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Original contribution

Primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement: a multi-institutional study of thirteen cases with a review of the literature☆,☆☆

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Summary Primary Ewing sarcoma (ES) of the urinary bladder is a rare and aggressive small blue round cell malignant neoplasm associated primarily with translocation involving *EWSR1* and *FLI1* genes located in the 22nd and 11th chromosomes, respectively. To date, 18 cases have been published in

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the literature as single-case reports, based chiefly on CD99 positivity (17 patients). Molecular confirmation by fluorescence in situ hybridization was performed in 9 patients, and FLI1 immunohistochemical (IHC) analysis was not performed in any of these published cases. Herein, we present thirteen patients of more comprehensive primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement. Clinicopathologic parameters including demographics; clinical presentation; histopathologic, IHC, and molecular profiles; and management and follow-up data of 13 patients with primary round cell sarcomas with *EWSR1* rearrangement (Ewing family of tumor) of the urinary bladder were analyzed. The studied patients (n = 13) included 6 females and 7 males; their age ranged from 4 years to 81 years (median = 30 years). The most common clinical presentation was hematuria (n = 7), followed by hydronephrosis (n = 2, one with renal failure). The tumor size ranged from 2.9 cm to 15 cm in maximum dimension. Conventional ES architecture and histology was observed in 6 cases, and diverse histology was observed in 7 cases (adamantinomatous pattern [n = 1], alveolar pattern [n = 1], *ganglioneuroblastoma-like* pattern [n = 2], and *small cell carcinoma—like* pattern [n = 3]). All the tumors were muscle invasive (invasion into the muscularis propria). IHC analysis showed that all tumors expressed FLI1, CD99, and at least one neuroendocrine marker. Focal cytokeratin staining was positive in 2 patients, and RB1 was retained in all patients. *EWSR1* rearrangement was seen in 12 of 12 tumors (in 12 patients) tested. A combined multimodal approach that included surgery with chemotherapy was instituted in all patients. Follow-up was available for 11 patients (ranging from 5 to 24 months). Six patients either died of disease (n = 3) or other causes (n = 3). Five patients were alive with metastases to the liver (n = 1), liver and lung (n = 2), liver and abdominal wall (n = 1), and kidney (n = 1). Based on our experience with the largest series to date and aggregate of the published data, ES/round cell sarcomas with *EWSR1* rearrangement occurring in the bladder have bimodal age distribution with poor prognosis despite aggressive therapy. Owing to its rarity and age distribution, the differential diagnosis is wide and requires a systematic approach for ruling out key age-dependent differential diagnoses aided with molecular confirmation.

1. Introduction

The Ewing family of tumors (EFTs) is aggressive round cell tumors with *EWSR1* rearrangement. These tumors primarily involve the bone and soft tissues and consist of Ewing sarcoma (ES; *FET-ETS* fusion genes) and other round cell sarcomas with *EWSR1-non-ETS* fusions, comprising 1% of all sarcomas [1]. These are believed to arise from the primitive neuroectoderm and share similar histopathologic features in different parts of the body regardless of their origin. They are divided into central and peripheral types. The central type has been recently renamed as embryonal tumors in the central nervous system tumor [2]. Initially, three major types of peripheral EFTs were described in the literature, which included ES of the bone, extraosseous ES, and primitive neuroectodermal tumor (PNET) [3]. These neoplasms are aggressive in nature and may occur within visceral organs such as the liver, uterus, parotid gland, kidney, pancreas, and urinary bladder [4,5]. EFTs of the urinary bladder are rare and are limited to anecdotal case reports [6—23]. Most patients present with hematuria, frequency, dysuria, hydronephrosis, and/or pelvic pain. Because of the rarity of this tumor, we performed a multi-institutional collaboration to comprehend the overarching clinical, gross, microscopic, immunohistochemical (IHC), and fluorescence in situ hybridization (FISH) features of 13 patients with EFT of the urinary bladder. Along with a thorough review of the literature, we attempt to elucidate the clinicopathologic characteristics unique to their occurrence in the urinary bladder. We discuss the differential diagnostic possibilities and the biologic potential of tumors occurring in the bladder.

2. Materials and methods

A retrospective computerized search was conducted at the respective institutions after the approval from the institutional review board. Thirteen patients with primary round cell sarcomas of the urinary bladder were identified based on their initial presentation at the primary site and the absence of another possible primary at the time of diagnosis; the patients were chosen with final pathologic diagnosis of either ES or PNET of the bladder. Demographics and clinical, histopathologic, IHC, and FISH data were recorded. The clinical profile, results of imaging studies, and prior pathology reports were reviewed. The hematoxylin and eosin (H&E)—stained and IHC slides were reviewed by five study pathologists (S.K.M., M.B., J.D., M.B.A., and B.L.B.) in a blinded fashion, and a consensus diagnosis was reached.

The IHC slides reviewed included pancytokeratin (CK), FLI1, CD45, CD99, desmin, myoD1, myogenin, WT1, PAX8, GATA3, uroplakin II, synaptophysin, chromogranin,

retinoblastoma 1 (RB1), and CD56. The results of IHC staining were recorded in a semiquantitative fashion as described in the following paragraph.

