Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/qcm4qy94

SOLVING DIAGNOSTIC CHALLENGES IN PEDIATRIC SMALL ROUND CELL TUMORS WITH IMMUNOHISTOCHEMISTRY

Dr. Divya Jain[1]*, Dr. Achin Gupta[2], Dr. Neeraj Raman[3], Dr. Amandeep[4]

[1*]Assistant Professor, Department of Pathology, Government Medical College, Alwar
[2]Assistant Professor, Department of Anaesthesia, Govt Medical College, Alwar
[3]Assistant Professor, Department of Microbiology, Govt Medical College, Alwar
[4]Assistant Professor, Department of Pediatrics, Government Medical College, Alwar

***Corresponding Author:** Dr. Divya Jain
*Email.divya0925jain@gmail.com

ABSTRACT

BACKGROUND: Pediatric small round cell tumors (SRCTs) are a group of aggressive cancers that look very similar under the microscope, making them difficult to tell apart based on appearance alone. An accurate diagnosis is critical because each type requires different treatment.

AIM: This study aimed to test how effective a standard panel of immunohistochemistry (IHC) stains is at providing a definitive diagnosis for these challenging tumors.

METHODS: We conducted a prospective study of 100 children with SRCTs. After an initial review under the microscope, all cases were tested with a targeted IHC panel designed to identify different tumor lineages (including CD99, myogenin, CD45, and synaptophysin).

RESULTS: Initial microscopic examination failed to provide a specific diagnosis in 71% of cases, labelling them only as "undifferentiated." The IHC panel successfully resolved 99% of all cases, providing a specific diagnosis. The Ewing sarcoma family (50%) was the most common tumor, followed by embryonal rhabdomyosarcoma (17%).

CONCLUSION: A systematic IHC panel is a highly effective and essential tool for diagnosing pediatric SRCTs. It resolves the vast majority of ambiguous cases, ensuring that children receive the correct diagnosis as the crucial first step towards appropriate therapy.

KEYWORDS: Pediatric Small Round Cell Tumors; Immunohistochemistry; Diagnostic Accuracy; Ewing Sarcoma; Rhabdomyosarcoma; Pathology.

INTRODUCTION

Small round cell tumors (SRCTs) in the pediatric population represent a morphologically overlapping group of aggressive neoplasms predominantly affecting children and adolescents[1]. These tumors typically exhibit sheets of undifferentiated, small round blue cells with a high nuclear-to-cytoplasmic ratio and scant cytoplasm, making diagnosis based solely on morphology challenging[2]. The principal entities in this spectrum include the Ewing sarcoma family of tumors, rhabdomyosarcoma, neuroblastoma, lymphoblastic lymphoma, and desmoplastic small round cell tumor (DSRCT)[3]. Accurate subtype identification is critical, as each tumor exhibits distinct biological behavioural, prognosis, and therapeutic requirements[4]. Immunohistochemistry (IHC) has emerged as an essential adjunct in the diagnostic workup, enabling precise lineage assignment through the detection of differentiation-specific antigens[5]. Despite its widespread use, diagnostic ambiguity persists,

particularly in resource-limited settings where antibody panels may be restricted or tissue preservation suboptimal[6]. Published series indicate that a significant proportion (40–70%) of SRCT cases remain ambiguous on haematoxylin and eosin (H&E) staining alone, highlighting the need for systematic IHC application[7]. Moreover, there exists a gap in the literature regarding prospective validation of compact, yet comprehensive, IHC panels across the full spectrum of pediatric SRCTs. Many prior studies are retrospective, limited to single tumor types, or do not quantify the diagnostic refinement achieved by IHC in real-world workflows[8].

We hypothesized that a systematic, lineage-oriented IHC approach would resolve the vast majority of initially ambiguous SRCT cases, allowing precise subclassification in most instances. Our results (detailed below) support this, demonstrating a high diagnostic yield and illuminating cases that remain undifferentiated despite IHC, with implications for future practice and molecular integration. In the following sections, our study will present the baseline clinicopathologic profile of our cohort, the diagnostic efficacy of IHC, the final distribution of tumor types, and a discussion of how our findings align with or differ from existing literature, concluding with recommendations and limitations.

