JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Evaluation of Some Haematological Parameters of Lassa Fever Patients In Virology Screening Centre Abakaliki, Ebonyi State

Alobu Clement Okah[1], Chukwurah F. Ejike [1], Boniface N. Ukwah[1], ChibuezeA. Okereke[1]

Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Ebonyi State University, Abakaliki, Ebonyi State.

ABSTRACT

Lassa fever remains a major public health challenge in West Africa, with Nigeria accounting for a substantial proportion of reported cases. This study evaluated conventional haematological parameters, platelet-derived biomarkers, derived systemic inflammatory indices, and ABO/Rh D blood group phenotypes among laboratory-confirmed Lassa fever patients to determine their diagnostic utility. A prospective hospital-based cross-sectional comparative study was conducted at the Virology Centre, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria. The study included 241 participants, comprising 133 laboratory-confirmed Lassa fever patients and 108 apparently healthy controls. Full blood count analysis was performed using SysmexXN-100 (Japan) while 22 derived haematological and systemic inflammatory indices_were calculated from routine haematological parameters. ABO and Rh D blood groups were determined using standard serological techniques. Data were analyzed using descriptive and inferential statistics. Patients with Lassa fever demonstrated significantly reduced red blood cell count, haemoglobin concentration, packed cell volume, and red cell indices, together with increased total white blood cell count, neutrophil percentage, and reduced lymphocyte percentage compared with controls ($p < 0.05$). In contrast, ABO/Rh D blood group phenotypes showed limited diagnostic significance. This study provides one of the most comprehensive evaluations of routine haematological biomarkers in laboratory-confirmed Lassa fever in southeastern Nigeria by integrating conventional haematological parameters, platelet-derived biomarkers, 22 derived systemic inflammatory indices, ABO/Rh D blood group phenotypes, and advanced statistical modelling.

Keywords: Lassa fever, Biomarkers, Incidence, Haematological parameters, Immune system

INTRODUCTION

Lassa fever is an acute viral haemorrhagic disease caused by Lassa virus (LASV), a single-stranded, bi-segmented RNA virus of the family Arenaviridae and genus Mammarena virus.[1] It was first identified in Lassa, Borno State, Nigeria, in 1969 following the deaths of two nursing missionaries [2], it has become one of the most important endemic viral infections in West Africa. Owing to its epidemic potential and the absence of an

approved vaccine, the World Health Organization (WHO) classifies Lassa fever as a priority disease requiring accelerated research and development.[3]

Transmission occurs primarily through direct or indirect contact with the urine, feaces, or saliva of the natural reservoir, *Mastomys natalensis* (the multimammate rat), which is widely distributed across West Africa.[4] The disease is endemic in Nigeria, Sierra Leone, Guinea, and Liberia, with evidence of transmission in Benin, Togo, Ghana, Mali, Côte d'Ivoire, and Senegal.[4,5] Nigeria consistently records the highest number of confirmed cases globally.[6] Although Lassa fever occurs year-round in Nigeria, incidence peaks during the dry season (November–April) because of increased human contact with infected rodents.[7] Between January and April 2023, the Nigeria Centre for Disease Control (NCDC) reported 1,03confirmed cases and 209 deaths (case fatality rate: 20.1%) across 26 states.[8] Edo, Ondo, and Ebonyi States remain the principal endemic areas.[6,7] Ebonyi State, particularly Abakaliki, lies within Nigeria's hyper endemic Lassa fever belt. The Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA), through its Virology Screening Centre (VSC), serves as a major referral centre for viral haemorrhagic fever diagnosis in southeastern Nigeria, processing approximately 150–200 Lassa fever screening samples monthly during peak transmission periods[6]. Clinically, Lassa fever presents with non-specific symptoms that overlap with malaria, typhoid fever, dengue fever, and bacterial sepsis, making early diagnosis difficult. [9,10] Initial manifestations include fever, malaise, headache, myalgia, and pharyngitis, while severe disease may progress to haemorrhage, encephalopathy, and multi-organ failure.[11,12] Case fatality ranges from about 1% in community infections to 15–20% among hospitalised patients and may exceed 50% in severe cases.[13,14] Sensor neural hearing loss is the most common long-term complication, affecting about one-third of survivors.[15]

Ribavirin remains the only established antiviral therapy, with greatest benefit when administered within six days of symptom onset[13] consequently, rapid recognition of suspected cases before confirmatory laboratory diagnosis is essential. Real-time reverse transcription polymerase chain reaction (RT-PCR), supported by Lassa virus-specific IgM enzyme-linked immunosorbent assay (ELISA), is the diagnostic gold standard.[9] However, these techniques require specialised laboratories, skilled personnel, and longer turnaround times, limiting their accessibility in many resource-limited settings.[16] In contrast, the full blood count (FBC) is inexpensive, widely available, and capable of detecting haematological abnormalities associated with systemic viral infection, making it a valuable adjunct for early patient assessment.[17] Lassa virus infection induces characteristic haematological changes through immune dysregulation and inflammatory activation. Viral replication in dendritic cells and macrophages stimulates cytokine release, resulting in leucocytosis, relative lymphopenia, platelet abnormalities, and coagulation disturbances detectable by routine FBC analysis.[18,19] The virus also suppresses type I interferon responses, promoting immune evasion and disease progression.[20] Previous studies have reported leucocytosis, lymphopenia, thrombocytopenia, and haemoconcentration as common haematological abnormalities in Lassa fever.[21] More recently, mean platelet volume (MPV) has been associated with mortality.[22]

Advances in automated haematology have introduced several derived inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), aggregate index of systemic inflammation (AISI), platelet-to-white blood cell ratio (PWR), plateletcrit (PCT), platelet distribution width (PDW), mean platelet volume (MPV), and red cell distribution width-to-lymphocyte ratio (RDWLR). These biomarkers have shown diagnostic value in other viral haemorrhagic fevers but remain inadequately studied in Lassa fever.[23,24,25,26] Likewise, the influence of ABO and RhD blood group phenotypes on susceptibility to Lassa fever and associated haematological changes has not been adequately investigated despite evidence linking blood group antigens to the severity of several viral infections.27,28 These unresolved issues highlight the need for comprehensive evaluation of routine haematological parameters, platelet-derived biomarkers, systemic inflammatory indices, and ABO/Rh D blood groups.

METHODS

Study Area

This study was conducted in Abakaliki, the capital of Ebonyi State, southeastern Nigeria (latitude 6°20′N, longitude 8°06′E). Abakaliki lies within the forest–savanna ecotone at an elevation of approximately 200 metres above sea level and experiences a humid tropical climate, with an average annual rainfall of about 1,750 mm and temperatures ranging from 25°C to 30°C.

The city is located within the Lassa fever hyperendemic belt of West Africa, where *Mastomys natalensis*, the principal reservoir of Lassa virus (LASV), is widespread in domestic and peridomestic environments. Lassa fever occurs throughout the year, with peak transmission during the dry season (November–February), when reduced vegetation and altered rodent foraging behavior increase human–rodent contact around homes and food storage areas (Ilori *et al*., 2019).

Between 2018 and 2023, Ebonyi State consistently contributed to Nigeria's annual Lassa fever burden and has been classified by the Nigeria Centre for Disease Control (NCDC) as a high-transmission endemic zone (Adesola *et al*., 2024).

Study Design

This study employed a prospective, hospital-based cross-sectional comparative design to evaluate selected haematological parameters and derived inflammatory indices in two independent groups: (1) laboratory-confirmed Lassa fever patients and (2) apparently healthy community controls. Participants were recruited consecutively from the Virology Screening Centre (VSC), Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA), Abakaliki, between October 2025 and May 2026, covering the peak Lassa fever transmission season and the subsequent transitional period. The final analytical sample comprised 241 participants, including 133 confirmed Lassa fever patients and 108 apparently healthy controls. Individual matching was not performed; instead, baseline comparability for age and sex was assessed statistically (Table 1), and both variables were included as pre-specified covariates in all multivariable analyses. Accordingly, unconditional binary logistic regression was used throughout, consistent with the unmatched cross-sectional design (Breslow & Day, 1980). References in earlier protocol drafts to a 1:1 matched case-control design were superseded by this final study design.

Study Population

The target population were Nigerians within the age range of 10 to 60 years old, who included all laboratory confirmed Lassa fever patients presenting for care at the AEFUTHA Virology Center, as well as apparently healthy individuals living within the Abakaliki metropolis who served as comparative controls. The accessible population, which formed the actual study sample, comprised 133 confirmed Lassa fever patients enrolled consecutively during the study period, and 108 apparently healthy controls recruited purposively and frequency-matched for age and sex. Collectively, these 241 participants constituted the study population from which all haematological data were obtained and analyzed in the Virology lab. of the hospital.

Selection Criteria

Inclusion Criteria

Participants were eligible for inclusion in the study if they:

1. Were 18 years or older at the time of blood collection.

2. Had laboratory-confirmed Lassa fever based on at least one of the following tests performed at the VSC:

- Real-time RT-PCR detecting LASV nucleoprotein RNA (TaqMan probe-based; Ct < 38 according to NCDC protocol);
- Lassa virus-specific IgM ELISA (Zalgen Labs; reactivity index ≥ 1.10); or
- Rapid LASV antigen detection test (Tulip Diagnostics) in a clinically compatible patient.

3. Presented during the acute phase of infection, defined as symptom onset within 14 days before specimen collection.

4. Provided written informed consent.

3.4.2 Inclusion Criteria for Apparently Healthy Controls

Apparently healthy controls were eligible if they:

1. Were **18 years or older**.

2. Had no confirmed or suspected Lassa fever, no febrile illness or antibiotic use within the preceding four weeks, and no history of chronic kidney disease, liver disease, malignancy, or haematological disorders.

3. Resided within the Abakaliki urban or peri-urban area, ensuring comparable environmental exposure and endemic malaria background.

4. Provided written informed consent.

Exclusion Criteria

The following participants were excluded from the study:

1. Pregnant or lactating women.

2. Individuals with haematological malignancies or those receiving cytotoxic chemotherapy within the preceding six months.

