62), 210	0.58, (0.48-0.67), 199	0.56, (0.48-0.64), 302	0.56, (0.48-0.63), 326	-	-	-	-
2	NGAL	0.49, (0.33-0.65), 65	0.62, (0.51-0.72), 110	0.53, (0.44-0.62), 175	0.55, (0.46-0.65), 196	0.54, (0.46-0.62), 296	0.53, (0.45-0.61), 320	-	-	-	-
3	LBP	0.56, (0.41-0.7), 66	0.56, (0.45-0.67), 112	0.53, (0.44-0.61), 177	0.65, (0.56-0.74), 195	0.6, (0.52-0.67), 298	0.56, (0.49-0.64), 322	-	-	-	-
4	C2	0.67, (0.53-0.81), 67	0.59, (0.49-0.69), 133	0.58, (0.51-0.66), 210	0.5, (0.4-0.6), 193	0.51, (0.43-0.58), 296	0.51, (0.44-0.59), 320	-	-	-	-
5	AGP	0.6, (0.45-0.75), 67	0.57, (0.47-0.67), 133	0.54, (0.46-0.62), 211	0.65, (0.55-0.74), 199	0.58, (0.5-0.66), 302	0.56, (0.49-0.64), 326	-	-	-	-
6	HBP	0.48, (0.25-0.71), 28	0.54, (0.36-0.72), 44	0.47, (0.31-0.63), 55	0.66, (0.51-0.81), 107	0.66, (0.53-0.79), 136	0.63, (0.5-0.76), 142	-	-	-	-
7	HP	0.53, (0.39-0.67), 67	0.58, (0.48-0.68), 132	0.5, (0.42-0.58), 209	0.56, (0.46-0.66), 196	0.47, (0.39-0.55), 299	0.48, (0.4-0.55), 323	-	-	-	-

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65) red (AUROC < 0.6)

Supplementary Table 12: Univariate analysis - age less than 6 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.64, (0.47-0.81), 50	0.71, (0.59-0.82), 148	0.7, (0.6-0.8), 178	0.62, (0.23-1), 11	0.62, (0.36-0.88), 44	0.62, (0.41-0.83), 56
RBC count	0.51, (0.33-0.68), 49	0.55, (0.44-0.65), 147	0.55, (0.44-0.65), 177	0.7, (0.34-1), 11	0.63, (0.42-0.84), 44	0.62, (0.45-0.8), 56
Lymphocyte count	0.45, (0.26-0.64), 49	0.58, (0.47-0.7), 147	0.55, (0.44-0.66), 177	0.57, (0.17-0.96), 11	0.6, (0.34-0.86), 44	0.63, (0.42-0.85), 56
Neutrophil count	0.59, (0.41-0.77), 49	0.65, (0.53-0.76), 140	0.66, (0.56-0.76), 169	0.7, (0.3-1), 11	0.49, (0.24-0.75), 44	0.55, (0.35-0.75), 56
IL-4	0.68, (0.5-0.86), 50	0.62, (0.52-0.71), 148	0.58, (0.49-0.67), 178	0.5, (0.5-0.5), 11	0.47, (0.42-0.51), 39	0.48, (0.44-0.51), 51
TRAIL	0.73, (0.56-0.89), 50	0.59, (0.48-0.69), 148	0.56, (0.47-0.66), 178	0.5, (0.5-0.5), 11	0.5, (0.5-0.5), 41	0.5, (0.5-0.5), 53
IL-6	0.6, (0.4-0.79), 49	0.64, (0.53-0.74), 147	0.63, (0.53-0.72), 175	0.47, (0.2-0.73), 11	0.48, (0.33-0.62), 37	0.48, (0.36-0.59), 49
CRP NycoCard	0.52, (0.33-0.7), 48	0.58, (0.48-0.69), 145	0.56, (0.46-0.66), 175	0.78, (0.47-1), 11	0.66, (0.41-0.91), 44	0.63, (0.42-0.84), 56
Gal-9	0.58, (0.37-0.79), 49	0.54, (0.43-0.65), 148	0.53, (0.43-0.64), 178	0.5, (0.05-0.95), 11	0.63, (0.45-0.82), 41	0.6, (0.44-0.76), 53
CHI3L1	0.53, (0.36-0.7), 50	0.6, (0.49-0.71), 148	0.57, (0.47-0.67), 178	0.47, (0.07-0.86), 11	0.54, (0.28-0.79), 40	0.56, (0.33-0.8), 51

IP-10	0.73, (0.57-0.9), 50	0.58, (0.47-0.69), 143	0.57, (0.47-0.67), 172	0.77, (0.38-1), 11	0.45, (0.26-0.64), 39	0.48, (0.32-0.64), 51
sPLA2	0.49, (0.3-0.69), 50	0.63, (0.52-0.75), 148	0.62, (0.52-0.72), 178	0.73, (0.38-1), 11	0.52, (0.27-0.78), 41	0.52, (0.31-0.73), 53
NGAL	0.61, (0.43-0.79), 47	0.56, (0.44-0.68), 118	0.54, (0.43-0.65), 141	0.87, (0.6-1), 11	0.62, (0.4-0.85), 40	0.61, (0.41-0.8), 52
LBP	0.55, (0.3-0.79), 48	0.48, (0.37-0.59), 122	0.52, (0.41-0.62), 147	0.45, (0.03-0.87), 11	0.58, (0.33-0.83), 41	0.61, (0.4-0.81), 53
C2	0.57, (0.38-0.76), 50	0.57, (0.47-0.68), 148	0.56, (0.46-0.67), 178	0.58, (0.2-0.97), 11	0.78, (0.6-0.96), 38	0.77, (0.6-0.93), 50
AGP	0.68, (0.52-0.84), 50	0.6, (0.49-0.71), 149	0.57, (0.47-0.68), 179	0.63, (0.24-1), 11	0.52, (0.32-0.73), 41	0.46, (0.27-0.65), 53
HBP	0.55, (0.27-0.84), 33	0.62, (0.49-0.76), 78	0.63, (0.49-0.76), 82
HP	0.72, (0.58-0.87), 50	0.59, (0.48-0.7), 147	0.56, (0.46-0.67), 177	0.57, (0.18-0.95), 11	0.45, (0.21-0.69), 40	0.47, (0.26-0.68), 52

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Supplementary Table 13: Univariate analysis - aged between 7 and 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives			
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict
WBC count	0.67, (0.51-0.82), 51	0.7, (0.6-0.8), 134	0.66, (0.57-0.75), 185	## unbalanced classes (24 non-bacterial, 1 bacterial) for 25 patients	## unbalanced classes (54 non-bacterial, 1 bacterial) for 55 patients	-	0.47, (0.03-0.91), 72
RBC count	0.74, (0.6-0.87), 51	0.55, (0.43-0.68), 134	0.53, (0.43-0.63), 185	-	-	-	0.67, (0.28-1), 73
Lymphocyte count	0.64, (0.49-0.79), 51	0.59, (0.47-0.7), 134	0.55, (0.46-0.64), 184	-	-	-	0.44, (0.14-0.75), 72
Neutrophil count	0.63, (0.47-0.79), 50	0.67, (0.56-0.78), 127	0.67, (0.58-0.76), 174	-	-	-	0.51, (0.17-0.86), 73
IL-4	0.53, (0.36-0.7), 51	0.54, (0.44-0.64), 134	0.53, (0.45-0.61), 184	-	-	-	0.62, (0.27-0.96), 65
TRAIL	0.51, (0.35-0.68), 51	0.52, (0.41-0.63), 134	0.54, (0.45-0.63), 184	-	-	-	0.62, (0.38-0.87), 72
IL-6	0.62, (0.46-0.78), 50	0.57, (0.46-0.68), 132	0.51, (0.41-0.6), 181	-	-	-	0.41, (0.37-0.46), 67
CRP NycoCard	0.55, (0.39-0.71), 51	0.52, (0.4-0.64), 134	0.51, (0.41-0.61), 185	-	-	-	0.59, (0.21-0.97), 73
Gal-9	0.6, (0.44-0.76), 51	0.53, (0.42-0.65), 134	0.55, (0.45-0.65), 185	-	-	-	0.64, (0.23-1), 72

CHI3L1	0.53, (0.36-0.69), 51	0.49, (0.38-0.6), 133	0.54, (0.45-0.64), 183	-	-	-	0.61, (0.08-1), 69
IP-10	0.63, (0.47-0.79), 50	0.56, (0.45-0.68), 133	0.53, (0.43-0.63), 184	-	-	-	0.55, (0.11-0.99), 67
NGAL	0.55, (0.38-0.71), 51	0.52, (0.41-0.64), 134	0.53, (0.44-0.63), 185	-	-	-	0.56, (0.13-0.99), 72
HNL	0.67, (0.48-0.85), 42	0.47, (0.35-0.59), 108	0.57, (0.48-0.67), 150	-	-	-	0.66, (0.33-1), 69
LBP	0.61, (0.44-0.78), 42	0.59, (0.47-0.71), 108	0.56, (0.46-0.66), 151	-	-	-	0.9, (0.77-1), 67
C2	0.62, (0.46-0.78), 51	0.57, (0.46-0.68), 133	0.54, (0.45-0.64), 184	-	-	-	0.73, (0.47-0.98), 70
AGP	0.6, (0.44-0.76), 51	0.55, (0.43-0.67), 134	0.52, (0.42-0.62), 185	-	-	-	0.53, (0.07-0.99), 72
HBP	0.64, (0.39-0.9), 21	0.46, (0.28-0.65), 50	0.49, (0.31-0.67), 55	-	-	-	-
HP	0.54, (0.37-0.7), 51	0.49, (0.38-0.59), 132	0.49, (0.40-0.59), 183	-	-	-	0.79, (0.6-0.98), 71

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 14: Univariate analysis - aged more than 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives			
	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose
WBC count	0.54, (0.32-0.76), 31	0.56, (0.37-0.75), 87	0.65, (0.51-0.78), 128	2 patients in total	11 patients in total	11 patients in total	
RBC count	0.42, (0.2-0.63), 31	0.58, (0.42-0.73), 86	0.57, (0.44-0.7), 126				
Lymphocyte count	0.77, (0.61-0.94), 31	0.64, (0.5-0.78), 87	0.66, (0.55-0.77), 127				
Neutrophil count	0.5, (0.28-0.73), 30	0.55, (0.35-0.74), 81	0.62, (0.48-0.77), 120				
IL-4	0.53, (0.33-0.73), 31	0.5, (0.34-0.66), 87	0.48, (0.37-0.59), 126				
TRAIL	0.62, (0.42-0.82), 31	0.6, (0.44-0.76), 87	0.63, (0.51-0.75), 126				
IL-6	0.67, (0.47-0.87), 32	0.52, (0.35-0.69), 88	0.54, (0.41-0.66), 129				
CRP NycoCard	0.57, (0.36-0.78), 32	0.52, (0.37-0.68), 88	0.52, (0.4-0.64), 129				
Gal-9	0.61, (0.4-0.82), 32	0.59, (0.44-0.73), 87	0.52, (0.39-0.65), 128				

CHI3L1	0.64, (0.43-0.85), 31	0.53, (0.37-0.69), 86	0.52, (0.4-0.65), 126	-	-	-	-
IP-10	0.66, (0.45-0.87), 32	0.52, (0.35-0.69), 87	0.58, (0.44-0.71), 128	-	-	-	-
sPLA2	0.62, (0.42-0.82), 32	0.53, (0.37-0.69), 88	0.56, (0.44-0.69), 129	-	-	-	-
NGAL	0.7, (0.48-0.92), 25	0.55, (0.35-0.75), 65	0.56, (0.41-0.7), 95	-	-	-	-
LBP	0.37, (0.14-0.6), 25	0.47, (0.29-0.66), 65	0.59, (0.46-0.73), 95	-	-	-	-
C2	0.64, (0.43-0.85), 32	0.59, (0.42-0.76), 88	0.47, (0.33-0.6), 129	-	-	-	-
AGP	0.68, (0.49-0.87), 32	0.47, (0.31-0.63), 88	0.52, (0.39-0.64), 129	-	-	-	-
HBP	0.8, (0.34-1), 7	0.62, (0.29-0.95), 23	0.62, (0.29-0.95), 24	-	-	-	-
HP	0.52, (0.31-0.73), 32	0.51, (0.35-0.67), 86	0.53, (0.41-0.64), 127	-	-	-	-

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 15: Multivariate analysis – non-malaria population; haematological biomarkers

Haematological biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
country neutrophil count, WBC count, lymphocyte count, fever duration, temperature, pulse rate, respiratory rate	country neutrophil count, fever duration	country neutrophil count fever duration respiratory rate		RF/SW/RFA: 0.75 (0.03)	WBC count: 0.7 (0.03)	+7%
		SW: 0.83 (0.04)		WBC count: 0.78 (0.03)	+6%	
		E SW/RFA: 0.83 (0.02)		WBC count: 0.77 (0.03)	+8%	
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.7 (0.12)	WBC count: 0.7 (0.03)	
			S	SW: 0.77 (0.12)	WBC count: 0.73 (0.03)	+5%
			E	RFA: 0.77 (0.08)	WBC count: 0.75 (0.03)	+3%
Malawi						
diastolic blood pressure, HAEMATO_C lymphocyte count, neutrophil count, neutrophil count, pulse rate, temperature, fever duration	fever duration neutrophil count	fever duration neutrophil count	L	RFA: 0.74(0.05)	neutrophil count: 0.72(.06)	+3%
			S	SW: 0.73(.06)	neutrophil count: 0.72(.07)	+1%
			E	RFA: 0.66(.16)	WBC count: 0.7 (0.05)	-6%

Brazil						
diastolic blood pressure, haematocrit lymphocyte count, neutrophil count, pulse rate, temperature, fever duration, respiratory rate, WBC count	WBC count respiratory rate neutrophil count	WBC count respiratory rate	L	RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%
			S	RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%
			E	RFA: 0.84 (0.07)	WBC count: 0.83 (0.07)	+1%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data due to the limited data.

Supplementary Table 16: Multivariate analysis – non-malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/model s: mean (SD) AUROC	Best host- biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
CRP AGP LBP NGAL pulse rate respiratory rate diastolic blood pressure temperature country	CRP country LBP NGAL pulse rate	CRP country NGAL pulse rate respiratory rate temperature		L RF/RFA/SW: 0.66 (0.05)	LBP: 0.62 (0.04)	+6%
				S RF: 0.74 (0.04)	LBP: 0.66 (0.05)	+12%
				E RFA: 0.76 (0.04)	LBP: 0.75 (0.04)	+1%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.64 (0.12)	LBP: 0.62 (0.04)	+3%
			S	RFA: 0.7 (0.11)	LBP: 0.66 (0.05)	+6%
			E	RFA: 0.7 (0.09)	LBP: 0.75 (0.04)	-7%
Malawi						
IP-10 Gal-9 NGAL temperature CRP respiratory rate fever duration pulse rate diastolic blood pressure	Gal-9 NGAL temperature	Gal-9 NGAL temperature pulse rate fever duration	L	SW: 0.7 (0.06)	Lipocalin. 2: 0.65 (0.06)	+8%
			S	RF/ SW: 0.67 (0.06)	Lipocalin. 2: 0.64 (0.06)	+5%
			E	RF: 0.71 (0.12)	IP-10: 0.69 (0.08)	+3%
Brazil						
CRP, AGP	Gal-9, TRAIL,	Gal-9, pulse rate, fever duration,	L	RF: 0.67 (0.04)	CRP: 0.65 (0.06)	+3%

pulse rate, diastolic blood pressure respiratory rate, temperature	NGAL	NGAL, temperature	S	SW/RFA: 0.66(.04)	CRP: 0.65 (0.05)	+1%
			E	SW/RFA: 0.65(.05)	CRP: 0.63 (0.08)	+3%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 17: Multivariate analysis – non-malaria population; haematological and protein biomarkers

Haematology + protein biomarkers								
Overall								
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%) ** multivariate and single host-biomarkers ratio		
Rulefit	Logistic - RFA	Logistic - SW	L	SW/RFA/RF:0.75(.03)	WBC count: 0.7 (.03)	+7%		
				SW:0.83(.04)	WBC count: 0.78(.03)	+6%		
				SW/RFA:0.83 (.03)	WBC count: 0.77 (0.04)	+8%		
Brazil								
Gal-9, neutrophil count, WBC count, CRP, sPLA, respiratory rate, temperature, diastolic rate, Gal-9 blood pressure, fever duration, pulse rate	neutrophil count, WBC count, respiratory rate	WBC count, Gal-9 respiratory rate	L	SW: 0.82 (0.06)	WBC count: 0.8 (0.06)	+2.5%		
				RFA: 0.82 (0.06)	WBC count: 0.8 (0.06)	+2.5%		
				SW: 0.85 (0.06)	WBC count: 0.83 (0.07)	+2%		
Gabon*								
Gabon performance evaluation using the overall model and Gabon data extracted from the Overall test sets			L	SW/RFA: 0.7 (0.12)	WBC count: 0.7 (.03)	-		
				SW/RFA: 0.76 (0.12)	WBC count: 0.78(.03)	-3%		
				RFA: 0.77 (0.07)	WBC count: 0.77 (0.04)	-		
Malawi								
IP-10 Gal-9 LBP neutrophil count	neutrophil count, WBC count	neutrophil count WBC count, fever duration,	L	SW/RFA: 0.74 (0.06)	neutrophil count: 0.72 (0.03)	+3%		

WBC count	duration, IP-10	IP-10, temperature	S	SW: 0.73 (0.06)	neutrophil count: 0.72 (0.07)	+1%
NGAL			E	RFA: 0.72 (0.6)	WBC count: 0.7 (0.)	+2%

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10 E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic
11 recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better
12 performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate
13 models).

14 * Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor
15 model and tested with Gabon data.

18 **Supplementary Table 18: Multivariate analysis – malaria population; haematological biomarkers**

Haematological biomarkers						
Multivariate models' variables			Classification group	Best multivariate model/models : mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
haematocrit	neutrophil count	lymphocyte count		L	RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)
lymphocyte count	WBC count	neutrophil count	S	SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%
neutrophil count	country	country		RFA: 0.69 (0.07)	neutrophil count: 0.61 (0.08)	+13%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.18)	neutrophil count: 0.65 (0.05)	+3%
			S	SW: 0.75 (0.2)	neutrophil count: 0.6 (0.08)	+25%
			E	Not sufficient data		
Malawi						
diastolic blood pressure	neutrophil count,	WBC count,	L	RFA: 0.7 (0.06)	WBC count: 0.69 (0.05)	+1%
lymphocyte count	WBC count,		S	SW: 0.69 (0.07)	WBC count: 0.69 (0.07)	-
neutrophil count	temperature		E	RFA: 0.6 (0.14)	lymphocyte count: 0.67 (0.05)	-10%
temperature						
WBC count						
haematocrit						
pulse rate						
respiratory rate						
fever duration						

51 E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic
52 recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better
53 performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate
54 models).

55 * Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor
56 model and tested with Gabon data.

Supplementary Table 19: Multivariate analysis – malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
AGP diastolic blood pressure Gal-9 C2 LBP pulse rate respiratory rate temperature fever duration	C2	country respiratory rate temperature AGP	L	SW: 0.62 (0.07)	CHI3L1: 0.57 (0.03)	+ 9%
			S	SW: 0.64 (0.04)	NGAL: 0.6 (0.06)	+ 7%
			E	SW: 0.67 (0.08)	C2: 0.63 (0.1)	+ 6%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.17)	CHI3L1: 0.57 (0.03)	+ 18%
			S	RFA: 0.81 (0.12)	NGAL: 0.6 (0.06)	+35% ^{\$}
			E	Not sufficient data		
Malawi						
diastolic blood pressure CHI3L1 IP-10 fever duration Gal-9 C2 pulse rate respiratory rate temperature			L	RFA/SW: 0.57 (0.06)	IP-10: 0.57 (0.05)	-
			S	SW/RFA: 0.62 (0.09)	HCC2_PL: 0.62 (0.06)	-
			E	SW/RFA: 0.61 (0.06)	IP-10: 0.66 (0.09)	-7%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model and tested with Gabon data. ^{\$}This output has to be considered an outlier due to biomarker data imbalance between pipeline data and the available Gabon data set.

Supplementary Table 20: Multivariate analysis – malaria population; haematological and protein biomarkers

Protein + haematological biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				

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AGP_Pl diastolic blood pressure Gal-9 C2 LBP. NGAL neutrophil count respiratory rate temperature pulse rate fever duration	country WBC count	country, Wbc_c,	L	SW/RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)	+5%
			S	RFA/SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%
			E	RFA/SW: 0.66 (0.11)	HCC2_PL: 0.63 (0.1)	+5%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	RFA/SW: 0.66 (0.18)	neutrophil count: 0.65 (0.05)	+1%
			S	RFA/SW: 0.7 (0.2)	neutrophil count: 0.6 (0.08)	+17%
			E	Not sufficient data		
Malawi						
CHI3L1 IP-10 Gal-9 C2 neutrophil count respiratory rate temperature diastolic blood pressure pulse rate fever duration	C2 neutrophil count WBC count	WBC count	L	SW: 0.69 (0.05)	WBC count: 0.69 (0.05)	-
			S	RFA: 0.73 (0.07)		WBC count: 0.69 (0.07)
			E	RFA: 0.72. (0.1)	lymphocyte count: 0.67 (0.05)	+7%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model and tested with Gabon data.

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Cross-Sectional Evaluation of Host Biomarkers for Guiding Antibiotic Use in Bacterial and Non-Bacterial Acute Febrile Illness in Low- and Middle-Income Tropical Settings

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1 1 **Cross-Sectional Evaluation of Host Biomarkers for Guiding Antibiotic**
2 2 **Use in Bacterial and Non-Bacterial Acute Febrile Illness in Low- and**
3 3 **Middle-Income Tropical Settings**

4
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46 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 47 • This study is the most diverse evaluations of host biomarkers across three settings in
48 low- and middle-income countries (LMICs) to differentiate bacterial from non-bacterial
49 infections.
- 50 • The study protocol aligns with FDA-approved classifications for distinguishing
51 between bacterial and non-bacterial infections, enhancing methodological rigor.
- 52 • The absence of a control group limits the ability to establish baseline biomarker
53 performance or to assess asymptomatic carriers.
- 54 • The two-step clinical classification process may introduce subjectivity, particularly as
55 clinicians had access to hematology biomarker results during classification, potentially
56 biasing results.

1 2 3 57 ABSTRACT 4 5

6 58 Objectives 7 8

9 59 To evaluate the effectiveness of 18 different host biomarkers in differentiating bacterial from
10 60 non-bacterial acute febrile illness (AFI) in resource-limited settings, specifically in Brazil,
11 61 Malawi, and Gabon.

12 62 Design 13 14

15 63 Multinational, cross-sectional study
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18 64 Setting 19 20

21 65 The study was carried out across multiple primary healthcare facilities, including urban and
22 66 rural settings, with a total of three participating centers. Recruitment took place from October
23 67 2018 to July 2019 in Brazil, May to November 2019 in Gabon, and April 2017 to April 2018
24 68 in Malawi.
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27 69 Participants 28 29

30 70 A total of 1,915 participants, including children and adults aged 21 to 65 years with a fever of
31 71 ≤ 7 days, were recruited through convenience sampling from outpatient clinics in Brazil, Gabon,
32 72 and Malawi. Individuals with signs of severe illness were excluded. Written consent was
33 73 obtained from all participants or their guardians.
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36 74 Intervention 37 38

39 75 Not applicable as the study primarily focused on biomarker evaluation without specific
40 76 therapeutic interventions.
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43 77 Primary and Secondary Outcome Measures 44 45

46 78 The primary outcome measure was the ability of each host biomarker to differentiate between
47 79 bacterial and non-bacterial AFI, as evaluated by area under the receiver operating characteristic
48 80 (AUROC) curves. Secondary outcomes included the performance of individual biomarkers
49 81 across the different study sites and in a multivariable setting.
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1 2 82 **Results**

3
4 83 A Kruskal-Wallis test, adjusted by Benjamini-Hochberg, was performed for each biomarker to
5 identify covariates significantly affecting biomarker values. The analysis revealed that country
6 of origin (Brazil, Gabon, Malawi), age, sex, and malaria status significantly impacted
7 biomarker distribution ($p \leq 0.001$). The most widely known biomarkers, such as white blood
8 cell count and C-reactive protein (CRP), demonstrated the best performance in distinguishing
9 between bacterial and non-bacterial infections, with AUROCs reaching up to 0.83 [0.77 - 0.88]
10 for white blood cell count and 0.71 [0.59 - 0.82] for CRP. However, none of the evaluated
11 novel host biomarkers exhibited high performance (AUROC < 0.70 in most cases), and
12 variations in biomarker performance were observed across the three settings. Multivariable
13 analyses demonstrated that while the best combination of biomarkers achieved higher
14 AUROCs, the increase was modest (1–13%), suggesting that the interaction of biomarkers
15 contributed minimally to predictive accuracy.

16 95 **Conclusions**

17 96 There is a continued need for innovation in the host-biomarker space as the available markers
18 do not meet the needs of diverse populations around the globe. This highlights the importance
19 of targeted evaluations in non-severe patients in multiple settings to understand true potentials
20 for real-life use. The findings highlight that not one-marker fits all settings and novel
21 innovations remain urgently needed.

22 101 **Trial Registration**

23 102 Clinical trial number: NCT03047642

24 103 **Keywords**

25 104 Antimicrobial Resistance, AMR, CRP, Host Biomarkers, Prospective study, biomarker, non-
26 malaria fever, primary health care, Malawi, Brazil, Gabon

27 106

107 INTRODUCTION

108 Globally, acute febrile illness (AFI) is one of the leading reasons individuals, particularly
109 children aged less than 5 years, present to primary healthcare facilities [1]. AFI has various
110 causes, both infectious and non-infectious, that vary according to geography, age group, and
111 season [1]. In malaria-endemic settings, malaria was long considered the primary cause of all
112 fevers; however, the introduction of rapid diagnostic tests (RDTs) for malaria in the past decade
113 has disproved this. Modelling estimates suggest that approximately 70% of all fevers can be
114 attributed to non-malarial causes, even in malaria-endemic settings [2]. In the Integrated
115 Management of Childhood Illness (IMCI), introduced by the World Health Organization
116 (WHO) and UNICEF in the mid-1990s and subsequently implemented in more than 100
117 countries, the standard “fever” algorithm currently includes a malaria RDT but no diagnostic
118 test for other infections [3]. Hence, at primary care level, the only evidence-based treatment
119 decision that can be made relies on the malaria RDT, resulting in extremely high levels of
120 antibiotic use in malaria-negative patients [4]. In this context of limited knowledge about the
121 causes of AFI and limited diagnostic and human capacity, it is unsurprising that healthcare
122 providers prescribe antibiotics to avoid negative outcomes in their patients.

123 To assist healthcare providers with clinical decision-making, a simple diagnostic tool is
124 required to differentiate patients with AFI of bacterial and non-bacterial aetiology and provide
125 appropriate care. In well-resourced settings, in both high-income countries (HICs) and low-
126 and middle-income countries (LMICs), some nonspecific host-biomarkers are used for this
127 purpose, most frequently C-reactive protein (CRP) and procalcitonin (PCT), although these
128 biomarkers are less useful in settings with a higher frequency of comorbidities [5]. Thus, in
129 2015, an international group of experts was convened to define the target product profile (TPP)
130 of such a tool, specifically for low-resource settings, to guide product development and
131 implementation as part of integrated treatment management guidelines [6]. Since then, the

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3 132 ongoing viral pandemic (SARS-CoV-2) has further highlighted the challenge of differential
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5 133 diagnosis and shows yet again that better antimicrobial stewardship interventions are needed
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7 134 to counter the overprescribing of antibiotics in patients with viral infections [7].
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11 135 Host biomarkers other than CRP and PCT have been evaluated for distinguishing bacterial
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13 136 from non-bacterial infections, including human neutrophil lipocalin (HNL), heparin-binding
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15 137 protein (HBP), and chitinase 3-like protein 1 (CHI3L1) [8]. There are also some commercially
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17 138 available tests. ImmunoXpert™, from MeMed, uses a biomarker combination comprising
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19 139 CRP, interferon gamma-inducible protein 10 (IP-10), and TNF-related apoptosis-inducing
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21 140 ligand (TRAIL), while FebriDx®, from Lumos Diagnostics, uses an MxA and CRP biomarker
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23 141 combination. While these biomarker signatures show promise, they have only been evaluated
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25 142 in limited settings. Any potential impact of co-infections or comorbidities, common in LMICs,
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27 143 on their effectiveness is unknown. Other characteristics of host-biomarker studies that hamper
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29 144 direct comparisons include: (i) just one/a few biomarkers in the study; (ii) small sample sizes,
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31 145 increasing the probability of recruiting unrepresentative study populations; (iii) narrow
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33 146 population subgroups (e.g. children only, hospitalised only, respiratory infections only, etc),
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35 147 limiting the generalisability of study results to the broader AFI population; (iv) studies
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37 148 conducted in one country, so co-infections/comorbidities may not be comparable with those of
38
39 149 other countries; (v) retrospective studies that used convenience sampling and case-control
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41 150 study designs, increasing the risk of bias; and (vi) the lack of a standard definitions for
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43 151 classifying bacterial versus non-bacterial infections [9].
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152 Here, we describe the Biomarker for Fever Diagnostic (BFF-Dx) study, specifically designed
153 to evaluate host biomarkers to distinguish bacterial from non-bacterial infections in line with
154 the published TPP and the final use case of such diagnostic tests. To our knowledge, this is the
155 only study to evaluate host biomarkers in the intended target population (non-severe patients),
156 prospectively, in multiple settings with a large sample set. We evaluated 18 host-biomarkers in

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3 157 three distinct settings, in Brazil, Gabon, and Malawi with the main objective to provide a
4 158 performance comparison of host biomarkers in the non-severe AFI population from resource-
5 159 limited settings, with the goal to overcome many of the previously described limitations (eg.
6 160 sample size, retrospective vs prospective, focused populations, biased analysis) [10]. The
7 161 described comparison was conducted within the pragmatic context of diagnostic product
8 162 development and aimed to identify host biomarkers or biomarker combinations for utilisation
9 163 in next-generation rapid diagnostic tests.

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3 164 **METHODOLOGY**
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6 165 **Study settings**
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9 166 This multinational, cross-sectional study was conducted in Brazil, Gabon, and Malawi; Gabon
10 and Malawi were selected as high-malaria endemicity settings, while Brazil was selected as a
11 low-malaria endemic setting. The study sites were UPA Manguinhos and Family Health Clinics
12 Armando Palhares in Rio de Janeiro, Brazil; the Clinical Trials Unit Center of Medical
13 Research Lambaréné (CERMEL), Lambaréné, Gabon; and Malawi Epidemiology and
14 Intervention Research Unit (MEIRU), Chilumba campus, Malawi. The enrollment sites were
15 an urban primary healthcare facility, a hospital in a semi-rural setting, and a rural primary
16 healthcare facility in Brazil, Gabon, and Malawi, respectively. Participants were recruited from
17 October 2018 to July 2019, May to November 2019, and April 2017 to April 2018, in Brazil,
18 Gabon, and Malawi, respectively. The study protocol was submitted to clinicaltrial.gov
19 (NCT03047642) and ethical approval was obtained from all relevant institutional committees
20 in Brazil (Research Ethics Committee of INI-FIOCRUZ and Comissão Nacional de Ética em
21 Pesquisa [Ref:2.235.565] ; National Research Ethics Committee), Gabon (Comité National
22 d'Ethique pour la Recherche [RefNr:N°0078/2019PR/SG/CNER]) and Malawi (National
23 Health Science Research Committee [ApprovalNr: 16/9/1668] ; Observational and
24 Intervention Research Ethics Committee of the London School of Hygiene and Tropical
25 Medicine , UK [LSTMH Ref: 11974]) and all details of the design have been previously
26 published [10]. Reporting complies with the STARD-15 checklist.
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185 **Study population and study procedure**

186 Participants were obtained through convenience sampling and included both children and
187 adults, aged between 2 and 65 years, who presented at the outpatient clinics with a history of
188 fever of ≤ 7 days duration (Brazil and Gabon) or fever at presentation (Malawi). Patients with

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3 signs of severe illness were not included in the study. The overarching study protocol was
4 slightly adapted to each site due to local requirements (logistical or ethical). Detailed criteria
5 for inclusion by study sites have been published previously [10]. Outcomes were based on the
6 TPP criteria and while no patient input was used, external expert input was used to define target
7 population and criteria. Only patients who met the eligibility criteria and who provided written
8 consent (patient or guardian for children) were enrolled in the study. Data and samples were
9 systematically collected and analysed as previously described. To ensure consistent quality and
10 comparability of data, the same standard operating procedures were used at all sites (for data
11 collection and laboratory testing) [10].
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199 **Patient and Public Involvement statement**

200 None

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202 **Bacterial/non-bacterial classification and biomarker selection and testing**

203 A two-step process was used to classify the patients into “bacterial” and “non-bacterial”
204 groups. First, the cause of fever (bacterial/non-bacterial) was classified according to laboratory-
205 determined parameters (“electronic group”). The electronic group was based on predefined and
206 widely accepted laboratory parameters, including direct pathogen detection, a fourfold increase
207 in anti- body titre, or a positive PCR or antigen RDT result. The list of tests performed is
208 described in detail in by Escadafal et al. [10]. Next, cases that could not be classified by
209 laboratory-determined parameters were assessed by a panel of three independent clinical
210 experts. Patient’s history and clinical and laboratory data was provided to the experts. Clinical
211 expert’s assessments were then compared. If the three panel members unanimously assigned a
212 diagnostic label, patients were considered to have “bacterial” or “non-bacterial” infections; if
213 two out of three panel members reported a classification of “bacterial” or “non-bacterial”, these

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3 214 patients were considered to have “probable bacterial infection” or “probable non-bacterial
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5 215 infection”, respectively.