MATERIALS AND METHODS

Study Design and Setting

This prospective diagnostic study was conducted over a 24-month period (August 2022–July 2024) in the Department of Histopathology. The study included both in-house and referral cases.

Patient Selection and Eligibility

A total of 120 cases of suspected small round cell tumors (SRCTs) in patients aged 15 years or younger were initially reviewed. Cases were included if they showed histological evidence of a malignant SRCT on haematoxylin and eosin (H&E)-stained sections, had adequate formalin-fixed paraffin-embedded (FFPE) tissue for a complete immunohistochemical (IHC) workup, and were accompanied by complete clinical details including age, sex, and tumor site. Cases were excluded if they represented central nervous system tumors other than medulloblastoma, lymphoproliferative disorders not classified as SRCTs (such as Hodgkin lymphoma), or specimens that were improperly fixed, autolyzed, or lacked sufficient tissue for IHC evaluation. After applying these criteria, 100 cases were included for final analysis. The remaining 20 cases were excluded due to inadequate tissue (n = 7), autolysis (n = 5), reclassification as a non-SRCT malignancy on IHC (n = 5), or patient age exceeding 15 years (n = 3).

Histopathological Processing

All tissue specimens were fixed in 10% neutral-buffered formalin for 18–24 hours. Bone-containing samples were decalcified before processing. After gross examination, tissues were dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin. Sections of 4–5 μm thickness were cut and stained with haematoxylin and eosin (H&E) using standard protocols. Each slide was independently reviewed by two pathologists to establish a preliminary morphological diagnosis.

Immunohistochemistry

The most representative tumor block was selected for IHC. Sections were mounted on gelatin-coated slides, deparaffinized, and rehydrated, followed by heat-induced epitope retrieval in citrate buffer (pH 6.0) using a BIOGENEX EZ-Retriever system. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol, and nonspecific binding was minimized using a commercial protein block. The slides were then incubated with primary antibodies (Table 1), followed by a super enhancer and polymer-HRP conjugate (BIOGENEX Super Sensitive Polymer-HRP Detection System). The reaction was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, and the sections were counterstained with Mayer's haematoxylin. Appropriate positive and negative controls were included with each batch.

Table 1: Diagnostic Antibody Panel

Antibody	Clone	Diagnostic Utility
CD45	LCA88	Lymphoid
CD99	12E7	Ewing Sarcoma
Vimentin	V9	Mesenchymal
Desmin	D33	Myogenic
Myogenin	F5D	Rhabdomyosarcoma
Synaptophysin	SNP88	Neural
Chromogranin	Polyclonal	Neuroendocrine
Cytokeratin	AE1/AE3	Epithelial
EMA	E-29	Epithelial
WT1	6F-H2	Wilms tumor / DSRCT

Interpretation and Diagnostic Criteria

IHC staining was assessed for its pattern (membranous, cytoplasmic, nuclear), intensity (weak, moderate, strong), and distribution (focal or diffuse). Staining in more than 5% of tumor cells was considered positive. The final diagnosis was established by correlating morphological features with the complete IHC profile, in accordance with the WHO classification of pediatric tumors.

Data Analysis

Patient demographic details, tumor sites, histopathological findings, IHC results, and final diagnoses were compiled in a master database. Data were analyzed descriptively and expressed as numbers and percentages.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee. All procedures were performed in accordance with the ethical standards of the institutional and national research committees and with the principles outlined in the Declaration of Helsinki and its subsequent amendments. As the study involved the use of archived, anonymized histopathological specimens without direct patient contact, the requirement for individual informed consent was waived by the committee.

RESULTS

Baseline Clinicopathological Characteristics: The study cohort of 100 patients had a male predominance (60%, M:F ratio 1.5:1). The age distribution showed a preponderance in older children, with 50% of cases in the 11–15-year age group. The most common primary sites were the musculoskeletal system (49%) and the head and neck region (21%), as detailed in Table 2.

