The Small Round Cell Sarcomas Complexities and Desmoplastic Presentation

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Abstract

Background: Small round cell sarcomas (SRCSs) account for most solid malignancies in the pediatric age group and are a part of group of malignant tumors characterized by heterogeneous clinical presentation and overlapping microscopic features of small, round, primitive cells. In addition to the recently established certain genetically defined subset of undifferentiated round cell sarcomas of soft tissue and bone, this group of sarcomas include desmoplastic small round cell tumor, poorly differentiated synovial sarcoma, alveolar rhabdomyosarcoma, mesenchymal chondrosarcoma, and small cell osteosarcoma. Although, those entities share clinical and cytomorphologic features and cannot be unambiguously classified based on clinical presentation and morphology alone. Most of SRCSs characterizes of particular patterns of protein expression or genetic changes and ancillary tests remain necessary to confirm or rule out a specific diagnosis. Subtle but occasionally distinctive cytotologic features narrows the number of differential diagnoses and helps to select appropriate ancillary tests necessary for the final diagnosis. Thus, when adequate fine needle aspiration (FNA) biopsy specimen is combined with ancillary tests, a specific histologic diagnosis can be made in almost all cases. However, due to complex cytoligic features of SRCS as well as various quality and diversity of FNA smears, there are cases that in that cytoplogic features which do not entirely match the known diagnostic criteria.

Summary: The aim of this review was to summarize cytomorphologic criteria and to present rare and divergent cytological features of SRCSs. Careful assessment of clinical presentation, cytological features, immunohistochemical patterns, and molecular alternations is necessary for an accurate diagnosis. Knowing of rare and divergent microscopic findings that does not fit with the known cytological criteria will help to avoid misdiagnosis.

Key Messages: The role of FNA biopsies diagnosing soft tissue and bone tumors has been increasing because of the ability of ancillary tests to assist in the diagnosis of specific tumors. SRCSs may be diagnosed accurately in cytology specimens. Access to clinical and radiographic presentation, utility of ancillary tests, understanding complexity of cytoplogic features, and awareness of the rare cytologic findings that differ from that of the established diagnostic criteria are essential to make correct diagnosis.
**Introduction**

Small round cell sarcomas (SRCS) are a part of larger group of small cell malignant tumors which includes lymphoma, blastemal tumors, and small cell carcinomas. SRCSs are high-grade sarcomas characterized by a predominantly small, round-to-oval, and relatively undifferentiated cells. The representative of this group is Ewing sarcoma (ES), which is characterized by gene fusions including EWSR1 or FUS and ETS transcription factors family, newly described CIC-rearranged sarcoma, sarcoma with BCOR genetic alterations, round cell sarcoma with EWSR1-non-ETS fusions, desmoplastic small round cell tumor (DSRCT), poorly differentiated synovial sarcoma (SS), alveolar rhabdomyosarcoma (RMS), mesenchymal chondrosarcoma, and small cell osteosarcoma. Thus, some of SRCS lack specific cytomorphic features and are difficult to clarify on morphology alone, most of those entities characterizes of subtle and occasionally distinctive cytologic features [1–5] (Table 1).

Fine needle aspiration (FNA) biopsy has been successfully used as an initial diagnostic procedure or as a complement to Trucut core needle biopsy (CNB) for the diagnosis and treatment of sarcomas, in the context of a multidisciplinary approach. Regarding diagnosing of SRCS, the role of cytology has been increasing because of the ability of ancillary tests to assist in the diagnosis of specific tumors. Despite the name, FNA smears of SRCS may display variable cell population and variety of nuclear and cytoplasmic features such as mixture of smaller, darker, larger, and paler cells, admixture of spindle cells, significant nuclear pleomorphism, cells with cytoplasmic vacuolations or with abundant cytoplasm, and cytoplasmic densities creating rhabdoid-like morphology. In addition, many of those entities characterize of specific architectural patterns such as pseudo-rosettes formations, papillary structures with vascular cores, the presence of “tigroid” or myxoid background, cartilaginous, fibrillar, and osteoid matrix or eosinophilic connective tissue likely associated with desmoplasia, presented intercellular or in the background of the smears. All those microscopic features narrow the number of differential diagnoses and help to select appropriate ancillary tests necessary for the final diagnosis.