3. Individuals with chronic autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis.

4. Participants receiving systemic corticosteroids (≥20 mg/day prednisolone equivalent) or biological immunosuppressive agents.

5. Individuals who had received a blood transfusion within the preceding four weeks.

6. Blood samples showing haemolysis, clotting, or inadequate volume.

7. Individuals unable or unwilling to provide written informed consent.

Sample size Determination

The sample size was estimated to detect clinically meaningful differences in the primary outcome measures (NLR, PLR and SII) between Lassa fever patients Controls. The calculation assumed a large effect size (Cohen's d= 0.80) based on previous studies (Okpunu *et al*., 2025; Yalcindag *et al*., 2024), two-sided significance level of 0,05, 80% statistical power, allow a 10% allowance for sample loss due to specimen quality issues. The minimum sample size was calculated using the formula for comparing two independent means with unequal groups (Snedecor and Cochran, 1989):

$$\Omega_1 = \frac{(Z\alpha/_2 + Z_\beta)^2 \times (\sigma^2_1 + \sigma^2_2)}{(\mu_{1-\mu 2})^2}$$

Where:

Ω_1 = minimum sample size for cases

$Z\alpha/2 = 1.96$ (for $\alpha = 0.05$, for two- sided)

$Z_\beta = 0.84$ (for $\beta = 0.20$, 80% statistical power)

$\mu_1 - \mu_2$ = estimated clinically meaningful difference in mean platelet count between Lassa fever patients and healthy controls = 50×10^9/L (based on previous studies, Olayinka *et al.,* 2020)

σ_1 = estimated standard deviation of platelet count in Lassa patients = 70×10^9/L

σ_2 = estimated standard deviation of platelet count in healthy controls = 60×10^9/L

r = ratio of controls to cases = 108/133 = 0.81

Substituting into the formula:

$$n_1 = \frac{(1.96 + 0.84)^2 \times (70^2 + 60^2/0.81)}{(50)^2}$$

$$n_1 = \frac{(7.84) \times (4900 + 3600/0.81)}{2500}$$

$$n_1 = \frac{7.84 \times (4900 + 4444.44)}{2500}$$

$$n_1 = \frac{7.84 \times 9344.44}{2500}$$

$n_1 = 7.84 \times 3.7378$

$n_1 = 29.30 \approx 30$ cases

n_2 (controls) = $30 \times 0.81 = 24.3 \approx 25$ controls

To account for incomplete data, instrument errors, and sample attrition, a 10% non-response rate was added:

Minimum required cases = $30 \div 0.90 = 34$ cases

Minimum required controls = $25 \div 0.90 = 28$ controls

However, to enhance statistical power, improve the precision of reference interval estimation, and allow for robust subgroup analyses (e.g., mild vs. severe disease, sex-based differences), consecutive recruitment during the approved study period resulted in the enrolment of 241 participants (133 confirmed Lassa fever cases and 108 apparently healthy controls), exceeding the minimum requirement.

Sampling Techniques

A Consecutive Sampling technique was adopted for this study. The study group were all patients within the age range of 10 to 60 years with laboratory confirmed lassa fever who were presented at the Virology Center, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, during the study period and satisfied the inclusion criteria were consecutively enrolled until 133 sample size was attained.

The control group comprised apparently heathy age and sex- matched subjects who tested negative to lassa fever and met the inclusion criteria, were enrolled until 108 sample size were attained following the same protocol.

Structured Data Collection forms

To ensure uniformity and consistency in data collection, a structured data collection form was designed and used to obtain relevant information from the subjects and the laboratory records including their demographic characteristics like age and sex, laboratory identification numbers, clinical information and results of the laboratory investigations.

Blood Sample Collection

A total of 241 venous blood samples were collected from patients within the age range 10 to 60 years by trained phlebotomists using standard viral haemorrhagic fever infection prevention and control precautions, including double gloves, fluid-resistant gowns, surgical masks, and protective goggles. 131 sample from laboratory confirm lassa fever patients and 108 samples from apparently healthy control participants. The sample collection was carried out in the ward of the Virology Screening Centre. The informed consent of each subjects was obtained in writing.

A volume of 5mls of a blood sample was aseptically collected from each participant by venipuncture. Each participant's arm was tied with turniquette and the site for blood collection was wiped with a cotton wool swab soaked in methylated spirit to clean and sterilize the area. A sterile 21-gauge needle attached to a 5ml k_2EDTA BD-Vacutainer® closed blood collection system was carefully used to puncture the vein and 5mls of blood sample were collected. A dry cotton wool was placed at the point of entrance of the needle into the vein and the needle was gentle removed, covered with its cov3r and properly disposed into the waste bin provided.

Each specimen was labelled with a unique participant study identification number immediately after collection and transported to the laboratory within 2 hours at an ambient temperature of 20–25°C. To ensure adequate mixing of blood with the anticoagulant, collection tubes were gently inverted 8–10 times immediately after venipuncture.

Complete blood count (CBC) analysis was performed within 4 hours of sample collection in accordance with the International Council for Standardization in Hematology (ICSH) recommendations for the pre-analytical stability of EDTA-anticoagulated whole blood (van Dongen *et al.*, 2012). Following completion of the CBC analysis, the same EDTA specimen was used for ABO and Rh D blood group determination, thereby minimizing repeated venipuncture and optimizing specimen utilization

Laboratory Analysis

Full Blood Count Analysis

Complete blood count (CBC) analysis was performed using the Sysmex XE-2100 Automated Haematology Analyzer (Sysmex Corporation, Kobe, Japan). Before each analytical session, tri-level quality control (Sysmex XE-Check) was performed, and analyses proceeded only when all control values were within ±2 standard deviations (SD) of the established target ranges, in accordance with the Westgard 12s and 13s quality control rules. Monthly calibration was performed by a certified Sysmex service engineer using manufacturer-traceable calibration materials.

The analyzer generated the following haematological parameters for each participant: total white blood cell count (TWBC), differential percentages and absolute counts of neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), and basophils (BASO); red blood cell (RBC) count; haemoglobin (Hb); packed cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); red cell distribution width (RDW-CV and RDW-SD); platelet count (PLT); mean platelet volume (MPV); platelet distribution width (PDW); and plateletcrit (PCT).

Basophil values were excluded from statistical analyses because more than 40% of measurements were below the analyser's minimum quantification threshold, in accordance with the pre-specified missing-data exclusion criterion (Section 3.4.3).

ABO and Rh D Blood Group Phenotype Determination

ABO and Rh D blood group phenotypes were determined using the direct agglutination tile (forward grouping) method with commercial monoclonal anti-A, anti-B, and anti-D antisera. Reverse (serum) grouping was performed for all samples with ambiguous or discrepant forward-grouping results. For forward grouping, 5 µL of EDTA-anticoagulated whole blood was placed into three wells of a clean ceramic serological tile, followed by one drop each of anti-A, anti-B, and anti-D antisera. The tile was gently rocked for 4–5 minutes at 20–22°C, and agglutination reactions were assessed visually against a white background under adequate illumination. Agglutination strength was graded according to the International Society of Blood Transfusion (ISBT) 0–4+ scale, with reactions of 2+ or greater regarded as positive.

Where required, reverse grouping was performed by incubating participant plasma with commercial reagent red cells (A_1, B, and O cells) to confirm the corresponding antibody pattern. ABO phenotype was assigned only when forward and reverse grouping results were concordant.

Samples initially typed as Rh D negative were further tested for weak-D antigen using the indirect antiglobulin test (IAT) in accordance with the British Committee for Standards in Haematology (BCSH) guidelines (Milkins *et al.*, 2013). Blood group phenotypes were subsequently classified into one of eight categories: A+, A−, B+, B−, AB+, AB−, O+, and O−.

Calculation of Derived Haematological and Systemic Inflammatory Indices

A total of 22 derived haematological and systemic inflammatory indices were calculated from the primary complete blood count (CBC) parameters for all 241 participants. These indices were categorized into three groups: leucocyte-based ratios (11 indices), composite systemic inflammatory indices (3 indices), and erythrocyte/platelet morphology indices (8 indices). Their formulae, units, classifications, and biological significance are presented in Table 3.2.

Leucocyte-based indices included the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), monocyte-to-lymphocyte ratio (MLR), neutrophil-to-monocyte ratio (NMR), eosinophil-to-lymphocyte ratio (ELR), eosinophil-to-neutrophil ratio (ENR), lymphocyte-to-eosinophil ratio (LER), platelet-to-monocyte ratio (PMR), platelet-to-total white blood cell ratio (PWR), and derived neutrophil-to-lymphocyte ratio (dNLR). Composite inflammatory indices comprised the systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), and aggregate index of systemic inflammation/pan-immune inflammation value (AISI/PIV). Morphological indices included the Mentzer index, Shine–Lal index, haemoglobin-to-red blood cell ratio (HRR), red blood cell-to-haemoglobin ratio (RHR), red blood cell-to-platelet ratio (RPR), red cell distribution width-to-lymphocyte ratio (RDWLR), mean platelet volume-to-platelet ratio (MPVPR), mean platelet volume-to-lymphocyte ratio (MPVLR), and haemoglobin-to-platelet ratio (HPR).

All leucocyte-derived ratios were calculated using the differential percentage values generated by the Sysmex XE-2100 analyzer. This approach is mathematically equivalent to calculations based on absolute NEUT cell counts because the total white blood cell count cancels during ratio computation: _abs / LYMPH_abs = (TWBC × NEUT%) / (TWBC × LYMPH%) = NEUT% / LYMPH%. This equivalence was verified computationally on the first 30 rows of the analytical dataset (maximum row-level discrepancy < 0.001), confirming the accuracy of all stored ratio values. Absolute count values (NEUT_abs, LYMPH_abs, MONO_abs) were additionally calculated and retained in the dataset as: abs = TWBC × percentage / 100, for use as covariates in regression modelling.

