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Frequency of Bone Marrow Infiltration by Non-Hematopoietic Small Round Blue Cell Tumors in the Pediatric Population

Fauzia Aamer[1], Omyya Baloch[2], Nazish Saqlain[3], Rabia Ahmad[4], Muhammad Abbas[5], Aamer Naseer Qureshi[6]

1 *Associate Professor, Department of Hematology, Children's Hospital / The University of Child Health, Lahore Pakistan*
Manuscript writing, Methodology

2 *Resident, Department of Hematology, Children's Hospital / The University of Child Health, Lahore Pakistan*
Conceptualization

3 *Professor, Department of Pathology, Post Graduate Medical Institute / Ameer-ud-Din Medical College / LGH, Lahore Pakistan*
Investigation

4 *Associate Professor, Department of Pathology, Allama Iqbal Medical College / Jinnah Hospital, Lahore Pakistan*
Data collection

5 *Associate Professor, Department of Pediatrics, RMTH / Rehbar Medical College, Lahore Pakistan*
Visualization

6 *Professor, Department of Pediatrics, Faisalabad Medical University / Allied Hospital, Faisalabad Pakistan*
Data curation

CORRESPONDING AUTHOR
Dr. Fauzia Aamer
Associate Professor, Department of Hematology, Children's Hospital / The University of Child Health, Lahore Pakistan
Email: drfauzia.haem@gmail.com

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ABSTRACT

Background: Bone marrow metastasis (BMM) of non-hematopoietic small round blue cell tumors poses significant diagnostic and therapeutic challenges in pediatric oncology. **Objective:** This study aimed to evaluate the frequency and characteristics of bone marrow infiltration by non-hematopoietic round blue cell tumors in a pediatric population, excluding lymphomas. **Study Design:** Retrospective cross-sectional descriptive study. **Settings:** Department of Hematology and Transfusion Medicine, University of Child Health Sciences and Children Hospital, Lahore Pakistan. **Duration:** Two-year period from October 2021 to October 2023. **Methods:** A total of 198 diagnosed cases of small round blue cell tumors, aged newborn to 16 years, were included. Data were collected on clinical findings, imaging results, and hematological parameters. Bone marrow aspirates and biopsies were performed, followed by histological examination and immunohistochemical analysis. **Results:** The study analyzed 198 pediatric patients with small round blue cell tumors, revealing that neuroblastoma was the most prevalent, comprising 90 cases with a 22.2% rate of positive bone marrow infiltration. Clinical findings indicated fever (75.8%) and pallor (65.7%) as common symptoms. Hematological parameters showed mean hemoglobin levels of 9.5 ± 1.5 g/dL, with neuroblastoma patients exhibiting the lowest levels at 8.5 ± 1.0 g/dL. CD56 positivity was observed in 66.7% of cases, mainly in neuroblastoma, emphasizing the role of histopathological evaluation in diagnosis and management. **Conclusion:** Early detection of bone marrow metastasis in non-hematopoietic small round blue cell tumors is vital for appropriate treatment planning.

Keywords: *Bone marrow metastasis, Non-hematopoietic tumors, Pediatric oncology, Small round blue cell tumors, Immunohistochemistry.*

INTRODUCTION

Malignant small round cell tumors constitute a heterogeneous category of neoplasms defined by the proliferation of malignant round cells. These cells are typically slightly larger than red blood cells (RBCs) and can be identified in air-dried smears as pattern-less sheets with high cellular density and a pronounced nuclear-to-cytoplasmic (N: C) ratio when examined under hematoxylin and eosin (H&E) staining.[1,2] This distinctive appearance is essential for the diagnosis and classification of malignant tumors, which often invade the bone marrow (BM). The presence of malignant cells in the BM signifies a more advanced stage of disease, often complicating treatment and prognosis.[3]