**Ewing Sarcoma**

ES is a primitive small round cell neoplasm in the group of neuroectodermal neoplasms characterized by gene fusions including EWSR1 and ETS transcription factors family. In the World Health Organization (WHO) Classification 2020, ES includes in larger group of undifferentiated SRCS of bone and soft tissue including round cell sarcomas with EWSR1-non-ETS fusions, CIC-rearranger sarcomas, and sarcomas with BCOR genetic alterations [1, 6]. The term peripheral neuroectodermal tumor which was traditionally used when ES displays neuroectodermal differentiation disappeared as both neoplasms share the same molecular profile.

ES is the third most frequent primary bone sarcoma after osteosarcoma and chondrosarcoma and second most common soft tissue and bone malignancies in children and adolescents after osteosarcoma. This neoplasm is rare before age 5 and after age 30 and extremely rare in elderly patients. Approximately, 80% of cases arise in patients younger than 20 years of age. Most common sites are the shafts of the long bones, pelvic bones, ribs, and spine. They may however occur in almost any bone. Sites of extraskeletal ES accounting 10–20% of neoplasm, include various parts of the body (cutaneous, subcutaneous, soft tissue, paraspinal muscles, the retroperitoneum, kidneys, and breasts) [4, 7–9]. The cytologic criteria of ES have been reported in several publications [4, 5, 8, 10–13].

Diagnosis of ES may be suggested from technically satisfactory and preferably both air-dried and wet-fixed smears. Aspirates are commonly hypercellular with dispersed cells admixed with clusters of loosely cohesive cells, occasional perivascular arrangement of tumor cells and rosette-like structures and (Fig. 1a, b). Double-cell population, large light cells with abundant, “thin” cytoplasm with clear spaces or vacuoles, round nuclei with finely chromatin texture, and small dark cells with scant cytoplasm and hyperchromatic nuclei are better appreciated in the air-dried smears so are the “tigroid” background and intracytoplasmic vacuoles (Fig. 1c, d). Cytoplasmic glycogen is associated with cytoplasmic vacuoles and clear spaces (Fig. 1e). Occasionally spindle-shaped cells or cells with rhabdoid morphology may be seen. Cytologic features of ES include uncommon divergent findings such as intranuclear inclusion and eosinophilic connective tissue fragments in the background of the smears (Fig. 1b, f).

More than 90% of ES show strong, cytoplasmic membrane positivity with CD99 and about 75%, predominantly cases with gene fusions, nuclear staining with the FLI-1, and ERG antibodies. Approximately, 25% of cases express keratin and differentiated subtype (previously peripheral neuroectodermal tumor) expresses neuroendocrine markers [14–17]. Nuclear marker NKKX2.2 is sensitive and moderately specific, while novel marker PAX7 is
Table 1. Common and rare, divergent cytologic features of SRCS