Statistical Analysis

All statistical analyses were performed using R (version ≥ 4.6.0; R Core Team, 2024) within the RStudio environment. The analytical workflow was pre-specified in a Statistical Analysis Plan (SAP version 2.0) before data unbinding and implemented using a version-controlled R script. The principal packages used

included tidy verse for data management and visualization, rstatix for statistical testing and effect-size estimation, car for variance homogeneity and multicollinearity assessment, FSA for Dunn's post-hoc comparisons, DescTools for odds ratios and Cramér's V, pROC for receiver operating characteristic (ROC) analysis, Resource Selection for the Hosmer–Lemeshow goodness-of-fit test, glmnet for LASSO-based feature selection, broom for regression output management, and naniar for missing-data assessment. Additional packages were used for spreadsheet output and figure preparation.

RESULTS

This chapter presents the results of the cross-sectional haematological characterisation of 133 confirmed Lassa fever patients compared to 108 apparently healthy controls (N = 241) at the Virology Screening Centre, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria. Results are organised sequentially around the five specific objectives of the study. All analyses were conducted in R (version 4.2.0) and are summarised in Tables 1–8 with accompanying figures.

Socio-Demographic Characteristics

The socio-demographic characteristics of the two groups are presented in Table 1. The median age of confirmed Lassa fever patients was 31.50 years (IQR: 26.00–40.00), compared with 34.00 years (IQR: 25.00–43.25) in apparently healthy controls. A Mann-Whitney U test indicated no statistically significant difference in age between the groups (U = 6514.5, 95% CI [−5.92, 1.05], p = .215), confirming successful age equivalence. Both groups were predominantly composed of females: 82 of 133 Lassa fever patients (61.7%) and 60 of 108 controls (55.6%). Pearson chi-square analysis with Yates' continuity correction revealed no significant difference in sex distribution ($\chi^2(1) = 0.68$, p = .409; Cramér's V = .062), confirming that the groups were comparable with respect to both age and sex.

Table 1

Socio-Demographic Characteristics of Lassa Fever Patients and Apparently Healthy Controls

Characteristics	Lassa (n = 133)	Control (n = 108)	Test statistic	95% CI	P
Age (years)	31.50 (26.00–40.00)	34.00 (25.00–43.25)	U = 6514.5	[−5.92, 1.05]	0.22
Sex, n (%)			$\chi^2(1) = 0.68$	OR [0.46, 1.30]	0.41
Female	82 (61.7%)	60 (55.6%)			
Male	51 (38.3%)	48 (44.4%)			

Note. Age is presented as median (IQR); sex as n (%). Mann-Whitney U used for age; Pearson chi-square with Yates' continuity correction for sex. CI = confidence interval; OR = odds ratio.

Full Blood Count Parameters

Full blood count analysis revealed statistically significant differences between confirmed Lassa fever patients and apparently healthy controls across all three haematological compartments—white blood cell, erythrocyte,

and platelet indices—as presented in Table 2. The majority of variables deviated significantly from normality (Shapiro-Wilk $p < .05$), necessitating non-parametric comparisons for most parameters.

White blood cell indices. Total white blood cell count (TWBC) was significantly elevated in Lassa fever patients (median 9.10 × 10⁹/L, IQR: 5.10–15.20) relative to controls (median 5.12 × 10⁹/L, IQR: 3.69–7.23; U = 9940.0, p < .001; r = 0.38), surviving Bonferroni correction. Lymphocyte percentage was significantly lower in Lassa fever patients (mean 31.14 ± 10.41% vs. 35.27 ± 13.51%; t(197.8) = −2.61, p = .010; d = −0.35), though this did not survive Bonferroni correction.

Erythrocyte indices. Mean corpuscular haemoglobin concentration (MCHC) was significantly higher in Lassa fever patients (median 34.30 g/dL vs. 32.60 g/dL; p < .001; r = 0.38), surviving Bonferroni correction. No significant differences were observed for RBC count, haemoglobin, PCV, or MCV (all p > .07), indicating that erythrocyte mass was largely preserved in this acute-phase cohort.

Platelet indices. Plateletcrit (PCT) demonstrated the strongest group difference (median 1.67% vs. 1.16%; p < .001; r = 0.41), surviving Bonferroni correction and representing the largest effect size among all platelet parameters. Platelet distribution width (PDW) was also significantly higher in Lassa patients (p < .001; r = 0.34), and mean platelet volume (MPV) was significantlly

lower (mean 9.36 ± 1.17 fL vs. 9.88 ± 1.18 fL; p < .001; d = −0.44), both surviving Bonferroni correction. Platelet count was not significantly different between groups (p = .215).

Table 2

Full Blood Count Parameters in Lassa Fever Patients and Apparently Healthy Controls

Parameter	Lassa (n=133)	Control (n=108)	Test statistic	95% CI	p	Effect size	B
White blood cell indices							
TWBC (×10⁹/L)	9.10 (5.10 – 15.20)	5.12 (3.69–7.23)	U = 9940.0	[1.96, 4.70]	<.001	r = 0.38	✓
Neutrophils (%)	57.0 (48.0–65.0)	53.0 (40.2–64.0)	U = 8272.5	[0.00, 8.00]	.043	r = 0.15	
Lymphocytes (%)	31.14 ± 10.41	35.27 ± 13.51	t(197.8) = −2.61	[−7.26, −1.01]	.010	d = −0.35	
Monocytes (%)	8.22 (5.51–12.16)	8.00 (6.35–10.00)	U = 7458.5	[−0.90, 1.40]	.608	r = 0.04	
Eosinophils (%)	2.00 (1.00–3.00)	2.00 (1.00–3.30)	U = 4890.0	[−0.72, 0.00]	.217	r = 0.10	

Erythrocyte indices						
RBC (×10¹²/L)	4.08 ± 0.94	4.23 ± 0.97	t(226.0) = −1.22	[−0.40, 0.09]	.223	d = −0.16
Haemoglobin (g/dL)	11.07 ± 2.59	10.99 ± 2.43	t(234.0) = 0.24	[−0.56, 0.72]	.809	d = 0.03
PCV (%)	33.00 (26.00–36.56)	34.00 (29.00–38.20)	U = 6211.5	[−3.50, 0.05]	.072	r = 0.14
MCV (fL)	78.95 ± 9.79	78.08 ± 9.29	t(233.2) = 0.70	[−1.56, 3.29]	.484	d = 0.09
MCH (pg)	27.26 ± 3.54	26.04 ± 3.06	t(238.1) = 2.87	[0.38, 2.06]	.004	d = 0.37
MCHC (g/dL)	34.30 (32.90–35.83)	32.60 (31.60–34.10)	U = 9915.5	[1.00, 2.10]	<.001	r = 0.38 ✓
RDW-CV (%)	15.56 (13.40–17.47)	14.35 (13.50–16.40)	U = 7816.5	[−0.24, 1.14]	.239	r = 0.09
RDW-SD (fL)	43.76 (39.00–49.03)	42.30 (38.98–45.78)	U = 7595.5	[−1.10, 2.47]	.443	r = 0.06
Platelet indices						
PLT (×10⁹/L)	219.11 (131.00–298.98)	193.00 (116.50–269.25)	U = 7849.5	[−10.00, 46.00]	.215	r = 0.09
MPV (fL)	9.36 ± 1.17	9.88 ± 1.18	t(228.7) = −3.39	[−0.81, −0.22]	<.001	d = −0.44 ✓
PDW (%)	16.20 (15.66–16.77)	15.60 (12.38–16.33)	U = 9655.0	[0.47, 1.43]	<.001	r = 0.34 ✓
PCT (%)	1.67 (1.22–1.96)	1.16 (0.23–1.72)	U = 10144.0	[0.35, 0.84]	<.001	r = 0.41 ✓

Note. Normally distributed variables presented as mean ± SD (Welch's t-test); non-normally distributed variables as median (IQR) (Mann-Whitney U). B = Bonferroni-corrected significance (α/17 = .003); ✓ = survives correction. TWBC = total white blood cell count; PCV = packed cell volume; MCV = mean

corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; PLT = platelet count; MPV = mean platelet volume; PDW = platelet distribution width; PCT = plateletcrit. Blank cells under B indicate no Bonferroni-significant findin

Figure 2. Full Blood Count Parameters — Lassa vs. Controls. Box: median + IQR; dots: individual observations; p-values from adaptive test (Welch's t-test or Mann-Whitney U). *** p < .001; ns = not significant.

Derived Haematological and Systemic Inflammatory Indices and Feature Selection

Twenty-two derived haematological and systemic inflammatory indices were calculated for all 241 participants and compared between groups. All indices departed from normality in at least one group (Shapiro-Wilk $p < .05$); all comparisons were therefore performed with Mann-Whitney U tests. Bonferroni correction was applied across 22 simultaneous comparisons (family-wise $\alpha = .002$), and BH-FDR adjustment was additionally computed. Full results are presented in Table 3.

Leucocyte-based ratios. The platelet-to-white-blood-cell ratio (PWR) demonstrated the most robust group difference, with Lassa fever patients showing significantly lower values (median 22.34 vs. 37.41; $p < .001$; $r = 0.29$)—the only leucocyte-based ratio to survive Bonferroni correction. The neutrophil-to-lymphocyte ratio (NLR; $p = .023$; $r = 0.17$) and platelet-to-lymphocyte ratio (PLR; $p = .019$; $r = 0.18$) were nominally significant but did not survive Bonferroni correction.**Composite systemic inflammation indices.** The systemic immune-inflammation index (SII) was nominally significantly elevated in Lassa fever patients (median372.62 vs. 277.36; $p = .015$; $r = 0.18$). SIRI and AISI/PIV approached but did not reach nominal significance ($p = .062$ and .069, respectively). None survived Bonferroni correction.

Erythrocyte and platelet morphology indices. The RDWLR was the only morphological index to achieve nominal statistical significance (median 0.52 vs. 0.43; $p = .019$; $r = 0.18$), without surviving Bonferroni correction. The Mentzer Index, Shine-Lal Index, MPVLR, and HPR were all non-significant (see also Figure 5).