Table 2: Baseline Characteristics of the Study Cohort (n=100)

Characteristic	Category	n	%
Age (years)	1-5	20	20.0
	6-10	30	30.0
	11-15	50	50.0
Gender	Male	60	60.0

Characteristic	Category	n	%
	Female	40	40.0
Primary Site	Musculoskeletal	49	49.0
	Head & Neck	21	21.0
	Abdomen/Pelvis	22	22.0
	Other	8	8.0

The cohort is characterized by a male predominance and a preponderance of older children (ages 11-15). Nearly half of all tumors originated in the musculoskeletal system, setting the stage for the high frequency of Ewing sarcoma and rhabdomyosarcoma later identified.

Table 3: Diagnostic Refinement by Immunohistochemistry

Initial Morphological Diagnosis	n	Final IHC Diagnosis	n (%)
Undifferentiated Round Cell Tumor	71	Ewing Sarcoma Family	41 (57.7%)
		Embryonal Rhabdomyosarcoma	15 (21.1%)
		Neuroblastoma	8 (11.3%)
		Desmoplastic Small Round Cell Tumor	4 (5.6%)
		Lymphoblastic Lymphoma	1 (1.4%)
		Remained Undifferentiated	1 (1.4%)
All Other Suspected Diagnoses	29	Confirmed by IHC	28 (96.6%)
		Changed after IHC	1 (3.4%)
TOTAL	100		100

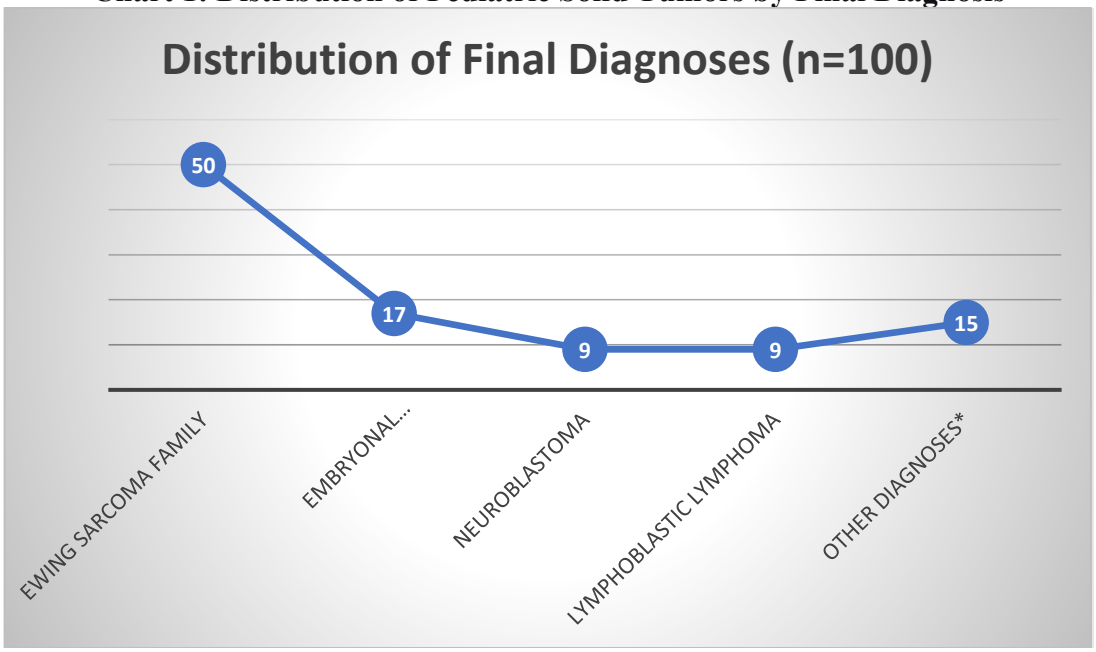
H&E morphology alone was non-diagnostic in 71% of cases. IHC was transformative, resolving 98.6% of these ambiguous cases and confirming or correcting nearly all preliminary diagnoses, achieving a definitive classification in 99% of the cohort.