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9 216 Data were analysed based on three groups of patients: 1) the “electronic group”, i.e. subjects
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11 217 with a cause of fever defined based on laboratory parameters; 2) the “strict group”, which
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13 218 comprised the electronic group and the patients that were unanimously classified by the clinical
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15 219 panel of three experts; and 3) the “loose group”, which comprised the electronic and strict
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17 220 groups as well as those patients for whom two of the clinical experts agreed they had either
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19 221 probable bacterial or probable non-bacterial infection. Subjects with undetermined cause of
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21 222 fever according to the three classification criteria considered (“electronic group”, “strict
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23 223 group”, “loose group”) were excluded from the statistical analysis. This outcome-oriented
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25 224 approach, based on methods previously developed for host-biomarker studies and described
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27 225 previously, was used to ensure the total intended-use population of any future test was
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29 226 represented in the final analysis [10, 11].
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36 227 The evaluated biomarkers were selected based on previously reported performances, and
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38 228 haematological markers as well as CRP were included as comparators (Table 1 and
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40 229 Supplementary Table 1 and 2) [8, 12].
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43 230 At the end of data collection, all biomarker data were analysed to assess the percentage of
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45 231 missing values and the percentage of values below the lower limit or above the upper limit of
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47 232 detection of the used tests. Biomarkers with more than 50% of missing data or more than 95%
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49 233 of saturated values below the lower limit of quantification of the used test, were excluded from
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51 234 the following statistical analysis.
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3 235 **Table 1.** Novel biomarkers identified in the literature and evaluated in the BFF-Dx study,
4 including sample type used, evaluation method, and sample origin.
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Abbreviation	Biomarker name	Sample type	Evaluation method	Sample origin
AGP	A-1-acid glycoprotein	EDTA-plasma	Luminex	B, G, M
C2	Complement 2	EDTA-plasma	Luminex	B, G, M
C4b	Complement C4b	EDTA-plasma	Luminex	B, G, M
CHI3L1	Chitinase-3-like protein 1	EDTA-plasma	Luminex	B, G, M
CRP	C-reactive protein	EDTA-plasma	CRP Nycocard/ NycoCardReader II, ELISA	B, G, M
Gal-9	Galectin-9	EDTA-plasma	Luminex	B, G, M
HBP	Heparin-binding protein	EDTA-plasma	ELISA	B, M
HNL	Human neutrophil lipocalin	Heparin-activated plasma time-controlled activation#	ELISA	M
		EDTA-plasma	ELISA	B, G, M
HP	Haptoglobin	EDTA-plasma	Luminex	B, G, M
IFN-gamma	Interferon gamma	EDTA-plasma	Luminex	B, G, M
IL-4	Interleukin-4	EDTA-plasma	Luminex	B, G, M
IL-6	Interleukin-6	EDTA-plasma	Luminex	B, G, M
IP-10	Gamma-induced protein 10	EDTA-plasma	Luminex	B, G, M
LBP	Lipopolysaccharide binding protein	EDTA-plasma	Luminex	B, G, M
NGAL	Neutrophil gelatinase-associated lipocalin	Frozen heparin-activated plasma	Luminex	M
		EDTA-plasma	Luminex	B, G, M
PCT	Procalcitonin	EDTA-plasma	Luminex; ELISA	B, G, M
sPLA2	Secretory phospholipase 2	EDTA-plasma	Luminex	B, G, M
sTREM-1	Soluble triggering receptor expressed on myeloid cells 1	EDTA-plasma	Luminex	B, G, M
TRAIL	TNF-related apoptosis-inducing ligand	EDTA-plasma	Luminex	B, G, M

237 B, Brazil; G, Gabon; M, Malawi
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239 # Whole blood samples were collected in lithium heparin tubes and activation was performed within 60 min prior to freezing
240 and subsequent ELISA testing [13]. All biomarkers were tested using the same standard operating procedures (SOPs) and all
241 sites were trained on the SOPs. For CRP and PCT different devices were used at different sites, repeat testing was performed
242 at the central facility (NMI).

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3 244 **Statistical analysis**

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5 245 a. Kruskal-Wallis Analysis and Definition of Covariates Influence on Biomarkers

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7 246 A Kruskal-Wallis test, adjusted by Benjamini-Hochberg, was performed for each biomarker to
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9 247 identify which covariates significantly affect the biomarker value. The covariates studied were
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11 248 country (i.e., the country of origin of the patients), age, sex, malaria status, comorbidities (i.e.,
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13 249 presence of one or more diseases among cardiovascular, neurological, respiratory, renal,
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15 250 genitourinary, connective tissue, cancer, or infectious diseases), malnutrition status calculated
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17 251 based on WHO body mass index criteria, self-reported use of antibiotics prior to visiting the
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19 252 health facility, axillary temperature $\geq 38^{\circ}\text{C}$, and positive result to Chikungunya test. The
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21 253 Kruskal-Wallis test was performed for each of the three patient groups defined in the previous
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23 254 section (“electronic”, “strict”, “loose”). The results of the Kruskal-Wallis test allowed the
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25 255 identification of covariates that most significantly impacted the biomarker distribution
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27 256 ($p \leq 0.001$, adjusted by Benjamini-Hochberg). The most significant covariates were considered
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29 257 for defining subgroups of patients in which the following univariate analyses were performed,
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31 258 or included as covariates in the multivariable analyses.

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33 259 b. Univariate analysis

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35 260 As an exploratory step, the ability of each biomarker to discriminate between bacterial and
36
37 261 non-bacterial infections was assessed by the area under the receiver operating characteristic
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39 262 curve (AUROC). In particular, subjects were ranked based on the values of the single variable
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41 263 of interest (i.e. based on ordered values) and, using this as score, calculated the ROC curve and
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43 264 the corresponding area under the curve. Such univariate analysis was conducted for each
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45 265 patient group (“electronic”, “strict”, “loose”) and specific patient subgroup (Malaria status,
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47 266 Country and Age).

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3 267 However, since the univariate analyses did not yield satisfactory results, we also explored
4 multivariable models to potentially improve the predictive capabilities by incorporating a
5 broader range of information.
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10 270 c. Multivariable analysis
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12 271 Multivariable classification models were developed to assess the discrimination ability of
13 combinations of biomarkers and covariates. For the multivariable analysis, both linear (logistic
14 regression) and non-linear classification models (RuleFit) were explored [14]. The candidate
15 features for each model included a group of host-biomarkers and some additional covariates
16 (age, temperature, fever duration, diastolic blood pressure, respiration rate, and pulse rate).
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18 274 Regarding host-biomarkers, three different groups of biomarkers were considered:
19 haematology biomarkers only (i.e. white blood cell, neutrophil, red blood cell, lymphocyte
20 counts), protein biomarkers only (i.e. novel biomarkers + CRP), and haematology plus protein
21 biomarkers (i.e. all biomarkers).
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33 280 For each patient subgroup and each candidate feature set, three multivariable models were
34 developed: i) a logistic regression model with stepwise (SW) feature selection; ii) a logistic
35 regression model with features selected based on recursive feature addition (RFA; a variant of
36 the method proposed in [15]); iii) RuleFit, a non-linear model in which a set of rules from an
37 ensemble of decision trees (typically from a tree-based model like a Random Forest or Gradient
38 Boosted Trees) is generated and then fit a sparse linear regression model (regularized with
39 LASSO), where the features are the rules generated from the trees [14, 15].
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287 To further tackle the number of biomarkers and variables included in the best models, we
288 introduced an additional selection step, employing a plateau seeking approach. The primary
289 objective of this approach was to pinpoint a concise set of variables capable of attaining an
290 AUROC score similar to that of our comprehensive model, which already incorporated the

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3 291 most impactful and previously selected variables. This was to ensure that our model is not only
4 effective in terms of performance but also efficient in its variable inclusion.
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7 293 Each model was trained and tested using the following pipeline. The data were randomly split
8 into training and test sets (80% and 20% of the data, respectively) stratifying by the outcome
9 variable. Missing data in the training and test sets were imputed using the MICE (multiple
10 imputation by chained equation) algorithm. The n_imp parameter for MICE imputation was
11 set to 1, resulting in a single imputed dataset; however, the imputation process was integrated
12 in a robust bootstrapping pipeline, generating ten independent datasets. This approach ensured
13 variability in our results, stemming not only from the MICE imputation but also from the
14 bootstrapping process. This dual approach guarantees that each imputed dataset is distinct [16].
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16 301 All quantitative variables were scaled into the range [0,1] by subtracting their minimum value
17 and dividing by the difference between the maximum and minimum values in the training set.
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19 303 The categorical variables with n categories were encoded using n-1 binary “dummy” variables.
20
21 304 The model was then trained on the imputed and scaled training set, and its performance was
22 assessed on the imputed and scaled test set by computing the AUROC. The AUROC on the
23 test set was also calculated for single host biomarkers, to allow a fair comparison of the
24 performance of the multivariable classification models vs. single host biomarkers.
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27 308 To assess the robustness and variability in the results of the developed models, the entire
28 pipeline were bootstrapped, i.e. it was run ten times with different random training-test set
29 splits. Finally, the mean and the standard deviation (SD) or the minimum and maximum
30 reached of the AUROC across the ten training-test splits were calculated for each multivariable
31 model and each single host biomarker.
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34 a. Software
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36 313 All statistical analyses and model development were performed using the R programming
37 language (version 4.1.2). Specifically, the *mice* package was used for data imputation, while
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3 316 the *pre* and *stats* packages were used for RuleFit and logistic regression model development,
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3 320 **RESULTS**
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8 321 **Study population**
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13 322 In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415;
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15 323 Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male.
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18 324 Children aged <5 years comprised 45/500 (9%), 182/415 (43·9%), and 367/1000 (36·7%)
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20 325 participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4)
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For peer review only

In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415; Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male. Children aged <5 years comprised 45/500 (9%), 182/415 (43·9%), and 367/1000 (36·7%) participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4) years (Table 2). Detailed baseline characteristics of patients and analyses of differences will be described in a separate manuscript (Alabi et al in preparation).

328 **Table 2: Baseline characteristics of patients.**

	Brazil	Gabon	Malawi	All
0–5 years (median, IQR, n)	3, [2-4], 45	3, [2-5], 182	3, [2-4], 137	3, [2-4], 594
5–15 years (median, IQR, n)	11, [8-14], 85	9, [7-12], 214	9 [7-12], 26	9, [7-12], 575
>15 years (median, IQR, n)	34, [24-45], 370	16, [16-16·5], 19	28, [21-33], 357	30, [21-42], 746
Male (%), n	49·6%, 248	45·1%, 187	42·7%, 217	45·0%, 862
Temperature, °C (median, IQR, n)	37·7, [36·7-38·4], 500	36·8, [36·4-37·4], 415	38·1, [37·8-38·8], 999	37·8, [37·3-38·5], 1914
WBC count, 10 ⁹ /L (median, IQR, n)	7·28, [5·47-10·39], 494	7·7, [5·7-10], 411	6·7, [5-8], 985	7·1, [5·3-9·8], 1890
Neutrophil count, 10 ⁹ /L (median, IQR, n)	4·97, [3·63-7·4], 494	2·77, [1·96-3·9], 408	4·3, [3·4-18], 906	4·1, [2·8-6], 1812
RBC count, 10 ⁹ /L (median, IQR, n)	40·1, [36·5-43·2], 494	33·2, [29·4-35·8], 412	36·2, [33·1-39·5], 984	36·3, [33-40·2], 1892
Lymphocyte count, 10 ⁹ /L (median, IQR, n)	1·15, [0·7-1·99], 493	2·73, [1·8-4·16], 411	1·5, [1-2·2], 982	1·63, [1-2·6], 1883
CRP NycoCard# – mg/L (median, IQR, n)	70·5, [35-98·75], 498	28, [5-73], 415	47, [12-16·5], 987	49, [13-98], 1900
Malaria-positive by RDT on-site (% all, n)	0·2%, 1	56·4%, 234	45·9%, 48	36·2%, 693
Malaria-positive by qPCR or microscopy (% all, n)	-	-	50·5%, 55	-
HIV-positive by RDT (% all, n)	1·4%, 7	1·2%, 5	4·2%, 4	2·8%, 54
History of antibiotic-use pre-presentation (% all, n)	8·8%, 44	2·41%, 10	7·2%, 7	6·5%, 124
History of antipyretic-use pre-presentation (% all, n)	83·2%, 416	79·76%, 331	55·1%, 51	62·2%, 1298
Cough (%), n	35·8%, 179	30·1%, 125	48·2%, 48	41%, 786

Diarrhea or vomiting (%), n	31·8%, 159	28·9%, 120	27·5%, 126	28·9%, 554
Dysuria or urinary urgency (%), n	0·9%, 45	5·12%, 21	7·6%, 33	7·4%, 142
Headache (%), n	76·4%, 382	46·5%, 193	71·1%, 338	67·2%, 1286
Sore throat or swallow pain (%), n	39%, 195	8·92%, 37	15·8%, 73	20%, 390
Rash (%), n	24·4%, 122	4·1%, 17	2·5%, 12	8·6%, 164

NycoCard was found to be equivalent to reference testing in the relevant range (Supplementary Figure 1). CRP, C-reactive protein; IQR, interquartile range; qPCR, quantitative PCR; RBC, red blood cell; RDT, rapid diagnostic test; WBC, white blood cell; -: data not available

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3 **331 Bacterial and non-bacterial outcomes by classification groups**

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5 332 Using the electronic classification grouping, 15·1% (290/1915) of cases were bacterial
6 infections, 20·2% (387/1915) were non-bacterial infections, and 64·5% (1238/1915) had an
7 undetermined cause of fever (Figure 1). Under the strict classification grouping, 24·3%
8 (366/1509), 66·9% (1010/1509), and 9·0% (133/1509) were classified as bacterial, non-
9 bacterial, and undetermined infections, respectively, while using the loose classification
10 grouping 25·7% (491/1915), 67·3% (1286/1915), and 7·0% (133/1915) were classified as
11 bacterial, non-bacterial, and undetermined infections, respectively (Figure 1). Subjects with
12 undetermined cause of fever/infections were excluded from the following univariate and
13 multivariable analyses.

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17 **342 Exclusion of biomarkers with too many missing or saturated values**

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19 343 The biomarkers C4b, HNL and PCT had more than 50% missing values and were therefore
20 excluded. The high number of missing values is due to fact that biomarkers were analysed in
21 groups based on the required dilution using Luminex platform. For some biomarkers the
22 dilution was not optimal, and it was only possible to re-measure biomarkers with a different
23 dilution a limited number of times. IFN-gamma and sTREM-1 were excluded due to more than
24 95% of values saturated to the minimum/maximum level detectable by the measurement
25 instrument. All the biomarkers retained in the analysis had less than 12% missing values
26 (Supplementary Table 3).

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3 352 **Identification of relevant subgroups for analyses**

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5 353 According to the Kruskal-Wallis analysis on the “electronic group”, the variables “country”,
6 “malaria status” and “age” had a strong ($p \leq 0.001$) or high ($0.001 \leq p < 0.01$) effect on many
7 of the host biomarkers (Supplementary Table 4). The variables “sex”, “comorbidities”,
8 “history of antibiotic use” showed no ($p > 0.05$) or slight ($p \leq 0.05$) associations with all the
9 host biomarkers. The effects of “chikungunya status” and “fever above 38°C” were generally
10 significant ($p \leq 0.01$), but the sample sizes for these groups were either too small or exhibited
11 an imbalance. Additionally, while we conducted subgroup analyses by clinical syndromes
12 (i.e. cough, diarrhea or vomiting, dysuria or urinary urgency, headache, sore throat or
13 swallow pain, rash), the resulting datasets were similarly limited in size, restricting our
14 ability to make robust interpretations from these analyses. The primary focus remained
15 centered on populations grouped by study country and malaria status variables - both of
16 which were strongly associated with the biomarker value in the “strict” and “loose” groups
17 (Supplementary Table 5, 6) - other significant covariates were also included in the
18 multivariable analysis. This inclusion was due to their influence, and factors like the study
19 country were considered as variables in the overall scenario.
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44 369 **Individual host-biomarker performance – univariate analysis**
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46 370 The performance of 18 host biomarkers was consistent across the three patient classification
47 groups in each of the settings (Table 3). White blood cell (WBC) and neutrophil counts were
48 the most effective biomarkers for differentiating bacterial and non-bacterial infections. For the
49 malaria-negative population, the mean (95% confidence interval) of AUROC for WBCs was
50 between 0.60 (0.48–0.72) and 0.83 (0.77–0.88) and for neutrophils it was between 0.67 (0.57–
51 0.77) and 0.80 (0.74–0.86) across the three countries and the three groups (“electronic”,
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376 “strict”, “loose”). Neutrophil and WBC counts showed the highest AUROCs in the Brazilian
 377 population, between 0·80 (0·74–0·86) and 0·83 (0·77–0·88), respectively. All protein
 378 biomarkers showed relatively poor performances (<0·7 in most cases, Table 4) in all three
 379 settings. Galactin-9, CRP, IP-10, and NGAL were the best-performing protein biomarkers
 380 across the three settings and criteria. Protein biomarkers showed better performances in Malawi
 381 and Gabon, as in Brazil most protein biomarkers showed performances of <0·6. When the
 382 biomarker results were stratified by age, the AUROCs were slightly higher for children (≤ 15
 383 years) compared with those seen for adults in the malaria-negative population (Supplementary
 384 Tables 9–11). Among the malaria-positive population, WBC, lymphocyte, and neutrophil
 385 counts were the best-performing biomarkers in both Gabon and Malawi (in most cases between
 386 0·6 and 0·7).

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389 **Table 3: Univariate analysis of 18 individual biomarkers[#] among malaria-negative patients for all three**
 390 **countries (a–c).**

391 Common biomarkers such as CRP and haematological biomarkers were included for reference. In this context we
 392 defined performance as follows: green (AUROC $\geq 0·7$), yellow (AUROC > 0·65 and <0·7), orange (AUROC 0·6–
 393 0·65), and red (AUROC <0·6).
 394 a) Brazil

	Brazil AUROC** (CI), N		
	Electronic	Strict	Loose
Haematological biomarkers			
Lymphocyte count	0·67 (0·59–0·74), 257	0·66 (0·59–0·72), 408	0·66 (0·6–0·72), 442
Neutrophil count	0·77 (0·7–0·84), 257	0·8 (0·74–0·86), 408	0·79 (0·73–0·84), 442
RBC count	0·61 (0·52–0·69), 258	0·58 (0·51–0·65), 408	0·58 (0·51–0·64), 442
WBC count	0·81 (0·75–0·87), 257	0·83 (0·77–0·88), 408	0·82 (0·77–0·87), 442
Protein biomarkers			
AGP	0·59 (0·51–0·68), 252	0·54 (0·47–0·61), 402	0·52 (0·46–0·59), 434
Chitinase 3-like 1	0·58 (0·5–0·66), 246	0·54 (0·47–0·6), 394	0·55 (0·49–0·61), 424
CRP*	0·61 (0·52–0·69), 259	0·61 (0·54–0·68), 412	0·62 (0·55–0·68), 446
IP-10/IP-10/CRG-2	0·6 (0·52–0·68), 252	0·53 (0·46–0·59), 402	0·53 (0·47–0·59), 434
Galectin-9	0·63 (0·55–0·71), 252	0·56 (0·49–0·63), 401	0·57 (0·5–0·63), 433
hCC2	0·51 (0·43–0·6), 244	0·51 (0·44–0·58), 392	0·52 (0·46–0·59), 424
HBP***	0·67 (0·52–0·81), 113	0·68 (0·55–0·8), 144	0·64 (0·51–0·76), 151

HPTGN	0·48 (0·4-0·57), 248	0·51 (0·44-0·58), 398	0·51 (0·45-0·58), 430
IL-4	0·58 (0·5-0·65), 249	0·53 (0·47-0·59), 398	0·54 (0·48-0·59), 429
IL-6	0·49 (0·43-0·54), 247	0·49 (0·44-0·54), 395	0·48 (0·43-0·52), 426
LBP	0·58 (0·5-0·66), 248	0·54 (0·48-0·61), 397	0·52 (0·46-0·58), 429
Lipocalin-2/NGAL	0·49 (0·41-0·57), 249	0·51 (0·44-0·57), 396	0·51 (0·44-0·57), 428
sPLA/Lp-PLA2	0·54 (0·46-0·62), 252	0·53 (0·46-0·59), 402	0·52 (0·45-0·58), 434
TRAIL	0·56 (0·49-0·64), 252	0·53 (0·47-0·59), 402	0·53 (0·48-0·59), 434

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396**b) Gabon**

	Gabon AUROC** (CI), N		
	Electronic	Strict	Loose
	Haematological biomarkers		
Lymphocyte count	0·58 (0·45-0·71), 81	0·52 (0·4-0·63), 167	0·55 (0·45-0·65), 222
Neutrophil count	0·78 (0·66-0·89), 80	0·72 (0·62-0·83), 165	0·67 (0·57-0·77), 219
RBC count	0·55 (0·41-0·68), 81	0·52 (0·41-0·63), 167	0·53 (0·43-0·63), 222
WBC count	0·67 (0·54-0·79), 81	0·6 (0·48-0·72), 167	0·61 (0·5-0·71), 222
Protein biomarkers			
AGP	0·77 (0·65-0·9), 80	0·7 (0·59-0·82), 163	0·65 (0·55-0·75), 220
Chitinase 3-like 1	0·6 (0·46-0·74), 79	0·6 (0·48-0·72), 162	0·62 (0·52-0·72), 217
CRP*	0·71 (0·59-0·82), 81	0·65 (0·55-0·75), 167	0·63 (0·53-0·72), 224
IP-10/IP-10/CRG-2	0·6 (0·48-0·73), 80	0·51 (0·4-0·62), 164	0·52 (0·43-0·62), 221
Galectin-9	0·7 (0·58-0·83), 80	0·6 (0·48-0·71), 163	0·54 (0·43-0·64), 219
hCC2	0·55 (0·41-0·69), 77	0·52 (0·4-0·64), 159	0·51 (0·41-0·61), 216
HBP***
HPTGN	0·64 (0·5-0·78), 77	0·62 (0·51-0·74), 159	0·55 (0·45-0·66), 214
IL-4	0·46 (0·4-0·52), 79	0·49 (0·45-0·53), 163	0·51 (0·47-0·55), 220
IL-6	0·51 (0·47-0·55), 80	0·51 (0·48-0·55), 164	0·51 (0·47-0·55), 221
LBP	0·69 (0·56-0·83), 78	0·67 (0·55-0·78), 160	0·6 (0·5-0·71), 217
Lipocalin-2/NGAL	0·67 (0·54-0·8), 79	0·6 (0·49-0·72), 163	0·58 (0·48-0·68), 219
sPLA/Lp-PLA2	0·58 (0·44-0·71), 80	0·54 (0·43-0·65), 164	0·58 (0·48-0·68), 221
TRAIL	0·5 (0·5-0·5), 74	0·5 (0·49-0·5), 156	0·49 (0·48-0·5), 212

397
398**c) Malawi**

	Malawi AUROC** (CI), N		
	Electronic	Strict	Loose
	Haematological biomarkers		
Lymphocyte count	0·56 (0·47-0·66), 154	0·51 (0·45-0·58), 303	0·52 (0·47-0·58), 461
Neutrophil count	0·67 (0·58-0·77), 143	0·73 (0·67-0·79), 273	0·7 (0·65-0·76), 414
RBC count	0·46 (0·36-0·56), 155	0·53 (0·46-0·59), 305	0·56 (0·5-0·61), 463
WBC count	0·69 (0·6-0·78), 155	0·72 (0·66-0·78), 304	0·68 (0·63-0·73), 461
Protein biomarkers			
AGP	0·56 (0·46-0·66), 158	0·54 (0·48-0·6), 309	0·54 (0·49-0·59), 466
Chitinase 3-like 1	0·49 (0·39-0·59), 155	0·5 (0·43-0·56), 304	0·5 (0·44-0·55), 462
CRP*	0·55 (0·45-0·65), 156	0·6 (0·54-0·67), 305	0·58 (0·53-0·63), 462
IP-10/IP-10/CRG-2	0·66 (0·56-0·75), 158	0·6 (0·53-0·66), 309	0·61 (0·56-0·66), 466

Galectin-9	0·71 (0·62-0·8), 158	0·61 (0·55-0·67), 309	0·63 (0·57-0·68), 466
hCC2	0·59 (0·49-0·69), 158	0·55 (0·49-0·62), 309	0·55 (0·5-0·6), 466
HBP***	0·53 (0·39-0·68), 63	0·55 (0·44-0·66), 106	0·52 (0·41-0·63), 124
HPTGN	0·54 (0·45-0·64), 157	0·51 (0·45-0·58), 307	0·51 (0·46-0·57), 464
IL-4	0·48 (0·4-0·57), 157	0·48 (0·42-0·53), 306	0·47 (0·42-0·51), 463
IL-6	0·56 (0·47-0·65), 158	0·61 (0·55-0·67), 307	0·59 (0·54-0·64), 465
LBP	0·52 (0·42-0·61), 157	0·54 (0·47-0·61), 267	0·53 (0·47-0·59), 394
Lipocalin-2/NGAL	0·56 (0·46-0·66), 156	0·65 (0·59-0·72), 265	0·61 (0·56-0·67), 392
sPLA/Lp-PLA2	0·58 (0·47-0·68), 158	0·55 (0·49-0·61), 308	0·56 (0·51-0·61), 466
TRAIL	0·61 (0·51-0·71), 157	0·62 (0·56-0·68), 306	0·62 (0·57-0·67), 463

*CRP was measured with a NycoCard device. **AUROC has a value between 0 and 1, where 1 corresponds to an effect classifier, 0·5 to one that assigns classes randomly. #Freeze-thaw experiments to evaluate the stability of the biomarkers after five cycles (referred to as “treated”) were performed with Luminex 9- and 2-plexes. Three samples each were freeze-thawed up to six times and compared with samples after the first thawing (referred to as “untreated”; biomarkers were considered stable with 80–120% recovery). Samples were analysed in triplicate and showed good stability up to five freeze-thaw cycles for all analytes showing acceptable results, except for the C2 and C4b biomarkers (C2: 2/3 [66·7%] samples were stable; C4b: two samples failed the sixth freeze-thaw cycle). As a result, these biomarkers were excluded as they would never be suitable as the basis of a diagnostic test. ***HBP was evaluated in a small group of patients in Malawi and Brazil; however, HBP did not show promise and was not evaluated further.

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3 **408 Combinations of host-biomarkers and additional covariates – multivariable analysis**

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5 409 The best-performing biomarkers in the univariate analysis were compared with the best
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7 410 performances from the multivariable analyses, which several feature-selected biomarkers and
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9 411 covariates (Table 4 and Supplementary Tables 15-20). In most cases the best combination of
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11 412 biomarkers showed higher AUROCs than the top-performing individual biomarkers, with a
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13 413 low/moderate “gain” (range 1–13%). The best-performing AUROCs were very similar,
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15 414 irrespective of the multivariable model used, especially for the “strict” and “loose” groups
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17 415 (difference in AUROC range 0·02–0·03 for Malawi and Brazil). Biomarkers identified as top
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19 416 performing by the multivariable analyses differed depending on the model used. While SW
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21 417 and RFA selected three to five biomarkers or combinations, RuleFit selected more biomarkers
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23 418 (ten variables on average) to be part of the signature. The relatively low increase in AUROC
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25 419 when comparing the top-performing single biomarker with multivariable models indicates that
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27 420 biomarkers in addition to the single best-performing biomarker do not make a major
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29 421 contribution.

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3 **Table 4: Multivariable analysis of biomarkers among malaria-negative patients, including the gain/loss of performance**
4 **when comparing multivariable analysis and single host-biomarkers comprising both haematological and protein host-**
5 **biomarkers.**

Classification group	Best multivariable model/models: mean (min-max) AUROC	Best host-biomarker: mean (min-max) AUROC	Multivariable AUROC gain/loss (%) *** multivariable and single host-biomarkers ratio
Overall (Brazil + Gabon + Malawi)*			
L	SW/RFA/RF:0·75 (0.69-0.81)	WBC count: 0·7 (0.64, 0.76)	+7%
S	SW:0.83 (0.75 - 0.91)	WBC count: 0·78 (0.72 - 0.84)	+6%
E	SW/RFA:0.83 (0.77 - 0.89)	WBC count: 0·77 (0.69 - 0.85)	+8%
Brazil			
L	SW: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
S	RFA: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
E	SW: 0·85 (0.73 - 0.97)	WBC count: 0·83 (0.69 - 0.97)	+2%
Gabon**			
L	SW/RFA: 0·7 (0.46 - 0.94)	WBC count: 0·7 (0.64 - 0.76)	..
S	SW/RFA: 0·76 (0.52 – 0.96)	WBC count: 0·78 (0.72 - 0.84)	-3%
E	RFA: 0·77 (0.63 - 0.91)	WBC count: 0·77 (0.69 - 0.85)	..
Malawi			
L	SW/RFA: 0·74 (0.62 - 0.86)	neutrophil count: 0·72 (0.66 - 0.78)	+3%
S	SW: 0·73 (0.61 - 0.85)	neutrophil count: 0·72 (0.58 - 0.86)	+ 1%
E	RFA: 0·72 (0.60 - 0.84)	WBC count: 0·7 (0.56, 0.84)	+ 2%

425 E, electronic classification group; S, strict classification group; L, loose classification group; RF, RuleFit; RFA, logistic
426 recursive feature addition; SW, stepwise logistic regression.
427

428 * In the “Overall” scenario, the model was developed using the data of all countries and the variable indicating the country
429 was used as a covariate in the model.

430 **Multivariable performances for Gabon were computed using as a predictor model the model trained in the “Overall” scenario
431 (all participants from the three analysed countries) then evaluated using Gabon data only. Indeed, the sample size of Gabon
432 data was not sufficient to allow the development of a reliable model specific for this country.

433 *** Performance comparison was computed as: [(multivariable AUROC – univariate AUROC) / univariate AUROC] * 100
434 Green (gain, i.e. the multivariable models show better performances than univariate models); red (loss, i.e. the univariate
435 models show better performances than multivariable models).

436 DISCUSSION

437 We present the most extensive and diverse host-biomarker evaluation study to differentiate
438 bacterial from non-bacterial infections in LMICs. The study aimed to identify if next-
439 generation host-biomarkers for distinguishing bacterial from non-bacterial cases of AFI, which
440 could replace existing biomarkers such as CRP, PCT, and WBC/neutrophil assessments. The
441 data show that none of the promising host-biomarkers exhibited high AUROCs in our non-
442 severe AFI population in either low malaria prevalence (Brazil) or high malaria prevalence
443 (Gabon, Malawi) settings. Haematology biomarkers and CRP were included a baseline to
444 identify better-performing markers; however, they remain those with the highest AUROC
445 values (approximately 0·60–0·70 AUROC) in our population.

446 Overall, the performance of all markers was underwhelming, yet not surprising. It aligns with
447 previous data where a marked reduction in performance was observed when shifting the
448 population from in- to outpatients [17-19]. Previously, it was hypothesised that the decrease in
449 performance in host biomarkers between HIC and LMIC settings, or even between Africa and
450 Asia, was due to the untreated comorbidities (e.g. diabetes, malaria, neglected tropical diseases)
451 which contribute to inflammation and the nonspecific triggering of host biomarkers, unrelated
452 to the current acute presentation [19, 20]. In our data the performance was indeed poorer in
453 malaria-positive patients (AUROC <0·6); however, even in the malaria-negative population,
454 biomarkers showed low performances (~0·6–0·7) in our cohort. Similarly, sex and arboviral
455 status appeared to have no major effect on biomarker performance. Our data notably indicated
456 that combining biomarkers can enhance performance. However, this improvement was not
457 consistently observed. When combining several biomarkers and additional covariates, the
458 “gain” in AUROC values was low/moderate (range 1–13%) compared to the top-performing
459 individual biomarkers. From a diagnostic development perspective, a low gain in performance
460 would not justify the additional complexity and cost of developing a simple multiplex test.

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3 461 Adding to the challenges of host-biomarker studies is the lack of consistent reference standards
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5 462 and that most studies have focused their analyses solely on the subpopulation of patients with
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7 463 a microbiologically confirmed diagnosis. This approach ignores the largest group (>70%) of
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9 464 patients and intended-use population of any future test [21]. The group with laboratory
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11 465 confirmed diagnosis will decrease further in the non-severe AFI population; presenting at
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13 466 primary care level. Going forward more clarity will likely follow as a recent host-biomarker
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15 467 test (BVtest, MeMed, Israel) was approved by the FDA and subsequent guidance will prescribe
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17 468 more clearly how studies have to be designed to standardize the classification of “bacterial”
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19 469 vs “non-bacterial” evaluated to guide prescribing for bacterial or non-bacterial infections [9,
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21 470 22]. Our protocol is aligned with the FDA approved classification hence we are confident our
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23 471 methodology is robust.

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26 472 While our study aimed to mitigate the challenges described, it still had several limitations. The
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28 473 study did not include a control group, so no baseline information was available for biomarker
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30 474 performance or asymptomatic carrier populations. The enrolment period in Brazil and Gabon
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32 475 lasted for less than one year and given the heterogeneity of causes of AFI across time a the
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34 476 performance of the biomarkers may not be generalisable to different times of the year and
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36 477 geographical settings, particularly in Asia. The study utilised a two-step process to classify
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38 478 outcomes, and the clinical classification based on recorded clinical information may have
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40 479 introduced subjectivity. Notably, clinicians had access to the haematology biomarker results
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42 480 (WBCs, neutrophils) during outcome classification, which might have introduced a bias in
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44 481 favour of these biomarkers. However, comparing AUROCs between all classification groups
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46 482 (E, L, S) suggests this potential bias had no major impact as the results are similar across
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48 483 groups. There were some heterogeneities in the inclusion criteria across the various study sites,
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50 484 including age groups and fever criteria. In Brazil and Gabon, the inclusion criterion was a
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52 485 history of fever in the past 7 days, while it was fever at presentation in Malawi. Studies have

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3 486 found that acute fever at presentation has implications for the interpretation of host biomarkers
4 487 [23]; however, our sub-analysis by acute fever showed no differences, so we do not consider
5 488 that these different inclusion criteria impacted interpretation. Despite best efforts to standardise
6 489 procedures, there was a level of adaptability required in the choice of testing methods by the
7 490 clinical teams in each country, for arbovirus and respiratory pathogen detection. Further, the
8 491 choice to follow the TPP and focus on non-severe patients in the recruitment was based on the
9 492 need's definition by the WHO and others, while this still holds as a major priority, in hindsight
10 493 this focus did not allow us to stratify by severity (eg. SOFA score).

