TYPES OF ANEMIA IN PEDIATRIC ONCOLOGY AND NON-ONCOLOGY PATIENTS: INSIGHTS FROM COULTER DIAGNOSIS AND PERIPHERAL BLOOD SMEAR ANALYSIS AT A TERTIARY CHILDREN'S HOSPITAL, KATHMANDU

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ABSTRACT

Anemia is a significant health burden for pediatric patients, particularly those undergoing cancer treatment in resource-limited settings like Nepal. This study evaluated the diagnostic performance of automated hematology systems by Coulter against manual peripheral blood smear (PBS) analysis for classifying anemia types in a pediatric population. A total of 454 pediatric patients were included, comprising 274 oncology (60.4%) and 180 non-oncology (39.6%) cases. The overall mean hemoglobin was 10.76 ± 2.11 g/dL, with moderate anemia (7.0–9.9 g/dL) being the most prevalent category (118 patients). Anemia severity was concentrated in the non-oncology group (95 of 118 moderate; 19 of 20 severe), while oncology patients predominantly had normal hemoglobin (125 cases). Significant differences in red cell indices were observed: oncology patients had higher mean MCV (8.7 fL higher, $p < 0.001$) and MCH (2.6 pg higher, $p < 0.001$) compared to non-oncology patients. A highly significant association was found between anemia type and clinical status ($\chi^2 = 207.012$, $p < 0.001$). Normocytic profiles (normocytic normochromic anemia, NNA / normocytic normochromic picture, NNP) dominated the oncology group (51.7% NNP), consistent with anemia of chronic disease. In contrast, microcytic hypochromic anemia (MHA) was more common in the non-oncology group, consistent with iron deficiency. The automated Coulter diagnosis showed a strong agreement with manual PBS findings ($r = 0.970$, $\chi^2 = 922.47$, $p < 0.001$). In contrast, MHA was significantly more prevalent in the non-oncology group, suggesting iron deficiency. Given the strong agreement between the systems, the automated Coulter diagnosis serves as a reliable initial screening tool for anemia classification, but PBS confirmation remains essential to differentiate anemia types and guide effective clinical management in developing country laboratories.

KEYWORDS

Anemia, automated hematology analyzer, pediatric oncology, peripheral blood smear, resource-limited settings

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INTRODUCTION

Anemia represents one of the most pervasive and debilitating public health challenges affecting children worldwide, particularly in developing nations. It is clinically defined as a state where the quantity or quality of circulating red blood cells or hemoglobin concentration and hematocrit level, falls below the established reference range for an individual's age, sex, and environment.[1] The WHO provides clear diagnostic thresholds for pediatric populations, defining anemia as a hemoglobin (Hb) level below 110 g/L for children aged 6–59 months and below 115 g/L for those aged 5–11 years.[2] Beyond a simple hematological reading, anemia is a major indicator of severe health consequences, including increased infant mortality, poor birth outcomes, and long-term cognitive and behavioral problems in children.[3, 4]

In this high-prevalence setting, children are divided into clinically distinct groups. Anemia in non-oncology patients is primarily characterized by microcytic hypochromic anemia (MHA), commonly resulting from severe nutritional iron deficiency.[5,6] In contrast, children undergoing cancer treatment represent a vulnerable group where anemia is a frequent complication caused by the malignancy itself, myelosuppressive chemotherapy, and coexisting malnutrition.[7-9] These factors often lead to normocytic normochromic anemia (NNA), a pattern linked to chronic disease or underlying bone marrow dysfunction.[10-12] The critical challenge lies in the diagnostic overlap, as nutritional deficiencies can coexist with cancer, making a simple MHA or NNA classification insufficient for tailored patient management.[13]

Effective anemia management relies on accurate and timely diagnosis, typically achieved through a synergistic approach: combining quantitative data (red blood cell indices like (MCV, MCH) from automated Coulter counters with qualitative morphological insights from manual peripheral blood smear (PBS) examination.[14-17]