| Tumor                                      | Diagnostically important microscopic features                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Divergent microscopic features                                                                                                                                                                                                                     |
|--------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ES                                         | Smears patterns and background: Hypercellular smears. Mixture of clustered and dispersed cells. Occasional perivascular arrangement of tumor cells and pseudorosettes. Tigroid background Tumor cells micromorphology: Monomorphous population of small round cells with high N:C ratio, bland nuclear morphology, and inconspicuous nucleoli. Double cell population large light and small dark cells (better appreciated in air-dried smears). Larger cells with cytoplasmic vacuoles that contain glycogen. Occasionally moderately pleomorphic cells, spindle-shaped cells, or cells with rhabdoid morphology | Desmoplasia (eosinophilic connective tissue) in the background. Intranuclear inclusions                                                                                                                                                              |
| CIC-rearranged sarcoma                      | Smears patterns and background: Hypercellular smears. Dispersed cells and loosely cohesive cell clusters and sheets. Perivascular arrangements and traversing capillaries evident in cell sheets. Necrotic debris and myxoid stroma Tumor cells micromorphology: Mild to moderate pleomorphism but more prominent variation in size and shape of nuclei, compared to that of ES. Centrally or eccentrically placed round-to-ovoid, occasional polygonal, angulated, and spindle-shaped nuclei with irregular nuclear membrane, coarse chromatin pattern and variably prominent nucleoli. Majority tumor cells with scant to moderate cytoplasm, occasionally small cytoplasmic vacuoles or poorly preserved cytoplasmic borders creating slender cytoplasmic processes connecting one cell to another | Desmoplasia (eosinophilic connective tissue) in the background and tigroid background. Cells with rhabdoid morphology                                                                                                                                  |
| Sarcoma with BCOR genetic alterations       | Smears patterns and background: Moderate cellular smears with dispersed cells and loosely cohesive cell clusters. Sometimes perivascular arrangements, or papillary clusters with vascular cores. Desmoplasia (connective eosinophilic tissue), myxoid stroma, and necrotic debris Tumor cells micromorphology: Round and spindled cells with evident pleomorphism. Double cell population: large, "light" cells and small, dark cells resembling those of ES |                                                                                                                                                                                                                                                     |
| DSRCT                                      | Smears patterns and background: Moderate cellular smears. Loosely cohesive sheets and clusters or less common, tight clusters. Desmoplastic stroma fragments and occasional acinar-like structures or pseudorosettes. Necrosis and apoptosis Tumor cells micromorphology: Uniform or slightly pleomorphic undifferentiated cells with round-to-oval, slightly angulated, irregular nuclei with granular chromatin, inconspicuous nucleoli and scant cytoplasm. Nuclear molding and paranuclear cytoplasmic densities | Hint of myxoid background matrix                                                                                                                                                                                                                     |
| Poorly differentiated SS of small cell type | Smears patterns and background: Hypercellular smears. Mixture of tight clusters, sheets, dispersed small round cells and stripped nuclei. Rarely cells forming rosette-like structures Tumor cells micromorphology: Small round uniform cells. Showing round-to-oval nuclei, and scant cytoplasm. Occasional mild nuclear pleomorphism | Double cell population: large, light cells and small, dark cells resembling those of ES                                                                                                                                                             |
| Alveolar RMS                                | Smears patterns and background: Hypercellular smears. Dispersed cells admixed with naked nuclei and clusters of loosely cohesive cells. Sometimes perivascular arrangement of tumor cells and lacy (tigroid) background Tumor cells micromorphology: Predominantly small- to medium-sized round or ovoid cells with scanty cytoplasm, hyperchromatic nuclei with coarse chromatin, often with large nucleoli. Occasionally Binucleated and multinucleated tumor cells and cells with eccentric nuclei and cytoplasmic densities |                                                                                                                                                                                                                                                     |
| Mesenchymal chondrosarcoma                  | Smears patterns and background: Predominantly cohesive cell clusters. Biphasic pattern. Fragments of fibrocartilaginous matrix and variable presentation of fibrillar and myxoid stroma and osteoclasts Tumor cells micromorphology: Tumor cells with sparse or moderate cytoplasm and round-to-oval, occasionally spindled nuclei with coarse chromatin and inconspicuous nucleoli | Desmoplasia (eosinophilic connective tissue) in the background                                                                                                                                                                                     |
| Small cell osteosarcoma                     | Smears patterns and background: Hypercellular aspirates. A mixture of cohesive fragments and dispersed small- to medium-sized cells. Rare osteoid Tumor cells micromorphology: Small- to medium- sized, slightly pleomorphic cells with high N:C ratios, round, occasionally oval or elongated nuclei, finely granular nuclear chromatin, scant cytoplasm or bare nuclei, fine cytoplasmic vacuoles | Chondromyxoid matrix                                                                                                                                                                                                                                 |

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a sensitive marker for ES but also stains several ES mimics [18–20].

