Review paper

Rhabdomyosarcoma in children – current pathologic and molecular classification

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The last 25 years have brought significant progress in the treatment of sarcomas in children, especially rhabdomyosarcoma (RMS). Nevertheless, treatment failure in some patients results from considerable biological heterogeneity noted in these tumours. RMS, the most common malignant soft tissue neoplasm in children, includes two main subtypes: embryonal (ERMS) and alveolar (ARMS). Due to greater aggressiveness and worse prognosis of ARMS in comparison to ERMS, discrimination between different rhabdomyosarcoma subtypes is of crucial clinical importance. This paper presents the current histological classification of RMS, up-to-date immunohistochemical and biological research regarding RMS, and its associated clinical and prognostic significance.

Key words: rhabdomyosarcoma, children, pathologic and molecular classification.

Introduction

Rhabdomyosarcoma (RMS) is the most common malignant solid tumour in children after neuroblastoma and nephroblastoma (Wilms tumour). This tumour accounts for 5% to 10% of all childhood tumours [1, 2, 3, 4, 5]. For all soft tissue sarcomas, RMS accounts for 19% of such cases in adults and 45% of cases in children. Rhabdomyosarcoma is the most common soft tissue malignant neoplasm in the latter age group. Rhabdomyosarcoma is derived from primary mesenchymal cells that show skeletal muscle differentiation. It was first described by Weber in 1854. About 90% of all RMS presentations are in individuals under 25 years of age, and almost 70% are in children under 10 years of age [1, 2, 3, 6]. The most common RMS location is in the head and neck region (35-40%), followed by the urogenital system, extremities, and torso [1, 2, 3, 6, 7].

Rhabdomyosarcoma aetiology and pathogenesis

The predisposing factors for the development of soft tissue sarcoma are unknown. Increased RMS morbidity is observed in individuals with genetic syndromes predisposing to carcinogenesis, such as Li-Fraumeni syndrome, Gardner syndrome, neurofibromatosis type I, and Beckwith-Wiedemann syndrome. The common co-occurrence of RMS with

defects of the central nervous system, urogenital, gastrointestinal, and circulatory systems, and melanocytic nevi has been reported [3, 5, 8, 9, 10, 11, 12].

Nomenclature and pathologic classification

In formerly used pathologic classifications, RMS was divided into two main types: alveolar and embryonal [3, 5, 7, 13]. The embryonal type includes the botryoides and spindle cell subtypes. However, in the current WHO classification (World Health Organisation; WHO 2013) [14], four histological RMS types are recognised and classified as follows:

1. Embryonal rhabdomyosarcoma:
   a. botryoides variant,
   b. anaplastic variant.
2. Alveolar rhabdomyosarcoma:
   a. solid variant,
   b. anaplastic variant.
3. Pleomorphic rhabdomyosarcoma.
4. Spindle cell/sclerosing rhabdomyosarcoma.

It should be noted that pleomorphic RMS occurs most commonly in adults, but rare cases have been observed in children [14].

The College of American Pathology (CAP) classification of skeletal muscle-derived tumours also recognises ectomesenchymoma as part of this group of malignant neoplasms. Ectomesenchymoma is a neoplasm consisting of rhabdomyoblasts resembling the myosarcoma of both embryonal and alveolar type, with a distinctly higher presence of ERMS elements observed. The histology of the tumour also shows the presence of ganglion cells and foci of neuroblastomatous dedifferentiation. The current WHO classification of soft tissue and bone tumours places ectomesenchymoma in the same group with neurogenic neoplasms [14].

The American group of physicians specialising in RMS, "Children's Oncology Group (COG)", distinguishes the classical and solid ARMS variant, and typical, botryoid, spindle cell, sclerosing, dense, and epithelioid ERMS. Additionally, rare mixed-type RMS is listed. The rhabdomyosarcoma, not otherwise specified (RMS-NOS) category is restricted to tumours that are small and showing sampling or fixation artefact, or are necrotic, making a specific classification impossible [15].

Pathologic appearance of rhabdomyosarcoma

Rhabdomyosarcoma diagnosis should be based not only on the histopathological appearance but also on the immunohistochemical and molecular profiles. The histology of RMS shows cellular elements that are related to the structures resembling the cells of developing striated muscle (Fig. 1). Rhabdomyoblasts with a diverse level of atypia are the key cells in RMS diagnosis. The highly differentiated rhabdomyoblast is a round or oval cell that contains abundant acidophilic granular or fibrillar cytoplasm, with eccentric or centrally-located circular nuclei. Binucleation is commonly noted. Occasional large nucleoli are visible within nuclei [16] (Fig. 2). Vacuoles containing glycogen are sometimes visible in the cytoplasm. Rhabdomyoblasts can take many different forms – ribbon or tadpole-like, appearing similar to tennis rackets or spiders (Fig. 3). In less than 30% of diagnosed RMS, rhabdomyoblasts with distinct striations are observed. Detection of such a characteristic RMS cytological feature is difficult using standard haematoxylin and eosin staining. The striations can be easily visualised using a tricolour histochemical staining method, such as phosphotungstic acid haematoxylin (PTAH) (Fig. 4). However, most of the tumours are composed of less differentiated or undifferentiated rhabdomyoblasts with scant cytoplasm, and round or oval nuclei. The cellular edges of such myoblasts are star shaped. Sometimes RMS cells fuse and generate

