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Anne Sorelle Nsang De Toue I
University of Montagnes
Claude Tayou Tagny
Universite de Yaounde I faculte des sciences biomedicales et Medical
Romaric TUONO DE MANFOUO
romatuono@yahoo.fr

University of Montagnes
Josué Simo Louokdom
University of Montagnes
Maryline Seuko Njopwouo
Universite de Yaounde I faculte des sciences biomedicales et Medical
Paul Koki Ndombo
Centre Mère Enfant de la Fondation Chantal Biya

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ERYTHROCYTE MORPHOLOGICAL ABNORMALITIES AND ASSOCIATIONS WITH CLINICAL PROFILES IN CHILDREN AGED 0 TO 5 YEARS, YAOUNDÉ-CAMEROON: AN ANALYTICAL STUDY

Anne Sorelle Nsang De Toue II[1], Claude Tayou Tagny[2], Romaric De Manfouo Tuono[1], Simo Josué Louokdom[1], Maryline Seuko Njopwouo[2], Koki Ndombo Paul[3]

[1] Higher Institute of Health Sciences, University of Montagnes, "*Bangangté",* Cameroon

[2] Faculty of Medicine and Biomedical Sciences, "*Université de Yaoundé 1*, *Yaoundé"*, Cameroon

[3]*Centre Mère Enfant de la Fondation Chantal Biya, Yaoundé"*, Cameroon

***** Corresponding author:
Romaric De Manfouo Tuono
Email: romatuono@yahoo.fr
Mobile phone: +237 695399394
https://orcid.org/ 0000-0002-2867-0538

SUMMARY

Background: Peripheral blood smears are an accessible diagnostic tool for identifying erythrocyte morphological abnormalities associated with hematological, infectious, or nutritional disorders. Despite their diagnostic value, their use remains limited in many resource-constrained settings. During early childhood, many inherited red cell disorders may present with nonspecific or absent morphological phenotypes, making genetic diagnosis challenging in low-resource settings. This study described the frequency and distribution of erythrocyte morphological abnormalities in children aged 0 to 5 years and evaluated their associations with clinical profiles.

Methods: A cross-sectional, analytical study was conducted from January 1 to June 30, 2025, at the Chantal Biya Foundation's Mother and Child Center. 158 children aged 0 to 60 months were enrolled. Blood smears were prepared from capillary or venous blood collected in EDTA and stained using May-Grünwald-Giemsa, according to International Council for Standardization in Haematology (ICSH) recommendations for blood smear review. Slides were blindly analyzed by three biologists. Ten consecutive immersion fields (×100) were examined per smear to identify and quantify erythrocyte morphological abnormalities. Sociodemographic, clinical, and biological data were correlated with the abnormalities using Pearson's Chi^2 or Fisher's exact test, with significance set at $p < 0.05$.

Results: Among the 158 children included, 78.6% (n = 125) were anemic. Shape abnormalities predominated (86.1%), notably anisopoikilocytosis (82.3%), schistocytes (44.9%), echinocytosis (42.4%), and polychromatophilia (41.1%). Three or more abnormalities were found in 63.3% of the children. The number of abnormalities was significantly associated with hemoglobin ($p = 0.001$), hematocrit ($p = 0.003$), and reticulocytes ($p < 0.001$). No significant association was found between the main morphological abnormalities and anemia or clinical parameters ($p > 0.05$).

Conclusion: Erythrocyte morphological abnormalities were highly prevalent in the children. Their accumulation was associated with more severe anemia and increased reticulocyte counts. Although individual abnormalities were not statistically associated with clinical profiles, specific findings such as sickle cells and target cells represent accessible and valuable diagnostic clues for underlying hemoglobinopathies and other inherited red cell disorders in resource-limited settings. Systematic blood smear examination, coupled with a stepwise diagnostic approach, can improve

early recognition of both common and rarer genetic conditions when advanced testing is not immediately available.

Keywords: erythrocyte abnormalities, blood smear, children, clinical profiles, Cameroon

1. INTRODUCTION

Red blood cells (RBCs), or erythrocytes, are anucleated cells produced in the bone marrow from hematopoietic stem cells. Their physiological lifespan is estimated at approximately 120 days. Thanks to their flexible lipid membrane and complex protein cytoskeleton, RBCs adopt a characteristic biconcave shape that optimizes gas exchange, facilitates their deformability in the microcirculation, and ensures efficient oxygen transport to the tissues. Any change in their morphology can therefore reflect a disruption of erythropoiesis, membrane damage, a hemoglobin abnormality, or significant oxidative stress. Morphological examination of erythrocytes is thus a valuable indicator in the evaluation of hematological and systemic diseases [1–3].