Table 3: Derived Haematological and Systemic Inflammatory Indices: Comparison BetweLassa Fever Patients and Controls

Index	Lassa (n=133)	Control (n=108)	Test statistic	95% CI	p	Effect size	B
Leucocyte-based ratios							
NLR	1.90 (1.28–2.80)	1.55 (0.87–2.54)	U = 8409.5	[0.05, 0.56]	.023	r = 0.17	
PLR	7.13 (4.46–11.24)	5.49 (3.64–8.94)	U = 8449.0	[0.23, 2.30]	.019	r = 0.18	
LMR	3.34 (2.28–5.50)	4.25 (2.70–6.27)	U = 6171.5	[−1.19, 0.02]	.061	r = 0.14	
MLR	0.28 (0.18–0.44)	0.24 (0.16–0.37)	U = 8041.0	[−0.01, 0.08]	.111	r = 0.12	
NMR	6.75 (3.98–10.52)	6.24 (4.20–9.37)	U = 7457.5	[−0.82, 1.35]	.609	r = 0.04	

PWR	22.34 (12.31–37.87)	37.41 (20.83–47.42)	U = 5121.0	[−15.89, −5.16]	<.001	r = 0.29	✓
Composite systemic inflammation indices							
SII	372.62 (199.79–636.63)	277.36 (135.86–529.61)	U = 8495.0	[16.03, 140.14]	.015	r = 0.18	
SIRI	15.77 (9.08–24.34)	11.82 (7.12–22.62)	U = 8185.5	[−0.15, 5.07]	.062	r = 0.14	
AISI/PIV	3271.95 (1273.68–5961.71)	1983.17 (1115.21–4891.70)	U = 8160.5	[−35.25, 1251.69]	.069	r = 0.14	
Erythrocyte and platelet morphology indices							
Mentzer Index	18.73 (15.53–25.14)	18.69 (15.68–21.56)	U = 7695.5	[−0.74, 2.35]	.341	r = 0.07	
Shine-Lal	1680.32 (1328.73–2147.88)	1587.77 (1194.63–1995.01)	U = 7869.0	[−50.99, 242.24]	.202	r = 0.10	
RDWLR	0.52 (0.39–0.68)	0.43 (0.32–0.63)	U = 8448.5	[0.01, 0.12]	.019	r = 0.18	
MPVLR	0.30 (0.24–0.41)	0.28 (0.22–0.39)	U = 7818.0	[−0.01, 0.05]	.238	r = 0.09	
HPR	0.05 (0.03–0.08)	0.06 (0.04–0.09)	U = 6530.0	[−0.01, 0.00]	.226	r = 0.09	

Note. All indices presented as median (IQR); all comparisons by Mann-Whitney U test. B = Bonferroni-corrected significance (α/22 = .002); ✓ = survives correction. NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; LMR = lymphocyte-to-monocyte ratio; MLR = monocyte-to-lymphocyte ratio; NMR = neutrophil-to-monocyte ratio; PWR = platelet-to-white-blood-cell ratio; SII = systemic immune-inflammation index; SIRI = systemic inflammation response index; AISI/PIV = aggregate index of systemic inflammation/pan-immune inflammation value; RDWLR = red cell distribution width-to-lymphocyte ratio; MPVLR = mean platelet volume-to-lymphocyte ratio; HPR = haemoglobin-to-platelet ratio.

Collinearity assessment and feature selection. Spearman rank correlation analysis identified substantial collinearity (rs > .85) among several indices that share component parameters, most notably NLR, SII, PLR, SIRI, RDWLR, and LMR. The corresponding correlation heatmap is displayed in Supplementary Figure S1 below. Complementary feature selection using LASSO regularisation and univariate logistic regression ranking identified PCT, TWBC, and PWR as the three most distinct, non-redundant biomarkers; these were carried forward to Objective 3 multivariable modelling.

Figure S1. *Spearman Rank Correlation Matrix of 22 Derived Haematological Indices.* Indices with rs > 0.85 (red shading) were flagged as collinear. Dark blue = strong negative correlation; dark red = strong positive correlation.

Figure 3. *Key Inflammatory Indices — Lassa vs. Controls.* Box: median + IQR; p-values from adaptive test (corrected dNLR used). * p < .05; ns = not significant.

Figure 5. *Erythrocyte and Platelet Morphology Indices — Lassa vs. Controls.* Box: median + IQR; p-values from adaptive test. * p < .05; ns = not significant.

Independent Predictive Value of Key Haematological Parameters

A multivariable binary logistic regression model was fitted with Lassa fever status as the binary outcome using all 241 complete cases. Age and sex were forced as pre-specified demographic covariates, and PCT, TWBC, and PWR were entered simultaneously as feature-selected haematological predictors. Model results are presented in Table 7.

Model fit and diagnostics. The Hosmer–Lemeshow goodness-of-fit test indicated acceptable model calibration ($\chi^2(8) = 15.0$, $p = .059$). Nagelkerke $R^2 = .225$; AIC = 299.1; BIC = 320.0. All VIFs were below 1.50, confirming the absence of problematic multicollinearity. The C-statistic for the full model was 0.746 (95% CI [0.683, 0.809]).

Independent predictor: Of the three haematological parameters, only PCT emerged as a statistically significant independent predictor of Lassa fever infection after adjustment for age and sex (adjusted OR = 2.49, 95% CI [1.73, 3.68], p < .001). TWBC (aOR = 1.04, p = .170) and PWR (aOR = 0.99, p = .128) showed directional trends only. Neither age (aOR = 0.99, p = .326) nor sex (aOR = 1.49, p = .182) was independently associated with Lassa fever status, confirming that neither variable confounded the haematological relationship

Table 7

Multivariable Logistic Regression: Independent Predictors of Lassa Fever Infection

Predictor	aOR	95% CI	Z	P	VIF	Sig.	Interpretation
Intercept	0.41	[0.13, 1.26]	−1.55	.122	—		
Age (years)	0.99	[0.97, 1.01]	−0.98	.326	1.02	ns	Not a confounder
Sex (female)	1.49	[0.83, 2.67]	1.34	.182	1.04	ns	Not a confounder
PCT (%)	**2.49**	[1.73, 3.68]	4.74	<.001	1.08	***	**Independent predictor**
TWBC ($\times 10^9$/L)	1.04	[0.99, 1.09]	1.37	.170	1.43	ns	Trend only
PWR	0.99	[0.98, 1.00]	−1.52	.128	1.46	ns	Trend only

Note. Outcome: Lassa fever status (1 = confirmed Lassa, 0 = control). Unconditional binary logistic regression, n = 241 complete cases. aOR = adjusted odds ratio (exponentiated coefficient); Sig. = significance category.

VIF = variance inflation factor (all < 1.50). Model fit: Nagelkerke R^2 = .225; AIC = 299.1; BIC = 320.0. Hosmer-Lemeshow goodness-of-fit: $\chi^2(8) = 15.0$, p = .059. *** p < .001; ns = not significant.

Diagnostic Performance and Optimal Clinical Cut-Off Values

ROC curve analysis was conducted for seven predictors — PCT, TWBC, PWR (model-selected) and NLR, PLR, SII, RDWLR (nominally significant in univariate comparison) — across all 241 participants. Diagnostic performance is summarised in Table 8 and visualised in Figure 6.

PCT. PCT demonstrated the strongest individual diagnostic discrimination (AUC = 0.706, 95% CI [0.639, 0.774])—the only individual predictor meeting the AUC ≥ .70 threshold for clinically useful discrimination. At a Youden-optimal cut-off of 0.428%, PCT achieved 97.0% sensitivity, 45.4% specificity, PPV of 68.6%, NPV of 92.5%, and LR+ of 1.78.

TWBC. TWBC showed the second-highest discrimination (AUC = 0.692, 95% CI [0.624, 0.760]). At a cut-off of 8.78 × 10^9/L, it achieved 53.4% sensitivity and 83.3% specificity (LR+ = 3.20; the highest of any single predictor). The AUC difference between PCT and TWBC was not statistically significant (DeLong Z = 0.30, p = .762).

PWR, SII, RDWLR, PLR, NLR. PWR demonstrated fair discrimination (AUC = 0.644). The four nominally significant univariate indices (NLR, PLR, SII, RDWLR) produced AUC values clustered between 0.586 and 0.591, below the clinical utility threshold. PCT significantly outperformed each of these indices (all DeLong p ≤ .011).

Full model. The full logistic regression model achieved a C-statistic of 0.746 (95% CI [0.683, 0.809]), exceeding the performance of any individual predictor, confirming that the combination of haematological and demographic information provides superior diagnostic discrimination.

Table 8

Diagnostic Performance of Haematological Predictors: ROC Curve Analysis

Predictor	AUC	95% CI	DeLong	Cut-off	Sn (%)	Sp (%)	PPV (%)	NPV (%)	LR+
PCT (%)	**0.706**	[0.639, 0.774]		0.428	97.0	45.4	68.6	92.5	1.78
TWBC (×10^9/L)	0.692	[0.624, 0.760]		8.780	53.4	83.3	79.8	59.2	3.20
PWR	0.644	[0.573, 0.714]		28.253	60.9	68.5	70.4	58.7	1.93
SII	0.591	[0.518, 0.664]		289.688	63.9	54.6	63.4	55.1	1.41
RDWLR	0.588	[0.514, 0.662]		0.449	63.9	58.3	65.4	56.8	1.53
PLR	0.588	[0.516, 0.661]		6.240	57.9	61.1	64.7	54.1	1.49
NLR	0.586	[0.511, 0.660]		0.955	88.7	30.6	61.1	68.7	1.28
Full LR model	**0.746**	[0.683, 0.809]		—	—	—	—	—	—

Note. All AUC values with 95% DeLong confidence intervals. Optimal cut-offs by Youden's J index. Sn = sensitivity; Sp = specificity; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; all computed at Youden-optimal threshold and observed sample prevalence (55.2%). Full LR model = AUC of the five-predictor logistic regression model. AUC ≥ .70 met by PCT (0.706) and the full LR model (0.746).

