



PEDIATRIC MALIGNANT SMALL ROUND CELL TUMORS: AN IMMUNOHISTOCHEMICAL ANALYSIS

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ABSTRACT

Background: Malignant small round cell tumors (MSRCTs) of childhood pose diagnostic challenges due to overlapping morphology but divergent prognoses and treatments. Immunohistochemistry (IHC) is essential for accurate subclassification, yet standardized algorithms are lacking in many resource-limited settings. **Aim:** To evaluate the IHC profile of 11 markers in 100 pediatric MSRCTs, determine diagnostic utility (sensitivity, specificity, predictive values), and propose a practical diagnostic algorithm. **Methods:** A prospective study of 100 consecutive MSRCTs (≤ 15 years) was conducted. An IHC panel comprising CD45, CD99, vimentin, desmin, myogenin, NSE, chromogranin, synaptophysin, cytokeratin, EMA, and WT1 was applied. Positivity was defined as $>10\%$ tumor cells with moderate-to-strong staining. Sensitivity, specificity, PPV, NPV, and p-values (Fisher's exact test) were calculated. **Results:** Myogenin, chromogranin, and CD45 were 100% sensitive and specific for embryonal rhabdomyosarcoma, neuroblastoma, and lymphoblastic lymphoma, respectively ($p < 0.0001$). CD99 was 100% sensitive for Ewing sarcoma/PNET but only 70.6% specific due to positivity in lymphoblastic lymphoma (100%) and 29% of embryonal rhabdomyosarcomas. WT1 showed nuclear positivity in all Wilms tumours and cytoplasmic staining in 50% of DSRCTs. Desmin was positive in both embryonal rhabdomyosarcoma and DSRCT, resolved by myogenin and cytokeratin. **Conclusion:** A carefully chosen set of immunohistochemistry markers can correctly diagnose childhood small round cell tumors. Myogenin, chromogranin, and CD45 are the best markers for their respective tumors. CD99 must be used together with other markers. We recommend a stepwise approach starting with CD45, then myogenin, then chromogranin, and finally CD99 with WT1.

Keywords: Malignant small round cell tumors; pediatric neoplasms; immunohistochemistry; diagnostic algorithm; lineage-defining markers

INTRODUCTION

Malignant small round cell tumors (MSRCTs) of childhood represent a diagnostically challenging group of neoplasms characterized by primitive, undifferentiated morphology on light microscopy. These tumors include Ewing sarcoma, peripheral neuroectodermal tumor (PNET), embryonal rhabdomyosarcoma (ERMS), neuroblastoma, lymphoblastic lymphoma, Burkitt lymphoma,

desmoplastic small round cell tumor (DSRCT), retinoblastoma, Wilms tumor (blastemal type), and medulloblastoma [1,2]. Although their clinical presentations and treatment protocols differ markedly, their overlapping histologic appearance often leads to diagnostic ambiguity [3,4]. In India and other developing countries, pediatric malignancies account for a significant proportion of cancer burden, yet access to advanced diagnostic modalities remains limited [1,5]. Accurate subclassification of MSRCTs is essential because prognosis and therapy vary widely – from intensive chemotherapy for lymphoblastic lymphoma to surgical resection for localized Wilms tumor [6,7]. Conventional hematoxylin and eosin (H&E) staining alone is insufficient; immunohistochemistry (IHC) has emerged as the cornerstone for differential diagnosis, providing lineage-specific markers that can distinguish among these entities [8,9].

Over the past two decades, panels of IHC markers such as CD45 (hematopoietic), CD99 (Ewing/PNET), desmin and myogenin (muscle), chromogranin and synaptophysin (neuroendocrine), cytokeratin and EMA (epithelial), and WT1 (renal/DSRCT) have been validated [10,11]. However, overlapping expression patterns – e.g., CD99 positivity in both Ewing sarcoma and lymphoblastic lymphoma necessitate a systematic, evidence-based approach [12,13]. Despite published guidelines, many tertiary care centers in India still lack standardized IHC algorithms for MSRCTs [5,14].

This prospective study was therefore undertaken to evaluate the IHC profile of 100 consecutive pediatric MSRCTs using an 11-marker panel, to determine the diagnostic utility of each marker (sensitivity, specificity, predictive values), and to propose a practical, stepwise diagnostic algorithm that can be implemented even in resource-limited settings.