Positive staining was defined as a cytoplasmic and membranous (CK, CD45, CD99, desmin, uroplakin II, synaptophysin, chromogranin, and CD56) or nuclear (FLI1, myoD1, myogenin, WT1, PAX8, RB1, and GATA3) staining pattern in the tumor cells, which can be easily observed at low-power magnification ($<\times 40$). Scant fine granular background staining of the tumor cells, which cannot be seen at low-power magnification, or no staining at all was considered negative. For each case, immunoreactivity was interpreted as follows: negative = <5% staining of tumor cells and positive = ≥5% staining of tumor cells. The percentages of immunoreactivity for these markers were evaluated in a semiquantitative fashion as follows: 0 = <5% staining of tumor cells; focal = 5−10% staining of tumor cells; multifocal = 11−50% staining of tumor cells; and diffuse = >50% staining of tumor cells. The intensity of immunoreactivity was graded as weak, moderate, and strong.

For the FISH study, the *EWSR1* break-apart FISH probe was used to detect rearrangements involving the human *EWSR1* gene located on the chromosome band 22q12.2. The following guidelines were followed for assessment and interpretation. The tumor areas (as highlighted by the pathologist on the H&E-stained slide) were evaluated on the hybridized slides for the specificity of hybridization, probe signal intensity, and signal-to-background ratio to determine whether the hybridization was optimum for analysis. The low-power ($\times 10$) analyses were based on abundance of abnormal cells, even distribution and the presence of very few overlapping abnormal nuclei, and the presence of heterogeneity (presence of subclonal changes), whereas the high-power ($\times 60$ or $\times 100$) analyses helped in the assessment of nonoverlapping, distinct, and nondisrupted nuclei with bright uniform 4',6-diamidino-2-phenylindole (DAPI) staining, a score of nuclei of a similar size to avoid truncation effect, and avoidance of autofluorescent structures. The slides and areas that passed the aforementioned criteria were enumerated for fluorescent signals. *EWSR1* break-apart (red/orange and green) signals were enumerated on their own using a single band-pass filter. It was started with one probe, followed by enumeration of the signals in each cell, and then was proceeded to the green filter for the other. This was followed by checking under the dual band-pass filter to look for a fused yellow signal. The number of signals in the nucleus was recorded on the score sheet. Inconclusive cells were not counted. Hundred abnormal cells were counted. Once the abnormal cells were scored, the number of fused (yellow, normal pattern) and discrete individual (red/orange and green, split signal) signals/cells were counted. If the average percentage of positive tumor cells with a split signal was <10% (<10/100), the sample was considered negative. If the average percentage of positive tumor cells with a split signal was ≥10% (≥10/100), the sample was considered positive. The FISH result was considered noninformative in the following cases: slides having less than 50 scorable abnormal cells, slides with no or patchy hybridization, and slides with high background or autofluorescence that interfered with signal enumeration [24,25].

3. Results

3.1. Clinical findings

The cohort included 13 patients. There were 6 (46%) females and 7 (54%) males, with a median age of 30 years (the age ranged from 4 years to 81 years) at the time of diagnosis. Eight of the patients aged <40 years (range from 4 years to 38 years with a mean age of 19 years) and five aged >50 years (range from 59 years to 81 years, with a mean age of 68 years). Hematuria was the most common presenting symptom, with five patients presenting having gross hematuria and three presenting with microscopic hematuria. One patient had unilateral hydronephrosis besides hematuria. One patient each presented with acute urinary retention, lower urinary tract symptoms, abdominal pain and mass, renal failure secondary to bilateral hydronephrosis, and lymphedema of the legs (Table 1). Five patients had history of other medical conditions, which included Hodgkin lymphoma (n = 2), chronic lymphocytic leukemia (n = 1), anemia (n = 1), and myocardial infarction (n = 1) (Table 1).

3.2. Histopathologic features

3.2.1. Macroscopic features

Tumors ranged in size from 2.9 cm up to 15 cm (median = 8.1 cm) in maximum dimension (on cystoscopy). The tumors were predominantly solid with well-circumscribed and well-demarcated interfaces (9 cases) to irregular borders (4 cases). The cut surfaces were variegated, fleshy, and tan to tan-white to tan-brown to tan-yellow with foci of hemorrhage. Additional elements, including grummous, waxy sebaceous, or gelatinous material, hair, bone, or teeth, were not seen in any of the tumors.

3.2.2. Microscopic features

The conventional histopathologic features of round blue cell histology were observed in all cases in varying proportions. In typical foci, the tumor was present in lobules that were separated by variably thick fibrous septae; in other areas, the tumor cells were present in sheets. The tumor cells were predominantly small and were round to oval; focal spindled morphology was also observed. Overt spindled histology was however not appreciated. They had scant to moderate amount of eosinophilic to amphophilic to clear cytoplasm with indistinct cell membranes. The nuclei

Table 1 Clinical, immunohistochemical, fluorescence in situ hybridization, and therapy details of the cases of primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement in the present cohort.

