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Original Article

BCOR overexpression in pediatric sarcomas- a morphologic continuum of mixed round and spindle cell tumors

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ABSTRACT

Objectives: The vast majority of BCOR (BCL6 corepressor) sarcomas occur in the pediatric population and include different clinico-pathologic entities. This study evaluates morphology, immunohistochemistry and clinical outcome in pediatric BCOR sarcomas.

Material and Methods: Children, aged ≤ 18yrs, diagnosed to have translocation negative Ewing-like sarcoma, clear cell sarcoma of the kidney and primitive myxoid mesenchymal tumor of infancy, over a period of five years were included. Immunohistochemical staining for BCOR antibody was done and the cases with BCOR overexpression were subjected to a further immunopanel comprising of special AT-rich sequence-binding protein 2 (SATB2), Transducin-Like enhancer of split-1 (TLE1), Cyclin D1 and NKX2.2. The clinical outcome of patients with BCOR overexpression was assessed.

Results: BCOR overexpression was seen in 16/42 cases; Five were primary soft tissue tumors, three were primary bone tumors, seven were clear cell sarcoma of the kidney and one primary renal sarcoma. The median age of this group was 3.5 years (range 2–18 years) with male predominance (75%). All the BCOR positive tumors showed statistically significant morphological and immunohistochemical overlap. 4/16 did not take treatment at our center. Of the 12 who received treatment, 8 are in Complete Remission 1 (CR1). The mean event-free survival (EFS) and overall survival (OS) were 51.89 months (95% CI: 37.36-66.42) and 62.08 months (95% confidence interval (CI): 52.85-71.30) respectively.

Conclusion: BCOR sarcomas did not show any statistically significant histological and immunohistochemical differences, thus reiterating the morphologic continuum of these clinically distinct tumors.

Keywords: BCOR, Pediatric sarcomas, Clear cell sarcoma of the kidney, Ewing-like sarcoma

INTRODUCTION

Undifferentiated small round cell sarcoma (USRCS) of soft tissue and bones in children and adolescents has always been a diagnostic challenge. The prototype of a small round cell tumor is Ewing sarcoma (ES), which is characterized by specific rearrangements in one of the five alternative ETS family member genes, i.e., FLI1, ERG, ETV1, E1AF, and FEV with EWSR1.[1,2] The detection of these rearrangements is diagnostic of Ewing sarcoma. The differential diagnosis of round-cell tumors is broad. It includes alveolar rhabdomyosarcoma, poorly differentiated synovial sarcoma, small cell osteosarcoma, neuroblastoma, desmoplastic small round cell tumors and mesenchymal chondrosarcoma[3] and diagnosis of these are confirmed using immunohistochemistry and detection of FOXO1(FKHR), SS18(SYT), EWSR1 and DDIT3(CHOP) gene rearrangements by FISH/RT–PCR.[4] However, a subset of tumors immunomorphologically resembling the Ewing sarcoma family of tumors (EFT) remain unclassified as they are negative for gene rearrangements for Ewing and the other round cell sarcomas listed above. The expanded spectrum of WHO 2020 classification of undifferentiated small round cell sarcomas of bone and soft tissue tumors includes round cell sarcomas with EWSR1 gene fusion with non-ETS family members, CIC-rearranged sarcomas, and BCOR(BCL-6 interacting co-repressor)-rearranged sarcomas.[5] Though all of them are rare compared to Ewing sarcoma, BCOR-rearranged sarcomas show a slightly higher incidence than the rest and are more common in the pediatric age group.[6–8]

BCOR-CCNB3 sarcomas (BCS) were first identified by Pierron *et al.*[6] in 2012 among a large group of undifferentiated round cell sarcomas lacking known genetic alterations. Sarcomas with BCOR alterations are divided into two groups, the first one being characterized by BCOR-related gene fusions and

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the second with internal tandem duplication (BCOR-ITD). Sarcomas with BCOR-CCNB3 fusion are predominantly seen in children and bones whereas the less common fusion partners BCOR-MAML3 or ZC3H7B-BCOR fusion are seen in young adults and present at variable anatomical locations. The BCOR-ITD, along with YWHAE-NUTM2B fusions, are seen in soft tissue undifferentiated round cell sarcomas (URCS), primitive myxoid mesenchymal tumors of infancy (PMMTI) and clear cell sarcoma of the kidney (CCSK), which are often noted in infants and young children.[7]

Molecular diagnostic tools that are needed to identify these BCOR genetic rearrangements are not routinely available in most centers in Low and Middle Income Countries (LMIC). However, immunohistochemically, all tumors with various BCOR gene alterations show solid and diffuse nuclear BCOR positivity.[9] Additional immunostains such as special AT-rich sequence-binding protein 2 (SATB2), transducin-Like enhancer of split-1 (TLE1), and Cyclin D1 also help to supplement the diagnosis of these tumors.[10]

The current study is undertaken to analyze the clinical characteristics, morphologic continuum, and prognosis in pediatric BCOR sarcomas. In this study, we have analyzed BCOR antibody overexpression in all Ewing translocation-negative USRCS in pediatric population. As BCOR-ITD tumors such as CCSK and PMMTI constitute the morphological spectrum of sarcoma with BCOR genetic alterations, these cases were included in the study. As our cohort was restricted to the pediatric population (<18 years), the study did not include high-grade endometrial stromal sarcoma.[11] The study would be the first of its kind in India to evaluate BCOR immunohistochemistry in the pediatric round and spindle cell sarcomas, with a subsequent panel comprising Cyclin D1, SATB2, and TLE1 to supplement the diagnosis. NKX2.2 is a recently described relatively specific nuclear marker for Ewing sarcoma, whose expression in BCOR sarcomas would be evaluated as well.[12]

MATERIAL AND METHODS

This study was approved by the institutional review board (IRB minute no: 13565) dated November 5, 2020. Informed consent in compliance with the Helsinki Declaration was obtained from the participants.

Children, aged ≤ 18 yrs, diagnosed to have translocation-negative Ewing like sarcoma, clear cell sarcoma of kidney ,and primitive myxoid mesenchymal tumor of infancy, from October 2015 to December 2020, were included. A total of 60 cases were identified, however, formalin fixed paraffin embedded blocks and slides of only 42 could be retrieved and these were included for further analysis. Clinical data was retrieved from the electronic database of the institution. Immunohistochemical analysis for BCOR antibody was done on retrieved slides/ blocks, and the immunohistochemical expression was recorded. For those cases positive for BCOR immunostain, further immunopanel comprising SATB2, TLE1, Cyclin D1 as well as NKX2.2 was done. CD99 which is a non-specific marker, but most often shows crisp strong membrane positivity in Ewing sarcoma, was also evaluated, where available.