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22 494 Overall, the results of this diverse study highlight the difficulties in identifying single host-
23 biomarkers or simple host-biomarker combinations that can help solve the problem of
24 undifferentiated prescribing at primary healthcare, particularly to be used across diverse global
25 settings. On the 8th birthday of the original TPP for a diagnostic assay to distinguish bacterial
26 and non-bacterial infections in resource-limited settings, a more recent consultation confirmed
27 that the need for such an assay remains and is in fact increasingly urgent [6, 24]. Yet again, the
28 499 consultation concluded primary healthcare clinics and their equivalents must have the ability
29 500 to perform tests other than just malaria RDTs [24]. The lack of diagnostics infrastructure at the
30 501 lower levels of health systems is well documented and requires urgent improvement to support
31 502 medical staff in their decision making.. While no novel host-biomarker assay meets these
32 503 needs, evidence for existing biomarkers, e.g. CRP, and various haematology biomarkers,
33 504 should be utilised to drive such improvements, albeit utilizing slightly different approaches and
34 505 cut-offs across settings. In addition to utilising existing tools, increased investment into lower
35 506 level health infrastructures are critical and the first step to improved care. Recent studies have
36 507 shown that even simple host-biomarkers, such as CRP, can have a major impact on how clinical
37 508 staff use antibiotics [25, 26, 27]. The current study confirms that the existing biomarkers are
38 509 imperfect and hence should only be used as guidance, in conjunction with expanded clinical
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3 511 algorithms [28, 29]. Such guidelines, alongside adopted policies, strengthened infrastructures
4 and accessible haematology/biochemistry data could enable healthcare workers to use simple
5 tools to gain additional data points to help form a more evidence-based diagnosis that has to be
6 guided by the local epidemiology. Optimising existing haematology or biochemistry tools and
7 their maintenance requirements to meet the needs of low resourced settings could be one step
8 towards more expanded use of these well-known markers. In conclusion, our study reinforces
9 the continued need for innovation in the host-biomarker space and highlights the importance
10 of targeted evaluations of such innovations, in diverse intended-use settings, to fully understand
11 their true value.

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3 520 **Acknowledgment**

4
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536
537 **Competing Interests**

538 SD, BLFC, CE, VH, SO, CH, AM, SL are or were employed by FIND, the global alliance for
539 diagnostic during the study period. All other authors do not declare any competing interests.

540
541 **Author contribution**

542 SD, CE, SO, AM, AMS, SG, STA, MML, ATA conceptualised the study and study design;
543 CE, AS, SG, STA, AMS, JKM, VH, JM, ALK, AA, JCBO, MML, PNE, JAM, PB, LB, AdRM,
544 BCC, MAMS, AMBdF, EAdS, RdS, MCSL, JH, AG, MJ, NSM, CH, SJL, implemented the

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3 545 study and data collection; MA, MV, SL, SO, BDC, BLFC, SD, SP, SG, AMS, STA conducted
4 data analysis and interpretation. BLFC, SD wrote the first draft of the manuscript and all
5
6 546 authors contributed to the final version of the manuscript. Guarantor is SD.
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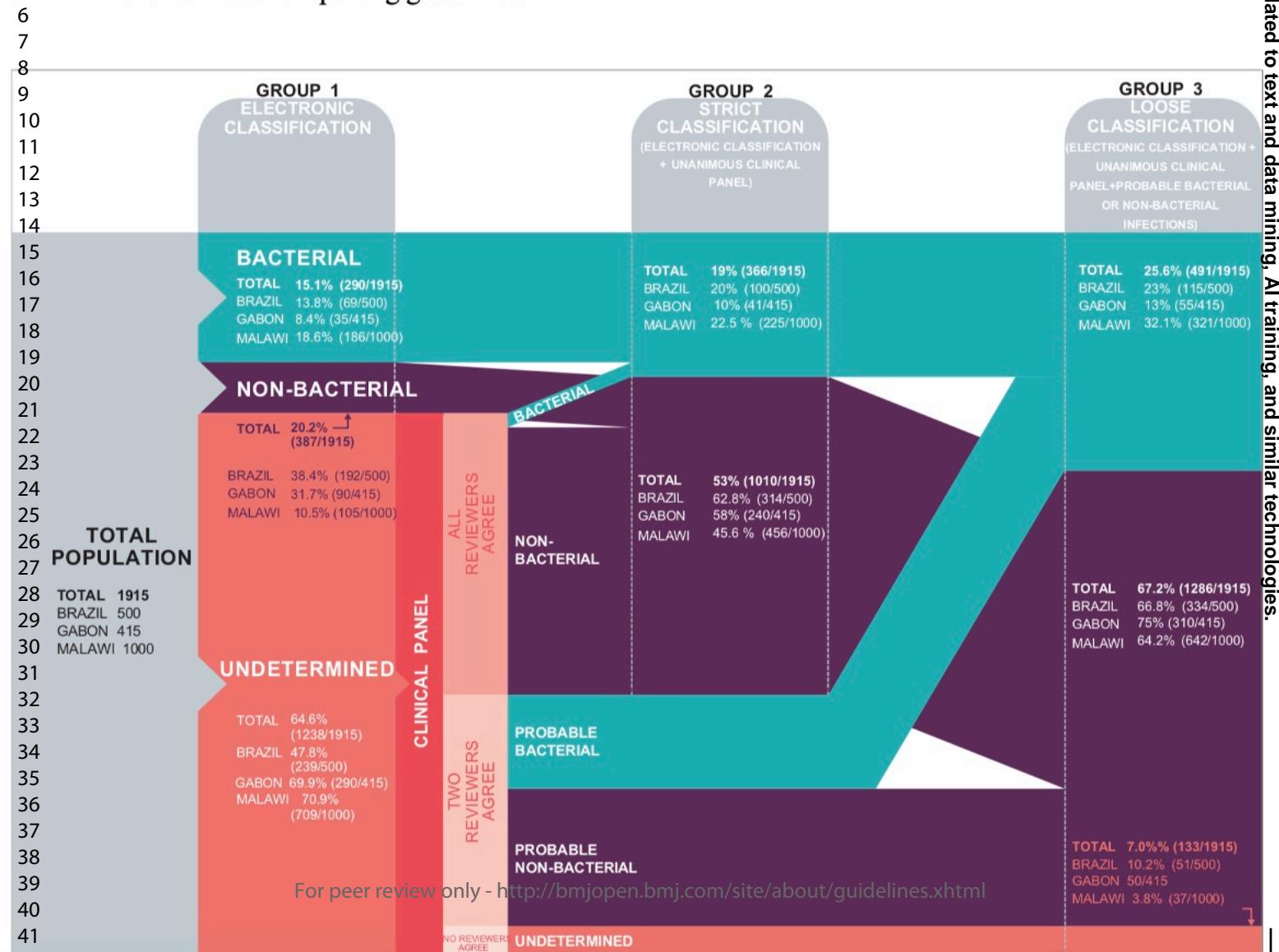
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3 **Figure 1: Classification criteria to assign bacterial versus non-bacterial infection categories for the analysis.**
4 The flows in different colours (turquoise=bacteria, purple=non-bacterial, red=undetermined) represent the
5 proportion of patients that were assigned into the respective group (bacteria/non-bacteria/undetermined) after each
6 classification step. Group 1 representing only patients assigned using laboratory data; group 2 representing
7 patients with a unanimous decision after review by the clinical panel; group 3 after clinical panel review and group
8 3 including all patients, even if only 2 panel members agreed on the probable cause. The study follows the
9 STARD-15 checklist and reporting guidelines.
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Figure 1: Classification criteria to assign bacterial versus non-bacterial infection categories for the analysis.

The flows in different colours (turquoise=bacteria, purple=non-bacterial, red=undetermined) represent the proportion of patients that were assigned into the respective group (bacteria/non-bacteria/undetermined) after each classification step. Group 1 representing only patients assigned using laboratory data; group 2 representing patients with a unanimous decision after review by the clinical panel; group 3 including all patients, even if only 2 panel members agreed on the probable cause. The study follows the STARD-15 checklist and reporting guidelines.



Supplementary Material

Evaluation of host biomarkers to support the development of a point-of-care diagnostic test to guide antibiotic use in bacterial/non-bacterial acute febrile illness cases

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Biomarker selection

Biomarkers evaluated were selected based on reported performances for distinguishing bacterial versus non-bacterial infections in prior publications, which were systematically reviewed in 2016 by Kapasi et al.¹ and other key publications (Supplementary Table 1). Biomarker performances reported in the 2016 systematic review were compared with reported performances in a later systematic review conducted in 2020.²

Supplementary Table 1. Biomarkers included based on Kapasi et al.'s (2016) systematic review and other key publications.

Biomarker	Performance, 2016 systematic review
C-reactive protein (CRP)	1
FebriDx (MxA+CRP)	2
Galectin-9	2
Gamma-induced protein 10 (IP-10)	2*
Haptoglobin	2 [#]
Heparin-binding protein (HBP)	3
Human neutrophil lipocalin (HNL)	2
Interferon gamma (IFN-gamma)	3
Interleukin-4 (IL-4)	2
Interleukin-6 (IL-6)	3
Lipopolysaccharide binding protein (LBP)	3 ^{\$}
Procalcitonin (PCT)	1
Secretory phospholipase 2 (sPLA2)	2
Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1)	3 ^{\$}
TNF-related apoptosis-inducing ligand (TRAIL)	2*
<i>Included based on key publications in the field</i>	
Biomarker	Publication
A-1-acid glycoprotein	Struck et al. ³
Chitinase-3-like protein 1 (CHI3L1)	Erdman et al. ⁴
Complement 2	Struck et al. ³
Complement C4b	Struck et al. ³
Neutrophil gelatinase-associated lipocalin (NGAL)	Huang et al. ⁵

Performances were scored as: 1, high-performing biomarker (meets the current TPP minimum diagnostic performance criteria, i.e. ≥ 0.90 and 0.80 sensitivity/specificity); 2, moderately performing biomarker (≥ 0.65 and 0.65 and < 0.90 and 0.80 sensitivity/specificity); 3, AUROC > 0.8 ; 4, low-performing biomarker; 5, not evaluated. *As part of the signature CRP+IP-10+TRAIL; # as part of the signature Haptoglobin+IL-10+TIMP1; \$ in respiratory tract infections as part of the signature CRP+LBP; § as part of the signature sTREM+CRP; 1 only in the context of meningitis, otherwise low performance.

Reference laboratory methodology

Materials, equipment, and software

All assay reagents used were delivered with the commercial kits and were used as described in the corresponding kit manuals. Supplementary Table 2 shows the commercial human multi-analyte kits and ELISA kits used.

Supplementary Table 2: Commercial human multi-analyte kits and ELISA kits used.

Analytes	Assay type	Provider	Reference laboratory that performed the analysis
CHI3L1, Gal-9, IL-4, IL-6, IP-10, IFN-gamma, sPLA2, sTREM-1, TRAIL	Luminex, 9-plex	Biotechne/ Systems R&D	NMI
NGAL, LBP	Luminex, 2-plex	Biotechne/ Systems R&D	NMI
C2, C4b	Luminex, 2-plex	Merck	NMI
HP, AGP	Luminex, 2-plex	Merck	NMI
PCT	Luminex, 1-plex	Biotechne/ Systems R&D	NMI
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HNL	ELISA	Diagnostics Development	NMI

CRP	ELISA	Biotechne/ Systems	R&D
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HBP	ELISA	Axis-Shield	on-site

NMI, The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany; MVZ Labor, Dr. Limbach & Kollegen, Heidelberg, Germany

For data generation, the Luminex FLEXMAP 3D instrument, operated with xPONENT Software V4.2, was used for the bead-based Luminex assays. The data evaluation was performed using Bio-Rad Bio-Plex Manager Software 6.1.1. To generate the data for the ELISAs at NMI a BioTek ELx 808 absorption reader was used. The embedded software Gen5 (BioTek) was used for data evaluation. At MVZ Limbach, a Cobas 8000 immunoanalyzer (Roche Diagnostics) was used for data generation.

Methods

All assays were processed according to the manufacturer's protocol. Standard curves, quality control (QC) samples, and blanks were analysed in duplicate; samples were assayed singly. Two or three QC samples were measured on each assay plate. QC samples were taken to cover the range of the standard curve (low, mid, and high level). All QC samples were prepared and aliquoted in larger quantities at the beginning of sample screening so that a fresh aliquot could be used for each measurement, and all QC samples underwent the same freeze–thaw cycle. The performance of the standard curves was controlled over the entire measurement period based on %CVs of the standard point duplicates (<20% and <25% for the last standard point) and percentage recovery on the basis of the nominal concentrations. If permitted by the dilution factor, samples out of the dynamic range were re-analysed with a lower or higher dilution factor.

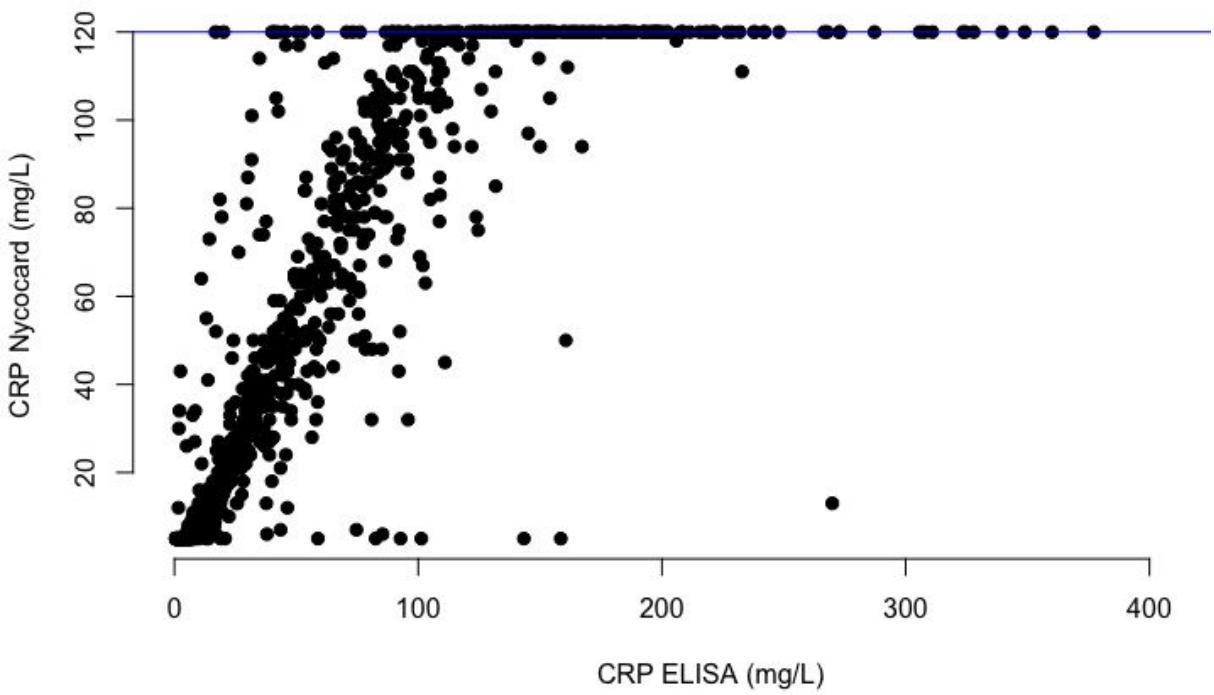
Heparin-binding protein (HBP) assay

The commercially available Axis-Shield heparin-binding protein ELISA for citrated plasma was validated for human EDTA plasma. Calibration curve, limit of detection (LOD), assay range, precision, parallelism, and spike-in recovery experiments were performed.

The ELISA was processed according to the assay protocol provided with the kit. Validation was performed using a fit-for-purpose approach and under consideration of the recommendations for assay validation given in guidelines from health authorities (European Medicine Agency (2011); Food and Drug Administration (2018)). This was a short validation with a limited number of samples.

Except for the percentage recovery, all analysed parameters met the criteria during the validation of the HBP ELISA using human EDTA plasma instead of the recommended citrated plasma matrix. The assay performance seemed to be stable for the sample evaluation using the kit.

Supplementary Figure 1: Analytical assessment of CRP Nycocard vs CRP ELISA



Statistical analysis

This section contains additional figures and tables related to the statistical analysis.

Supplementary Table 3: Number and percentage of missing values for the biomarkers included in the statistical analysis

	Electronic group [¶] [n (%)]	Strict group [§] [n (%)]	Loose group [#] [n (%)]
White blood cells	6 (0.8%)	11 (0.8%)	15 (0.8%)
HAEMATO COUNT	6 (0.8%)	11 (0.8%)	15 (0.8%)
Lymphocytes	6 (0.8%)	12 (0.9%)	17 (1%)
Neutrophils	22 (3%)	64 (5%)	90 (5%)
CRP NYCOCARD	5 (0.7%)	10 (0.7%)	14 (0.8%)
IL-6	10 (1.5%)	20 (1%)	24 (1%)
Gal-9	10 (1.5%)	20 (1%)	24 (1%)
CHI3L1	10 (1.5%)	20 (1%)	25 (1%)
IP-10	10 (1.5%)	20 (1%)	24 (1%)
TRAIL	10 (1.5%)	20 (1%)	24 (1%)
IL-4	13 (2%)	24 (2%)	29 (2%)
sPLA2	10 (1.5%)	20 (1%)	24 (1%)
NGAL	29 (4%)	138 (10%)	197 (11%)
LBP	30 (4%)	139 (10%)	198 (11%)
C2	10 (1.5%)	21 (1.5%)	25 (1%)
AGP	10 (1.5%)	21 (1.5%)	25 (1%)
HP	11 (1.6%)	24 (2%)	29 (2%)

¶ Total number of subjects in the Electronic group: 677

§ Total number of subjects in the Strict group: 1376

Total number of subjects in the Loose group: 1777

Kruskal-Wallis tables

Supplementary Table 4: Kruskal-Wallis table results for the electronic classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	1.214 5E-13	1.980 8E-01	1.098 5E-02	3.440 8E-01	8.4018E-01	2.7154E-01	4.3535E-01	3.4408E-01	5.4183E-09
HAEMA TO COUNT	2.804 0E-45	1.044 6E-09	4.346 1E-28	1.318 5E-36	6.8045E-02	9.1321E-01	6.9000E-01	9.9455E-01	3.6951E-08
Lymphocytes	1.385 0E-45	8.068 0E-03	3.156 2E-29	4.541 4E-32	1.0022E-05	4.4874E-01	4.5900E-01	5.4198E-08	1.9910E-11
Neutrophils	5.649 5E-03	3.914 7E-01	1.133 7E-04	1.867 4E-17	1.5980E-02	4.2719E-01	4.3608E-01	3.0003E-08	6.5439E-04
CRP NYCOCARD	1.448 5E-03	4.229 7E-01	1.386 1E-15	3.033 2E-07	2.1171E-01	4.6667E-01	8.4615E-01	3.0231E-03	2.1171E-01
IL-6	9.262 6E-06	2.527 7E-01	4.668 6E-34	4.281 0E-21	6.1106E-03	7.1615E-01	5.8674E-02	2.0177E-10	9.2626E-06
Gal-9	7.808 4E-11	3.329 6E-01	1.273 1E-07	2.247 1E-07	4.3173E-01	5.3845E-01	9.9020E-02	3.6659E-01	8.5282E-04
CHI3L1	3.687 4E-01	1.542 7E-01	2.259 3E-04	3.594 2E-05	9.0961E-01	8.0977E-01	7.9973E-01	2.5264E-02	2.5264E-02
IP-10	7.023 5E-01	7.023 5E-01	4.042 9E-09	7.048 6E-10	4.9729E-01	7.0235E-01	4.0169E-01	3.6086E-08	3.3476E-01
TRAIL	1.410 8E-03	1.542 9E-02	6.771 0E-19	6.947 3E-56	9.2177E-01	2.2485E-02	9.5591E-01	9.7926E-04	1.8702E-06
IL-4	1.419 0E-03	8.956 6E-02	1.789 6E-25	1.117 9E-73	4.2256E-01	8.9341E-03	8.9692E-01	3.0403E-03	2.2958E-09
sPLA2	9.599 3E-05	9.212 7E-01	2.847 7E-20	5.681 0E-03	1.5011E-01	9.2127E-01	6.1633E-01	7.4323E-03	7.4323E-03
NGAL	2.684 1E-02	7.192 4E-01	1.249 8E-05	6.460 4E-21	7.1924E-01	2.6841E-02	5.1387E-01	1.2498E-05	9.6273E-03
LBP	2.265 8E-11	5.148 1E-02	1.852 7E-54	2.154 4E-101	8.2974E-02	5.3837E-03	1.1745E-01	3.5938E-09	6.0583E-19
C2	1.721 9E-02	3.006 3E-01	6.862 8E-13	6.862 8E-13	6.2951E-02	8.5874E-01	5.6324E-01	4.4637E-01	6.2045E-03
AGP	5.188 8E-03	2.027 4E-01	3.674 7E-16	1.344 5E-16	1.5176E-01	9.8963E-01	6.3154E-01	2.3325E-01	3.1922E-05
HP	2.942 0E-07	2.739 0E-01	1.839 3E-25	2.499 7E-25	2.7390E-01	2.7390E-01	4.0178E-01	7.2077E-01	2.9140E-03
C4b	5.615 9E-19	6.701 0E-02	4.504 1E-81	1.949 1E-84	6.7179E-03	6.7179E-03	3.3168E-01	1.8052E-01	8.0363E-18

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 5: Kruskal-Wallis table results for the strict classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	3.114 9E-20	2.409 1E-01	3.674 9E-09	9.399 7E-03	3.1632E-01	6.3502E-02	6.3502E-02	9.1443E-01	1.7973E-08
HAEMA TO COUNT	6.183 5E-100	1.999 4E-04	5.630 4E-55	3.785 2E-68	1.6199E-04	8.0189E-01	7.1282E-01	2.9137E-01	1.7149E-10
Lymphocytes	8.477 8E-84	1.529 1E-01	2.677 9E-44	2.740 4E-58	6.3047E-07	6.1980E-03	4.5554E-01	7.1024E-22	8.6226E-15
Neutrophils	8.951 3E-04	1.715 2E-01	7.983 8E-14	1.913 4E-37	4.5549E-02	5.2789E-01	4.5549E-02	3.0001E-19	4.1217E-02
CRP NYCOCA RD	1.654 7E-02	5.765 6E-02	2.457 0E-38	6.299 1E-11	7.4370E-01	3.0220E-01	7.4370E-01	9.7289E-15	3.0220E-01
IL-6	2.570 4E-02	1.288 8E-01	2.513 1E-68	3.475 8E-27	1.4641E-01	8.1220E-01	6.6933E-02	4.3924E-26	2.5371E-04
Gal-9	7.442 4E-19	3.545 5E-03	1.343 2E-11	1.375 7E-08	1.1615E-01	3.9116E-01	1.3397E-01	2.2573E-01	2.4249E-03
CHI3L1	2.833 5E-01	1.543 3E-01	3.678 7E-11	7.431 9E-16	2.8335E-01	2.8335E-01	2.8335E-01	8.7744E-06	1.5017E-03
IP-10	2.452 1E-01	6.871 6E-01	8.565 6E-31	1.550 3E-36	2.1157E-01	3.0336E-01	3.2906E-01	4.1236E-22	3.2906E-01
TRAIL	6.435 8E-04	2.420 6E-01	3.746 7E-46	4.580 6E-127	7.7652E-01	8.3869E-04	7.7652E-01	2.8337E-17	1.7642E-08
IL-4	4.210 8E-04	5.985 8E-01	2.594 9E-55	2.708 3E-159	3.3368E-01	8.0705E-05	6.5563E-01	2.2888E-11	2.2888E-11
sPLA2	3.000 5E-14	1.126 4E-01	4.135 5E-60	4.705 5E-09	6.7473E-04	2.2676E-01	3.6531E-01	1.0844E-09	4.7059E-05
NGAL	7.746 2E-02	1.130 0E-01	6.092 7E-16	1.372 0E-35	5.9955E-01	4.9221E-02	4.4419E-01	1.4382E-19	8.8808E-03
LBP	1.350 9E-14	3.412 3E-01	6.066 0E-94	1.936 0E-197	2.1248E-02	3.6673E-05	3.0644E-01	2.3473E-28	7.4289E-21
C2	7.267 4E-07	4.315 7E-01	2.314 5E-26	4.532 4E-25	6.8236E-03	4.3157E-01	4.3157E-01	8.8206E-03	2.1062E-03
AGP	4.851 3E-04	1.737 9E-01	5.058 7E-21	7.149 6E-23	1.5900E-01	7.9521E-01	9.7767E-01	1.1305E-01	1.4880E-05
HP	1.212 7E-13	6.331 1E-01	1.636 6E-46	3.005 3E-46	2.9299E-03	5.6523E-01	5.6523E-01	9.0316E-01	4.8596E-04

C4b	6.319 3E-21	1.923 1E-02	1.666 4E-139	3.199 9E-147	1.9749E-04	2.6638E-04	9.3349 E-01	8.0678E-03	3.0903E-25
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Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 6: Kruskal-Wallis table results for the loose classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^\circ\text{C}$	Chikungunya
White blood cells	2.057 4E-28	9.875 9E-01	1.848 4E-08	4.526 0E-03	9.0171E-02	4.8259E-02	1.0890 E-01	7.4007E-01	1.8484E-08
HAEMA TO COUNT	1.308 3E-126	1.861 9E-04	6.283 5E-56	7.796 2E-76	1.1102E-06	7.8862E-01	7.9391 E-01	2.9434E-01	1.2853E-10
Lymphocytes	4.965 1E-101	2.946 1E-01	4.679 6E-45	1.637 2E-67	4.8743E-07	6.6823E-04	2.9461 E-01	2.4236E-29	4.3110E-15
Neutrophils	1.131 0E-04	7.267 7E-01	7.274 2E-15	1.612 7E-46	2.0313E-01	4.6743E-01	2.0038 E-01	1.2920E-24	2.9723E-02
CRP NYCOCARD	1.361 4E-01	4.412 3E-03	1.034 7E-57	2.470 3E-15	4.0226E-01	5.2068E-01	5.9738 E-01	6.7648E-18	1.3614E-01
IL-6	9.525 0E-02	4.873 6E-02	8.630 3E-95	1.968 8E-31	1.5356E-01	8.2374E-01	9.3076 E-02	6.1774E-34	2.1766E-05
Gal-9	2.046 3E-27	1.443 1E-03	1.931 8E-13	6.827 3E-10	2.3586E-01	2.3586E-01	3.6447 E-02	2.3586E-01	3.0166E-03
CHI3L1	2.748 3E-01	5.354 1E-02	3.612 8E-14	3.612 8E-14	2.8535E-01	7.9359E-01	3.0946 E-01	1.4718E-04	7.1655E-04
IP-10	4.138 4E-01	7.867 4E-01	6.519 3E-43	4.220 2E-47	7.9605E-02	3.6101E-01	4.1384 E-01	1.4436E-34	4.1902E-01
TRAIL	2.472 2E-02	1.391 8E-01	6.282 8E-56	2.918 5E-156	8.2684E-01	6.2797E-05	8.2684 E-01	2.4486E-17	1.1148E-09
IL-4	1.144 8E-02	3.191 1E-01	3.084 4E-69	4E-206	3.9276E-01	4.7672E-08	5.7785 E-01	2.1611E-12	1.2664E-13
sPLA2	8.375 3E-18	2.731 7E-01	1.589 0E-82	1.270 2E-09	1.2356E-04	3.7225E-01	4.1002 E-01	8.1232E-15	4.0213E-05

NGAL	1.570 6E-01	2.065 0E-02	3.748 6E-27	2.284 8E-43	3.7129E- 01	1.4239E- 01	3.9957 E-01	1.3734E- -24	5.3057E- 03
LBP	1.656 7E-10	4.386 5E-01	2.110 116	2.427 254	8.2765E- 03	5.4993E- 07	6.1624 E-01	1.4861E- -39	1.4254E- 24
C2	2.103 5E-04	1.459 3E-01	7.600 5E-28	2.186 5E-27	4.8543E- 02	2.9326E- 01	3.8932 E-01	9.8425E- -03	1.2901E- 03
AGP	2.507 6E-03	9.527 3E-02	1.987 0E-26	3.272 6E-28	9.3140E- 02	8.9492E- 01	9.5756 E-01	9.5273E- -02	3.2225E- 06
HP	5.764 0E-15	7.268 5E-01	2.837 6E-51	7.966 7E-51	7.2760E- 03	6.9555E- 01	6.9555 E-01	9.7145E- -01	1.7228E- 04
C4b	3.907 7E-15	9.303 7E-03	9.356 160	3.444 171	6.9926E- 04	2.2357E- 03	8.6228 E-01	2.2357E- -03	1.0351E- 29

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Supplementary Table 7: Univariate analysis – Overall (malaria-positive and malaria-negative) population

	Overall - Malaria negatives			Overall - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.74, (0.7- 0.79), 493	0.75, (0.71- 0.78), 880	0.72, (0.68- 0.75), 1127	0.65, (0.57- 0.73), 174	0.65, (0.58- 0.71), 481	0.64, (0.59- 0.7), 630
RBC count	0.58, (0.53- 0.63), 494	0.52, (0.48- 0.56), 880	0.51, (0.47- 0.54), 1127	0.58, (0.5- 0.67), 175	0.5, (0.44- 0.56), 481	0.51, (0.46- 0.57), 630
Lymphocyte count	0.66, (0.61- 0.71), 491	0.57, (0.53- 0.61), 877	0.55, (0.51- 0.58), 1123	0.63, (0.54- 0.71), 174	0.57, (0.5- 0.63), 480	0.54, (0.49- 0.6), 627
Neutrophil count	0.71, (0.66- 0.75), 480	0.75, (0.71- 0.79), 847	0.73, (0.69- 0.76), 1079	0.67, (0.59- 0.75), 172	0.65, (0.58- 0.71), 461	0.65, (0.59- 0.71), 603
IL-4	0.36, (0.31- 0.42), 486	0.4, (0.35- 0.44), 868	0.61, (0.57- 0.64), 1113	0.66, (0.58- 0.74), 175	0.59, (0.53- 0.65), 478	0.58, (0.53- 0.63), 624
TRAIL	0.36, (0.3- 0.41), 489	0.63, (0.59- 0.67), 871	0.63, (0.59- 0.67), 1117	0.68, (0.6- 0.76), 175	0.6, (0.54- 0.66), 478	0.58, (0.53- 0.64), 625
IL-6	0.61, (0.55- 0.66), 489	0.49, (0.45- 0.53), 873	0.49, (0.45- 0.53), 1120	0.42, (0.33- 0.5), 175	0.57, (0.5- 0.63), 478	0.53, (0.48- 0.59), 626
CRP NycoCard	0.52, (0.47- 0.57), 496	0.57, (0.53- 0.61), 884	0.57, (0.53- 0.6), 1132	0.52, (0.43- 0.6), 175	0.49, (0.43- 0.56), 481	0.5, (0.44- 0.55), 630
Gal-9	0.52, (0.47- 0.57), 490	0.54, (0.5- 0.58), 875	0.56, (0.52- 0.59), 1122	0.57, (0.48- 0.65), 176	0.54, (0.48- 0.6), 480	0.53, (0.48- 0.59), 629
CHI3L1	0.56, (0.51- 0.62), 489	0.55, (0.51- 0.59), 873	0.55, (0.51- 0.59), 1119	0.5, (0.41- 0.59), 176	0.52, (0.45- 0.58), 480	0.5, (0.44- 0.55), 627
IP-10	0.53, (0.48- 0.58), 489	0.52, (0.48- 0.56), 874	0.52, (0.49- 0.56), 1120	0.56, (0.47- 0.64), 176	0.53, (0.47- 0.59), 478	0.51, (0.45- 0.56), 627
sPLA2	0.52, (0.47- 0.57), 490	0.52, (0.48- 0.56), 874	0.52, (0.49- 0.56), 1121	0.49, (0.4- 0.58), 176	0.54, (0.48- 0.61), 479	0.54, (0.49- 0.6), 628

NGAL	0.61, (0.56-0.66), 489	0.62, (0.57-0.66), 833	0.6, (0.57-0.64), 1049	0.61, (0.52-0.7), 157	0.56, (0.49-0.62), 403	0.56, (0.51-0.62), 527
LBP	0.74, (0.69-0.78), 488	0.69, (0.65-0.73), 832	0.67, (0.64-0.71), 1048	0.67, (0.58-0.76), 158	0.58, (0.52-0.64), 404	0.57, (0.51-0.62), 529
C2	0.59, (0.54-0.64), 483	0.56, (0.52-0.6), 866	0.56, (0.52-0.59), 1113	0.63, (0.55-0.72), 176	0.59, (0.53-0.66), 480	0.56, (0.5-0.61), 629
AGP	0.67, (0.62-0.72), 490	0.6, (0.56-0.64), 874	0.58, (0.55-0.62), 1120	0.52, (0.43-0.6), 176	0.52, (0.45-0.59), 480	0.53, (0.47-0.59), 629
HBP	0.67, (0.57-0.76), 179	0.64, (0.56-0.72), 254	0.61, (0.53-0.68), 280	0.55, (0.37-0.72), 57	0.52, (0.42-0.63), 141	0.53, (0.43-0.64), 149
HP	0.55, (0.49-0.6), 489	0.5, (0.46-0.54), 871	0.52, (0.48-0.56), 1116	0.58, (0.49-0.66), 175	0.55, (0.48-0.61), 473	0.54, (0.48-0.59), 622

Supplementary Table 8: Univariate analysis – malaria-positive population

	Malawi - Malaria positives			Gabon - Malaria positives				
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose
WBC count	0.67 (0.58-0.76), 132	0.68 (0.61 – 0.75), 369	0.67 (0.61-0.72), 491	0.67 (0.44-0.91), 42	0.61 (0.38-0.83), 112	0.61 (0.44-0.78), 139		
RBC count	0.69 (0.6-0.79), 131	0.55 (0.48-0.61), 367	0.53 (0.47-0.59), 488	0.56 (0.31-0.81), 43	0.51 (0.3-0.71), 113	0.49 (0.33-0.65), 140		
Lymphocyte count	0.7 (0.61-0.79), 131	0.59 (0.53-0.66), 368	0.57 (0.51-0.62), 488	0.72 (0.51-0.93), 42	0.66 (0.47-0.85), 112	0.67 (0.52-0.82), 139		
Neutrophil count	0.62 (0.52-0.72), 129	0.65 (0.57-0.72), 348	0.66 (0.6-0.72), 463	0.53 (0.31-0.76), 43	0.59 (0.39-0.79), 113	0.59 (0.43-0.75), 140		
IL-4	0.46 (0.36-0.56), 132	0.47 (0.4-0.53), 369	0.48 (0.42-0.53), 488	0.44 (0.38-0.5), 40	0.46 (0.44-0.49), 103	0.5 (0.42-0.57), 127		
TRAIL	0.6 (0.51-0.7), 132	0.55 (0.49-0.62), 369	0.54 (0.48-0.59), 488	0.5 (0.5-0.5), 43	0.5 (0.5-0.5), 109	0.53 (0.47-0.6), 136		
IL-6	0.6 (0.5-0.7), 131	0.58 (0.51-0.65), 367	0.54 (0.48-0.6), 485	0.45 (0.32 - 0.57), 42	0.47 (0.37-0.57), 103	0.45 (0.37-0.53), 127		
CRP NycoCard	0.48 (0.38-0.58), 131	0.54 (0.47-0.61), 367	0.53 (0.47-0.59), 489	0.59 (0.32-0.86), 44	0.59 (0.36-0.82), 114	0.57 (0.4-0.75), 141		
Gal-9	0.58 (0.48-0.69), 132	0.56 (0.49-0.62), 369	0.54 (0.47-0.6), 491	0.57 (0.34-0.8), 43	0.5 (0.32-0.68), 109	0.56 (0.42-0.71), 136		
CHI3L1	0.56 (0.46-0.66), 132	0.55 (0.48-0.62), 367	0.55 (0.49-0.61), 487	0.52 (0.26-0.79), 43	0.53 (0.31-0.75), 106	0.63 (0.44-0.81), 131		
IP-10	0.67 (0.58-0.76), 132	0.56 (0.49-0.63), 363	0.52 (0.46-0.59), 484	0.51 (0.33-0.69), 40	0.49 (0.35-0.63), 104	0.48 (0.35-0.61), 129		
sPLA2	0.53 (0.43-0.64), 133	0.56 (0.48-0.63), 370	0.56 (0.5-0.62), 492	0.49 (0.24-0.74), 43	0.56 (0.34-0.77), 109	0.49 (0.32-0.67), 136		
NGAL	0.5 (0.39-0.61), 114	0.5 (0.43-0.58), 291	0.49 (0.42-0.55), 386	0.65 (0.44-0.91), 41	0.59 (0.41-0.77), 106	0.54 (0.38-0.7), 131		