Age (years)	Gender	Presenting symptoms	Tumor size (cm)	Tumor location	Metastasis	Medical illness	Surgical intervention	Chemotherapy	Radiation therapy	Urinary diversion	FLI1	CD99	CD45	pan CK	Desmin	Myo D1	RB1	Myo genin	WT1	PAX8	GATA3	Uroplakin II	Synapto physin	Chromo granin	CD56	EWSR1 BA FISH	Follow-up	Dead or alive
4	M	Gross hematuria	3.7	Dome of the bladder	Liver, abdominal wall	None	TURBT, partial cystectomy	Adjuvant chemotherapy	Yes	ND	+	+	−	−	−	−	NL	−	−	−	Focal and weak +	−	+	−	+	ND	DOD, 14 months	Dead
7	M	Gross hematuria	15	Dome, trigone, and both ureteric orifices	None	Anemia	TURBT, radical cystectomy	Palliative chemotherapy	Yes	Ileal conduit	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (45%)	Alive, 5 months	Alive
15	F	Microscopic hematuria	11	Dome, trigone, neck, and both ureteric orifices	Kidney	None	TURBT, radical cystectomy	Adjuvant chemotherapy	No	Ileal conduit	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (70%)	Alive, 9 months	Alive
18	F	Microscopic hematuria	3.5	Dome of the bladder	None	None	TURBT, partial cystectomy	Adjuvant chemotherapy	No	ND	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (52%)	LTF; last follow-up was 5 months ago	Unknown
21	F	Fever, acute urinary retention	4.4	Dome of the bladder	Liver	None	TURBT, partial cystectomy	Adjuvant chemotherapy	No	ND	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (48%)	DOD, 11 months	Dead
21	F	Lower abdominal pain, gross hematuria	7	Left lateral wall	None	None	Partial cystectomy	Adjuvant chemotherapy	No	ND	+	+	−	−	Focal and weak +	−	NL	−	−	−	Focal and weak +	−	+	−	−	+ (64%)	Disease free for 2 years	Dead
30	F	Gross hematuria	4.8	Trigone and neck	None	HL	TURBT, radical cystectomy	Adjuvant chemotherapy	No	Ileal conduit	+	+	−	Focal and weak +	−	−	NL	−	−	−	−	−	+	−	+	+ (45%)	Alive 7 months after diagnosis	Alive
38	M	Fatigue, incontinence, urgency	6	Left lateral wall and dome	None	MI	TURBT, partial cystectomy	Adjuvant chemotherapy	No	ND	+	+	−	−	−	−	NL	−	−	−	Focal and weak +	−	+	−	+	+ (53%)	Dead after 19 months	Dead
59	F	Lymphedema of the legs	9	Dome, trigone, and both ureteric orifices	None	None	Radical cystectomy	Adjuvant chemotherapy	No	Ileal conduit	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (78%)	LTF; last follow-up was 11 months after surgery, and the patient was disease free.	Unknown
61	M	Microscopic hematuria	14	Dome, trigone, neck, and both ureteric orifices	None	None	TURBT, radical cystopro statectomy	Adjuvant chemotherapy	No	Ileal conduit	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (80%)	Alive, 2 years	Alive
62	M	Fever, dull aching lower abdomen pain, lump	8.3	Right lateral wall and dome	None	CLL	TURBT, radical cystopro statectomy	Adjuvant chemotherapy	No	Ileal conduit	+	+	−	−	−	−	NL	−	−	−	−	−	+	+	+	+ (58%)	Alive, 18 months	Alive

(*continued on next page*)

Table 1 (*continued*)

Age (years)	Gender	Presenting symptoms	Tumor size (cm)	Tumor location	Metastasis	Medical illness	Surgical intervention	Chemotherapy	Radiation therapy	Urinary diversion	FLI1	CD99	CD45	pan CK	Desmin	Myo D1	RB1	Myo genin	WT1	PAX8	GATA3	Uroplakin II	Synapto physin	Chromo granin	CD56	EWSR1 BA FISH	Follow-up	Dead or alive
72	M	Renal failure, B/L hydronephrosis	2.9	Left lateral wall	Lung, liver	None	Radical cystoprostatectomy	Palliative chemotherapy, neoadjuvant chemotherapy	Yes	B/L nephr ostomy	+	+	−	−	Focal and weak +	−	NL	−	−	−	−	−	+	−	+	+ (36%)	DOD, 19 months	Dead
81	M	Dark urine, U/L hydronephrosis	6.4	Left lateral wall extending to the neck	Lung, liver	HL	Partial cystectomy	Palliative chemotherapy, neoadjuvant chemotherapy	Yes	U/L nephr ostomy	+	+	−	Focal and weak +	−	−	NL	−	−	−	−	−	+	+	+	+ (51%)	Died of pulmonary embolism 7 months after diagnosis	Dead

Abbreviations: FISH, fluorescence in situ hybridization; CK, cytokeratin; ND, not done; BA, break-apart; HL, Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; MI, myocardial infarction; +, positive; -, negative; NL, no loss/retained; DOD, died of disease; NED, no evidence of disease; LTF, lost to follow-up; TURBT, transurethral resection of bladder tumor; U/L, Unilateral; B/L, bilateral.

were round with smooth contours; some had focal indentations and inconspicuous to small visible nucleoli. The neoplastic cells encircled either neurofibrillary cores forming Homer Wright rosettes (in 7 cases) or rarely around central lumens forming Flexner-Wintersteiner rosettes (2 cases). Rare, large polygonal cells with pleomorphic nuclei were scattered in between the small blue round cells. The focal organoid pattern of arrangement and foci of coagulative tumor cell necrosis were present in 4 tumors. Mitotic figures and apoptotic activity were moderate to focally high in all tumors, except for two cases wherein they were uniformly high throughout the tumors. Round cell sarcomas with adamantinomatous features, large polygonal cells arranged in the alveolar pattern reminiscent of an alveolar rhabdomyosarcoma, and EFT with ganglion cells and schwannian stroma (*ganglioneuroblastoma-like*) were also observed in one case each in our cohort. Three tumors had focal nuclear molding, perivascular coagulative tumor cell necrosis, and brisk mitotic activity reminiscent of a small cell carcinoma (Fig. 1). Rhabdomyosarcomatous, chondroid, osseous, or melanocytic differentiation was not identified in any of the cases. All the tumors were invasive into the muscularis propria. Perivesical fat and lymphovascular invasion were present in nine (pT2a, as per staging of soft tissue sarcoma, 8th Edition, AJCC Staging Manual) and eleven cases, respectively. None of the patients had urothelial carcinoma (in situ, papillary, or variant histology).