Majority of ES harbor the chromosomal translocation t(11;22)(q24;q12) involving the EWSR1 gene on chromosome 22 and the FLI-1 gene on chromosome 11, corresponding to EWSR1-FLI1 fusion (85%), or EWSR1-ERG fusion (10%). In the remaining cases EWSR1 or FUS is rearrangement with other ETS family members (like ETV1, ETV4, FEV). Finally, small subset of tumors may show fusions with FUS instead of EWSR1 with ERG or FEV [1, 7, 9, 21–23].

**CIC-Rearranged Sarcoma**

*CIC*-rearranged sarcoma shows slight male predominance, and age range presentation from children to adults with a peak incidence in the third decade of life. Most cases arise in the soft tissue of the trunk and limbs with a small subset of cases affects bones [24–26] *CIC*-rearranged sarcoma have highly aggressive course and the reported 5-year overall survival rate is 17%–44% [25, 26].

To date, there are only a few reports describing cytological features of these tumors [27–31]. Smears are usually hypercellular with tumor cells dispersed or arranged in tight (Fig. 2a) or loosely cohesive clusters and sheets. Small capillaries surrounded by the tumor cells or traversing vessels are evident in cell sheets (Fig. 2c). Tumor cells show mild to moderate pleomorphism but more prominent variation in size and shape of nuclei, compared to that of ES. While nuclei in *CIC*-rearranged sarcoma cases are commonly round-to-ovoid, occasional cells with polygonal, angulated, and spindle-shaped nuclei with irregular nuclear membrane are also seen in smears. Centrally or eccentrically placed nuclei show coarse chromatin pattern, irregular nuclear contours, and variably prominent nucleoli (Fig. 2b). Majority tumor cells show scant to moderate cytoplasm, some with small cytoplasmic vacuoles or poorly preserved cytoplasmic borders creating slender cytoplasmic processes connecting one cell to another (Fig. 2b). Necrotic debris in the background is a frequent finding in smears as is presence of myxoid stroma (Fig. 2d).

No pseudorosettes are found in published *CIC*-rearranged sarcoma cases [27–31], but unpublished data...
mentioned presence of pseudorosettes in some cases [31]. In addition, not previously reported divergent findings in smears include desmoplastic background matrix, tigroid background and mono-, bi-, and multinucleated cells with eccentrically placed nuclei and cytoplasmic densities creating rhabdoid morphology (Fig. 2e–g).

By immunohistochemistry, CIC-rearranged sarcomas express CD99 with diffuse and membranous pattern in approximately 20% of cases, while in most cases, multifocal, patchy pattern or even completely absent expression. Nuclear immunoreactivity for FLI1, WT1, and ETV4 is present in most cases while NKKX2.2 and PAX7 are negative [24, 25, 32–34]. Calretinin and ERG can be positive, while expression of keratin, S-100, and myogenic markers is rarely detected [25, 26]. Two translocations have been described for majority of CIC-DUX4 fusion sarcomas: t(4;19)(q35;q13) and t(10;19)(q26;q13), resulting with CIC-DUX4 fusion [24, 25, 32–34]. Non-DUX4 partner genes can be rarely detected, including NUTM1, NUT-M2A, LEUTX, or FOXO4 [36–38]. Cases with CIC-NUTM1 express NUT protein [38].

**Sarcoma with BCOR Genetic Alterations**

BCOR-rearranged sarcomas are uncommon and consist of a group of tumors, characterized by genetic alterations of BCOR gene, most often BCOR-CCNB3. This neoplasm occurs in bone and soft tissue (ratio 1.5:1) in the pelvis, lower limbs, and paraspinal region in children with a male sex predominance and over 90% of patients younger than 20 [39–42]. Clinical course is similar to ES with reported 5-year overall survival 75% [39–41]. Other sarcomas with BCOR genetic alterations are undifferenti-
ated round cell sarcoma of infancy and primitive myxoid mesenchymal tumor of infancy characterized by BCOR-ITD and very rare neoplasms with lack of well-established clinicopathological features [43, 44].