Table 4: Distribution of Final Diagnoses (n=100)

Final Diagnosis	n	%
Ewing Sarcoma Family	50	50.0
Embryonal Rhabdomyosarcoma	17	17.0
Neuroblastoma	9	9.0
Lymphoblastic Lymphoma	9	9.0
Other Diagnoses*	15	15.0
TOTAL	100	100.0
*Includes Desmoplastic Small Round Cell Tumor (4), Burkitt Lymphoma (3), Retinoblastoma (3), Wilms Tumor (3), Medulloblastoma (1), Undifferentiated Tumor (1).		

After IHC analysis, the Ewing sarcoma family of tumors emerges as the single most common entity, accounting for half of all cases. This distribution, dominated by bone/soft tissue tumors, likely reflects the study's inclusion criteria and institutional referral patterns**.**

Chart 1: Distribution of Pediatric Solid Tumors by Final Diagnosis



Ewing Sarcoma was most common (50%), followed by Embryonal (17%), while others were less frequent.

Table 5: Essential IHC Profile for Major Pediatric SRCTs

Final Diagnosis	Key Positive Markers	Key Negative Markers
Ewing Sarcoma Family	CD99 (Membranous), Vimentin	CD45, Myogenin
Embryonal Rhabdomyosarcoma	Myogenin (Nuclear), Desmin	CD45, CD99
Neuroblastoma	Synaptophysin, Chromogranin	CD99, Desmin
Lymphoblastic Lymphoma	CD45, CD99 (Cytoplasmic)	Myogenin
Desmoplastic Small Round Cell Tumor	Desmin, Cytokeratin, WT1	Myogenin, CD45

Distinct marker patterns reliably differentiated major SRCTs, confirming IHC's pivotal diagnostic role.

DISCUSSION

The diagnostic odyssey of pediatric small round cell tumors (SRCTs) represents a central challenge in surgical pathology, where morphological ambiguity meets clinical urgency. Our prospective study of 100 cases unequivocally demonstrates that this diagnostic impasse is best resolved through the systematic application of immunohistochemistry (IHC). The finding that 71% of cases were initially classified as Undifferentiated Round Cell Tumors (URCTs) on H&E staining alone (**Table 3**) is a stark testament to the profound morphological overlap within this group. This high percentage of diagnostically ambiguous cases lies between the rates reported by Sajid H. Shah et al[9] (45.2%) and Lawerence D Cruze et al[10] (93.02%). The biological basis for this "small blue round cell" phenotype is rooted in the shared origin of these tumors from primitive, rapidly proliferating progenitor cells, leading to a common histologic appearance of high nuclear-to-cytoplasmic ratio and hyperchromasia. The variation across studies likely reflects differences in pathologist expertise, the specific case mix (e.g., proportion of site-specific tumors like retinoblastoma that offer morphological clues), and institutional referral patterns.

The paramount contribution of IHC is highlighted by its ability to resolve 99% of cases in our cohort, a success rate that aligns with studies by Mandakini M Patel[11] (96.25%) and J.O Thomas et al[12] (96%). This transformative power is most evident in the reclassification of URCTs, where IHC revealed that the majority were Ewing sarcoma (57.7%) or embryonal rhabdomyosarcoma (21.1%). This shift from a non-specific morphological label to a precise lineage-based diagnosis is the critical first step in initiating tumor-specific, often multimodal therapy. The single undifferentiated case in our series, which required referral for molecular studies, exemplifies a known diagnostic limitation. The mechanisms for such IHC failures can include poor antigen preservation, aberrant or null phenotypic expression, or the existence of truly undifferentiated or novel neoplasms that lack expression of known lineage-specific markers.

The final distribution of tumors in our cohort (**Table 4**), dominated by the Ewing sarcoma family (50%), contrasts with several other series that reported lymphoma as the most common SRCT.[13,14,10,15] This discordance is not random but has a clear mechanistic explanation rooted in our rigorous methodology. We explicitly excluded leukemic presentations and non-SRCT lymphomas (e.g.,