3.3. IHC findings

All thirteen patients expressed CD99 and FLI1 in a multifocal to diffuse pattern with strong intensity. FLI1 staining intensity of the tumor cells was like that of the intratumoral and peritumoral endothelial cells. Synaptophysin positivity and CD56 positivity were observed in ten and eight patients, respectively, which were either focal to diffuse with moderate to strong intensity. Chromogranin was positive in five patients (focal to multifocal with weak to moderate intensity). Weak and focal expression for GATA3, CK, and desmin was observed in three, two, and two tumors, respectively. The tumors with GATA3 positivity (typical PNET histology) were negative for CK (mutually exclusive). RB1 protein was retained in all tumors. MyoD1, myogenin, WT1, PAX8, and uroplakin II were negative in all the tumors (Table 1 and Fig. 2).

One case, an 81-year-old patient with adamantinomatous histology, depicted focal and weak CK positivity. However, *EWSR1* break-apart FISH and FLI1+/CD99+ phenotype helped establish the diagnosis. The tumor with ganglion-like cells and schwannian stroma in a 7-year-old male child did not express GATA3, a marker of neuroblastic neoplasms, excluding a poorly differentiated neuroblastoma or ganglioneuroblastoma, which was further supported by *EWSR1* break-apart FISH positivity.

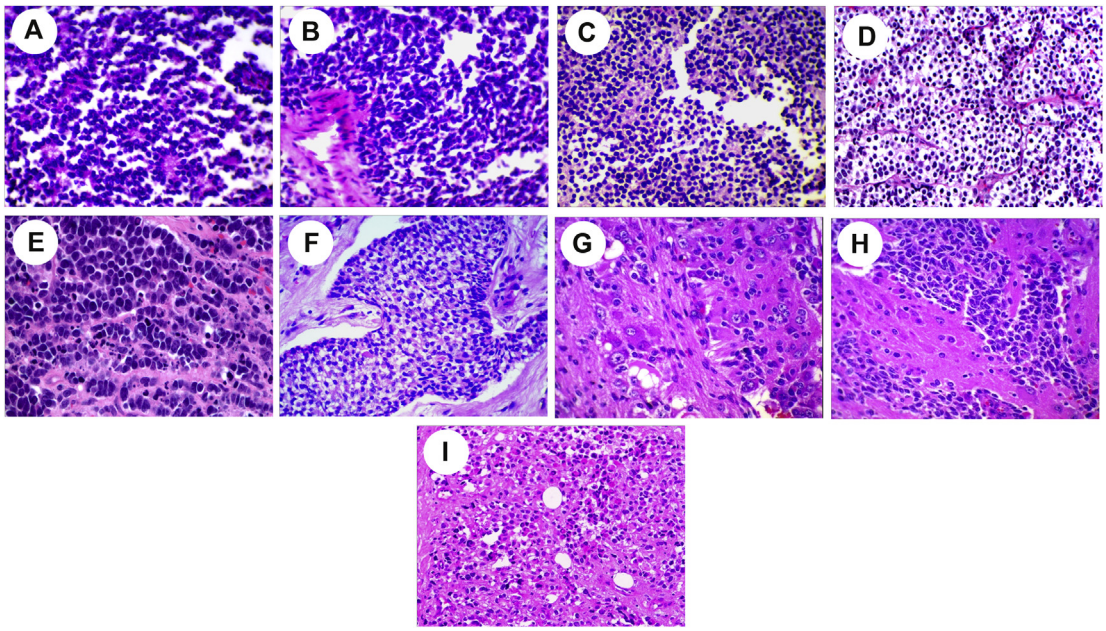

Fig. 1 Primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement: A, malignant small blue round cells encircling neurofibrillary cores forming Homer Wright rosettes (H&E, original magnification, ×400); B, spindle cell histology (H&E, original magnification, ×400); C, round cell histology (H&E, original magnification, ×400); D, clear cell features (H&E, original magnification, ×400); E, small cell−like histology with nuclear molding (H&E, original magnification, ×400); F, adamantinomatous pattern (H&E, original magnification, ×400); G, ganglion-like cells in a schwannian stroma (H&E, original magnification, ×400); H, ganglioneuroblastoma-like histology (H&E, original magnification, ×400); I, large polygonal cells arranged in an alveolar pattern (H&E, original magnification, ×400). H&E, hematoxylin and eosin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Three tumors had focal nuclear molding reminiscent of a small cell carcinoma; however, complete absence of CK staining, retention of RB1, and *EWSR1* break-apart assay positivity excluded the diagnosis of small cell carcinoma.

