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Association of haemato-biochemical indices and blood composite ratios with microfilaridermia in Onchocerciasis patients

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Abstract

Background Onchocerciasis causes chronic systemic inflammation. Several studies have used markers such as haemato-biochemical indices to predict the occurrence of systemic inflammation. This study assessed the variability and predictability of haemato-biochemical indices and blood composite ratios (BCRs) in microfilariae positive (MF+) and microfilariae negative (MF-) subgroups of onchocercomata participants.

Methods One hundred and five (105) MF+ and 34 MF- participants were retrospectively recruited into the study. Screening for the presence of *O. volvulus* microfilariae was done from skin snips taken from the left and right iliac crests of participants using established and approved protocols. Haematological and biochemical indices were measured using standard laboratory automated analyzers. Blood composite ratios (BCRs) were calculated as ratios of the absolute parameters involved.

Results A significantly increased total WBC, absolute eosinophil, eosinophil percent and absolute basophil were observed in the MF+ participants compared to MF- participants. Reduced gamma-glutamyl transferase (GGT) with increased estimated glomerular filtration rate (eGFR) was significantly associated with MF+ participants compared to MF- participants. BCRs were significantly higher for eosinophil-to-neutrophil ratio (ENR), eosinophil-to-monocyte ratio (EMR), eosinophil-to-basophil ratio (EBR) and eosinophil-to-lymphocyte ratio (ELR) in MF+ participants compared to MF- participants. After multivariate adjustment, onchocercomata participants with increased eosinophil counts (aOR = 13.86, 95% CI [2.07–92.90], $p = 0.007$), ENR x10 (aOR = 1.42, 95% CI [1.05–1.93], $p = 0.025$), EMR (aOR = 2.64, 95% CI [1.25–5.60], $p = 0.011$), EBR (aOR = 1.07, 95% CI [1.01–1.10], $p = 0.020$) and ELR x10 (aOR = 1.69, 95% CI [1.14–2.51], $p = 0.009$) were more likely to have microfilaridermia.

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Conclusions Elevated eosinophil counts with higher ENR, EMR, EBR and ELR levels are significantly associated with microfilaridermia in onchocercosoma participants. Combining BCRs with eosinophil count significantly led to an improvement in the conventional model for predicting microfilaridermia.

Keywords Onchocerciasis, Onchocercosoma, Microfilaria, Haemato-biochemical indices, Blood composite ratios, Eosinophil, Eosinophil-to-monocyte ratio, Eosinophil-to-Neutrophil ratio.

Background

Onchocerciasis, a chronic helminth infection, is one of the leading causes of morbidity and mortality worldwide [1, 2]. The infection is caused by a filarial nematode *Onchocerca volvulus* and transmitted by blackfly vectors [3]. In humans, onchocerciasis can lead to severe dermal and ocular pathologies often as a result of an acute inflammatory response to dead microfilariae (MF) [4]. Community-directed treatment with ivermectin (CDTI) remains the World Health Organization (WHO) strategy for achieving high therapeutic coverage, and elimination of the infection with a global target of 12 (31%) endemic countries verified for having interrupted transmission by 2030 [5]. CDTI approach has improved treatment coverage and subsequently broke transmission cycles—radically altering the consequences of *O. volvulus* infection [6, 7]. However, recent reports show that CDTI alone is unlikely to eliminate onchocerciasis in areas where fear of ivermectin (IVM) adverse events and death are detrimentally anchored in the population [1, 8]. Alternative strategies are therefore needed for onchocerciasis elimination [8, 9], and research on diagnostic factors in this group is of special interest.

The clinical spectrum of human onchocerciasis manifests as two polar forms among infected participants who reside in areas that have undergone none or only a few rounds of mass drug administration (MDA) [10]. The hyporesponsive form, characterized by participants with palpable onchocercosomas (nodules) but no strong pathology amidst high MF skin loads, is more common than the hyperresponsive form [10] which manifests as sowda with few or no skin MF. In addition to these clinical manifestations, a subgroup of the hyporesponsive form has been reported in hyperendemic areas that have undergone multiple rounds of ivermectin (IVM) [11, 12]. This subgroup, believed to stem from the effects of repeated preventive chemotherapy with IVM, has palpable nodules with adult worms but are a-microfilaridermic and manifest little or no dermal/skin and ocular pathology(-ies) [13]. To date, there remains a clinical dearth of understanding between this population of a-microfilaridermic (MF-) and hyporesponsive forms (MF+).

A strong inflammatory response through the activation of inflammatory cells is characteristic of helminth infection [14]. Onchocerciasis is known to activate both the innate and adaptive immune system cells in the host [15, 16]. *O. volvulus* infection is characterized by type 2

immune response with changes in lymphocytes, eosinophils, basophils, neutrophils and mast cell distributions [17]. Several studies have shown that the vast majority of incoming L3 are killed by innate immune cells [14, 17, 18]. Also, evidence from other reports shows that granulocytes are key mediators of microfilariae killing following antihelminthic treatment [13, 14].

High neutrophil levels after multiple rounds of MDA have been implicated in a-microfilaridermias [13, 19]. The neutrophils in these participants correlated negatively with eosinophils [13]. Also, high eosinophil levels with low neutrophil and basophil levels have been implicated in MF+ participants [13, 20]. Thus, suggesting a potential interaction among granulocytes in onchocerciasis infection. Further to this, without concomitant neutrophil involvement, high eosinophil counts alone may not predict distinction among onchocercosoma participants with or without skin MF [16, 19].

Blood composite ratios (BCRs) of inflammatory cells are novel and stable biomarkers of vast clinical relevance in inflammatory diseases, mortality and prognosis [21–23]. A growing number of studies suggest that BCRs may serve as an independent discriminatory predictor of chronic diseases [24–26]. Thus, BCRs such as eosinophil-to-neutrophil ratio (ENR), eosinophil-to-basophil ratio (EBR), eosinophil-to-monocyte ratio (EMR), neutrophil-to-lymphocyte ratio (NLR) and eosinophil-to-lymphocyte ratio (ELR) may be stable biomarkers in onchocerciasis disease. However, the variability and predictability of these ratios have not been previously elucidated in MF+ and MF- onchocerciasis participants. To resolve this, we examined the haemato-biochemical profile and selected BCRs among MF+ and MF- participants in the Sefwi Akontombra district of Ghana.

Materials and methods

Ethical consideration

Ethical approval of the study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Community leaders were consulted at the beginning of the study for their support and permission, and written approval was sought from the Western North Regional and the Sefwi Akontombra District Health Directorates. Informed consent was obtained from all participants and/or their legal guardians either

by thumb printing or signing. This study was part of other larger studies titled: “The efficacy of rifampicin 35 mg/kg/d plus albendazole 400 mg/d given for 7 or 14 days against onchocerciasis– a randomized, controlled, parallel-group, open-label, phase II pilot trial” with registration number PACTR202009704006025, and “Prevalence assessment for onchocerciasis in some selected districts in Ghana”. The ethical approvals received for these studies were CHRPE/AP/359/20 and CHRPE/AP/492/20.

Study population

A total of 139 participants, made up of 105 MF+ and 34 MF- participants (males and females) aged between 18 to 68 years were retrospectively recruited from November 2020 to April 2021. Recruitment of the *O. volvulus* infected participants was done from 25 communities located adjacent to and along the Tano river in the Sefwi Akontombra district of Ghana. The Sefwi Akontombra district lies between latitudes 6° N and 6° 30' N and longitudes 2° 45' W and 2° 15' W in the north-eastern part of the Western North Region [27]. It is characterized by moderate to heavy rainfall of between 1,524 mm and 1,780 mm per annum, with the peak in September-October. The communities within the districts are intersected by the Tano River and other major tributaries like Suhein, Kunuma, Sui, and the Yoyo [27]. Agricultural activities primarily along the Tano river play a major role in the

district's economy through the production of cocoa, palm trees, and other food crops [27].

Data collection

Demographic characteristics, clinical data, IVM intake status and laboratory findings for each participant were extracted from electronic records (REDCap). Electronic data were double entered by two independent data entry persons and were validated with original paper case report forms (pCRF). Participants were assured of the safety and confidentiality of their responses and laboratory data. Participants with a history of chronic diseases, haematological disorders, pregnancy, non-steroidal anti-inflammatory drug (NSAID) usage, recent antimalarial drug intake, incomplete or no laboratory results, intestinal helminths and other parasites, history of smoking and alcoholism were excluded from the study. Figure 1. shows the participant enrollment flowchart of the study. Participants were classified as MF+ and MF- as previously reported [13, 20].