Immunohistochemistry was done using an automated immunohistochemistry staining system (Ventana Benchmark ULTRA) on the representative sections (5um) from the tumor tissue sections of small biopsies after antigen retrieval using rabbit monoclonal antibodies directed against SATB2 (clone EP281, RTU, path in situ), TLE1 (clone IF5, RTU, path in situ), NKX2.2 (clone NX2/294, RTU, path in situ) and Cyclin D1 (clone, SP4, 1:200 dilution, DAKO). BCOR immunohistochemistry was performed on Ventana Benchmark (OPTI VIEW), clone C-10 (dilution of 1:50) by Santacruz.

The evaluation of BCOR immunohistochemistry was based on the intensity (strong, moderate, weak, and negative) and the estimated percentage of positive tumor cells.[9] Tumors with moderate to strong nuclear staining in more than 10% of the tumor cells were considered positive. Evaluation of SATB2, TLE1, CyclinD1, NKX2.2 and CD99 were done according to published protocols.[9,13–15]

Clinical and laboratory data of those positive for BCOR antibody were compared with the negative cohort. Treatment received and outcome of those with BCOR sarcoma were assessed in detail including event-free survival (EFS) and overall survival (OS). The clinical outcome was defined as alive in complete remission/on palliation or as died of disease. EFS was defined from the date of diagnosis to the date of documentation of relapse/ disease progression whereas OS was defined from the date of diagnosis to the date of death/ the last follow-up. Patients not experiencing an event were censored at the date of their last follow-up.

Statistical Package for the Social Sciences (SPSS) software International Business Machines (IBM) SPSS Statistics v25 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Chi-square or Fisher's exact test was used for categorical variables. Kaplan-Meier analysis was used to estimate survival, and the log-rank test was used to assess differences by groups.

RESULTS

Of the 42 cases included in this study, 34 were undifferentiated round cell tumors of the bone and soft tissues which were negative for EWS-FLI 1, 2, EWS-ERG, EWS-FEV translocation. There were 8 primary renal tumors. The median age of the study population was 9.5 years (range 1–18 years), and there was a male preponderance (69%). 16/42 cases were

positive for BCOR antibody; Five were primary soft tissue tumors [Figures 1a–1d], three primary bone tumors [Figures 2a-2d], seven were clear cell sarcoma of the kidney (CCSK) of kidney [Figures 3a–3d] and one primary renal sarcoma [Figures 4a–4d]. The median age of this group was 3.5 years (range 2-18 years)– and 75% were males.

The clinical characteristics and histological features of BCOR positive tumors were compared with BCOR negative [Table 1]. BCOR positive tumors showed statistically significant morphological overlap displaying specific histologic features such as hypo and hypercellular areas with spindle and round cells set in a myxoid stroma with a distinct vasculature [Figures 1a–1d], which was prominent in some to rich and branching in most. The tumor cells were commonly arranged in sheets, and exhibited hyperchromatic nuclei with irregular nuclear membranes, inconspicuous nucleoli, and scant to moderate amounts of cytoplasm. This morphology overlapped with that of other round cell sarcomas, and these variables were not statistically significant. Mitotic activity was inconspicuous in more than 50% of the tumors, while necrosis was noted in 50% of them. The other unique but rare histology we observed was the presence of rosettes, in a soft tissue tumor (retroperitoneum) in an 18-year-old male.

Further immunomarkers were performed on the 16 sarcomas that were positive for BCOR antibody [Table 2, Figures 2–4], and it showed positivity for cyclinD1(93.7%) in all the cases except one; positivity for SATB2 in 11 cases (68.7%) and positivity for TLE1 in only three cases (18.7%). NKX2.2 was consistently negative in all the cases, making it a helpful marker to differentiate Ewing sarcoma from sarcoma with

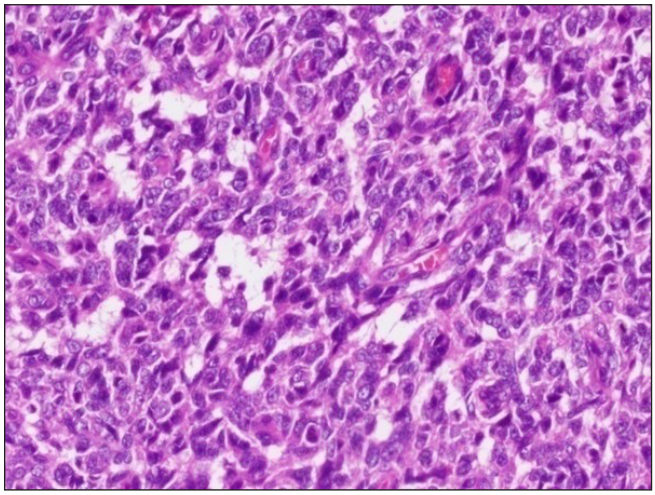

Figure 1a: BCOR-CCNB3 positive neck mass with round and spindle cells, (Hematoxylin and eosin, 200x).

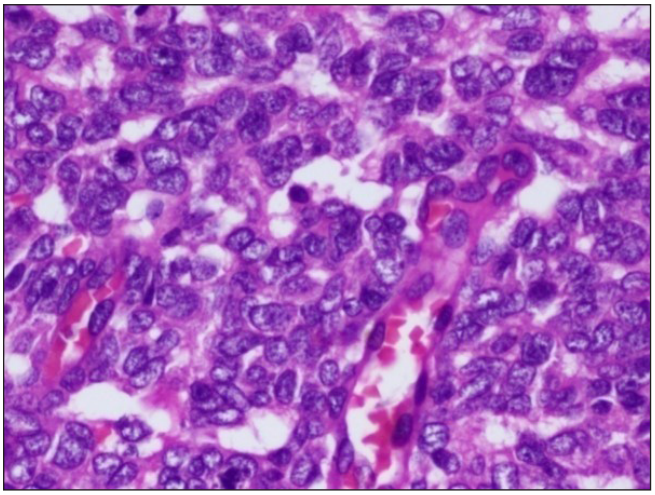

Figure 1b: BCOR-CCNB3 positive neck mass with spindle and round cells, (Hematoxylin and eosin, 400x).