3.4. Molecular findings

The FISH study for *EWSR1* showed break-apart in 12 of 12 (100%) patients, confirming the diagnosis. Partner-specific probes were not used to determine the exact fusion partner (Table 1 and Fig. 2). Molecular analysis could not be performed in one patient as there was not enough viable tumor tissue left for testing. However, this tumor had a typical histology. The tumor had lobules separated by variably thick fibrous septae. The tumor cells were predominantly small and were round to oval. They had scant to moderate amount of eosinophilic to amphophilic to clear cytoplasm with indistinct cell membranes. The nuclei were round with smooth contours and inconspicuous nucleoli. The neoplastic cells encircled neurofibrillary cores forming Homer Wright rosettes. FLI1 and CD99 were diffusely and strongly positive along with neuroendocrine markers (synaptophysin and CD56). RB1 was retained in the tumor cells. Therefore, this case was included in the study, despite *EWSR1* FISH not being performed.

3.5. Treatment and follow-up

Surgery was performed on all tumors. Six patients underwent partial cystectomy, and seven had radical cystectomy or cystoprostatectomy. Ten (77%) patients received adjuvant chemotherapy after surgical resection, and 3 (23%) patients received chemotherapy with a palliative intent. The chemotherapy included cyclophosphamide, vincristine, and dactinomycin for all cases and addition of ifosfamide and etoposide for nonmetastatic cases. Four patients also received radiation therapy, of which 3 patients had received palliative chemotherapy. Five patients had metastases to the liver (n = 1), liver and lung (n = 2), liver and abdominal wall (n = 1), and kidney (n = 1). Of these five patients, three patients had presented with metastasis, and two developed metastases on the follow-up. Follow-up information, available for 11 patients, ranged from 5 months to 24 months. Six patients died with a median survival period of 16.5 months (ranged from 7 months to 24 months), and five patients were alive with a median follow-up period of 9 months (ranged from 5