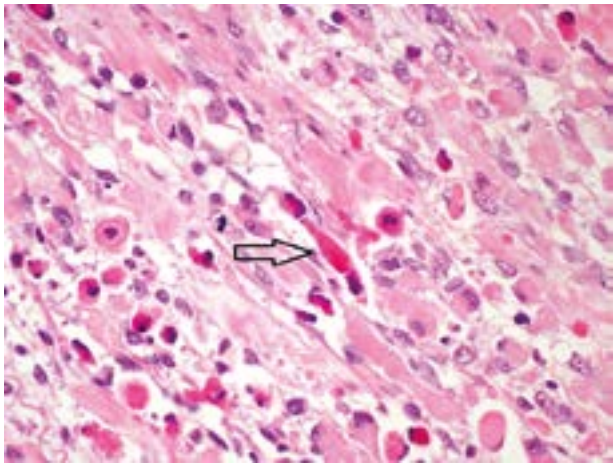

Fig. 1. Embryonal rhabdomyosarcoma. Neoplastic cell resembles developing striated skeletal muscle (=>) (HE stain, magnification 400 ×)

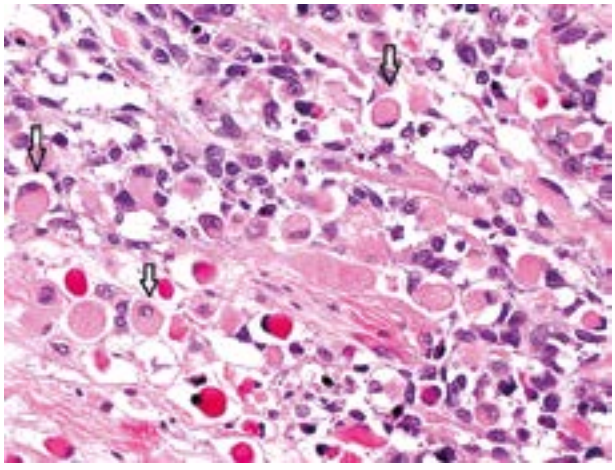

Fig. 2. Embryonal rhabdomyosarcoma. Rhabdomyoblasts: round and oval shapes of neoplastic cells (=>) (HE stain, magnification 400 ×)

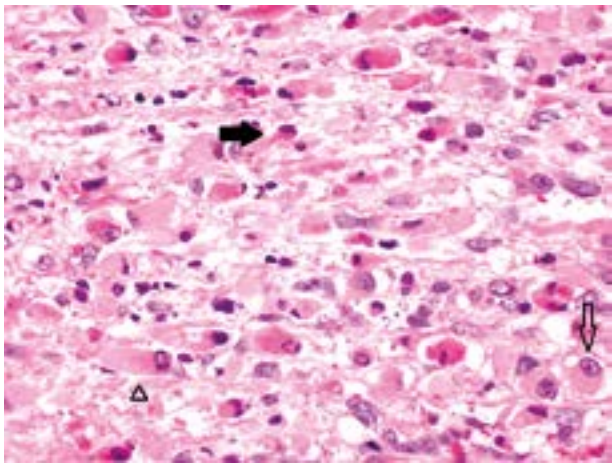

Fig. 3. Embryonal rhabdomyosarcoma. Rhabdomyoblasts: various shapes of neoplastic cells (>, =>, →) (HE stain, magnification 400 ×)

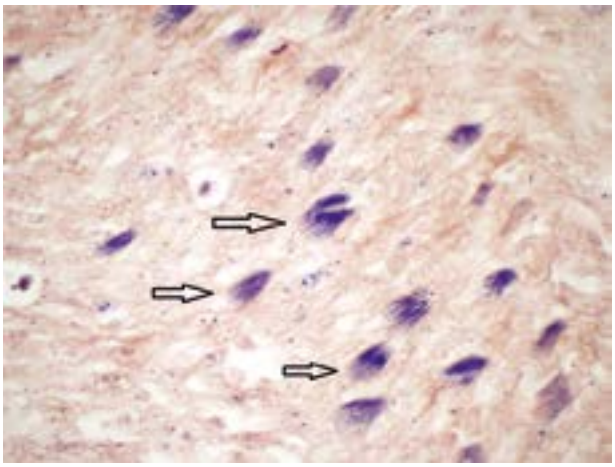

Fig. 4. Embryonal rhabdomyosarcoma. Cross-striations in cytoplasm of rhabdomyoblasts (=>) (PTAH stain, magnification 400 ×)

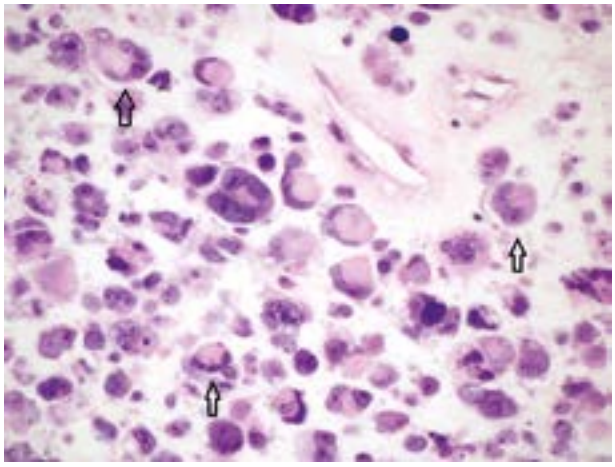

Fig. 5. Rhabdomyosarcoma, pleomorphic type. Numerous cells with multiple nuclei resembling multinucleated giant cells (=>) (stain HE, magnification 600 ×)

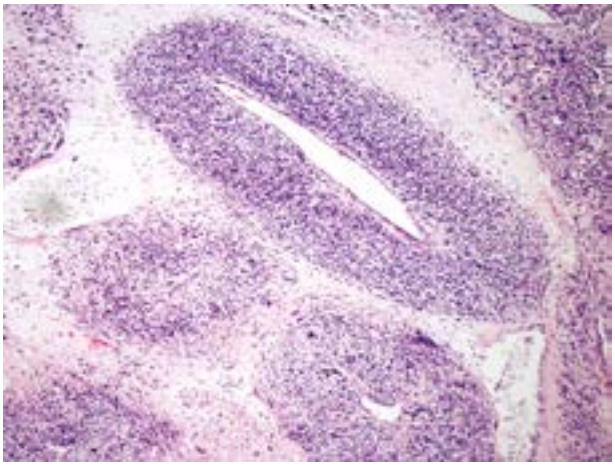

Fig. 6. Embryonal rhabdomyosarcoma. Hypercellular zones around blood vessels (HE stain, magnification 400 ×)

polynuclear cells that resemble multinucleated giant cells (Fig. 5) [17].