Erythrocyte morphological abnormalities include changes in size (anisocytosis, microcytosis, macrocytosis), color (hypochromia, polychromatophilia), and shape (poikilocytosis). Some abnormalities have significant diagnostic value. For example, sickle cells suggest sickle cell disease, schistocytes suggest mechanical hemolysis or thrombotic microangiopathy, while target cells are frequently observed in hemoglobinopathies and iron deficiency. Similarly, the presence of spherocytes, elliptocytes, or dacryocytes may indicate constitutional or acquired red blood cell disorders. Thus, morphological analysis of the peripheral blood smear remains an essential examination in hematology, allowing clinicians to quickly identify certain etiologies that are sometimes missed by the complete blood count (CBC) alone [4].

In children aged 0 to 5 years, this analysis is of particular importance. This age group corresponds to a period of rapid growth characterized by increased micronutrient requirements, progressive maturation of the immune system, and intense erythropoietic activity. Young children also exhibit increased vulnerability to infections, nutritional deficiencies, and genetic diseases that can alter red blood cell morphology. Furthermore, their still-immature antioxidant defense mechanisms promote greater susceptibility to oxidative stress and hemolysis. In this context, peripheral blood smears constitute a simple, inexpensive, and particularly useful diagnostic tool in resource-limited settings [5].

In sub-Saharan Africa, and particularly in Cameroon, several common pediatric diseases are associated with erythrocyte morphological abnormalities. Malaria remains one of the leading causes of morbidity and mortality in children under five years of age in endemic areas. [6,7] This infection leads to significant changes in red blood cells related to hemolysis and inflammatory stress [8,9]. Nutritional deficiencies, primarily iron deficiency, also represent a major public health problem, promoting the occurrence of microcytosis and sometimes severe hypochromia [10,11]. In addition, hemoglobinopathies, particularly sickle cell disease, are very common in Cameroon, where the prevalence of homozygous sickle cell disease is estimated at between 2 and 3% of births [12]. In these patients, blood smears may reveal sickle cells, target cells, anisopoikilocytes, and other signs of chronic hemolysis [12].

Despite the diagnostic importance of red blood cell morphology, its use remains insufficient in many African healthcare facilities. In some hospital settings, blood smear interpretation is limited by a lack of equipment, qualified personnel, or the priority given to automated tests. However, several studies have shown that combining complete blood count (CBC) parameters with morphological analysis significantly improves the diagnostic approach to anemias and infectious syndromes in children [13]. In Cameroon, little data is available regarding the frequency of erythrocyte morphological abnormalities in young children and their associations with clinical profiles observed in hospital practice.

The aim of this study was therefore not only to describe the frequency and distribution of erythrocyte morphological abnormalities in children aged 0 to 5 years, but also to explore their potential value as accessible diagnostic clues for underlying genetic and acquired disorders in a resource-constrained pediatric setting. By highlighting both the limitations of nonspecific findings and the orientation value of more specific abnormalities (such as sickle cells and target cells), this work seeks to support a pragmatic, stepwise diagnostic approach that can be implemented where advanced genetic testing is not routinely available.

2. METHODS

2.1.Design, Framework and Study Period

We conducted a descriptive and analytical cross-sectional study with prospective recruitment over a six-month period from January 1 to June 30, 2025. The study took place in the pediatric emergency department of the Mother and Child Center of the Chantal Biya Foundation , a leading hospital for the management of pediatric illnesses in Cameroon. This department receives a large number of children daily from Yaoundé and other regions of the country, presenting with a variety of pathologies, including infectious, nutritional, hematological, and genetic disorders.

The biological analyses focused primarily on complete blood counts (CBCs) and morphological evaluation of red blood cells from peripheral blood smears. Morphological analyses were performed in two reference laboratories: the hematology laboratory of the Pasteur Center of Cameroon and the laboratory of the Mother and Child Center of the Chantal Biya Foundation . Both facilities have qualified medical biology staff and implement standardized procedures that comply with the requirements of ISO 15189, the standard for the quality and competence of medical biology laboratories.

2.2.Study population

The study population consisted of children aged 0 to 60 months who were seen or hospitalized at the Chantal Biya Foundation's Mother and Child Center during the study period and for whom a complete blood count had been prescribed as part of their medical care. Only children who had undergone a complete clinical evaluation by a general practitioner or pediatrician were included to ensure the reliability of the clinical data collected. Participation in the study required prior informed consent signed by the parents or legal guardians.

Children with blood samples that did not conform to the biological analysis were excluded from the study, particularly in cases of sample coagulation, significant hemolysis, insufficient blood volume, incomplete biological data, or blood smears of poor technical quality. Children who had received a blood transfusion or a bone marrow transplant within the three months preceding enrollment were also excluded, as these situations can artificially alter hematological parameters and erythrocyte morphology. Finally, any withdrawal of parental consent during the study automatically resulted in the exclusion of the participant concerned.

