Section: Pathology

Original Research Article

DIAGNOSTIC UTILITY OF IMMUNOHISTOCHEMISTRY IN SMALL ROUND BLUE CELL TUMORS: AT A TERTIARY CARE INSTITUTION

K. Umamaheswari[1], R. Raj Kumar[2], G. Ilanthendral[2]

[1]*Associate Professor, Department of Pathology, Government Medical College & Hospital, Pudukkottai, India.*
[2]*Assistant Professor, Department of Pathology, Government Medical College & Hospital, Pudukkottai, India.*

Received *: 11/01/2026*
Received in revised form *: 10/03/2026*
Accepted *: 29/03/2026*

Corresponding Author:
Dr. G. Ilanthendral,
Assistant Professor, Department of Pathology, Government Medical College & Hospital, Pudukkottai, India.
Email: breeze.krm10@gmail.com

DOI: 10.70034/ijmedph.2026.2.177

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (2); 1047-1052

ABSTRACT

Background: Small round blue cell tumors (SRBCTs) of bone and soft tissues constitute a heterogeneous group of malignant neoplasms characterized by overlapping histomorphological features, posing significant diagnostic challenges on routine hematoxylin and eosin sections. Accurate classification is essential for appropriate therapeutic management and prognostication. Immunohistochemistry (IHC) plays a crucial role in differentiating these entities.

Materials and Methods: This retrospective descriptive study was conducted in our institution, Department of Pathology from January 2023 to December 2023. Formalin-fixed, paraffin-embedded tissue blocks of tumors diagnosed histomorphologically as small round cell tumors on biopsy and resection specimens were retrieved from archival records. Only tumors arising in bone and soft tissues were included, while tumors involving bone marrow, spleen, and lymph nodes were excluded. Bony specimens were decalcified prior to routine processing. Immunohistochemistry was performed using a panel comprising CD45/LCA, CD20, CD3, CD99 (MIC2), Desmin, epithelial membrane antigen (EMA), Cytokeratin (CK), Synaptophysin, Chromogranin, and glial fibrillary acidic protein (GFAP). A total of 11 cases fulfilling inclusion criteria were analyzed.

Results: Eleven cases initially diagnosed as small round cell tumors were evaluated. The cohort comprised 6 cases of Ewing sarcoma/primitive neuroectodermal tumor (PNET), 2 cases of cutaneous T-cell lymphoma, 1 case of alveolar rhabdomyosarcoma, and 1 case of Wilms tumor. One case was reclassified as an inflammatory lesion following immunohistochemical analysis. Immunohistochemistry enabled definitive diagnosis and categorization in all cases and was essential in distinguishing morphologically overlapping entities. The majority of patients were in the 60–70-year age group, with Ewing sarcoma being the most frequent tumor in the present series.

Conclusion: Immunohistochemistry is an indispensable adjunct to histopathology in the accurate diagnosis and subclassification of small round cell tumors of bone and soft tissues. A comprehensive antibody panel facilitates definitive differentiation among morphologically similar lesions and guides appropriate clinical management.

Keywords: Small round blue cell tumor; Immunohistochemistry; Ewing sarcoma; Primitive neuroectodermal tumor; Rhabdomyosarcoma.

INTRODUCTION

Small round blue cell tumors (SRBCTs) constitute a heterogeneous group of highly aggressive malignant neoplasms characterized by primitive, undifferentiated morphology. Histologically, these tumors are composed of sheets of small, round to oval cells with scant cytoplasm, hyperchromatic nuclei, and a high nucleocytoplasmic ratio. On routine hematoxylin and eosin (H&E) stained sections, they

typically appear as "small round blue cells," a descriptive term that reflects their basophilic staining characteristics. However, this morphological similarity often obscures their diverse biological origins, posing significant diagnostic challenges, particularly in small biopsies and poorly preserved specimens.

The spectrum of SRBCTs of bone and soft tissues is broad and includes entities such as Ewing sarcoma/primitive neuroectodermal tumor (PNET), extraskeletal Ewing sarcoma, rhabdomyosarcoma, neuroblastoma, desmoplastic small round cell tumor, non-Hodgkin lymphoma, small cell osteosarcoma, mesenchymal chondrosarcoma, and small cell carcinoma, among others. Despite distinct histogenesis, these tumors frequently share overlapping clinical presentations and histomorphological features, making definitive diagnosis based solely on routine microscopy difficult in a subset of cases.