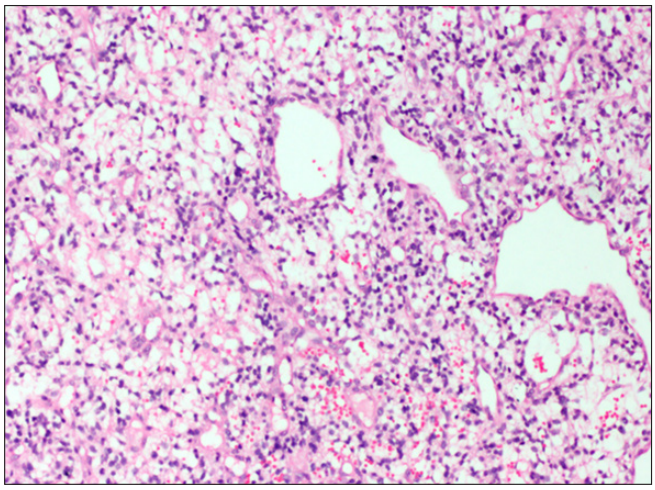

Figure 1c: Primitive myxoid mesenchymal tumor infancy, neck. Myxoid to edematous stroma and conspicuous vasculature, (Hematoxylin and eosin, 100x).

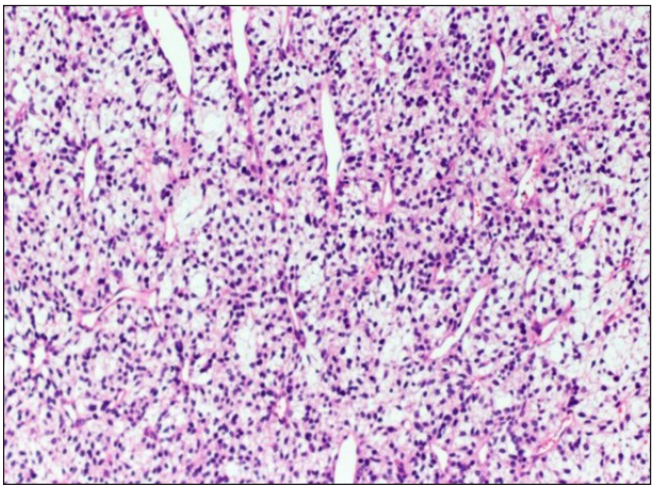

Figure 1d: Primitive myxoid mesenchymal tumor of Infancy, neck. Myxoid to edematous stroma and conspicuous vasculature, (Hematoxylin and eosin, 100x)

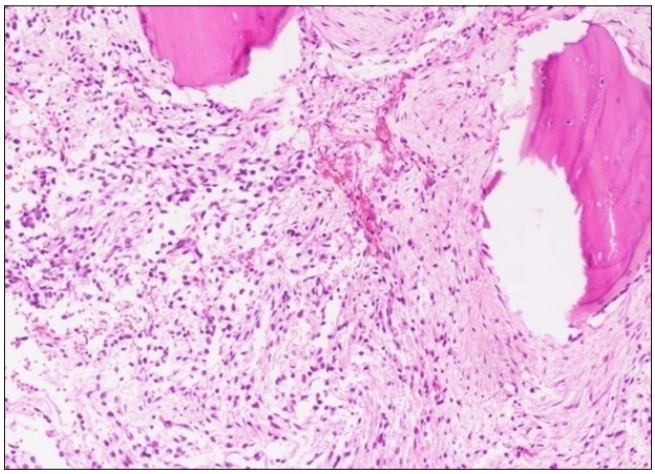

Figure 2a: BCOR-CCNB3 positive sarcoma – calcaneum: BCOR sarcoma of bone with spindle and round cells in a myxoid stroma, Hematoxylin and eosin, 100x.

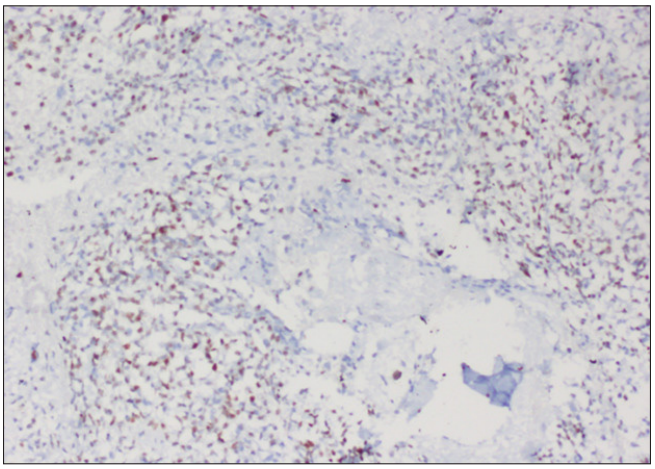

Figure 2b: Moderate to intense nuclear staining in >10% of tumor cells, BCOR, 100x.

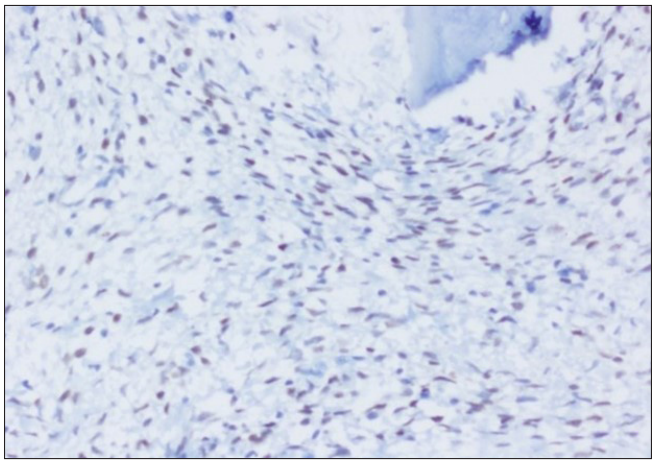

Figure 2c: Moderate to intense nuclear staining of tumor cells, SATB2, 200x.

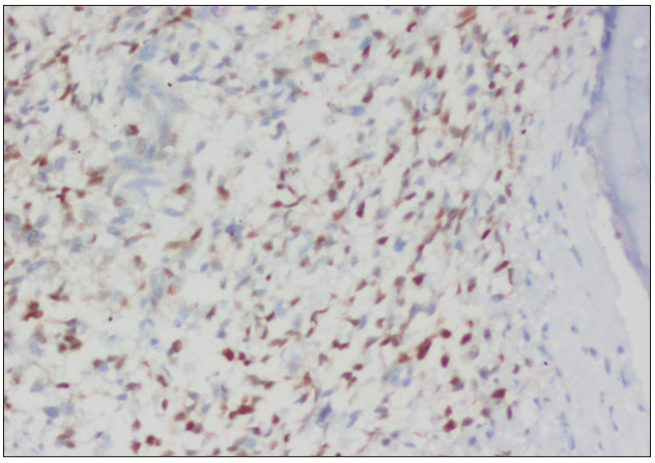

Figure 2d: Moderate to intense nuclear staining of tumor cells, CyclinD1, 200x.