MDA rounds/intake assessment

MDA has been implemented by the Ministry of Health in Ghana through the district's disease control office for over 20 years in the district with considerable compliance. Therefore, at the time of sampling, most of the participants self-reported having taken part in the MDA

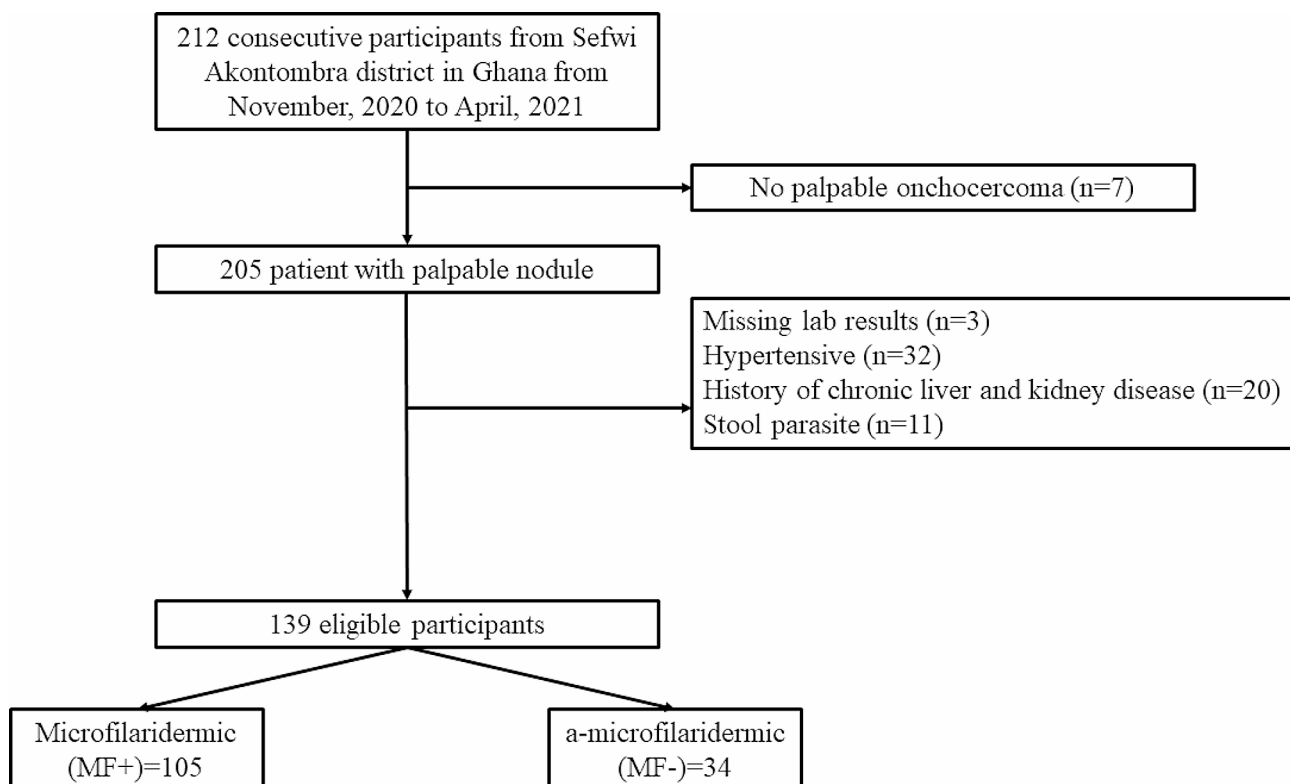


Fig. 1 Flowchart of participant inclusion in the study

programme from the district. The self-reported intake and participation in MDA programmes were used to assess individual IVM intake for each participant. To prevent MDA intake/treatment rounds recall bias, responses from participants were re-checked/cross-checked from the treatment records with the community health volunteers in the communities who had the MDA records for every household. This was done before the numbers were used in any analyses.

Distance to the nearest river

Distances to the nearest river were measured using eTrex 10 (Garmin International, Inc., USA). Briefly, the coordinates of the communities were acquired using the GPS function of the eTrex 10 and referenced to the nearest river around the communities. The distance from a focal point in the community to the nearest river was then measured and recorded in kilometres (km).

Parasitological and laboratory assessment

Participants with *O. volvulus* infection who had at least one palpable nodule were recruited for the study [28]. Screening for the presence of skin microfilariae (MF/mg of skin) was done as previously reported [13]. Infection with *Plasmodium* parasite(s), intestinal helminths and other parasites (protozoans and flagellates) — were diagnosed using standard laboratory methods. Venous blood was collected by venipuncture from the median cubital superficial vein of the upper limb by sterile disposable hypodermic vacutainer needles into tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (for haematological analytes) and gel clot activators (for biochemistry analytes). Haematological indices were then measured using a standard laboratory automated analyzer (Sysmex XN 350, USA). Serum alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and creatinine were measured using an automated biochemical analyzer (Selectra ProS ELITech Clinical Chemistry, Germany) according to the manufacturer's instructions. The calculation of eGFR was based on the formulae proposed by Levey et al. [29]. Blood Composite ratios were calculated as a ratio of the absolute parameters involved. Early morning stool samples from participants were collected and analyzed using wet mount and Kato-Katz techniques. In the Kato-Katz method, counting and recording of stool parasites were carried out according to the manufacturer's instructions by experienced laboratory scientists.

Statistical analysis

Statistical analysis was done using GraphPad Prism version 8.4.3 (GraphPad Software, Inc., San Diego, CA) and SPSS Statistics 25.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as median

and interquartile ranges whereas categorical variables were expressed as frequencies and percentages. Comparisons between continuous variables were done using the Mann-Whitney U-test. The chi-square test and Fisher's exact tests were performed for categorical variables where appropriate. The receiver operating characteristics (ROC) curve analyses were done to assess the predictability of microfilaridemia by the BCRs. Test for multicollinearity were conducted to identify and address high correlations between predictor variables, enhance model stability and improve interpretability. Predictors with Variable Inflation Factor (VIF) greater than 10 were excluded from further analysis. Univariate and multivariate logistic regression analyses were done to determine the association between BCRs and demographic characteristics. Furthermore, C-statistics were done to assess the incremental predictability of blood cell ratios. To optimize BCRs for clinical practice, their cut-off values with their specificity, sensitivity, negative predictive values (NPV) and positive predictive values (PPV) were assessed. Statistical significance was set at $p < 0.05$.

Results

Baseline characteristics of study participants

Among all enrolled onchocercosomata participants, 105 were microfilaria-positive (MF+) and 34 were microfilaria-negative (MF-). MF+ participants were younger, having a higher proportion of males and farmers than MF- participants (Table 1). The median years lived in endemic area for MF+ participants were significantly lower (25.0 [15.0–30.0] vs. 30.0 [22.0–35.0], $p = 0.007$) than MF- participants (Table 1). A higher percentage of the MF- participants lived 1–2 km from the nearest river (58.8% vs. 38.1%, $p = 0.046$) compared to MF+ participants (Table 1). Participants who lived <1 km (4.0 [4.0–5.0]) from the nearest river had significantly taken higher rounds of MDA compared to those who either lived 1–2 km (4.0 [3.0–4.0]) or >2 km (3.0 [2.0–4.8]) from the nearest river (Fig. 2).

A p -value less than 0.05 was considered statistically significant. ns; not significant, km; kilometre. Distance to the nearest river was measured as a community reference point to the nearest river using eTrex10 (Garmin International, Inc., USA).

