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A malaria-exposure antibody signature in Rwandan non-Burkitt acute lymphoblastic leukemia

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Ethics: ethical approval was obtained from the University of Rwanda College of Medicine and Health Sciences Institutional Review Board (ethical clearance 158/CMHS IRB/2016). Written informed consent or assent was obtained from each participant or guardian, respectively.

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To the Editor,

Malaria remains endemic in Rwanda, where exposure to *Plasmodium falciparum* is common. Although the relationship between *P. falciparum* malaria and endemic Burkitt lymphoma is well established,[1,2] considerably less is known about potential interactions between malaria exposure and non-Burkitt acute lymphoblastic leukemia (ALL). In Rwandan patients with non-Burkitt ALL, we identified a malaria-exposure antibody signature characterized by selective enrichment of high anti-histidine-rich protein 2 (HRP-2) IgG3 reactivity, comparatively lower anti-SE36 IgG1 reactivity and a higher anti-HRP-2 IgG3/anti-SE36 IgG1 ratio. This profile was evaluated using acute myeloid leukemia (AML) as a disease comparator from the same diagnostic setting and Rwandan adult blood donors as available healthy controls.

We analyzed anti-HRP-2 IgG3 and anti-SE36 IgG1 because these antibody responses capture complementary aspects of malaria exposure and humoral immunity. HRP-2 is a soluble parasite protein released during blood-stage infection. The anti-HRP-2 assay measured IgG3, an antibody subclass with a shorter serum half-life than IgG1, IgG2 and IgG4. Consistent with this shorter persistence, malaria-specific IgG3 responses may be rapidly boosted after antigen exposure, supporting the interpretation of anti-HRP-2 IgG3 as a marker compatible with recent or recurrent antigenic stimulation.[3-6] By contrast, the anti-SE36 assay measured IgG1 directed against SE36, a recombinant antigen derived from the N-terminal domain of *P. falciparum* serine repeat antigen 5, a blood-stage antigen implicated in naturally acquired and vaccine-induced malaria immunity.[7,8] Anti-SE36 IgG1 antibodies were therefore interpreted as markers of more persistent SE36-directed humoral immunity.

Anti-HRP-2 IgG3 and anti-SE36 IgG1 antibody titers were measured in diagnostic serum samples obtained before initiation of leukemia-directed treatment from patients with newly diagnosed ALL and AML enrolled in a nationwide acute leukemia study in Rwanda, along with serum samples from 94 Rwandan blood donors. The primary biomarker analysis included Rwandan residents diagnosed between 2016 and 2019 with available measurements for both markers, comprising 156 patients with ALL and 101 patients with AML. Baseline characteristics of patients and controls are summarized in Supplementary Table S1.

Ethical approval was obtained from the University of Rwanda College of Medicine and Health Sciences Institutional Review Board (ethical clearance 158/CMHS IRB/2016). Approval to collect samples and access patient information was obtained from the participating institutions and the Ministry of Healthy in Rwanda. Written informed consent or assent was obtained from each participant or guardian, respectively.

Anti-HRP-2 IgG3 and anti-SE36 IgG1 antibody titers were quantified by ELISA. Plates were coated with HRP-2 or SE36 antigen (East Coast Biologics), blocked with 5% BSA and incubated with serum samples overnight, with washes performed between each step. Bound antibodies were detected using horseradish peroxidase-conjugated anti-human IgG3 or IgG1 antibodies (Invitrogen, Eugene, OR, USA), followed by colorimetric development. A pooled serum sample from malaria-exposed Rwandan individuals was included on each plate as a reference standard, and pooled serum from malaria-naive Swedish blood donors served as negative control. Samples were analyzed in duplicate, and optical density values were converted to arbitrary units using calibration curves.

Continuous antibody levels were compared using Mann-Whitney U tests, threshold proportions using Fisher exact tests and multivariable models as specified below. Multivariable linear regression used ln(value+1)-transformed outcomes and adjusted for age, sex and Rwandan geographic region. The ratio was calculated as anti-HRP-2 IgG3/(anti-SE36 IgG1+1), with 1 added to the denominator to avoid division by zero and preserve observations with very low or undetectable anti-SE36 IgG1. An exploratory comparison with Rwandan blood donor controls was restricted to the age range represented among controls and analyzed using Fisher exact tests and age-adjusted logistic regression.

Compared with AML, ALL showed a divergent malaria antibody profile: higher anti-HRP-2 IgG3, lower anti-SE36 IgG1 and a higher anti-HRP-2 IgG3/anti-SE36 IgG1 ratio (Figure 1A-C; Table 1). In multivariable analysis, ALL remained associated with higher anti-HRP-2 IgG3 and a higher anti-HRP-2 IgG3/anti-SE36 IgG1 ratio after adjustment for age, sex and region, indicating a selective HRP-2 IgG3-skewed profile with comparatively lower SE36 IgG1 reactivity (Table 2).