To date, there are only three papers which reported cytological features of BCOR-CCNB3 sarcoma [31, 45, 46]. Smears are usually hypercellular with mixture of dispersed cells and cell sheets and papillary clusters with vascular cores (Fig. 3a) but no rosettes formations. In one cytology report, small tumor cell clusters with thin and delicate vascular cores and tiny vascular fragments were conspicuous findings [45]. Double-cell population, large light cells, and small dark cells with hyperchromatic nuclei remaining smears of ES are common findings (Fig. 3b, c), as is admixture of spindle cells (Fig. 3d). Most nuclei show inconspicuous nucleoli. Smears of BCOR-CCNB3 sarcoma present significant nuclear pleomorphisms and single cells with rhabdoid-like features. Background necrosis, eosinophilic connective tissue (Fig. 3b, c), and myxoid stroma can be found in most cases [31].

By immunohistochemistry, BCOR-CCNB3 sarcoma express cyclin B3, BCOR protein, TLE1, and SATB2. Reactivity for CD99 occurs in approximately 50% of cases with staining pattern similar to CIC-rearranged sarcomas [39–41].
Round Cell Sarcoma with EWSR1-Non-ETS Fusions

Round cell sarcoma with EWSR1-non-ETS fusions is the least common entity of undifferentiated SRCS of bone and soft tissue and is defined as a sarcoma with EWSR1 or FUS fusions involving non-ETS gene family partners [1]. Clinically round cell sarcomas with FUS-NFATC2 and EWSR1-NFATC2 occur in children and adults with striking male predilection while EWSR1-PATZ1 sarcomas occur in broad range of age and affects both sexes equally. FUS-NFATC2 and EWSR1-NFATC2 affect exclusively or mainly bones respectively while most cases of EWSR1-PATZ1 sarcomas arise in the deep soft tissue of chest wall and abdomen, and the brain. To date, only one paper mentioned cytologic description of EWSR1-PATZ1 sarcoma exists describing hypercellular smears of round cells with marked cellular pleomorphism intermixed with small, short spindle cells.

Desmoplastic Small Round Cell Tumor

DSRCT is an aggressive malignant mesenchymal tumor of unknown histogenesis. It is predominantly found in the abdominal cavity in adolescents and young adults with a striking male predominance and peak incidence in the third decade of life. Clinical presentation is often dramatic, with a large intra-abdominal or retroperitoneal/pelvic mass with multiple serosal implants and subsequent metastasis to lymph nodes, liver, and lungs. An extra-abdominal location is rare, with the thorax and paraortic area being the most common sites. The prognosis of DSRCT is extremely poor and the average survival is less than 3 years [1, 47, 48].

Aspirates show commonly loosely cohesive sheets and clusters or less common, tight clusters of uniform or slightly pleomorphic cells with high N:C ratios; scant or poorly preserved cytoplasm; round-to-oval, occasionally angulated, irregular nuclei with granular chromatin and inconspicuous nucleoli (Fig. 4a, b). Nuclear molding and paranuclear cytoplasmic densities are common findings (Fig. 4b, c). Mitosis, apoptosis, necrosis, and crushing ar-
Tifacts are common findings in smears [49–52]. The cytomorphology of DSRCT, though similar to other small blue round cell sarcomas, displays characteristic findings of nests of tumor cells with some cohesiveness retained, associated with desmoplastic stroma and occasional acinar-like structures or pseudorosettes (Fig. 4b, d). Rarely, cytologic features of DSRCT include divergent finding such as myxoid matrix in the background of the smears (Fig. 4e). DSRCT exhibits polyphenotypic differentiation, and neoplastic cells typically express epithelial (keratin and EMA), myogenic (desmin), mesenchymal (vimentin), neural (neuroendocrine), and WT1 markers [49, 53, 54]. DSRCT is characterized by a unique translocation t(11;22)(p13;q12) with EWSR1-WT1 gene fusion [48].