LBP	0.47 (0.35-0.59), 115	0.54 (0.46-0.61), 295	0.54 (0.48-0.6), 393	0.6 (0.34 - 0.85), 42	0.58 (0.37-0.8), 105	0.65 (0.48-0.81), 131
C2	0.62 (0.52-0.72), 133	0.57 (0.5-0.64), 369	0.54 (0.48-0.6), 491	0.72 (0.54-0.9), 43	0.72 (0.57-0.87), 105	0.64 (0.48-0.8), 131
AGP	0.54 (0.44 - 0.64), 133	0.52 (0.44-0.59), 371	0.48 (0.42-0.54), 493	0.51 (0.27-0.75), 43	0.53 (0.33-0.74), 109	0.58 (0.41-0.76), 136
HBP	0.55, (0.37-0.72), 57	0.53, (0.43-0.64), 143	0.54, (0.44-0.64), 151
HP	0.58 (0.48-0.68), 133	0.54 (0.47-0.61), 365	0.51 (0.45-0.57), 487	0.57 (0.33-0.8), 42	0.56 (0.36-0.76), 107	0.61 (0.46-0.77), 134

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65,) red (AUROC < 0.6)

Univariate analysis – age subgroups

Supplementary Table 9: Univariate analysis - age less than 6 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.83, (0.73-0.94), 61	0.79, (0.71-0.87), 122	0.76, (0.69-0.84), 170	0.52, (0.25-0.78), 21	0.65, (0.46-0.85), 34	0.69, (0.51-0.86), 38	0.78, (0.62-0.94), 32	0.68, (0.52-0.83), 75	0.65, (0.52-0.79), 105
RBC count	0.65, (0.49-0.8), 62	0.58, (0.48-0.68), 123	0.58, (0.5-0.67), 172	0.6, (0.33-0.86), 21	0.56, (0.35-0.77), 33	0.59, (0.39-0.78), 37	0.6, (0.4-0.81), 32	0.56, (0.4-0.72), 75	0.53, (0.38-0.67), 105
Lymphocyte count	0.58, (0.43-0.72), 60	0.53, (0.42-0.64), 121	0.48, (0.38-0.57), 170	0.63, (0.36-0.89), 21	0.67, (0.44-0.91), 34	0.7, (0.5-0.9), 38	0.71, (0.53-0.89), 32	0.6, (0.44-0.76), 75	0.63, (0.49-0.76), 105
Neutrophil count	0.82, (0.7-0.93), 57	0.79, (0.7-0.88), 108	0.77, (0.69-0.86), 148	0.58, (0.32-0.85), 21	0.56, (0.36-0.77), 34	0.6, (0.41-0.79), 38	0.86, (0.72-0.99), 32	0.79, (0.67-0.92), 74	0.7, (0.58-0.83), 103
IL-4	0.54, (0.39-0.68), 63	0.5, (0.41-0.59), 125	0.48, (0.41-0.56), 174	0.63, (0.38-0.88), 20	0.66, (0.49-0.84), 31	0.62, (0.44-0.8), 33	0.43, (0.31-0.55), 30	0.49, (0.43-0.56), 72	0.51, (0.44-0.57), 103
TRAIL	0.57, (0.39-0.75), 63	0.6, (0.5-0.69), 125	0.59, (0.51-0.67), 174	0.5, (0.23-0.77), 20	0.63, (0.43-0.82), 31	0.59, (0.4-0.79), 33	0.5, (0.5-0.5), 28	0.5, (0.5-0.5), 69	0.49, (0.48-0.51), 99
IL-6	0.59, (0.44-0.73), 63	0.61, (0.52-0.7), 125	0.6, (0.52-0.68), 174	0.41, (0.29-0.53), 20	0.39, (0.29-0.49), 29	0.39, (0.3-0.49), 31	0.5, (0.5-0.5), 31	0.5, (0.5-0.5), 73	0.49, (0.47-0.5), 104
CRP NycoCard	0.56, (0.37-0.74), 61	0.61, (0.51-0.71), 121	0.59, (0.5-0.68), 169	0.49, (0.22-0.76), 21	0.59, (0.38-0.79), 34	0.6, (0.42-0.79), 38	0.76, (0.57-0.95), 32	0.62, (0.49-0.76), 75	0.57, (0.45-0.69), 106

Gal-9	0.79, (0.66- 0.92), 63	0.59, (0.49- 0.69), 125	0.57, (0.48- 0.66), 173	0.47, (0.2- 0.75), 20	0.5, (0.28- 0.72), 31	0.52, (0.3- 0.73), 33	0.66, (0.45- 0.87), 31	0.6, (0.43- 0.76), 72	0.54, (0.4- 0.69), 102
CHI3L1	0.56, (0.4- 0.72), 62	0.52, (0.42- 0.63), 124	0.54, (0.45- 0.63), 173	0.61, (0.35- 0.87), 20	0.66, (0.47- 0.86), 31	0.67, (0.49- 0.86), 33	0.68, (0.49- 0.88), 31	0.62, (0.45- 0.79), 73	0.61, (0.47- 0.75), 102
IP-10	0.67, (0.51- 0.83), 63	0.62, (0.52- 0.72), 125	0.6, (0.51- 0.68), 174	0.65, (0.39-0.9), 20	0.7, (0.51- 0.89), 31	0.64, (0.45- 0.84), 33	0.71, (0.53-0.9), 31	0.52, (0.38- 0.67), 73	0.51, (0.38- 0.63), 104
sPLA2	0.66, (0.5- 0.82), 63	0.55, (0.45- 0.66), 125	0.56, (0.47- 0.65), 174	0.65, (0.38- 0.91), 20	0.69, (0.48- 0.9), 31	0.68, (0.48- 0.88), 33	0.58, (0.37- 0.78), 31	0.57, (0.41- 0.72), 73	0.59, (0.45- 0.73), 104
NGAL	0.61, (0.44- 0.77), 63	0.68, (0.58- 0.78), 109	0.67, (0.59- 0.76), 144	0.67, (0.41- 0.93), 20	0.58, (0.38- 0.79), 31	0.52, (0.31- 0.72), 33	0.63, (0.43- 0.83), 31	0.6, (0.44- 0.77), 73	0.57, (0.43- 0.71), 103
LBP	0.47, (0.31- 0.63), 63	0.5, (0.39- 0.62), 109	0.53, (0.43- 0.63), 144	0.47, (0.2- 0.75), 20	0.46, (0.25- 0.68), 30	0.48, (0.27- 0.7), 32	0.73, (0.53- 0.93), 30	0.7, (0.53- 0.86), 70	0.59, (0.44- 0.75), 101
C2	0.51, (0.34- 0.69), 63	0.56, (0.45- 0.66), 125	0.52, (0.44- 0.61), 174	0.47, (0.18- 0.76), 19	0.64, (0.41- 0.87), 29	0.62, (0.4- 0.83), 31	0.51, (0.29- 0.73), 30	0.48, (0.32- 0.64), 71	0.5, (0.36- 0.64), 102
AGP	0.54, (0.38-0.7), 63	0.56, (0.45- 0.66), 125	0.57, (0.48- 0.66), 174	0.72, (0.48- 0.96), 20	0.57, (0.34- 0.81), 31	0.61, (0.39- 0.82), 33	0.8, (0.63- 0.98), 31	0.72, (0.56- 0.88), 72	0.62, (0.48- 0.76), 103
HBP	0.67, (0.45 -0.89), 26	0.55, (0 .37- 0.73), 4 5	0.54, (0 .37- 0.71), 4 8
HP	0.64, (0.49- 0.78), 62	0.57, (0.46- 0.67), 124	0.57, (0.48- 0.66), 173	0.68, (0.42- 0.93), 20	0.61, (0.38- 0.84), 31	0.62, (0.41- 0.84), 33	0.78, (0.59- 0.97), 28	0.72, (0.57- 0.88), 69	0.63, (0.49- 0.77), 100

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65), red (AUROC < 0.6)

Supplementary Table 10: Univariate analysis - aged between 7 and 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives				
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	AUROC (CI), N	Electronic	Strict	Loose
WBC count	0.49, (0.26- 0.73), 28	0.69, (0.54- 0.84), 50	0.75, (0.64- 0.86), 81	0.79, (0.61- 0.96), 34	0.83, (0.71- 0.95), 69	0.82, (0.71- 0.94), 75	0.46, (0.27- 0.65), 47	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112
RBC count	0.62, (0.41- 0.84), 28	0.54, (0.37- 0.7), 51	0.57, (0.44- 0.7), 82	0.7, (0.51- 0.88), 34	0.61, (0.45- 0.78), 69	0.6, (0.44- 0.75), 75	0.56, (0.38- 0.75), 47	0.55, (0.4- 0.7), 87	0.48, (0.35- 0.62), 112	0.48, (0.35- 0.62), 112	0.48, (0.35- 0.62), 112

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	Lymphocyte count	0.76, (0.58-0.94), 28	0.67, (0.51-0.83), 51	0.62, (0.49-0.74), 82	0.6, (0.37-0.83), 34	0.69, (0.54-0.85), 69	0.71, (0.56-0.86), 75	0.59, (0.42-0.76), 47	0.61, (0.48-0.74), 87	0.55, (0.43-0.68), 112
	Neutrophil count	0.46, (0.23-0.7), 26	0.7, (0.54-0.86), 45	0.76, (0.64-0.87), 73	0.73, (0.53-0.93), 34	0.82, (0.69-0.95), 69	0.8, (0.68-0.93), 75	0.66, (0.46-0.86), 46	0.61, (0.43-0.8), 86	0.61, (0.44-0.78), 111
	IL-4	0.56, (0.34-0.78), 28	0.46, (0.31-0.6), 50	0.48, (0.37-0.6), 80	0.73, (0.53-0.92), 33	0.62, (0.47-0.77), 69	0.59, (0.45-0.74), 75	0.46, (0.41-0.5), 47	0.48, (0.46-0.5), 86	0.51, (0.45-0.57), 112
	TRAIL	0.48, (0.23-0.73), 28	0.6, (0.45-0.76), 50	0.57, (0.45-0.7), 80	0.55, (0.34-0.77), 33	0.53, (0.38-0.68), 69	0.52, (0.38-0.66), 75	0.5, (0.5-0.5), 45	0.49, (0.48-0.51), 83	0.49, (0.47-0.5), 109
	IL-6	0.45, (0.21-0.69), 28	0.56, (0.4-0.71), 51	0.55, (0.44-0.67), 82	0.46, (0.34-0.58), 33	0.44, (0.33-0.56), 69	0.43, (0.33-0.53), 75	0.53, (0.44-0.62), 47	0.53, (0.46-0.6), 86	0.54, (0.46-0.62), 112
	CRP NycoCard	0.56, (0.34-0.78), 28	0.61, (0.46-0.77), 51	0.62, (0.5-0.74), 82	0.57, (0.33-0.81), 34	0.52, (0.35-0.68), 71	0.51, (0.35-0.68), 77	0.75, (0.59-0.92), 47	0.71, (0.55-0.87), 87	0.69, (0.56-0.83), 113
	Gal-9	0.67, (0.43-0.9), 28	0.68, (0.53-0.84), 51	0.66, (0.54-0.78), 82	0.71, (0.52-0.9), 33	0.57, (0.41-0.73), 69	0.54, (0.39-0.7), 75	0.79, (0.62-0.95), 47	0.61, (0.44-0.77), 86	0.55, (0.39-0.71), 112
	CHI3L1	0.53, (0.28-0.78), 28	0.6, (0.44-0.76), 51	0.61, (0.49-0.73), 82	0.69, (0.5-0.87), 32	0.66, (0.52-0.79), 67	0.59, (0.44-0.73), 71	0.53, (0.32-0.73), 46	0.58, (0.41-0.74), 84	0.62, (0.47-0.77), 110
	IP-10	0.64, (0.42-0.86), 28	0.56, (0.39-0.72), 51	0.59, (0.46-0.72), 82	0.73, (0.53-0.92), 33	0.62, (0.46-0.78), 69	0.58, (0.42-0.73), 75	0.6, (0.41-0.78), 47	0.48, (0.31-0.66), 86	0.52, (0.37-0.67), 112
	sPLA2	0.47, (0.21-0.72), 28	0.55, (0.39-0.72), 51	0.56, (0.43-0.68), 82	0.54, (0.33-0.76), 33	0.49, (0.35-0.64), 69	0.56, (0.43-0.7), 75	0.46, (0.28-0.64), 47	0.52, (0.36-0.67), 86	0.44, (0.29-0.59), 112
	NGAL	0.56, (0.32-0.8), 28	0.68, (0.52-0.85), 46	0.73, (0.61-0.85), 73	0.71, (0.52-0.9), 33	0.68, (0.54-0.82), 69	0.64, (0.5-0.78), 75	0.7, (0.52-0.89), 46	0.6, (0.44-0.77), 85	0.59, (0.44-0.74), 111
	LBP	0.54, (0.3-0.77), 28	0.59, (0.42-0.75), 46	0.58, (0.45-0.72), 73	0.68, (0.5-0.87), 33	0.66, (0.52-0.8), 69	0.67, (0.54-0.8), 75	0.71, (0.52-0.9), 46	0.66, (0.48-0.84), 85	0.63, (0.46-0.79), 111
	C2	0.62, (0.34-0.9), 28	0.53, (0.36-0.7), 51	0.53, (0.41-0.66), 82	0.54, (0.31-0.76), 32	0.57, (0.4-0.74), 67	0.61, (0.45-0.77), 73	0.62, (0.42-0.81), 45	0.46, (0.27-0.65), 83	0.52, (0.36-0.68), 109
	AGP	0.57, (0.3-0.83), 28	0.55, (0.39-0.71), 51	0.52, (0.39-0.65), 81	0.53, (0.3-0.76), 33	0.6, (0.44-0.75), 69	0.61, (0.46-0.75), 75	0.75, (0.56-0.94), 47	0.68, (0.5-0.86), 86	0.67, (0.52-0.83), 112
	HBP	0.76, (0.28-1), 10	0.58, (0.29-0.87), 19	0.65, (0.39-0.91), 23	## Unbalance d classes	0.92, (0.69-1), 8	0.72, (0.28-1), 9

HP	0.5, (0.25-0.76), 28	0.51, (0.35-0.67), 51	0.5, (0.37-0.63), 82	0.52, (0.3-0.75), 32	0.62, (0.46-0.78), 68	0.6, (0.45-0.76), 74	0.53, (0.33-0.73), 47	0.54, (0.37-0.7), 85	0.53, (0.38-0.67), 109
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Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Supplementary Table 11: Univariate analysis - aged more than 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.67, (0.53-0.82), 66	0.71, (0.62-0.8), 132	0.68, (0.6-0.75), 210	0.84, (0.77-0.91), 202	0.84, (0.77-0.9), 305	0.83, (0.77-0.89), 329	2 patients in total	5 patients in total	5 patients in total
RBC count	0.59, (0.44-0.73), 65	0.53, (0.43-0.63), 131	0.51, (0.43-0.59), 209	0.56, (0.45-0.67), 203	0.56, (0.47-0.64), 306	0.55, (0.47-0.63), 330	-	-	-
Lymphocyte count	0.5, (0.34-0.66), 66	0.53, (0.43-0.63), 131	0.49, (0.41-0.57), 209	0.67, (0.58-0.76), 202	0.65, (0.57-0.72), 305	0.64, (0.57-0.71), 329	-	-	-
Neutrophil count	0.65, (0.49-0.81), 60	0.7, (0.6-0.8), 120	0.66, (0.59-0.74), 193	0.82, (0.74-0.9), 202	0.82, (0.76-0.89), 305	0.82, (0.75-0.88), 329	-	-	-
IL-4	0.4, (0.28-0.52), 66	0.47, (0.39-0.54), 131	0.45, (0.39-0.52), 209	0.56, (0.47-0.65), 196	0.53, (0.46-0.6), 298	0.54, (0.47-0.6), 321	-	-	-
TRAIL	0.68, (0.54-0.82), 66	0.65, (0.56-0.73), 131	0.66, (0.59-0.73), 209	0.57, (0.48-0.65), 199	0.54, (0.47-0.61), 302	0.54, (0.48-0.61), 326	-	-	-
IL-6	0.59, (0.46-0.72), 67	0.63, (0.54-0.72), 131	0.59, (0.52-0.66), 209	0.51, (0.44-0.58), 194	0.51, (0.45-0.58), 297	0.5, (0.44-0.56), 320	-	-	-
CRP NycoCard	0.53, (0.38-0.68), 67	0.6, (0.5-0.7), 133	0.57, (0.49-0.64), 211	0.66, (0.57-0.76), 204	0.65, (0.57-0.73), 307	0.66, (0.58-0.73), 331	-	-	-
Gal-9	0.72, (0.59-0.86), 67	0.6, (0.5-0.7), 133	0.63, (0.56-0.71), 211	0.61, (0.52-0.71), 199	0.56, (0.48-0.65), 301	0.57, (0.5-0.65), 325	-	-	-
CHI3L1	0.52, (0.36-0.67), 65	0.51, (0.41-0.61), 129	0.53, (0.45-0.61), 207	0.66, (0.58-0.75), 194	0.62, (0.54-0.69), 296	0.62, (0.55-0.69), 320	-	-	-
IP-10	0.64, (0.48-0.79), 67	0.59, (0.49-0.69), 133	0.61, (0.53-0.68), 210	0.59, (0.5-0.68), 199	0.52, (0.44-0.6), 302	0.53, (0.45-0.6), 326	-	-	-

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sPLA2	0.53, (0.37-0.69), 67	0.54, (0.44-0.64), 132	0.54, (0.46-0.62), 210	0.58, (0.48-0.67), 199	0.56, (0.48-0.64), 302	0.56, (0.48-0.63), 326	-	-	-
NGAL	0.49, (0.33-0.65), 65	0.62, (0.51-0.72), 110	0.53, (0.44-0.62), 175	0.55, (0.46-0.65), 196	0.54, (0.46-0.62), 296	0.53, (0.45-0.61), 320	-	-	-
LBP	0.56, (0.41-0.7), 66	0.56, (0.45-0.67), 112	0.53, (0.44-0.61), 177	0.65, (0.56-0.74), 195	0.6, (0.52-0.67), 298	0.56, (0.49-0.64), 322	-	-	-
C2	0.67, (0.53-0.81), 67	0.59, (0.49-0.69), 133	0.58, (0.51-0.66), 210	0.5, (0.4-0.6), 193	0.51, (0.43-0.58), 296	0.51, (0.44-0.59), 320	-	-	-
AGP	0.6, (0.45-0.75), 67	0.57, (0.47-0.67), 133	0.54, (0.46-0.62), 211	0.65, (0.55-0.74), 199	0.58, (0.5-0.66), 302	0.56, (0.49-0.64), 326	-	-	-
HBP	0.48, (0.25-0.71), 28	0.54, (0.36-0.72), 44	0.47, (0.31-0.63), 55	0.66, (0.51-0.81), 107	0.66, (0.53-0.79), 136	0.63, (0.5-0.76), 142	-	-	-
HP	0.53, (0.39-0.67), 67	0.58, (0.48-0.68), 132	0.5, (0.42-0.58), 209	0.56, (0.46-0.66), 196	0.47, (0.39-0.55), 299	0.48, (0.4-0.55), 323	-	-	-

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65) red (AUROC < 0.6)

Supplementary Table 12: Univariate analysis - age less than 6 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.64, (0.47-0.81), 50	0.71, (0.59-0.82), 148	0.7, (0.6-0.8), 178	0.62, (0.23-1), 11	0.62, (0.36-0.88), 44	0.62, (0.41-0.83), 56
RBC count	0.51, (0.33-0.68), 49	0.55, (0.44-0.65), 147	0.55, (0.44-0.65), 177	0.7, (0.34-1), 11	0.63, (0.42-0.84), 44	0.62, (0.45-0.8), 56
Lymphocyte count	0.45, (0.26-0.64), 49	0.58, (0.47-0.7), 147	0.55, (0.44-0.66), 177	0.57, (0.17-0.96), 11	0.6, (0.34-0.86), 44	0.63, (0.42-0.85), 56
Neutrophil count	0.59, (0.41-0.77), 49	0.65, (0.53-0.76), 140	0.66, (0.56-0.76), 169	0.7, (0.3-1), 11	0.49, (0.24-0.75), 44	0.55, (0.35-0.75), 56
IL-4	0.68, (0.5-0.86), 50	0.62, (0.52-0.71), 148	0.58, (0.49-0.67), 178	0.5, (0.5-0.5), 11	0.47, (0.42-0.51), 39	0.48, (0.44-0.51), 51
TRAIL	0.73, (0.56-0.89), 50	0.59, (0.48-0.69), 148	0.56, (0.47-0.66), 178	0.5, (0.5-0.5), 11	0.5, (0.5-0.5), 41	0.5, (0.5-0.5), 53
IL-6	0.6, (0.4-0.79), 49	0.64, (0.53-0.74), 147	0.63, (0.53-0.72), 175	0.47, (0.2-0.73), 11	0.48, (0.33-0.62), 37	0.48, (0.36-0.59), 49
CRP NycoCard	0.52, (0.33-0.7), 48	0.58, (0.48-0.69), 145	0.56, (0.46-0.66), 175	0.78, (0.47-1), 11	0.66, (0.41-0.91), 44	0.63, (0.42-0.84), 56
Gal-9	0.58, (0.37-0.79), 49	0.54, (0.43-0.65), 148	0.53, (0.43-0.64), 178	0.5, (0.05-0.95), 11	0.63, (0.45-0.82), 41	0.6, (0.44-0.76), 53
CHI3L1	0.53, (0.36-0.7), 50	0.6, (0.49-0.71), 148	0.57, (0.47-0.67), 178	0.47, (0.07-0.86), 11	0.54, (0.28-0.79), 40	0.56, (0.33-0.8), 51

IP-10	0.73, (0.57-0.9), 50	0.58, (0.47-0.69), 143	0.57, (0.47-0.67), 172	0.77, (0.38-1), 11	0.45, (0.26-0.64), 39	0.48, (0.32-0.64), 51
sPLA2	0.49, (0.3-0.69), 50	0.63, (0.52-0.75), 148	0.62, (0.52-0.72), 178	0.73, (0.38-1), 11	0.52, (0.27-0.78), 41	0.52, (0.31-0.73), 53
NGAL	0.61, (0.43-0.79), 47	0.56, (0.44-0.68), 118	0.54, (0.43-0.65), 141	0.87, (0.6-1), 11	0.62, (0.4-0.85), 40	0.61, (0.41-0.8), 52
LBP	0.55, (0.3-0.79), 48	0.48, (0.37-0.59), 122	0.52, (0.41-0.62), 147	0.45, (0.03-0.87), 11	0.58, (0.33-0.83), 41	0.61, (0.4-0.81), 53
C2	0.57, (0.38-0.76), 50	0.57, (0.47-0.68), 148	0.56, (0.46-0.67), 178	0.58, (0.2-0.97), 11	0.78, (0.6-0.96), 38	0.77, (0.6-0.93), 50
AGP	0.68, (0.52-0.84), 50	0.6, (0.49-0.71), 149	0.57, (0.47-0.68), 179	0.63, (0.24-1), 11	0.52, (0.32-0.73), 41	0.46, (0.27-0.65), 53
HBP	0.55, (0.27-0.84), 33	0.62, (0.49-0.76), 78	0.63, (0.49-0.76), 82
HP	0.72, (0.58-0.87), 50	0.59, (0.48-0.7), 147	0.56, (0.46-0.67), 177	0.57, (0.18-0.95), 11	0.45, (0.21-0.69), 40	0.47, (0.26-0.68), 52

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Supplementary Table 13: Univariate analysis - aged between 7 and 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	AUROC (CI), N	AUROC (CI), N	AUROC (CI), N
WBC count	0.67, (0.51-0.82), 51	0.7, (0.6-0.8), 134	0.66, (0.57-0.75), 185	## unbalanced classes (24 non-bacterial, 1 bacterial) for 25 patients	## unbalanced classes (54 non-bacterial, 1 bacterial) for 55 patients	0.47, (0.03-0.91), 72
RBC count	0.74, (0.6-0.87), 51	0.55, (0.43-0.68), 134	0.53, (0.43-0.63), 185	-	-	0.67, (0.28-1), 73
Lymphocyte count	0.64, (0.49-0.79), 51	0.59, (0.47-0.7), 134	0.55, (0.46-0.64), 184	-	-	0.44, (0.14-0.75), 72
Neutrophil count	0.63, (0.47-0.79), 50	0.67, (0.56-0.78), 127	0.67, (0.58-0.76), 174	-	-	0.51, (0.17-0.86), 73
IL-4	0.53, (0.36-0.7), 51	0.54, (0.44-0.64), 134	0.53, (0.45-0.61), 184	-	-	0.62, (0.27-0.96), 65
TRAIL	0.51, (0.35-0.68), 51	0.52, (0.41-0.63), 134	0.54, (0.45-0.63), 184	-	-	0.62, (0.38-0.87), 72
IL-6	0.62, (0.46-0.78), 50	0.57, (0.46-0.68), 132	0.51, (0.41-0.6), 181	-	-	0.41, (0.37-0.46), 67
CRP NycoCard	0.55, (0.39-0.71), 51	0.52, (0.4-0.64), 134	0.51, (0.41-0.61), 185	-	-	0.59, (0.21-0.97), 73
Gal-9	0.6, (0.44-0.76), 51	0.53, (0.42-0.65), 134	0.55, (0.45-0.65), 185	-	-	0.64, (0.23-1), 72

CHI3L1	0.53, (0.36-0.69), 51	0.49, (0.38-0.6), 133	0.54, (0.45-0.64), 183	-	-	0.61, (0.08-1), 69
IP-10	0.63, (0.47-0.79), 50	0.56, (0.45-0.68), 133	0.53, (0.43-0.63), 184	-	-	0.55, (0.11-0.99), 67
NGAL	0.55, (0.38-0.71), 51	0.52, (0.41-0.64), 134	0.53, (0.44-0.63), 185	-	-	0.56, (0.13-0.99), 72
HNL	0.67, (0.48-0.85), 42	0.47, (0.35-0.59), 108	0.57, (0.48-0.67), 150	-	-	0.66, (0.33-1), 69
LBP	0.61, (0.44-0.78), 42	0.59, (0.47-0.71), 108	0.56, (0.46-0.66), 151	-	-	0.9, (0.77-1), 67
C2	0.62, (0.46-0.78), 51	0.57, (0.46-0.68), 133	0.54, (0.45-0.64), 184	-	-	0.73, (0.47-0.98), 70
AGP	0.6, (0.44-0.76), 51	0.55, (0.43-0.67), 134	0.52, (0.42-0.62), 185	-	-	0.53, (0.07-0.99), 72
HBP	0.64, (0.39-0.9), 21	0.46, (0.28-0.65), 50	0.49, (0.31-0.67), 55	-	-	-
HP	0.54, (0.37-0.7), 51	0.49, (0.38-0.59), 132	0.49, (0.40-0.59), 183	-	-	0.79, (0.6-0.98), 71

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 14: Univariate analysis - aged more than 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.54, (0.32-0.76), 31	0.56, (0.37-0.75), 87	0.65, (0.51-0.78), 128	2 patients in total	11 patients in total	11 patients in total
RBC count	0.42, (0.2-0.63), 31	0.58, (0.42-0.73), 86	0.57, (0.44-0.7), 126	-	-	-
Lymphocyte count	0.77, (0.61-0.94), 31	0.64, (0.5-0.78), 87	0.66, (0.55-0.77), 127	-	-	-
Neutrophil count	0.5, (0.28-0.73), 30	0.55, (0.35-0.74), 81	0.62, (0.48-0.77), 120	-	-	-
IL-4	0.53, (0.33-0.73), 31	0.5, (0.34-0.66), 87	0.48, (0.37-0.59), 126	-	-	-
TRAIL	0.62, (0.42-0.82), 31	0.6, (0.44-0.76), 87	0.63, (0.51-0.75), 126	-	-	-
IL-6	0.67, (0.47-0.87), 32	0.52, (0.35-0.69), 88	0.54, (0.41-0.66), 129	-	-	-
CRP NycoCard	0.57, (0.36-0.78), 32	0.52, (0.37-0.68), 88	0.52, (0.4-0.64), 129	-	-	-
Gal-9	0.61, (0.4-0.82), 32	0.59, (0.44-0.73), 87	0.52, (0.39-0.65), 128	-	-	-

CHI3L1	0.64, (0.43-0.85), 31	0.53, (0.37-0.69), 86	0.52, (0.4-0.65), 126	-	-	-	-
IP-10	0.66, (0.45-0.87), 32	0.52, (0.35-0.69), 87	0.58, (0.44-0.71), 128	-	-	-	-
sPLA2	0.62, (0.42-0.82), 32	0.53, (0.37-0.69), 88	0.56, (0.44-0.69), 129	-	-	-	-
NGAL	0.7, (0.48-0.92), 25	0.55, (0.35-0.75), 65	0.56, (0.41-0.7), 95	-	-	-	-
LBP	0.37, (0.14-0.6), 25	0.47, (0.29-0.66), 65	0.59, (0.46-0.73), 95	-	-	-	-
C2	0.64, (0.43-0.85), 32	0.59, (0.42-0.76), 88	0.47, (0.33-0.6), 129	-	-	-	-
AGP	0.68, (0.49-0.87), 32	0.47, (0.31-0.63), 88	0.52, (0.39-0.64), 129	-	-	-	-
HBP	0.8, (0.34-1), 7	0.62, (0.29-0.95), 23	0.62, (0.29-0.95), 24	-	-	-	-
HP	0.52, (0.31-0.73), 32	0.51, (0.35-0.67), 86	0.53, (0.41-0.64), 127	-	-	-	-

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 15: Multivariate analysis – non-malaria population; haematological biomarkers

Haematological biomarkers							
Overall							
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC		
country neutrophil count, WBC count, lymphocyte count, fever duration, temperature, pulse rate, respiratory rate	country neutrophil count, fever duration	country neutrophil count, fever duration, respiratory rate	L	RF/SW/RFA: 0.75 (0.03)	WBC count: 0.7 (0.03)		
			S	SW: 0.83 (0.04)	WBC count: 0.78 (0.03)		
			E	SW/RFA: 0.83 (0.02)	WBC count: 0.77 (0.03)		
Gabon*							
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.7 (0.12)	WBC count: 0.7 (0.03)		
			S	SW: 0.77 (0.12)	WBC count: 0.73 (0.03)		
			E	RFA: 0.77 (0.08)	WBC count: 0.75 (0.03)		
Malawi							
diastolic blood pressure, HAEMATO_C lymphocyte count, neutrophil count, pulse rate, temperature, fever duration	fever duration, neutrophil count	fever duration, neutrophil count	L	RFA: 0.74(0.05)	neutrophil count: 0.72(.06)		
			S	SW: 0.73(.06)	neutrophil count: 0.72(.07)		
			E	RFA: 0.66(.16)	WBC count: 0.7 (0.05)		

Brazil						
diastolic blood pressure, haematocrit lymphocyte count, neutrophil count, pulse rate, temperature, fever duration, respiratory rate, WBC count	WBC count respiratory rate	WBC count respiratory rate	L	RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%
			S	RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%
			E	RFA: 0.84 (0.07)	WBC count: 0.83 (0.07)	+1%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data due to the limited data.