The difference was most apparent in the upper tail of anti-HRP-2 IgG3 reactivity. High anti-HRP-2 IgG3 reactivity was enriched in ALL across adjacent upper-tail thresholds, with the strongest separation at >4,000 AU/µL and concordant findings at >3,000 and >5,000 AU/µL (Figure 1D; Supplementary Table S2). Among leukemia patients with available diagnosis dates, sampling month did not differ between ALL and AML (χ^2 p=0.29), and the association between

ALL and anti-HRP-2 IgG3 >4,000 AU/μL persisted after adjustment for age, sex, region and rainy season (March-May or September-December; OR 4.48; p=0.023).

Available healthy controls were Rwandan adult blood donors aged 19-58 years. In an adult age-overlap analysis, high anti-HRP-2 IgG3 reactivity was more frequent in ALL than controls (20.6% versus 1.1%; Fisher exact p=0.00037; age-adjusted p=0.0025), whereas AML did not differ from controls (1.9% versus 1.1%; Fisher exact p=1.00) (Supplementary Table S3). Month of sampling was unavailable for controls, precluding season-matched control analysis.

Additional analyses supported the specificity of the ALL-associated antibody profile. The anti-HRP-2 IgG3/anti-SE36 IgG1 ratio was higher in ALL than AML among children 14 years or younger (p=0.034) and among older patients (p=0.041). Anti-HRP-2 IgG3 and anti-SE36 IgG1 were not significantly correlated within patients (Spearman ρ=0.087; p=0.17). Available immunophenotype data suggested that the HRP-2-skewed profile may be more pronounced in B-ALL than T-ALL, although subgroup numbers were limited. Anti-HRP-2 IgG3 thus tended to be higher in B-ALL than T-ALL with borderline significance (median 1,278 versus 341 AU/μL; p=0.063), while anti-SE36 IgG1 did not differ significantly (p=0.79). Available morphology did not support Burkitt-type disease as the driver of the signal: among the 25 ALL patients with anti-HRP-2 IgG3 >4,000 AU/μL, FAB morphology was documented in 15 cases, of which 13 were L1, 2 were L2 and none were L3.

Together, these findings identify a distinct malaria-associated antibody profile in Rwandan non-Burkitt ALL, characterized by selective enrichment of high anti-HRP-2 IgG3 reactivity together

with comparatively lower anti-SE36 IgG1. The findings should be interpreted as acute leukemia subtype-associated serologic differences rather than direct evidence of causality. Nevertheless, the ALL-associated pattern was observed in comparison with contemporary AML cases, remained evident after multivariable adjustment, persisted after accounting for seasonality and was supported by the adult control analysis. Available morphology data argued against Burkitt-type disease as an explanation for the observed HRP-2 IgG3 enrichment. These results support further investigation of malaria-exposure signatures in non-Burkitt ALL arising in malaria-endemic settings.

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Tables

Table 1. Malaria-associated antibody profile in Rwandan acute leukemia patients

Biomarker	ALL (n=156)	AML (n=101)	Raw p-value	Holm-adjusted p-value
Anti-HRP-2 IgG3, AU/µl	788 (234-1750)	500 (60.0-1145)	0.024	0.049
Anti-SE36 IgG1 antibody, AU/µl	49.8 (25.5-160)	85.4 (29.7-257)	0.027	0.049
Anti-HRP-2 IgG3/(anti-SE36 IgG1+1)	11.8 (2.00-38.6)	4.20 (0.34-18.5)	0.0012	0.0035

Values are median (interquartile range). P-values were calculated using the Mann-Whitney U test. Holm adjustment was applied across the three primary biomarker comparisons. AU: arbitrary units; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HRP-2: histidine-rich protein 2; SE36: serine repeat antigen 5-derived antigen.