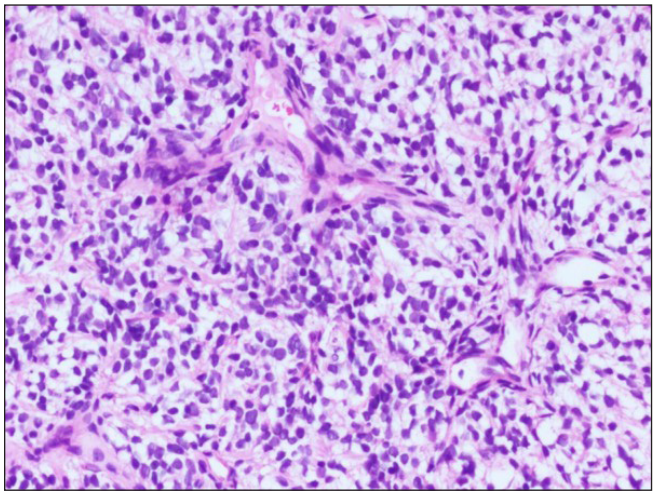

Figure 3a: Clear cell sarcoma of the kidney: Tumor cells with clear cytoplasm with a rich and branching vasculature, H&E, 200x.

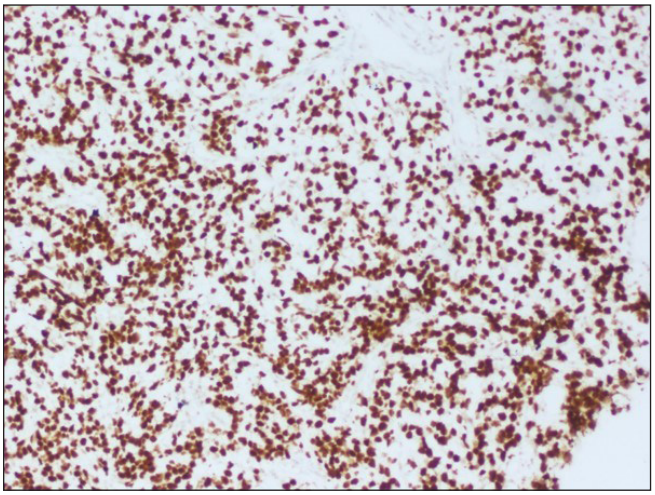

Figure 3b: Diffuse strong nuclear staining of BCOR, 100x.

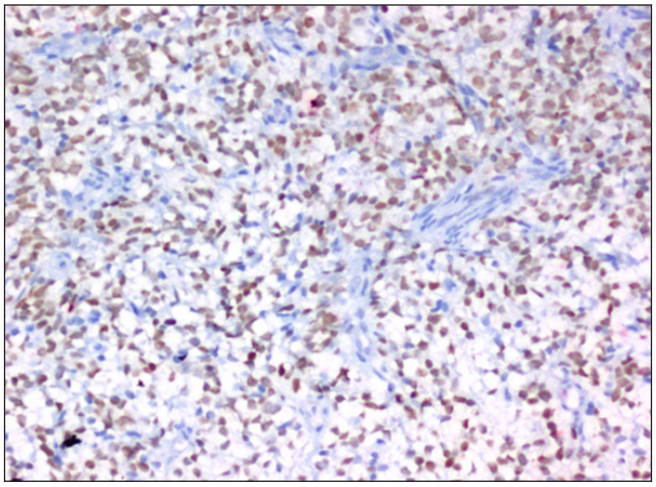

Figure 3c: Strong nuclear staining of SATB2, 200x. SATB2: Special AT-rich sequence-binding protein 2.

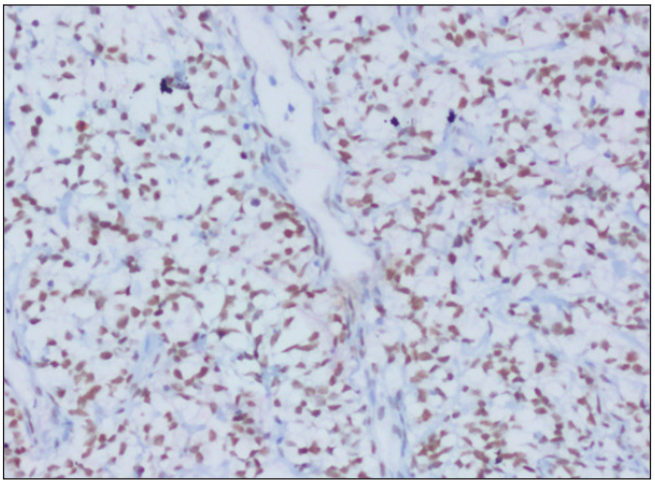

Figure 3d: Strong nuclear staining of TLE1, 200x. TLE1: Transducin-Like enhancer of split-1.

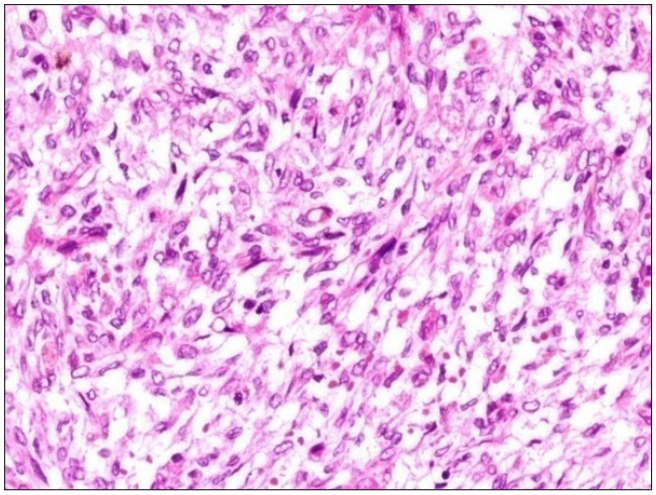

Figure 4a: Primary Renal BCOR sarcoma: Primary visceral (renal) BCOR sarcoma with predominant spindle cell morphology, H&E, 200x.