Embryonal rhabdomyosarcoma

The microscopic appearance of embryonal rhabdomyosarcoma (ERMS) shows rhabdomyoblasts of heterogeneous appearance. Undeveloped, round cells with a hyperchromatic nucleus and basophilic cytoplasm are common in low-cell-density regions embedded in a myxoid submucosa. High-cell-concentration regions are present around vessels and are organised in characteristic perivascular thickenings (Fig. 6). In association with poorly differentiated cells, better differentiated rhabdomyoblasts showing acidophilic cytoplasm, sometimes with cross-striation, are commonly observed. Overall, ERMS histology resembles a combination of the stages of the embryonal development of striated muscle: from the small, round, undifferentiated cells, through tadpole-like cells, ribbon-shaped striated cells, to fully differentiated rhabdomyoblasts (Fig. 7).

Embryonal rhabdomyosarcoma – botryoid variant

In the botryoid variant of ERMS, a so-called compact cambium layer (appearance analogous to the layer of plant cells that are present between xylem and phloem and cause the thickening of the plant) can be seen. These cells form the group of densely packed undifferentiated neoplastic cells just under the epithelium. The more hypocellular and mucoid areas of the neoplasm are observed under this layer (Figs. 8, 9).

Embryonal rhabdomyosarcoma – anaplastic variant

The anaplastic variant of ERMS is composed of large, anaplastic rhabdomyoblasts with hyperchromatic nuclei. These cells are often present as single cells between rhabdomyoblasts with various types

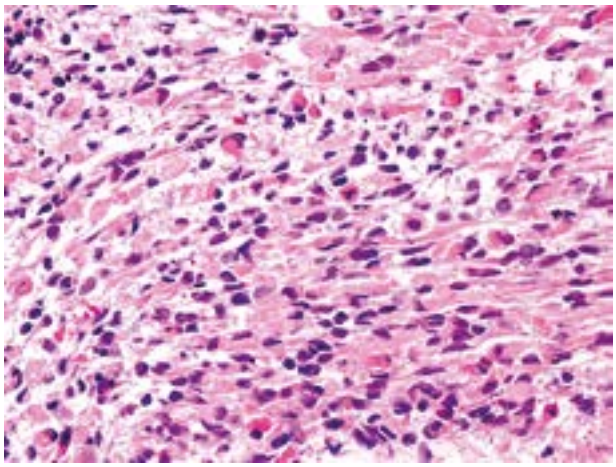

Fig. 7. Embryonal rhabdomyosarcoma. Characteristic pleomorphic rhabdomyoblasts (HE stain, magnification 400 ×)

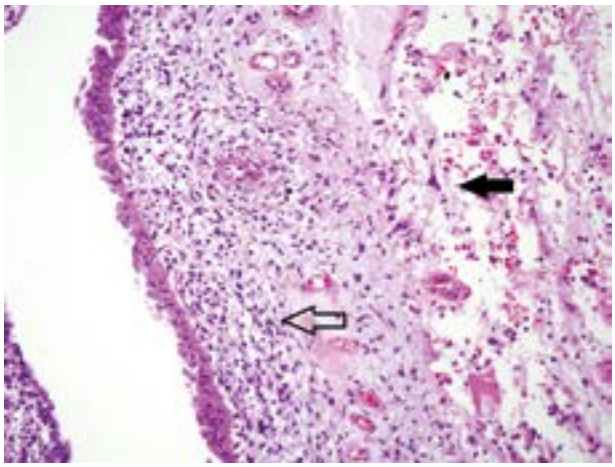

Fig. 8. Embryonal rhabdomyosarcoma, botryoides variant. Epithelium covering the cambial layer (=>) with loosely arranged cells underneath (→) (HE stain, magnification 200 ×)

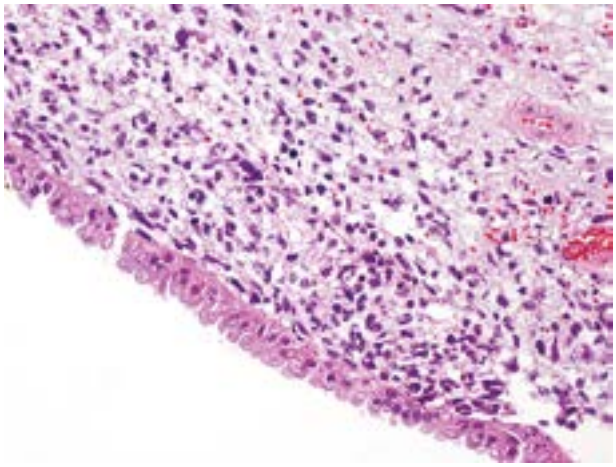

Fig. 9. Embryonal rhabdomyosarcoma, botryoides variant. A hypercellular cambial layer with epithelial layer underneath (HE stain, magnification 400 ×)

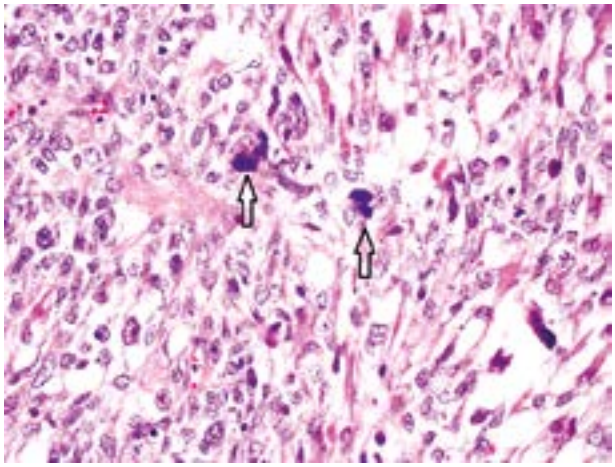

Fig. 10. Embryonal rhabdomyosarcoma, anaplastic variant. Single cells with features of anaplasia (=>) (HE stain, magnification 200 ×)

of atypia (Fig. 10). They are highly polymorphous and contain a small amount of cytoplasm. This last characteristic is distinct from the pleomorphic type, where cells often contain abundant acidophilic cytoplasm. Atypical mitotic figures are also present.