Recent advances in tumor classification, particularly the WHO Classification of Soft Tissue and Bone Tumours, have significantly refined the diagnostic framework of SRBCTs. The updated classification emphasizes the integration of histomorphology with immunohistochemistry (IHC) and molecular genetics.

From a clinical perspective, accurate identification and subclassification of SRBCTs are of paramount importance, as treatment strategies and prognostic outcomes vary considerably among different entities. For instance, Ewing sarcoma typically requires multimodal therapy including chemotherapy, surgery, and radiotherapy, whereas lymphomas are primarily managed with systemic chemotherapy and immunotherapy. Similarly, rhabdomyosarcoma and other sarcomas follow distinct therapeutic protocols. Therefore, an accurate and timely diagnosis directly influences patient management and survival outcomes.

While clinical features and anatomical location provide useful diagnostic clues, they are often insufficient for definitive diagnosis. In this context, immunohistochemistry has emerged as a crucial adjunct to routine histopathology. By employing a panel of lineage-specific markers, IHC facilitates differentiation between morphologically similar tumors, enabling accurate classification. The use of markers such as CD99, desmin, leukocyte common antigen (LCA), cytokeratin, and neuroendocrine markers allows for systematic evaluation and exclusion of differential diagnoses.

Despite these advances, challenges persist due to immunophenotypic overlap and limited availability of molecular diagnostic techniques in many centers. Consequently, a pragmatic, panel-based IHC approach remains indispensable, particularly in resource-constrained settings.

In this context, the present study aims to evaluate the role of immunohistochemistry in the diagnosis and subclassification of small round blue cell tumors of bone and soft tissues, highlighting its utility in resolving diagnostic dilemmas and guiding appropriate clinical management.

Aims and Objectives

- To analyze the spectrum of small round blue cell tumors of bone and soft tissues over a one-year period.
- To evaluate the role and diagnostic utility of immunohistochemistry in the accurate classification of small round cell tumors.

MATERIALS AND METHODS

This retrospective descriptive study was conducted in our institution, Department of Pathology, over a period of one year from January 2023 to December 2023. Formalin-fixed, paraffin-embedded tissue blocks of tumors diagnosed histomorphologically as small round blue cell tumors on small biopsies and resection specimens were retrieved from departmental archives. Only tumors arising in bone and soft tissues were included in the study. Bony specimens underwent decalcification prior to routine tissue processing.

Cases from all age groups and both sexes were included. Tumors primarily involving the bone marrow, spleen, and lymph nodes were excluded.

Immunohistochemistry was performed on representative sections to aid in differentiation and categorization. The antibody panel comprised CD45/LCA, CD20, CD3, CD99 (MIC2), Desmin, epithelial membrane antigen (EMA), Cytokeratin (CK), Synaptophysin, Chromogranin, and glial fibrillary acidic protein (GFAP). Appropriate positive and negative controls were used.

A total of 11 cases fulfilling inclusion criteria were identified and analyzed.

RESULTS

A total of 11 cases initially diagnosed histomorphologically as small round cell tumors were evaluated. The cohort included 6 cases of Ewing sarcoma/primitive neuroectodermal tumor (PNET), 2 cases of cutaneous T-cell lymphoma, 1 case of alveolar rhabdomyosarcoma, and 1 case of Wilms tumor. One case was subsequently reclassified as an inflammatory lesion following immunohistochemical evaluation.

Immunohistochemistry facilitated definitive categorization of all cases and was essential in distinguishing morphologically overlapping entities within the small round blue cell tumor spectrum. The majority of patients were in the 60–70-year age group with a male predominance.

Table 1: age wise distribution

Age in years	No. Of cases	Percentage
<10	2	18
10 -20	2	18
21-30	1	9
31-40	1	9
41-50	0	0
51-60	2	18
>60	3	27

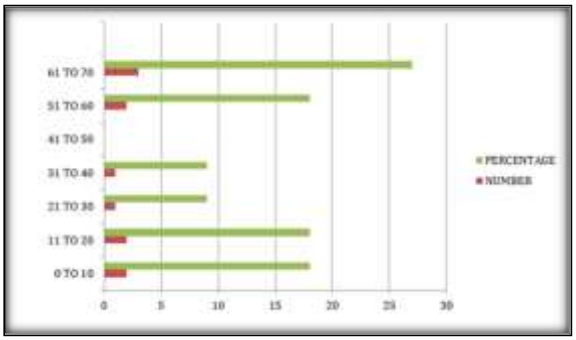

Figure 1: age distribution

Most common age group reported in this study comes over 60 years.