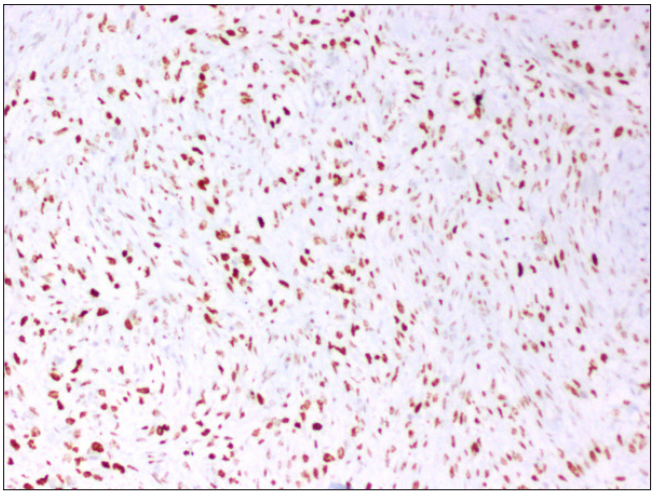

Figure 4b: Moderate to intense staining for BCOR in >10% of tumor cells, 100x.

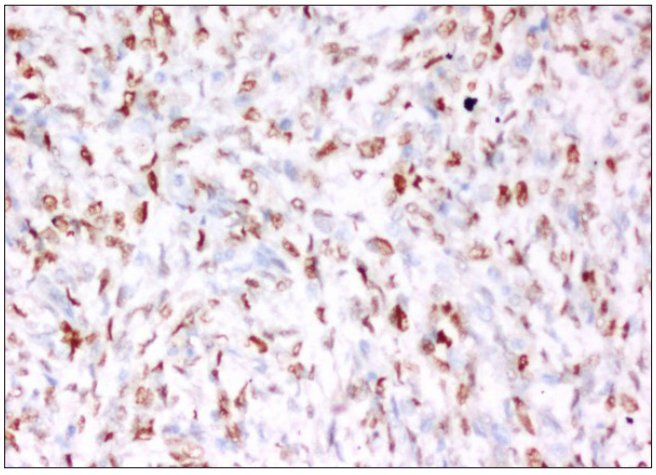

Figure 4c: Moderate to intense staining of CyclinD1, 200x.

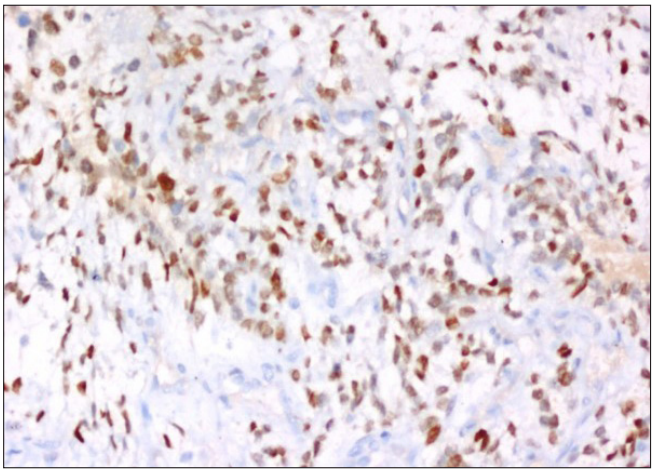

Figure 4d: Moderate to intense staining of SATB2, 200x, SATB2: Special AT-rich sequence-binding protein 2.

Table 1: Comparison of clinical profile and histology of BCOR positive and negative tumors.

Variables	BCOR positive (n = 16)	BCOR negative (n = 26)	p-value
Age in yrs (Mean ± SD)	5.94 ± 5.2	9.39 ± 5.22	0.040
Gender (M/F)	12/4	19/9	0.617
Site of tumor (Bone/ST/kidney)	3/5/8	22/4/2	0.001
Metastases at diagnosis	4	8	0.631
Components (spindle/ round)	Spindle & round (87.5%)	Spindle and round (19.3%)	<0.001
	Spindle (12.5%)	Spindle (3.8%)	
		Round (76.9%)	
Pattern of arrangement	Sheets (50%)	Sheets (50%)	0.47
	Sheets and lobules (31.3%)	Sheets and lobules (15.4%)	
	Others* (18.7%)	Others* (34.6%)	
Cellularity	Both hyper and hypocellular (75%)	Both hyper and hypocellular (3.8%)	<0.001
	Hypercellular (25%)	Hypercellular (96.2%)	
Nuclear details	Chromatin	Chromatin	0.75
	Hyperchromasia (75%)	Hyperchromasia (57.6%)	
	Fine (25%)	Fine (34.8%)	
		Vesicular (7.6%)	
	Nucleoli	Nucleoli	0.39
	Present (12.5%)	Present (23%)	
	Inconspicuous (87.5%)	Inconspicuous (77%)	
	Nuclear membranes	Nuclear membranes	0.5
	Regular (24%)	Regular (32%)	
	Irregular (76%)	Irregular (68%)	
Cytoplasm	Scant (37.5%)	Scant (42%)	0.7
	Moderate (12.5%)	Moderate (15.3%)	
	Scant to moderate (50%)	Scant to moderate (38.7%)	
	Clear (56.2%)	Clear (57.6%)	0.9
	Eosinophilic (43.8%)	Eosinophilic (42.4%)	
Stroma	Myxoid (50%)	Myxoid (3.8%)	<0.001
	Collagenous (25%)	Collagenous (75%)	
	Edematous (25%)	Edematous (7.6%)	
		Scant (11.6%)	
Mitosis	Not evident (56.3%)	Not evident (34.6%)	---
	Brisk (31.2%)	Brisk (57.6%)	
	Occasional (12.5%)	Occasional (7.8%)	
Necrosis	Present (50%)	Present (60.7%)	0.4
	Absent (50%)	Absent (39.3%)	
Vascularity	Rich and branching (63%)	Rich and branching (3.8%)	<0.001
	Prominent (37%)	Prominent (46.2%)	
		Not conspicuous (50%)	

*Others – fascicles, cords, trabeculae, micropapillae, SD: Standard Deviation, M: Male, F: Female.

BCOR genetic alterations, particularly in small biopsies. CD99 was available for 12 cases, and it was positive in only one case (8.3%) that showed diffuse membranous staining. It was either negative or showed non-specific cytoplasmic granular staining in the rest, unlike Ewing sarcoma, where CD99 often shows crisp, strong membranous staining.

Histological features of clear cell sarcoma of the kidney were compared with that of BCOR sarcomas arising in bone and soft tissue [Table 3]. The features that were more common in CCSK were clear cytoplasm with a rich and branching vasculature. Interestingly a brisk mitosis and a myxoid stroma were noted as more often in BCOR sarcomas arising in bone [Figure 2a] and soft tissue compared to CCSK [Figure 3a]. However, none of the variables were statistically significant.

Table 4 illustrates the treatment and outcome of BCOR sarcoma in this group. Various treatment protocols were used, depending on the primary site of the tumor, the period of diagnosis, and where they received initial treatment. Some of these patients received treatment elsewhere and then brought to our center for continuation of treatment and some others were transferred to the local hospital for further treatment. 4/16 did not take treatment at our center. Of the 12 who

Table 2: Immunohistochemistry of sarcomas with BCOR genetic alterations.