Hodgkin lymphoma), which constitute a significant proportion of pediatric lymphoid malignancies. Furthermore, a potential referral bias towards a well-equipped orthopaedic center at our institution likely enriched our cohort for bone and soft tissue tumors, thereby elevating the relative proportion of Ewing sarcoma. This highlights how patient population, institutional expertise, and strict inclusion criteria can significantly shape the perceived epidemiology of these tumors. The IHC profiles we established (**Table 5**) are both robust and clinically practical, yet they also reveal the pitfalls of over-relying on single markers. The sensitivity of CD99 for Ewing sarcoma, seen in 100% of our cases, is linked to the pervasive overexpression of the MIC2 glycoprotein, a direct downstream target of the EWSR1-FLI1 fusion oncoprotein that defines this disease.[16] However, the critical pitfall of CD99 positivity in 100% of our lymphoblastic lymphoma cases underscores its lack of absolute specificity. The mechanism here is unrelated to EWSR1 rearrangements and is thought to be part of the immature T-cell phenotype, a well-documented diagnostic trap.[10,18] This cross-reactivity mandates that CD99 never be used in isolation but always as part of a interpretive panel, most importantly including CD45 to exclude hematopoietic malignancies.

Conversely, myogenin proved to be a highly specific and reliable nuclear marker for rhabdomyosarcoma. Its nuclear localization is a direct reflection of its role as a transcription factor master regulator of myogenic differentiation, making it a definitive lineage-decision marker.[11,15] The complex, polyphenotypic immunoprofile of Desmoplastic Small Round Cell Tumor (DSRCT)—co-expressing desmin (mesenchymal), cytokeratin (epithelial), and WT1 (nuclear)—is a classic example of "divergent differentiation." This is mechanistically driven by the EWSR1-WT1 fusion oncogene, which aberrantly regulates genes from multiple lineages, forcing the tumor to express conflicting lineage markers.[17] This very confusion becomes a diagnostic signature when interpreted correctly.

Our data on marker expression also provides practical insights. The variable expression of vimentin across multiple tumor types confirms its role as a sensitive but non-specific marker of mesenchymal lineage, consistent with studies by J.O Thomas[12] and Mandakini M Patel.[11] Similarly, the expression of neural markers (Synaptophysin, Chromogranin) in neuroblastoma, while characteristic, was not 100% for all markers, reinforcing the established practice of using a panel of at least two neural markers to confirm neuroendocrine differentiation reliably.[19,20] The consistent negativity for CD99 in neuroblastoma, a neural crest-derived tumor, provides a crucial discriminatory feature from Ewing sarcoma, a tumor of putative mesenchymal origin.

Clinical significance

This study holds direct clinical significance by demonstrating that a systematic, panel-based immunohistochemistry (IHC) approach is indispensable for diagnosing pediatric small round cell tumors. By resolving diagnostic ambiguity in 99% of cases, this method enables the critical first step of accurate lineage assignment. This precision is not academic; it directly dictates the choice of tumor-specific, often vastly different, chemotherapeutic regimens and surgical or radiation strategies. Consequently, the implementation of this accessible diagnostic protocol ensures children receive the correct, potentially curative therapy from the outset, thereby avoiding the delays and toxicities of inappropriate treatment and directly working to improve overall survival outcomes.

Limitations

Our findings should be interpreted in the context of certain limitations. The single-center, prospective design may introduce selection bias, as evidenced by our high proportion of bone and soft tissue tumors. Furthermore, the primary diagnostic standard was IHC itself. While this is the standard of care in most diagnostic settings, the study would be strengthened by correlative molecular confirmation (e.g., FISH for EWSR1, FOXO1 fusions) in a larger subset of cases, which represents the contemporary gold standard for definitive diagnosis of many of these entities.

CONCLUSION

Pathological diagnosis of pediatric SRCTs requires a paradigm shift from reliance on morphology alone to the mandatory integration of a targeted IHC panel. A systematic, antibody-based approach is

highly effective, resolving diagnostic uncertainty in the vast majority of cases by leveraging the distinct molecular pathways that define each tumor type. The interpretation of these markers, however, must be nuanced, acknowledging both their powerful specificity and their potential pitfalls. This diagnostic precision is the non-negotiable first step toward delivering appropriate, potentially curative, and risk-stratified therapy to children with these malignancies.

AUTHOR CONTRIBUTIONS: All authors contributed equally to this work**.** Each author was involved in the conception and design of the study, acquisition and analysis of data, drafting and critical revision of the manuscript, and has given final approval for the version to be published.