Anemia has been defined according to World Health Organization criteria as a hemoglobin level below 11 g/dL in children aged 6 to 59 months [14]. However, for the purposes of comparative analysis, a threshold of 12 g/dL was chosen to improve the sensitivity of detecting early erythrocyte abnormalities. This choice is based on studies showing that hemoglobin concentrations below 12 g/dL may be associated with decreased red blood cell function, impaired oxygen transport, and increased susceptibility to infections and developmental disorders in children [11].

2.3. Clinical evaluation of participants

Before undergoing biological tests, all children included in the study received a complete clinical evaluation by a general practitioner or pediatrician from the Mother and Child Center of the Chantal Biya Foundation. This evaluation aimed to guide medical management and identify clinical situations likely to be associated with hematological or morphological abnormalities of red blood cells, thus justifying the prescription of a complete blood count combined with a peripheral blood smear.

Sociodemographic data included age, sex, and mode of admission (outpatient or inpatient). Interviews with parents or legal guardians sought information on the child's main medical history, including previous episodes of anemia, transfusion history, repeated hospitalizations, recurrent infections, a family history of sickle cell disease or other hemoglobinopathies, as well as nutritional and vaccination history.

The clinical examination included an assessment of the child's general condition, vital signs, and the main functional and physical signs that might suggest a hematological or infectious disorder. Particular attention was paid to the presence of pallor of the skin and mucous membranes, jaundice, fever, asthenia, dyspnea, tachycardia, hemorrhagic syndrome, edema, lymphadenopathy, hepatomegaly, or splenomegaly. Signs suggestive of malnutrition, dehydration, or severe infection were also systematically sought.

The prescription of peripheral blood smears was primarily motivated by the presence of clinical or laboratory abnormalities suggesting an underlying hematological disorder. The main indications included anemic syndromes, prolonged febrile states, suspected severe malaria, jaundice, abnormal complete blood counts, suspected hemoglobinopathies, and the investigation

of cytopenias or hemolysis. In some patients, the blood smear also allowed for the detection of specific morphological abnormalities suggestive of iron deficiency, sickle cell disease, parasitic infection, or another systemic condition.

The clinical diagnoses established following the medical evaluation were grouped into different pathological categories, including infectious diseases, nutritional disorders, anemic syndromes, hemoglobinopathies, and other pediatric conditions. These clinical data were then compared with the morphological findings observed on blood smears to investigate the associations between erythrocyte abnormalities and the clinical profiles of the children included in the study.

2.4.Morphological analysis procedure

Blood smears were prepared from capillary or venous blood samples collected in EDTA tubes, within a maximum of two hours of collection to minimize secondary morphological changes related to sample storage. After gentle homogenization of the sample, a thin drop of blood was spread onto a clean, degreased slide according to the standard peripheral blood smear technique. The slides were then air-dried before staining.

Morphological examination of blood smears was performed after May-Grünwald-Giemsa staining, in accordance with the standardized procedures recommended in hematology (ICSH recommendations for blood smear review) [15]. This method allows for optimal differentiation of cellular structures and facilitates the identification of erythrocyte morphological abnormalities, including size abnormalities (anisocytosis, microcytosis, macrocytosis), color abnormalities (hypochromia, polychromatophilia), and shape abnormalities (poikilocytosis, target cells, sickle cells, schistocytes, spherocytes). Morphological analysis of the blood smear remains an essential tool in the diagnostic guidance of anemias and hematological disorders.

Each slide was examined independently and blindly by three experienced medical biologists, each with at least five years of experience in morphological hematology. The observers were not provided with the patients' clinical data or laboratory results to minimize interpretation bias. Microscopic examination was performed using an optical microscope equipped with a 100x immersion objective, after prior selection of the optimal viewing area corresponding to a homogeneous distribution of blood cells.

For each smear, ten consecutive immersion fields were systematically analyzed, corresponding to the evaluation of approximately 1000 to 2000 erythrocytes per observer. Morphological abnormalities were described qualitatively and then quantified according to their frequency of observation. Particular attention was paid to abnormalities in size, shape, and staining, as well as the presence of intraerythrocytic inclusions.

To ensure the reproducibility and reliability of the morphological data, an inter-observer quality control procedure was implemented. In cases of discrepancies exceeding 15% between readers regarding the quantification of observed anomalies, a peer review of the slide was conducted until a diagnostic consensus was reached. This approach helped to limit variability in interpretation and improve the accuracy of the morphological results obtained [4].

A complete blood count (CBC), including hemoglobin concentration, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width, was performed using a Mindray automated hematology analyzer. These parameters were obtained to provide a comprehensive quantitative assessment of erythrocyte characteristics and to facilitate comparison and concordance with the morphological findings obtained from peripheral blood smear examination. The automated indices were considered complementary to microscopic assessment and were used to support the interpretation, completeness, and consistency of the observed erythrocyte morphological abnormalities.