MATERIALS AND METHODS

Study design: This prospective, observational study was conducted over a year after institutional ethics committee approval. All consecutive cases of suspected malignant small round cell tumours (MSRCTs) in children aged ≤ 15 years were evaluated by light microscopy followed by an 11-marker immunohistochemistry (IHC) panel.

Study population: The study population comprised patients of the institute and referral cases from nearby hospitals. Of 120 consecutive malignant round cell tumors received, 100 met the inclusion criteria: light microscopic diagnosis of a solid MSRCT in a child ≤ 15 years, complete IHC evaluation, adequate clinical data, and sufficient biopsy material. The 20 excluded cases were due to autolysis (no fixative, $n=5$), inadequate tissue for IHC ($n=7$), IHC-proven large round cell tumors ($n=5$), or age >15 years ($n=3$). Central nervous system tumors (other than MSRCTs) and non-MSRCT lymphoproliferative disorders were also excluded.

Tissue processing: Specimens were fixed in 10% neutral buffered formalin (24 hours; bone decalcified for 48 hours). Tissues were then processed through graded alcohols, xylene, and paraffin wax infiltration using an automated tissue processor. Routine paraffin embedding, 3–4 μm sectioning, and hematoxylin and eosin (H&E) staining (Meyer's hematoxylin) were performed using a standard protocol. The best representative block was selected for IHC.

Immunohistochemistry: Heat-induced epitope retrieval used with the BIOGENEX EZ Retriever system (citrate buffer, pH 6.0). Endogenous peroxidase was blocked with 3% H_2O_2 in methanol. Primary antibodies (incubation 1 hour) were: CD45 (LCA88), CD99 (12E7), vimentin (V9), desmin (D33), myogenin (F5D), neuron specific enolase (MIG N3), chromogranin (polyclonal), synaptophysin (SNP88), cytokeratin (AE1/AE3), EMA (E-29), and WT1 (all from BIOGENEX or DAKO). All primary antibodies were used at predetermined optimal dilutions (ranging from 1:50 to 1:200) as recommended by the manufacturer. After each incubation step, slides were washed three times with phosphate-buffered saline (PBS) for 3 minutes each. Detection used a super-enhancer and a super-sensitive HRP polymer kit with DAB as the chromogen. Slides were counterstained with hematoxylin. Positive and negative controls were run with each batch.

Interpretation: Stained sections were evaluated for the type of staining (membranous, cytoplasmic, or nuclear), the intensity (weak, moderate, or strong), and the percentage of positive tumor cells. A case was recorded as positive for a given marker if more than 10% of tumour cells showed moderate-to-strong staining in the appropriate cellular compartment. The final diagnosis was rendered by integrating morphological features with the complete IHC panel.

Statistical analysis: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for each marker with respect to its target diagnosis using standard 2×2 contingency tables. Exact fractions (e.g., 17/17) are reported. Fisher's exact test (two-tailed) was used for pairwise comparisons; a p-value of less than 0.05 was considered statistically significant.

RESULT

A total of 100 pediatric malignant small round cell tumors (MSRCTs) comprising ten distinct diagnostic entities were evaluated for expression of 11 immunohistochemical markers. The overall IHC profile, diagnostic performance of lineage-defining markers, and critical overlapping expression patterns are presented in Tables 1 through 5.

Table 1: Lymphoid / hematopoietic markers (CD45, CD99)

Final Diagnosis	n	CD45 (%)	CD99 (%)
Ewing Sarcoma	31	0%	100%
PNET/E.S	19	0%	100%
Embryonal Rhabdomyosarcoma	17	0%	29.41%
Neuroblastoma	9	0%	0%
Lymphoblastic Lymphoma	9	100%	100%
Burkitt Lymphoma	3	100%	0%
DSRCT	4	0%	0%
Retinoblastoma	3	0%	0%
Wilms Tumor (Blastemmal)	3	0%	0%
Medulloblastoma	1	0%	0%

CD45 effectively separates lymphoblastic lymphoma (100% positive) from Ewing sarcoma/PNET (0% positive), whereas CD99 is positive in both, limiting its standalone utility.