Cases	Site	BCOR	SATB2	TLE1	CyclinD1	NKX2.2	CD99	Diagnosis
3/M	Left kidney	positive	positive	negative	positive	negative	cytoplasmic granular	CCSK
4/F	Right kidney	positive	negative	negative	positive	negative	cytoplasmic granular	CCSK
3/F	Right kidney	positive	positive	negative	positive	negative	cytoplasmic granular	CCSK
2/M	Left kidney	positive	negative	negative	positive	negative	NA	CCSK
1/M	Left kidney	positive	positive	positive	positive	negative	cytoplasmic granular	CCSK
4/M	Left kidney	positive	positive	positive	positive	negative	cytoplasmic granular	CCSK
4/M	Right abdominopelvic mass	positive	positive	positive	positive	negative	cytoplasmic granular	Recurrent CCSK
14/F	Right kidney	positive	negative	negative	positive	negative	negative	BCOR sarcoma
5/M	Right retroperitoneum	positive	positive	negative	positive	negative	NA	BCOR sarcoma
2.5/M	Nape of neck	positive	negative	negative	positive	negative	Cytoplasmic granular	BCOR sarcoma
4.9/M	Left calcaneum	positive	positive	negative	positive	negative	negative	BCOR sarcoma
2/M	Right cervical region	positive	positive	negative	positive	negative	negative	BCOR sarcoma
18/M	Left retroperitoneum	positive	negative	negative	negative	negative	diffuse membranous	BCOR sarcoma
3/M	Left thigh	positive	positive	negative	positive	negative	NA	BCOR sarcoma
17/F	Left proximal tibia	positive	positive	negative	positive	negative	cytoplasmic granular	BCOR sarcoma
11/M	Sacrum	positive	positive	positive	positive	negative	NA	BCOR sarcoma

SATB2: special AT-rich sequence-binding protein 2, TLE1: transducin-Like enhancer of split-1, CD99: Cluster of Differentiation 99, CCSK: Clear Cell Sarcoma of the Kidney, NA: Not available.

received treatment, 8 are in CR1. The mean EFS and OS were 51.89 months (95% CI: 37.36-66.42) and 62.08 months (95% CI: 52.85-71.30) respectively.

DISCUSSION

Undifferentiated small round cell sarcomas of bone and soft tissue (USRCS) has undergone significant revision in the 2020 WHO classification of tumors with introduction of new entities that lack translocations characteristic of Ewing sarcoma. It includes round-cell sarcomas with EWSR1 gene fusion with non-ETS family members, CIC-rearranged sarcomas, and BCOR-rearranged sarcomas.[5] These tumors differ from Ewing sarcoma by the molecular signature they carry and the variable responses they show when treated on Ewing protocol. So, it becomes imperative to accurately diagnose them, enabling us to characterize, classify, prognosticate, and plan standardized protocols to treat this group of sarcomas appropriately. BCOR-related gene fusion was first described in 2012 by Pierron *et al.* among Ewing translocation negative round cell sarcomas.[6] Thereafter, many studies have been published unraveling this new entity of BCOR-rearranged sarcoma. In 35% of cases of USRCS negative for Ewing translocation, diagnosis have been apprised to sarcoma with BCOR genetic alterations with the use of molecular techniques.[16] However, these techniques are not routinely available in most centers in LMIC. Kao *et al*, has published studies where strong and diffuse BCOR expression by immunohistochemistry was noted in all-round cell sarcomas with BCOR genetic alterations, including BCOR-ITD, while CCNB3 over expression were seen only in BCS.[7,9] They also noted consistent immunoreactivity for SATB2, TLE1 and CyclinD1 which matched the mRNA upregulation at gene expression level.[10]

The current study describes clinicopathological features of 42 pediatric patients with USRCS of bone/soft tissue and primary renal sarcomas over a period of five years. BCOR over expression was present in 16 of 42 (38%) patients. On exclusion of seven clear cell sarcoma of the kidney, 9 of 35 (25.7%) bone and soft tissue tumors were positive for BCOR antibody, whereas Li *et al* reported 32%, Rekhi *et al* reported 16% and Matsuyama *et al* reported 12% either by BCOR immunohistochemistry or molecular methods.[16–18]

Table 3: Comparison of histology between BCOR-positive Clear cell sarcoma of kidney (CCSK) and BCOR sarcoma of bone and soft tissue.

Histological features	Clear cell sarcoma of kidney (n = 7)	Sarcoma with BCOR genetic alterations (n = 9)	p-value
Components (spindle/ round)	Spindle & round (100%)	Spindle and round (77.7%) Spindle (22.3%)	---
Pattern of arrangement	Sheets (71.4%) Sheets and lobules (28.6%)	Sheets (33.4%) Sheets and lobules (33.3%) Others* (33.3%)	0.22
Cellularity	Both hyper and hypocellular (85.7%) Hypercellular (14.3%)	Both hyper and hypocellular (66.7%) Hypercellular (33.3%)	0.58
Nuclear details	Chromatin Hyperchromasia (85.7%) Fine (14.3%)	Chromatin Hyperchromasia (66.6%) Fine (33.4%)	---
	Nucleoli Present (0%) Inconspicuous (100%)	Nucleoli Present (22.2%) Inconspicuous (77.8%)	0.47
	Nuclear membranes Regular (42.9%) Irregular (57.1%)	Nuclear membranes Regular (22.2%) Irregular (77.8%)	0.59
Cytoplasm	Moderate (100%)	Scant (66.6%) Moderate (33.4%)	0.01
	Clear (71.4%) Eosinophilic (28.6%)	Clear (44.4%) Eosinophilic (55.6%)	0.35
Stroma	Myxoid (42.8%) Collagenous (42.8%) Edematous (14.4%)	Myxoid (55.5%) Collagenous (11.1%) Edematous (33.4%)	0.53
Mitosis	Not evident (85.7%) Brisk (14.3%)	Not evident (33.3%) Brisk (44.4%) Occasional (22.3%)	---
Necrosis	Present (57.1%) Absent (42.9%)	Present (44.4%) Absent (55.6%)	1
Vascularity	Rich and branching (71.4%) Prominent (28.6%)	Rich and branching (55.5%) Prominent (44.5%)	0.6

*Others – fascicles, cords, trabeculae, micropapillae, SD: Standard Deviation, M: Male, F: Female.