MF+ participants exhibit disproportionate abnormalities in haematological and biochemical indices

Table 2 shows the abnormalities in haematological and biochemical indices of MF+ and MF- participants based on reference ranges set by the manufacturers of the analyzers. MF+ participants showed disproportionate abnormalities in haematological indices for RBC (6.7%), absolute eosinophil (51.4%), eosinophil percent (58.1%), absolute lymphocyte (6.7%), lymphocyte percent (18.1%),

Table 1 Baseline socio-demographic characteristics of study participants

Variable	N	a-Microfilaridermic (n=34)	N	Microfilaridermic (n=105)	p-value
Age (years)	34	41.5 [34.5–45.0]	105	37.0 [30.0–44.0]	0.070 [†]
Year in an endemic area (years)	34	30.0 [22.0–35.0]	105	25.0 [15.0–30.0]	0.007[†]
Gender (male, n.%)	34	22 [64.7]	105	70 [66.7]	0.834 ^a
Distance to the nearest river (n.%)					
< 1.00 km	34	12 [35.3]	105	43 [41.0]	0.046^a
1.00–2.00 km		20 [58.8]		40 [38.1]	
> 2.00 km		2 [5.9]		22 [21.0]	
Occupation	34		105		0.001^b
Farmer		23 [67.7]		89 [84.8]	
Trader		8 [23.5]		3 [2.8]	
Others		3 [8.8]		13 [12.4]	
Has taken IVM before, n (%)	34	34 [100.0]	105	95 [90.5]	0.062 ^b
Individual IVM intake	34	4.0 [3.75–5.0]	105	4.0 [3.0–5.0]	0.056 [†]
total nodule sites	34	1.0 [1.0–2.0]	105	2.0 [1.0–2.0]	0.089 [†]
total nodule counts	34	1.0 [1.0–2.3]	105	2.0 [1.0–3.0]	0.169 [†]
Mf skin load (Mf/mg)	34	0.00 [0.00–0.00]	105	0.62 [0.25–2.00]	<0.001[†]

^a: chi-square test; ^b: Fishers' exact test; [†]: Mann-Whitney U-test analysis; km: kilometres; Mf/mg: microfilariae per milligram of skin weight

absolute monocyte (9.5%), monocyte percent (5.7%) and basophil percent (2.9%). Biochemical indices such as ALT (5.7%), AST (9.5%), GGT (7.6%), creatinine (2.9%) and eGFR (37.1%) were also disproportionately abnormal in MF+ participants. Disproportionate abnormalities in RBC (8.8%), absolute eosinophil (14.7%), eosinophil percent (29.4%), absolute lymphocyte (2.9%), lymphocyte percent (26.5%), monocyte percent (2.9%), ALT (11.8%), AST (5.9%), GGT (11.8%), creatinine (8.8%) and eGFR (50.0%) were also observed in MF- participants.

MF+ participants show significant variabilities in haematological and biochemical indices

MF+ participants had significantly higher Total WBC (5.39 [4.78–6.60] vs. 4.71 [3.97–5.50], $p=0.001$), absolute eosinophil (0.42 [0.19–0.84] vs. 0.14 [0.08–0.36], $p<0.0001$), absolute basophil (0.03 [0.02–0.04] vs. 0.02 [0.01–0.03], $p=0.001$) and eosinophil percent (8.0 [3.8–14.1] vs. 3.2 [1.9–7.5], $p<0.0001$) with reduced GGT (25.3 [20.1–32.8] vs. 33.4 [21.9–39.6], $p=0.013$) compared to MF- participants (Table 3). Strikingly, MF- participants had significantly lower eGFR levels (89.7 [82.8–94.8] vs. 94.8 [84.4–107.5], $p=0.040$) compared to MF+ participants (Table 3). However, RBC, HGB,

PLT, absolute neutrophil, absolute lymphocyte, absolute monocyte, neutrophil percent, lymphocyte percent, basophil percent, monocyte percent, ALT, AST, and creatinine did not differ significantly ($p>0.05$) between MF+ and MF- participants (Table 3).

Increased blood composite ratios are significantly associated with MF+ participants

Blood composite ratios (BCR) were significantly higher for ENR x10 (2.30 [0.87–4.14] vs. 0.73 [0.40–1.81], $p<0.0001$), EMR (0.90 [0.43–1.84] vs. 0.36 [0.22–0.57], $p<0.0001$), EBR (14.67 [7.38–24.07] vs. 8.20 [5.25–12.00], $p=0.0006$) and ELR x10 (1.76 [0.87–3.55] vs. 0.72 [0.40–1.70], $p=0.0001$) in MF+ participants compared to MF- participants (Fig. 3). Although MF+ participants showed a lower NLR (0.88 [0.64–1.14] vs. 0.92 [0.69–1.29]) compared to MF- participants, no statistically significant difference was observed ($p=0.361$) (Fig. 3).

BCRs correlate positively with eosinophil but negatively with neutrophil levels in MF+ participants

Among the MF- participants, ENR x10, EMR, EBR and ELR x10 showed a significant positive correlation with absolute eosinophil and eosinophil percent (Fig. 4A). ENR x10, EMR, ELR x10 and not EBR showed a significant positive correlation with absolute basophil and basophil percent (Fig. 4A). ENR showed a significant negative correlation with neutrophil percent while ELR x10 showed a negative correlation with lymphocyte percent (Fig. 4A). ENR and ELR showed a significant positive correlation with creatinine (Fig. 4A). The numerical outcome of Spearman's correlation analysis can be found in the supplementary sheet as supporting information.

Among the MF+ participants, ENR x10, EMR, EBR and ELR x10 showed a significant positive correlation with absolute eosinophil and eosinophil percent but a negative correlation with absolute neutrophil and neutrophil percent (Fig. 4B). ENR x10, EMR and ELR x10 showed a significant positive correlation with absolute basophil and basophil percent (Fig. 4B). EMR showed a significant negative correlation with absolute monocyte and monocyte percent while ELR x10 showed a negative correlation with lymphocyte percent (Fig. 4B). Both absolute eosinophil and eosinophil percent showed a significant negative correlation with absolute neutrophil and neutrophil percent (Fig. 4B). Absolute eosinophil and eosinophil percent showed a significant positive correlation with absolute basophil and basophil percent (Fig. 4B). The numerical outcome of Spearman's correlation analysis can be found in the supplementary sheet as supporting information.

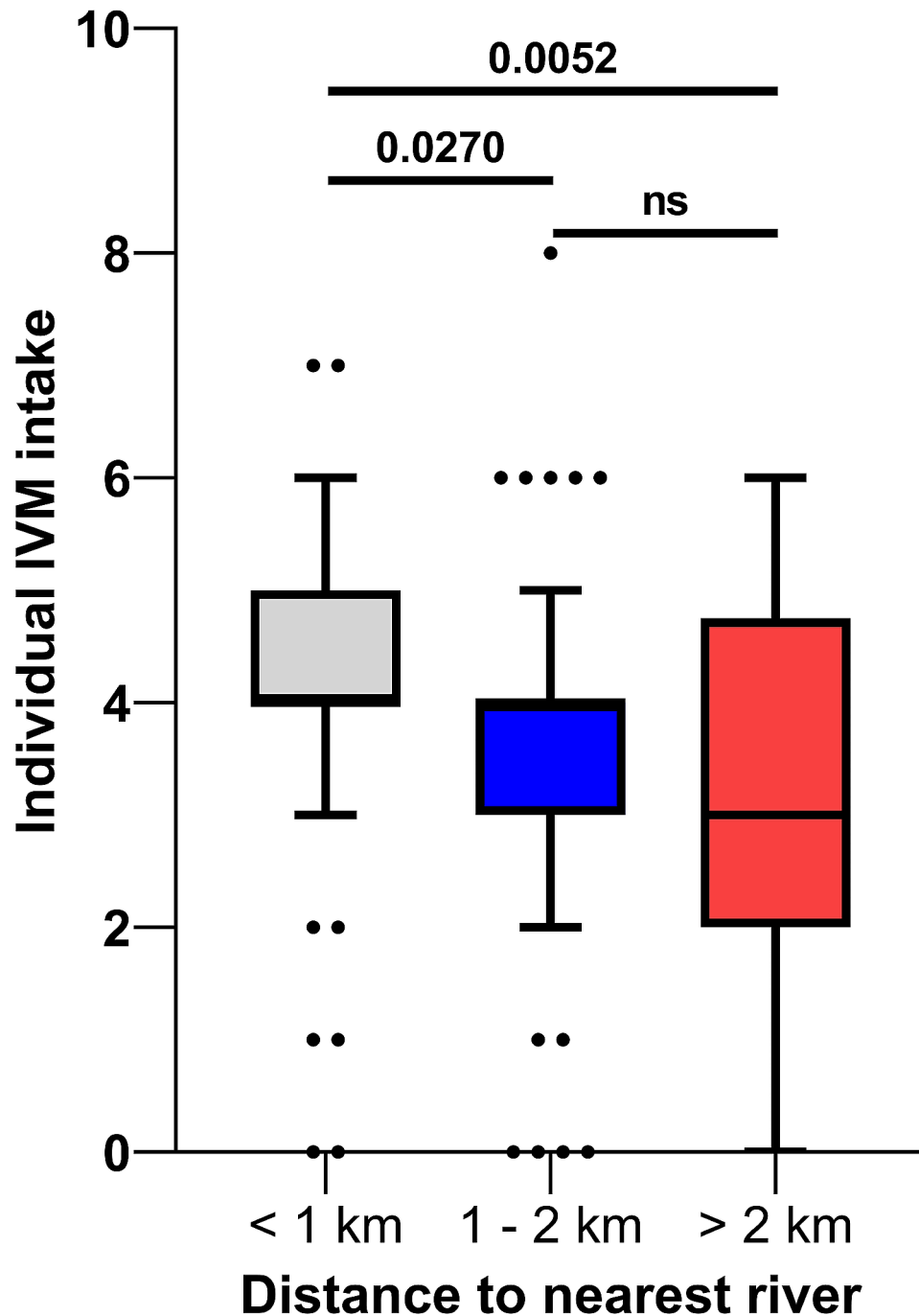


Fig. 2 Distribution of ivermectin intake among study participants by distance to the nearest river