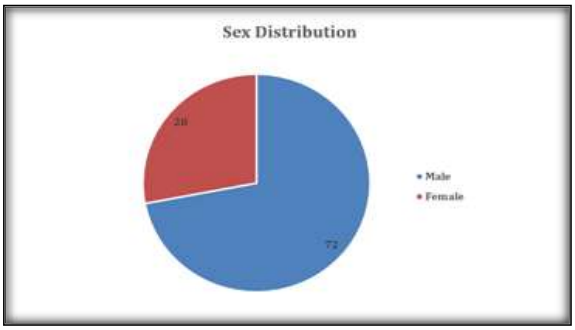

Figure 2: sex distribution

In this study males are more commonly affected compared to females.

Table 2: Sex wise distribution

S.no	Sex	Number	Percentage
1.	Male	8	72
2.	Female	3	28

Table 3: histopathological diagnosis

S.no	HPE diagnosis	No.of cases	Percentage
1	Ewing sarcoma	6	54.4
2	Wilms tumor	1	9
3	Alveolar RMS	1	9
4	Cutaneous T cell lymphoma	2	18
5	Inflammatory lesions	1	9

Table 4: Histopathological Spectrum of Small Round Cell Tumors

Tumor type	Number of cases	Percentage
Ewing sarcoma/PNET	6	54.5%
Cutaneous T-cell lymphoma	2	18%
Alveolar rhabdomyosarcoma	1	9%
Wilms tumor	1	9%
Inflammatory lesion	1	9%

Table 5: immunohistochemical panel of markers

S. No	Age /Sex	SITE	HPE Diagnosis	CD 45	CD 3	CD20	CD99	GFAP	Vimentin	Others (PAN CK/SYNAPTO)	Final Diagnosis
1	35/F	D10 Vertebra	Epidural SOL	--			+	--			Ewing sarcoma
2	23/ M	Iliac bone	Malignant tumor	--			+				Ewing sarcoma
3	64/ M	L5 Vertebra	Lymphoma		--	--	+				Ewing sarcoma
4	17/ M	Iliac muscle	RMS				+		--	--	
5	52/F	Slide received	SRBCT		+	+	--			--	Inflammatory lesion
6	2.5/ F	Left forearm	Alveolar RMS							MyoD1 +	Alveolar RMS
7	61/ M	Sacrum	Metastasis / SRBCT				+				Ewing sarcoma
8	7/F	Left kidney	Wilms tumor								Wilms tumor

9	15/ M	D4 vertebra	Lymphoma	--	--	--	+				Ewing sarcoma
10	63/ M	Retroperitoneal mass	SRBCT		+	--	--	--	--	--	T cell lymphoma
11	57/ M	Skin	Mycosis fungoides		+	--					Cutaneous T cell lymphoma

Figure 3: histopathological diagnosis

In this study Ewings sarcoma contributes maximum percentage of cases.

Color Plate

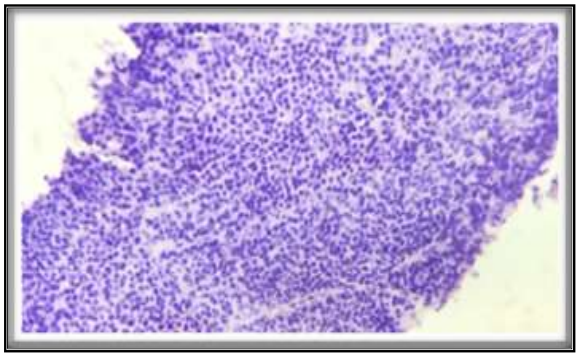

Figure 4: Ewing sarcoma/primitive neuroectodermal tumor (PNET), bone.
(A) Hematoxylin and eosin (H&E) section showing sheets of uniform small round cells with scant cytoplasm

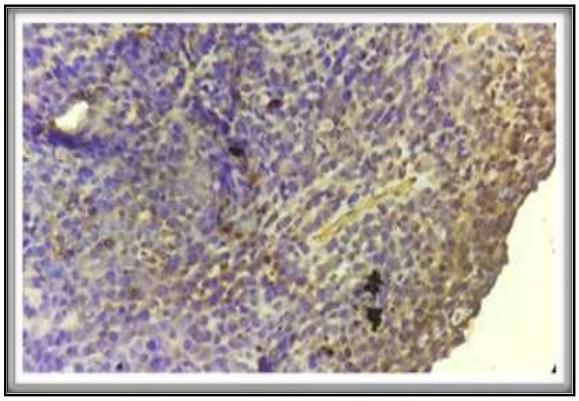

Figure 5: (B) Diffuse membranous positivity for CD99 on immunohistochemistry confirming Ewing sarcoma (×200).