Therefore, the critical study gap is not just the difference in anemia patterns, but the formal validation of the diagnostic agreement between these two essential tools in this specific environment. A comprehensive correlation study is required to ensure that the rapid, automated Coulter diagnosis accurately reflects the gold-standard morphological classification provided by PBS, particularly when distinguishing between the subtle yet critical anemia types NNA vs MHA) prevalent in mixed oncology and non-oncology pediatric populations.[18] Hence, this study utilizes both automated Coulter and manual PBS analysis to generate essential comparative data, aiming to delineate the critical hematological differences between oncology and non-oncology pediatric populations and validate the reliability of Coulter indices against PBS morphology to update evidence-based care and diagnostic protocols in resource-limited laboratories.

MATERIALS AND METHODS

This was a hospital-based cross-sectional study conducted at Kanti Children's Hospital (KCH) Maharajgunj, Kathmandu till a year starting from October 2023 - September 2024. The study involved consecutive pediatric patients under 15 years who presented to the outpatient or inpatient services during the study period. The patient cohort was divided into two groups: oncology patients (defined as having a confirmed malignancy diagnosis) and non-oncology patients (all other presenting complaints).

Ethical approval for the study was obtained from the Institutional Review Committee (IRC) of Kanti Children's Hospital, Maharajgunj, Kathmandu (Ref. no: 1434). Peripheral venous blood was collected under aseptic conditions using standard phlebotomy techniques. Approximately 1–3 mL of blood was drawn into an EDTA tube and labeled with patient identifier codes to maintain blinding during the analysis phase.

Complete blood counts (CBC), including Hb, total leukocyte count (TLC), platelet count, and absolute neutrophil count (ANC), were performed using an automated hematology analyzer; thin blood films were prepared for PBS examination within one hour of collection, air-dried, and stained using Wright stain. The stained smears were examined under a light microscope by a pathologist to assess red blood cell morphology. Abnormal findings were reviewed and confirmed.

Anemia classification in this study was based on both Hb levels and PBS examination. Hb concentration was measured using an automated hematology analyzer, with anemia defined according to the WHO age-specific cutoffs values. The classification was further refined by evaluating red blood cell (RBC) morphology on PBS. Microcytic hypochromic anemia was identified by the presence of small,

pale RBCs, commonly associated with iron deficiency whereas normocytic normochromic anemia was characterized by normal-sized and normal-colored RBCs. Macrocytic anemia, marked by larger RBCs, and dimorphic anemia, identified by two distinct RBC populations. The severity of anemia (mild, moderate, or severe) according to WHO was graded by correlating RBC indices (MCV, MCH, MCHC) from the CBC with PBS findings (Table 1). The data were accumulated in MS Excel and analyzed using SPSS-25. The significance was determined by applying the Chi-square test and a p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 454 pediatric patients were included in the study, comprising 288 males (63.4%) and 166 females (36.6%), demonstrating a clear male predominance. The mean age of the cohort was 2.1 ± 0.0567 years. The clinical population was categorized into oncology patients (n = 274; 60.4%) and non-oncology patients (n = 180; 39.6%) to compare hematological

Table 1: WHO classification of severity of anemia

Age	Mild anemia	Moderate anemia	Severe anemia
6–59 months	10.0 – 10.9 g/dL	7.0 – 9.9 g/dL	< 7.0 g/dL
5–11 years	11.0 – 11.4 g/dL	8.0 – 10.9 g/dL	< 8.0 g/dL
12–14 years	11.0 – 11.9 g/dL	8.0 – 10.9 g/dL	< 8.0 g/dL

Table 2: Demographics of study population

Information of patients		Gender		Total
		Male	Female	
Age	< 1 year	39	42	81
	1-5 years	149	64	213
	6-10 years	62	36	98
	11-15 year	38	24	62
Clinical Group	Oncology Patients	173	101	274
	Non oncology patients	115	65	180
Total		**288**	**166**	**454**