2.5.Supplementary Material

Representative microscopic images of the erythrocyte morphological abnormalities observed in the peripheral blood smears are provided in the Supplementary Material. These images illustrate the different morphological abnormalities identified during microscopic examination.

Supplementary Figure S1. Representative microscopic images of acanthocytes.

Supplementary Figure S2. Representative microscopic images of anisopoikilocytosis.

Supplementary Figure S3. Representative microscopic images of target cells and sickle cells.

Supplementary Figure S4. Representative microscopic images of target cells.

Supplementary Figure S5. Representative microscopic images of sickle cells.

Supplementary Figure S6. Representative microscopic images of echinocytes.

Supplementary Figure S7. Representative microscopic images of Parasitized erythrocytes showing Plasmodium trophozoites.

2.6.Statistical analyses

The variables studied included sociodemographic characteristics, clinical data, hematological parameters of the complete blood count (CBC) as well as morphological abnormalities observed on the peripheral blood smear.

Sociodemographic variables included the participants' age and sex. Age was grouped into three categories: 0–1 month, 2–12 months, and 13–60 months. Clinical variables included the reason for consultation or hospitalization, the diagnoses made (malaria, sickle cell disease, acute chest syndrome, infections, anemic syndromes, etc.), medical history, and treatments administered.

The biological variables analyzed were primarily hemoglobin level (HGB), mean corpuscular volume (MCV/MCV), mean corpuscular hemoglobin concentration (MCHC/MCHC) and erythroid cell count when available.

The morphological analysis of blood smears focused on identifying the main erythrocyte abnormalities, including anisopoikilocytosis, schistocytes , echinocytes , polychromatophilia , target cells, sickle cells, and intraerythrocyte parasitic inclusions. The observed abnormalities were described qualitatively and then quantified using a semi-quantitative scale: low abundance (+) when less than 25% of cells were affected, moderate abundance (++) between 25% and 50%, and high abundance (+++) above 50%.

The data were entered using Microsoft Excel 2013 and then analyzed with R software version 4.3.2 (R Foundation for Statistics). Computing, Vienna, Austria). Qualitative variables were expressed as counts and percentages, while quantitative variables were presented as mean ± standard deviation or median [interquartile range] according to their distribution. Associations between morphological abnormalities and clinical or biological variables were assessed using Pearson's chi-squared test or Fisher's exact test, depending on the conditions of application. The threshold for statistical significance was set at $p < 0.05$.

2.7.Ethical considerations

The study was carried out after obtaining administrative authorizations and approval from the The studies involving humans were approved by Institutional Ethics Committee of Université des Montagnes (No. 2024/102). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin; as well as agreements from the Mother and Child Centre of the Chantal Biya Foundation and the Pasteur Centre of Cameroon. Informed consent was obtained from all medical biologists/experts who participated in the blind evaluation of the blood smears.

The confidentiality and anonymity of the data collected were rigorously respected throughout the study, in accordance with the ethical principles of biomedical research and the requirements of the General Data Protection Regulation (GDPR).

3. RESULTS

3.1.Characteristics of the population

One hundred and fifty-eight children were included in the study. The median age was 12 months [6–36], with a mean of 21 ± 19 months. The age distribution of the children was as follows: 0–1 month: 16.5% (n = 26), 2–12 months: 41.1% (n = 65), and 13–60 months: 42.4% (n = 67). The male-to-female ratio was 1.14, with a male predominance of 84 boys (53.2%) and 74 girls (46.8%). Table I below describes the main clinical characteristics of the participants:

Table I: Summary of the main characteristics of the studied population

Settings	***Terms and conditions***	***Number of employees N=158 (n)***	***Frequency 100.0 (%)***
Age	Median age	—	12 months [6–36]
	Middle age	—	21 ± 19 months
Age ranges	0–1 month	26	16.5
	2–12 months	65	41.1
	13–60 months	67	42.4
Sex	Male	84	53.2
	Female	74	46.8
	Male/female sex ratio	—	1.14
Hematological profile	Average hemoglobin	—	9.7 ± 3.1 g/dL
	Extreme hemoglobin values	—	2.4–19.3 g/dL
	Anemia (Hb < 12 g/dL)	125	78.6
	Average GMV	—	77 ± 15 fL
	average CCMH	—	34.0 ± 1.2 g/dL
	medium reticulocytes	—	3.1 ± 2.4%
Reasons for consultation	General demonstrations	79	49.7
	Neurological disorders	21	13.2
	Respiratory symptoms	20	12.6
Prior self-medication	Yes	43	27.2
	No	115	72.8
Types of self-medication *	Level I analgesics	19	44.2
	Antibiotics	24	55.8
Hospital treatments	Painkillers	—	28.4

Settings	*Terms and conditions*	*Number of employees N=158 (n)*	*Frequency 100.0 (%)*
	Anti-infective	—	25.3
	Antimalarials	—	24.2

The mean hemoglobin level was 9.7 ± 3.1 g/dL, with a range of 2.4 to 19.3 g/dL. Anemia (Hb < 12 g/dL) was observed in 78.6% of the children (n = 125). The mean values of the main erythrocyte parameters were 77 ± 15 fL for MCV, 34.0 ± 1.2 g/dL for MCHC, and 3.1 ± 2.4 g/dL for reticulocyte count.