BCRs show fair predictability in classifying MF+ and a-MF participants

Receiver operator characteristic (ROC) curve analysis were performed to determine the diagnostic ability of BCRs in classifying MF+ participants and MF- participants (Fig. 5). The analysis showed that ENR x10 (AUC=72.5%; $p<0.001$), EMR (AUC=73.0%; $p<0.001$) and ELR x10 (AUC=71.8%; $p<0.001$) had a fair predictive value in classifying MF+ and MF- participants. Absolute

eosinophil (AUC=73.8%; $p<0.001$) had the highest predictive values in discriminating onchocerciasis patients with microfilaridermia and a-microfilaridermia than either of the blood composite ratios (Fig. 5). EBR and basophil had the lowest predictive values of 69.3% and 67.4% respectively. For optimal cutoff values of BCRs that best distinguished between MF+ and MF-, an ENR x10 > 2.265, EBR > 14.25, and absolute eosinophil > 0.415 showed higher specificity but lower sensitivity (Table 4).

Table 2 Haematological and biochemical ranges and abnormalities of study participants

Variable	unit	Classification	a-Microfilaridermics (MF-, n = 34)	Microfilaridermics (MF+, n = 105)	Total n (%)	Reference range
RBC	x10 ⁶ /L	Normal	31 (91.2)	98 (93.3)	129 (92.8)	2.50–
		Abnormal	3 (8.8)	7 (6.7)	10 (7.2)	5.50
HGB	g/dL	Normal	34 (100.0)	105 (100.0)	139 (100.0)	8.0–
		Abnormal	0 (0.0)	0 (0.0)	0 (0.0)	17.0
HCT	%	Normal	34 (100.0)	105 (100.0)	139 (100.0)	26.0–
		Abnormal	0 (0.0)	0 (0.0)	0 (0.0)	50.0
PLT	x10 ³ /L	Normal	33 (97.1)	104 (99.0)	137 (98.6)	50–400
		Abnormal	1 (2.9)	1 (1.0)	2 (1.4)	
Total WBC	x10 ⁹ /L	Normal	34 (100.0)	104 (99.0)	138 (99.3)	3.00–
		Abnormal	0 (0.0)	1 (1.0)	1 (0.7)	15.00
Neutrophil Abs	x10 ⁹ /L	Normal	34 (100.0)	104 (99.0)	138 (99.3)	1.50–
		Abnormal	0 (0.0)	1 (1.0)	1 (0.7)	7.00
Lymphocyte Abs	x10 ⁹ /L	Normal	33 (97.1)	98 (93.3)	131 (94.2)	1.00–
		Abnormal	1 (2.9)	7 (6.7)	8 (5.8)	3.70
Monocyte Abs	x10 ⁹ /L	Normal	34 (100.0)	95 (90.5)	129 (92.8)	0.00–
		Abnormal	0 (0.0)	10 (9.5)	10 (7.2)	0.70
Eosinophil Abs	x10 ⁹ /L	Normal	29 (85.3)	51 (48.6)	80 (57.6)	0.00–
		Abnormal	5 (14.7)	54 (51.4)	59 (42.4)	0.40
Basophil Abs	x10 ⁹ /L	Normal	34 (100.0)	105 (100.0)	139 (100.0)	0.00–
		Abnormal	0 (0.0)	0 (0.0)	0 (0.0)	0.10
Neutrophil %	%	Normal	34 (100.0)	104 (99.0)	138 (99.3)	37.0–
		Abnormal	0 (0.0)	1 (1.0)	1 (0.7)	72.0
Lymphocyte %	%	Normal	25 (73.5)	86 (81.9)	111 (79.9)	20.0–
		Abnormal	9 (26.5)	19 (18.1)	28 (20.1)	50.0
Monocyte %	%	Normal	33 (97.1)	99 (94.3)	132 (95.0)	0.0–
		Abnormal	1 (2.9)	6 (5.7)	7 (5.0)	14.0
Eosinophil %	%	Normal	24 (70.6)	44 (41.9)	68 (48.9)	0.0
		Abnormal	10 (29.4)	61 (58.1)	71 (51.1)	–6.0
Basophil %	%	Normal	34 (100.0)	102 (97.1)	136 (97.8)	0.0–1.0
		Abnormal	0 (0.0)	3 (2.9)	3 (2.2)	
ALT	U/L	Normal	30 (88.2)	99 (94.3)	129 (92.8)	0.0–
		Abnormal	4 (11.8)	6 (5.7)	10 (7.2)	40.0
AST	U/L	Normal	32 (94.1)	95 (90.5)	127 (91.4)	0.0–
		Abnormal	2 (5.9)	10 (9.5)	12 (8.6)	40.0
GGT	U/L	Normal	30 (88.2)	97 (92.4)	127 (91.4)	0.0–
		Abnormal	4 (11.8)	8 (7.6)	12 (8.6)	55.0
Creatinine	μmol/L	Normal	31 (91.2)	102 (97.1)	133 (95.7)	53.0–
		Abnormal	3 (8.8)	3 (2.9)	6 (4.3)	124.0
eGFR	mL/min/1.73m ²	Normal	17 (50.0)	66 (62.9)	83 (59.7)	> 90.00
		Abnormal	17 (50.0)	39 (37.1)	56 (40.3)	

RBC; red blood cell count, HGB; haemoglobin concentration, PLT; platelet count, WBC; white blood cell count, ALT; alanine transaminase, AST; aspartate transaminase, GGT; gamma-glutamyl transferase, eGFR; estimated glomerular filtration rate

The optimal cutoff values of EMR that best distinguished between MF+ and MF- were values > 0.395 with 79.1% sensitivity and 61.8% specificity (Table 4). ELR x10 cutoff value > 1.160 showed 63.8% sensitivity and 70.6% specificity in predicting onchocerciasis patients with microfilaridemia (Table 4).

Eosinophil count and BCRs are independently associated with increased odds of microfilaridemia in Onchocerciasis

Univariate analysis showed that ENR x10 (cOR=1.60, 95% CI [1.18–2.19, *p*=0.003), EMR (cOR=3.68, 95% CI [1.64–8.26], *p*=0.002), EBR (cOR=1.09, 95% CI [1.03–1.15], *p*=0.003) and ELR x10 (cOR=1.93, 95% CI [1.29–2.88], *p*=0.001) were significantly associated with increased odds of microfilariae positivity (Table 5). After adjusting for age, gender, years lived in endemic

Table 3 Biochemical and Haematological Variabilities among Study Participants

Variable	unit	N	a-Microfilaridermic (n=34) median (IQR)	N	Microfilaridermic (n=105) median (IQR)	p-value
RBC	x10 ⁹ /L	34	4.73 [4.24–5.24]	105	4.57 [4.20–4.95]	0.209
HGB	g/dL	34	13.5 [12.1–14.6]	105	13.1 [12.1–14.2]	0.390
HCT	%	34	41.2 [38.2–43.6]	105	40.1 [37.1–42.8]	0.253
PLT	x10 ⁹ /L	34	196 [123–246]	105	200 [161–247]	0.343
Total WBC	x10 ⁹ /L	34	4.71 [3.97–5.50]	105	5.39 [4.78–6.60]	0.001
Neutrophil Abs	x10 ⁹ /L	34	1.78 [1.54–2.54]	105	1.99 [1.46–2.69]	0.602
Lymphocyte Abs	x10 ⁹ /L	34	2.07 [1.79–2.44]	105	2.28 [1.85–2.85]	0.069
Monocyte Abs	x10 ⁹ /L	34	0.43 [0.32–0.53]	105	0.47 [0.35–0.59]	0.110
Eosinophil Abs	x10 ⁹ /L	34	0.14 [0.08–0.36]	105	0.42 [0.19–0.84]	<0.0001
Basophil Abs	x10 ⁹ /L	34	0.02 [0.01–0.03]	105	0.03 [0.02–0.04]	0.001
Neutrophil %	%	34	40.4 [35.6–48.4]	105	37 [31.2–44.7]	0.055
Lymphocyte %	%	34	44.9 [38.9–53.6]	105	42.2 [36.3–49.0]	0.198
Monocyte %	%	34	9.0 [7.7–9.8]	105	8.0 [6.5–10.5]	0.216
Eosinophil %	%	34	3.2 [1.9–7.5]	105	8.0 [3.8–14.1]	<0.0001
Basophil %	%	34	0.4 [0.3–0.6]	105	0.5 [0.4–0.7]	0.057
ALT	U/L	34	24.0 [20.1–29.6]	105	22.4 [17.5–30.2]	0.513
AST	U/L	34	22.3 [18.5–29.0]	105	23.0 [17.8–28.5]	0.745
GGT	U/L	34	33.4 [21.9–39.6]	105	25.3 [20.1–32.8]	0.013
Creatinine	μmol/L	34	95.0 [79.0–103.0]	105	91.0 [79.5–101.0]	0.289
eGFR	mL/min/1.73m ²	34	89.7 [82.8–94.8]	105	94.8 [84.4–107.5]	0.040