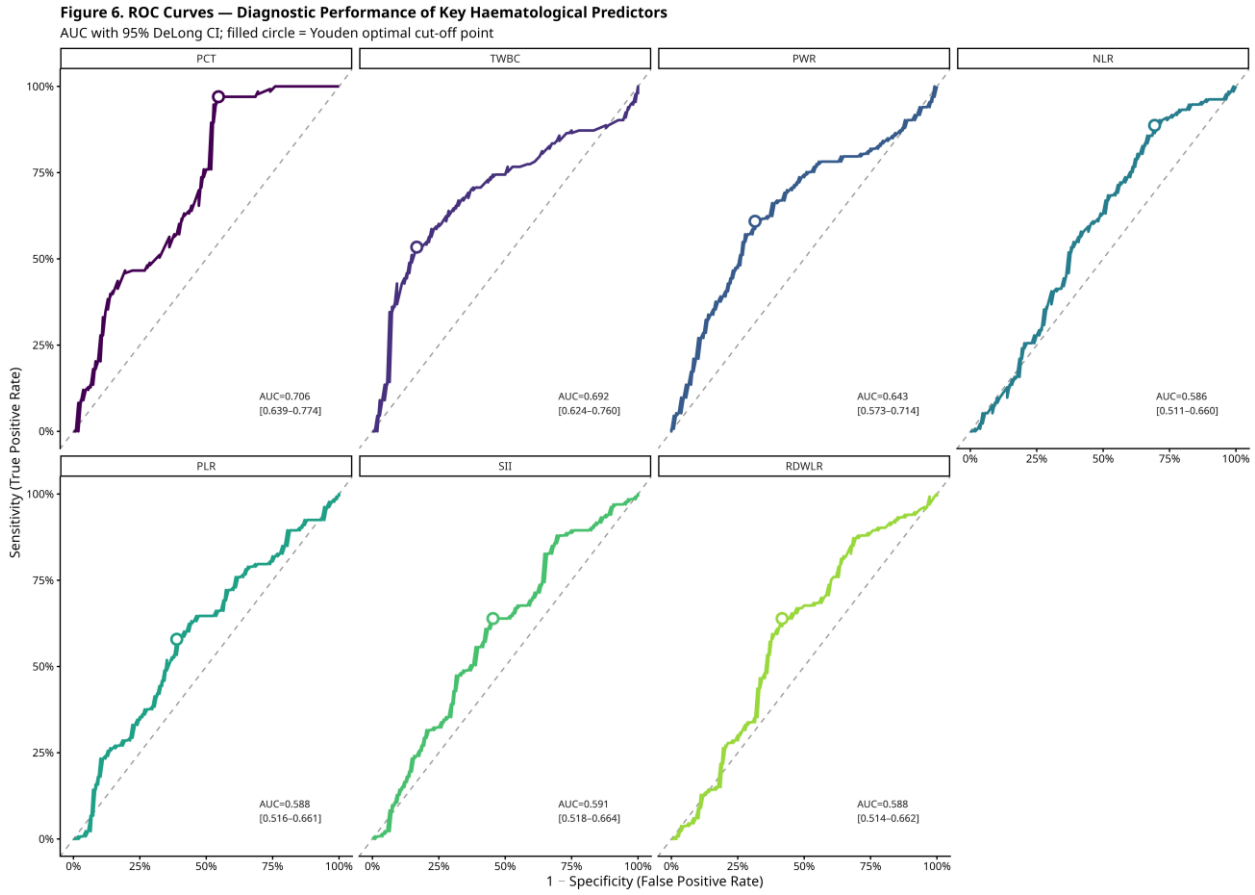

Figure 6. *ROC Curves — Diagnostic Performance of Key Haematological Predictors.* AUC with 95% DeLong CI shown for each predictor. Open circle = Youden-optimal cut-off point. Dashed diagonal = line of no discrimination. Upper row (left to right): PCT, TWBC, PWR, NLR. Lower row: PLR, SII, RDWLR.

ABO and RhD Blood Group Phenotype Distribution and Haematological Associations

ABO Blood Group Phenotype Frequency Distribution

Blood group O was the most prevalent phenotype in both groups: 73 of 133 Lassa fever patients (54.9%) and 60 of 108 controls (55.6%). Blood group A was the second most common in Lassa patients (33 of 133; 24.8%), followed by blood group B (20 of 133; 15.0%) and blood group AB (7 of 133; 5.3%). Pearson chi-square analysis revealed no statistically significant difference in the overall ABO distribution ($\chi^2(3) = 6.02$, $p = .111$). Individually, none of the four ABO contrasts reached statistical significance after Bonferroni correction (threshold $p < .013$), including blood group A (OR = 0.53, $p = .055$) and blood group B (OR = 1.88, $p = .052$). These results provide no evidence of ABO blood group-dependent susceptibility to or protection from Lassa fever.

4.5.2 RhD Blood Group Phenotype Frequency Distribution

RhD-positive status was the predominant phenotype in both Lassa fever patients (123 of 133; 92.5%) and controls (98 of 108; 90.7%). Fisher's exact test revealed no statistically significant difference in RhD phenotype distribution between groups ($p = .152$; OR = 0.35, 95% CI [0.06, 1.42]). The distribution and group

comparisons for both ABO and RhD phenotypes are presented in Table 4 and Figure 1. ABO-specific odds ratios are visualised in Figure 4.

Table 4

ABO and RhD Blood Group Phenotype Distribution and Association With Lassa Fever Status

Blood group	Lassa n (%) (n=133)	Control n (%) (n=108)	OR	95% CI	P
ABO blood group					
A	33 (24.8%)	16 (14.8%)	0.53	[0.27, 1.02]	.055
B	20 (15.0%)	27 (25.0%)	1.88	[0.99, 3.59]	.052
AB	7 (5.3%)	5 (4.6%)	0.87	[0.27, 2.84]	.822
O	73 (54.9%)	60 (55.6%)	1.03	[0.62, 1.71]	.917
ABO overall: $\chi^2(3) = 6.02$, $p = .111$					
RhD blood group					
RhD-positive	123 (92.5%)	98 (90.7%)	0.35	[0.06, 1.42]	.152
RhD-negative	10 (7.5%)	10 (9.3%)	—	—	.152

Note. OR = odds ratio (each group vs. all others combined). ABO distribution compared by Pearson chi-square (minimum expected cell = 5.38). RhD distribution compared by Fisher's exact test. No blood group phenotype showed a statistically significant association with Lassa fever status after Bonferroni correction ($\alpha/4 = .013$ for ABO contrasts).

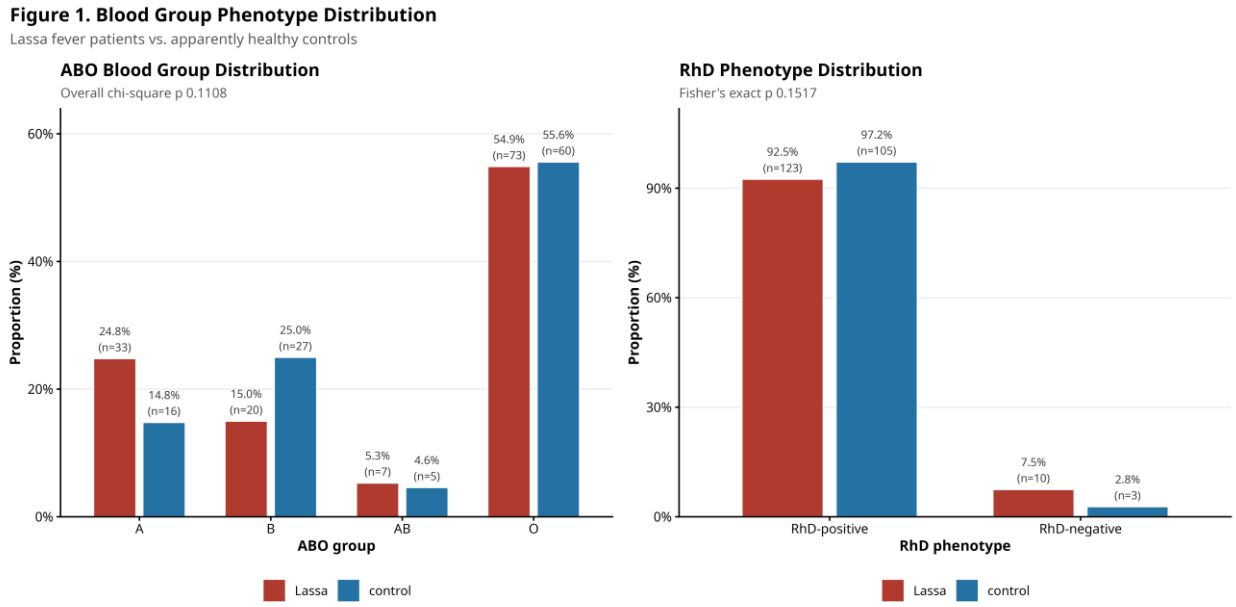

Figure 1. *Blood Group Phenotype Distribution — Lassa Fever Patients vs. Apparently Healthy Controls.* Left panel: ABO blood group distribution (overall chi-square p = .1108). Right panel: RhD phenotype distribution (Fisher's exact p = .1517). Red = Lassa (n = 133); Blue = Control (n = 108). Proportions within each group are displayed above bars.