The main reasons for consultation were general symptoms, including fever, asthenia, and anorexia, found in 49.7% of cases (n = 79), followed by neurological disorders in 13.2% of cases (n = 21) and respiratory symptoms in 12.6% of cases (n = 20). Prior self-medication was reported in 27.2% of children (n = 43), primarily with level I analgesics (44.2%) and antibiotics (55.8%). In the hospital, the most frequently administered treatments were analgesics (28.4%), anti-infectives (25.3%), and antimalarials (24.2%).

3.2.Frequency and distribution of morphological anomalies

Analysis of the blood smears revealed a variety of erythrocyte morphological abnormalities. Table II below describes the distribution and profiles of the morphological abnormalities observed in the blood smears:

Table II: Distribution and profiles of morphological abnormalities observed in blood smears

Section	Setting	Number (n)	Frequency (%)
Number of morphological anomalies	1 anomaly	18	11.4
	2 anomalies	40	25.3
	3 anomalies	61	38.6
	≥ 4 anomalies	39	24.7
Morphological profiles	Size anomalies	26	16.5
	Shape anomalies	136	86.1

Section	Setting	Number (n)	Frequency (%)
Individual morphological anomalies	Immaturity/regeneration	67	42.4
	Inclusion anomalies	1	0.6
	Rolls	4	2.5
	Parasitemia	9	5.7
	Anisopoikilocytosis	130	82.3
	Schizocytes	71	44.9
	Echinocytosis	67	42.4
	Polychromatophilia	65	41.1
	target red blood cells	35	22.2
	Anisocytosis	26	16.5
	Sickle cells	22	13.9
	Trophozoites of *Plasmodium falciparum*	8	5.1
	Erythroblasts	6	3.8
	Dacryocytes	6	3.8

The majority of children presented with at least three abnormalities, with 38.6% having three abnormalities and 24.7% having four or more. Shape abnormalities were the most frequently observed (86.1%), followed by immaturity or regeneration abnormalities (42.4%) and size abnormalities (16.5%). Among individual abnormalities, anisopoikilocytosis was the most frequent (82.3%), followed by schistocytes (44.9%), echinocytosis (42.4%), and polychromatophilia (41.1%). Target cells and anisocytosis accounted for 22.2% and 16.5% of the observed abnormalities, respectively, while sickle cells were found in 13.9% of the children. Erythroblasts and dacryocytes were less frequently observed (3.8% each). Plasmodium falciparum trophozoites were also identified in 5.1% of children.

Table III describes sociodemographic, clinical and hematological characteristics according to the number of morphological abnormalities

Table III. Sociodemographic, clinical and hematological characteristics according to the number of morphological abnormalities

Features	1 anomaly (n=18)	2 anomalies (n=40)	3 anomalies (n=61)	≥4 anomalies (n=39)	p
Age, month, average ± AND	20.8 ± 20.2	17.8 ± 17.3	20.9 ± 19.7	25.1 ± 19.3	0.312
Female sex, n (%)	8 (44.4)	22 (55.0)	22 (36.1)	22 (56.4)	0.145
Age 0–1 month, n (%)	2 (11.1)	9 (22.5)	10 (16.4)	5 (12.8)	0.574
Age 2–12 months, n (%)	10 (55.6)	15 (37.5)	26 (42.6)	13 (33.3)	
Age 13–60 months, n (%)	6 (33.3)	16 (40.0)	25 (41.0)	21 (53.8)	
Anemia, n (%)	11 (61.1)	30 (75.0)	50 (82.0)	33 (84.6)	0.186
Hemoglobin, g/dL, mean ± ET	11.62 ± 2.48	10.31 ± 3.48	9.43 ± 2.77	8.53 ± 2.92	**0.001**
Hematocrit, %, mean ± ET	33.64 ± 7.56	30.07 ± 10.46	27.66 ± 7.95	25.44 ± 8.29	**0.003**
VGM, fL, mean ± ET	72.22 ± 18.96	79.47 ± 15.69	75.25 ± 13.40	79.37 ± 15.67	0.325
Platelets, G/L, mean ± ET	289.6 ± 130.9	288.2 ± 192.8	349.1 ± 182.4	335.1 ± 179.0	0.166
Reticulocytes, %, mean ± ET	0.43 ± 1.84	0.00 ± 0.00	0.22 ± 1.08	3.49 ± 6.91	**<0.001**
Leukocytes, G/L, mean ± AND	10.82 ± 11.08	15.15 ± 21.14	11.48 ± 5.54	14.22 ± 7.74	0.058
≥1 sign/symptom, n (%)	5 (27.8)	10 (25.0)	24 (39.3)	10 (25.6)	0.352
≥1 clinical diagnosis, n (%)	5 (27.8)	10 (25.0)	22 (36.1)	10 (25.6)	0.584

From Table III, the number of morphological abnormalities is significantly associated with hemoglobin and hematocrit. Children with ≥4 abnormalities have the lowest mean Hb and Ht

concentrations. Anemia is also more frequent as the number of abnormalities increases, but this trend does not reach statistical significance. The number of abnormalities is not significantly associated with age, sex, age group, the presence of at least one sign/symptom, or the presence of a clinical diagnosis.