RBC=red blood cell; HGB=haemoglobin concentration; HCT=haematocrit; PLT=platelet; WBC=white blood cell; abs=absolute; ALT=alanine transaminase; AST=aspartate transaminase; GGT=gamma-glutamyl transferase; eGFR=estimated glomerular filtration; IQR=inter-quartile range. Mann-Whitney U-test was used for all analyses. *p*-value<0.05 was considered statistically significant

area, distance to the nearest river, occupation, individual IVM intake, total nodule counts, total nodule sites, ENR x10 (aOR=1.42, 95% CI [1.05–1.93], *p*=0.025), EMR (aOR=2.64, 95% CI [1.25–5.60], *p*=0.011), EBR (aOR=1.07, 95% CI [1.01–1.10], *p*=0.020) and ELR x10 (aOR=1.69, 95% CI [1.14–2.51], *p*=0.009) were independently associated with increased odds of microfilariae positivity (Table 6). High eosinophil counts were independently associated with microfilariae positivity (aOR=13.86, 95% CI [2.07–92.90], *p*=0.007) (Table 6).

ENR, EMR, EBR and ELR significantly improve the conventional model for predicting microfilaridermia

To verify whether the addition of BCRs to a model containing conventional predictors could improve the classification of MF+ and MF- onchocercosoma participants, C-statistics were used (Table 7). The results show that eosinophil, ENR, EMR, EBR and ELR significantly improved the model by 4.7%, 4.2%, 4.3%, 3.3% and 3.6% respectively (Table 7). Combining all the BCRs significantly led to the improvement of the model by 4.0% (AUC=81.8%, 95% CI [74.2–89.4], *p*<0.0001) for predicting microfilaridermic participants. Strikingly, adding eosinophil to the combined BCRs showed the best discriminating ability of the model with an AUC of 82.9% (95% CI, 75.6–90.2, *p*<0.0001).

Discussion

Attempts are being made to distinguish between onchocerciasis subgroups that have unique prognostic characteristics or display distinct responses to treatment due to the ongoing effects of CDTI [13, 16, 20]. Onchocerciasis causes chronic systemic inflammation even in patients with few pathologies. Various diagnostic markers have been used to detect systemic inflammation in patients including haemato-biochemical indices [30, 31]. Previous studies have shown that total WBC, neutrophil, eosinophil, basophil and monocytes play an important role and are used as indicators of an inflammatory response [30, 32]. In our study, we observed significantly higher total WBC, eosinophil and basophil counts in MF+ participants implying an ongoing inflammatory reaction. Again, we observed that MF+ participants had significantly higher eosinophils but less neutrophil counts compared to MF- participants corroborating previous findings [13, 20].

The enzyme gamma-glutamyl transferase (GGT) is mostly present in the liver and is important for maintaining intracellular glutathione levels [33, 34]. It is frequently employed as a marker for liver or biliary disorders [33]. Elevated GGT is linked to an increased risk of many chronic diseases, including cardiovascular disease, diabetes, metabolic syndrome and chronic infection [35–37]. However, our study found that reduced GGT levels were significantly associated with MF+ participants

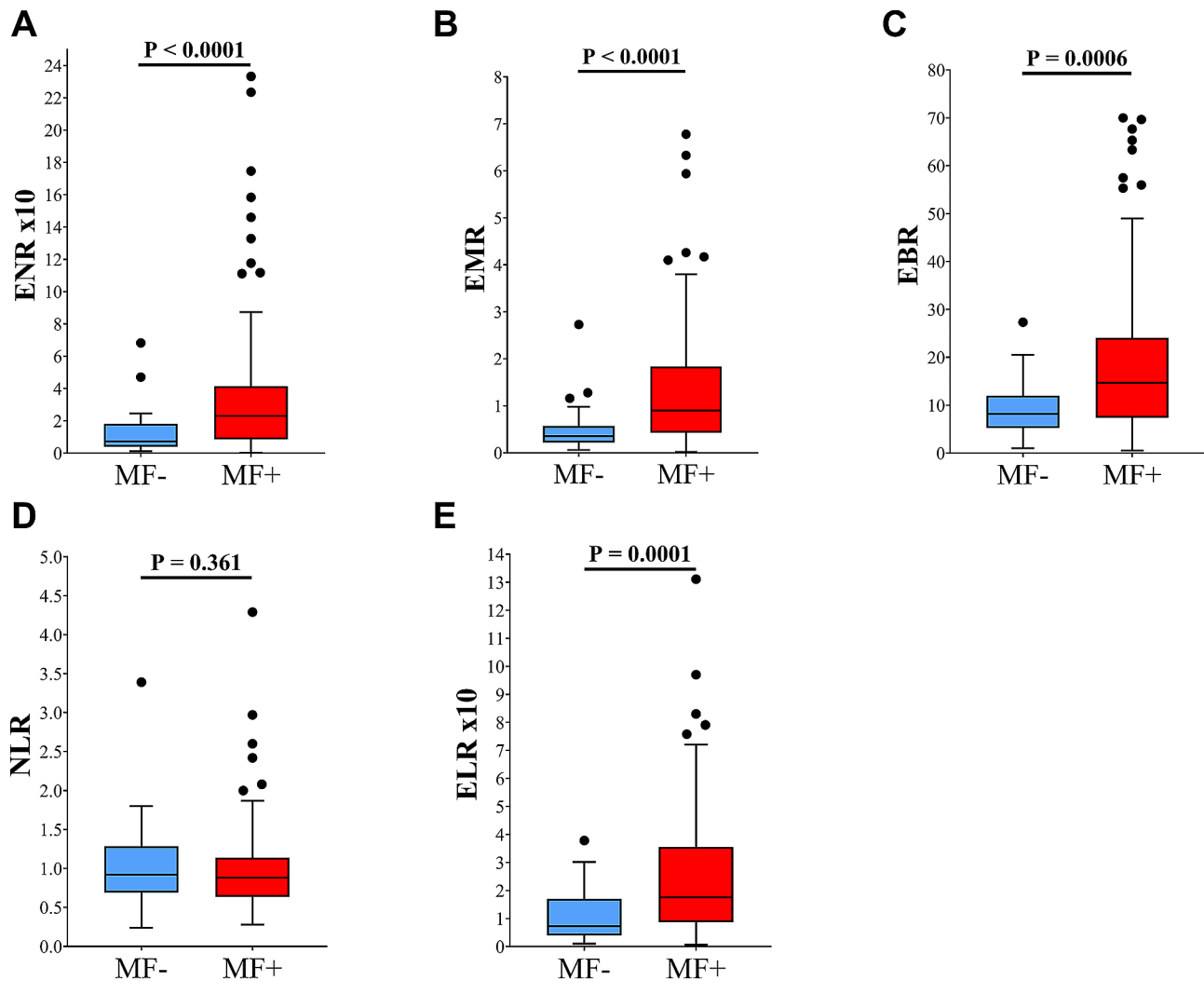


Fig. 3 Variabilities in blood composite ratios among study participants

ENR: eosinophil-to-neutrophil ratio; EBR: eosinophil-to-basophil ratio; EMR: eosinophil-to-monocyte ratio; NLR: neutrophil-to-lymphocyte ratio; ELR: eosinophil-to-lymphocyte ratio

compared to MF- participants. The mechanism underlying this is unknown, and further research is needed to better understand the function of GGT in onchocerciasis subgroups and how it affects the inflammatory immune response.