The phenomenon of tumor cells metastasizing bone marrow was first documented in 1834, marking a pivotal moment in our understanding of cancer spread. Metastasis is a significant contributor to cancer-related mortality. This process requires tumor cells to adapt to various microenvironments, and the bone marrow is a particularly conducive environment for certain types of cancer.[4] The BM not only serves as a site for hematopoiesis but also plays a role in cancer dormancy and potential relapse. Many solid tumors, such as Ewing sarcoma, retinoblastoma, and rhabdomyosarcoma,

frequently exhibit bone marrow involvement, which poses significant therapeutic challenges for clinicians treating patients with metastatic disease.[5]

The metastatic spread of non-hematopoietic small round cell tumors, including but not limited to primitive neuroectodermal tumors, Ewing sarcoma, rhabdomyosarcoma, retinoblastoma, mesenchymal chondrosarcoma, nephroblastoma, poorly differentiated chordoma, small cell osteosarcoma, desmoplastic small round cell tumors, germ cell tumors, and melanotic neuroectodermal tumors, underscores the necessity of early detection. Recognizing bone marrow metastasis is critical for several reasons: it aids in initial clinical staging, informs therapeutic decision-making, stratifies prognostic risk, evaluates response to treatment, and predicts potential relapse.[6]

Currently, the diagnosis and classification of pediatric non-hematopoietic (solid) tumors are primarily based on clinical manifestations, imaging studies, and laboratory results. These findings are typically confirmed through invasive procedures, such as tissue biopsies, which facilitate conventional histopathological examination, immunohistochemistry (IHC), and molecular or cytogenetic analysis of tumor tissues. These diagnostic modalities are essential for accurately identifying tumor types and guiding treatment strategies.[7] From a hematological perspective, the most common peripheral blood smear finding in patients with bone marrow metastasis is a leucoerythroblastic blood picture. This abnormality indicates the presence of immature white blood cells and may suggest associated bone marrow fibrosis. While both procedures are valuable, bone marrow biopsy is often superior to the aspiration for the detection of metastatic disease, providing a more comprehensive view of the marrow architecture and the extent of infiltration by malignant cells.[8]

The morphology of metastatic cells typically reflects the characteristics of the primary tumor site. In many cases, metastatic carcinoma growth within the bone marrow leads to trabecular bone destruction, resulting in osteolytic lesions. Conversely, certain carcinomas can induce the formation of new woven bone, leading to osteosclerotic lesions. The pattern of bone involvement can be complex, with some tumors displaying a mixed lytic-sclerotic appearance, particularly in late-phase disease when sclerotic features become more prominent.[9] Bone marrow metastasis can often present as cytopenias, mimicking primary hematopoietic disorders. This overlap can lead to diagnostic challenges, as clinicians must differentiate between the effects of metastatic disease and primary hematological conditions. Therefore, a thorough understanding of the clinical and pathological features of malignant small round cell tumors and their potential for bone marrow involvement is vital for optimizing patient management and improving outcomes in those affected by these aggressive malignancies. Overall, the interplay between these tumors and the bone marrow microenvironment remains a critical area of research, with ongoing studies aimed at elucidating the mechanisms of metastasis and developing targeted therapeutic strategies.[10]

The rationale for our study focuses on analyzing the hematological findings associated with non-hematopoietic malignancies that metastasize to the bone marrow in pediatric patients. We aim to determine the frequency of bone marrow infiltration by non-hematopoietic round blue cell tumors, excluding lymphomas. Emphasizing histomorphology and immunohistochemistry (IHC), our study seeks to enhance diagnostic accuracy and establish primary diagnoses in clinically suspected cases. By identifying the unique characteristics of these tumors, we hope to improve clinical management and treatment outcomes. Ultimately, our research aims to contribute valuable insights to pediatric oncology and address existing gaps in the literature.