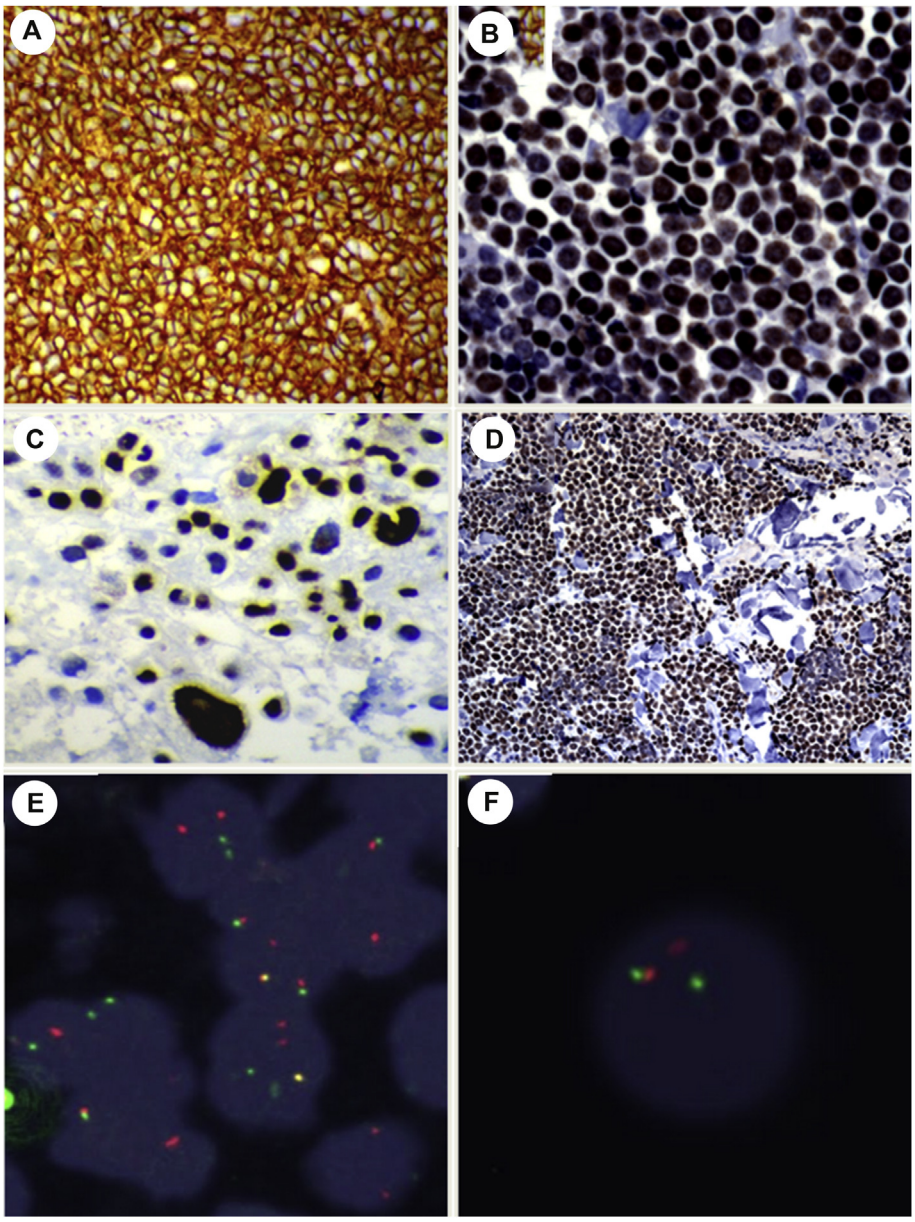

Fig. 2 Primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement: A, diffuse and strong membranous immunoreactivity for CD99 (original magnification, ×400); B, diffuse and strong nuclear immunoreactivity for FLI1 (original magnification, ×400); C, ganglion-like cell with strong FLI1 expression (original magnification, ×400); D, retention of rentinoblastoma 1 (RB1) (original magnification, ×100); E, tumor with translocation affecting the *EWSR1* gene (locus 22q12.2) as indicated by one orange/green fusion signal (nonrearranged), one orange signal (*EWSR1* gene), and one separate green signal (FISH, magnification, ×60); F, A tumor cell at higher magnification showing translocation affecting the EWSR1 gene (locus 22q12.2) as indicated by one orange/green fusion signal (nonrearranged), one orange signal (*EWSR1* gene), and one separate green signal (*FISH*, magnification 100×). FISH, fluorescence in situ hybridization. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

months to 24 months). Four of five patients with metastases died (Table 1).

4. Discussion

Primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement are rare tumors, with previously reported eighteen patients with primary tumor of the bladder in the literature over the years [6–23] (Table 2). Much of the current understanding of the disease is derived from similar tumors occurring at other sites [3,26,27]. Herein, we include thirteen additional patients to the literature, with a focus to understand the histological features including variations, immunoprofiles, diagnostic criteria, and age-dependent differential diagnoses at this anatomic location. The biologic potential of tumors

Table 2 Clinical, immunohistochemical, fluorescence in situ hybridization, and therapy details of the published cases of primary round cell sarcomas of the urinary bladder with or without *EWSR1* rearrangement.

Age (years)	Gender	Presenting symptoms	Tumor size (cm)	Metastasis	Medical history	Surgical intervention	Chemotherapy	Radiation therapy	FLI1	CD99	EWSR1 BA FISH	Follow-up	Dead/ alive	Reference
10	M	Polyuria, lower abdominal swelling, bilateral hydronephrosis	13	None	None	Partial cystectomy	Neoadjuvant chemotherapy	No	ND	+	+	NED after 11 months	Alive	Sueyoshi et al., 2014 [8]
14	F	Progressive abdominal lump, pain	15	None	None	Sleeve resection of the bladder	Adjuvant chemotherapy	NA	ND	+	ND	LTF after three chemotherapy cycles	NA	Rao et al., 2011 [9]
15	F	Gross hematuria	3	None	None	TURBT, partial cystectomy	Adjuvant chemotherapy	NA	ND	+	+	NED, 18 months	Alive	Gousse et al., 1997 [21]
16	M	Gross hematuria, dysuria	NA	None	ALL	TURBT	Adjuvant chemotherapy	No	ND	+	+	NED, 24 months	Alive	Osone et al., 2007 [10]
21	M	Microscopic hematuria, dysuria	11	None	Kidney transplantation, immunosuppression, heroin abuse	TURBT, partial cystectomy	Adjuvant chemotherapy	NA	ND	+	ND	NED, 18 months	Alive	Banerjee et al., 1997 [18]
21	F	Developed frequency, gross hematuria, dysuria	9	None	None	TURBT, radical cystectomy	Adjuvant chemotherapy	NA	ND	+	+	NED, 36 months	Alive	Lopez-Beltran et al., 2006 [12]
30	F	Polyuria, gross hematuria	9.4	None	None	TURBT, radical cystectomy	Neoadjuvant chemotherapy	No	ND	+	+	NED after 14 months	Alive	Lam et al., 2016 [20]
38	F	Gross hematuria	12	None	HL	TURBT, radical cystectomy	NA	NA	ND	+	ND	NA	NA	Desai et al., 1998 [14]
38	F	Gross hematuria	4	None	None	TURBT, radical cystectomy	Adjuvant chemotherapy	No	ND	+	+	NED after 14 months	Alive	Tonyali et al., 2016 [7]
57	F	Pelvic pain, dysuria, urinary frequency, macroscopic hematuria	3.3	None	None	TURBT	Adjuvant chemotherapy	NA	ND	+	+	NED after 27 months	Alive	Busato et al., 2011 [17]
61	F	Bilateral hydronephrosis	NA	Lung	Diabetes mellitus, hypertension,	Bladder biopsy	NA	NA	ND	ND	+	NA	NA	Colecchia et al.,

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Table 2 (*continued*)