Alveolar rhabdomyosarcoma

The alveolar RMS (ARMS) is characterised by the presence of poorly differentiated rhabdomyoblasts, which are slightly larger than the undifferentiated cells in ERMS. These cells are characterised by scant cytoplasm and large nuclei (Fig. 11). The histology of ARMS also shows differentiated rhabdomyoblasts with abundant acidophilic cytoplasm and characteristic neoplastic multinucleated giant cells. The neoplastic cells cover the thick strands of connective tissue with regressive changes in the form of sclerosis.

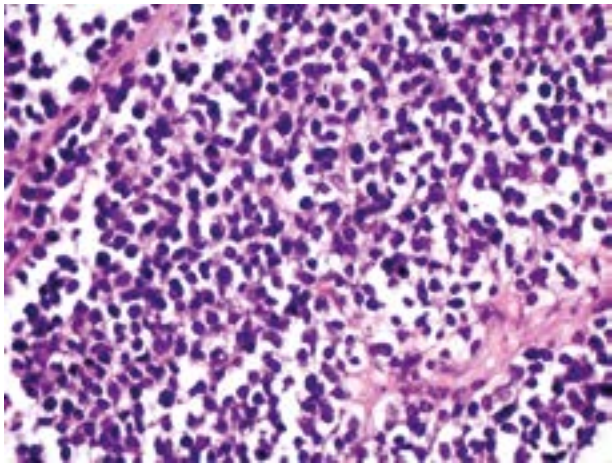

Fig. 11. Alveolar rhabdomyosarcoma, cells with big nuclei and scant cytoplasm (HE stain, magnification 400 ×)

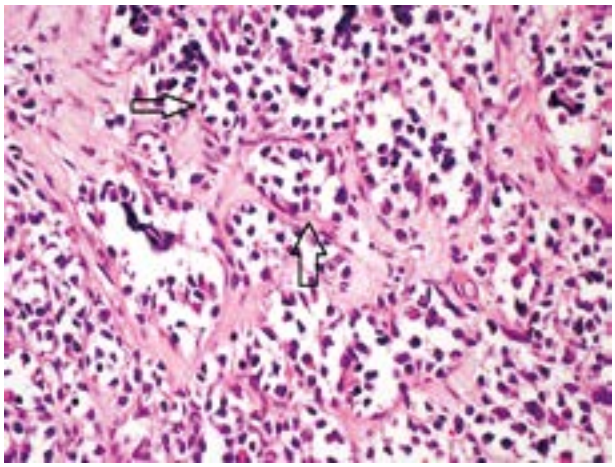

Fig. 12. Alveolar rhabdomyosarcoma, cells forming a pattern resembling pulmonary alveoli (=>) (HE stain, magnification 400 ×)

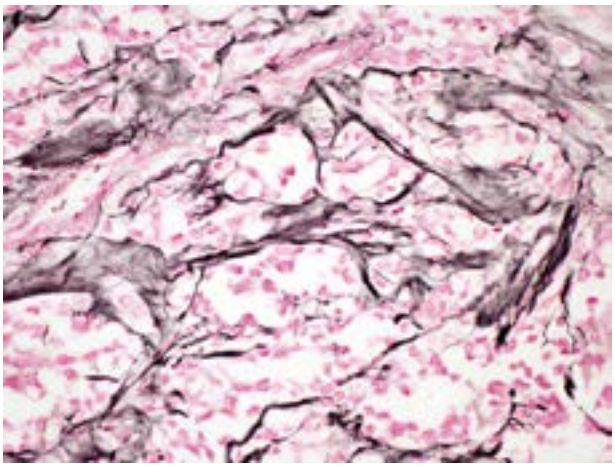

Fig. 13. Alveolar rhabdomyosarcoma. Alveolar-like spaces formed by primitive round cells separated with connective tissue septa (silver impregnation stain, magnification 400 ×)

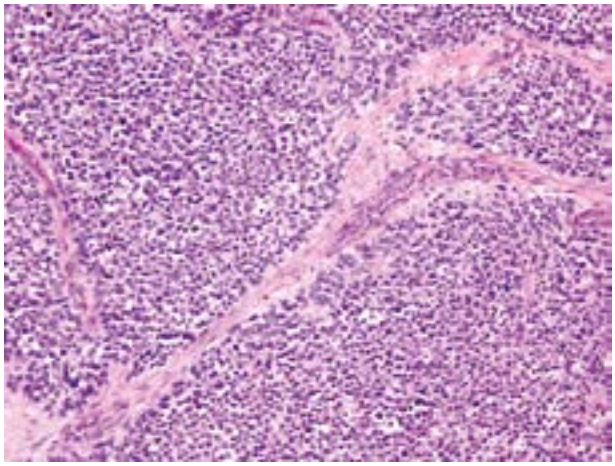

Fig. 14. Alveolar rhabdomyosarcoma, solid variant. Sheets of small round blue cells (HE stain, magnification 400 ×)