The mean age of our cohort of sarcomas with BCOR genetic alterations was 5.9 yrs (Range 2–18yrs). Of the 7 CCSK patients, 5 (71%) were males, concordant with published reports wherein CCSK had a male-to-female ratio of 2:1.[19] The remaining nine cases were male-predominant USRCS of bone/soft tissue where 7/9 (77.75%), which was similar to published results.[8,10] We observed a predilection for soft tissue (5, 55.5%) than bone (3, 33.3%). While BCS are noted more often in bone, BCOR fusion with non-CCNB3 fusion partners and sarcomas with BCOR-ITD are common in soft tissue.[20]

We had one case of primary renal BCOR sarcoma in a 14-year-old girl. Rare visceral locations including lung and kidney have been described in literature.[8,10] Primary renal BCOR sarcomas differ from CCSK, in that they affect slightly older children, demonstrate predominant spindle morphology, and show extensive dilation of native renal tubules resulting in extensive cystic change.[21] Histology of our case showed only spindle cells, however as the diagnosis was made on small biopsy, cystic change was not observed in the limited material examined.

Though BCS was initially identified in a subset of undifferentiated small round cell sarcomas, subsequent studies have found them to be composed of round to spindle cells with variable cellularity, without a distinct architectural pattern, set in varying amounts of myxoid or collagenous stroma with delicate capillary network.[8,10,16,18] All the cases in our study population had the same reproducible histology described in the literature. The distinct and statistically significant morphological features of BCOR-positive sarcomas were, variable cellularity with a combination of round and spindle cell morphology, and rich vascularity, set in a myxoid to the collagenous stroma. The aforementioned histology should hence strongly raise the possibility of BCOR sarcomas in an appropriate clinical setting.

When our cohort was further subdivided to compare histology between bone and soft tissue BCOR sarcomas vs. CCSK,

Table 4: Summary of Clinical profile, treatment, and outcome of children with BCOR sarcoma

S. No.	Age, Gender	Primary Site	Metastases at diagnosis	Histo pathological diagnosis	Neo-adjuvant/ adjuvant chemotherapy	Surgery	Tumor response to chemo TV/N/M	Radiotherapy	CR1	Relapse/ Disease progression	Status	Follow up in months
1.	3, F	Left Kidney	No	CCSK	AVD X 4/4 drugs x 34 wks	Yes	15% ↓ LS- I	Flank 2520 cGy in 14#	Yes	No	Alive &in CR	35
2.	4, F	Right kidney	No	CCSK	Cyclo/Etop Vinc/Dox 24wks	Yes	Upfront LS-I	Flank 10.8 Gyin 6#	Yes	No	Alive &in CR	39
3.	3, F	Right kidney	Lungs, Abdominal wall	CCSK	AVD X 6wks 4drugs X 34wks	Yes	7.7%↓ No N Clear	Abd Flank 10.8Gy in 6# Lung 12Gy in 8#	Yes	No	Alive &in CR	51
4.	2, M	Left kidney	Lungs	CCSK	CE, CD X 1 CD, CE, ID, VI X 31wks	Yes	50% ↓ No N Clear LS-I	Flank 10.8Gy in 6#	Yes	No	Alive &in CR	39
5.	4, M	Right kidney	No	CCSK	AVD X 24wks	Yes Else-where	Upfront	Tumor bed 25.2Gy in 14#	Yes	Local Bones Lymph nodes	Sent home on palliation	18
6.	14, F	Right kidney	No	Primary Renal BCOR sarcoma	AVD X 4 ICE X 1, CD/ CE/ID 28wks	Yes	84.6%↓ No N Positive LS-II	Flank 25.2Gy in 14#	Yes	No	Alive &in CR	34
7.	2, M	Right Cervical ST	No	PMMTI	VIDE X 6 VAI X 1, VAC X7	Yes	Upfront	Neck 59.4Gy in 33#	Yes	No	Alive &in CR	60
8.	4, M	Left calcaneum	No	Sarcoma with BCOR genetic alterations	VIDE X 6/ VAI X 8	Yes	33%↓ >90% Clear	Local 10#	Yes	No	Alive &in CR	67
9.	14, F	Left tibia	No	Sarcoma with BCOR genetic alterations	CE X 6/ Transferred out for adjuvant	Yes	- <90% N Positive	No	No	No	Alive at last FU & TO	10
10.	11, M	Sacrum	No	Sarcoma with BCOR genetic alterations	VIDE X 6 VAC X8	No	-	Pelvis 46Gy in 23#, 14Gy in 7#	Yes	No	Alive & in CR	14

(*Continued*)

(*Continued*)

S. No.	Age, Gender	Primary Site	Metastases at diagnosis	Histo pathological diagnosis	Neo-adjuvant/ adjuvant chemotherapy	Surgery	Tumor response to chemo TV/N/M	Radiotherapy	CR1	Relapse/ Disease progression	Status	Follow up in months
11.	5, M	Right retro-peritoneum	Lungs Bone	Sarcoma with BCOR genetic alterations	CD (1), CE(2) ID(1)	No Inope-rable	58.7%↓	No	No	Yes Local	Died of disease	8
12.	18, M	Left retro-peritoneum	No	Sarcoma with BCOR genetic alterations	VDC/IE X4 VC X2 VAC/IE X1 Defaulted	Yes	Upfront	Retro-peritoneum 41.4Gy in 23#, 9Gy in 5#	No	Yes Ascites Peritoneal deposits	Sent home on palliation	16
13.	2, M	Left kidney	Liver	CCSK	-	-	-	-	-	-	TO	-
14.	4, M	Left kidney	-	CCSK	-	-	-	-	-	-	LTFU	-
15.	2, M	Nape of neck	No	RCT With BCOR-CCNB3 gene fusion	-	Yes	Upfront Excision biopsy	-	-	-	TO	1
16.	3, M	Left Thigh ST	Lymph nodes	Sarcoma with BCOR genetic alterations	VIDE X 1	-	-	-	-	-	TO	1

M: Male, F: Female, ST: Soft tissue, CCSK: Clear cell sarcoma of the kidney, PMMTI: Primary myxoid mesenchymal tumor of infancy, AVD: Actinomycin, Vincristine, Doxorubicin, 4drugs: Cyclophosphamide/Doxorubicin alternate with Carboplatin/Etoposide, VIDE: Vincristine, Ifosfamide, Doxorubicin, Etoposide, VAI: Vincristine, Actinomycin, Ifosfamide, VAC: Vincristine, Actinomycin, Cyclophosphamide, TV: Tumor volume, N: Necrosis, M: Margins, LS: Local stage CR: Complete remission after diagnosis, TO: Transferred out, LTFU: Lost to follow up

the features that were more common in BCOR sarcomas were brisk mitosis and myxoid stroma while the prominent features in the latter were clear cytoplasm with a rich and branching vasculature. However, none of these variables were statistically significant. A study by Kao *et al* in 2016 compared the clinicopathologic and genetic features of infantile URCS and PMMTI with that of CCSK, and proposed that PMMTI and a subset of infantile URCS might represent the soft tissue counterpart of CCSK due to the significant overlap in morphology and molecular genetics.[7]