METHODS

The study employed a retrospective cross-sectional descriptive design, conducted at the Department of Hematology and Transfusion Medicine at the University of Child Health Sciences and Children Hospital, Lahore, over two years from October 2021 to October 2023. A total sample size of 198 was calculated using the WHO calculator (www.openepi.com) with a 5% level of significance and 80% power of the test, based on the expected frequency of bone marrow infiltration observed in 15.2% of cases.[15] The study employed a non-probability consecutive sampling technique. The inclusion criteria consisted of documented cases of round blue cell tumors in participants aged between newborn and 16 years, including both male and female genders. Cases that did not meet these criteria, such as those outside the specified age range or diagnosed with other hematological diseases, were excluded. There were a total of 198 cases that were sent for bone marrow investigation due to tiny round blue cell tumors (excluding lymphomas). Each patient's demographic information (age, sex), clinical results, and imaging studies (ultrasound, X-rays, CT scans, MRI, and primary tumor biopsy results) were carefully recorded. A blood sample containing ethylenediamine tetra acetic acid (EDTA) was collected for complete blood count analysis and peripheral blood smear inspection. Peripheral blood smears were stained with Giemsa stain, and bilateral bone marrow aspirates were obtained per the study protocol during standard diagnostics at initial diagnosis, employing a 16G lumbar puncture needle and then stained with Giemsa.

Bone marrow biopsies (BMBx) were obtained using the standard method with a Jamshidi needle from the posterior superior iliac spine under local anesthesia. The biopsies measured approximately 1.5–2 cm in length, were preserved in 10% formalin solution, and subjected to decalcification with 10% formal-formic acid for 4–6 hours before standard processing and paraffin embedding. Serial slices of 4–6 µm in thickness were prepared and stained with hematoxylin and eosin (H&E), with additional immunostaining applied as necessary.

Small round blue cell tumors include neuroblastoma (NB), rhabdomyosarcoma, non-Hodgkin's lymphoma, Ewing's sarcoma, primitive neuroectodermal tumor (PNET), and the blastemic component of Wilms' tumor. Metastasis is described as the process by which cancer disseminates from the main tumor to establish itself at anatomically separate locations. Bone marrow metastasis (BMM) denotes the infiltration of malignant tumors from non-hematopoietic tissues into the bone marrow, causing the invasion of cancer cells, which ultimately results in structural damage and the emergence of hematopoietic disorders. Hematological parameters, such as hemoglobin concentration, total leukocyte count, and platelet count, were recorded, together with the findings from peripheral blood film analyses emphasizing red blood cell shape and the detection of atypical cells. A thorough analysis of bone marrow smears was performed, with particular emphasis on the identification of unusual cells. Sections of bone marrow biopsy containing a minimum of five well-preserved marrow spaces were analyzed for cellularity, normal hematological components, infiltration by non-hematopoietic round cells, histological patterns, the morphology of infiltration (islands/nests, diffuse sheets), reticulin fibrosis, necrosis, and other secondary alterations. The immunohistochemistry (IHC) panel comprised markers including CD56, CD99, synaptophysin, chromogranin, S100, desmin, myogenin, and vimentin, chosen according to the observed morphology.

Data analysis was performed using SPSS version 20, where the distribution of data was considered. Qualitative data, such as gender and blood group, were expressed as frequency and percentage, while quantitative data, such as age, were presented as mean ± standard deviation (SD). The odds ratio for non-hematopoietic small round blue cell tumors was calculated, and data were stratified by age and gender. Post-stratification, the odds ratio was recalculated, with an odds ratio greater than 1 considered significant, and a p-value of less than 0.05 deemed statistically significant.

RESULTS

In Table 1, the demographics and clinical findings of the 198 patients were summarized. The age distribution indicated that 60 patients (30.3%) were in the birth to 5 years group, 72 patients (36.4%) were in the 6 to 10 years group, and 66 patients (33.3%) were in the 11 to 13 years group. Fever was the most common clinical finding, observed in 150 patients (75.8%), followed by pallor in 130 patients (65.7%). Jaundice was reported in 40 patients (20.2%), hepatosplenomegaly in 85 patients (42.9%), and suspicious body mass was noted in 105 patients (53.0%).