Age (years)	Gender	Presenting symptoms	Tumor size (cm)	Metastasis	Medical history	Surgical intervention	Chemotherapy	Radiation therapy	FLI1	CD99	EWSR1 BA FISH	Follow-up	Dead/ alive	Reference
		and renal failure			ischemic heart disease, and Mediterranean anemia									2002 [16]
62	M	Back ache, acute urinary retention	NA	Lung	Anemia	At autopsy	NA	NA	ND	+	ND	DOD after 3 weeks of diagnosis	Dead	Mentzel et al., 1998 [23]
65	M	Gross hematuria, dysuria	5	Pelvic lymph node (8 months after the operation)	None	TURBT, radical cystoprostatectomy	Adjuvant chemotherapy (after recurrence)	Yes (after recurrence)	ND	+	+	DOD after 22 months	Dead	Okada et al., 2011 [11]
67	F	Repeated hematuria, fever	3	None	None	TURBT, partial cystectomy	NA	NA	ND	+	ND	Dead after 8 months	Dead	Al Meshaan et al., 2009 [19]
70	M	Hematuria and irritative urinary symptoms	4.4	None	None	TURBT	Adjuvant chemotherapy	No	ND	+	ND	Alive 3 years after diagnosis	Alive	Parizi et al., 2019 [22]
72	M	Gross hematuria, oliguria	NA	Abdominal wall	Cancer of unknown primary in the left femoral muscle	PLND, ileum resection, abdominal metastasis removed	NA	NA	ND	+	ND	LTF; last follow-up was two months to diagnosis, discharged in poor general condition	NA	Ellinger et al., 2006 [15]
74	M	Frequency, dysuria, hematuria	NA	None	None	NA	Palliative chemotherapy	NA	ND	+	ND	DOD after 4 months	Dead	Zheng et al., 2011 [6]
81	M	Lymphedema of the lower extremities, fatigue, urge of incontinence	NA	None	None	TURBT	NA	NA	ND	+	ND	DOD after 2 weeks of diagnosis	Dead	Kruger et al., 2003 [13]

Abbreviations: FISH, fluorescence in situ hybridization; +, positive; ND, not done; HL, Hodgkin lymphoma; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; TURBT, transurethral resection of bladder tumor; PLND, pelvic lymph node dissection; DOD, died of disease; NED, no evidence of disease; NA, not available; LTF, lost to follow-up.

occurring in the bladder and comparison of cases published in the literature are discussed as well. Our study is unique as it is the largest case series of primary EFT of the bladder reported in the literature thus far with molecular confirmation of cases in the majority of the cases. These tumors were worked up in a complete and systematic manner, taking into account a broad range of differential diagnoses of EFTs and other round blue cell tumors in the bladder.

EFT of the urinary bladder occurs in both children and adults. Our study has a bimodal distribution: patients can be divided into two groups depending on age as children and young adults (<40 years of age) and middle-aged patients to the elderly (≥59 years). Cases reported in the literature ranged in age from 10 years to 81 years, with a median age of 47.5 years [6−23]. In the reported cases, 4 of 18 patients were younger than 18 years, which is in concordance with our data [6−23]. Disease occurrence is equivalent in both the genders (Tables 1 and 2). Major presenting symptoms observed in EFT of the urinary bladder across our study and prior reports are hematuria, dysuria, fatigue, lymphedema, abdominal lump, and frequency (Tables 1 and 2) [6−23]. Comparative analyses of the presenting symptoms between this study and the reported cases in the literature have a lot of similarities. In most of the studies, the detection and evaluation of tumor was made by imaging modalities, such as cystoscopy, computed tomography, magnetic resonance imaging, bone scintigraphy, and positron emission tomography. Although any particular risk factors cannot be listed for EFT development, earlier reports have suggested that immunosuppression and chemotherapy for other prior malignancies not related to EFT may be responsible for development of this tumor. Three patients in our cohort had a prior history of chemotherapy for Hodgkin lymphoma (n = 2) and chronic lymphocytic lymphoma (n = 1). This has been described in earlier cases reported in the literature. Other nontumorous conditions such as anemia and myocardial infarction present in this cohort were also reported in other cases. Other nontumorous medical conditions reported in the literature include conditions associated with immunosuppression such as diabetes, renal transplantation, and heroine abuse [16,18].

The tumor size ranged from 2.9 cm to 15 cm, with a mean size of 8.1 cm, which is comparable with that in the prior reports (size ranged from 3 cm to 15 cm, with a mean size of 7.6 cm) [7−9,11,12,14,17−22]. The tumors were well to poorly circumscribed and demarcated, had solid tan to tan-white cut surfaces with areas of hemorrhage, and showed necrosis.

Histopathologic examination revealed a malignant small round cell tumor with a typical immunoprofile and genetic profile in most cases. EFT must be distinguished from other primary and metastatic tumors with similar morphology, including other types of high-grade neuroendocrine carcinomas (small cell carcinoma) that are rare in the bladder. Round cell sarcomas with variant morphologic features such as adamantinomatous features, an alveolar pattern of arrangement, and EFT with *ganglioneuroblastoma-like* with ganglion-like cells and schwannian stroma were observed in one tumor each. All tumors with variant morphologies were positive for *EWSR1* break-apart FISH, which helped in correctly identifying them. In addition, pertinent IHC staining results, eg, GATA3 negativity in *ganglioneuroblastoma-like* with ganglion-like cells and schwannian stroma and myoD1 and myogenin negativity in EFT with an alveolar pattern, helped exclude other morphologically similar entities such as neuroblastoma/ganglioneuroblastoma and alveolar rhabdomyosarcoma, respectively, in these tumors [28]. Although the tumor with adamantinomatous features was focally positive for CK, thus raising carcinoma as a differential diagnosis, it was positive for CD99 and FLI1 IHC staining, favoring it to be an EFT, which was subsequently confirmed by a positive *EWSR1* break-apart FISH test result. Three tumors had focal nuclear molding reminiscent of a small cell carcinoma; however, the complete absence of CK staining, retention of RB1, and the *EWSR1*/break-apart FISH result excluded the diagnosis of small cell carcinoma [29].