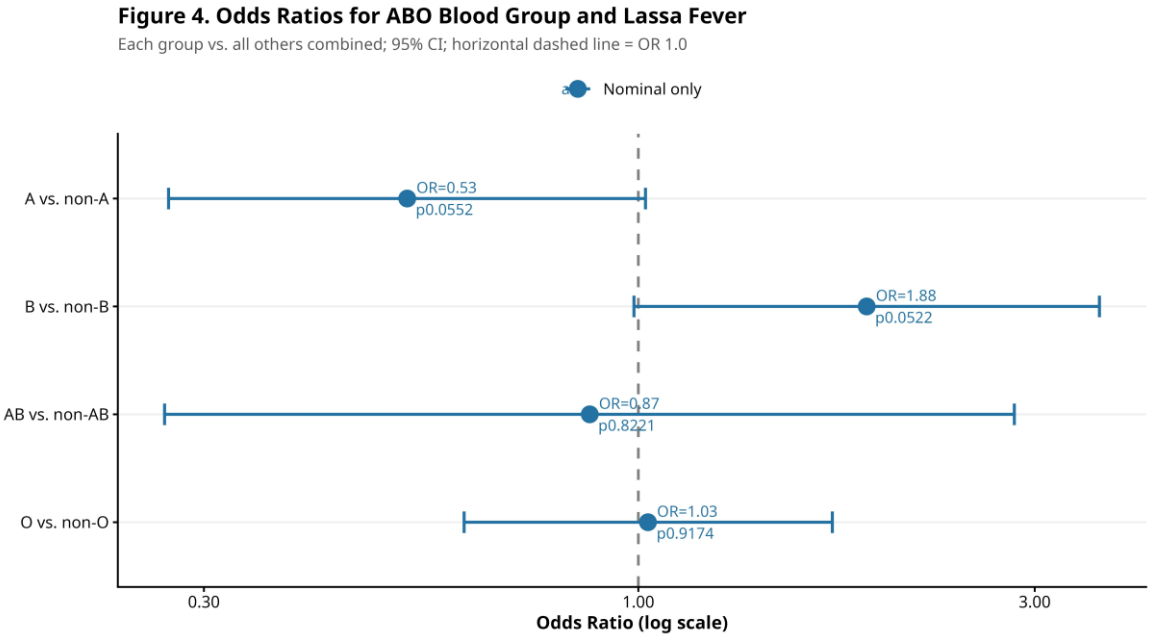

Figure 4. *Odds Ratios for ABO Blood Group and Lassa Fever.* Each group vs. all others combined. 95% CI shown. Horizontal dashed line = OR 1.0. Log scale x-axis. All ORs are nominal only; none survived Bonferroni correction (α/4 = .013).

4.5.3 Association Between ABO Blood Group and Haematological Parameters in Lassa Fever Patients

Omnibus comparisons across the four ABO groups were conducted for all FBC parameters and derived indices within the Lassa cohort (n = 133). Results are presented in Table 5. None of the 15 parameters tested demonstrated a statistically significant omnibus difference across ABO groups (all $p > .06$; all effect sizes ε^2 or $\eta^2 \leq .054$). Full post-hoc pairwise comparisons (234 tests) yielded no Bonferroni-significant pairwise contrasts. These results indicate that ABO blood group phenotype does not significantly modulate the haematological manifestations of Lassa fever within this cohort.

Table 5

Association Between ABO Blood Group and Haematological Parameters in Lassa Fever Patients (Omnibus Tests)

Parameter	Test	Statistic	p (omnibus)	Post-hoc	Effect size	N
TWBC	Kruskal-Wallis H	H(3) = 1.56	.669	Dunn (Bonf.)	$\varepsilon^2 = .012$	133
NEUT	One-way ANOVA	F(3,129) = 0.10	.962	Tukey HSD	$\eta^2 = .002$	133
LYMPH	One-way ANOVA	F(3,129) = 0.28	.837	Tukey HSD	$\eta^2 = .007$	133
PLT	One-way ANOVA	F(3,129) = 2.20	.091	Tukey HSD	$\eta^2 = .049$	133
MPV	One-way ANOVA	F(3,129) = 0.37	.774	Tukey HSD	$\eta^2 = .009$	133
PDW	Kruskal-Wallis H	H(3) = 6.45	.092	Dunn (Bonf.)	$\varepsilon^2 = .049$	133
PCT	One-way ANOVA	F(3,129) = 0.94	.422	Tukey HSD	$\eta^2 = .022$	133
NLR	Kruskal-Wallis H	H(3) = 0.26	.968	Dunn (Bonf.)	$\varepsilon^2 = .002$	133
PLR	Kruskal-Wallis H	H(3) = 4.60	.203	Dunn (Bonf.)	$\varepsilon^2 = .035$	133
SII	Kruskal-Wallis H	H(3) = 3.23	.358	Dunn (Bonf.)	$\varepsilon^2 = .025$	133
SIRI	Kruskal-Wallis H	H(3) = 1.41	.703	Dunn (Bonf.)	$\varepsilon^2 = .011$	133
RDWLR	Kruskal-Wallis H	H(3) = 0.25	.970	Dunn (Bonf.)	$\varepsilon^2 = .002$	133
Mentzer Index	Kruskal-Wallis H	H(3) = 7.02	.071	Dunn (Bonf.)	$\varepsilon^2 = .053$	133
PWR	Kruskal-Wallis H	H(3) = 4.80	.187	Dunn (Bonf.)	$\varepsilon^2 = .036$	133

HPR	Kruskal-Wallis H	H(3) = 7.10	.069	Dunn (Bonf.)	ε^2 = .054	133

Note. Analyses restricted to confirmed Lassa fever patients (n = 133). Omnibus tests: Kruskal-Wallis H (non-normal distributions) or one-way ANOVA (normal distributions with homogeneous variances). Post-hoc: Dunn's test with Bonferroni correction or Tukey HSD. Effect sizes: ε^2 (Kruskal-Wallis), η^2 (ANOVA). No parameter differed significantly across ABO groups. NEUT = neutrophil percentage; LYMPH = lymphocyte percentage; Bonf. = Bonferroni-corrected post-hoc.

Association Between RhD Blood Group and Haematological Parameters in Lassa Fever Patients (Exploratory)

Given the small number of Rh D-negative individuals in the Lassa fever cohort (n = 10), the following analysis is strictly exploratory and is presented for descriptive completeness rather than as hypothesis-testing evidence. Mann-Whitney U comparisons were conducted between Rh D-positive (n = 123) and RhD-negative (n = 10) Lassa fever patients across ten selected parameters, with BH-FDR adjustment (Table 6). No haematological parameter differed significantly between Rh D groups after BH-FDR correction (all adjusted p > .80). These findings are insufficient to draw inferential conclusions about Rh D-dependent haematological effects.

Table 6

Association Between RhD Blood Group and Selected Haematological Parameters in Lassa Fever Patients (Exploratory)

Parameter	RhD-positive (n=123)	RhD-negative (n=10)	Test statistic	p	BH-FDR p
TWBC	8.90 (5.00–15.04)	11.72 (8.79–16.62)	U = 455.0	.174	.802
PLT	218.00 (127.61–298.47)	232.88 (205.39–340.25)	U = 479.5	.249	.802
MPV	9.38 ± 1.18	9.17 ± 1.03	t(11.0) = 0.62	.547	.929
PDW	16.20 (15.66–16.76)	16.25 (15.62–17.02)	U = 597.0	.881	.929
PCT	1.67 (1.23–1.93)	1.93 (1.13–2.61)	U = 477.0	.241	.802
NLR	1.89 (1.28–2.64)	2.00 (1.23–3.55)	U = 576.5	.746	.929
PLR	7.13 (4.24–10.88)	9.06 (5.98–12.60)	U = 468.0	.211	.802
SII	360.37 (198.93–597.35)	606.30 (266.26–779.92)	U = 470.0	.218	.802
RDWLR	0.52 (0.39–0.68)	0.50 (0.40–0.66)	U = 650.5	.765	.929
RPR	0.07 (0.05–0.13)	0.06 (0.04–0.08)	U = 805.5	.105	.802

Note. Analyses restricted to confirmed Lassa fever patients (n = 133: RhD-positive n = 123, RhD-negative n = 10). All comparisons by Mann-Whitney U test. BH-FDR p = Benjamini-Hochberg false discovery rate-adjusted p-value. All findings are exploratory given the small RhD-negative subsample.

DISCUSSION

The observed reductions in erythrocyte parameters are biologically plausible because Lassa virus infection is characterized by widespread systemic inflammation, endothelial dysfunction, increased vascular permeability, and, in severe cases, haemorrhage. Elevated concentrations of pro-inflammatory cytokines, particularly interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), suppress erythropoietin activity and inhibit erythroid progenitor cell proliferation within the bone marrow, thereby reducing red cell production. In addition, capillary leakage, haemodilution following fluid resuscitation, nutritional deficiencies during prolonged illness, and occasional bleeding episodes may further contribute to reductions in haemoglobin concentration and packed cell volume.

These findings agree with those reported by [21, 29, 22, 16], who similarly observed reductions in haemoglobin concentration, packed cell volume, and other erythrocyte indices among patients with Lassa fever. The consistency of these findings across different endemic populations suggests that anemia is a reproducible manifestation of the disease. However, the magnitude of the reductions varies among studies, probably because of differences in disease severity, nutritional status, and timing of specimen collection, patient selection, and laboratory methodologies. Earlier studies also relied on less sophisticated haematology analyzers and often lacked appropriate control groups, limiting direct comparison with the present investigation.

Clinically, these findings suggest that routine assessment of erythrocyte parameters may assist in evaluating disease progression and identifying patients at increased risk of complications. Although these parameters are not sufficiently specific to establish a diagnosis independently, they provide valuable supportive information when interpreted alongside clinical findings and other laboratory investigations.

The present study also demonstrated significantly higher total white blood cell (TWBC) counts and neutrophil percentages, together with reduced lymphocyte percentages, among patients with Lassa fever. These changes reflect activation of the innate immune response accompanied by suppression of adaptive immunity during acute infection.

The increase in total white blood cell count and neutrophilia is consistent with cytokine-driven emergency granulopoiesis stimulated by inflammatory mediators such as IL-6 and granulocyte colony-stimulating factor (G-CSF). Activated neutrophils contribute to viral clearance through phagocytosis, cytokine production, and interactions with vascular endothelium. Conversely, lymphopenia probably results from impaired T-cell activation, lymphocyte apoptosis, and redistribution of lymphocytes to infected tissues following suppression of type I interferon responses by the virus.

These findings are consistent with reports by [21, 18, 22, 16], all of whom described leucocytosis accompanied by relative lymphopenia in patients with severe Lassa fever. Nevertheless, some investigators have reported normal or even reduced total white blood cell counts, particularly among patients presenting early in the course of infection. Such discrepancies may reflect differences in disease stage, viral load, host immune responses, and inclusion criteria across studies.