Table 3: Overall hematological parameter of study population

Parameter	Mean ± SD	Range (Min–Max)	Median	Unit
Hemoglobin	10.76 ± 2.11	2.3 – 19.8	10.90	g/dL
MCV	79.66 ± 11.29	35.30 – 110.00	80.45	fL
MCH	26.17 ± 4.19	12.40 – 51.30	26.45	pg
MCHC	32.58 ± 2.35	14.70 – 51.60	32.30	g/dL

Table 4: Distribution of Hb levels by severity, sex, and patient type

Category	Hb range (g/dL)	Male	Female	Cancer	Non-cancer
Severe anemia	<7.0	15	5	1	19
Moderate anemia	7.0 to 9.9	63	55	23	95
Mild anemia	10.0 to 10.9	58	41	18	81
Normal	11.0 to 14.5	142	62	125	79
High Hb	>14.5	10	3	13	0

Table 5: Difference in RBC indices (MCV, MCH, MCHC) based on oncology status

Parameter	Oncologic (n=180)			Non-oncologic patients (n=274)			Unit
	Mean ± SD	range (Min–Max)	Median	Mean ± SD	range (Min–Max)	Median	
Hb	12.01±1.91	6.3-19.8	12.01	9.94±1.81	2.3-13.8	10.2	g/dL
MCV	84.93± 9.13	35.30-110.0	84.65	76.20±11.26	45.30-108	77.3	fL
MCH	27.75 ±3.06	21.30-36.20	27.80	25.14±4.50	12.4-51.3	25.6	pg
MCHC	32.19±1.38	29.80-37.30	31.90	32.83±2.78	14.7-51.6	32.85	g/dL

characteristics between the two groups. Most children were within the 1–5 years age (n = 213; 46.9%), followed by those aged 6–10 years (n = 98; 21.6%). Preschool children (<5 years) represented 64.7% of the total cohort (n = 294). Infants <1 year accounted for 81 cases (17.8%), whereas children aged 11–15 years comprised 62 cases (13.7%). Among children aged 1–5 years, males were more common (149; 70%) compared with females (64; 30%) (Table 2).

The mean Hb level was 10.76 ± 2.11 g/dL (range: 2.3–19.8; median 10.90). The mean MCV was 79.66 ± 11.29 fL, and the mean MCH and MCHC were 26.17 ± 4.19 pg and 32.58 ± 2.35 g/dL, respectively (Table 3).

The prevalence of anemia was highest in the moderate (7.0–9.9 g/dL) category (118 patients) and lowest in the severe (<7.0 g/dL) category (20 patients). The majority of severe and moderate anemia cases were observed in non-cancer patients (19/20 and 95/118, respectively), while cancer patients predominantly had normal hemoglobin (125 cases) (Table 4).

Table 5 compares key hematological parameters between oncologic patients and non-oncologic patients. The oncologic group shows higher mean levels of hemoglobin (12.01 g/dL), MCV (84.93 fL), and MCH (27.75 pg) than the non-oncologic group. Conversely, the non-oncologic group has a slightly higher mean MCHC (32.83 g/dL) compared to the oncologic group (32.19 g/dL) (Table 5).

MCV was significantly higher in oncology patients compared to non-oncology patients ($t = 9.07$, $p < 0.001$). On average, oncology patients had an MCV that was 8.7 fL higher (95% CI: 6.83–10.62 fL). Similarly, MCH was significantly higher in oncology patients ($t = 7.37$, $p < 0.001$), with an average increase of 2.6 pg (95% CI: 1.92–3.31 pg). In contrast, MCHC was significantly lower in oncology patients ($t = –3.22$, $p = 0.001$), showing a mean reduction of 0.63 g/dL (95% CI: –1.02 to –0.25 g/dL) (Table 6).