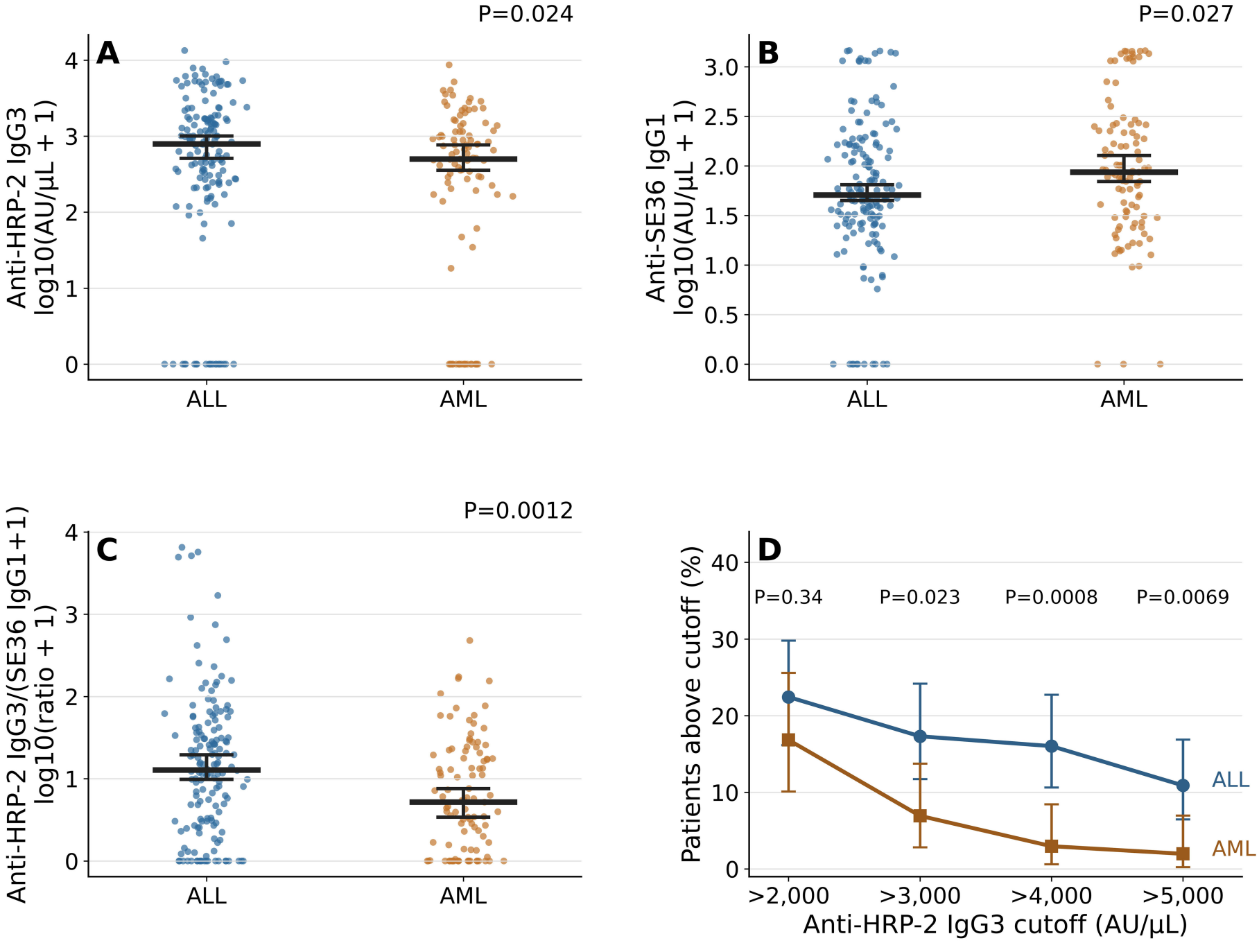
Table 2. Multivariable analysis

Outcome	n	Beta for ALL vs AML	95% CI	Raw p-value	Holm-adjusted p-value
Anti-HRP-2 IgG3	252	0.97	0.18 to 1.75	0.016	0.032
Anti-SE36 IgG1	252	-0.40	-0.87 to 0.08	0.099	0.099
Anti-HRP-2 IgG3/(anti-SE36 IgG1+1)	252	0.88	0.36 to 1.40	0.0010	0.0031

Coefficients derive from linear regression models using log-transformed antibody values and adjusted for age, sex and Rwandan geographic region. Positive β values indicate higher levels in ALL relative to AML; negative β values indicate lower levels in ALL relative to AML. The smaller n reflects missing age values. Holm adjustment was applied across the three prespecified multivariable biomarker models. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CI: confidence interval; HRP-2: histidine-rich protein 2; SE36: serine repeat antigen 5-derived antigen.

Figure legend

Figure 1. Divergent malaria antibody profile in acute leukemia subtypes. Anti-HRP-2 IgG3 (A), anti-SE36 IgG1 (B) and the anti-HRP-2 IgG3/(anti-SE36 IgG1+1) antibody-profile ratio (C) are shown for Rwandan ALL and AML patients. Individual values are shown; horizontal bars indicate medians with bootstrapped 95% confidence intervals. Values in panels A-C are plotted on log10(value+1) axes. Panel D shows the proportion of patients exceeding adjacent upper-tail anti-HRP-2 IgG3 thresholds; error bars indicate 95% binomial confidence intervals. P values in panels A-C were calculated using Mann-Whitney U tests and P values in panel D using Fisher exact tests. The constant of 1 in the denominator was added to avoid division by zero and preserve observations with very low or undetectable anti-SE36 IgG1 values.