**Poorly Differentiated SS of Small Cell Type**

SS is characterized by a pathognomonic translocation t(X;18) which is present in >95% of cases. SS accounts for 5–10% of soft tissue sarcomas and is a highly aggressive sarcoma with common local recurrences and metastases in approximately 50% of cases. SS affects both sexes equally and may occur at any age thus most patients are between the ages of 10 and 40 years. Most SS cases are deep seated and arise in the extremities, trunk, and head and neck region, but they may occur elsewhere in the body, including rare locations in visceral organs, genital tracts, the retroperitoneum, and the mediastinum [1, 55–57]. Based on their morphology, SS are divided on monophasic, biphasic, or poorly differentiated subtypes, the last divided on three morphologic variants: the small cell variant resembling ES, the spindle cell variant resembling malignant peripheral nerve sheath tumor, and the epithelioid, pleomorphic variant with rhabdoid features [1, 58–63]. The cytologic criteria of SS have been reported in several publications [58–60, 62–68]. FNA smears of SS are usually cellular and in low magnification show a distinctive pattern of tight clusters of bland spindle cells alternating with dispersed cells or bare nuclei. Small cell variant of SS shares cytomorphological features of small round primitive cells with high N:C ratio with those of other malignant SRCSs. The cytologic smears are highly cellular, consisting of a mixture of loose or tight clusters and dispersed uniform and monotonous cells, showing round-to-ovoid nuclei and scant cytoplasm (Fig. 5a, b). Mitotic figures, occasional mild nuclear pleomorphism, and cells forming rosette-like structures may be also observed [58, 59]. Aspirates of morphologic variants of poorly differentiated SS showing double-cell population, large light cells, and small dark cells resembling that of ES (Fig. 5c) may cause diagnostic difficulties and a definitive diagnosis requires immunocytochemical and molecular genetics examinations [60, 61]. Neoplastic cells of SS stain diffusely and strongly for TLE1. Most of the SS stain positively for CD99 and Bcl-2 and are often focally positive for EMA and cytokeratins while focal S100 expression occurs in approximately 40% of cases [1, 57, 63, 66]. SS has a specific and often sole genetic anomaly of the t(X;18)(p11;q11) translocation which creates a SS18/SSX fusion gene [67–70].

**Alveolar RMS**

RMS is a malignant tumor with differentiation towards skeletal muscle and is the most common sarcoma of childhood. Pediatric RMS belongs to the group of neo-
plasms that are commonly examined by FNAB [2, 5, 71–76]. The sensitivity and specificity for those tumors exceed 90% and with an adequate sampling and the use of ancillary techniques a diagnosis of a specific histologic subtype is establish in the majority of cases [5, 76, 77]. Four main subtypes of RMS are recognized in the current WHO classification: embryonal, alveolar, spindle cell/sclerosing, and pleomorphic [1]. The embryonal subtype accounts for approximately 60% of cases. Alveolar RMS accounts for about 20% of all pediatric RMS and occurs most frequently in adolescents and young adults between 10 and 25 years of age. Alveolar RMS is a neoplasm composed of a monomorphic population of primitive round cells with skeletal muscle differentiation and belongs to the group of small, round, blue-cell sarcomas. Aspirates are commonly hypercellular with dispersed cells admixed with clusters of loosely cohesive cells and occasionally perivascular arrangement of tumor cells (Fig. 6a). In contrast to embryonal RMS, smears are more uniform with predominantly small- to medium-sized round or ovoid cells with hyperchromatic nuclei, often with large nucleoli (Fig. 6b). Binucleated and multinucleated tumor cells and cells with eccentric nuclei and cytoplasmic densities are frequent findings in smears (Fig. 6c, d).

Similar to embryonal RMS, tumor cells are immunoreactive with desmin, myogenin, MyoD1, and PAX7.

Fig. 6. Alveolar RMS. a Loosely cohesive cluster with perivascular arrangement of tumor cells (H&E stain). b Loosely and dispersed, single, small-, and medium-sized cells with hyperchromatic nuclei and prominent nucleoli (H&E stain). c Loosely sheets of medium-sized cells with irregular hyperchromatic nuclei and occasional tumor giant cells (H&E stain). d Rhabdomyoblastic morphology of tumor cells (H&E stain).
Aberrant expression of keratins, CD99, S100 protein, neuron-specific enolase, and CD56 may present diagnostic challenges and confusion with other small round blue-cell tumors [77, 78]. Cytogenetically, alveolar RMS shows recurrent translocations, with t(2;13)(q35;q14) being the most common and t(1;13)(p36;q14) occurring in fewer cases. These translocations result in chimeric genes encoding fusion proteins with strong transcriptional activation and oncogenic effects. The genes involved are PAX3 located on chromosome 2 and PAX7 on chromosome 1. They fuse with FOXO1 on chromosome 13 resulting in the PAX3/PAX7-FOXO1 fusion genes [79–81]. The MYCN oncogene in 2p24 can also be amplified and overexpressed in fusion-positive cases [82].