From a clinical perspective, alterations in leucocyte parameters provide useful evidence of systemic immune activation but should be interpreted cautiously because similar changes occur in several bacterial and viral infections. Their greatest value lies in combination with other haematological and inflammatory biomarkers rather than as isolated diagnostic indicators.

Unlike erythrocyte and leucocyte parameters, conventional platelet count did not differ significantly between patients and controls. This observation suggests that platelet quantity alone may not accurately reflect the haemostatic disturbances associated with Lassa fever. Viral haemorrhagic fevers are characterized by platelet

activation, endothelial injury, and immunothrombosis, processes that may alter platelet function without producing marked thrombocytopenia.

Interestingly, although platelet count remained relatively stable, significant alterations were observed in platelet-derived biomarkers, including plateletcrit (PCT), platelet distribution width (PDW), and mean platelet volume (MPV). This finding supports the concept that platelet function and platelet turnover are more profoundly affected than platelet numbers during acute Lassa fever. Increased platelet activation and peripheral consumption are likely accompanied by compensatory thrombopoiesis, resulting in the release of larger, more heterogeneous platelets into the circulation while maintaining overall platelet counts within the normal range.

This observation agrees with recent evidence from [22], who identified mean platelet volume as an independent predictor of mortality, and with studies of other viral haemorrhagic fevers demonstrating that platelet-derived indices are more sensitive indicators of disease activity than platelet count alone. Consequently, platelet-derived biomarkers may represent more informative laboratory indicators of disease severity and inflammatory activation than conventional platelet enumeration.

Overall, the findings of the present study demonstrate that routine haematological parameters provide important insights into the pathophysiological changes occurring during Lassa fever. The observed alterations in erythrocyte indices reflect impaired erythropoiesis and inflammatory anemia, whereas leucocyte changes indicate activation of innate immunity and suppression of adaptive immune responses. In contrast, the preservation of platelet count despite significant changes in platelet-derived indices suggests that platelet function rather than platelet quantity is a more sensitive marker of disease-related haemostatic dysfunction. Collectively, these findings support the continued use of routine full blood count analysis as an accessible and cost-effective component of the laboratory evaluation of patients with suspected Lassa fever, particularly in resource-limited settings where access to molecular diagnostics may be delayed.

The second objective of this study was to evaluate the diagnostic significance of derived haematological and systemic inflammatory indices in patients with laboratory-confirmed Lassa fever. Unlike conventional full blood count parameters, these composite biomarkers integrate information from multiple haematological variables and provide a more comprehensive assessment of the balance between inflammation, immune activation, erythropoiesis, and haemostasis. The present study demonstrated that several derived indices differed significantly between patients with Lassa fever and apparently healthy controls, indicating that these biomarkers reflect the complex host response to acute Lassa virus infection.

A major strength of this study is the simultaneous evaluation of twenty-two derived inflammatory and haematological indices generated from routine full blood count parameters. While previous investigations of Lassa fever have largely concentrated on conventional haematological variables, the present study extends current knowledge by systematically assessing composite biomarkers using advanced statistical approaches, including Least Absolute Shrinkage and Selection Operator.

The third objective of this study was to evaluate the distribution of ABO and Rh D blood group phenotypes among patients with laboratory-confirmed Lassa fever and apparently healthy controls and to determine whether these blood group systems were associated with susceptibility to infection. Although the ABO and Rh D blood group systems have been implicated in the pathogenesis and susceptibility of several infectious diseases, the present study found limited evidence to support a strong association between blood group phenotype and Lassa fever. These findings suggest that routine haematological and inflammatory biomarkers may have greater diagnostic value than ABO and Rh D blood group phenotypes in the laboratory assessment of Lassa fever.

The ABO blood group system is one of the most extensively studied genetic factors in infectious disease susceptibility. Previous investigations have demonstrated associations between ABO blood groups and several viral infections, including severe acute respiratory syndrome (SARS), coronavirus disease 2019 (COVID-19),

norovirus infection, and hepatitis B virus infection [27, 30]. Proposed mechanisms include differences in pathogen adhesion, immune recognition, inflammatory responses, and coagulation pathways mediated by ABO antigens expressed on erythrocytes, endothelial cells, and other tissues. However, these associations are highly pathogen-specific and depend largely on the biological mechanisms through which individual microorganisms interact with host cells.

Unlike viruses such as SARS-CoV-2, which may be influenced by ABO-related immunological mechanisms, Lassa virus primarily gains cellular entry through α-dystroglycan and lysosome-associated membrane protein 1 (LAMP1) receptors. These receptors are not directly determined by ABO antigen expression. Consequently, there is no strong biological basis to expect a major direct effect of ABO phenotype on viral entry or replication. Any observed association would therefore more likely reflect indirect influences on inflammatory responses, endothelial function, coagulation pathways, or population genetic factors rather than a direct effect on susceptibility to infection.

In the present study, the distribution of ABO blood groups among patients with confirmed Lassa fever generally reflected the pattern expected within the local population. Although some variations in blood group frequencies were observed between cases and controls, these differences were not sufficiently strong to support a clinically meaningful association between ABO phenotype and Lassa fever susceptibility. This finding is consistent with the limited and inconclusive evidence currently available in the literature. Most previous investigations have reported variable results, often influenced by small sample sizes, geographical differences, ethnic variations in blood group distribution, and methodological limitations.

The absence of a strong association in the present study may indicate that ABO blood group frequencies among infected individuals largely mirror the underlying distribution of blood groups within the general population. This interpretation is biologically plausible because the primary determinants of Lassa fever transmission are environmental exposure, rodent contact, healthcare-associated transmission, and host immune responses rather than inherited blood group characteristics. Consequently, factors such as viral load, immune competence, and access to healthcare are likely to exert a substantially greater influence on disease occurrence and outcome than ABO phenotype.

Similarly, evaluation of the Rh D blood group system revealed no compelling evidence of a significant relationship between Rh D status and susceptibility to Lassa fever. Although Rh D antigens are clinically important in transfusion medicine and haemolytic disease of the newborn, their role in infectious disease susceptibility remains poorly understood. Previous studies investigating Rh D status in viral infections have produced inconsistent findings, and there is currently no established biological mechanism linking Rh D expression to Lassa virus infection. The findings of the present study therefore support the view that Rh D status is unlikely to be a major determinant of disease susceptibility.

An important strength of this investigation is that ABO and RhD phenotypes were evaluated alongside conventional haematological parameters, platelet-derived biomarkers, and derived inflammatory indices within the same study population. This approach allowed direct comparison of their relative diagnostic contributions. While several haematological and inflammatory biomarkers demonstrated significant associations with laboratory-confirmed Lassa fever, ABO and Rh D blood group phenotypes contributed comparatively little to disease prediction. This observation suggests that blood group systems have limited value as standalone biomarkers for identifying infected individuals.

The findings of the present study also highlight an important methodological limitation within previous research. Many published studies examining blood group associations in infectious diseases have relied primarily on descriptive comparisons without adjusting for potential confounding variables. Such approaches may overestimate the importance of observed associations. By contrast, the present study evaluated blood group phenotypes within a broader analytical framework that included multivariable statistical modelling and

assessment of multiple laboratory parameters. This provides a more robust evaluation of their potential contribution to disease prediction.

From a clinical perspective, the results indicate that ABO and Rh D blood group phenotypes should not be considered reliable diagnostic markers for Lassa fever. Their routine determination remains essential for transfusion support and patient management, particularly in severely ill patients who may require blood products. However, the findings suggest that greater emphasis should be placed on conventional haematological parameters, platelet-derived biomarkers, and derived systemic inflammatory indices when assessing patients with suspected infection.

Overall, the present study demonstrates that although ABO and Rh D blood group systems remain important biological characteristics, their contribution to the diagnosis and prediction of Lassa fever appears limited. In contrast, routine haematological parameters and derived inflammatory biomarkers provide more direct reflections of the host response to infection and therefore offer greater potential for clinical application. These findings contribute to the limited body of evidence regarding blood group phenotypes and Lassa fever and help clarify their relative importance within the broader spectrum of laboratory biomarkers evaluated in this study.

Conclusion

This study provides a comprehensive evaluation of the haematological profile of patients with confirmed Lassa fever in the Abakaliki endemic zone of Ebonyi State, Nigeria, and demonstrates the diagnostic value of routine full blood count parameters in the early identification of the disease.

The findings showed that Lassa fever is characterised by a distinct haematological pattern involving leucocytosis with relative neutrophilia and lymphopenia, preserved erythrocyte indices despite elevated mean corpuscular haemoglobin concentration, and characteristic platelet abnormalities marked by increased plateletcrit (PCT) and platelet distribution width (PDW) with reduced mean platelet volume (MPV). These changes indicate that platelet indices provide more useful diagnostic information than platelet count alone and that normal haemoglobin concentration should not be used to exclude Lassa fever during the early stage of infection.

Among the derived inflammatory indices evaluated, the platelet-to-white blood cell ratio (PWR) was the only marker that remained statistically significant after correction for multiple comparisons. Multivariable analysis further identified plateletcrit as the only independent haematological predictor of confirmed Lassa fever, while receiver operating characteristic analysis demonstrated that PCT had the highest diagnostic performance. A PCT threshold of 0.428% provided high sensitivity for screening suspected cases, whereas a total white blood cell count above 8.78×10^9/L offered greater specificity and complemented PCT in clinical assessment before RT-PCR confirmation.

The study also found no significant association between ABO or RhD blood group phenotypes and susceptibility to Lassa fever or the observed haematological abnormalities, indicating that blood group typing has no diagnostic or prognostic value in the management of the disease.