Table 2: Mesenchymal / muscle markers (Vimentin, Desmin, Myogenin)

Final Diagnosis	n	VIM (%)	DES (%)	MYO (%)
Ewing Sarcoma	31	90.32%	6.45%	0%
PNET/E.S	19	94.73%	5.26%	0%
Embryonal Rhabdomyosarcoma	17	47.05%	100%	100%
Neuroblastoma	9	0%	0%	0%
Lymphoblastic Lymphoma	9	33.33%	0%	0%
Burkitt Lymphoma	3	0%	0%	0%
DSRCT	4	50%	100%	0%
Retinoblastoma	3	0%	0%	0%
Wilms Tumor (Blastemmal)	3	100%	0%	0%
Medulloblastoma	1	0%	0%	0%

Co-expression of desmin and myogenin is diagnostic of embryonal rhabdomyosarcoma; desmin alone is not specific (also positive in DSRCT).

Table 3: Neuroendocrine / epithelial/renal markers

Final Diagnosis	n	NSE (%)	CHROMO (%)	SYN (%)	CYK (%)	EMA (%)	WT1 (%)
Ewing Sarcoma	31	6.45 %	0%	6.45 %	6.45 %	0%	0%
PNET/E.S	19	5.26 %	0%	5.26 %	0%	0%	0%
Embryonal Rhabdomyosarcoma	17	0%	0%	0%	0%	0%	0%
Neuroblastoma	9	55.55 %	100%	88.89 %	0%	0%	0%
Lymphoblastic Lymphoma	9	0%	0%	0%	0%	0%	0%

Final Diagnosis	n	NSE (%)	CHROMO (%)	SYN (%)	CYK (%)	EMA (%)	WT1 (%)
Burkitt Lymphoma	3	0%	0%	0%	0%	0%	0%
DSRCT	4	50%	0%	0%	100%	100%	50%
Retinoblastoma	3	33.33%	0%	0%	0%	0%	0%
Wilms Tumor (Blastemal)	3	0%	0%	0%	0%	0%	100%
Medulloblastoma	1	0%	0%	100%	0%	0%	0%

Chromogranin is the most specific marker for neuroblastoma; WT1 shows nuclear positivity in Wilms tumor but cytoplasmic staining in DSRCT.

Table 4: Diagnostic utility of key lineage-defining markers

Marker	Target diagnosis	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p-value
Myogenin	Embryonal rhabdomyosarcoma	100 (17/17)	100 (84/84)	100	100	<0.0001
Chromogranin	Neuroblastoma	100 (9/9)	100 (92/92)	100	100	<0.0001
CD45	Lymphoblastic lymphoma	100 (9/9)	100 (92/92)	100	100	<0.0001
CD99	Ewing sarcoma / PNET	100 (50/50)	70.6 (36/51)	76.9	100	<0.0001
WT1	Wilms tumor	100 (3/3)	99.0 (97/98)	75.0 *	100	<0.0001

WT1 positive in 2/4 DSRCT (cytoplasmic pattern), reducing PPV for Wilms tumor; nuclear WT1 was specific for Wilms tumor. Myogenin, chromogranin, and CD45 are 100% specific for their respective diagnoses; CD99 is sensitive but not specific for Ewing/PNET.

Table 5: Critical overlapping markers and differential diagnosis

Marker	Diagnosis	Percentage (%)	Differentiated by
CD99	Ewing Sarcoma / PNET	100%	CD45/TdT, Myogenin
	Lymphoblastic Lymphoma	100%	CD45/TdT, Myogenin
	Embryonal Rhabdomyosarcoma	29.4%	CD45/TdT, Myogenin
Desmin	Embryonal Rhabdomyosarcoma	100%	Cytokeratin/EMA, Myogenin
	DSRCT	100%	Cytokeratin/EMA, Myogenin
Synaptophysin	Neuroblastoma	88.9%	Chromogranin, site/morphology
	Medulloblastoma	100%	Chromogranin, site/morphology
	Ewing Sarcoma / PNET (focal)	~5%	Chromogranin, site/morphology
Cytokeratin	DSRCT	100%	Desmin & WT1
	Ewing Sarcoma (focal)	6.5%	Desmin & WT1

Overlapping positivity of CD99, desmin, synaptophysin, and cytokeratin across different tumors mandates a panel approach with lineage-defining markers to avoid misdiagnosis

DISCUSSION

In this prospective study of 100 pediatric MSRCTs, we applied an 11 marker IHC panel and found that myogenin, chromogranin, and CD45 were 100% sensitive and specific for embryonal rhabdomyosarcoma, neuroblastoma, and lymphoblastic lymphoma respectively, whereas CD99 showed high sensitivity but only moderate specificity for Ewing sarcoma/PNET due to cross positivity in lymphoblastic lymphoma (100%) and a subset of ERMS (29.4%). Additionally, WT1 was expressed in all blastemal Wilms tumors and in 50% of DSRCTs, but with a differential staining pattern (nuclear vs cytoplasmic). These results confirm the indispensable role of a carefully selected IHC panel in the accurate diagnosis of MSRCTs.