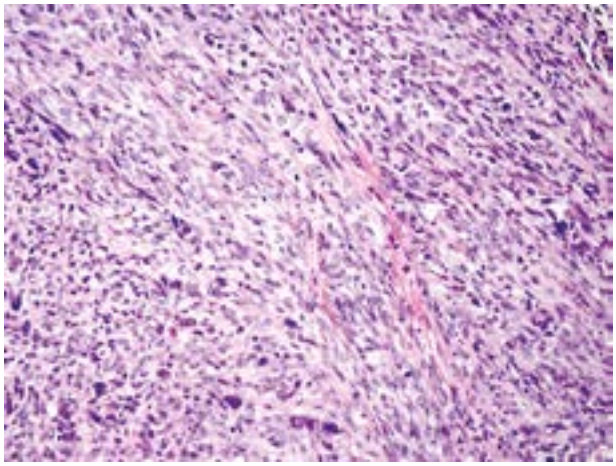

Fig. 15. Rhabdomyosarcoma, pleomorphic type. Chaotically arranged bizarre cells in the bottom left-hand corner of the Fig. (HE stain, magnification 400 ×)

The neoplastic cells aggregate in areas at the edges of fibrous septa to which the rhabdomyoblasts adhere in a single layer. In the central portion of such lesion, the cells lose this connection, undergoing necrosis and degeneration. Such foci can resemble a pulmonary alveolar structure (Figs. 12, 13).

Alveolar rhabdomyosarcoma – solid variant

In this variant of the tumour, the neoplastic cells have no connective tissue submucosa, and rhabdomyoblasts are present in various stages of differentiation forming extensive lobular structures. The cytological features of the cells are the same as in the classic form [14] (Fig. 14).

The WHO classification of bone and soft tissue tumours additionally lists rare ARMS variants without detailed characteristics including mixed alveolar and embryonal rhabdomyosarcoma and anaplastic alveolar rhabdomyosarcoma [14].

Pleomorphic rhabdomyosarcoma

Pleomorphic rhabdomyosarcoma is composed of the following cells: polymorphic, spindle, and multinucleated giant cells with abundant acidophilic cytoplasm. Sometimes cells with bizarre atypia are haphazardly arranged in the connective tissue submucosa (Fig. 15). Highly differentiated striated rhabdomyoblasts are rarely observed.

Spindle cell/sclerosing rhabdomyosarcoma

The spindle-cell variant is characterised by extensive histologic heterogeneity. Cells can be ribbon-shaped, embedded in sclerotic submucosa, or can form fascicular or elongated interwoven arrangements composing a so-called herring-bone pattern (Figs. 16, 17). The sclerosing subtype rarely occurs in children [18]. There is evidence that suggests a different biology and prognosis for the spindle-cell RMS subtype in children in comparison with ERMS [19].

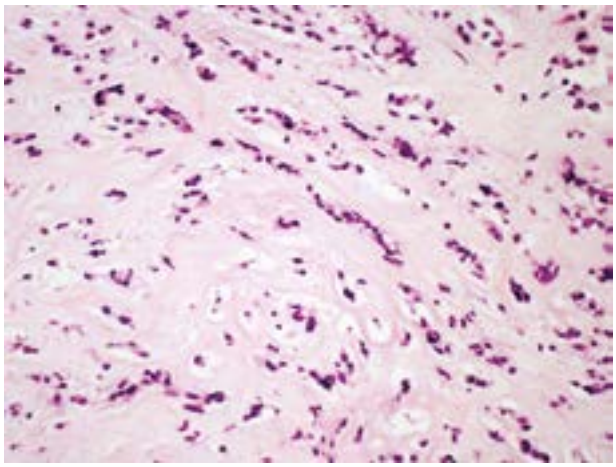

Fig. 16. Rhabdomyosarcoma, spindle cell/sclerosing variant. Cords of cells in hyalising stroma (HE stain, magnification 400 ×)

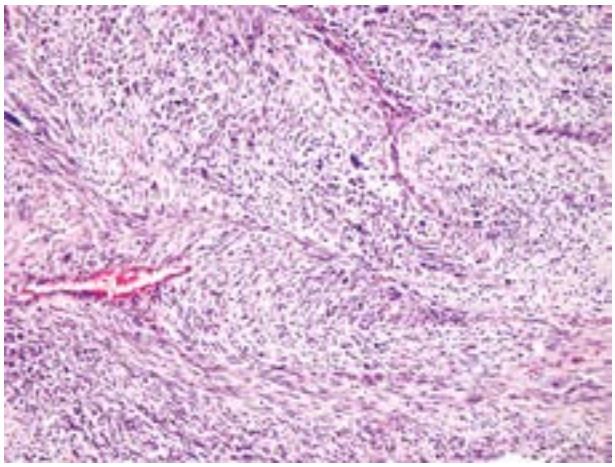

Fig. 17. Rhabdomyosarcoma, spindle cell/sclerosing variant. Spindle cells forming herringbone pattern (HE stain, magnification 400 ×)

Table I. The morphological differences between ERMS and spindle-cell RMS [20, 21]

Morphological characteristic	ERMS	Spindle-cell RMS
Cell shape	Oval, star-shaped, spindle-like	Regular, elongated, spindle-like
Cell patterns	Lobes with various cellular density, from loose, low-cell-concentration to high-cell-concentration	fascicles, cords, storiform patterns
Rhabdomyoblasts	Frequently present, but in varying amounts	Can be present, usually in small amounts
Mitotic figures	Frequent	Frequent
Submucosa	Often loose, myxoid	Various amount of collagenised submucosa organised in fascicles

Therefore, distinguishing between these two RMS groups is of crucial importance (Table I) [20, 21].

Immunohistochemical diagnostics

The diversity in RMS morphology often leads to significant difficulty in correct diagnosis. Therefore, utilisation of integrated diagnostic methods, including immunohistochemical and molecular methods, is necessary.