Overall, this study demonstrates that routinely available haematological parameters, particularly plateletcrit and total white blood cell count, can provide valuable support for the early identification and risk stratification of suspected Lassa fever patients in resource-limited settings. Incorporating these findings into clinical practice and laboratory reporting, following further external validation, has the potential to improve early diagnosis, guide timely treatment, and strengthen Lassa fever case management in endemic area

References

1.Salvato, M. S., Clegg, J. C. S., Buchmeier, M. J., Charrel, R. N., Gonzalez, J. P., Lukashevich, I. S., Peters, C. J., Rico-Hesse, R., & Romanowski, V. (2005). Family Arenaviridae. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, & L. A. Ball (Eds.), *Virus taxonomy: Eighth report of the International Committee on Taxonomy of Viruses* (pp. 725–733). Academic Press.

2. Andersen, K. G., Shapiro, B. J., Matranga, C. B., Sealfon, R., Lin, A. E., Moses, L. M., Folarin, O. A., Goba, A., Odia, I., Ehiane, P. E., Momoh, M., England, E. M., Winnicki, S., Branco, L. M., Gire, S. K., Phelan, E., Tariyal, R., Tewhey, R., Omoniwa, O., & Sabeti, P. C. (2015). Clinical sequencing uncovers origins and evolution of Lassa virus. *Cell*, *162*(4), 738–750. https://doi.org/10.1016/j.cell.2015.07.020

3. World Health Organization. (2018). *WHO list of blueprint priority diseases*. https://www.who.int/blueprint/priority-diseases/en/

4. Olayemi, A., Cadar, D., Magassouba, N., Obadare, A., Kourouma, F., Oyeyiola, A., Fasogbon, S., Igbokwe, J., Rieger, T., Bockholt, S., Jerónimo, B., Fichet-Calvet, E., & Schmidt-Chanasit, J. (2016). New hosts of the Lassa virus. *Scientific Reports*, *6*, Article 25280. https://doi.org/10.1038/srep25280

5. Sogoba, N., Feldmann, H., & Safronetz, D. (2012). Lassa fever in West Africa: Evidence for an expanded region of endemicity. *Zoonoses and Public Health*, *59*(Suppl. 2), 43–47. https://doi.org/10.1111/j.1863-2378.2012.01469.x

6. Adesola, R. O., Olusegun, A., Oguntola, F., & Yusuf, M. (2024). Challenges associated with re-emergence of Lassa fever in Nigeria: An exploratory study of epidemiology, phylogenomics, and recommendations toward its eradication. *Health Science Reports*, *7*(11), e70225. https://doi.org/10.1002/hsr2.70225

7. Ilori, E. A., Furuse, Y., Ipadeola, O. B., Dan-Nwafor, C. C., Abubakar, A., Womi-Eteng, O. E., Ogbaini-Emovon, E., Okogbenin, S., Unigwe, U., Ogah, E., Ayodeji, O., Abejegah, C., Liasu, A. A., Musa, E. O., Woldetsadik, S. F., Lasuba, C. L. P., Alemu, W., & Ihekweazu, C. (2019). Epidemiologic and clinical features of Lassa fever outbreak in Nigeria, January 1–May

8. Nigeria Centre for Disease Control. (2023). *Lassa fever situation report, week 1–17, 2023*. NCDC. https://ncdc.gov.ng/diseases/sitreps

9. Brosh-Nissimov, T. (2016). Lassa fever: Lessons for the next decade. *Internal and Emergency Medicine*, *11*(2), 169–178. https://doi.org/10.1007/s11739-015-1342-5

10. Signorell, A. (2024). *DescTools: Tools for descriptive statistics* (R package version 0.99.54). https://CRAN.R-project.org/package=DescTools

11. Ly, H. (2020). Lassa fever: Viral replication, disease pathogenesis, and host immune modulations. *Pathogens*, *9*(6), Article 437. https://doi.org/10.3390/pathogens9060437

12. Terüg, J. C., Ducomble, T., Miranda, O., Grais, R. F., & Staderini, N. (2021). Lassa fever outcomes and prognostic factors in Nigeria (LASCOPE): A prospective cohort study. *Lancet Global Health*, *9*(4), e469–e478. https://doi.org/10.1016/S2214-109X(20)30518-0

13. McCormick, J. B., King, I. J., Webb, P. A., Scribner, C. L., Craven, R. B., Johnson, K. M., Elliott, L. H., & Belmont-Williams, R. (1986). Lassa fever: Effective therapy with ribavirin. *New England Journal of Medicine*, *314*(1), 20–26. https://doi.org/10.1056/NEJM198601023140104

14. Okokhere, P., Colubri, A., Azige, S., Iruolagbe, C., Adomeh, D., Odia, I., Ehimuan, J., Aire, C., Ibeh, M., Peel, T., & Shabman, R. (2018). Clinical and laboratory predictors of Lassa fever outcome in a dedicated treatment facility in Nigeria: An observational cohort study. *The Lancet Infectious Diseases*, *18*(6), 684–695. https://doi.org/10.1016/S1473-3099(18)30121-X

15. Cummins, D., McCormick, J. B., Bennett, D., Samba, J. A., Farrar, B., Machin, S. J., & Fisher-Hoch, S. P. (1990). Acute sensorineural deafness in Lassa fever. *JAMA*, *264*(16), 2093–2096. https://doi.org/10.1001/jama.1990.03450160077033

16. Ireneh, R. U., Temitope, A., Chukwuemeka, O., & Akindele, B. (2025). Lassa hemorrhagic fever and haematologic dysregulation: Insights from Federal Medical Center, Owo, Ondo State, Nigeria. *Journal of Interventional Epidemiology and Public Health*, *28*(2). https://doi.org/10.11604/pamj-ph.2025.28.2.47095

17. Afolabi, O. K., Wusu, A. D., Ogunlana, O. O., Adeagbo, B. A., & Badmus, J. A. (2022). Reference intervals for haematological parameters in apparently healthy adults in Southwest Nigeria. *Pan African Medical Journal*, *43*, Article 184. https://doi.org/10.11604/pamj.2022.43.184.35521

18. Baize, S., Pannetier, D., Faure, C., Marianneau, P., Marendat, I., Georges-Courbot, M.-C., & Deubel, V. (2012). Immune responses and Lassa virus infection. *Viruses*, *4*(11), 2766–2785. https://doi.org/10.3390/v4112766

19. Connolly-Andersen, A. M., Whitaker, H., Klingström, J., & Ahlm, C. (2014). The role of platelets in the pathogenesis of viral hemorrhagic fevers. *PLOS Neglected Tropical Diseases*, *8*(6), Article e2858. https://doi.org/10.1371/journal.pntd.0002858

20. Hastie, K. M., Kimberlin, C. R., Zandonatti, M. A., MacRae, I. J., & Saphire, E. O. (2011). Structure of the Lassa virus nucleoprotein C terminus reveals a unique fold important for protein function. *Journal of Biological Chemistry*, *286*(44), 38208–38217. https://doi.org/10.1074/jbc.M111.268953

21. Fisher-Hoch, S. P., McCormick, J. B., Sasso, D., & Craven, R. B. (1988). Hematologic dysfunction in Lassa fever. *Journal of Medical Virology*, *26*(2), 127–135. https://doi.org/10.1002/jmv.1890260204

22. Okpunu, E. C., Olanrewaju, D. O., Otumu, O. S., Dic-Ijiewere, E. O., Awodu, O. A., & Nwankwo, C. C. (2025). Haematological profile and mortality in patients with Lassa fever in Irrua Specialist Teaching Hospital, Irrua, Nigeria. *International Blood Research & Reviews*, *16*(4), 70–81. https://doi.org/10.9734/ibrr/2025/v16i4371

23. Muhammad, S., Fischer, I., Naderi, S., Faghih Jouibari, M., Abdolrezaee, S., & Karimialavijeh, E. (2021). Systemic inflammatory index is a novel predictor of intubation requirement and mortality after SARS-CoV-2 infection. *Pathogens*, *10*(1), Article 58. https://doi.org/10.3390/pathogens10010058

24. Wang, R. H., Wen, W. X., Jiang, Z. P., Du, Z. P., Ma, Z. H., Lu, A. L., Li, H. P., Yuan, F., Wu, S. B., Guo, J. W., Cai, Y. F., Huang, Y., Wang, L. X., & Lu, H. J. (2023). The clinical value of neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), platelet-to-lymphocyte ratio (PLR) and systemic inflammation response index (SIRI) for predicting the occurrence and severity of pneumonia in patients with intracerebral hemorrhage. *Frontiers in Immunology*, *14*, Article 1115031. https://doi.org/10.3389/fimmu.2023.1115031

25. Yalcindag, A., Celebi, S., Gultepe, B., Gencoglu, M., Goksen, M., Kisa, U., Erduran, E., Kalagara, I., Karadag, A., & Turan, N. (2024). Multi-inflammatory index as a novel predictive and prognostic marker in patients with Crimean-Congo haemorrhagic fever. *Pathogens*, *13*(3), 189. https://doi.org/10.3390/pathogens13030189

26. Korniluk, A., Koper-Lenkiewicz, O. M., Kaminska, J., Kemona, H., & Dymicka-Piekarska, V. (2019). Mean platelet volume (MPV): New perspectives for an old marker in the course and prognosis of inflammatory conditions. *Mediators of Inflammation*, *2019*, Article 9213074. https://doi.org/10.1155/2019/9213074

27. Cooling, L. (2015). Blood groups in infection and host susceptibility. *Clinical Microbiology Reviews*, *28*(3), 801–870. https://doi.org/10.1128/CMR.00109-14

28. Samra, S., Habeb, M., & Nafae, R. (2021). ABO groups can play a role in susceptibility and severity of COVID-19. *Egyptian Journal of Immunology*, *28*(1), 51–61. https://doi.org/10.1186/s43168-020-00051

29. Owhin, S. O., Abejegah, C., Olatunde, L., Akhideno, P. E., Emorinken, A., Adelabu, Y., & Boma, P. O. (2020). The impact and morphology of anaemia among Lassa fever patients treated in a dedicated treatment center in south-west Nigeria. *Journal of Tropical Medicine and Health*, *4*, 148.

30. Engelmann, B., & Massberg, S. (2013). Thrombosis as an intravascular effector of innate immunity. *Nature Reviews Immunology*, *13*(1), 34–45. https://doi.org/10.1038/nri3345.