Supplementary Table 16: Multivariate analysis – non-malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/model s: mean (SD) AUROC	Best host- biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
CRP AGP LBP NGAL pulse rate respiratory rate diastolic blood pressure temperature country	CRP country LBP NGAL pulse rate	CRP country NGAL pulse rate respiratory rate temperature		RF/RFA/SW: 0.66 (0.05)	LBP: 0.62 (0.04)	+6%
				RF: 0.74 (0.04)	LBP: 0.66 (0.05)	+12%
				RFA: 0.76 (0.04)	LBP: 0.75 (0.04)	+1%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.64 (0.12)	LBP: 0.62 (0.04)	+3%
			S	RFA: 0.7 (0.11)	LBP: 0.66 (0.05)	+6%
			E	RFA: 0.7 (0.09)	LBP: 0.75 (0.04)	-7%
Malawi						
IP-10 Gal-9 NGAL temperature CRP respiratory rate fever duration pulse rate diastolic blood pressure	Gal-9 NGAL temperature	Gal-9 NGAL temperature pulse rate fever duration	L	SW: 0.7 (0.06)	Lipocalin. 2: 0.65 (0.06)	+8%
				RF/ SW: 0.67 (0.06)	Lipocalin. 2: 0.64 (0.06)	+5%
				RF: 0.71 (0.12)	IP-10: 0.69 (0.08)	+3%
Brazil						
CRP, AGP	Gal-9, TRAIL,		L	RF: 0.67 (0.04)	CRP: 0.65 (0.06)	+3%

pulse rate, diastolic blood pressure respiratory rate, temperature	NGAL	Gal-9, pulse rate, fever duration, NGAL, temperature	S	SW/RFA: 0.66(.04)	CRP: 0.65 (0.05)	+1%
			E	SW/RFA: 0.65(.05)	CRP: 0.63 (0.08)	+3%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 17: Multivariate analysis – non-malaria population; haematological and protein biomarkers

Haematology + protein biomarkers								
Overall								
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%) ** multivariate and single host-biomarkers ratio		
Rulefit	Logistic - RFA	Logistic - SW	L	SW/RFA/RF:0.75(.03)	WBC count: 0.7 (.03)	+7%		
				SW:0.83(.04)	WBC count: 0.78(.03)	+6%		
				SW/RFA:0.83 (.03)	WBC count: 0.77 (0.04)	+8%		
Brazil								
Gal-9, neutrophil count, WBC count, CRP, sPLA, respiratory rate, temperature, diastolic rate, Gal-9 blood pressure, fever duration, pulse rate	neutrophil count, WBC count, respiratory rate	WBC count, Gal-9 respiratory rate	L	SW: 0.82 (0.06)	WBC count: 0.8 (0.06)	+2.5%		
				RFA: 0.82 (0.06)	WBC count: 0.8 (0.06)	+2.5%		
				SW: 0.85 (0.06)	WBC count: 0.83 (0.07)	+2%		
Gabon*								
Gabon performance evaluation using the overall model and Gabon data extracted from the Overall test sets			L	SW/RFA: 0.7 (0.12)	WBC count: 0.7 (.03)	-		
				SW/RFA: 0.76 (0.12)	WBC count: 0.78(.03)	-3%		
				RFA: 0.77 (0.07)	WBC count: 0.77 (0.04)	-		
Malawi								
IP-10 Gal-9 LBP neutrophil count	neutrophil count, WBC count	neutrophil count WBC count, fever duration,	L	SW/RFA: 0.74 (0.06)	neutrophil count: 0.72 (0.03)	+3%		

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WBC count	fever	IP-10, duration, IP-10	S	SW: 0.73 (0.06)	neutrophil count: 0.72 (0.07)	+1%
NGAL		temperature	E	RFA: 0.72 (0.6)	WBC count: 0.7 (0.)	+2%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 18: Multivariate analysis – malaria population; haematological biomarkers

Haematological biomarkers						
Multivariate models' variables			Classification group	Best multivariate model/models : mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
haematocrit	neutrophil count	lymphocyte count		RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)	+5%
lymphocyte count	WBC count	neutrophil count	S	SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%
neutrophil count	country	country		RF: 0.69 (0.07)	neutrophil count: 0.61 (0.08)	+13%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.18)	neutrophil count: 0.65 (0.05)	+3%
			S	SW: 0.75 (0.2)	neutrophil count: 0.6 (0.08)	+25%
			E	Not sufficient data		
Malawi						
diastolic blood pressure	neutrophil count,	WBC count,	L	RFA: 0.7 (0.06)	WBC count: 0.69 (0.05)	+1%
lymphocyte count	WBC count,		S	SW: 0.69 (0.07)	WBC count: 0.69 (0.07)	-
neutrophil count	temperature		E	RFA: 0.6 (0.14)	lymphocyte count: 0.67 (0.05)	-10%
temperature						
WBC count						
haematocrit						
pulse rate						
respiratory rate						
fever duration						

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 19: Multivariate analysis – malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
AGP diastolic blood pressure Gal-9 C2 LBP pulse rate respiratory rate temperature fever duration	C2	country respiratory rate temperature AGP	L	SW: 0.62 (0.07)	CHI3L1: 0.57 (0.03)	+ 9%
			S	SW: 0.64 (0.04)	NGAL: 0.6 (0.06)	+ 7%
			E	SW: 0.67 (0.08)	C2: 0.63 (0.1)	+ 6%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.17)	CHI3L1: 0.57 (0.03)	+ 18%
			S	RFA: 0.81 (0.12)	NGAL: 0.6 (0.06)	+35% ^{\$}
			E	Not sufficient data		
Malawi						
diastolic blood pressure CHI3L1 IP-10 fever duration Gal-9 C2 pulse rate respiratory rate temperature			L	RFA/SW: 0.57 (0.06)	IP-10: 0.57 (0.05)	-
			S	SW/RFA: 0.62 (0.09)	HCC2_PL: 0.62 (0.06)	-
			E	SW/RFA: 0.61 (0.06)	IP-10: 0.66 (0.09)	-7%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model and tested with Gabon data. ^{\$}This output has to be considered an outlier due to biomarker data imbalance between pipeline data and the available Gabon data set.

Supplementary Table 20: Multivariate analysis – malaria population; haematological and protein biomarkers

Protein + haematological biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				

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AGP_Pl diastolic blood pressure Gal-9 C2 LBP. NGAL neutrophil count respiratory rate temperature pulse rate fever duration	country WBC count	country, Wbc_c,	L	SW/RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)	+5%
			S	RFA/SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%
			E	RFA/SW: 0.66 (0.11)	HCC2_PL: 0.63 (0.1)	+5%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	RFA/SW: 0.66 (0.18)	neutrophil count: 0.65 (0.05)	+1%
			S	RFA/SW: 0.7 (0.2)	neutrophil count: 0.6 (0.08)	+17%
			E	Not sufficient data		
Malawi						
CHI3L1 IP-10 Gal-9 C2 neutrophil count respiratory rate temperature diastolic blood pressure pulse rate fever duration	C2 neutrophil count WBC count	WBC count	L	SW: 0.69 (0.05)	WBC count: 0.69 (0.05)	-
			S	RFA: 0.73 (0.07)		WBC count: 0.69 (0.07)
			E	RFA: 0.72. (0.1)	lymphocyte count: 0.67 (0.05)	+7%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model and tested with Gabon data.

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1 1 **Cross-Sectional Evaluation of Host Biomarkers for Guiding Antibiotic**
2 2 **Use in Bacterial and Non-Bacterial Acute Febrile Illness in Low- and**
3 3 **Middle-Income Tropical Settings**

4
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3 46 **ABSTRACT**
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6 47 **Objectives**
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8 48 To evaluate the effectiveness of 18 different host biomarkers in differentiating bacterial from
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10 49 non-bacterial acute febrile illness (AFI) in resource-limited settings, specifically in Brazil,
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12 50 Malawi, and Gabon.

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14 51 **Design**
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17 52 Multinational, cross-sectional study
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19 53 **Setting**
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21 54 The study was carried out across multiple primary healthcare facilities, including urban and
22
23 55 rural settings, with a total of three participating centers. Recruitment took place from October
24
25 56 2018 to July 2019 in Brazil, May to November 2019 in Gabon, and April 2017 to April 2018
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27 57 in Malawi.

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29 58 **Participants**
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31 59 A total of 1,915 participants, including children and adults aged 21 to 65 years with a fever of
32
33 60 ≤7 days, were recruited through convenience sampling from outpatient clinics in Brazil, Gabon,
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35 61 and Malawi. Individuals with signs of severe illness were excluded. Written consent was
36
37 62 obtained from all participants or their guardians.

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39 63 **Intervention**
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41 64 Not applicable as the study primarily focused on biomarker evaluation without specific
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43 65 therapeutic interventions.

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45 66 **Primary and Secondary Outcome Measures**
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48 67 The primary outcome measure was the ability of each host biomarker to differentiate between
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50 68 bacterial and non-bacterial AFI, as evaluated by area under the receiver operating characteristic
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52 69 (AUROC) curves. Secondary outcomes included the performance of individual biomarkers
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54 70 across the different study sites and in a multivariable setting.

71 **Results**

72 A Kruskal-Wallis test, adjusted by Benjamini-Hochberg, was performed for each biomarker to
73 identify covariates with a significant difference in the distribution of biomarker values. The
74 analysis revealed that country of origin (Brazil, Gabon, Malawi), age, sex, and malaria status
75 significantly impacted biomarker distribution ($p \leq 0.001$). The most widely known biomarkers,
76 such as white blood cell count and C-reactive protein (CRP), demonstrated the best
77 performance in distinguishing between bacterial and non-bacterial infections, with AUROCs
78 reaching up to 0.83 [0.77 - 0.88] for white blood cell count and 0.71 [0.59 - 0.82] for CRP.
79 However, none of the evaluated novel host biomarkers exhibited high performance (AUROC
80 < 0.70 in most cases), and variations in biomarker performance were observed across the three
81 settings. Multivariable analyses demonstrated that while the best combination of biomarkers
82 achieved higher AUROCs, the increase was modest (1–13%), suggesting that the interaction
83 of biomarkers contributed minimally to predictive accuracy.

84 **Conclusions**

85 There is a continued need for innovation in the host-biomarker space as the available markers
86 do not meet the needs of diverse populations around the globe. This highlights the importance
87 of targeted evaluations in non-severe patients in multiple settings to understand true potentials
88 for real-life use. The findings highlight that not one-marker fits all settings and novel
89 innovations remain urgently needed.

90 **Trial Registration**

91 Clinical trial number: NCT03047642

92 **Keywords**

93 Antimicrobial Resistance, AMR, CRP, Host Biomarkers, Prospective study, biomarker, non-
94 malaria fever, primary health care, Malawi, Brazil, Gabon

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96 STRENGTHS AND LIMITATIONS OF THIS STUDY

- 97 • **Diverse Evaluation:** This study is an extensive evaluation of 18 host biomarkers across low-
98 and middle-income countries (LMICs) to differentiate bacterial from non-bacterial infections.
- 99 • **Methodological Alignment:** The study protocol aligns with FDA-approved classifications for
100 distinguishing between bacterial and non-bacterial infections, enhancing methodological rigor.
- 101 • **No Control Group:** The absence of a control group limits the ability to establish baseline
102 biomarker performance or to assess asymptomatic carriers.
- 103 • **Time and Geographic Variability:** The short enrollment period and heterogeneity of acute
104 febrile illness causes may limit the generalizability of findings across different times and
105 geographical contexts, particularly in Asia.
- 106 • **Subjectivity in Classification:** The two-step clinical classification process may introduce
107 subjectivity, particularly as clinicians had access to hematology biomarker results during
108 classification, potentially biasing results.

109 INTRODUCTION

110 Globally, acute febrile illness (AFI) is one of the leading reasons individuals, particularly
111 children aged less than 5 years, present to primary healthcare facilities [1]. AFI has various
112 causes, both infectious and non-infectious, that vary according to geography, age group, and
113 season [1]. In malaria-endemic settings, malaria was long considered the primary cause of all
114 fevers; however, the introduction of rapid diagnostic tests (RDTs) for malaria in the past decade
115 has disproved this. Modelling estimates suggest that approximately 70% of all fevers can be
116 attributed to non-malarial causes, even in malaria-endemic settings [2]. In the Integrated
117 Management of Childhood Illness (IMCI), introduced by the World Health Organization
118 (WHO) and UNICEF in the mid-1990s and subsequently implemented in more than 100
119 countries, the standard “fever” algorithm currently includes a malaria RDT but no diagnostic
120 test for other infections [3]. Hence, at primary care level, the only evidence-based treatment
121 decision that can be made relies on the malaria RDT, resulting in extremely high levels of
122 antibiotic use in malaria-negative patients [4]. In this context of limited knowledge about the
123 causes of AFI and limited diagnostic and human capacity, it is unsurprising that healthcare
124 providers prescribe antibiotics to avoid negative outcomes in their patients.

125 To assist healthcare providers with clinical decision-making, a simple diagnostic tool is
126 required to differentiate patients with AFI of bacterial and non-bacterial aetiology and provide
127 appropriate care. In well-resourced settings, in both high-income countries (HICs) and low-
128 and middle-income countries (LMICs), some nonspecific host-biomarkers are used for this
129 purpose, most frequently C-reactive protein (CRP) and procalcitonin (PCT), although these
130 biomarkers are less useful in settings with a higher frequency of comorbidities [5]. Thus, in
131 2015, an international group of experts was convened to define the target product profile (TPP)
132 of such a tool, specifically for low-resource settings, to guide product development and
133 implementation as part of integrated treatment management guidelines [6]. Since then, the

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3 134 ongoing viral pandemic (SARS-CoV-2) has further highlighted the challenge of differential
4 diagnosis and shows yet again that better antimicrobial stewardship interventions are needed
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6 135 to counter the overprescribing of antibiotics in patients with viral infections [7].
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10 137 Host biomarkers other than CRP and PCT have been evaluated for distinguishing bacterial
11 from non-bacterial infections, including human neutrophil lipocalin (HNL), heparin-binding
12 protein (HBP), and chitinase 3-like protein 1 (CHI3L1) [8]. There are also some commercially
13 available tests. ImmunoXpert™, from MeMed, uses a biomarker combination comprising
14 CRP, interferon gamma-inducible protein 10 (IP-10), and TNF-related apoptosis-inducing
15 ligand (TRAIL), while FebriDx®, from Lumos Diagnostics, uses an MxA and CRP biomarker
16 combination. While these biomarker signatures show promise, they have only been evaluated
17 in limited settings. Any potential impact of co-infections or comorbidities, common in LMICs,
18 on their effectiveness is unknown. Other characteristics of host-biomarker studies that hamper
19 direct comparisons include: (i) just one/a few biomarkers in the study; (ii) small sample sizes,
20 increasing the probability of recruiting unrepresentative study populations; (iii) narrow
21 population subgroups (e.g. children only, hospitalised only, respiratory infections only, etc),
22 limiting the generalisability of study results to the broader AFI population; (iv) studies
23 conducted in one country, so co-infections/comorbidities may not be comparable with those of
24 other countries; (v) retrospective studies that used convenience sampling and case-control
25 study designs, increasing the risk of bias; and (vi) the lack of a standard definitions for
26 classifying bacterial versus non-bacterial infections [9].
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30 154 Here, we describe the Biomarker for Fever Diagnostic (BFF-Dx) study, specifically designed
31 to evaluate host biomarkers to distinguish bacterial from non-bacterial infections in line with
32 the published TPP and the final use case of such diagnostic tests. To our knowledge, this is the
33 only study to evaluate host biomarkers in the intended target population (non-severe patients),
34 prospectively, in multiple settings with a large sample set. We evaluated 18 host-biomarkers in
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3 159 three distinct settings, in Brazil, Gabon, and Malawi with the main objective to provide a
4 160 performance comparison of host biomarkers in the non-severe AFI population from resource-
5 161 limited settings, with the goal to overcome many of the previously described limitations (eg.
6 162 sample size, retrospective vs prospective, focused populations, biased analysis) [10]. The
7 163 described comparison was conducted within the pragmatic context of diagnostic product
8 164 development and aimed to identify host biomarkers or biomarker combinations for utilisation
9 165 in next-generation rapid diagnostic tests.

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3 166 **METHODOLOGY**
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6 167 **Study settings**
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9 168 This multinational, cross-sectional study was conducted in Brazil, Gabon, and Malawi; Gabon
10 and Malawi were selected as high-malaria endemicity settings, while Brazil was selected as a
11 low-malaria endemic setting. The study sites were UPA Manguinhos and Family Health Clinics
12 Armando Palhares in Rio de Janeiro, Brazil; the Clinical Trials Unit Center of Medical
13 Research Lambaréné (CERMEL), Lambaréné, Gabon; and Malawi Epidemiology and
14 Intervention Research Unit (MEIRU), Chilumba campus, Malawi. The enrollment sites were
15 an urban primary healthcare facility, a hospital in a semi-rural setting, and a rural primary
16 healthcare facility in Brazil, Gabon, and Malawi, respectively. Participants were recruited from
17 October 2018 to July 2019, May to November 2019, and April 2017 to April 2018, in Brazil,
18 Gabon, and Malawi, respectively. The study protocol was submitted to clinicaltrial.gov
19 (NCT03047642) and ethical approval was obtained from all relevant institutional committees
20 in Brazil (Research Ethics Committee of INI-FIOCRUZ and Comissão Nacional de Ética em
21 Pesquisa ; National Research Ethics Committee), Gabon (Comité National d'Ethique pour la
22 Recherche) and Malawi (National Health Science Research Committee ; Observational and
23 Intervention Research Ethics Committee of the London School of Hygiene and Tropical
24 Medicine , UK) and all details of the design have been previously published [10]. Reporting
25 complies with the STARD-15 checklist.

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50 186 **Study population and study procedure**
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Participants were obtained through convenience sampling and included both children and adults, aged between 2 and 65 years, who presented at the outpatient clinics with a history of fever of ≤ 7 days duration (Brazil and Gabon) or fever at presentation (Malawi). Patients with signs of severe illness were not included in the study. The overarching study protocol was

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3 191 slightly adapted to each site due to local requirements (logistical or ethical). Detailed criteria
4 192 for inclusion by study sites have been published previously [10]. Outcomes were based on the
5 193 TPP criteria and while no patient input was used, external expert input was used to define target
6 194 population and criteria. Only patients who met the eligibility criteria and who provided written
7 195 consent (patient or guardian for children) were enrolled in the study. Data and samples were
8 196 systematically collected and analysed as previously described. To ensure consistent quality and
9 197 comparability of data, the same standard operating procedures were used at all sites (for data
10 198 collection and laboratory testing) [10].
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24 200 **Patient and Public Involvement statement**
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26 201 None
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31 203 **Bacterial/non-bacterial classification and biomarker selection and testing**
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34 204 A two-step process was used to classify the patients into “bacterial” and “non-bacterial”
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36 205 groups. First, the cause of fever (bacterial/non-bacterial) was classified according to laboratory-
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38 determined parameters (“electronic group”). The electronic group was based on predefined and
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40 widely accepted laboratory parameters, including direct pathogen detection, a fourfold increase
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42 in anti- body titre, or a positive PCR or antigen RDT result. The list of tests performed is
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44 described in detail in by Escadafal et al. [10]. Next, cases that could not be classified by
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46 laboratory-determined parameters were assessed by a panel of three independent clinical
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48 experts. Patient’s history and clinical and laboratory data was provided to the experts. Clinical
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50 expert’s assessments were then compared. If the three panel members unanimously assigned a
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52 diagnostic label, patients were considered to have “bacterial” or “non-bacterial” infections; if
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54 two out of three panel members reported a classification of “bacterial” or “non-bacterial”, these
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3 215 patients were considered to have “probable bacterial infection” or “probable non-bacterial
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5 216 infection”, respectively.

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9 217 Data were analysed based on three groups of patients: 1) the “electronic group”, i.e. subjects
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11 218 with a cause of fever defined based on laboratory parameters; 2) the “strict group”, which
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13 219 comprised the electronic group and the patients that were unanimously classified by the clinical
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15 220 panel of three experts; and 3) the “loose group”, which comprised the electronic and strict
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17 221 groups as well as those patients for whom two of the clinical experts agreed they had either
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19 222 probable bacterial or probable non-bacterial infection. Subjects with undetermined cause of
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21 223 fever according to the three classification criteria considered (“electronic group”, “strict
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23 224 group”, “loose group”) were excluded from the statistical analysis. This outcome-oriented
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25 225 approach, based on methods previously developed for host-biomarker studies and described
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27 226 previously, was used to ensure the total intended-use population of any future test was
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29 227 represented in the final analysis [10, 11].
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36 228 The evaluated biomarkers were selected based on previously reported performances, and
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38 229 haematological markers as well as CRP were included as comparators (Table 1 and
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40 230 Supplementary Table 1 and 2) [8, 12].
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43 231 At the end of data collection, all biomarker data were analysed to assess the percentage of
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45 232 missing values and the percentage of values below the lower limit or above the upper limit of
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47 233 detection of the used tests. Biomarkers with more than 50% of missing data or more than 95%
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49 234 of saturated values below the lower limit of quantification of the used test, were excluded from
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51 235 the following statistical analysis.
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3 236 **Table 1.** Novel biomarkers identified in the literature and evaluated in the BFF-Dx study,
4 including sample type used, evaluation method, and sample origin.
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Abbreviation	Biomarker name	Sample type	Evaluation method	Sample origin
AGP	A-1-acid glycoprotein	EDTA-plasma	Luminex	B, G, M
C2	Complement 2	EDTA-plasma	Luminex	B, G, M
C4b	Complement C4b	EDTA-plasma	Luminex	B, G, M
CHI3L1	Chitinase-3-like protein 1	EDTA-plasma	Luminex	B, G, M
CRP	C-reactive protein	EDTA-plasma	CRP Nycocard/ NycoCardReader II, ELISA	B, G, M
Gal-9	Galectin-9	EDTA-plasma	Luminex	B, G, M
HBP	Heparin-binding protein	EDTA-plasma	ELISA	B, M
HNL	Human neutrophil lipocalin	Heparin-activated plasma time-controlled activation#	ELISA	M
		EDTA-plasma	ELISA	B, G, M
HP	Haptoglobin	EDTA-plasma	Luminex	B, G, M
IFN-gamma	Interferon gamma	EDTA-plasma	Luminex	B, G, M
IL-4	Interleukin-4	EDTA-plasma	Luminex	B, G, M
IL-6	Interleukin-6	EDTA-plasma	Luminex	B, G, M
IP-10	Gamma-induced protein 10	EDTA-plasma	Luminex	B, G, M
LBP	Lipopolysaccharide binding protein	EDTA-plasma	Luminex	B, G, M
NGAL	Neutrophil gelatinase-associated lipocalin	Frozen heparin-activated plasma	Luminex	M
		EDTA-plasma	Luminex	B, G, M
PCT	Procalcitonin	EDTA-plasma	Luminex; ELISA	B, G, M
sPLA2	Secretory phospholipase 2	EDTA-plasma	Luminex	B, G, M
sTREM-1	Soluble triggering receptor expressed on myeloid cells 1	EDTA-plasma	Luminex	B, G, M
TRAIL	TNF-related apoptosis-inducing ligand	EDTA-plasma	Luminex	B, G, M

238 B, Brazil; G, Gabon; M, Malawi

239 # Whole blood samples were collected in lithium heparin tubes and activation was performed within 60 min prior to freezing
240 and subsequent ELISA testing [13]. All biomarkers were tested using the same standard operating procedures (SOPs) and all
241 sites were trained on the SOPs. For CRP and PCT different devices were used at different sites, repeat testing was performed
242 at the central facility (NMI).

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3 245 **Statistical analysis**

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5 246 a. Kruskal-Wallis Analysis and Definition of Covariates Influence on Biomarkers

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7 247 A Kruskal-Wallis test, adjusted by Benjamini-Hochberg, was conducted for each biomarker to
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9 248 determine which covariates exhibited statistically significant differences in the distribution of
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11 249 biomarker values. The covariates studied were country (i.e., the country of origin of the
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13 250 patients), age, sex, malaria status, comorbidities (i.e., presence of one or more diseases among
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15 251 cardiovascular, neurological, respiratory, renal, genitourinary, connective tissue, cancer, or
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17 252 infectious diseases), malnutrition status calculated based on WHO body mass index criteria,
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19 253 self-reported use of antibiotics prior to visiting the health facility, axillary temperature $\geq 38^{\circ}\text{C}$,
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21 254 and positive result to Chikungunya test. The Kruskal-Wallis test was performed for each of the
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23 255 three patient groups defined in the previous section (“electronic”, “strict”, “loose”). The results
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25 256 of the Kruskal-Wallis test allowed the identification of covariates that most significantly
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27 257 impacted the biomarker distribution ($p \leq 0.001$, adjusted by Benjamini-Hochberg). The most
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29 258 significant covariates were considered for defining subgroups of patients in which the
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31 259 following univariate analyses were performed, or included as covariates in the multivariable
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33 260 analyses.

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35 261 b. Univariate analysis

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37 262 As an exploratory step, the ability of each biomarker to discriminate between bacterial and
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39 263 non-bacterial infections was assessed by the area under the receiver operating characteristic
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41 264 curve (AUROC). In particular, subjects were ranked based on the values of the single variable
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43 265 of interest (i.e. based on ordered values) and, using this as score, calculated the ROC curve and
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45 266 the corresponding area under the curve. Such univariate analysis was conducted for each
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47 267 patient group (“electronic”, “strict”, “loose”) and specific patient subgroup (Malaria status,
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49 268 Country and Age).

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3 269 However, since the univariate analyses did not yield satisfactory results, we also explored
4 270 multivariable models to potentially improve the predictive capabilities by incorporating a
5 271 broader range of information.
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10 272 c. Multivariable analysis
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12 273 Multivariable classification models were developed to assess the discrimination ability of
13 274 combinations of biomarkers and covariates. For the multivariable analysis, both linear (logistic
14 275 regression) and non-linear classification models (RuleFit) were explored [14]. The candidate
15 276 features for each model included a group of host-biomarkers and some additional covariates
16 277 (age, temperature, fever duration, diastolic blood pressure, respiration rate, and pulse rate).
17
18 278 Regarding host-biomarkers, three different groups of biomarkers were considered:
19 279 haematology biomarkers only (i.e. white blood cell, neutrophil, red blood cell, lymphocyte
20 280 counts), protein biomarkers only (i.e. novel biomarkers + CRP), and haematology plus protein
21 281 biomarkers (i.e. all biomarkers).
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24 282 For each patient subgroup and each candidate feature set, three multivariable models were
25 283 developed: i) a logistic regression model with stepwise (SW) feature selection; ii) a logistic
26 284 regression model with features selected based on recursive feature addition (RFA; a variant of
27 285 the method proposed in [15]); iii) RuleFit, a non-linear model in which a set of rules from an
28 286 ensemble of decision trees (typically from a tree-based model like a Random Forest or Gradient
29 287 Boosted Trees) is generated and then fit a sparse linear regression model (regularized with
30 288 LASSO), where the features are the rules generated from the trees [14, 15].
31 289 To further tackle the number of biomarkers and variables included in the best models, we
32 290 introduced an additional selection step, employing a plateau seeking approach. The primary
33 291 objective of this approach was to pinpoint a concise set of variables capable of attaining an
34 292 AUROC score similar to that of our comprehensive model, which already incorporated the
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3 293 most impactful and previously selected variables. This was to ensure that our model is not only
4 effective in terms of performance but also efficient in its variable inclusion.
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7 295 Each model was trained and tested using the following pipeline. The data were randomly split
8 into training and test sets (80% and 20% of the data, respectively) stratifying by the outcome
9 variable. Missing data in the training and test sets were imputed using the MICE (multiple
10 imputation by chained equation) algorithm. The n_imp parameter for MICE imputation was
11 set to 1, resulting in a single imputed dataset; however, the imputation process was integrated
12 in a robust bootstrapping pipeline, generating ten independent datasets. This approach ensured
13 variability in our results, stemming not only from the MICE imputation but also from the
14 bootstrapping process. This dual approach guarantees that each imputed dataset is distinct [16].
15
16 300 All quantitative variables were scaled into the range [0,1] by subtracting their minimum value
17 and dividing by the difference between the maximum and minimum values in the training set.
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19 301 The categorical variables with n categories were encoded using n-1 binary “dummy” variables.
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21 302 The model was then trained on the imputed and scaled training set, and its performance was
22 assessed on the imputed and scaled test set by computing the AUROC. The AUROC on the
23 test set was also calculated for single host biomarkers, to allow a fair comparison of the
24 performance of the multivariable classification models vs. single host biomarkers.
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27 310 To assess the robustness and variability in the results of the developed models, the entire
28 pipeline were bootstrapped, i.e. it was run ten times with different random training-test set
29 splits. Finally, the mean and the standard deviation (SD) or the minimum and maximum
30 reached of the AUROC across the ten training-test splits were calculated for each multivariable
31 model and each single host biomarker.
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34 315 a. Software
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37 316 All statistical analyses and model development were performed using the R programming
38 language (version 4.1.2). Specifically, the *mice* package was used for data imputation, while
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3 318 the *pre* and *stats* packages were used for RuleFit and logistic regression model development,
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For peer review only

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3 322 **RESULTS**
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8 323 **Study population**
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13 324 In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415;
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15 325 Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male.
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18 326 Children aged <5 years comprised 45/500 (9%), 182/415 (43·9%), and 367/1000 (36·7%)
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20 327 participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4)
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In total, 1915 patients with AFI were included in the study (Brazil: n=500; Gabon: n=415; Malawi: n=1000). Just under half (862/1915, 45%) of participants at each study site were male. Children aged <5 years comprised 45/500 (9%), 182/415 (43·9%), and 367/1000 (36·7%) participants in Brazil, Gabon, and Malawi, respectively; the median (range) age was 3 (2–4) years (Table 2). Detailed baseline characteristics of patients and analyses of differences will be described in a separate manuscript (Alabi et al in preparation).

330 **Table 2: Baseline characteristics of patients.**

	Brazil	Gabon	Malawi	All
0–5 years (median, IQR, n)	3, [2-4], 45	3, [2-5], 182	3, [2-4], 137	3, [2-4], 594
5–15 years (median, IQR, n)	11, [8-14], 85	9, [7-12], 214	9 [7-12], 26	9, [7-12], 575
>15 years (median, IQR, n)	34, [24-45], 370	16, [16-16·5], 19	28, [21-33], 357	30, [21-42], 746
Male (%), n	49·6%, 248	45·1%, 187	42·7%, 217	45·0%, 862
Temperature, °C (median, IQR, n)	37·7, [36·7-38·4], 500	36·8, [36·4-37·4], 415	38·1, [37·8-38·8], 999	37·8, [37·3-38·5], 1914
WBC count, 10 ⁹ /L (median, IQR, n)	7·28, [5·47-10·39], 494	7·7, [5·7-10], 411	6·7, [5-8], 985	7·1, [5·3-9·8], 1890
Neutrophil count, 10 ⁹ /L (median, IQR, n)	4·97, [3·63-7·4], 494	2·77, [1·96-3·9], 408	4·3, [3·4-18], 906	4·1, [2·8-6], 1812
RBC count, 10 ⁹ /L (median, IQR, n)	40·1, [36·5-43·2], 494	33·2, [29·4-35·8], 412	36·2, [33·1-39·5], 984	36·3, [33-40·2], 1892
Lymphocyte count, 10 ⁹ /L (median, IQR, n)	1·15, [0·7-1·99], 493	2·73, [1·8-4·16], 411	1·5, [1-2·2], 982	1·63, [1-2·6], 1883
CRP NycoCard# – mg/L (median, IQR, n)	70·5, [35-98·75], 498	28, [5-73], 415	47, [12-16·5], 987	49, [13-98], 1900
Malaria-positive by RDT on-site (% all, n)	0·2%, 1	56·4%, 234	45·9%, 48	36·2%, 693
Malaria-positive by qPCR or microscopy (% all, n)	-	-	50·5%, 55	-
HIV-positive by RDT (% all, n)	1·4%, 7	1·2%, 5	4·2%, 4	2·8%, 54
History of antibiotic-use pre-presentation (% all, n)	8·8%, 44	2·41%, 10	7·2%, 7	6·5%, 124
History of antipyretic-use pre-presentation (% all, n)	83·2%, 416	79·76%, 331	55·1%, 51	62·2%, 1298
Cough (%), n	35·8%, 179	30·1%, 125	48·2%, 48	41%, 786

Diarrhea or vomiting (%), n	31·8%, 159	28·9%, 120	27·5%, 126	28·9%, 554
Dysuria or urinary urgency (%), n	0·9%, 45	5·12%, 21	7·6%, 33	7·4%, 142
Headache (%), n	76·4%, 382	46·5%, 193	71·1%, 338	67·2%, 1286
Sore throat or swallow pain (%), n	39%, 195	8·92%, 37	15·8%, 73	20%, 390
Rash (%), n	24·4%, 122	4·1%, 17	2·5%, 12	8·6%, 164

NycoCard was found to be equivalent to reference testing in the relevant range (Supplementary Figure 1). CRP, C-reactive protein; IQR, interquartile range; qPCR, quantitative PCR; RBC, red blood cell; RDT, rapid diagnostic test; WBC, white blood cell; -: data not available

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3 **333 Bacterial and non-bacterial outcomes by classification groups**

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5 334 Using the electronic classification grouping, 15·1% (290/1915) of cases were bacterial
6 infections, 20·2% (387/1915) were non-bacterial infections, and 64·5% (1238/1915) had an
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8 335 undetermined cause of fever (Figure 1). Under the strict classification grouping, 24·3%
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10 336 (366/1509), 66·9% (1010/1509), and 9·0% (133/1509) were classified as bacterial, non-
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12 337 bacterial, and undetermined infections, respectively, while using the loose classification
13 grouping 25·7% (491/1915), 67·3% (1286/1915), and 7·0% (133/1915) were classified as
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15 338 bacterial, non-bacterial, and undetermined infections, respectively (Figure 1). Subjects with
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17 339 undetermined cause of fever/infections were excluded from the following univariate and
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19 340 multivariable analyses.

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344 Exclusion of biomarkers with too many missing or saturated values

345 The biomarkers C4b, HNL and PCT had more than 50% missing values and were therefore
346 excluded. The high number of missing values is due to fact that biomarkers were analysed in
347 groups based on the required dilution using Luminex platform. For some biomarkers the
348 dilution was not optimal, and it was only possible to re-measure biomarkers with a different
349 dilution a limited number of times. IFN-gamma and sTREM-1 were excluded due to more than
350 95% of values saturated to the minimum/maximum level detectable by the measurement
351 instrument. All the biomarkers retained in the analysis had less than 12% missing values
352 (Supplementary Table 3).

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3 354 **Identification of relevant subgroups for analyses**

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5 355 According to the Kruskal-Wallis analysis on the “electronic group”, the variables “country”,
6 “malaria status” and “age” showed statistically significant differences in the distributions of
7 many host biomarkers ($p \leq 0.001$ for strong differences, $0.001 \leq p < 0.01$ for high differences;
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9 357 Supplementary Table 4). The variables “sex”, “comorbidities”, “history of antibiotic use”
10 showed no ($p > 0.05$) or slight ($p \leq 0.05$) differences in all the host biomarkers. The effects of
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12 358 “chikungunya status” and “fever above 38°C” were generally significant ($p \leq 0.01$), but the
13 sample sizes for these groups were either too small or exhibited an imbalance. Additionally,
14 while we conducted subgroup analyses by clinical syndromes (i.e. cough, diarrhea or
15 vomiting, dysuria or urinary urgency, headache, sore throat or swallow pain, rash), the
16 resulting datasets were similarly limited in size, restricting our ability to make robust
17 interpretations from these analyses. The primary focus remained centered on populations
18 grouped by study country and malaria status variables - both of which showed strong
19 statistical differences with the value of the biomarkers in the “strict” and “loose” groups
20 (Supplementary Table 5, 6) - other significant covariates were also included in the
21 multivariable analysis. This inclusion was due to their influence, and factors like the study
22 country were considered as variables in the overall scenario.