FUNDING: No external funding was received for this study.

CONFLICT OF INTEREST: NIL

REFERENCES

1. Sharma S, Sharma M. Round Cell Tumors: Classification and Diagnostic Approach. J Pathol Transl Med. 2017;51(3):205-217.
2. Rudzinski ER. Introduction to small round-cell tumors. In: Essentials of Surgical Pediatric Pathology. Cambridge: Cambridge University Press; 2019. p. 45–60.
3. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol. 2009;27(2):289-97.
4. Rekhi B. Small round cell lesions of the bone: Diagnostic approach, differential diagnoses and impact on treatment. Indian J Pathol Microbiol. 2019;62(2):123-134.
5. Sebire NJ, Gibson S, Rampling D, Williams E, Malone M, Ramsay AD. Immunohistochemical findings in embryonal small round cell tumors of childhood. Histopathology. 2005;46(5):498-505.
6. Gawash A, El Gohary G, Elsaid H. Desmoplastic small round cell tumor: current understanding and challenges. J Pediatr Hematol Oncol. 2025;47(3):215-223.
7. Barroca H. Pediatric small round blue cell tumors: cytopathological puzzle or an intriguing window. Acta Cytol. 2022;66(4):319-330.
8. Thomas JO, Mandakini MP. Small round cell tumors in children: immunohistochemical refinement of diagnosis. J Pediatr Pathol. 2018;41(1):12-20.
9. Shah SH, Soomro IN, Siddiqui MS, Pervez S, Hassan SH. Immunohistochemical Evaluation Of Small Round Cell Tumors of Childhood. J Pak Med Assoc. 1999;49(4):87-9.
10. D'cruze L, Dutta R, Rao S, R A, Varadarajan S, Kuruvilla S. The role of immunohistochemistry in the analysis of the spectrum of small round cell tumours at a tertiary care centre. J Clin Diagn Res. 2013;7(7):1377-82.
11. Patel MM, Dhandha ZB, Italiya SL, Shah MB, Kaptan KR, Mansuri BM. Role of Immunohistochemistry In Differential Diagnosis Of Round Cell Tumor. Natl J Med Res. 2013;3(5):207-210.
12. Thomas JO, Olu-Eddo AA. Immunocytochemistry in the diagnosis of small blue cell tumours of childhood. West Afr J Med. 2006;25(3):199-204.
13. Jabeen S, Haque M, Islam MJ, Talukder MH. Profile of Paediatric Malignancies: A Five Year Study. Dhaka Med Coll. 2010;19(1):33-38.
14. Malik FR, Tahir A, Bashir H, Akhtar P. Spectrum of pediatric malignancies. J Rawalpindi Med Coll. 2012;16(1):47-49.
15. Bashyal R, Pathak TB, Shrestha S, Pun CB, Banstola S, Neupane S, et al. Role Of Immunohistochemistry In the Diagnosis of Malignant Small Round Cell Tumors. J Pathol Nepal. 2011;1:87-91.
16. Ordóñez NG. Role of immunohistochemistry in differentiating eosinophilic mesothelioma from lung adenocarcinoma. Adv Anat Pathol. 1998;5(4):216-23.

17. Gerald WL, Rosai J, Ladanyi M. Characterization of the EWS-WT1 fusion gene in desmoplastic small round cell tumor. Cancer Res. 1995;55(6):1385-92.
18. Folpe AL, Goldblum JR, Rubin BP, Shehata BM, Liu W, Dei Tos AP, et al. Morphologic and immunophenotypic diversity in Ewing family tumors: a study of 66 genetically confirmed cases. Am J Surg Pathol. 2005;29(8):1025-33.
19. Hachitanda Y, Tsuneyoshi M, Enjoji M. An ultrastructural and immunohistochemical evaluation of cytodifferentiation in neuroblastic tumors. Mod Pathol. 1989;2(1):13-9.
20. Miettinen M, Rapola J. Synaptophysin - an immunohistochemical marker for childhood neuroblastoma. Acta Pathol Microbiol Immunol Scand A. 1987;95(4):167-70.