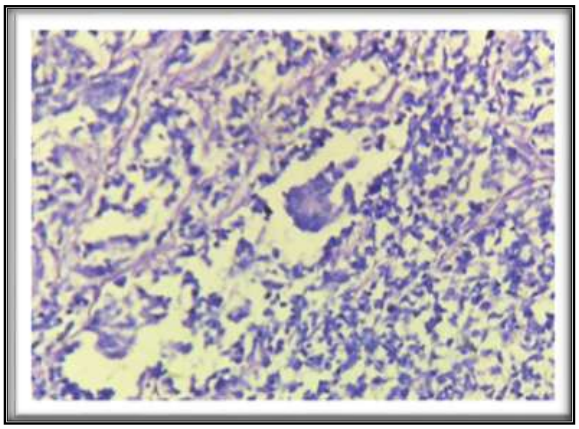

Figure 6: Alveolar rhabdomyosarcoma, soft tissue.
(A) H&E section showing nests of dyscohesive small round tumor cells separated by fibrous septae (alveolar pattern) (×200).

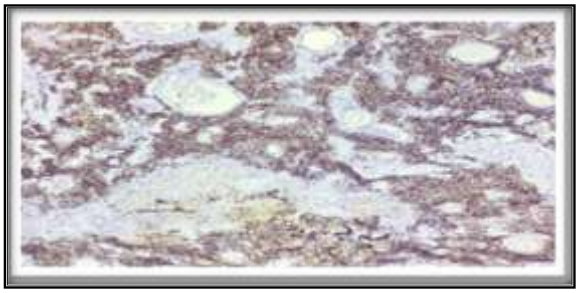

Figure 7: (B) Nuclear positivity for MyoD1 in tumor cells on immunohistochemistry (×200).

Figure 8: Gross image of Wilms tumor

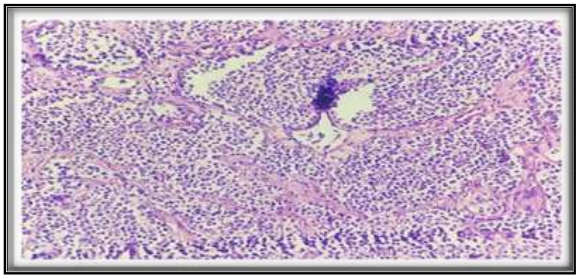

Figure 9: Wilms tumor, kidney.(A) H&E section showing predominant blastemal component with focal epithelial differentiation (×200).

DISCUSSION

Small round blue cell tumors (SRBCTs) of bone and soft tissues represent a diagnostically complex and heterogeneous group of malignancies unified by their primitive cytomorphology and overlapping histological features. The diagnostic challenge is particularly pronounced in small biopsy specimens, where architectural patterns may be limited. The WHO Classification of Soft Tissue and Bone Tumours has significantly redefined this group by integrating molecular genetics into routine classification, thereby shifting the diagnostic paradigm from morphology-based to molecularly driven taxonomy.[1]

A key conceptual advancement in the WHO 5th edition is the recognition of undifferentiated small round cell sarcomas as a distinct category, encompassing Ewing sarcoma, CIC-rearranged sarcoma, BCOR-associated sarcoma, and other rare fusion-driven entities.[1,2] This reclassification underscores the biological heterogeneity within tumors that were previously grouped under "Ewing-like sarcomas." Importantly, these entities demonstrate differences in clinical behavior, response to therapy, and prognosis, making accurate subclassification clinically imperative.

In the present study, Ewing sarcoma/PNET constituted the predominant tumor type, accounting for 54.5% of cases. This finding is consistent with prior reports that identify Ewing sarcoma as one of the most frequent SRBCTs of bone and soft tissue.[3] The diagnosis in our series relied primarily on diffuse membranous CD99 expression, a sensitive but not entirely specific marker. As highlighted in contemporary literature, CD99 positivity may also be observed in other neoplasms, including lymphoblastic lymphoma and synovial sarcoma, necessitating cautious interpretation in conjunction with morphology and additional markers.[3,4] In resource-limited settings, where molecular diagnostics such as EWSR1 rearrangement testing may not be readily available, a judicious IHC panel remains the cornerstone of diagnosis.