Evaluation of glomerular filtration rate (GFR) is critical for the diagnosis and treatment of kidney diseases [38, 39]. The estimated GFR (eGFR), one of the several ways to measure GFR, is recommended for the initial evaluation of GFR [40]. Observational studies have shown that declining eGFR values are associated with an increased risk of kidney complications, cardiovascular diseases and mortality [39, 41]. Several parasitic infections have been associated with glomerular disease [42–44]. Direct parasite destruction, immunological consequences, and systemic symptoms all play a role in the mechanism underlying parasite-induced kidney disease [45]. A

study showed that onchocerciasis patients have a high prevalence of glomerular and tubular disturbances [46]. In this study, we observed a significantly lower eGFR among MF- participants compared to MF+ participants. This could be due to immune complex-mediated damage caused by dead parasites among MF- participants [44, 45, 47].

Studies show that occupation, distance to the nearest river, age, compliance to the MDA programme, presence of nodules and gender are conventional predictors of onchocerciasis infections [13, 28, 48, 49]. We observed that farming was associated with an increased likelihood of microfilaridermia in our study before and after adjusting for covariables. Infectious bites from vectors may occur repeatedly during active farming periods, which typically are reported in endemic areas [50]. Kifle et al. showed that participants who lived <2 km from the

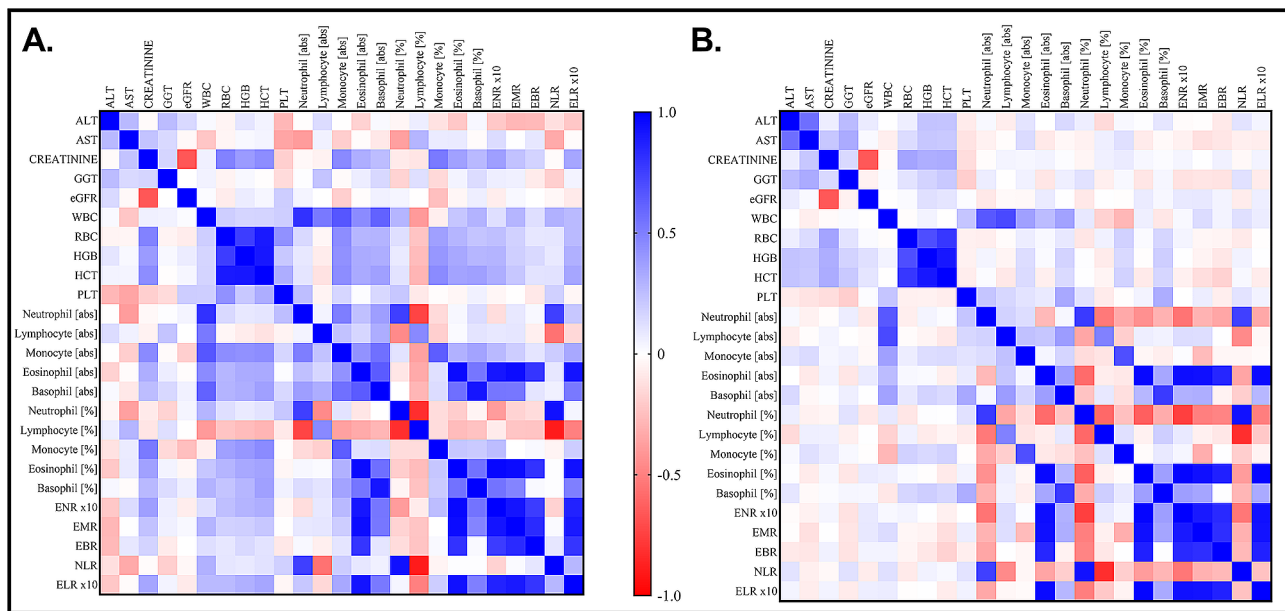


Fig. 4 Spearman's correlation analysis of BCRs with haemato-biochemical indices. **(A)** Correlation analysis among MF+ participants **(B)** Correlation analysis among MF- participants. Blue-red coloration represents max (+) to min (-) correlation coefficient. RBC=red blood cell; HGB=haemoglobin concentration; HCT=haematocrit; PLT=platelet; WBC=white blood cell; ALT=alanine transaminase; AST=aspartate transaminase; GGT=gamma-glutamyl transferase; eGFR=estimated glomerular filtration; ENR=eosinophil-to-neutrophil ratio; EBR=eosinophil-to-basophil ratio; EMR=eosinophil-to-monocyte ratio; NLR=neutrophil-to-lymphocyte ratio; ELR=eosinophil-to-lymphocyte ratio (See details in Additional file 1 and Additional file 2)

nearest river were more likely to have microfilaridermia compared to those who lived ≥ 2 km from the nearest river [51]. Our study observed that individuals who lived 1–2 km from the nearest river were less likely to have microfilaridermia compared to those who lived > 2 km away from the river. This could be due to the observed significantly higher intake of IVM among participants who lived nearer to the river than those far away in our study. Treatment coverage and compliance are highly effective at reducing the microfilariae burden at both community and individual levels [1, 52].

Centering on onchocerciasis infection, Tamarozzi et al., reported that inflammatory reaction to dead skin MF and MF in the eyes alongside the subsequent release of their bacterial endosymbiont *Wolbachia* was the basis of *Onchocerca* dermatitis and ocular keratitis immunopathology [18]. Microfilariae from *Onchocerca* dermatitis tissues have been reported to be surrounded by neutrophils, eosinophils or macrophages [53, 54]. Previous studies have emphasized the association between eosinophilia and the severity of onchocerciasis [55–57]. Eosinophil infiltration into onchocercosmata is triggered by MF and it has been shown that eosinophils actively attack MF [58, 59]. Evidence shows that live adult *O. volvulus* does not elicit eosinophilic invasion so long as they do not produce MF [58]. In chronically infected and untreated participants, eosinophil and macrophages infiltration cause permanent tissue damage [18]. Our study corroborates these hypotheses where we observed disproportionate

abnormalities in eosinophil levels (51.4%) which were significantly associated with increased odds of microfilaridermia in MF+ participants.

Focusing on basophil involvement in onchocerciasis infection, our findings corroborate the evidence that basophil numbers are increased in helminth infection [60, 61]. Basophils have been shown to release histamine and interleukin 4 (IL-4) in response to filarial nematodes where they function to amplify the ongoing type 2 immune response [62]. Furthermore, it has been shown that depletion of basophils results in a drastic reduction of eosinophils proliferation [62]. Labadah et al. reported a significantly lower basophil count with high eosinophil count in MF+ participants compared to MF- participants [20]. In our study, MF+ participants had significantly higher basophil compared to MF- participants. This may be because their participants were relatively younger than those observed in our study and a study showed that age significantly influences changes in neutrophil, basophil and eosinophil immunity [63].

Neutrophils are the major effector cells of early inflammatory infiltrate around damaged MF in the cornea and skin or attached to *Wolbachia*-containing adult worms [64, 65]. Their recruitment, activation and subsequent development of tissue pathology or systemic adverse events to a microfilaricidal drug depend on the endosymbiont *Wolbachia* [18]. Brattig et al. found that following doxycycline depletion of *Wolbachia*, the neutrophil infiltrates in onchocercosmata were drastically reduced