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26 372 **Individual host-biomarker performance – univariate analysis**
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28 373 The performance of 18 host biomarkers was consistent across the three patient classification
29 groups in each of the settings (Table 3 and Supplementary Tables 7-9). White blood cell (WBC)
30 and neutrophil counts were the most effective biomarkers for differentiating bacterial and non-
31 bacterial infections. For the malaria-negative population, the mean (95% confidence interval)
32 of AUROC for WBCs was between 0.60 (0.48–0.72) and 0.83 (0.77–0.88) and for neutrophils
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 3 378 it was between 0·67 (0·57–0·77) and 0·80 (0·74–0·86) across the three countries and the three
 4 groups (“electronic”, “strict”, “loose”). Neutrophil and WBC counts showed the highest
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 6 379 AUROCs in the Brazilian population, between 0·80 (0·74–0·86) and 0·83 (0·77–0·88),
 7 respectively. All protein biomarkers showed relatively poor performances (<0·7 in most cases,
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 9 380 Table 4) in all three settings. Galactin-9, CRP, IP-10, and NGAL were the best-performing
 10 protein biomarkers across the three settings and criteria. Protein biomarkers showed better
 11 performances in Malawi and Gabon, as in Brazil most protein biomarkers showed
 12 performances of <0·6. When the biomarker results were stratified by age, the AUROCs were
 13 slightly higher for children (≤ 15 years) compared with those seen for adults in the malaria-
 14 negative population (Supplementary Tables 10–15). Among the malaria-positive population,
 15 WBC, lymphocyte, and neutrophil counts were the best-performing biomarkers in both Gabon
 16 and Malawi (in most cases between 0·6 and 0·7).

32 390 **Table 3: Univariate analysis of 18 individual biomarkers# among malaria-negative patients for all three
 33 countries (a-c).**

34 391 Common biomarkers such as CRP and haematological biomarkers were included for reference. In this context we
 35 392 defined performance as follows: dark blue (AUROC $\geq 0·7$), light blue (AUROC $> 0·65$ and $< 0·7$), orange (AUROC
 36 393 $0·6$ – $0·65$), and red (AUROC $< 0·6$).
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38 395 **a) Brazil**

	Brazil AUROC** (CI), N		
	Electronic	Strict	Loose
Haematological biomarkers			
Lymphocyte count	0·67 (0·59–0·74), 257	0·66 (0·59–0·72), 408	0·66 (0·6–0·72), 442
Neutrophil count	0·77 (0·7–0·84), 257	0·8 (0·74–0·86), 408	0·79 (0·73–0·84), 442
RBC count	0·61 (0·52–0·69), 258	0·58 (0·51–0·65), 408	0·58 (0·51–0·64), 442
WBC count	0·81 (0·75–0·87), 257	0·83 (0·77–0·88), 408	0·82 (0·77–0·87), 442
Protein biomarkers			
AGP	0·59 (0·51–0·68), 252	0·54 (0·47–0·61), 402	0·52 (0·46–0·59), 434
Chitinase 3-like 1	0·58 (0·5–0·66), 246	0·54 (0·47–0·6), 394	0·55 (0·49–0·61), 424
CRP*	0·61 (0·52–0·69), 259	0·61 (0·54–0·68), 412	0·62 (0·55–0·68), 446
IP-10/IP-10/CRG-2	0·6 (0·52–0·68), 252	0·53 (0·46–0·59), 402	0·53 (0·47–0·59), 434
Galectin-9	0·63 (0·55–0·71), 252	0·56 (0·49–0·63), 401	0·57 (0·5–0·63), 433
hCC2	0·51 (0·43–0·6), 244	0·51 (0·44–0·58), 392	0·52 (0·46–0·59), 424
HBP***	0·67 (0·52–0·81), 113	0·68 (0·55–0·8), 144	0·64 (0·51–0·76), 151
HPTGN	0·48 (0·4–0·57), 248	0·51 (0·44–0·58), 398	0·51 (0·45–0·58), 430
IL-4	0·58 (0·5–0·65), 249	0·53 (0·47–0·59), 398	0·54 (0·48–0·59), 429

IL-6	0·49 (0·43-0·54), 247	0·49 (0·44-0·54), 395	0·48 (0·43-0·52), 426
LBP	0·58 (0·5-0·66), 248	0·54 (0·48-0·61), 397	0·52 (0·46-0·58), 429
Lipocalin-2/NGAL	0·49 (0·41-0·57), 249	0·51 (0·44-0·57), 396	0·51 (0·44-0·57), 428
sPLA/Lp-PLA2	0·54 (0·46-0·62), 252	0·53 (0·46-0·59), 402	0·52 (0·45-0·58), 434
TRAIL	0·56 (0·49-0·64), 252	0·53 (0·47-0·59), 402	0·53 (0·48-0·59), 434

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397**b) Gabon**

	Gabon AUROC** (CI), N		
	Electronic	Strict	Loose
Haematological biomarkers			
Lymphocyte count	0·58 (0·45-0·71), 81	0·52 (0·4-0·63), 167	0·55 (0·45-0·65), 222
Neutrophil count	0·78 (0·66-0·89), 80	0·72 (0·62-0·83), 165	0·67 (0·57-0·77), 219
RBC count	0·55 (0·41-0·68), 81	0·52 (0·41-0·63), 167	0·53 (0·43-0·63), 222
WBC count	0·67 (0·54-0·79), 81	0·6 (0·48-0·72), 167	0·61 (0·5-0·71), 222
Protein biomarkers			
AGP	0·77 (0·65-0·9), 80	0·7 (0·59-0·82), 163	0·65 (0·55-0·75), 220
Chitinase 3-like 1	0·6 (0·46-0·74), 79	0·6 (0·48-0·72), 162	0·62 (0·52-0·72), 217
CRP*	0·71 (0·59-0·82), 81	0·65 (0·55-0·75), 167	0·63 (0·53-0·72), 224
IP-10/IP-10/CRG-2	0·6 (0·48-0·73), 80	0·51 (0·4-0·62), 164	0·52 (0·43-0·62), 221
Galectin-9	0·7 (0·58-0·83), 80	0·6 (0·48-0·71), 163	0·54 (0·43-0·64), 219
hCC2	0·55 (0·41-0·69), 77	0·52 (0·4-0·64), 159	0·51 (0·41-0·61), 216
HBP***
HPTGN	0·64 (0·5-0·78), 77	0·62 (0·51-0·74), 159	0·55 (0·45-0·66), 214
IL-4	0·46 (0·4-0·52), 79	0·49 (0·45-0·53), 163	0·51 (0·47-0·55), 220
IL-6	0·51 (0·47-0·55), 80	0·51 (0·48-0·55), 164	0·51 (0·47-0·55), 221
LBP	0·69 (0·56-0·83), 78	0·67 (0·55-0·78), 160	0·6 (0·5-0·71), 217
Lipocalin-2/NGAL	0·67 (0·54-0·8), 79	0·6 (0·49-0·72), 163	0·58 (0·48-0·68), 219
sPLA/Lp-PLA2	0·58 (0·44-0·71), 80	0·54 (0·43-0·65), 164	0·58 (0·48-0·68), 221
TRAIL	0·5 (0·5-0·5), 74	0·5 (0·49-0·5), 156	0·49 (0·48-0·5), 212

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399**c) Malawi**

	Malawi AUROC** (CI), N		
	Electronic	Strict	Loose
Haematological biomarkers			
Lymphocyte count	0·56 (0·47-0·66), 154	0·51 (0·45-0·58), 303	0·52 (0·47-0·58), 461
Neutrophil count	0·67 (0·58-0·77), 143	0·73 (0·67-0·79), 273	0·7 (0·65-0·76), 414
RBC count	0·46 (0·36-0·56), 155	0·53 (0·46-0·59), 305	0·56 (0·5-0·61), 463
WBC count	0·69 (0·6-0·78), 155	0·72 (0·66-0·78), 304	0·68 (0·63-0·73), 461
Protein biomarkers			
AGP	0·56 (0·46-0·66), 158	0·54 (0·48-0·6), 309	0·54 (0·49-0·59), 466
Chitinase 3-like 1	0·49 (0·39-0·59), 155	0·5 (0·43-0·56), 304	0·5 (0·44-0·55), 462
CRP*	0·55 (0·45-0·65), 156	0·6 (0·54-0·67), 305	0·58 (0·53-0·63), 462
IP-10/IP-10/CRG-2	0·66 (0·56-0·75), 158	0·6 (0·53-0·66), 309	0·61 (0·56-0·66), 466
Galectin-9	0·71 (0·62-0·8), 158	0·61 (0·55-0·67), 309	0·63 (0·57-0·68), 466
hCC2	0·59 (0·49-0·69), 158	0·55 (0·49-0·62), 309	0·55 (0·5-0·6), 466

HBP***	0·53 (0·39-0·68), 63	0·55 (0·44-0·66), 106	0·52 (0·41-0·63), 124
HPTGN	0·54 (0·45-0·64), 157	0·51 (0·45-0·58), 307	0·51 (0·46-0·57), 464
IL-4	0·48 (0·4-0·57), 157	0·48 (0·42-0·53), 306	0·47 (0·42-0·51), 463
IL-6	0·56 (0·47-0·65), 158	0·61 (0·55-0·67), 307	0·59 (0·54-0·64), 465
LBP	0·52 (0·42-0·61), 157	0·54 (0·47-0·61), 267	0·53 (0·47-0·59), 394
Lipocalin-2/NGAL	0·56 (0·46-0·66), 156	0·65 (0·59-0·72), 265	0·61 (0·56-0·67), 392
sPLA/Lp-PLA2	0·58 (0·47-0·68), 158	0·55 (0·49-0·61), 308	0·56 (0·51-0·61), 466
TRAIL	0·61 (0·51-0·71), 157	0·62 (0·56-0·68), 306	0·62 (0·57-0·67), 463

*CRP was measured with a NycoCard device. **AUROC has a value between 0 and 1, where 1 corresponds to an effect classifier, 0·5 to one that assigns classes randomly. #Freeze-thaw experiments to evaluate the stability of the biomarkers after five cycles (referred to as “treated”) were performed with Luminex 9- and 2-plexes. Three samples each were freeze-thawed up to six times and compared with samples after the first thawing (referred to as “untreated”; biomarkers were considered stable with 80–120% recovery). Samples were analysed in triplicate and showed good stability up to five freeze-thaw cycles for all analytes showing acceptable results, except for the C2 and C4b biomarkers (C2: 2/3 [66·7%] samples were stable; C4b: two samples failed the sixth freeze-thaw cycle). As a result, these biomarkers were excluded as they would never be suitable as the basis of a diagnostic test. ***HBP was evaluated in a small group of patients in Malawi and Brazil; however, HBP did not show promise and was not evaluated further.

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3 **409 Combinations of host-biomarkers and additional covariates – multivariable analysis**

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5 410 The best-performing biomarkers in the univariate analysis were compared with the best
6 performances from the multivariable analyses, which several feature-selected biomarkers and
7 covariates (Table 4 and Supplementary Tables 16-21). In most cases the best combination of
8 biomarkers showed higher AUROCs than the top-performing individual biomarkers, with a
9 low/moderate “gain” (range 1–13%). The best-performing AUROCs were very similar,
10 irrespective of the multivariable model used, especially for the “strict” and “loose” groups
11 (difference in AUROC range 0·02–0·03 for Malawi and Brazil). Biomarkers identified as top
12 performing by the multivariable analyses differed depending on the model used. While SW
13 and RFA selected three to five biomarkers or combinations, RuleFit selected more biomarkers
14 (ten variables on average) to be part of the signature. The relatively low increase in AUROC
15 when comparing the top-performing single biomarker with multivariable models indicates that
16 biomarkers in addition to the single best-performing biomarker do not make a major
17 contribution.

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3 **Table 4: Multivariable analysis of biomarkers among malaria-negative patients, including the gain/loss of performance**
4 **when comparing multivariable analysis and single host-biomarkers comprising both haematological and protein host-**
5 **biomarkers.**

Classification group	Best multivariable model/models: mean (min-max) AUROC	Best host-biomarker: mean (min-max) AUROC	Multivariable AUROC gain/loss (%) *** multivariable and single host-biomarkers ratio
Overall (Brazil + Gabon + Malawi)*			
L	SW/RFA/RF:0·75 (0.69-0.81)	WBC count: 0·7 (0.64, 0.76)	+7%
S	SW:0.83 (0.75 - 0.91)	WBC count: 0·78 (0.72 - 0.84)	+6%
E	SW/RFA:0.83 (0.77 - 0.89)	WBC count: 0·77 (0.69 - 0.85)	+8%
Brazil			
L	SW: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
S	RFA: 0·82 (0.70 - 0.94)	WBC count: 0·8 (0.68 - 0.92)	+2·5%
E	SW: 0·85 (0.73 - 0.97)	WBC count: 0·83 (0.69 - 0.97)	+2%
Gabon**			
L	SW/RFA: 0·7 (0.46 - 0.94)	WBC count: 0·7 (0.64 - 0.76)	..
S	SW/RFA: 0·76 (0.52 – 0.96)	WBC count: 0·78 (0.72 - 0.84)	-3%
E	RFA: 0·77 (0.63 - 0.91)	WBC count: 0·77 (0.69 - 0.85)	..
Malawi			
L	SW/RFA: 0·74 (0.62 - 0.86)	neutrophil count: 0·72 (0.66 - 0.78)	+3%
S	SW: 0·73 (0.61 - 0.85)	neutrophil count: 0·72 (0.58 - 0.86)	+ 1%
E	RFA: 0·72 (0.60 - 0.84)	WBC count: 0·7 (0.56, 0.84)	+ 2%

426 E, electronic classification group; S, strict classification group; L, loose classification group; RF, RuleFit; RFA, logistic
427 recursive feature addition; SW, stepwise logistic regression.
428

429 * In the “Overall” scenario, the model was developed using the data of all countries and the variable indicating the country
430 was used as a covariate in the model.

431 **Multivariable performances for Gabon were computed using as a predictor model the model trained in the “Overall” scenario
432 (all participants from the three analysed countries) then evaluated using Gabon data only. Indeed, the sample size of Gabon
433 data was not sufficient to allow the development of a reliable model specific for this country.

434 *** Performance comparison was computed as: [(multivariable AUROC – univariate AUROC) / univariate AUROC] * 100
435 Green (gain, i.e. the multivariable models show better performances than univariate models); red (loss, i.e. the univariate
436 models show better performances than multivariable models).

437 DISCUSSION

438 We present the most extensive and diverse host-biomarker evaluation study to differentiate
439 bacterial from non-bacterial infections in LMICs. The study aimed to identify if next-
440 generation host-biomarkers for distinguishing bacterial from non-bacterial cases of AFI, which
441 could replace existing biomarkers such as CRP, PCT, and WBC/neutrophil assessments. The
442 data show that none of the promising host-biomarkers exhibited high AUROCs in our non-
443 severe AFI population in either low malaria prevalence (Brazil) or high malaria prevalence
444 (Gabon, Malawi) settings. Haematology biomarkers and CRP were included a baseline to
445 identify better-performing markers; however, they remain those with the highest AUROC
446 values (approximately 0·60–0·70 AUROC) in our population.

447 Overall, the performance of all markers was underwhelming, yet not surprising. It aligns with
448 previous data where a marked reduction in performance was observed when shifting the
449 population from in- to outpatients [17-19]. Previously, it was hypothesised that the decrease in
450 performance in host biomarkers between HIC and LMIC settings, or even between Africa and
451 Asia, was due to the untreated comorbidities (e.g. diabetes, malaria, neglected tropical diseases)
452 which contribute to inflammation and the nonspecific triggering of host biomarkers, unrelated
453 to the current acute presentation [19, 20]. In our data the performance was indeed poorer in
454 malaria-positive patients (AUROC <0·6); however, even in the malaria-negative population,
455 biomarkers showed low performances (~0·6–0·7) in our cohort. Similarly, sex and arboviral
456 status appeared to have no major effect on biomarker performance. Our data notably indicated
457 that combining biomarkers can enhance performance. However, this improvement was not
458 consistently observed. When combining several biomarkers and additional covariates, the
459 “gain” in AUROC values was low/moderate (range 1–13%) compared to the top-performing
460 individual biomarkers. From a diagnostic development perspective, a low gain in performance
461 would not justify the additional complexity and cost of developing a simple multiplex test.

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3 462 Adding to the challenges of host-biomarker studies is the lack of consistent reference standards
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5 463 and that most studies have focused their analyses solely on the subpopulation of patients with
6
7 464 a microbiologically confirmed diagnosis. This approach ignores the largest group (>70%) of
8 patients and intended-use population of any future test [21]. The group with laboratory
9 confirmed diagnosis will decrease further in the non-severe AFI population; presenting at
10 primary care level. Going forward more clarity will likely follow as a recent host-biomarker
11 test (BVtest, MeMed, Israel) was approved by the FDA and subsequent guidance will prescribe
12 more clearly how studies have to be designed to standardize the classification of “bacterial”
13 vs “non-bacterial” evaluated to guide prescribing for bacterial or non-bacterial infections [9,
14 22]. Our protocol is aligned with the FDA approved classification hence we are confident our
15 methodology is robust.

16
17 473 While our study aimed to mitigate the challenges described, it still had several limitations. The
18 study did not include a control group, so no baseline information was available for biomarker
19 performance or asymptomatic carrier populations. The enrolment period in Brazil and Gabon
20 lasted for less than one year and given the heterogeneity of causes of AFI across time a the
21 performance of the biomarkers may not be generalisable to different times of the year and
22 geographical settings, particularly in Asia. The study utilised a two-step process to classify
23 outcomes, and the clinical classification based on recorded clinical information may have
24 introduced subjectivity. Notably, clinicians had access to the haematology biomarker results
25 (WBCs, neutrophils) during outcome classification, which might have introduced a bias in
26 favour of these biomarkers. However, comparing AUROCs between all classification groups
27 (E, L, S) suggests this potential bias had no major impact as the results are similar across
28 groups. There were some heterogeneities in the inclusion criteria across the various study sites,
29 including age groups and fever criteria. In Brazil and Gabon, the inclusion criterion was a
30 history of fever in the past 7 days, while it was fever at presentation in Malawi. Studies have
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3 487 found that acute fever at presentation has implications for the interpretation of host biomarkers
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5 488 [23]; however, our sub-analysis by acute fever showed no differences, so we do not consider
6
7 489 that these different inclusion criteria impacted interpretation. Despite best efforts to standardise
8
9 procedures, there was a level of adaptability required in the choice of testing methods by the
10
11 clinical teams in each country, for arbovirus and respiratory pathogen detection. Further, the
12
13 491 choice to follow the TPP and focus on non-severe patients in the recruitment was based on the
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15 need's definition by the WHO and others, while this still holds as a major priority, in hindsight
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17 493 this focus did not allow us to stratify by severity (eg. SOFA score).
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22 495 Overall, the results of this diverse study highlight the difficulties in identifying single host-
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24 biomarkers or simple host-biomarker combinations that can help solve the problem of
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26 undifferentiated prescribing at primary healthcare, particularly to be used across diverse global
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28 settings. On the 8th birthday of the original TPP for a diagnostic assay to distinguish bacterial
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30 and non-bacterial infections in resource-limited settings, a more recent consultation confirmed
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32 500 that the need for such an assay remains and is in fact increasingly urgent [6, 24]. Yet again, the
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34 consultation concluded primary healthcare clinics and their equivalents must have the ability
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36 501 to perform tests other than just malaria RDTs [24]. The lack of diagnostics infrastructure at the
37
38 502 lower levels of health systems is well documented and requires urgent improvement to support
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40 503 medical staff in their decision making.. While no novel host-biomarker assay meets these
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42 needs, evidence for existing biomarkers, e.g. CRP, and various haematology biomarkers,
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44 505 should be utilised to drive such improvements, albeit utilizing slightly different approaches and
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46 506 cut-offs across settings. In addition to utilising existing tools, increased investment into lower
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48 507 level health infrastructures are critical and the first step to improved care. Recent studies have
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50 509 shown that even simple host-biomarkers, such as CRP, can have a major impact on how clinical
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52 510 staff use antibiotics [25, 26, 27]. The current study confirms that the existing biomarkers are
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54 511 imperfect and hence should only be used as guidance, in conjunction with expanded clinical
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3 512 algorithms [28, 29]. Such guidelines, alongside adopted policies, strengthened infrastructures
4 and accessible haematology/biochemistry data could enable healthcare workers to use simple
5 tools to gain additional data points to help form a more evidence-based diagnosis that has to be
6 guided by the local epidemiology. Optimising existing haematology or biochemistry tools and
7 their maintenance requirements to meet the needs of low resourced settings could be one step
8 towards more expanded use of these well-known markers. In conclusion, our study reinforces
9 the continued need for innovation in the host-biomarker space and highlights the importance
10 of targeted evaluations of such innovations, in diverse intended-use settings, to fully understand
11 their true value.

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529 530 **Competing Interests**

531 SD, BLFC, CE, VH, SO, CH, AM, SL are or were employed by FIND, the global alliance for
532 diagnostic during the study period. All other authors do not declare any competing interests.

533 534 **Author contribution**

535 SD, CE, SO, AM, AMS, SG, STA, MML, ATA conceptualised the study and study design;
536 CE, AS, SG, STA, AMS, JKM, VH, JM, ALK, AA, JCBO, MML, PNE, JAM, PB, LB, AdRM,
537 BCC, MAMS, AMBdF, EAdS, RdS, MCSL, JH, AG, MJ, NSM, CH, SJL, implemented the
538 study and data collection; MA, MV, SL, SO, BDC, BLFC, SD, SP, SG, AMS, STA conducted
539 data analysis and interpretation. BLFC, SD wrote the first draft of the manuscript and all
540 authors contributed to the final version of the manuscript. Guarantor is SD.

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3 546 data collection, analysis and interpretation of data. Further they had no role in writing of the
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5 547 report or decision to submit for publication.
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3 **638 Figure 1: Classification criteria to assign bacterial versus non-bacterial infection categories for the analysis.**
4 **639** The flows in different colours (turquoise=bacteria, purple=non-bacterial, red=undetermined) represent the
5 **640** proportion of patients that were assigned into the respective group (bacteria/non-bacteria/undetermined) after each
6 **641** classification step. Group 1 representing only patients assigned using laboratory data; group 2 representing
7 **642** patients with a unanimous decision after review by the clinical panel; group 3 after clinical panel review and group
8 **643** 3 including all patients, even if only 2 panel members agreed on the probable cause. The study follows the
9 **644** STARD-15 checklist and reporting guidelines.
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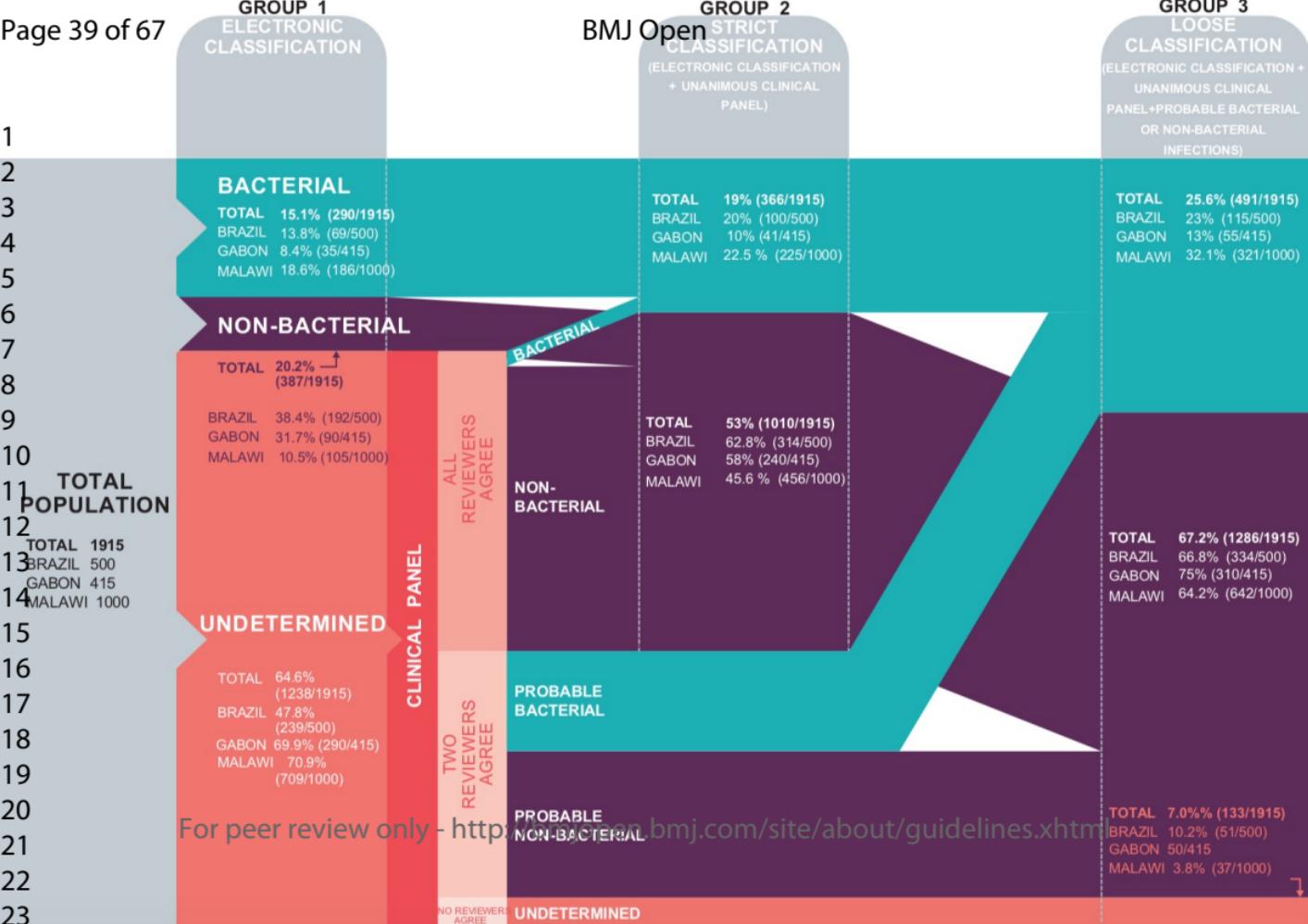
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Supplementary Material

Evaluation of host biomarkers to support the development of a point-of-care diagnostic test to guide antibiotic use in bacterial/non-bacterial acute febrile illness cases

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Biomarker selection

Biomarkers evaluated were selected based on reported performances for distinguishing bacterial versus non-bacterial infections in prior publications, which were systematically reviewed in 2016 by Kapasi et al.¹ and other key publications (Supplementary Table 1). Biomarker performances reported in the 2016 systematic review were compared with reported performances in a later systematic review conducted in 2020.²

Supplementary Table 1. Biomarkers included based on Kapasi et al.'s (2016) systematic review and other key publications.

Biomarker	Performance, 2016 systematic review
C-reactive protein (CRP)	1
FebriDx (MxA+CRP)	2
Galectin-9	2
Gamma-induced protein 10 (IP-10)	2*
Haptoglobin	2 [#]
Heparin-binding protein (HBP)	3
Human neutrophil lipocalin (HNL)	2
Interferon gamma (IFN-gamma)	3
Interleukin-4 (IL-4)	2
Interleukin-6 (IL-6)	3
Lipopolysaccharide binding protein (LBP)	3 ^{\$}
Procalcitonin (PCT)	1
Secretory phospholipase 2 (sPLA2)	2
Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1)	3 ^{\$}
TNF-related apoptosis-inducing ligand (TRAIL)	2*
<i>Included based on key publications in the field</i>	
Biomarker	Publication
A-1-acid glycoprotein	Struck et al. ³
Chitinase-3-like protein 1 (CHI3L1)	Erdman et al. ⁴
Complement 2	Struck et al. ³
Complement C4b	Struck et al. ³
Neutrophil gelatinase-associated lipocalin (NGAL)	Huang et al. ⁵

Performances were scored as: 1, high-performing biomarker (meets the current TPP minimum diagnostic performance criteria, i.e. ≥ 0.90 and 0.80 sensitivity/specificity); 2, moderately performing biomarker (≥ 0.65 and 0.65 and < 0.90 and 0.80 sensitivity/specificity); 3, AUROC > 0.8 ; 4, low-performing biomarker; 5, not evaluated. *As part of the signature CRP+IP-10+TRAIL; # as part of the signature Haptoglobin+IL-10+TIMP1; \$ in respiratory tract infections as part of the signature CRP+LBP; § as part of the signature sTREM+CRP; 1 only in the context of meningitis, otherwise low performance.

Reference laboratory methodology

Materials, equipment, and software

All assay reagents used were delivered with the commercial kits and were used as described in the corresponding kit manuals. Supplementary Table 2 shows the commercial human multi-analyte kits and ELISA kits used.

Supplementary Table 2: Commercial human multi-analyte kits and ELISA kits used.

Analytes	Assay type	Provider	Reference laboratory that performed the analysis
CHI3L1, Gal-9, IL-4, IL-6, IP-10, IFN-gamma, sPLA2, sTREM-1, TRAIL	Luminex, 9-plex	Biotechne/ Systems R&D	NMI
NGAL, LBP	Luminex, 2-plex	Biotechne/ Systems R&D	NMI
C2, C4b	Luminex, 2-plex	Merck	NMI
HP, AGP	Luminex, 2-plex	Merck	NMI
PCT	Luminex, 1-plex	Biotechne/ Systems R&D	NMI
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HNL	ELISA	Diagnostics Development	NMI

CRP	ELISA	Biotechne/ Systems	R&D
	Immunoassay	Elecsys BRAHMS, Roche	MVZ Limbach
HBP	ELISA	Axis-Shield	on-site

NMI, The Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany; MVZ Labor, Dr. Limbach & Kollegen, Heidelberg, Germany

For data generation, the Luminex FLEXMAP 3D instrument, operated with xPONENT Software V4.2, was used for the bead-based Luminex assays. The data evaluation was performed using Bio-Rad Bio-Plex Manager Software 6.1.1. To generate the data for the ELISAs at NMI a BioTek ELx 808 absorption reader was used. The embedded software Gen5 (BioTek) was used for data evaluation. At MVZ Limbach, a Cobas 8000 immunoanalyzer (Roche Diagnostics) was used for data generation.

Methods

All assays were processed according to the manufacturer's protocol. Standard curves, quality control (QC) samples, and blanks were analysed in duplicate; samples were assayed singly. Two or three QC samples were measured on each assay plate. QC samples were taken to cover the range of the standard curve (low, mid, and high level). All QC samples were prepared and aliquoted in larger quantities at the beginning of sample screening so that a fresh aliquot could be used for each measurement, and all QC samples underwent the same freeze–thaw cycle. The performance of the standard curves was controlled over the entire measurement period based on %CVs of the standard point duplicates (<20% and <25% for the last standard point) and percentage recovery on the basis of the nominal concentrations. If permitted by the dilution factor, samples out of the dynamic range were re-analysed with a lower or higher dilution factor.

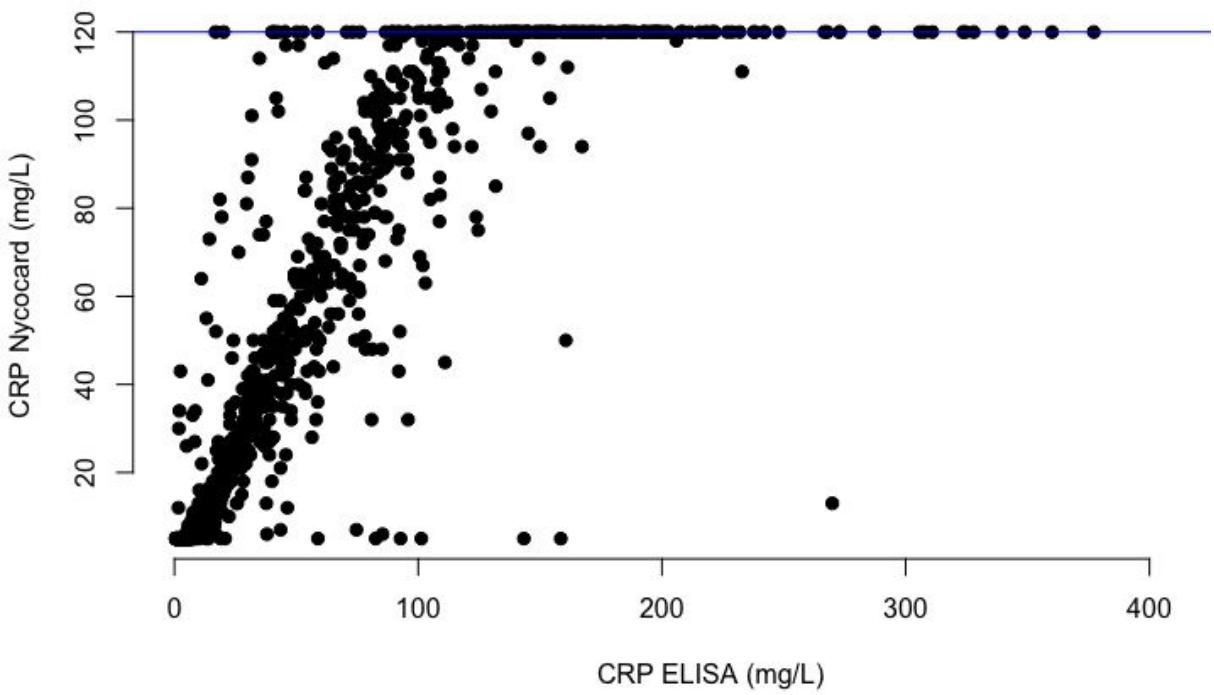
Heparin-binding protein (HBP) assay

The commercially available Axis-Shield heparin-binding protein ELISA for citrated plasma was validated for human EDTA plasma. Calibration curve, limit of detection (LOD), assay range, precision, parallelism, and spike-in recovery experiments were performed.

The ELISA was processed according to the assay protocol provided with the kit. Validation was performed using a fit-for-purpose approach and under consideration of the recommendations for assay validation given in guidelines from health authorities (European Medicine Agency (2011); Food and Drug Administration (2018)). This was a short validation with a limited number of samples.

Except for the percentage recovery, all analysed parameters met the criteria during the validation of the HBP ELISA using human EDTA plasma instead of the recommended citrated plasma matrix. The assay performance seemed to be stable for the sample evaluation using the kit.

Supplementary Figure 1: Analytical assessment of CRP Nycocard vs CRP ELISA



Statistical analysis

This section contains additional figures and tables related to the statistical analysis.