3.3. Association between profiles and key clinical characteristics

3.3.1. Association between erythrocyte morphological profiles and key clinical characteristics

Table IV below describes the morphological profiles grouped according to the main clinical characteristics:

Table IV: Association between erythrocyte morphological profiles and the main clinical characteristics

Morphological profile	Number n (%)	Anemia n (%)	p	≥1 sign/symptom n (%)	p	≥1 clinical diagnosis n (%)	p
Size anomalies	26 (16.5)	17 (65.4)	> 0.05	10 (38.5)	> 0.05	8 (30.8)	> 0.05
Shape anomalies	136 (86.1)	109 (80.1)	> 0.05	41 (30.1)	> 0.05	40 (29.4)	> 0.05
Regeneration abnormalities/immaturity	67 (42.4)	51 (78.5)	> 0.05	22 (33.8)	> 0.05	23 (35.4)	> 0.05
Inclusion anomalies	1 (0.6)	1 (100,0)	—	0 (0,0)	—	0 (0,0)	—
Red blood cells in rolls	4 (2.5)	4 (100,0)	—	1 (25.0)	—	1 (25.0)	—
Parasitemia	9 (5.7)	7 (77.8)	> 0.05	3 (33.3)	> 0.05	1 (11,1)	> 0.05
target red blood cells	35 (22.2)	29 (82.9)	> 0.05	11 (31.4)	> 0.05	9 (25.7)	> 0.05

Note: *The frequencies of clinical variables are calculated within each morphological profile. The p -values correspond to the association tests between each morphological profile and the clinical variables considered. Categories with very small sample sizes, particularly inclusion abnormalities and rolled red blood cells, were not included in inferential analysis.*

Table IV shows that shape abnormalities were the most frequent (86.1%), followed by regeneration/immaturity abnormalities (41.1%). Anemia was particularly common in children with target cell abnormalities (82.9%), shape abnormalities (80.1%), and regeneration/immaturity

abnormalities (78.5%). However, no statistically significant association was observed between the different morphological profiles and anemia, signs or symptoms, or clinical diagnoses ($p > 0.05$).

3.3.2. **Association between major morphological anomalies and clinical parameters**

Table V describes the association between the main morphological anomalies and clinical parameters:

Table V Main morphological anomalies and clinical parameters

Morphological anomaly	n (%)	Anemia n (%)	p	≥1 sign/symptom n (%)	p	≥1 clinical diagnosis n (%)	p
Anisopoikilocytosis	130 (82.3)	104 (80.0)	0.214	—	—	—	—
Schizocytes	71 (44.9)	59 (83.1)	0.481	—	—	—	—
Echinocytes	67 (42.4)	55 (82.1)	0.563	—	—	—	—
Polychromatophilia	65 (41.1)	51 (78.5)	0.792	—	—	—	—
target red blood cells	35 (22.2)	29 (82.9)	0.601	11 (31.4)	0.742	9 (25.7)	0.681
Anisocytosis	26 (16.5)	17 (65.4)	0.091	10 (38.5)	0.528	8 (30.8)	0.744
Sickle cells	22 (13.9)	19 (86.4)	0.392	—	—	—	—
Trophozoites of *Plasmodium falciparum*	8 (5.1)	6 (75.0)	0.716	—	—	—	—
Dacryocytes	6 (3.8)	5 (83.3)	—	—	—	—	—
Erythroblasts	6 (3.8)	5 (83.3)	—	—	—	—	—
Red blood cells in rolls	4 (2.5)	4 (100,0)	—	1 (25.0)	—	1 (25.0)	—

Note: *Morphological anomalies are not mutually exclusive; a single child may present with several anomalies. The frequencies of anemia, signs/symptoms, and clinical diagnoses are calculated within each morphological category. Categories with very small sample sizes were not included in inferential analysis.*

Table V Anemia was frequently observed in children with these abnormalities, particularly those with sickle cells (86.4%), schistocytes (83.1%), target cells (82.9%), and echinocytes (82.1%).