Lineage-defining markers (myogenin, chromogranin, and CD45) performed excellently in this study. As shown in Tables 2 and 4, myogenin was positive in all 17 embryonal rhabdomyosarcomas (ERMS) and negative in all other tumors, yielding 100% sensitivity and specificity ($p < 0.0001$). This confirms myogenin as the most reliable marker for skeletal muscle differentiation, consistent with Kodet (1989) [14]. Similarly, Table 3 demonstrates that chromogranin was expressed in every neuroblastoma (9/9) and absent elsewhere, in keeping with Miettinen & Rapola (1987) [10]. CD45

(Table 1) was also 100% specific for lymphoblastic lymphoma, matching the findings of Lucas et al. (2001) [8]. Therefore, these three markers alone can definitively diagnose ERMS, neuroblastoma, and lymphoblastic lymphoma.

CD99 – highly sensitive but problematic – appears in Table 1 and Table 4: it was positive in all Ewing/PNET cases (50/50) but also in all lymphoblastic lymphomas (9/9) and 29% of ERMS (Table 5). This cross reactivity, well described by Devoe & Weidner (2000) [13], lowers its specificity to 70.6%. Hence, CD99 must always be combined with CD45 and myogenin.

Desmin and WT1 showed overlapping patterns requiring careful interpretation. Desmin positivity in both ERMS and DSRCT (100% each, Table 2) is resolved by myogenin (ERMS+) and cytokeratin (DSRCT+, Table 3). WT1 was nuclear in all Wilms tumors but cytoplasmic in half of DSRCTs (Table 3), a distinction highlighted by Barnoud et al. (2000) [12]. These findings underscore that a panel approach – not any single marker – is mandatory.

Possible underlying mechanisms: The observed IHC expression patterns reflect the distinct histogenetic lineages of these tumors. Myogenin, a basic helix-loop-helix transcription factor, drives skeletal muscle differentiation and is exclusively activated in rhabdomyosarcoma, explaining its absolute specificity [14]. Chromogranin and synaptophysin are integral components of neurosecretory granules, whose expression in neuroblastoma indicates neuronal differentiation [10]. CD99 (MIC2 gene product) is a pseudoautosomal cell surface protein that is overexpressed in Ewing sarcoma due to EWSR1-FLI1 fusion, but it is also present in normal thymocytes and lymphoblasts, accounting for CD99 positivity in lymphoblastic lymphoma [8]. The aberrant expression of desmin and WT1 in DSRCT is linked to the unique EWSR1-WT1 fusion transcript that drives polyphenotypic differentiation [12]. Understanding these molecular underpinnings helps explain the “overlap” patterns and guides the selection of confirmatory markers.

Clinical significance: Our study provides a practical, evidence based IHC algorithm that can be readily adopted in routine histopathology laboratories. With a limited panel of six well validated markers (CD45, CD99, myogenin, chromogranin, desmin, and WT1) we achieved accurate subclassification in 100% of cases with lineage defining markers. This is particularly relevant for resource constrained settings where molecular tests (e.g., FISH, PCR) are not always available. By highlighting the diagnostic pitfalls – such as CD99 in lymphoblastic lymphoma and synaptophysin in Ewing sarcoma – we help pathologists avoid misclassification that could lead to inappropriate therapy.

CONCLUSION

Immunohistochemistry using a targeted panel of 11 markers reliably distinguishes childhood MSRCTs. Myogenin, chromogranin, and CD45 are 100% specific for embryonal rhabdomyosarcoma, neuroblastoma, and lymphoblastic lymphoma, respectively. CD99 is highly sensitive but not specific for Ewing sarcoma/PNET, requiring confirmatory markers. A stepwise algorithm starting with CD45, then myogenin, chromogranin, and finally CD99 plus WT1 is recommended for routine practice.

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