Supplementary Table 3: Number and percentage of missing values for the biomarkers included in the statistical analysis

	Electronic group [¶] [n (%)]	Strict group [§] [n (%)]	Loose group [#] [n (%)]
White blood cells	6 (0.8%)	11 (0.8%)	15 (0.8%)
HAEMATO COUNT	6 (0.8%)	11 (0.8%)	15 (0.8%)
Lymphocytes	6 (0.8%)	12 (0.9%)	17 (1%)
Neutrophils	22 (3%)	64 (5%)	90 (5%)
CRP NYCOCARD	5 (0.7%)	10 (0.7%)	14 (0.8%)
IL-6	10 (1.5%)	20 (1%)	24 (1%)
Gal-9	10 (1.5%)	20 (1%)	24 (1%)
CHI3L1	10 (1.5%)	20 (1%)	25 (1%)
IP-10	10 (1.5%)	20 (1%)	24 (1%)
TRAIL	10 (1.5%)	20 (1%)	24 (1%)
IL-4	13 (2%)	24 (2%)	29 (2%)
sPLA2	10 (1.5%)	20 (1%)	24 (1%)
NGAL	29 (4%)	138 (10%)	197 (11%)
LBP	30 (4%)	139 (10%)	198 (11%)
C2	10 (1.5%)	21 (1.5%)	25 (1%)
AGP	10 (1.5%)	21 (1.5%)	25 (1%)
HP	11 (1.6%)	24 (2%)	29 (2%)

¶ Total number of subjects in the Electronic group: 677

§ Total number of subjects in the Strict group: 1376

Total number of subjects in the Loose group: 1777

Kruskal-Wallis tables

Supplementary Table 4: Kruskal-Wallis table results for the electronic classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	1.214 5E-13	1.980 8E-01	1.098 5E-02	3.440 8E-01	8.4018E-01	2.7154E-01	4.3535E-01	3.4408E-01	5.4183E-09
HAEMA TO COUNT	2.804 0E-45	1.044 6E-09	4.346 1E-28	1.318 5E-36	6.8045E-02	9.1321E-01	6.9000E-01	9.9455E-01	3.6951E-08
Lymphocytes	1.385 0E-45	8.068 0E-03	3.156 2E-29	4.541 4E-32	1.0022E-05	4.4874E-01	4.5900E-01	5.4198E-08	1.9910E-11
Neutrophils	5.649 5E-03	3.914 7E-01	1.133 7E-04	1.867 4E-17	1.5980E-02	4.2719E-01	4.3608E-01	3.0003E-08	6.5439E-04
CRP NYCOCARD	1.448 5E-03	4.229 7E-01	1.386 1E-15	3.033 2E-07	2.1171E-01	4.6667E-01	8.4615E-01	3.0231E-03	2.1171E-01
IL-6	9.262 6E-06	2.527 7E-01	4.668 6E-34	4.281 0E-21	6.1106E-03	7.1615E-01	5.8674E-02	2.0177E-10	9.2626E-06
Gal-9	7.808 4E-11	3.329 6E-01	1.273 1E-07	2.247 1E-07	4.3173E-01	5.3845E-01	9.9020E-02	3.6659E-01	8.5282E-04
CHI3L1	3.687 4E-01	1.542 7E-01	2.259 3E-04	3.594 2E-05	9.0961E-01	8.0977E-01	7.9973E-01	2.5264E-02	2.5264E-02
IP-10	7.023 5E-01	7.023 5E-01	4.042 9E-09	7.048 6E-10	4.9729E-01	7.0235E-01	4.0169E-01	3.6086E-08	3.3476E-01
TRAIL	1.410 8E-03	1.542 9E-02	6.771 0E-19	6.947 3E-56	9.2177E-01	2.2485E-02	9.5591E-01	9.7926E-04	1.8702E-06
IL-4	1.419 0E-03	8.956 6E-02	1.789 6E-25	1.117 9E-73	4.2256E-01	8.9341E-03	8.9692E-01	3.0403E-03	2.2958E-09
sPLA2	9.599 3E-05	9.212 7E-01	2.847 7E-20	5.681 0E-03	1.5011E-01	9.2127E-01	6.1633E-01	7.4323E-03	7.4323E-03
NGAL	2.684 1E-02	7.192 4E-01	1.249 8E-05	6.460 4E-21	7.1924E-01	2.6841E-02	5.1387E-01	1.2498E-05	9.6273E-03
LBP	2.265 8E-11	5.148 1E-02	1.852 7E-54	2.154 4E-101	8.2974E-02	5.3837E-03	1.1745E-01	3.5938E-09	6.0583E-19
C2	1.721 9E-02	3.006 3E-01	6.862 8E-13	6.862 8E-13	6.2951E-02	8.5874E-01	5.6324E-01	4.4637E-01	6.2045E-03
AGP	5.188 8E-03	2.027 4E-01	3.674 7E-16	1.344 5E-16	1.5176E-01	9.8963E-01	6.3154E-01	2.3325E-01	3.1922E-05
HP	2.942 0E-07	2.739 0E-01	1.839 3E-25	2.499 7E-25	2.7390E-01	2.7390E-01	4.0178E-01	7.2077E-01	2.9140E-03
C4b	5.615 9E-19	6.701 0E-02	4.504 1E-81	1.949 1E-84	6.7179E-03	6.7179E-03	3.3168E-01	1.8052E-01	8.0363E-18

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 5: Kruskal-Wallis table results for the strict classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^{\circ}\text{C}$	Chikungunya
White blood cells	3.114 9E-20	2.409 1E-01	3.674 9E-09	9.399 7E-03	3.1632E-01	6.3502E-02	6.3502E-02	9.1443E-01	1.7973E-08
HAEMA TO COUNT	6.183 5E-100	1.999 4E-04	5.630 4E-55	3.785 2E-68	1.6199E-04	8.0189E-01	7.1282E-01	2.9137E-01	1.7149E-10
Lymphocytes	8.477 8E-84	1.529 1E-01	2.677 9E-44	2.740 4E-58	6.3047E-07	6.1980E-03	4.5554E-01	7.1024E-22	8.6226E-15
Neutrophils	8.951 3E-04	1.715 2E-01	7.983 8E-14	1.913 4E-37	4.5549E-02	5.2789E-01	4.5549E-02	3.0001E-19	4.1217E-02
CRP NYCOCA RD	1.654 7E-02	5.765 6E-02	2.457 0E-38	6.299 1E-11	7.4370E-01	3.0220E-01	7.4370E-01	9.7289E-15	3.0220E-01
IL-6	2.570 4E-02	1.288 8E-01	2.513 1E-68	3.475 8E-27	1.4641E-01	8.1220E-01	6.6933E-02	4.3924E-26	2.5371E-04
Gal-9	7.442 4E-19	3.545 5E-03	1.343 2E-11	1.375 7E-08	1.1615E-01	3.9116E-01	1.3397E-01	2.2573E-01	2.4249E-03
CHI3L1	2.833 5E-01	1.543 3E-01	3.678 7E-11	7.431 9E-16	2.8335E-01	2.8335E-01	2.8335E-01	8.7744E-06	1.5017E-03
IP-10	2.452 1E-01	6.871 6E-01	8.565 6E-31	1.550 3E-36	2.1157E-01	3.0336E-01	3.2906E-01	4.1236E-22	3.2906E-01
TRAIL	6.435 8E-04	2.420 6E-01	3.746 7E-46	4.580 6E-127	7.7652E-01	8.3869E-04	7.7652E-01	2.8337E-17	1.7642E-08
IL-4	4.210 8E-04	5.985 8E-01	2.594 9E-55	2.708 3E-159	3.3368E-01	8.0705E-05	6.5563E-01	2.2888E-11	2.2888E-11
sPLA2	3.000 5E-14	1.126 4E-01	4.135 5E-60	4.705 5E-09	6.7473E-04	2.2676E-01	3.6531E-01	1.0844E-09	4.7059E-05
NGAL	7.746 2E-02	1.130 0E-01	6.092 7E-16	1.372 0E-35	5.9955E-01	4.9221E-02	4.4419E-01	1.4382E-19	8.8808E-03
LBP	1.350 9E-14	3.412 3E-01	6.066 0E-94	1.936 0E-197	2.1248E-02	3.6673E-05	3.0644E-01	2.3473E-28	7.4289E-21
C2	7.267 4E-07	4.315 7E-01	2.314 5E-26	4.532 4E-25	6.8236E-03	4.3157E-01	4.3157E-01	8.8206E-03	2.1062E-03
AGP	4.851 3E-04	1.737 9E-01	5.058 7E-21	7.149 6E-23	1.5900E-01	7.9521E-01	9.7767E-01	1.1305E-01	1.4880E-05
HP	1.212 7E-13	6.331 1E-01	1.636 6E-46	3.005 3E-46	2.9299E-03	5.6523E-01	5.6523E-01	9.0316E-01	4.8596E-04

C4b	6.319 3E-21	1.923 1E-02	1.666 4E-139	3.199 9E-147	1.9749E-04	2.6638E-04	9.3349 E-01	8.0678E-03	3.0903E-25
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Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Kruskal-Wallis tables

Supplementary Table 6: Kruskal-Wallis table results for the loose classification

	Age	Sex	Malaria	Country	Comorbidities	Malnutrition*	Prior antibiotics	Temperature $\geq 38^\circ\text{C}$	Chikungunya
White blood cells	2.057 4E-28	9.875 9E-01	1.848 4E-08	4.526 0E-03	9.0171E-02	4.8259E-02	1.0890 E-01	7.4007E-01	1.8484E-08
HAEMA TO COUNT	1.308 3E-126	1.861 9E-04	6.283 5E-56	7.796 2E-76	1.1102E-06	7.8862E-01	7.9391 E-01	2.9434E-01	1.2853E-10
Lymphocytes	4.965 1E-101	2.946 1E-01	4.679 6E-45	1.637 2E-67	4.8743E-07	6.6823E-04	2.9461 E-01	2.4236E-29	4.3110E-15
Neutrophils	1.131 0E-04	7.267 7E-01	7.274 2E-15	1.612 7E-46	2.0313E-01	4.6743E-01	2.0038 E-01	1.2920E-24	2.9723E-02
CRP NYCOCARD	1.361 4E-01	4.412 3E-03	1.034 7E-57	2.470 3E-15	4.0226E-01	5.2068E-01	5.9738 E-01	6.7648E-18	1.3614E-01
IL-6	9.525 0E-02	4.873 6E-02	8.630 3E-95	1.968 8E-31	1.5356E-01	8.2374E-01	9.3076 E-02	6.1774E-34	2.1766E-05
Gal-9	2.046 3E-27	1.443 1E-03	1.931 8E-13	6.827 3E-10	2.3586E-01	2.3586E-01	3.6447 E-02	2.3586E-01	3.0166E-03
CHI3L1	2.748 3E-01	5.354 1E-02	3.612 8E-14	3.612 8E-14	2.8535E-01	7.9359E-01	3.0946 E-01	1.4718E-04	7.1655E-04
IP-10	4.138 4E-01	7.867 4E-01	6.519 3E-43	4.220 2E-47	7.9605E-02	3.6101E-01	4.1384 E-01	1.4436E-34	4.1902E-01
TRAIL	2.472 2E-02	1.391 8E-01	6.282 8E-56	2.918 5E-156	8.2684E-01	6.2797E-05	8.2684 E-01	2.4486E-17	1.1148E-09
IL-4	1.144 8E-02	3.191 1E-01	3.084 4E-69	4E-206	1.748 0E-01	4.7672E-08	5.7785 E-01	2.1611E-12	1.2664E-13
sPLA2	8.375 3E-18	2.731 7E-01	1.589 0E-82	1.270 2E-09	1.2356E-04	3.7225E-01	4.1002 E-01	8.1232E-15	4.0213E-05

1	NGAL	1.570 6E-01	2.065 0E-02	3.748 6E-27	2.284 8E-43	3.7129E- 01	1.4239E- 01	3.9957 E-01	1.3734E- -24	5.3057E- 03
2	LBP	1.656 7E-10	4.386 5E-01	2.110 116	2.427 254	8.2765E- 03	5.4993E- 07	6.1624 E-01	1.4861E- -39	1.4254E- 24
3	C2	2.103 5E-04	1.459 3E-01	7.600 5E-28	2.186 5E-27	4.8543E- 02	2.9326E- 01	3.8932 E-01	9.8425E- -03	1.2901E- 03
4	AGP	2.507 6E-03	9.527 3E-02	1.987 0E-26	3.272 6E-28	9.3140E- 02	8.9492E- 01	9.5756 E-01	9.5273E- -02	3.2225E- 06
5	HP	5.764 0E-15	7.268 5E-01	2.837 6E-51	7.966 7E-51	7.2760E- 03	6.9555E- 01	6.9555 E-01	9.7145E- -01	1.7228E- 04
6	C4b	3.907 7E-15	9.303 7E-03	9.356 160	3.444 171	6.9926E- 04	2.2357E- 03	8.6228 E-01	2.2357E- -03	1.0351E- 29

Different colours based on significance: green ($p < 0.05$, slight significance); orange ($p < 0.01$, high significance); red ($p < 0.001$, strong significance). * Malnutrition status calculated based on WHO body mass index criteria.

Supplementary Table 7: Univariate analysis of 18 individual biomarkers[#] among malaria-negative patients with all reference groups (electronic, strict, loose). Common biomarkers such as CRP and haematological biomarkers were included for reference. In this context we defined performance as follows: green (AUROC ≥ 0.7), yellow (AUROC > 0.65 and < 0.7), orange (AUROC 0.6–0.65), and red (AUROC < 0.6).

	Brazil AUROC ^{**} (CI), N			Gabon AUROC ^{**} (CI), N			Malawi AUROC ^{**} (CI), N		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
Haematological biomarkers									
Lymphocyte count	0.67 (0.59-0.74), 257	0.66 (0.59-0.72), 408	0.66 (0.6-0.72), 442	0.58 (0.45-0.71), 81	0.52 (0.4-0.63), 167	0.55 (0.45-0.65), 222	0.60 (0.57-0.66), 254	0.51 (0.45-0.58), 303	0.52 (0.47-0.58), 461
Neutrophil count	0.77 (0.7-0.84), 257	0.8 (0.74-0.86), 408	0.79 (0.73-0.84), 442	0.78 (0.66-0.89), 80	0.72 (0.62-0.83), 165	0.67 (0.57-0.77), 219	0.77 (0.58-0.77), 243	0.73 (0.67-0.79), 273	0.7 (0.65-0.76), 414
RBC count	0.61 (0.52-0.69), 258	0.58 (0.51-0.65), 408	0.58 (0.51-0.64), 442	0.55 (0.41-0.68), 81	0.52 (0.41-0.63), 167	0.53 (0.43-0.63), 222	0.60 (0.36-0.66), 255	0.53 (0.46-0.59), 305	0.56 (0.5-0.61), 463
WBC count	0.81 (0.75-0.87), 257	0.83 (0.77-0.88), 408	0.82 (0.77-0.87), 442	0.67 (0.54-0.79), 81	0.6 (0.48-0.72), 167	0.61 (0.5-0.71), 222	0.66 (0.56-0.66), 255	0.72 (0.66-0.78), 304	0.68 (0.63-0.73), 461
Protein biomarkers									
AGP	0.59 (0.51-0.68), 252	0.54 (0.47-0.61), 402	0.52 (0.46-0.59), 434	0.77 (0.65-0.9), 80	0.7 (0.59-0.82), 163	0.65 (0.55-0.75), 220	0.60 (0.46-0.66), 258	0.54 (0.48-0.6), 309	0.54 (0.49-0.59), 466
Chitinase 3-like 1	0.58 (0.5-0.66), 246	0.54 (0.47-0.6), 394	0.55 (0.49-0.61), 424	0.6 (0.46-0.74), 79	0.6 (0.48-0.72), 162	0.62 (0.52-0.72), 217	0.59 (0.39-0.59), 255	0.5 (0.43-0.56), 304	0.5 (0.44-0.55), 462
CRP*	0.61 (0.52-0.69), 259	0.61 (0.54-0.68), 412	0.62 (0.55-0.68), 446	0.71 (0.59-0.82), 81	0.65 (0.55-0.75), 167	0.63 (0.53-0.72), 224	0.55 (0.45-0.55), 256	0.6 (0.54-0.67), 305	0.58 (0.53-0.63), 462
IP-10/IP-10/CRG-2	0.6 (0.52-0.68), 252	0.53 (0.46-0.59), 402	0.53 (0.47-0.59), 434	0.6 (0.48-0.73), 80	0.51 (0.4-0.62), 164	0.52 (0.43-0.62), 221	0.56 (0.56-0.58), 258	0.6 (0.53-0.66), 309	0.61 (0.56-0.66), 466
Galectin-9	0.63 (0.55-0.71), 252	0.56 (0.49-0.63), 401	0.57 (0.5-0.63), 433	0.7 (0.58-0.83), 80	0.6 (0.48-0.71), 163	0.54 (0.43-0.64), 219	0.61 (0.52-0.68), 258	0.61 (0.55-0.67), 309	0.63 (0.57-0.68), 466
hCC2	0.51 (0.43-0.6), 244	0.51 (0.44-0.58), 392	0.52 (0.46-0.59), 424	0.55 (0.41-0.69), 77	0.52 (0.4-0.64), 159	0.51 (0.41-0.61), 216	0.59 (0.49-0.69), 258	0.55 (0.49-0.62), 309	0.55 (0.5-0.6), 466
HBP***	0.67 (0.52-0.81), 113	0.68 (0.55-0.8), 144	0.64 (0.51-0.76), 151	0.53 (0.39-0.68), 63	0.55 (0.44-0.66), 106	0.52 (0.41-0.63), 124
HPTGN	0.48 (0.4-0.57), 248	0.51 (0.44-0.58), 398	0.51 (0.45-0.58), 430	0.64 (0.5-0.78), 77	0.62 (0.51-0.74), 159	0.55 (0.45-0.66), 214	0.54 (0.45-0.64), 257	0.51 (0.45-0.58), 307	0.51 (0.46-0.57), 464

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IL-4	0·58 (0·5-0·65), 249	0·53 (0·47-0·59), 398	0·54 (0·48-0·59), 429	0·46 (0·4-0·52), 79	0·49 (0·45-0·53), 163	0·51 (0·47-0·55), 220	0·48 (0·44-0·57), 157	0·48 (0·42-0·53), 306	0·47 (0·42-0·51), 463
IL-6	0·49 (0·43-0·54), 247	0·49 (0·44-0·54), 395	0·48 (0·43-0·52), 426	0·51 (0·47-0·55), 80	0·51 (0·48-0·55), 164	0·51 (0·47-0·55), 221	0·66 (0·47-0·55), 158	0·61 (0·55-0·67), 307	0·59 (0·54-0·64), 465
LBP	0·58 (0·5-0·66), 248	0·54 (0·48-0·61), 397	0·52 (0·46-0·58), 429	0·69 (0·56-0·83), 78	0·67 (0·55-0·78), 160	0·6 (0·5-0·71), 217	0·62 (0·42-0·57), 157	0·54 (0·47-0·61), 267	0·53 (0·47-0·59), 394
Lipocalin-2/NGAL	0·49 (0·41-0·57), 249	0·51 (0·44-0·57), 396	0·51 (0·44-0·57), 428	0·67 (0·54-0·8), 79	0·6 (0·49-0·72), 163	0·58 (0·48-0·68), 219	0·66 (0·46-0·56), 156	0·65 (0·59-0·72), 265	0·61 (0·56-0·67), 392
sPLA/Lp-PLA2	0·54 (0·46-0·62), 252	0·53 (0·46-0·59), 402	0·52 (0·45-0·58), 434	0·58 (0·44-0·71), 80	0·54 (0·43-0·65), 164	0·58 (0·48-0·68), 221	0·58 (0·47-0·58), 158	0·55 (0·49-0·61), 308	0·56 (0·51-0·61), 466
TRAIL	0·56 (0·49-0·64), 252	0·53 (0·47-0·59), 402	0·53 (0·48-0·59), 434	0·5 (0·5-0·5), 74	0·5 (0·49-0·5), 156	0·49 (0·48-0·5), 212	0·51 (0·51-0·57), 157	0·62 (0·56-0·68), 306	0·62 (0·57-0·67), 463

Supplementary Table 8: Univariate analysis – Overall (malaria-positive and malaria-negative) population

	Overall - Malaria negatives			Overall - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.74, (0.7-0.79), 493	0.75, (0.71-0.78), 880	0.72, (0.68-0.75), 1127	0.65, (0.57-0.73), 174	0.65, (0.58-0.71), 481	0.64, (0.59-0.7), 630
RBC count	0.58, (0.53-0.63), 494	0.52, (0.48-0.56), 880	0.51, (0.47-0.54), 1127	0.58, (0.5-0.67), 175	0.5, (0.44-0.56), 481	0.51, (0.46-0.57), 630
Lymphocyte count	0.66, (0.61-0.71), 491	0.57, (0.53-0.61), 877	0.55, (0.51-0.58), 1123	0.63, (0.54-0.71), 174	0.57, (0.5-0.63), 480	0.54, (0.49-0.6), 627
Neutrophil count	0.71, (0.66-0.75), 480	0.75, (0.71-0.79), 847	0.73, (0.69-0.76), 1079	0.67, (0.59-0.75), 172	0.65, (0.58-0.71), 461	0.65, (0.59-0.71), 603
IL-4	0.36, (0.31-0.42), 486	0.4, (0.35-0.44), 868	0.61, (0.57-0.64), 1113	0.66, (0.58-0.74), 175	0.59, (0.53-0.65), 478	0.58, (0.53-0.63), 624
TRAIL	0.36, (0.3-0.41), 489	0.63, (0.59-0.67), 871	0.63, (0.59-0.67), 1117	0.68, (0.6-0.76), 175	0.6, (0.54-0.66), 478	0.58, (0.53-0.64), 625
IL-6	0.61, (0.55-0.66), 489	0.49, (0.45-0.53), 873	0.49, (0.45-0.53), 1120	0.42, (0.33-0.5), 175	0.57, (0.5-0.63), 478	0.53, (0.48-0.59), 626
CRP NycoCard	0.52, (0.47-0.57), 496	0.57, (0.53-0.61), 884	0.57, (0.53-0.6), 1132	0.52, (0.43-0.6), 175	0.49, (0.43-0.56), 481	0.5, (0.44-0.55), 630
Gal-9	0.52, (0.47-0.57), 490	0.54, (0.5-0.58), 875	0.56, (0.52-0.59), 1122	0.57, (0.48-0.65), 176	0.54, (0.48-0.6), 480	0.53, (0.48-0.59), 629
CHI3L1	0.56, (0.51-0.62), 489	0.55, (0.51-0.59), 873	0.55, (0.51-0.59), 1119	0.5, (0.41-0.59), 176	0.52, (0.45-0.58), 480	0.5, (0.44-0.55), 627
IP-10	0.53, (0.48-0.58), 489	0.52, (0.48-0.56), 874	0.52, (0.49-0.56), 1120	0.56, (0.47-0.64), 176	0.53, (0.47-0.59), 478	0.51, (0.45-0.56), 627
sPLA2	0.52, (0.47-0.57), 490	0.52, (0.48-0.56), 874	0.52, (0.49-0.56), 1121	0.49, (0.4-0.58), 176	0.54, (0.48-0.61), 479	0.54, (0.49-0.6), 628
NGAL	0.61, (0.56-0.66), 489	0.62, (0.57-0.66), 833	0.6, (0.57-0.64), 1049	0.61, (0.52-0.7), 157	0.56, (0.49-0.62), 403	0.56, (0.51-0.62), 527
LBP	0.74, (0.69-0.78), 488	0.69, (0.65-0.73), 832	0.67, (0.64-0.71), 1048	0.67, (0.58-0.76), 158	0.58, (0.52-0.64), 404	0.57, (0.51-0.62), 529
C2	0.59, (0.54-0.64), 483	0.56, (0.52-0.6), 866	0.56, (0.52-0.59), 1113	0.63, (0.55-0.72), 176	0.59, (0.53-0.66), 480	0.56, (0.5-0.61), 629
AGP	0.67, (0.62-0.72), 490	0.6, (0.56-0.64), 874	0.58, (0.55-0.62), 1120	0.52, (0.43-0.6), 176	0.52, (0.45-0.59), 480	0.53, (0.47-0.59), 629
HBP	0.67, (0.57-0.76), 179	0.64, (0.56-0.72), 254	0.61, (0.53-0.68), 280	0.55, (0.37-0.72), 57	0.52, (0.42-0.63), 141	0.53, (0.43-0.64), 149
HP	0.55, (0.49-0.6), 489	0.5, (0.46-0.54), 871	0.52, (0.48-0.56), 1116	0.58, (0.49-0.66), 175	0.55, (0.48-0.61), 473	0.54, (0.48-0.59), 622

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Supplementary Table 9: Univariate analysis – malaria-positive population

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.67 (0.58-0.76), 132	0.68 (0.61-0.75), 369	0.67 (0.61-0.72), 491	0.67 (0.44-0.91), 42	0.61 (0.38-0.83), 112	0.61 (0.44-0.78), 139
RBC count	0.69 (0.6-0.79), 131	0.55 (0.48-0.61), 367	0.53 (0.47-0.59), 488	0.56 (0.31-0.81), 43	0.51 (0.3-0.71), 113	0.49 (0.33-0.65), 140
Lymphocyte count	0.7 (0.61-0.79), 131	0.59 (0.53-0.66), 368	0.57 (0.51-0.62), 488	0.72 (0.51-0.93), 42	0.66 (0.47-0.85), 112	0.67 (0.52-0.82), 139
Neutrophil count	0.62 (0.52-0.72), 129	0.65 (0.57-0.72), 348	0.66 (0.6-0.72), 463	0.53 (0.31-0.76), 43	0.59 (0.39-0.79), 113	0.59 (0.43-0.75), 140
IL-4	0.46 (0.36-0.56), 132	0.47 (0.4-0.53), 369	0.48 (0.42-0.53), 488	0.44 (0.38-0.5), 40	0.46 (0.44-0.49), 103	0.5 (0.42-0.57), 127
TRAIL	0.6 (0.51-0.7), 132	0.55 (0.49-0.62), 369	0.54 (0.48-0.59), 488	0.5 (0.5-0.5), 43	0.5 (0.5-0.5), 109	0.53 (0.47-0.6), 136
IL-6	0.6 (0.5-0.7), 131	0.58 (0.51-0.65), 367	0.54 (0.48-0.6), 485	0.45 (0.32-0.57), 42	0.47 (0.37-0.57), 103	0.45 (0.37-0.53), 127
CRP NycoCard	0.48 (0.38-0.58), 131	0.54 (0.47-0.61), 367	0.53 (0.47-0.59), 489	0.59 (0.32-0.86), 44	0.59 (0.36-0.82), 114	0.57 (0.4-0.75), 141
Gal-9	0.58 (0.48-0.69), 132	0.56 (0.49-0.62), 369	0.54 (0.47-0.6), 491	0.57 (0.34-0.8), 43	0.5 (0.32-0.68), 109	0.56 (0.42-0.71), 136
CHI3L1	0.56 (0.46-0.66), 132	0.55 (0.48-0.62), 367	0.55 (0.49-0.61), 487	0.52 (0.26-0.79), 43	0.53 (0.31-0.75), 106	0.63 (0.44-0.81), 131
IP-10	0.67 (0.58-0.76), 132	0.56 (0.49-0.63), 363	0.52 (0.46-0.59), 484	0.51 (0.33-0.69), 40	0.49 (0.35-0.63), 104	0.48 (0.35-0.61), 129
sPLA2	0.53 (0.43-0.64), 133	0.56 (0.48-0.63), 370	0.56 (0.5-0.62), 492	0.49 (0.24-0.74), 43	0.56 (0.34-0.77), 109	0.49 (0.32-0.67), 136
NGAL	0.5 (0.39-0.61), 114	0.5 (0.43-0.58), 291	0.49 (0.42-0.55), 386	0.65 (0.44-0.91), 41	0.59 (0.41-0.77), 106	0.54 (0.38-0.7), 131
LBP	0.47 (0.35-0.59), 115	0.54 (0.46-0.61), 295	0.54 (0.48-0.6), 393	0.6 (0.34-0.85), 42	0.58 (0.37-0.8), 105	0.65 (0.48-0.81), 131
C2	0.62 (0.52-0.72), 133	0.57 (0.5-0.64), 369	0.54 (0.48-0.6), 491	0.72 (0.54-0.9), 43	0.72 (0.57-0.87), 105	0.64 (0.48-0.8), 131
AGP	0.54 (0.44-0.64), 133	0.52 (0.44-0.59), 371	0.48 (0.42-0.54), 493	0.51 (0.27-0.75), 43	0.53 (0.33-0.74), 109	0.58 (0.41-0.76), 136
HBP	0.55 (0.37-0.72), 57	0.53 (0.43-0.64), 143	0.54 (0.44-0.64), 151
HP	0.58 (0.48-0.68), 133	0.54 (0.47-0.61), 365	0.51 (0.45-0.57), 487	0.57 (0.33-0.8), 42	0.56 (0.36-0.76), 107	0.61 (0.46-0.77), 134

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Univariate analysis – age subgroups

Supplementary Table 10: Univariate analysis - age less than 6 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives		
	Electronic	Strict	Loose	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.83, (0.73- 0.94), 61	0.79, (0.71- 0.87), 122	0.76, (0.69- 0.84), 170	0.52, (0.25- 0.78), 21	0.65, (0.46- 0.85), 34	0.69, (0.51- 0.86), 38	0.78, (0.62- 0.94), 32	0.68, (0.52- 0.83), 75	0.65, (0.52- 0.79), 105
RBC count	0.65, (0.49-0.8), 62	0.58, (0.48- 0.68), 123	0.58, (0.5- 0.67), 172	0.6, (0.33- 0.86), 21	0.56, (0.35- 0.77), 33	0.59, (0.39- 0.78), 37	0.6, (0.4- 0.81), 32	0.56, (0.4- 0.72), 75	0.53, (0.38- 0.67), 105
Lymphocyte count	0.58, (0.43- 0.72), 60	0.53, (0.42- 0.64), 121	0.48, (0.38- 0.57), 170	0.63, (0.36- 0.89), 21	0.67, (0.44- 0.91), 34	0.7, (0.5- 0.9), 38	0.71, (0.53- 0.89), 32	0.6, (0.44- 0.76), 75	0.63, (0.49- 0.76), 105
Neutrophil count	0.82, (0.7- 0.93), 57	0.79, (0.7- 0.88), 108	0.77, (0.69- 0.86), 148	0.58, (0.32- 0.85), 21	0.56, (0.36- 0.77), 34	0.6, (0.41- 0.79), 38	0.86, (0.72- 0.99), 32	0.79, (0.67- 0.92), 74	0.7, (0.58- 0.83), 103
IL-4	0.54, (0.39- 0.68), 63	0.5, (0.41- 0.59), 125	0.48, (0.41- 0.56), 174	0.63, (0.38- 0.88), 20	0.66, (0.49- 0.84), 31	0.62, (0.44- 0.8), 33	0.43, (0.31- 0.55), 30	0.49, (0.43- 0.56), 72	0.51, (0.44- 0.57), 103
TRAIL	0.57, (0.39- 0.75), 63	0.6, (0.5- 0.69), 125	0.59, (0.51- 0.67), 174	0.5, (0.23- 0.77), 20	0.63, (0.43- 0.82), 31	0.59, (0.4- 0.79), 33	0.5, (0.5- 0.5), 28	0.5, (0.5- 0.5), 69	0.49, (0.48- 0.51), 99
IL-6	0.59, (0.44- 0.73), 63	0.61, (0.52- 0.7), 125	0.6, (0.52- 0.68), 174	0.41, (0.29- 0.53), 20	0.39, (0.29- 0.49), 29	0.39, (0.3- 0.49), 31	0.5, (0.5- 0.5), 31	0.5, (0.5- 0.5), 73	0.49, (0.47- 0.5), 104
CRP NycoCard	0.56, (0.37- 0.74), 61	0.61, (0.51- 0.71), 121	0.59, (0.5- 0.68), 169	0.49, (0.22- 0.76), 21	0.59, (0.38- 0.79), 34	0.6, (0.42- 0.79), 38	0.76, (0.57- 0.95), 32	0.62, (0.49- 0.76), 75	0.57, (0.45- 0.69), 106
Gal-9	0.79, (0.66- 0.92), 63	0.59, (0.49- 0.69), 125	0.57, (0.48- 0.66), 173	0.47, (0.2- 0.75), 20	0.5, (0.28- 0.72), 31	0.52, (0.3- 0.73), 33	0.66, (0.45- 0.87), 31	0.6, (0.43- 0.76), 72	0.54, (0.4- 0.69), 102
CHI3L1	0.56, (0.4- 0.72), 62	0.52, (0.42- 0.63), 124	0.54, (0.45- 0.63), 173	0.61, (0.35- 0.87), 20	0.66, (0.47- 0.86), 31	0.67, (0.49- 0.86), 33	0.68, (0.49- 0.88), 31	0.62, (0.45- 0.79), 73	0.61, (0.47- 0.75), 102
IP-10	0.67, (0.51- 0.83), 63	0.62, (0.52- 0.72), 125	0.6, (0.51- 0.68), 174	0.65, (0.39-0.9), 20	0.7, (0.51- 0.89), 31	0.64, (0.45- 0.84), 33	0.71, (0.53-0.9), 31	0.52, (0.38- 0.67), 73	0.51, (0.38- 0.63), 104
sPLA2	0.66, (0.5- 0.82), 63	0.55, (0.45- 0.66), 125	0.56, (0.47- 0.65), 174	0.65, (0.38- 0.91), 20	0.69, (0.48- 0.9), 31	0.68, (0.48- 0.88), 33	0.58, (0.37- 0.78), 31	0.57, (0.41- 0.72), 73	0.59, (0.45- 0.73), 104
NGAL	0.61, (0.44- 0.77), 63	0.68, (0.58- 0.59), 125	0.67, (0.41- 0.93), 20	0.67, (0.41- 0.93), 20	0.58, (0.38- 0.93), 20	0.52, (0.31- 0.93), 20	0.63, (0.43- 0.83), 20	0.6, (0.44- 0.83), 20	0.57, (0.43- 0.83), 20

		0.78), 109	0.76), 144		0.79), 31	0.72), 33		0.77), 73	0.71), 103
LBP	0.47, (0.31- 0.63), 63	0.5, (0.39- 0.62), 109	0.53, (0.43- 0.63), 144	0.47, (0.2- 0.75), 20	0.46, (0.25- 0.68), 30	0.48, (0.27- 0.7), 32	0.73, (0.53- 0.93), 30	0.7, (0.53- 0.86), 70	0.59, (0.44- 0.75), 101
C2	0.51, (0.34- 0.69), 63	0.56, (0.45- 0.66), 125	0.52, (0.44- 0.61), 174	0.47, (0.18- 0.76), 19	0.64, (0.41- 0.87), 29	0.62, (0.4- 0.83), 31	0.51, (0.29- 0.73), 30	0.48, (0.32- 0.64), 71	0.5, (0.36- 0.64), 102
AGP	0.54, (0.38-0.7), 63	0.56, (0.45- 0.66), 125	0.57, (0.48- 0.66), 174	0.72, (0.48- 0.96), 20	0.57, (0.34- 0.81), 31	0.61, (0.39- 0.82), 33	0.8, (0.63- 0.98), 31	0.72, (0.56- 0.88), 72	0.62, (0.48- 0.76), 103
HBP	0.67, (0.45 -0.89), 26	0.55, (0 .37- 0.73), 4 5	0.54, (0 .37- 0.71), 4 8
HP	0.64, (0.49- 0.78), 62	0.57, (0.46- 0.67), 124	0.57, (0.48- 0.66), 173	0.68, (0.42- 0.93), 20	0.61, (0.38- 0.84), 31	0.62, (0.41- 0.84), 33	0.78, (0.59- 0.97), 28	0.72, (0.57- 0.88), 69	0.63, (0.49- 0.77), 100