All the 16 cases showed strong and diffuse nuclear expression of BCOR antibody. The supplementary immunopanel, previously described in literature, showed most consistent immunopositivity for cyclinD1 (~93.7%) in our study. SATB2 (11 cases; 68.7%) was more commonly expressed than TLE1. Similar studies conducted have shown variable positivity for these markers. The expression of cyclinD1 ranged from 30% to 90%.[10,22] There was 83% to 100% positivity noted for SATB2 and 80% to 100% positivity for TLE1.[10,16,22,23]

In our study, we found that there was no significant difference in expression of immunohistochemical markers between CCSK and BCOR sarcoma of bone and soft tissue. This lack of statistically significant histological and immunohistochemical differences between BCOR-ITD tumors like CCSK and PMMTI vs BCOR sarcoma of bone and soft tissue in our study, reiterates the fact that, irrespective of the different clinico-pathological entities that fall under this spectrum, with different treatment protocols and outcomes, these tumors form a morphologic continuum.[7,10,24]

The differential diagnosis of round cell tumors is broad and includes alveolar rhabdomyosarcoma, neuroblastoma, desmoplastic small round cell tumor, poorly differentiated synovial sarcoma, small cell osteosarcoma, and mesenchymal chondrosarcoma.

Ewing sarcoma (ES) is the most important differential to consider among round cell sarcomas of bone in children and adolescents, the diagnosis of which is best confirmed by molecular methods. Though there is an overlap of clinical and demographic presentation between ES and BCOR sarcomas, the previously mentioned distinct histology which is characteristic of BCOR sarcomas, helps in distinguishing between the two entities.[8,10,16,18] Though not specific, diffuse strong membranous staining for CD99 and strong nuclear staining for NKX2.2 favor a diagnosis of Ewing sarcoma. Most of our cases were either negative or showed nonspecific cytoplasmic granular staining for CD99, similar to documented literature.[10] In our study we observed that NKX2.2 was consistently negative in the entire cohort, suggesting that it is a useful marker to distinguish the two entities.

A major diagnostic pitfall for BCOR sarcomas in adolescents is synovial sarcoma (SS), known to express BCOR by immunohistochemistry.[9,18] TLE1 was introduced as a diagnostic immunohistochemical marker for synovial sarcoma. However, its expression has also been described in BCOR sarcomas.[9,13,25] In our cohort of 16 BCOR sarcomas, TLE1 was found to be positive in three cases (18.7%). Kao *et al.* found that about 49% of their synovial sarcomas express BCOR nuclear immunoreactivity, irrespective of the histologic subtypes or the fusion types detected either SS18-SSX1 or SS18-SSX2.[9] In such scenarios, confirmation of the diagnosis is by molecular methods. In the light of the recent discovery of SS18-SSX fusion-specific (E9X9V) and SSX C-terminus (E5A2C) immunohistochemistry for the diagnosis of synovial sarcoma, these antibodies could also be utilized to confirm or refute the diagnosis of synovial sarcoma.[26]

Small cell osteosarcoma also enters the differential diagnosis when considering poorly differentiated intra-osseous round cell sarcomas with SATB2 expression.[25] BCOR sarcomas are known to express variable SATB2.[9,18] Hence SATB2 and TLE1 stains should be always interpreted with caution when challenged with a poorly differentiated bone or soft tissue sarcoma with round cell and/or spindle cell morphology, especially in limited biopsy material. Therefore, performing BCOR immunohistochemistry is warranted in such biopsies.

The other differential that may be considered in a CD99 negative small round cell tumor of infancy and children is undifferentiated neuroblastoma. However, it can be distinguished by its diffuse staining for neuroendocrine markers and absence/dotlike pattern of CD99 immuno-staining.

Less frequently, embryonal/alveolar rhabdomyosarcoma could also be confused with undifferentiated round cell tumor of children, which can be differentiated by its positivity for myogenic markers.

The less common differentials include CIC-rearranged sarcoma and Round cell sarcoma with EWSR1-non-ETS fusions; the latter is further divided into EWSR1/FUS-NFATC2 and EWSR1-PATZ1 sarcomas. These tumors could be distinguished from BCOR sarcomas by their different clinical profile including age and site of presentation, morphology and immunoprofile including expression of CD99, WT1, ETV4, NKX3.1, CD138, myogenic and neurogenic markers.[27–29] However, definitive diagnosis is by demonstrating the genetic rearrangements by molecular techniques.

Of the 12 children who received treatment, 8 are in CR1. The mean EFS and OS were 51.89months (95% CI: 37.36-66.42) and 62.08 months (95% CI: 52.85-71.30) respectively. 4 of 5

children with CCSK, 2 of 3 with bone tumors, and both cases of primary renal sarcoma and PMMTI are in CR1, whereas both children with retroperitoneal soft tissue BCOR sarcoma progressed and died of the disease.

CONCLUSION

BCOR antibody has been proven to be a sensitive marker in identifying all BCOR genetic rearrangements and thus we have found its utility as a valuable surrogate to molecular testing in our center. Although we had a small cohort of sarcomas with BCOR overexpression, emphasis on the need for teasing out this group from the undifferentiated sarcomas was brought out. Our limitation, however, was the inability to perform molecular testing for BCOR, due to limited resources. Large multi-centric studies are needed to identify BCOR genetic rearrangements using molecular testing and assessing immunomarkers, that can potentially be used to screen these patients in a resource-limited setting, which can translate into better management and prognostication of these patients.

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Author contribution

Dr. Ponmar Madhurima: Data collection, analysis and interpretation of results, and manuscript preparation. Dr. Hema N Srinivasan: Data collection, analysis and interpretation of results, and manuscript preparation. Drs. Naina Mary Simon, Leenu Lizbeth Joseph, Rikki Rorima John, Deepthi Boddu: Data collection, analysis and interpretation of results.

Dr. Leni Mathew: Data collection, analysis and interpretation of results, and manuscript preparation. Dr. Jennifer Prabhu: Study conception and design, analysis, and interpretation of results, and manuscript preparation.

Ethical approval

This study was approved by the institutional review board (IRB minute no: 13565) dated November 5, 2020.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that no artificial intelligence (AI)-assisted technology was used to assist in the writing or editing of the manuscript, and no images were manipulated using AI.

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