In Table 2, the complete blood count (CBC) and peripheral smear findings were presented. The mean hemoglobin level was 9.5 ± 1.5 g/dL, with a range from 7.0 to 12.0 g/dL. The mean white blood cell count was 10.5 ± 2.0 x10^9/L, ranging from 4.0 to 15.0 x10^9/L. The mean platelet count was reported as 220 ± 30 x10^9/L, with a range of 150 to 300. RBC morphology showed an abnormality in 30 patients (15%).

In Table 3, the distribution of small round blue cell tumors and bone marrow infiltration was analyzed. Among the total cases, neuroblastoma accounted for 90 cases, with 20 (22.2%) showing positive bone marrow infiltration. Infiltration patterns included diffuse sheets in 15 patients (75%) and island/nest in 5 patients (25%). For Ewing sarcoma, out of 70 cases, 7 (10.0%) demonstrated positive bone marrow infiltration, with a similar distribution of infiltration patterns. Rhabdomyosarcoma had the lowest infiltration rate, with 3 (7.9%) of 38 cases showing positive infiltration.

In Table 4, immunohistochemistry (IHC) results for cases with positive bone marrow infiltration were summarized. The marker CD56 was positive in 20 cases (66.7%), with neuroblastoma accounting for 15 (75.0%) of these. CD99 positivity was found in 7 cases (23.3%), predominantly in Ewing sarcoma. Synaptophysin and chromogranin were positive in 15 (50.0%) and 10 (33.3%) cases, respectively, both exclusively in neuroblastoma. S100 and desmin markers were positive in 3 cases (10.0%) each, all from rhabdomyosarcoma.

In Table 5, the summary of hematological parameters in cases with bone marrow infiltration indicated that neuroblastoma had a mean hemoglobin of 8.5 ± 1.0 g/dL, while Ewing sarcoma had a mean of 9.0 ± 1.2 g/dL, and rhabdomyosarcoma had a mean of 9.5 ± 1.3 g/dL. The total leukocyte count was similar across the tumor types, with neuroblastoma showing a mean of 11.0 ± 1.8. Platelet counts varied slightly, with neuroblastoma at 210 ± 20 x10^9/L and rhabdomyosarcoma at 225 ± 30 x10^9/L.

In Table 6, the odds ratios for tumor types by age and gender were presented. For the age group of birth to 5 years, neuroblastoma had a significant odds ratio of 2.11

with a p-value of 0.02, indicating a higher risk compared to the other tumor types. In the 6 to 10 years group, the odds ratio for neuroblastoma was lower at 0.67 with a p-value of 0.55, suggesting no significant risk. For gender, males were more likely to have neuroblastoma, as indicated by an odds ratio of 1.5 with a p-value of 0.03, while females showed an odds ratio of 0.75, also significant with a p-value of 0.03

Table 1: Patient demographics and clinical findings

Parameters	Category	Total Patients (n=198)	Male (n=128)	Female (n=70)
Age Group	Birth - 5 years	60 (30.3%)	38 (29.7%)	22 (31.4%)
	6 - 10 years	72 (36.4%)	48 (37.5%)	24 (34.3%)
	11 - 13 years	66 (33.3%)	42 (32.8%)	24 (34.3%)
Clinical Findings	Fever	150 (75.8%)	98 (76.6%)	52 (74.3%)
	Pallor	130 (65.7%)	82 (64.1%)	48 (68.6%)
	Jaundice	40 (20.2%)	25 (19.5%)	15 (21.4%)
	Hepatosplenomegaly	85 (42.9%)	54 (42.2%)	31 (44.3%)
	Suspicious Body Mass	105 (53.0%)	68 (53.1%)	37 (52.9%)

Table 2: CBC and peripheral smear findings

Parameter	Mean ± SD	Range
Hemoglobin (Hb)	9.5 ± 1.5 g/dL	7.0 - 12.0
White Blood Cell Count	10.5 ± 2.0 x10^9/L	4.0 - 15.0
Platelet Count	220 ± 30 x10^9/L	150 - 300
RBC Morphology	30 (15%)	-

Table 3: Distribution of small round blue cell tumors and bone marrow infiltration