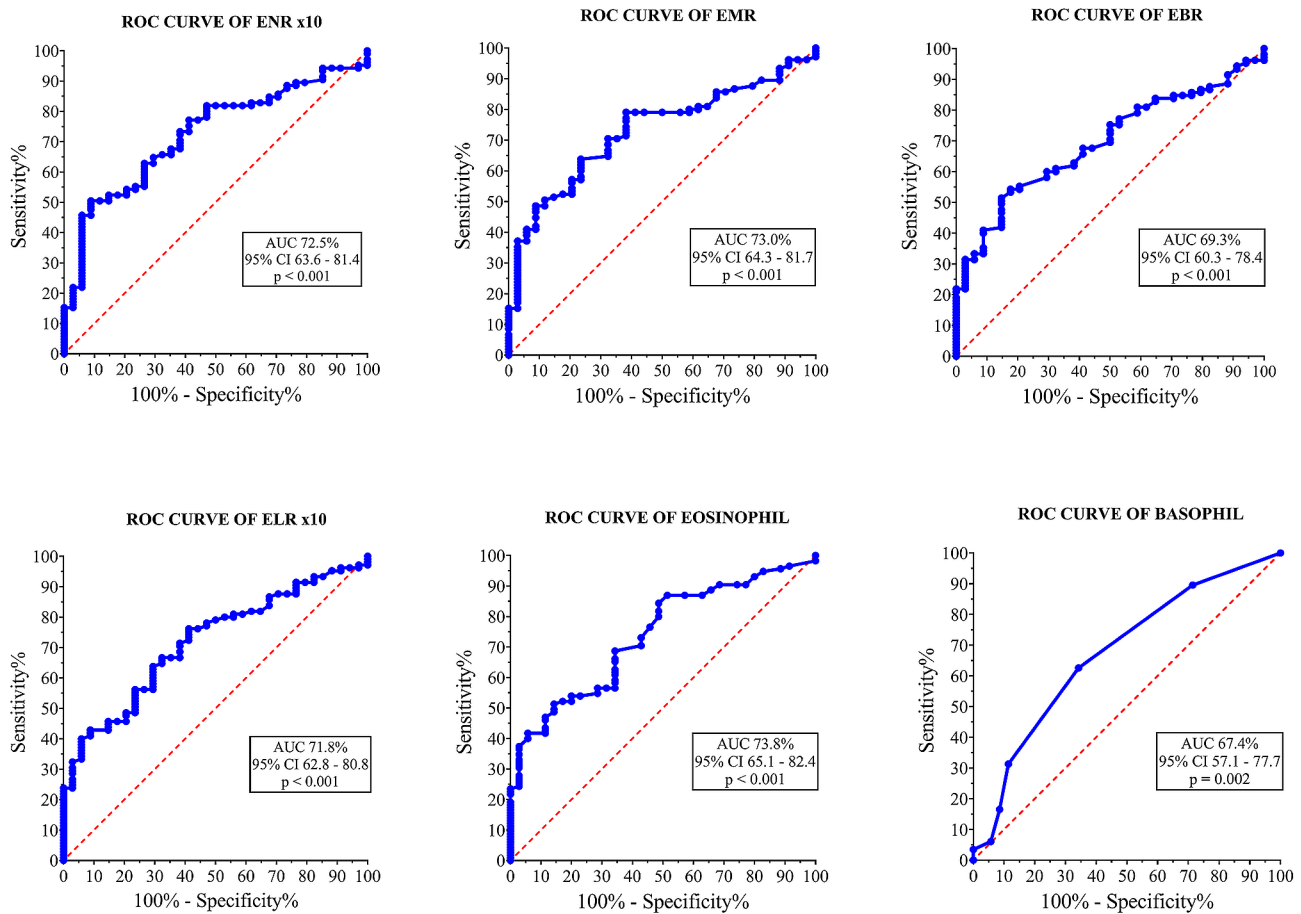


Fig. 5 Predictability of blood composite ratios and inflammatory cells in *O. volvulus* infection

ROC was based on binary logistic regression and discriminant classification analysis for MF+ and MF- participants. AUC: area under the curve of ROC; CI: confidence interval

Table 4 Cutoff values of blood composite ratios in predicting microfilaridemia

Parameter	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	PPV (95% CI)	NPV (95% CI)	p-Value
Eosinophil [Abs]	> 0.415	50.5 [40.6–60.4]	88.2 [72.6–96.7]	59.7 [51.1–67.9]	93.0 [83.8–97.1]	36.6 [31.5–42.0]	< 0.001
Basophil [Abs]	> 0.025	63.8 [53.9–73.0]	67.7 [49.5–82.6]	64.8 [56.2–72.7]	85.9 [78.6–91.0]	37.7 [30.0–46.1]	0.001
ENR x10	> 2.265	50.5 [40.6–60.4]	91.2 [76.3–98.1]	60.4 [51.8–68.6]	94.6 [85.5–98.2]	37.4 [32.4–42.6]	< 0.001
EMR	> 0.395	79.1 [70.0–86.4]	61.8 [43.6–77.8]	74.8 [66.8–81.8]	86.5 [80.5–90.8]	48.8 [37.7–60.1]	< 0.001
EBR	> 14.25	51.4 [41.5–61.3]	85.3 [68.9–95.1]	59.7 [51.1–67.9]	91.5 [82.5–96.1]	36.3 [30.9–42.0]	< 0.001
ELR x10	> 1.160	63.8 [53.9–73.0]	70.6 [52.5–84.9]	65.5 [56.9–73.3]	87.0 [79.6–92.0]	38.5 [31.1–46.9]	< 0.001

PPV; positive predictive value, NPV; negative predictive value, CI; confidence interval, abs; absolute count, EBR: eosinophil-to-basophil ratio; EMR: eosinophil-to-monocyte ratio; NLR: neutrophil-to-lymphocyte ratio; ELR: eosinophil-to-lymphocyte ratio. The Chi-square test was used for all analyses. *p*-value less than 0.05 was considered statistically significant

[64]. Although their role remains debatable, recent studies suggest that they may be involved in host protective immunity through *Wolbachia*-induced neutrophil extracellular trap formation (NET) [65, 66]. The function of this NETosis limits the dissemination and prevention of inflammatory damage induced by *Wolbachia*; provides an anti-parasitic response to limit MF production; and also limits the penetration of more damaging immune cells such as degranulating eosinophils [66]. Previously, we reported significantly higher levels of neutrophils among

MF- participants compared to MF+ participants [13]. A similar trend was observed in this study but was not statistically significant. This could be because of the homeostatic restoration of neutrophil activation after a limited period of IVM treatment [67, 68]. Neutrophil activation is observed during the adverse reaction following treatment with IVM and correlates with MF load, presence of *Wolbachia* DNA and proinflammatory cytokines [69]. Levels of neutrophils increase shortly after IVM and

Table 5 Univariate logistics regression analysis for microfilaridermia among study participants

Variable	Univariate analysis	
	OR (95 CI %)	p-value
Age (years)	0.97 [0.93–1.01]	0.121
Year in an endemic area (years)	0.96 [0.92–0.99]	0.011
gender (male)	1.09 [0.48–2.46]	0.834
Distance to the river (< 1 km)	0.33 [0.07–1.59]	0.165
Distance to the river (1–2 km)	0.18 [0.04–0.85]	0.030
Occupation (Farmer)	2.66 [1.09–6.51]	0.032
Individual IVM intake	0.79 [0.61–1.02]	0.080
Total nodule sites	1.54 [0.89–2.66]	0.124
Total nodule counts	1.11 [0.85–1.45]	0.425
Total WBC	1.74 [1.20–2.51]	0.003
Basophil	1.65 [1.16–2.34]	0.005
Eosinophil	28.18 [3.97–200.13]	0.001
GGT	0.98 [0.96–1.00]	0.067
eGFR	1.02 [1.00–1.05]	0.062
ENR x10	1.60 [1.18–2.19]	0.003
EMR	3.68 [1.64–8.26]	0.002
EBR	1.09 [1.03–1.15]	0.003
ELR x10	1.93 [1.29–2.88]	0.001

WBC; white blood cell count, GGT; gamma glutamyl transferase; ENR; eosinophil-to-neutrophil ratio, EMR; eosinophil-to-monocyte ratio, EBR; eosinophil-to-basophil ratio, ELR; eosinophil-to-lymphocyte ratio

are reduced to baseline after 4–6 months of *Wolbachia* depletion [68–70].

Different from previous studies, we evaluated the blood composite ratios (BCRs) of MF+ and MF- participants based on reported interaction between eosinophil and other inflammatory cells in discriminating onchocerciasis subgroups [13, 20]. We observed significantly higher levels of ENR, EBR, EMR and ELR in the MF+ participants compared to MF- participants. We also found that elevated ENR, EMR, EBR and ELR correlated positively with eosinophil but negatively with neutrophil in MF+ participants. The clinical implications of these composite ratios have been reported particularly in inflammatory diseases, allergies and cancers. Higher ELR values were associated with worse survival results, according to Holub and Biete [26], who reported an AUROC of 0.61 for utilizing ELR to predict survival outcomes. Brescia et al., also observed that EBR was significantly higher in patients with inflammatory diseases [71]. These ratios have proved to be better prediction biomarkers than established biomarkers [72]. The increased ENR, EBR, EMR and ELR in this study suggest that they might serve as stable complementary biomarkers to eosinophils in predicting microfilariae in *O. volvulus* infection. ENR > 0.2265, EBR > 14.25 and ELR > 0.116 showed significantly higher specificity (> 80%) in diagnosing microfilaridermias. This is important to the current elimination goals of onchocerciasis, where ensuring a very high degree of specificity is paramount [73]. EMR is a stable biomarker [74] and proved

to be very robust in discriminating between MF+ and MF- participants in this study. At a level of > 0.395, EMR showed considerably higher specificity and sensitivity in detecting skin microfilariae. The reported specificity and sensitivity of these biomarkers corroborate reports on sensitivity and specificity by other researchers who explored these biomarkers [26, 74, 75]. Interestingly, we found that eosinophil alone had the highest AUROC value (73.8%) and presented much better accuracy than all the BCRs. The C-statistics analysis revealed that these markers should be considered as part of the conventional model of diagnosing microfilaridermia among onchocercomata patients and not as a standalone biomarker. In endemic areas with variable levels of medical resources, a routine complete blood count test is offered as part of the diagnosis. BCRs could be obtained from a simple calculation of absolute cell counts and may assist clinicians to judge whether patients will harbour microfilariae.