Normocytic profiles were highly prevalent in children with cancer, with 93 (51.7%) showing a normocytic normochromic picture and 82 (45.6%) having normocytic normochromic anemia. In contrast, non-oncology patients most frequently presented with normocytic normochromic anemia (161 cases) and microcytic hypochromic anemia (104 cases). Notably, dimorphic and macrocytic anemia appeared exclusively in the non-oncology group. These distinct hematological profiles were confirmed by a significant association in 454 cases (Pearson $\chi^2 = 207.012$, $df = 4$, $p < 0.001$) and by correlation analyses (Pearson $r = 0.553$, $p < 0.001$; Spearman's $\rho = 0.321$, $p < 0.001$), highlighting the diagnostic relevance of PBS in pediatric oncology. A significant association was observed between Coulter-

Table 6: RBC indices between oncology and non-oncology groups

Parameter	Levene's test F	Levene's p-value	t test statistics	df	p-value (2-tailed)	Mean difference	SE difference	95% CI (Lower–upper)
MCV	7.961	0.005	9.070	432.332	<0.001	8.73	0.96	6.83 – 10.62
MCH	12.501	<0.001	7.370	451.439	<0.001	2.62	0.35	1.92 – 3.31
MCHC	25.001	<0.001	−3.216	424.536	0.001	−0.63	0.20	−1.02– 0.25

Mean corpuscular volume (MCV), Mean corpuscular Hb (MCH), Mean corpuscular Hb concentration (MCHC)

Table 7: PBS and Coulter findings with oncology status

Anemia types	PBS findings			Coulter diagnosis		
	Child without cancer	Child with cancer	Total	Child without cancer	Child with cancer	Total
NNA	82	161	253	75	161	236
MHA	5	104	109	12	108	120
DA	--	6	6	--	--	-0
MA	--	3	3	--	5	5
NNP	93	--	93	93	--	93
Total	180	274	454	180	274	454
p-Value		0.0001			0.0001	

NNA: Normocytic Normochromic Anemia, MHA: Microcytic Hypochromic Anemia, MA: Macrocytic Anemia, DA: Dimorphic Anemia and NNP: Normocytic normochromic picture

Table 8: Coulter diagnosis versus PBS analysis

Findings		PBS Analysis						p -Value
		NNA	MHA	DA	MA	NNP	Total	
Coulter diagnosis	NNA	209	23	4	--	--	236	0.0001
	MHA	33	86	1	--	--	120	
	MA	1	--	1	3	--	5	
	NNP	--	--	--	--	93	93	
	Total	243	109	6	3	93	454	

diagnosed anemia types and cancer status (p = 0.0001), with NNA more common in children without cancer (n=161) and a distinct NNP exclusive to children with cancer (n=93). MHA was predominantly seen in non-cancer patients (n=108), and MA was rare overall (n=5), appearing only in non-oncology cases. The strength of association between variables was moderate (Pearson's R = 0.542, p = 0.0001), with a weaker but significant correlation (Spearman's rho = 0.355, p = 0.0001). These findings highlight a statistically significant and clinically relevant difference in anemia profiles between the two pediatric groups (Table 7).

Among 454 cases, 236 were diagnosed with NNA by Coulter, of which 209 (88.6%) were confirmed by PBS. Of the 120 cases identified as microcytic hypochromic anemia by Coulter, 86 (71.7%) matched the PBS findings. All 93 cases classified as having a normal picture on Coulter were confirmed by PBS. In 33 cases labeled as microcytic hypochromic by Coulter, PBS showed a NNP. Pearson's correlation coefficient was r =0.970 (p <0.001), and Spearman's rho was ρ =0.843 (p <0.001). The chi-square test between Coulter diagnosis and PBS findings showed $\chi^2 = 922.47$, df = 12, p <0.001.

Among 180 children with cancer, 93 (51.7%) had NNA. Among 274 children without cancer, 108 (39.4%) had MHA, and 161 (58.8%) had NNA. Macrocytic anemia was observed in 5 of 274 non-oncology cases. Pearson's correlation between anemia type and oncology status was r = 0.542 (p <0.001), and Spearman's rho was ρ = 0.355 (p <0.001). The association between Coulter diagnosis and oncology status yielded $\chi^2 = 195.038$, df = 3, p < 0.001 (Table 8).