**Mesenchymal Chondrosarcoma**

Mesenchymal chondrosarcoma is a rare high-grade bimorphic malignant tumor composed of islands of low-grade hyaline cartilage and malignant small round cells. Most cases occur in the second and third decades of life. The craniofacial bones (jawbones), ribs, vertebrae, and
the ilium are the most common sites [1, 83]. Approximately, 30% of mesenchymal chondrosarcomas arise primarily in extraskeletal sites, commonly in meninges and somatic soft tissues [1, 84].

The cytologic features of mesenchymal chondrosarcoma have been reported in a few case reports [85–89]. Small, round monomorphic (ES-like) tumor cells in cohesive clusters, some embedded in a fibrillar matrix, have been described [85–90] (Fig. 7a). Tumor cells contain sparse or moderate cytoplasm and round-to-oval, occasionally spindled nuclei with coarse chromatin and inconspicuous nucleoli (Fig. 7b). Fragments of fibrocartilaginous matrix and eosinophilic connective tissue, osteoclasts, and myxoid stroma are variable presented in smears (Fig. 7b–d). A biphasic pattern of cartilaginous fragments and small blue round cells support the diagnosis mesenchymal chondrosarcoma in FNA smears. When a biphasic pattern is not obvious and smears only show a small round cell population, the differential diagnosis includes other small cell malignancies and correlation to clinical and radiographic data as well as ancillary techniques is crucial for a correct diagnosis.

The typical immunophenotype of mesenchymal chondrosarcoma is S100 protein positivity in the cartilaginous

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**Fig. 8.** Small-cell osteosarcoma. a, b Dispersed small- to medium-sized tumor cells with scanty cytoplasm, rounded nuclei with granular chromatin and moderate anisonucleosis resembling those of ES (H&E and MGG staining). c Chondromyxoid matrix and dispersed slightly pleomorphic cells. Subsequent histologic section disclosed areas of cartilage in small cell osteosarcoma (MGG stain). d Dispersed tumor cells and small fragment of eosinophilic tissue (MGG stain).
component and CD99, SOX9, and NKX2.2 positivity in the small cell population. Variable positivity for desmin and negative staining for FLI-1 and CD45 help in distinguishing mesenchymal chondrosarcoma from ES and non-Hodgkin lymphoma. Small cell osteosarcoma may exhibit CD99 membrane positivity in some cases, which may cause a problem in distinguishing it from mesenchymal chondrosarcoma. A recurrent and specific HEY1-NCOA2 gene fusion is found in this subtype of chondrosarcoma and it is notably missing in the other variants of the disease. The pathogenetic mechanism of the fusion is not fully elucidated, but it could upregulate transcription [91]. The IDH1 and IDH2 mutations seen in the conventional chondrosarcomas are usually missing [92].

**Small Cell Osteosarcoma**

Small cell osteosarcoma, a rare histological subtype of osteosarcoma, accounts for less than 1% of all cases of osteosarcoma [93, 94]. The cytology of rare small cell osteosarcoma has been only briefly described [95–97] Aspirates from rare small cell osteosarcomas are hypercellular and tumor cells resemble those of ES. A distinction between small cell osteosarcoma and ES based on cytologic findings alone is difficult. The main cytologic feature of small cell osteosarcoma is a mixture of cohesive fragments and dispersed small- to medium-sized cells with scant cytoplasm and bare nuclei (Fig. 8a, b). Fine cytoplasmic vacuoles were reported. Tumor cells are slightly pleomorphic with round, occasionally oval or elongated nuclei, finely granular chromatin, and a high nuclear/cytoplasmic ratio. Identification of osteoid matrix is the key to the diagnosis but can be difficult in smears [94–96]. In addition, osteoid in FNA smears may display different appearance and can be difficult to distinguish it from eosinophilic desmoplasmic tissue or cartilaginous and fibrillary matrix presented in smears of other small round cell neoplasm (Fig. 8c, d).