Our study is not without limitations. Even though according to the Ghana NTD Control Programme and other studies there may not be other filarial infections in the district [76–78], we cannot exclusively reject the possibility of Onchocerciasis co-infection with other filarial diseases such as LF and Mansonellosis in the participants recruited, but the chances are very low considering the site (Sefwi Akontombra district) where this study was carried out, which is the western North Region of Ghana. Also, as a retrospective study, it is burdened by all the associated limitations that accompany this type of data sampling method including an absence of data on potential confounders. Although the observed changes in blood ratios do not solely indicate microfilarial presence, they reflect the intricate interplay between eosinophils and the immune response. Therefore, their interpretation should be context specific. Notwithstanding, the results of our study should be considered as hypothesis generating as it has not been previously elucidated in this study population.

We do recommend that further confirmatory study in a sufficiently powered prospective analysis be done which considers the above-mentioned limitations.

Conclusion

To conclude, we observed a significantly higher total WBC, absolute eosinophil, absolute basophil, ENR, EBR, EMR and ELR levels in the MF+ participants compared to MF- participants. We also found that ENR, EBR, EMR and ELR were significantly associated with increased odds of MF positivity in participants with onchocercomata. Combining BCRs with eosinophil significantly led to improvement in the conventional model for predicting microfilaridermia.

Table 6 Multivariate logistics regression analysis for microfilaridermia among study participants

Variable	Multivariate analysis					
	Model 1 + ENR		Model 1 + EMR		Model 1 + EBR	
	aOR (95 CI %)	p-value	aOR (95 CI %)	p-value	aOR (95 CI %)	p-value
Age (years)	0.99 [0.93–1.05]	0.687	0.99 [0.93–1.05]	0.676	0.99 [0.93–1.05]	0.688
gender (male)	0.61 [0.20–1.80]	0.367	0.65 [0.22–1.92]	0.438	0.60 [0.20–1.80]	0.361
Year in an endemic area (years)	0.97 [0.92–1.01]	0.162	0.97 [0.92–1.02]	0.174	0.96 [0.92–1.01]	0.105
Distance to the river (< 1 km)	0.29 [0.05–1.68]	0.167	0.26 [0.04–1.50]	0.130	0.31 [0.05–1.76]	0.185
Distance to the river (1–2 km)	0.18 [0.03–1.02]	0.052	0.15 [0.03–0.84]	0.031	0.17 [0.03–0.97]	0.046
Occupation (Farmer)	5.67 [1.58–20.33]	0.008	4.97 [1.41–17.56]	0.013	6.01 [1.67–21.59]	0.006
Individual IVM intake	0.90 [0.65–1.25]	0.526	0.92 [0.51–1.49]	0.563	0.92 [0.66–1.28]	0.619
Total nodule counts	0.94 [0.58–1.52]	0.791	0.92 [0.57–1.49]	0.745	0.91 [0.56–1.46]	0.687
Total nodule sites	1.52 [0.57–4.08]	0.408	1.48 [0.55–4.02]	0.441	1.48 [0.55–3.99]	0.436
ENR x10	1.42 [1.05–1.93]	0.025				
EBR			2.64 [1.25–5.60]	0.011		
EMR x10					1.07 [1.01–1.10]	0.020
	Multivariate analysis					
	Model 1 + ELR		Model 1 + Eosinophil			
	aOR (95 CI %)	p-value	aOR (95 CI %)	p-value		
Age (years)	0.99 [0.93–1.06]	0.654	0.99 [0.93–1.05]	0.696		
gender (male)	0.56 [0.19–1.69]	0.304	0.55 [0.18–1.71]	0.300		
Year in an endemic area (years)	0.97 [0.92–1.02]	0.177	0.97 [0.92–1.02]	0.196		
Distance to the river (< 1 km)	0.28 [0.05–1.66]	0.163	0.25 [0.04–1.50]	0.126		
Distance to the river (1–2 km)	0.16 [0.03–0.91]	0.039	0.16 [0.03–0.93]	0.041		
Occupation (Farmer)	5.94 [1.65–21.39]	0.006	5.74 [1.55–21.25]	0.009		
Individual IVM intake	0.91 [0.65–1.27]	0.566	0.92 [0.66–1.30]	0.646		
Total nodule counts	0.93 [0.57–1.49]	0.751	0.91 [0.56–1.49]	0.709		
Total nodule sites	1.43 [0.53–3.87]	0.484	1.52 [0.56–4.15]	0.411		
ELR x10	1.69 [1.14–2.51]	0.009				
Eosinophil [abs]			13.86 [2.07–92.90]	0.007		

Model 1 included age, gender, years lived in an endemic area, distance to the nearest river, occupation, total nodule counts, total nodule sites, ENR; eosinophil-to-neutrophil ratio, EMR; eosinophil-to-monocyte ratio, EBR; eosinophil-to-basophil ratio, ELR; eosinophil-to-lymphocyte ratio

Table 7 C-statistics for blood ratios to improve the predictability of microfilaridermia among study participants

Parameter	C-statistics		
	Estimate	95% CI	p-value
Model 1	0.778	0.692–0.864	< 0.0001
Model 1 + Eosinophil	0.825	0.752–0.899	< 0.0001
Model 1 + ENR	0.820	0.740–0.899	< 0.0001
Model 1 + EMR	0.821	0.745–0.897	< 0.0001
Model 1 + EBR	0.811	0.733–0.889	< 0.0001
Model 1 + ELR	0.814	0.739–0.889	< 0.0001
Model 1 + ENR + EMR + EBR + ELR	0.818	0.742–0.894	< 0.0001
Model 1 + Eosinophil + Combined ratios	0.829	0.756–0.902	< 0.0001

Model 1 was defined to include age, gender (male), years lived in an endemic area, distance to the nearest river, occupation (farmer), individual intake of MDA (ivermectin), total nodule counts and total nodule sites. ENR; eosinophil-to-neutrophil ratio, EMR; eosinophil-to-monocyte ratio, EBR; eosinophil-to-basophil ratio, ELR; eosinophil-to-lymphocyte ratio; combined ratios included ENR, EMR, EBR and ELR

Abbreviations

MF+	Microfilaria positive
MF-	Microfilaria negative
BCRs	Blood composite ratios
MDA	Mass Drug Administration

GGT	Gamma-glutamyl transferase
eGFR	estimated glomerular filtration rate
ENR	Eosinophil-to-neutrophil ratio
EMR	Eosinophil-to-monocyte ratio
EBR	Eosinophil-to-basophil ratio
ELF	Eosinophil-to-lymphocyte ratio
CDTI	Community-directed treatment with ivermectin
WHO	World Health Organization
IVM	Ivermectin
ALT	Alanine transaminase
AST	Aspartate transaminase
NPV	Negative predictive values
PPV	Positive predictive values

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-024-09278-0>.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

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Author contributions

AYD and LBD conceived and acquired funding for the study. LBD, DAM, ARA, CG, PO, JOM, VSO AYD, LBD and MAO carried out the field/recruitment surveys. ARA, MAO, PO, DAM, CG, MA, VSO and JOM worked on the laboratory investigations. LBD, AYD, CG, DAM, MA, CG, PO, JOM and VSO did the data curation and formal analysis. The project administration was overseen by AYD, LBD, JOM and DAM. AYD, LBD, JOM, DAM and VSO supervised and validated the study. The first and original manuscript draft preparation was done by LBD and CG. Review and Editing of the manuscript draft were done by AYD, LBD, CG, JOM and DAM. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval of the study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Community leaders were consulted at the beginning of the study for their support and permission, and written approval was sought from the Western North Regional and the Sefwi Akontombra District Health Directorates. Informed consent was obtained from all participants and/or their legal guardians either by thumb printing or signing. This study was part of other larger studies titled: "The efficacy of rifampicin 35 mg/kg/d plus albendazole 400 mg/d given for 7 or 14 days against onchocerciasis—a randomized, controlled, parallel-group, open-label, phase II pilot trial" with registration number PACTR202009704006025, and "Prevalence assessment for onchocerciasis in some selected districts in Ghana". The ethical approvals received for these studies were CHRPE/AP/359/20 and CHRPE/AP/492/20. The study was undertaken according to the principles and guidelines of the Helsinki Declaration of 1964, most recently amended in October 2013.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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