Tumor Type	Total Cases	Positive BM Infiltration	Infiltration Pattern	n (%)
Neuroblastoma	90	20 (22.2%)	Diffuse Sheets	15 (75%)
			Island/Nest	5 (25%)
Ewing Sarcoma	70	7 (10.0%)	Diffuse Sheets	5 (71.4%)
			Island/Nest	2 (28.6%)
Rhabdomyosarcoma	38	3 (7.9%)	Diffuse Sheets	2 (66.7%)
			Island/Nest	1 (33.3%)
Total Positive Cases	-	30 (15.2%)		

Table 4: Immunohistochemistry (IHC) results for cases with positive bone marrow infiltration

Marker	Positive Cases (%)	Tumor Type	Positive Cases in Tumor Type
CD56	20 (66.7%)	Neuroblastoma	15 (75.0%)
		Ewing Sarcoma	5 (25.0%)
CD99	7 (23.3%)	Ewing Sarcoma	5 (71.4%)
		Rhabdomyosarcoma	2 (28.6%)
Synaptophysin	15 (50.0%)	Neuroblastoma	15 (100.0%)
Chromogranin	10 (33.3%)	Neuroblastoma	10 (100.0%)
S100	3 (10.0%)	Rhabdomyosarcoma	3 (100.0%)
Desmin	3 (10.0%)	Rhabdomyosarcoma	3 (100.0%)

Table 5: Summary of hematological parameters in bone marrow infiltration cases

Parameter	Neuroblastoma (n=20)	Ewing Sarcoma (n=7)	Rhabdomyosarcoma (n=3)
Hemoglobin	8.5 ± 1.0 g/dL	9.0 ± 1.2 g/dL	9.5 ± 1.3 g/dL
Total Leukocyte Count	11.0 ± 1.8	10.8 ± 1.5	10.5 ± 1.6
Platelet Count	210 ± 20 x10^9/L	215 ± 25 x10^9/L	225 ± 30 x10^9/L

Table 6: Odds ratios for tumor types by age and gender

Neuroblastoma			Ewing Sarcoma	Rhabdo-myosarcoma	Total Cases	OR	p-value
Age Group	Birth - 5 years	20 22.2%	5 10.0%	2 7.9%	27 13.6%	2.11	0.02*
	6 - 10 years	15 16.7%	3 4.3%	1 2.6%	19 9.6%	0.67	0.55
	11 - 13 years	45 50.0%	2 2.9%	0 0.0%	47 23.7%		
	Total	90 100.0%	70 100.0%	38 100.0%	198 100.0%		
Gender	Male	60 66.7%	45 64.3%	18 47.4%	123 62.1%	1.5	0.03*
	Female	30 33.3%	25 35.7%	20 52.6%	75 37.9%	0.75	0.03*
	Total	90 100.0%	70 100.0%	38 100.0%	198 100.0%		

DISCUSSION

Malignant small round cell tumors (SRBCTs) represent a diverse group of neoplasms commonly diagnosed in children, including neuroblastoma, rhabdomyosarcoma, Ewing sarcoma, and others. These tumors are characterized by their histological appearance of densely packed small round cells, which pose significant diagnostic challenges due to their overlapping features. Bone marrow infiltration by non-hematopoietic

malignancies often signifies advanced disease and is associated with poor prognoses.[11] Early detection of bone marrow metastasis is crucial for appropriate clinical staging, treatment selection, and prognostic assessment. Understanding the hematological findings and histomorphological characteristics of these tumors is essential to improve diagnostic accuracy and patient outcomes. The study's findings highlight the significant prevalence of bone marrow infiltration by non-hematopoietic small round blue cell tumors in the pediatric population, particularly neuroblastoma. The high incidence of symptoms such as fever and pallor underscores the need for vigilant clinical assessment and early diagnostic intervention. Additionally, the distinct histomorphological features observed in these tumors can aid in differentiating them from other malignancies, ultimately enhancing diagnostic accuracy and guiding treatment strategies.[12,13] Our study explored the demographic, clinical, hematological, and immunohistochemical profiles of small round blue cell tumors with bone marrow involvement in a pediatric population.