Green (AUROC ≥ 0.7), yellow (AUROC ≥ 0.65 and < 0.7), orange (AUROC 0.6-0.65), red (AUROC < 0.6)

Supplementary Table 11: Univariate analysis - aged between 7 and 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives				
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict
WBC count	0.49, (0.26- 0.73), 28	0.69, (0.54- 0.84), 50	0.75, (0.64- 0.86), 81	0.79, (0.61- 0.96), 34	0.83, (0.71- 0.95), 69	0.82, (0.71- 0.94), 75	0.46, (0.27- 0.65), 47	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112	0.51, (0.34- 0.67), 87	0.47, (0.31- 0.62), 112
RBC count	0.62, (0.41- 0.84), 28	0.54, (0.37- 0.7), 51	0.57, (0.44- 0.7), 82	0.7, (0.51- 0.88), 34	0.61, (0.45- 0.78), 69	0.6, (0.44- 0.75), 75	0.56, (0.38- 0.75), 47	0.55, (0.4- 0.7), 87	0.48, (0.35- 0.62), 112	0.55, (0.4- 0.7), 87	0.48, (0.35- 0.62), 112
Lymphocyte count	0.76, (0.58- 0.94), 28	0.67, (0.51- 0.83), 51	0.62, (0.49- 0.74), 82	0.6, (0.37- 0.83), 34	0.69, (0.54- 0.85), 69	0.71, (0.56- 0.86), 75	0.59, (0.42- 0.76), 47	0.61, (0.48- 0.74), 87	0.55, (0.43- 0.68), 112	0.61, (0.48- 0.74), 87	0.55, (0.43- 0.68), 112
Neutrophil count	0.46, (0.23-0.7), 26	0.7, (0.54- 0.86), 45	0.76, (0.64- 0.87), 73	0.73, (0.53- 0.93), 34	0.82, (0.69- 0.95), 69	0.8, (0.68- 0.93), 75	0.66, (0.46- 0.86), 46	0.61, (0.43- 0.8), 86	0.61, (0.44- 0.78), 111	0.61, (0.44- 0.78), 111	0.51, (0.45- 0.57), 112
IL-4	0.56, (0.34- 0.78), 28	0.46, (0.31- 0.6), 50	0.48, (0.37- 0.6), 80	0.73, (0.53- 0.92), 33	0.62, (0.47- 0.77), 69	0.59, (0.45- 0.74), 75	0.46, (0.41-0.5), 47	0.48, (0.46- 0.5), 86	0.51, (0.45- 0.57), 112	0.49, (0.45- 0.57), 112	0.49, (0.45- 0.57), 112
TRAIL	0.48, (0.23- 0.73), 28	0.6, (0.45- 0.76), 50	0.57, (0.45- 0.7), 80	0.55, (0.34- 0.77), 33	0.53, (0.38- 0.68), 69	0.52, (0.38- 0.66), 75	0.5, (0.5- 0.5), 45	0.49, (0.48- 0.51), 83	0.49, (0.47- 0.5), 109	0.49, (0.47- 0.5), 109	0.49, (0.47- 0.5), 109
IL-6	0.45, (0.21- 0.69), 28	0.56, (0.4- 0.44),	0.55, (0.44- 0.58),	0.46, (0.34- 0.58), 33	0.44, (0.33- 0.53),	0.43, (0.33- 0.53),	0.53, (0.44- 0.62), 47	0.53, (0.44- 0.62), 86	0.54, (0.46- 0.6), 86	0.54, (0.46- 0.6), 86	0.54, (0.46- 0.6), 86

		0.71), 51	0.67), 82		0.56), 69	0.53), 75			0.62), 112
CRP NycoCard	0.56, (0.34- 0.78), 28	0.61, (0.46- 0.77), 51	0.62, (0.5- 0.74), 82	0.57, (0.33- 0.81), 34	0.52, (0.35- 0.68), 71	0.51, (0.35- 0.68), 77	0.75, (0.59- 0.92), 47	0.71, (0.55- 0.87), 87	0.69, (0.56- 0.83), 113
Gal-9	0.67, (0.43-0.9), 28	0.68, (0.53- 0.84), 51	0.66, (0.54- 0.78), 82	0.71, (0.52-0.9), 33	0.57, (0.41- 0.73), 69	0.54, (0.39- 0.7), 75	0.79, (0.62- 0.95), 47	0.61, (0.44- 0.77), 86	0.55, (0.39- 0.71), 112
CHI3L1	0.53, (0.28- 0.78), 28	0.6, (0.44- 0.76), 51	0.61, (0.49- 0.73), 82	0.69, (0.5- 0.87), 32	0.66, (0.52- 0.79), 67	0.59, (0.44- 0.73), 71	0.53, (0.32- 0.73), 46	0.58, (0.41- 0.74), 84	0.62, (0.47- 0.77), 110
IP-10	0.64, (0.42- 0.86), 28	0.56, (0.39- 0.72), 51	0.59, (0.46- 0.72), 82	0.73, (0.53- 0.92), 33	0.62, (0.46- 0.78), 69	0.58, (0.42- 0.73), 75	0.6, (0.41- 0.78), 47	0.48, (0.31- 0.66), 86	0.52, (0.37- 0.67), 112
sPLA2	0.47, (0.21- 0.72), 28	0.55, (0.39- 0.72), 51	0.56, (0.43- 0.68), 82	0.54, (0.33- 0.76), 33	0.49, (0.35- 0.64), 69	0.56, (0.43- 0.7), 75	0.46, (0.28- 0.64), 47	0.52, (0.36- 0.67), 86	0.44, (0.29- 0.59), 112
NGAL	0.56, (0.32-0.8), 28	0.68, (0.52- 0.85), 46	0.73, (0.61- 0.85), 73	0.71, (0.52-0.9), 33	0.68, (0.54- 0.82), 69	0.64, (0.5- 0.78), 75	0.7, (0.52- 0.89), 46	0.6, (0.44- 0.77), 85	0.59, (0.44- 0.74), 111
LBP	0.54, (0.3- 0.77), 28	0.59, (0.42- 0.75), 46	0.58, (0.45- 0.72), 73	0.68, (0.5- 0.87), 33	0.66, (0.52- 0.8), 69	0.67, (0.54- 0.8), 75	0.71, (0.52-0.9), 46	0.66, (0.48- 0.84), 85	0.63, (0.46- 0.79), 111
C2	0.62, (0.34-0.9), 28	0.53, (0.36- 0.7), 51	0.53, (0.41- 0.66), 82	0.54, (0.31- 0.76), 32	0.57, (0.4- 0.74), 67	0.61, (0.45- 0.77), 73	0.62, (0.42- 0.81), 45	0.46, (0.27- 0.65), 83	0.52, (0.36- 0.68), 109
AGP	0.57, (0.3- 0.83), 28	0.55, (0.39- 0.71), 51	0.52, (0.39- 0.65), 81	0.53, (0.3- 0.76), 33	0.6, (0.44- 0.75), 69	0.61, (0.46- 0.75), 75	0.75, (0.56- 0.94), 47	0.68, (0.5- 0.86), 86	0.67, (0.52- 0.83), 112
HBP	0.76, (0.28- -1), 10	0.58, (0. 29- 0.87), 1 9	0.65, (0. 39- 0.91), 2 3	## Unbalance d classes	0.92, (0. .69- 1), 8	0.72, (0. .28- 1), 9
HP	0.5, (0.25- 0.76), 28	0.51, (0.35- 0.67), 51	0.5, (0.37- 0.63), 82	0.52, (0.3- 0.75), 32	0.62, (0.46- 0.78), 68	0.6, (0.45- 0.76), 74	0.53, (0.33- 0.73), 47	0.54, (0.37- 0.7), 85	0.53, (0.38- 0.67), 109

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

Supplementary Table 12: Univariate analysis - aged more than 15 years (non-malaria)

	Malawi - Malaria negatives			Brazil - Malaria negatives			Gabon - Malaria negatives		
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N
WBC count	0.67, (0.53- 0.82), 66	0.71, (0.62- 0.62), 66	0.68, (0.6- 0.6), 202	0.84, (0.77- 0.91), 202	0.84, (0.77- 0.77), 202	0.83, (0.77- 0.77), 202	2 patients in total	5 patients in total	5 patients in total

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		0.8), 132	0.75), 210		0.9), 305	0.89), 329			
1	RBC count	0.59, (0.44- 0.73), 65	0.53, (0.43- 0.63), 131	0.51, (0.43- 0.59), 209	0.56, (0.45- 0.67), 203	0.56, (0.47- 0.64), 306	0.55, (0.47- 0.63), 330	-	-
2	Lymphocyte count	0.5, (0.34- 0.66), 66	0.53, (0.43- 0.63), 131	0.49, (0.41- 0.57), 209	0.67, (0.58- 0.76), 202	0.65, (0.57- 0.72), 305	0.64, (0.57- 0.71), 329	-	-
3	Neutrophil count	0.65, (0.49- 0.81), 60	0.7, (0.6- 0.8), 120	0.66, (0.59- 0.74), 193	0.82, (0.74-0.9), 202	0.82, (0.76- 0.89), 305	0.82, (0.75- 0.88), 329	-	-
4	IL-4	0.4, (0.28- 0.52), 66	0.47, (0.39- 0.54), 131	0.45, (0.39- 0.52), 209	0.56, (0.47- 0.65), 196	0.53, (0.46- 0.6), 298	0.54, (0.47- 0.6), 321	-	-
5	TRAIL	0.68, (0.54- 0.82), 66	0.65, (0.56- 0.73), 131	0.66, (0.59- 0.73), 209	0.57, (0.48- 0.65), 199	0.54, (0.47- 0.61), 302	0.54, (0.48- 0.61), 326	-	-
6	IL-6	0.59, (0.46- 0.72), 67	0.63, (0.54- 0.72), 131	0.59, (0.52- 0.66), 209	0.51, (0.44- 0.58), 194	0.51, (0.45- 0.58), 297	0.5, (0.44- 0.56), 320	-	-
7	CRP NycoCard	0.53, (0.38- 0.68), 67	0.6, (0.5- 0.7), 133	0.57, (0.49- 0.64), 211	0.66, (0.57- 0.76), 204	0.65, (0.57- 0.73), 307	0.66, (0.58- 0.73), 331	-	-
8	Gal-9	0.72, (0.59- 0.86), 67	0.6, (0.5- 0.7), 133	0.63, (0.56- 0.71), 211	0.61, (0.52- 0.71), 199	0.56, (0.48- 0.65), 301	0.57, (0.5- 0.65), 325	-	-
9	CHI3L1	0.52, (0.36- 0.67), 65	0.51, (0.41- 0.61), 129	0.53, (0.45- 0.61), 207	0.66, (0.58- 0.75), 194	0.62, (0.54- 0.69), 296	0.62, (0.55- 0.69), 320	-	-
10	IP-10	0.64, (0.48- 0.79), 67	0.59, (0.49- 0.69), 133	0.61, (0.53- 0.68), 210	0.59, (0.5- 0.68), 199	0.52, (0.44- 0.6), 302	0.53, (0.45- 0.6), 326	-	-
11	sPLA2	0.53, (0.37- 0.69), 67	0.54, (0.44- 0.64), 132	0.54, (0.46- 0.62), 210	0.58, (0.48- 0.67), 199	0.56, (0.48- 0.64), 302	0.56, (0.48- 0.63), 326	-	-
12	NGAL	0.49, (0.33- 0.65), 65	0.62, (0.51- 0.72), 110	0.53, (0.44- 0.62), 175	0.55, (0.46- 0.65), 196	0.54, (0.46- 0.62), 296	0.53, (0.45- 0.61), 320	-	-
13	LBP	0.56, (0.41-0.7), 66	0.56, (0.45- 0.67), 112	0.53, (0.44- 0.61), 177	0.65, (0.56- 0.74), 195	0.6, (0.52- 0.67), 298	0.56, (0.49- 0.64), 322	-	-
14	C2	0.67, (0.53- 0.81), 67	0.59, (0.49- 0.69), 133	0.58, (0.51- 0.66), 210	0.5, (0.4- 0.6), 193	0.51, (0.43- 0.58), 296	0.51, (0.44- 0.59), 320	-	-
15	AGP	0.6, (0.45- 0.75), 67	0.57, (0.47- 0.67), 132	0.54, (0.46- 0.67), 210	0.65, (0.55- 0.74), 199	0.58, (0.5- 0.67), 307	0.56, (0.49- 0.67), 331	-	-

		0.67), 133	0.62), 211		0.66), 302	0.64), 326		
HBP	0.48, (0.25- -0.71), 28	0.54, (0 .36- -0.72), 44	0.47, (0 .31- -0.63), 55	0.66, (0.51- -0.81), 107	0.66, (0 .53- -0.79), 136	0.63, (0 .5- -0.76), 142	-	-
HP	0.53, (0.39- -0.67), 67	0.58, (0.48- -0.68), 132	0.5, (0.42- -0.58), 209	0.56, (0.46- -0.66), 196	0.47, (0.39- -0.55), 299	0.48, (0.4- -0.55), 323	-	-

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 13: Univariate analysis - age less than 6 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives				
	AUROC (CI), N	Electronic	Strict	Loose	AUROC (CI), N	Electronic	Strict	Loose
WBC count	0.64, (0.47- -0.81), 50	0.71, (0.59- -0.82), 148	0.7, (0.6-0.8), 178	0.62, (0.23-1), 11	0.62, (0.36- -0.88), 44	0.62, (0.41- -0.83), 56		
RBC count	0.51, (0.33- -0.68), 49	0.55, (0.44- -0.65), 147	0.55, (0.44- -0.65), 177	0.7, (0.34-1), 11	0.63, (0.42- -0.84), 44	0.62, (0.45- -0.8), 56		
Lymphocyte count	0.45, (0.26- -0.64), 49	0.58, (0.47- -0.7), 147	0.55, (0.44- -0.66), 177	0.57, (0.17- -0.96), 11	0.6, (0.34- -0.86), 44	0.63, (0.42- -0.85), 56		
Neutrophil count	0.59, (0.41- -0.77), 49	0.65, (0.53- -0.76), 140	0.66, (0.56- -0.76), 169	0.7, (0.3-1), 11	0.49, (0.24- -0.75), 44	0.55, (0.35- -0.75), 56		
IL-4	0.68, (0.5- -0.86), 50	0.62, (0.52- -0.71), 148	0.58, (0.49- -0.67), 178	0.5, (0.5-0.5), 11	0.47, (0.42- -0.51), 39	0.48, (0.44- -0.51), 51		
TRAIL	0.73, (0.56- -0.89), 50	0.59, (0.48- -0.69), 148	0.56, (0.47- -0.66), 178	0.5, (0.5-0.5), 11	0.5, (0.5-0.5), 41	0.5, (0.5-0.5), 53		
IL-6	0.6, (0.4-0.79), 49	0.64, (0.53- -0.74), 147	0.63, (0.53- -0.72), 175	0.47, (0.2- -0.73), 11	0.48, (0.33- -0.62), 37	0.48, (0.36- -0.59), 49		
CRP NycoCard	0.52, (0.33- -0.7), 48	0.58, (0.48- -0.69), 145	0.56, (0.46- -0.66), 175	0.78, (0.47-1), 11	0.66, (0.41- -0.91), 44	0.63, (0.42- -0.84), 56		
Gal-9	0.58, (0.37- -0.79), 49	0.54, (0.43- -0.65), 148	0.53, (0.43- -0.64), 178	0.5, (0.05- -0.95), 11	0.63, (0.45- -0.82), 41	0.6, (0.44- -0.76), 53		
CHI3L1	0.53, (0.36- -0.7), 50	0.6, (0.49- -0.71), 148	0.57, (0.47- -0.67), 178	0.47, (0.07- -0.86), 11	0.54, (0.28- -0.79), 40	0.56, (0.33- -0.8), 51		
IP-10	0.73, (0.57- -0.9), 50	0.58, (0.47- -0.69), 143	0.57, (0.47- -0.67), 172	0.77, (0.38-1), 11	0.45, (0.26- -0.64), 39	0.48, (0.32- -0.64), 51		
sPLA2	0.49, (0.3- -0.69), 50	0.63, (0.52- -0.75), 148	0.62, (0.52- -0.72), 178	0.73, (0.38-1), 11	0.52, (0.27- -0.78), 41	0.52, (0.31- -0.73), 53		
NGAL	0.61, (0.43- -0.79), 47	0.56, (0.44- -0.68), 118	0.54, (0.43- -0.65), 141	0.87, (0.6-1), 11	0.62, (0.4- -0.85), 40	0.61, (0.41- -0.8), 52		
LBP	0.55, (0.3- -0.79), 48	0.48, (0.37- -0.59), 122	0.52, (0.41- -0.62), 147	0.45, (0.03- -0.87), 11	0.58, (0.33- -0.83), 41	0.61, (0.4- -0.81), 53		
C2	0.57, (0.38- -0.76), 50	0.57, (0.47- -0.68), 148	0.56, (0.46- -0.67), 178	0.58, (0.2- -0.97), 11	0.78, (0.6- -0.96), 38	0.77, (0.6- -0.93), 50		
AGP	0.68, (0.52- -0.84), 50	0.6, (0.49- -0.71), 149	0.57, (0.47- -0.68), 179	0.63, (0.24-1), 11	0.52, (0.32- -0.73), 41	0.46, (0.27- -0.65), 53		
HBP	0.55, (0.27- -0.84), 33	0.62, (0.49- -0.76), 78	0.63, (0.49- -0.76), 82		
HP	0.72, (0.58- -0.87), 50	0.59, (0.48- -0.7), 147	0.56, (0.46- -0.67), 177	0.57, (0.18- -0.95), 11	0.45, (0.21- -0.69), 40	0.47, (0.26- -0.68), 52		

Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$), red ($AUROC < 0.6$)

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Supplementary Table 14: Univariate analysis - aged between 7 and 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	AUROC (CI), N			AUROC (CI), N		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.67, (0.51-0.82), 51	0.7, (0.6-0.8), 134	0.66, (0.57-0.75), 185	## unbalanced classes (24 non-bacterial, 1 bacterial) for 25 patients	## unbalanced classes (54 non-bacterial, 1 bacterial) for 55 patients	0.47, (0.03-0.91), 72
RBC count	0.74, (0.6-0.87), 51	0.55, (0.43-0.68), 134	0.53, (0.43-0.63), 185	-	-	0.67, (0.28-1), 73
Lymphocyte count	0.64, (0.49-0.79), 51	0.59, (0.47-0.7), 134	0.55, (0.46-0.64), 184	-	-	0.44, (0.14-0.75), 72
Neutrophil count	0.63, (0.47-0.79), 50	0.67, (0.56-0.78), 127	0.67, (0.58-0.76), 174	-	-	0.51, (0.17-0.86), 73
IL-4	0.53, (0.36-0.7), 51	0.54, (0.44-0.64), 134	0.53, (0.45-0.61), 184	-	-	0.62, (0.27-0.96), 65
TRAIL	0.51, (0.35-0.68), 51	0.52, (0.41-0.63), 134	0.54, (0.45-0.63), 184	-	-	0.62, (0.38-0.87), 72
IL-6	0.62, (0.46-0.78), 50	0.57, (0.46-0.68), 132	0.51, (0.41-0.6), 181	-	-	0.41, (0.37-0.46), 67
CRP NycoCard	0.55, (0.39-0.71), 51	0.52, (0.4-0.64), 134	0.51, (0.41-0.61), 185	-	-	0.59, (0.21-0.97), 73
Gal-9	0.6, (0.44-0.76), 51	0.53, (0.42-0.65), 134	0.55, (0.45-0.65), 185	-	-	0.64, (0.23-1), 72
CHI3L1	0.53, (0.36-0.69), 51	0.49, (0.38-0.6), 133	0.54, (0.45-0.64), 183	-	-	0.61, (0.08-1), 69
IP-10	0.63, (0.47-0.79), 50	0.56, (0.45-0.68), 133	0.53, (0.43-0.63), 184	-	-	0.55, (0.11-0.99), 67
NGAL	0.55, (0.38-0.71), 51	0.52, (0.41-0.64), 134	0.53, (0.44-0.63), 185	-	-	0.56, (0.13-0.99), 72
HNL	0.67, (0.48-0.85), 42	0.47, (0.35-0.59), 108	0.57, (0.48-0.67), 150	-	-	0.66, (0.33-1), 69
LBP	0.61, (0.44-0.78), 42	0.59, (0.47-0.71), 108	0.56, (0.46-0.66), 151	-	-	0.9, (0.77-1), 67
C2	0.62, (0.46-0.78), 51	0.57, (0.46-0.68), 133	0.54, (0.45-0.64), 184	-	-	0.73, (0.47-0.98), 70

1	AGP	0.6, (0.44-0.76), 51	0.55, (0.43-0.67), 134	0.52, (0.42-0.62), 185	-	-	0.53, (0.07-0.99), 72
2	HBP	0.64, (0.39-0.9), 21	0.46, (0.28-0.65), 50	0.49, (0.31-0.67), 55	-	-	-
3	HP	0.54, (0.37-0.7), 51	0.49, (0.38-0.59), 132	0.49, (0.40-0.59), 183	-	-	0.79, (0.60-0.98), 71

13 Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

17 Supplementary Table 15: Univariate analysis - aged more than 15 years (malaria)

	Malawi - Malaria positives			Gabon - Malaria positives		
	Electronic	Strict	Loose	Electronic	Strict	Loose
WBC count	0.54, (0.32-0.76), 31	0.56, (0.37-0.75), 87	0.65, (0.51-0.78), 128	2 patients in total	11 patients in total	11 patients in total
RBC count	0.42, (0.2-0.63), 31	0.58, (0.42-0.73), 86	0.57, (0.44-0.7), 126	-	-	-
Lymphocyte count	0.77, (0.61-0.94), 31	0.64, (0.57-0.78), 87	0.66, (0.55-0.77), 127	-	-	-
Neutrophil count	0.5, (0.28-0.73), 30	0.55, (0.35-0.74), 81	0.62, (0.48-0.77), 120	-	-	-
IL-4	0.53, (0.33-0.73), 31	0.5, (0.34-0.66), 87	0.48, (0.37-0.59), 126	-	-	-
TRAIL	0.62, (0.42-0.82), 31	0.6, (0.44-0.76), 87	0.63, (0.51-0.75), 126	-	-	-
IL-6	0.67, (0.47-0.87), 32	0.52, (0.35-0.69), 88	0.54, (0.41-0.66), 129	-	-	-
CRP NycoCard	0.57, (0.36-0.78), 32	0.52, (0.37-0.68), 88	0.52, (0.40-0.64), 129	-	-	-
Gal-9	0.61, (0.4-0.82), 32	0.59, (0.44-0.73), 87	0.52, (0.39-0.65), 128	-	-	-
CHI3L1	0.64, (0.43-0.85), 31	0.53, (0.37-0.69), 86	0.52, (0.40-0.65), 126	-	-	-
IP-10	0.66, (0.45-0.87), 32	0.52, (0.35-0.69), 87	0.58, (0.44-0.71), 128	-	-	-
sPLA2	0.62, (0.42-0.82), 32	0.53, (0.37-0.69), 88	0.56, (0.44-0.69), 129	-	-	-
NGAL	0.7, (0.48-0.92), 25	0.55, (0.35-0.75), 65	0.56, (0.41-0.7), 95	-	-	-
LBP	0.37, (0.14-0.6), 25	0.47, (0.29-0.66), 65	0.59, (0.46-0.73), 95	-	-	-
C2	0.64, (0.43-0.85), 32	0.59, (0.42-0.76), 88	0.47, (0.33-0.6), 129	-	-	-
AGP	0.68, (0.49-0.87), 32	0.47, (0.31-0.63), 88	0.52, (0.39-0.64), 129	-	-	-
HBP	0.8, (0.34-1), 7	0.62, (0.29-0.95), 23	0.62, (0.29-0.95), 24	-	-	-
HP	0.52, (0.31-0.73), 32	0.51, (0.35-0.67), 86	0.53, (0.41-0.64), 127	-	-	-

56 Green ($AUROC \geq 0.7$), yellow ($AUROC \geq 0.65$ and < 0.7), orange ($AUROC 0.6-0.65$) red ($AUROC < 0.6$)

Supplementary Table 16: Multivariate analysis – non-malaria population; haematological biomarkers

Haematological biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
country neutrophil count, WBC count, lymphocyte count, fever duration, temperature, pulse rate, respiratory rate	country neutrophil count, fever duration	country neutrophil count fever duration respiratory rate		L RF/SW/RFA: 0.75 (0.03)	WBC count: 0.7 (0.03)	+7%
				S SW: 0.83 (0.04)	WBC count: 0.78 (0.03)	+6%
				E SW/RFA: 0.83 (0.02)	WBC count: 0.77 (0.03)	+8%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.7 (0.12)	WBC count: 0.7 (0.03)	
			S	SW: 0.77 (0.12)	WBC count: 0.73 (0.03)	+5%
			E	RFA: 0.77 (0.08)	WBC count: 0.75 (0.03)	+3%
Malawi						
diastolic blood pressure, HAEMATO_C lymphocyte count, neutrophil count, pulse rate, temperature, fever duration	fever duration neutrophil count	fever duration neutrophil count	L RFA: 0.74(0.05)	neutrophil count: 0.72(.06)	+3%	
			S SW: 0.73(0.06)	neutrophil count: 0.72(.07)	+1%	
			E RFA: 0.66(0.16)	WBC count: 0.7 (0.05)	-6%	
Brazil						
diastolic blood pressure, haematocrit lymphocyte count, neutrophil count, pulse rate, temperature, fever duration, respiratory rate, WBC count	WBC count respiratory rate neutrophil count	WBC count respiratory rate	L RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%	
			S RFA: 0.82 (0.08)	WBC count: 0.81 (0.08)	+1%	
			E RFA: 0.84 (0.07)	WBC count: 0.83 (0.07)	+1%	

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data due to the limited data.

Supplementary Table 17: Multivariate analysis – non-malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/model s: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
CRP AGP LBP NGAL pulse rate respiratory rate diastolic blood pressure temperature country	CRP country LBP NGAL pulse rate	CRP country NGAL pulse rate respiratory rate temperature	L	RF/RFA/SW: 0.66 (0.05)	LBP: 0.62 (0.04)	+6%
			S	RF: 0.74 (0.04)	LBP: 0.66 (0.05)	+12%
			E	RFA: 0.76 (0.04)	LBP: 0.75 (0.04)	+1%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.64 (0.12)	LBP: 0.62 (0.04)	+3%
			S	RFA: 0.7 (0.11)	LBP: 0.66 (0.05)	+6%
			E	RFA: 0.7 (0.09)	LBP: 0.75 (0.04)	-7%
Malawi						
IP-10 Gal-9 NGAL temperature CRP respiratory rate fever duration pulse rate diastolic blood pressure			L	SW: 0.7 (0.06)	Lipocalin. 2: 0.65 (0.06)	+8%
			S	RF/ SW: 0.67 (0.06)	Lipocalin. 2: 0.64 (0.06)	+5%
			E	RF: 0.71 (0.12)	IP-10: 0.69 (0.08)	+3%
Brazil						
CRP, Gal-9, AGP pulse rate, diastolic blood pressure respiratory rate, temperature			L	RF: 0.67 (0.04)	CRP: 0.65 (0.06)	+3%
			S	SW/RFA: 0.66 (0.04)	CRP: 0.65 (0.05)	+1%
			E	SW/RFA: 0.65 (0.05)	CRP: 0.63 (0.08)	+3%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 18: Multivariate analysis – non-malaria population; haematological and protein biomarkers

Haematology + protein biomarkers				
Overall				
Multivariate models' variables				

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Rulefit	Logistic - RFA	Logistic - SW	Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%) ** multivariate and single host-biomarkers ratio
AGP LBP NGAL neutrophil count WBC count Country temperature fever duration pulse rate respiratory rate	Country neutrophil count fever duration respiratory rate	L S E	SW/RFA/RF:0.75(.03) SW:0.83(.04) SW/RFA:0.83 (.03)	WBC count: 0.7 (.03) WBC count: 0.78(.03) WBC count: 0.77 (0.04)	+7% +6% +8%	
Brazil						
Gal-9, neutrophil count, WBC count, CRP, sPLA, respiratory rate, temperature, diastolic blood pressure, fever duration, pulse rate	neutrophil count, WBC count, Gal-9 respiratory rate	L S E	WBC count, Gal-9 respiratory rate	SW: 0.82 (0.06) RFA: 0.82 (0.06) SW: 0.85 (0.06)	WBC count: 0.8 (0.06) WBC count: 0.8 (0.06) WBC count: 0.83 (0.07)	+2.5% +2.5% +2%
Gabon*						
Gabon performance evaluation using the overall model and Gabon data extracted from the Overall test sets		L S E		SW/RFA: 0.7 (0.12) SW/RFA: 0.76 (0.12) RFA: 0.77 (0.07)	WBC count: 0.7 (.03) WBC count: 0.78(.03) WBC count: 0.77 (0.04)	- -3% -
Malawi						
IP-10 Gal-9 LBP neutrophil count WBC count NGAL pulse rate respiratory rate temperature diastolic blood pressure fever duration	neutrophil count, WBC count fever duration, IP-10 temperature	L S E	neutrophil count WBC count, fever duration, IP-10, temperature	SW/RFA: 0.74 (0.06) SW: 0.73 (0.06) RFA: 0.72 (0.6)	neutrophil count: 0.72 (0.03) neutrophil count: 0.72 (0.07) WBC count: 0.7 (0.)	+3% +1% +2%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 19: Multivariate analysis – malaria population; haematological biomarkers

Haematological biomarkers

Overall							
Multivariate models' variables			Classification group	Best multivariate model/models : mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)	
Rulefit	Logistic - RFA	Logistic - SW					
haematocrit lymphocyte count neutrophil count diastolic blood pressure fever duration pulse rate respiratory rate country temperature	neutrophil count WBC count country	lymphocyte count neutrophil count country	L	RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)	+5%	
				SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%	
				RF: 0.69 (0.07)	neutrophil count: 0.61 (0.08)	+13%	
Gabon*							
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.18)	neutrophil count: 0.65 (0.05)	+3%	
			S	SW: 0.75 (0.2)	neutrophil count: 0.6 (0.08)	+25%	
			E	Not sufficient data			
Malawi							
diastolic blood pressure lymphocyte count neutrophil count temperature WBC count haematocrit pulse rate respiratory rate fever duration	neutrophil count, WBC count, temperature	WBC count,	L	RFA: 0.7 (0.06)	WBC count: 0.69 (0.05)	+1%	
SW: 0.69 (0.07)	WBC count: 0.69 (0.07)	-					
RFA: 0.6 (0.14)	lymphocyte count: 0.67 (0.05)	-10%					

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

* Multivariate performances for Gabon were computed using the Overall population-trained model as a predictor model and tested with Gabon data.

Supplementary Table 20: Multivariate analysis – malaria population; protein biomarkers

Protein biomarkers						
Overall						
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)
Rulefit	Logistic - RFA	Logistic - SW				
AGP diastolic blood pressure Gal-9	C2	country respiratory rate temperature AGP	L	SW: 0.62 (0.07)	CHI3L1: 0.57 (0.03)	+9%
			S	SW: 0.64 (0.04)	NGAL: 0.6 (0.06)	+7%

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C2 LBP pulse rate respiratory rate temperature fever duration			E	SW: 0.67 (0.08)	C2: 0.63 (01)	+ 6%
Gabon*						
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	SW: 0.67 (0.17)	CHI3L1: 0.57 (0.03)	+ 18%
			S	RFA: 0.81 (0.12)	NGAL: 0.6 (0.06)	+35%\$
			E	Not sufficient data		
Malawi						
diastolic blood pressure CHI3L1 IP-10 fever duration Gal-9 C2 pulse rate respiratory rate temperature	respiratory rate, sPLA	respiratory rate, sPLA	L	RFA/SW: 0.57 (0.06)	IP-10: 0.57 (0.05)	-
			S	SW/RFA: 0.62 (0.09)	HCC2_PL: 0.62 (0.06)	-
			E	SW/RFA: 0.61 (0.06)	IP-10: 0.66 (0.09)	-7%

E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate models).

*Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model and tested with Gabon data. \$This output has to be considered an outlier due to biomarker data imbalance between pipeline data and the available Gabon data set.

Supplementary Table 21: Multivariate analysis – malaria population; haematological and protein biomarkers

Protein + haematological biomarkers								
Overall								
Multivariate models' variables			Classification group	Best multivariate model/models: mean (SD) AUROC	Best host-biomarker: mean (SD) AUROC	Multivariate AUROC gain/loss (%)		
Rulefit	Logistic - RFA	Logistic - SW	country, Wbc_c,	L	SW/RFA: 0.68 (0.04)	neutrophil count: 0.65 (0.05)	+5%	
				S	RFA/SW: 0.66 (0.05)	neutrophil count: 0.6 (0.08)	+10%	
				E	RFA/SW: 0.66 (0.11)	HCC2_PL: 0.63 (0.1)	+5%	
Gabon*								
Gabon performance evaluation using the Overall model and Gabon data extracted from the Overall test sets			L	RFA/SW: 0.66 (0.18)	neutrophil count: 0.65 (0.05)	+1%		
			S	RFA/SW: 0.7 (0.2)	neutrophil count: 0.6 (0.08)	+17%		
			E	Not sufficient data				
Malawi								

CHI3L1	C2 neutrophil count WBC count	WBC count	L	SW: 0.69 (0.05)	WBC count: 0.69 (0.05)	-
IP-10			S	RFA: 0.73 (0.07)	WBC count: 0.69 (0.07)	+6%
Gal-9			E	RFA: 0.72. (0.1)	lymphocyte count: 0.67 (0.05)	+7%

13 E, electronic classification group; S, strict classification group; L, loose classification group; RF, Rulefit; RFA, logistic
14 recursive feature addition; SW, stepwise logistic regression. Green (gain, i.e. multivariate models have better
15 performances than univariate models); red (loss, i.e. univariate models have better performances than multivariate
16 models).

17 *Multivariate performances for Gabon are computed using the Overall population-trained model as a predictor model
18 and tested with Gabon data.

Supplementary Material References

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