Anisocytosis was associated with a lower frequency of anemia (65.4%). Other abnormalities, including dacryocytes, erythroblasts, and roll cells, were less frequent but also associated with a high proportion of anemia. Overall, these results highlight the frequency high incidence of erythrocyte morphological abnormalities and their frequent coexistence with anemia in the studied population.

4. DISCUSSION

The aim of this study was to describe the frequency and distribution of erythrocyte morphological abnormalities in children aged 0 to 5 years and to evaluate their associations with clinical and hematological characteristics. It included 158 children, with a median age of 12 months [6–36]. The main findings show a high frequency of anemia and erythrocyte morphological abnormalities. Shape abnormalities constituted the predominant morphological profile, with a frequency of 86.1%, while anisopoikilocytosis was the most frequent individual abnormality (82.3%). Furthermore, the number of morphological abnormalities observed on the blood smear was significantly associated with hemoglobin concentrations, hematocrit, and reticulocyte count. In contrast, no statistically significant association was found between the main morphological abnormalities considered individually and anemia or the clinical characteristics studied.

One of the first important findings of this study is the high prevalence of anemia, found in 78.6% of the children. However, this proportion must be interpreted in light of the hospital setting of the study population. Indeed, the children included were being treated in a pediatric hospital and therefore likely had more acute or chronic illnesses than children in the general population. This selection could partly explain the particularly high prevalence of anemia observed. The high prevalence of anemia found in our study is nonetheless consistent with the situation described in young children in sub-Saharan Africa. The World Health Organization considers anemia a major public health problem in children and estimates that it affects a significant proportion of children aged 6 to 59 months worldwide. In these children, the commonly used reference threshold is a hemoglobin concentration below 11 g/dL at sea level [16].

In our series, the mean hemoglobin level was 9.7 ± 3.1 g/dL, with values ranging from 2.4 to 19.3 g/dL, indicating a wide dispersion of values. This frequency of anemia is comparable to that reported in several Cameroonian studies. A study conducted in children aged 12 to 59 months in Yaoundé and Douala found an anemia prevalence of 45%, with an independent association between anemia and several factors, including malaria, iron status, and certain hemoglobinopathy profiles [17]. Other studies conducted in Cameroon have reported higher prevalences in populations exposed to malaria. In the Mount Cameroon region, a prevalence of 72.8% of anemia was observed in children under 5 years of age [18]. The difference between these results and

those of our study can be explained by several factors, including the recruitment setting, the age of the children, whether the studies were community-based or hospital-based, and the criteria used to define anemia. It should also be noted that our population included infants under 6 months of age, for whom hemoglobin interpretation requires consideration of age-related physiological values. Therefore, direct comparisons of prevalence with studies focusing solely on children aged 6 to 59 months should be made with caution.

Morphological analysis showed a clear predominance of shape abnormalities, observed in 86.1% of the children. Anisopoikilocytosis alone accounted for 82.3% of cases, making it the most frequently observed individual abnormality. This finding reflects significant morphological heterogeneity of erythrocytes in the studied population. Anisopoikilocytosis refers to a combination of variations in the size and shape of red blood cells. It is a nonspecific morphological sign that can be observed in various situations, including nutritional anemias, hemoglobinopathies, hemolytic states, and certain inflammatory conditions. Its high frequency in our study does not, therefore, allow us to identify a specific etiology on its own. This caution is all the more important given that our study did not aim to determine the etiological cause of each morphological abnormality. The high frequency of morphological abnormalities observed in our population can also be compared to available Cameroonian data on anisocytosis. In a study of 628 children aged 5 years or younger in the Mount Cameroon region, anisocytosis affected 54.1% of the children and was particularly frequent in children aged 24 months or younger, in whom it reached 68.8% [18]. The higher frequency of anisopoikilocytosis in our series could be explained by the fact that our analysis was based directly on microscopic examination of cell morphology, whereas some studies rely mainly on indices provided by the automated system, notably the RDW.

It is essential to recognize that many inherited disorders of the red cell membrane or metabolism may present in early childhood with only mild or nonspecific morphological changes such as anisopoikilocytosis or polychromatophilia, or even with a near-normal blood film. In the absence of targeted investigations (hemoglobin electrophoresis, enzyme assays, or molecular testing), these conditions are easily overlooked or attributed solely to iron deficiency or infection. This diagnostic challenge is particularly acute in resource-limited settings, where advanced testing is often unavailable outside specialized centers. Consequently, the blood smear, despite its

limitations, remains one of the few accessible tools that can raise suspicion of an underlying genetic disorder and orient subsequent investigations.

The second most frequent abnormality was the presence of schistocytes, observed in 44.9% of children. This frequency warrants cautious interpretation. Indeed, the presence of schistocytes on a blood smear does not necessarily mean that the child has thrombotic microangiopathy. According to the recommendations of the International Council for Standardization in Haematology (ICSH), the diagnostic value of schistocytes depends in particular on their quantification, their morphological context, and their clinical presentation; a proportion greater than 1% is considered suspicious for thrombotic microangiopathy in the absence of other severe abnormalities in red blood cell shape [19,20].