A
P=0.024
Anti-HRP-2 IgG3
log10(AU/µL + 1)
ALL
AML
B
P=0.027
Anti-SE36 IgG1
log10(AU/µL + 1)
ALL
AML
C
P=0.0012
Anti-HRP-2 IgG3/(SE36 IgG1+1)
log10(ratio + 1)
ALL
AML
D
P=0.34
P=0.023
P=0.0008
P=0.0069
Patients above cutoff (%)
ALL
AML
>2,000
>3,000
>4,000
>5,000
Anti-HRP-2 IgG3 cutoff (AU/µL)

Supplementary Data

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Supplementary Table S1. Characteristics of patients and controls

Variable	ALL (n=156)	AML (n=101)	Controls (n=94)
Age, years	11.0 (1-82)	26.0 (1-65)	34 (19-58)
Pediatric age (≤14 years)	98/153 (64.1%)	33/99 (33.3%)	0/94 (0.0%)
Male sex	95/156 (60.9%)	53/101 (52.5%)	81/94 (86.2%)
Kigali	35/156 (22.4%)	14/101 (13.9%)	17/94 (18.1%)
Eastern region	24/156 (15.4%)	16/101 (15.8%)	69/94 (73.4%)
Southern region	42/156 (26.9%)	34/101 (33.7%)	0/94 (0.0%)
Western region	31/156 (19.9%)	13/101 (12.9%)	8/94 (8.5%)
Northern region	24/156 (15.4%)	24/101 (23.8%)	0/94 (0.0%)
WBC, $\times 10^3/\mu L$	14.8 (0.5-910)	23.4 (0.9-459)	NA
Platelets, $\times 10^3/\mu L$	33.8 (1.0-491)	39.0 (0.0-1051)	NA

Values are median (range) or n/N (%). The table includes Rwandan residents with available anti-HRP-2 IgG3 and anti-SE36 IgG1 measurements, and Rwandan blood donor controls with available measurements. Age was available for 153 ALL and 99 AML patients; WBC for 152 ALL and 99 AML patients; platelet count for 152 ALL and 77 AML patients. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; WBC: white blood cell count. WBC and platelet values were not available (NA) for controls.

Supplementary Table S2. High anti-HRP-2 IgG3 threshold analysis

Cutoff, AU/µl	ALL > cutoff, n/N (%)	AML > cutoff, n/N (%)	Raw p-value	Holm-adjusted p-value
2,000	35/156 (22.4%)	17/101 (16.8%)	0.34	0.34
3,000	27/156 (17.3%)	7/101 (6.9%)	0.023	0.045
4,000	25/156 (16.0%)	3/101 (3.0%)	0.0008	0.003
5,000	17/156 (10.9%)	2/101 (2.0%)	0.0068	0.02

P-values were calculated using Fisher exact test. Holm adjustment was applied across the four anti-HRP-2 IgG3 thresholds. AU: arbitrary units; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; HRP-2: histidine-rich protein 2

Supplementary Table S3. Adult age-overlap comparison with Rwandan blood donors

Variable	Adult ALL (n=34), n/N (%)	Adult AML (n=53) n/N (%)	Controls (n=94), n/N (%)	ALL vs control Fisher p-value	AML vs control Fisher p-value	ALL vs control Age-adjusted OR (95% CI); p-value
Anti-HRP-2 IgG3 >4,000 AU/µl	7/34 (20.6%)	1/53 (1.9%)	1/94 (1.1%)	0.00037	1.00	30.4 (3.3-278); p=0.0025
High anti-HRP-2 IgG3/(anti-SE36 IgG1+1) ratio	11/34 (32.4%)	6/53 (11.3%)	12/94 (12.8%)	0.018	1.00	3.60 (1.3-9.9); p=0.013

The analysis for ALL, AML and controls was restricted to individuals aged 19-58 years, corresponding to the age range represented among controls. A high antibody-profile ratio was defined as >38.6, corresponding to the upper quartile of the Rwandan ALL cohort. Fisher p-values were calculated using Fisher exact test. Age-adjusted odds ratios and p-values derive from logistic regression models adjusted for age. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; AU: arbitrary units; CI: confidence interval; HRP-2: histidine-rich protein 2; OR: odds ratio; SE36: serine repeat antigen 5-derived antigen.

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