Dimorphic anemia was the most severe, with the lowest median Hb (7–8 g/dL). MHA also showed low levels with greater variability. In contrast, normocytic and macrocytic anemia had similar medians around 11 g/dL, while the NNP had the highest, most stable levels (13–14 g/dL), reflecting near-normal cases. Statistical analysis confirmed a significant association between coulter-diagnosed anemia types and Hb levels (p = 0.0001). This relationship was moderately strong (Pearson's R =0.539, p =0.0001) and showed a significant linear trend (value =131.505, p =0.0001). Spearman's correlation also confirmed a weaker, positive ordinal relationship (rho =0.352, p =0.0001).

DISCUSSION

This study aimed to determine the prevalence and types of anemia in oncology and non-oncology children at Kanti Children's Hospital in Nepal. We compared results from an automated Coulter analyzer with manual PBS examinations to gain insight into pediatric blood conditions. Our demographic findings show a higher prevalence in males (63.4%), consistent with other studies on pediatric hospitalization.[19] The largest patient group was aged 1–5 years (46.9%), aligning with global trends of higher illness rates in young children.[20] A significant majority (60.4%) were oncology patients, also with a male predominance. This finding is similar to other research on pediatric cancers, possibly because some cancers are more common in boys.[21]

The average Hb level was 10.76 g/dL, similar to other studies on pediatric anemia.[9] Most anemic cases were classified as mild (73.3%), with fewer being moderate (20.9%) or severe (5.7%), confirming that mild-to-moderate anemia is most common. This study shows strong agreement between the automated Coulter DX and manual PBS methods. For NNA, 88.6% of automated results were confirmed by PBS, which is consistent with previous findings.[22] For MHA, the agreement was 71.7%, also aligning with other studies.[23] While the automated system is reliable, PBS is crucial for identifying specific morphological details, making it essential for confirming complex or unclear cases.

Coulter analyzer results may vary due to sample particle size which is less than 1 micron or calibration sensitivity. Peripheral smear correlation is recommended to confirm findings.[24] Coulter analyzers primarily measure cell counts and sizes based on

electrical impedance. If the instrument is not properly calibrated, or if the sample contains small particles (e.g., blood dust or fragmented cells), it may misclassify them as smaller cell populations (such as platelets or microcytes). This can lead to report variations, especially in parameters like RBC count, MCV, and platelet count. To verify such findings, a PBS examination should be performed for morphological correlation and confirmation of any abnormal or doubtful results. Children with cancer had significantly higher MCV and MCH, indicating larger red blood cells with more Hb per cell. In contrast, MCHC was lower, showing reduced hemoglobin concentration within each cell. These differences were statistically significant, suggesting true hematologic alterations in the oncology group and this finding agrees with the previous research.[25]

The type of anemia differed significantly between the groups. Oncology patients most commonly had NNA (51.7%), whereas non-oncology patients primarily had MHA (39.4%) and NNA (58.8%). Dimorphic anemia, on the other hand, is a mixed condition featuring both microcytic and macrocytic RBCs. The Coulter machine often misidentifies it because it calculates the MCV as an average of both large and small cells, which can misleadingly appear normal. It is reliably diagnosed by an expert pathologist or hematologist through the manual examination of a PBS.[26] This highlights that cancer-related anemia is distinct and helps guide the development of tailored diagnostic and treatment protocols for each group. Furthermore, dimorphic anemia was associated with the lowest Hb levels (7–8 g/dL), indicating greater severity. This is a critical insight for pediatric oncology, as it may signal issues like bone marrow suppression from chemotherapy or complex nutritional deficiencies, thereby informing the need for careful monitoring and potential transfusion therapy.

In conclusion, children with cancer predominantly exhibit NNP consistent with anemia of chronic disease, whereas non-oncology patients commonly show MHA suggestive of iron deficiency. The strong agreement between automated Coulter analysis and PBS examination confirms the reliability of automated screening. Nevertheless, peripheral smear evaluation is essential for accurate morphological differentiation, severity assessment, and informed clinical management in resource-limited healthcare settings.

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