Although a wide range of histopathologic variations may be observed in EFT, depending on the differential diagnosis, judicious use of appropriate IHC markers and FISH assay helps in correct diagnosis. In the genitourinary tract, the possibility of carcinomas, such as urothelial carcinoma, small cell carcinoma, or prostatic adenocarcinoma with neuroendocrine differentiation, should always be excluded before the diagnosis of a primary EFT is considered. Metastasis of EFT from other sites such as soft tissue, bone, skin, or other visceral organs should be considered before diagnosing a primary EFT of the bladder owing to rarity of the tumor at this location. In addition, nonepithelial tumors with similar morphology including melanoma, lymphoma, rhabdomyosarcoma, Merkel cell carcinoma, and leukemia should be excluded. This is critical as the management and prognosis of these entities are different. Although several histologic and imaging features may help differentiate EFT from its mimics, the diagnosis often requires IHC and molecular confirmation. Characteristic paranuclear dot positivity with cytokeratin 20 is seen in Merkel cell carcinoma. Similarly, typical histology, FISH negativity, and TTF1 and CK expression (focal to diffuse pattern) support the diagnosis of a small cell carcinoma. RB1 protein, as analyzed by immunohistochemistry, is either completely or partially lost in small cell carcinoma, whereas retention of RB1 is a feature of EFT [29]. Negativity for myogenic markers (desmin, myoD1, and myogenin), WT1, and leukocyte common antigen argues against rhabdomyosarcoma, desmoplastic small round cell tumor, and hematolymphoid malignancies [30,31]. Absence of urothelial carcinoma in situ, papillary or variant urothelial carcinoma histology, and negative urothelial markers such as GATA3, uroplakin, p63, and CK5/6 rule out urothelial carcinoma. PNETs are negative for prostatic markers (NKX3.1, P501s, androgen receptor, and prostate-specific antigen).

Neuroblastic neoplasms, another morphologic mimic of PNET, express GATA3, FLI1, CD99, and neuron-specific enolase. However, GATA3 negativity/weak expression supported by *EWSR1* FISH positivity in EFT aids in excluding diagnosis of a neuroblastic tumor [26–28]. Poorly differentiated synovial sarcoma comes under the differential diagnostic consideration of EFT, which exhibits a variable degree of FLI1, CD99, and CK positivity with expression of synaptophysin, wherein a break-apart FISH assay for SYT-SSX1/2 helps in arriving at a correct diagnosis [26,27,32]. We acknowledge the limitation in our study of our inability to determine the fusion partners via sequencing, which may shed light in further segregating these tumors as ES (*FET-ETS* fusion genes) and round cell sarcomas with *EWSR1-non-ETS* fusions such as *EWSR1-NFATC2, FUS-NFATC2,* and *EWSR1-PATZ1*, besides other possible potential fusion partners.

The treatment of primary round cell sarcomas of the urinary bladder with *EWSR1* fusion includes a multimodality approach comprising surgical intervention supported by chemotherapy and radiation therapy [33]. In our study, cystectomy followed by adjuvant chemotherapy was the most common treatment regimen. Three of our patients were given palliative chemotherapy. EFT of the bladder is an aggressive tumor, with many patients presenting with or developing metastases soon after diagnosis. Four of the eighteen patients reported in the literature developed metastases to the lung (2 patients), pelvic lymph node (1 patient), and abdominal wall (1 patient). Five patients from our cohort had either presented with or soon developed metastases primarily to the liver and lungs.

Comparisons between our study and prior case reports have also revealed that prognosis is different in our cohort. Prior reports showed a poor survival trend in older patients compared with the young ones, which was not seen in our series. We observed a similar type of survival pattern across both age-groups. This finding may be attributed to a more detailed workup considering and ruling out different diagnostic possibilities, followed by confirmation with FISH. We used a broad IHC panel and *EWSR1* break-apart FISH assay with retention of RB1 to diagnose the cases. IHC promiscuity of CD99 and FLI1 across the small round cell tumors is well established in the literature. Moreover, neuroendocrine marker expression in rhabdomyosarcomas and CD56 expression in lymphomas are well known [27,29–31]. Limited utilization of IHC analysis and FISH (performed in nine of eighteen patients) in the previously published case reports could be the reason for difference in survival as we hypothesize that all the differential diagnostic entities of an EFT were not completely excluded (Table 2) [6–23].

Primary round cell sarcomas of the urinary bladder with *EWSR1* rearrangement are an extremely unusual malignant tumor. A definitive diagnosis is based on histomorphologic findings, the IHC profile, and molecular analysis for translocation involving the *EWSR1* gene. Appropriate distinction of this entity from its morphologic mimics, including other round cell sarcomas/tumors, carcinomas, melanomas, and lymphomas, is of paramount importance as it carries distinctive therapeutic and prognostic implications.

Authors' contributions

J.D. and S.K.M. contributed to conception and design. J.D. and S.K.M. contributed to development of methodology. M.B., B.L.B., S.N., D.P., S.J., M.G., J.S.G., J.D., and S.K.M. contributed to acquisition of data. M.B., A.T., J.D., J.S.G., and S.K.M. contributed to analysis of data. S.K.M., A.T., M.B., J.D., and J.S.G. contributed to interpretation of data. S.K.M., A.T., J.S.G., J.D., M.B., B.L.B., and. M.B.A. contributed to writing and review/revision of the manuscript. S.K.S., P.K.S., and K.P. contributed to technical support. S.K.M., J.D., and M.B.A. contributed to study supervision.

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