In our study, schistocytes were reported as a morphological finding, without quantification as a percentage. The frequency of 44.9% therefore corresponds to the proportion of children with schistocytes and cannot be directly compared to the threshold of >1% recommended by the ICSH for suspecting thrombotic microangiopathy. It would thus be inappropriate to equate their presence with a high frequency of thrombotic microangiopathies [19,20].

Echinocytosis was also frequent (42.4%). Echinocytes can be observed in certain pathological conditions, but their appearance can also be influenced by storage conditions and pre-analytical factors. The frequency observed in our study should therefore be interpreted with caution and does not, on its own, allow us to identify a particular etiology [21,22].

The coexistence of several morphological abnormalities was a significant finding of our study: 63.3% of children presented with at least three abnormalities, including 24.7% with at least four. The number of abnormalities was significantly associated with hemoglobin ($p = 0.001$) and hematocrit ($p = 0.003$), with the lowest values observed in children with at least four abnormalities (Hb: 8.53 ± 2.92 g/dL; Ht : 25.44 ± 8.29%). These results suggest that a greater diversity of morphological abnormalities may be associated with more extensive erythrocyte involvement, although a causal relationship cannot be established. The number of abnormalities was also significantly associated with the reticulocyte count ($p < 0.001$), which reached 3.49 ± 6.91% in children with at least four abnormalities. This increase may reflect heightened stimulation of erythropoiesis, but is not sufficient on its own to conclude that hemolysis is present. The interpretation of reticulocytes must be carried out in light of the degree of anemia

and the clinical and biological context [23]. These results must be considered within the multifactorial context of pediatric anemia in Cameroon. A study conducted in Yaoundé and Douala notably demonstrated an association between anemia and malaria, iron deficiency, inflammation, and certain hemoglobin genotypes [17]. Similarly, research carried out in the Mount Cameroon region has shown a significant relationship between malaria and anemia in children [24,25].

However, our study does not allow us to determine the mechanisms responsible for the association between multiple abnormalities and decreased hemoglobin. This could reflect the coexistence of several pathophysiological mechanisms. Further investigations into iron status, hemolysis markers, hemoglobinopathies, inflammation, and nutritional status would be necessary to better characterize these mechanisms.

The main strengths of this study are the inclusion of children aged 0 to 5 years and the evaluation of smears by three experienced biologists, independently and blinded, according to a standardized procedure. These elements enhance the quality and reproducibility of the morphological analysis.

Its main limitations are its single-center, hospital-based nature, which restricts the generalizability of the results, and the relatively small sample size for certain rare abnormalities. The cross-sectional design does not allow for the establishment of causal relationships. Finally, the lack of systematic investigation of iron status, hemoglobinopathies, hemolysis, and inflammation limits the identification of underlying etiologies. In summary, it highlights a high frequency of erythrocyte morphological abnormalities, dominated by anisopoikilocytosis and shape abnormalities. The multiplicity of abnormalities was associated with a decrease in hemoglobin and hematocrit, as well as an increase in reticulocytes. However, the abnormalities considered individually were not significantly associated with anemia or clinical characteristics. These results underscore the value of the blood smear as a complementary tool for diagnosis and characterization, although it cannot, on its own, establish an etiology.

CONCLUSION

This study highlights the high frequency and wide diversity of erythrocyte morphological abnormalities in the children studied. Anisopoikilocytosis, schistocytes, and echinocytes were particularly common, while multiple morphological abnormalities were significantly associated with decreased hemoglobin and hematocrit levels, as well as increased reticulocyte counts. In contrast, individual morphological abnormalities were not significantly associated with anemia or clinical characteristics.

These results highlight the value of blood smears as a complementary examination to the complete blood count (CBC) for the detection and characterization of erythrocyte abnormalities in children. However, their often nonspecific nature necessitates interpretation integrated with clinical and biological data and does not, on its own, allow for the establishment of an etiology. In the Cameroonian pediatric context, strengthening the quality of morphological examination and training healthcare professionals in its interpretation could contribute to improving diagnostic guidance and the management of hematological abnormalities, particularly in facilities with limited diagnostic resources.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.

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Authors' Contributions

Romaric Tuono De Manfouo, Anne Sorelle Nsang De Toue II, and Maryline Seuko Njopwouo contributed to the study design, statistical analysis, data interpretation, and manuscript revision. They also contributed to data collection and the drafting of the manuscript. Josue Simo performed the statistical analysis of the extracted data. Claude Tayou Tagny and Paul Koki supervised the

work and critically reviewed the manuscript. Romaric Tuono De Manfouo had full access to all the data in the study and took complete responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the manuscript.

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Transparency Statement

The lead author, Romaric Tuono De Manfouo, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Supplementary material:

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

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