The use of immunohistochemical methods for RMS diagnosis in order to identify rhabdomyoblasts is a routine procedure. In cases of less differentiated tumours, the easiest method to detect rhabdomyoblastic differentiation of the sarcoma is the demonstration of expression of MyoD1 protein and myogenin (Myf4) (Figs. 18, 19). Positive nuclear staining in both markers is an important diagnostic criterion for RMS and is the gold standard in differential diagnosis with other neoplasms.

Furthermore, MyoD1 and myogenin have additional practical significance for distinguishing the ARMS from other RMS subtypes. Myogenin expression obtained in more than 50% of the neoplastic cells is highly suggestive of a diagnosis of ARMS [22, 23, 24]. Myogenin and MyoD1 are useful for distinguishing the classical ARMS and sclerosing RMS. Myogenin expression in sclerosing RMS is weak and focal, and

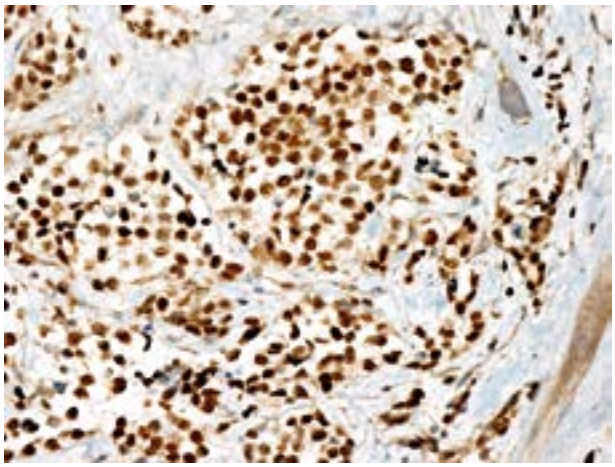

Fig. 18. Alveolar rhabdomyosarcoma. Positive nuclear staining for MyoD1 (magnification 400 ×)

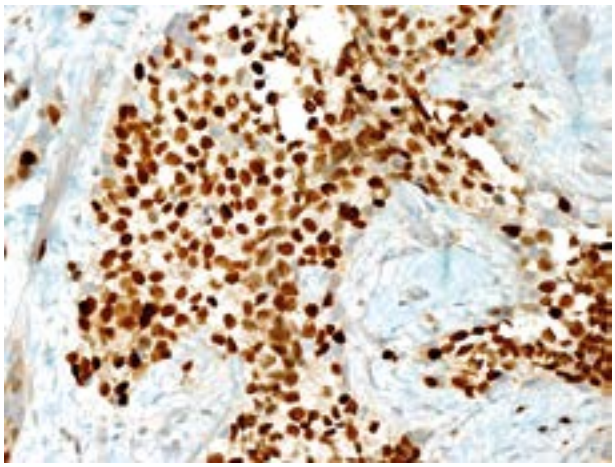

Fig. 19. Alveolar rhabdomyosarcoma. Positive nuclear staining for myogenin (Myf-4) (magnification 400 ×)

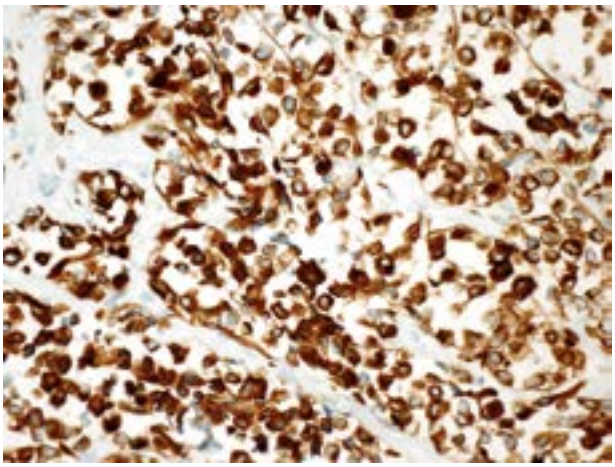

Fig. 20. Alveolar rhabdomyosarcoma. Positive cytoplasmatic staining for desmin (magnification 400 ×)

in the case of ARMS, strong and diffuse. In contrast, the MyoD1 expression is strong and diffuse in sclerosing RMS. ARMS, however, exhibits variable MyoD1 expression [15]. Cytoplasmic expression of vimentin and desmin can be observed in poor or undifferentiated cells (Fig. 20), and expression of muscle actin and myoglobin, in differentiated rhabdomyoblasts.

Recently the importance of novel immunohistochemical markers as prognostic factors for RMS has been reported. Among others, the role of p53, bcl-2, MDR-1, and MIB1 (Ki67) expression is highlighted. The DNA ploidy status in cerralion with the clinical course of RMS was also analysed – hyperdiploid ERMS tumours showing a more favourable prognosis [25, 26].

Expression of other proteins evaluated the use of immunohistochemical techniques such as AP2i and P-cadherin appeared to be a selective ARMS marker. On the other hand, the expression of epidermal growth factor receptor (EGFR) and fibrillin-2 are characteristic for ERMS. EGFR and fibrillin-2 expression are correlated with the favourable course of the disease, while the presence of AP2i and P-cadherin is associated with a poor prognosis [27].

Differential diagnosis

The differential diagnosis with other tumours showing small cells with round and spindled configurations is based not only on the morphology of the cells but, above all, also on additional tests, particularly immunophenotyping [21] (Tables II, III). Various neoplasms can exhibit differentiation towards skeletal muscle, which can additionally hamper obtaining a correct diagnosis [28, 29, 30]. The differential diagnosis in this group of tumours includes: malignant Triton tumour, spindle cell carcinomas, sarcomatoid carcinomas, melanoma, liposarcoma, malignant teratoma, teratocarcinosarcoma, salivary carcinosarcoma, anaplastic thyroid carcinoma, nephroblastoma, and some tumours of the central nervous system.