**Summary and Conclusion**

Cytologic differential diagnosis of SRCS is broad and may be difficult due to their undifferentiated or poorly differentiated micromorphology. Main differentials are discussed above. Sarcomas with a round cell pattern examined frequently by FNA biopsy are ES family and alveolar RMS, while poorly differentiated SS, DSRCT mesenchymal chondrosarcoma, and small cell osteosarcoma are less common.

Differential diagnosis of undifferentiated round cell sarcomas includes other SRCSs [10, 14, 31, 45] but also non-sarcomatous malignancies such as lymphoblastic lymphoma and small cell or neuroendocrine carcinoma. Poorly differentiated SS of small cell type showing double-cell population, resembling that of ES characterizes by specific immunoprofile compared to the ES such as TLE1, EMA, and keratins positivity. Poorly differentiated SS can also mimic BCOR-CCNB3 sarcoma because coexpression of PAX7 and BCOR and occasional expression of TLE1 in the last. EMA and keratins positivity and specific gene fusions are helpful in differential diagnosis.

Cytologic features of ES and other newly described entities of this group such are CIC-rearranged and sarcoma with BCOR genetic alterations may closely mimic alveolar RMS that constitute the main differential diagnosis in FNA smears [31, 45]. Some specific cytologic features described above and immunoprofile of RMS, mainly nuclear myogenic markers such as MYOD1 and myogenin as well as specific gene fusions allows to distinguish these entities. Other differential diagnosis of alveolar RMS includes embryonal RMS, poorly differentiated SS and precursor lymphoblastic lymphoma, aberrant expression of keratins, CD99, S100 protein, neuron-specific enolase, and CD56 in alveolar RMS may present diagnostic challenges. Like other small round blue-cell neoplasms, the correct diagnosis of alveolar RMS requires ancillary techniques such as immunocytochemistry and molecular genetics examinations [4, 71].

DSRCT commonly affects young patients and shows predilection to abdominal cavity. Smears display frequently desmoplastic stroma fragments [49–52]. DSRCT exhibits polyphenotypic differentiation, but majority of cases show both keratins and desmin positivity [49, 53, 54]. Typical clinical presentation in addition to a unique translocation t(11;22)(p13;q12) with EWSRI-WT1 gene fusion [48, 49] helps to distinguish DSRCT from other small blue round cell tumors and small cell carcinomas.

A biphasic pattern of cartilaginous fragments and small blue round cells in smears of mesenchymal chondrosarcoma support the diagnosis in FNA smears. Mesenchymal chondrosarcoma is a differential diagnostic problem when a biphasic pattern is not obvious in those cases where the small cell population predominates and fragments of chondroid tissue are difficult to find. When smears only show a small round cell population, the differential diagnosis includes other small cell malignancies, and correlations to clinical and radiographic data as well as ancillary techniques are necessary for a correct diagnosis. The immunophenotype of mesenchymal chondrosar-
The FNA biopsy ideally should be the first biopsy method in the examination of a suspected recurrence or metastasis of a previously treated SRCS followed by CNB or excision biopsy when necessary. Dependent on local expertise and the site of the lesion, FNAB can be used for the evaluation of primary SRCS by itself or simultaneously with CNB or followed by CNB or subsequent open biopsy when necessary. Ancillary tests are essential for the accurate determination of the “line of differentiation” or histopathological subtype of SRCS and the methods used on histopathological specimens are also applicable to cytopathological material. Immunocytochemistry and molecular genetic tests on FNA biopsy material can be used to confirm or rule out a specific diagnosis as particular genetic changes have been described in many types of SRCS. In summary, FNA biopsy for diagnosis of SRCSs can be recommended as the cytologic examination of these neoplasms gives excellent results both in the evaluation of recurrent and metastatic tumors and primary sarcomas.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

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