An intriguing observation in our study was the predominance of cases in the 60–70-year age group, which deviates from the classical age distribution of Ewing sarcoma, typically affecting children and adolescents. This discrepancy may reflect institutional referral patterns, small sample size, or potential inclusion of morphologically similar entities within the spectrum of undifferentiated round cell sarcomas. The WHO classification cautions against over-reliance on age as a diagnostic criterion, particularly in tumors with ambiguous morphology, thereby reinforcing the need for a comprehensive diagnostic approach.

The identification of cutaneous T-cell lymphoma in two cases highlights a critical diagnostic pitfall, as lymphomas can closely mimic SRBCTs histologically. The use of lymphoid markers such as CD45, CD3, and CD20 was instrumental in establishing the diagnosis. This distinction has profound therapeutic implications, as lymphomas are managed with systemic chemotherapy and immunotherapy rather than surgical excision.[5] Similarly, the diagnosis of alveolar rhabdomyosarcoma in our study was supported by Desmin positivity, indicative of myogenic differentiation. Current evidence further emphasizes the prognostic significance of molecular alterations such as FOXO1 gene fusions in alveolar rhabdomyosarcoma, which are associated with more aggressive clinical behavior.[6]

The inclusion of epithelial markers (CK, EMA) and neuroendocrine markers (synaptophysin, chromogranin) in our IHC panel facilitated exclusion of other differential diagnoses such as poorly differentiated carcinoma and neuroblastoma. This highlights the importance of a panel-based, algorithmic approach in evaluating SRBCTs, as no single immunomarker is entirely specific. The stepwise use of lineage-specific markers significantly enhances diagnostic accuracy and reduces the likelihood of misclassification.

A noteworthy finding in our study was the reclassification of one case as an inflammatory lesion following IHC analysis. This underscores the potential for overdiagnosis of malignancy in small round cell lesions, particularly in the presence of dense inflammatory infiltrates. Such diagnostic pitfalls can lead to overtreatment, further emphasizing the indispensable role of IHC in confirming malignancy and lineage differentiation.

Despite the strengths of IHC, its limitations must be acknowledged. Immunophenotypic overlap among SRBCTs remains a significant challenge, and certain entities may exhibit aberrant or focal marker expression. The WHO 5th edition strongly advocates the incorporation of molecular diagnostics, particularly in cases with atypical morphology or inconclusive immunoprofiles.[1,2] However, limited access to advanced molecular techniques in many centers, especially in developing countries, remains a practical constraint.

CONCLUSION

From a clinical perspective, accurate subclassification of SRBCTs is essential, as treatment protocols and prognosis vary widely among different entities. For instance, Ewing sarcoma requires multimodal therapy including chemotherapy, surgery, and radiotherapy, whereas lymphomas are primarily treated with systemic regimens. Therefore, precise diagnosis directly impacts patient management and outcomes.

this study reinforces the pivotal role of immunohistochemistry as an adjunct to histopathology in the evaluation of small round blue cell tumors. While morphology remains the initial diagnostic step, a systematic approach integrating

IHC and, where feasible, molecular diagnostics is essential for accurate classification. The evolving WHO framework underscores a shift toward precision diagnostics, which is crucial for guiding appropriate therapy and improving patient outcomes.

REFERENCES

1. WHO Classification of Tumours Editorial Board. Soft Tissue and Bone Tumours. 5th ed. Lyon: World Health Organization; 2020.
2. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F, editors. WHO Classification updates on undifferentiated small round cell sarcomas. IARC Press; 2020.
3. Wei S, Siegal GP. Small round cell tumors of soft tissue and bone. Arch Pathol Lab Med. 2022;146(1):47–59.
4. Thway K, Fisher C. Histopathological diagnostic approach to round cell tumors. Semin Diagn Pathol. 2019;36(3):152–166.
5. National Comprehensive Cancer Network. NCCN Guidelines for Non-Hodgkin Lymphoma. 2023.
6. Skapek SX, Ferrari A, Gupta AA, et al. Rhabdomyosarcoma classification and molecular advances. Lancet Oncol. 2019;20(1):e36–e48.