Molecular diagnostics

The traditional clinical and pathological parameters are sometimes not sufficient to adequately define the clinical course and prognosis. Additionally, determining the RMS subtype is not always possible based on the pathologic examination alone. Hence, much recent attention has been devoted to the molecular distinctions of rhabdomyosarcoma. The analyses of cytogenetic changes have shown their usefulness in distinguishing subtypes of RMS. The most common translocations that are selectively characteristic for ARMS are t(2;13)(q35;q14) and t(1;13)(p36;q14), which lead to the generation of fusion genes, *PAX3* (2q35) or *PAX7* (1p36), respectively, with the gene encoding fork-head-region transcription factor – foxo1 (previous name fkhr) (13q14) [31]. The translocations mentioned above are present, respectively, in 56% to 85%, and 6% to 10% of all ARMS-type tumours, and these are either not observed or are only occasionally present in ERMS [31, 32]. Preliminary data shows that ARMS tumours with a PAX7/FKHR translocation exhibit a less aggressive course of disease in comparison with tumours having PAX3/FKHR translocation. However, ERMS-type tumours show changes in a number of chromosome pairs – 2, 7, 8, 11, 12, 13, and 20 – in as much as 25% to 50% of cases. The loss of 9 and 10 chromosome pairs is described in 20% to 30% of cases. Genome amplification is another change observed relatively often in RMS – in as many as 16% to 56% of tumours. In case of ERMS, the amplification covers the 12q13-q15 region, and in the case of ARMS it involves the 1p36 (PAX7-FOXO1), 2p24 (MYCN),12q13-q14, 13q14 (PAX7-FOXO1), and 13q31 (MIR17HG, encoding miR-17-92 microRNA) regions. RMS-type tumours

Table II. Differentiation of small, round, blue cell tumours (table modified) [21]

Tumour	Typical morphological characteristics	Keratin	CD99	NB84	CD45	Desmin	Myogenin	Synaptophysin	Other markers and remarks
Alveolar RMS	Presence of rhabdomyoblasts	+/–	–	–	–	+++	+++	+/–	MyoD1(+)
Desmoplastic small round cell tumour	Small, monomorphic cells organised in nests, cords, spicules, lobes with prominent desmoplastic submucosa	+++	+/–	+/–	–	+++ (dot)	–	+/–	WT1(+) if the antibody recognises carboxy terminus; very rare in adults
Ewing sarcoma	Areas of small monomorphic cells; Homer Wright rosettes	+/–	+++	+/–	–	–	–	+/–	FLI-1(+); notable negative CD56; rarer in adults
Extrarenal rhabdoid tumour	Polygonal, various in size, rhomboidal cells with weak cohesion, organised in lobes or solid spicules; frequent necrosis	+++	+/–	–	–	–	–	+/–	Characteristic loss of nuclear INI1; very rare in adults
Lymphoma	Different morphological characteristics dependent on the type of lymphoma	–	+/–	–	+++	–	–	–	TdT(+) to help exclude CD45(-) lymphoblastic lymphomas
Melanoma	Cells various in size and pleomorphism with weak cohesion, sometimes with presence of brown pigment	–	–	–	–	–	–	–	S100(+), SOX10(+); variable melanocytic
Neuroblastoma	Presence of neural differentiation, e.g. so-called neuropile	–	–	+++	–	–	–	+++	Notable CD99 negativity
Poorly differentiated synovial sarcoma	The arrangements with high cell concentration of cells with high pleomorphism and numerous mitoses	+/–	+++	–	–	–	–	–	TLE1(+); also commonly expresses CD56
Small cell neuroendocrine carcinoma	Salt-and-pepper nuclei	+++	–	–	–	–	–	+++	TTF-1(+), very rare in children

(+++) – typically positive; (+/–) – variably positive or negative; (–) – typically negative

Table III. Differentiation of spindle-cell tumours (table modified) [21]

Tumour	Typical morphological characteristics	Keratin	S100 protein	SMA	Desmin	CD34	Other markers
Spindle-cell RMS	Presence of rhabdomyoblasts, cells organised in storiform or herring-bone pattern	–	–	+/–	+++	–	Myogenin(+), MyoD1(+)
Cellular angiofibroma	Cells with bipolar nodules, small and medium-size vessels present with characteristic hyalinisated wall	–	–	–	–	+++	Loss of nuclear Rb protein expression; typically in population of middle-aged and older adults
Dermatofibrosarcoma protuberans (DFSP)	High-cell-density histology with poorly defined borders	–	–	–	–	+++	CD34 expression decreased or absent in fibrosarcomatous DFSP; can be present in children, even as a congenital lesion
Fibromatosis		–	–	+/–	–	–	Nuclear β-catenin expression in majority of tumours
Inflammatory myofibroblastic tumour	Myofibroblasts and inflammatory infiltration with various degree of severity	+/–	–	+++	+/–	–	ALK(+) in > 50% of cases
Leiomyosarcoma	Cells organised in fascicles or cords, frequent necrosis	–	–	+++	+/–	–	Caldesmon(+); occasional focal keratin(+); rare in children
Low-grade myofibroblastic sarcoma	High-cell-density histology with cells organised in fascicles and storiform patterns; collagenised submucosa	–	–	+/–	+/–	–	Desmin(+) and absence of nuclear beta-catenin favors this diagnosis over fibromatosis; rare in children
Low-grade fibromyxoid sarcoma	Typically present zones of fibrous and mixoid histology	–	–	+/–	–	–	MUC4 most specific marker; focal EMA expression common; about 20% of cases in patients < 18 years old
Malignant peripheral nerve sheath tumour	Variable cellularity within the lesion, cells organised in fascicle, whorled, herring-bone patterns	–	+++	–	–	+/–	S100(+) usually focal or patchy; desmin expression seen in rhabdomyoblastic elements (malignant Triton tumour); can occur in various age
Mammary-type myofibroblastoma	Variable in size fascicles of fragile spindle cells	–	–	–	+++	+++	Loss of nuclear Rb protein expression; present in 4th-7th decade of life
Neurofibroma	Few different types of organisation: spread, localised, plexifrom	–	+++	–	–	+++	Contains mixture of S100(+) Schwann cells; can be present in children CD34(+) stromal cells and EMA/claudin-1(+) perineural cells
Nodular fasciitis	Loose fascicles and storifrom patterns, often with extravasated erythrocytes	–	–	+++	–	–	Rare focal desmin(+); can occur in children
PEComa	Nests, spicules, and lobes of epithelioid and spindle-shape cells	–	–	+++	+/–	–	Myomelanocytic immunophenotype with additional variable expression of HMB-45, melan-A, and/or MITF

Table III. Cont.