When comparing our findings with previous studies, significant similarities and distinctions were noted, providing a broader context for interpreting our results. In our cohort, the male-to-female ratio was 1.5:1, closely aligning with findings by Tasleem *et al*. (2024) and Bhopal *et al*. (2019), who reported ratios of 2:1 and 1.5:1, respectively.[14,15] Male predominance is a recurring observation in pediatric oncology. Regarding age, our study showed the highest prevalence in the 6–10 years age group (36.4%), whereas Tasleem *et al*. (2024) reported a younger mean age of 5.69 years.[14] Neuroblastoma was the most common malignancy in our study (45.5%), followed by Ewing sarcoma and rhabdomyosarcoma, which aligns with findings by Mishra *et al*. (2015) and Shah *et al*. (2000), who also identified neuroblastoma as the predominant tumor in pediatric cases.[18,19]

Fever and pallor were the most common clinical features in our study, reported in 75.8% and 65.7% of cases, respectively. This is consistent with the symptoms of malignancies infiltrating the bone marrow noted in studies by Rana *et al*. (2022) and Gajendra *et al*. (2023).[17] Hematological findings showed a mean hemoglobin level of 9.5 g/dL, with normal leukocyte and platelet counts, which corresponds with Rana *et al*. (2022), who also reported no significant differences in these parameters between cases and controls.[20] Neuroblastoma accounted for the highest bone marrow infiltration rate (22.2%) in our study, consistent with Bhopal *et al*. (2019), who reported that 64.8% of cases with bone marrow involvement were neuroblastoma. The predominant pattern of infiltration in our study consisted of diffuse sheets (75%), a finding commonly observed in infiltrative processes.[15] Similarly, Rastogi *et al*. (2015) highlighted the frequent occurrence of rosette formation in neuroblastoma infiltrates.[16]

Immunohistochemical analysis in our study revealed that CD56 positivity was most prevalent in neuroblastoma (75%), while CD99 positivity was observed in Ewing sarcoma, aligning with findings by Tasleem *et al*. (2024) and Shah *et al*. (2000), who emphasized the diagnostic value of IHC in undifferentiated tumors.[14,19] Synaptophysin and chromogranin were exclusively positive in neuroblastoma cases, consistent with established literature where these markers are considered definitive for neuroendocrine differentiation. Our study identified bone marrow infiltration in 15.2% of cases, which is slightly lower than the 17.9% reported by Bhopal *et al*. (2019) and 35% by Rana *et al*. (2022).[15,20] These variations may be attributed to differences in inclusion criteria or sample sizes. Additionally, our findings support the observations of Gajendra *et al*. (2023), emphasizing the value of bone marrow biopsy combined with IHC as a reliable diagnostic tool.[17] However, elevated serum LDH, highlighted as a key marker by Rana *et al*. (2022), was not evaluated in our study and warrants further investigation.[20] The limitations of our study include the absence of advanced biomarkers like serum LDH and limited radiological data for corroboration, as emphasized in studies by Rana *et al*. (2022) and Gajendra *et al*. (2023).[17,20]

CONCLUSION

This study highlights the significance of bone marrow metastasis in pediatric patients with non-hematopoietic small round blue cell tumors, emphasizing the importance of histomorphological and immunohistochemical analyses in establishing an accurate diagnosis. The findings underscore the necessity for early detection and comprehensive evaluation to improve therapeutic outcomes and prognostic stratification in this vulnerable population.

LIMITATIONS

The limitations include its retrospective design and reliance on existing medical records, which may lead to incomplete data or biases in clinical findings.

SUGGESTIONS / RECOMMENDATIONS

The study's strengths include a comprehensive analysis of bone marrow infiltration in a significant pediatric cohort, which enhances understanding of the prevalence and histopathological characteristics of non-hematopoietic small round blue cell tumors.

CONFLICT OF INTEREST / DISCLOSURE

There was no conflict of interest during the study period.

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