Tumour	Typical morphological characteristics	Keratin	S100 protein	SMA	Desmin	CD34	Other markers
Perineurioma	Slightly elongated cells in various patterns: storiform, whorled, lamellar, fascicular	–	–	–	–	+/–	EMA(+), claudin-1(+); rare in children
Schwannoma	Presence of two cellular patterns: Antoni A and Antoni B; Verocay bodies	–	+++	–	–	+/–	CD34 expression usually subcapsular; focal keratin(+) in retroperitoneal schwannomas; can occur in children
Solitary fibrous tumour	Classic pattern without characteristic various cellularity	–	–	–	–	+++	Nuclear STAT6(+) is highly specific; can occur in children
Spindle cell lipoma	Identical, fragile cells with the tendency to form "shoals", developed adipocytes present	–	–	–	–	+++	Loss of nuclear Rb protein expression; very rare < 20 years of life
Synovial sarcoma	Can occur as a biphasic (spindle cells and epithelial elements) or monophasic lesion (spindle cells)	+++	+/–	–	–	–	TLE1(+); also expresses CD56 and calretinin; most commonly between 10 and 40 years of age

(+++) – typically positive; (+/–) – variably positive or negative; (–) – typically negative

also show relatively common loss of heterozygosity of the region located on chromosome 11p15.5 [33, 34].

Analysis of gene mutations have shown their common occurrence in RMS, potentially indicating their contribution in neoplastic pathogenesis. As many as 28% of ERMS tumours are found to have point mutations in *KRAS, TP53, FGFR4, EGRF, PIK3CA, CTNNB1, CDKN2A, BRAF,* and *PTPN11* genes, indicating frequent occurrence in this tumour [33, 34]. However, in ARMS tumours these mutations occur only occasionally. Mutation of the *MyoD1 – myogenic differentiation 1* – gene was observed in the spindle-cell subtype localised in head and neck and limb regions. This mutation is present in the DNA-binding element of the *MyoD1* transcription factor, which leads to the generation of a protein product acting as a *MYC* oncogene. The mutation in *MyoD1* is assosiated with poor prognosis [33, 34]. Although chemotherapy remains the primary treatment for child patients, the identification of point mutations in the previously mentioned genes may in the future be a useful diagnostic element in the targeted therapy of RMS. These therapies offer a new approach to increase the efficacy of RMS treatment. The most important of these are those blocking the signalling pathways of the epidermal growth factor receptor (*EGFR, HER-1, ERBB1*), which is a member of the group of tyrosine kinase receptors, consisting of three additional receptors that are similar in structure:

EGFR2/HER2/HER-2-NEU/ERBB2, EGFR3/HER-3/ERBB3, and ERBB4/HER4. Phosphorylated tyrosine kinase stimulates intracellular signal transduction by a cascade of other pathways such as *RAS-RAF-MEK-MAPK-PI3K-AKT-JAK-STAT*, which regulate processes of proliferation, apoptosis, and angiogenesis. Others are those that participate in the mTOR pathway distorting the integration of signals from proteins such as *PI3K, AKT*, and *PTEN*. This pathway harmonises with other *IGF1-R-PI3K/AKT-mTOR* and *MAPK* pathways.

The summary of molecular changes in RMS and possible targeted therapies can be seen in Table IV.

Clinico-surgical-pathologic classification

All types of RMS should be accepted as high-grade sarcomas [35]. The exception is pleomorphic RMS in adults, for which the grading system was established by Fédération Nationale des Centres de Lutte Contre le Cancer/American Joint Committee on Cancer (FNCLCC/AJCC). This system is based on the analysis of the histological type, the mitotic activity, and the level of necrosis in the tumour tissue [36]. The Paediatric Oncology Group (POG) introduced its own system for assessment of the malignancy level based on the histological type, presence, and amount of necrosis, and mitotic activity. However, the POG

Table IV. Potential molecular targets present in RMS

Target	Therapeutic factors
EGFR – epidermal growth factor receptor	Erlotinib
	Gefitinib
RAS – small GTP-ases family	Afatinib
	Cetuximab
	Panitumumab
	Sorafenib
ALK – anaplastic lymphoma kinase	Crizotinib
BCR/ABL – fusion protein with tyrosin kinase activity (break point cluster region/Abelson murine leukaemia viral oncogene homologue)	Imatinib
	Dasatinib
MEK – mitogen-activated protein kinase	Sorafenib
	Selumetinib
IGF1R – insulin-like growth factor 1 receptor	Cixutumumab
PDGFR – platelet-derived growth factor receptor	Olaratumab
	Dasatinib
	Pazopanib
	Sorafenib
mTOR – mammalian target of rapamycin kinase	Sirolimus
	Everolimus
PI3KCA – gene encoding the catalytic subunit of PI3K kinase (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α)	Temsirolimus
	Ridaforolimus
RAF – Raf serine-threonine kinases	Sorafenib
VEGFR – vascular endothelial growth factor receptor	Sunitinib
	Apatinib
AKT – serine-threonine protein kinase B	Bevacizumab
	Pazopanib
EZH2 – polycomb-group protein (PCG)	Tazemetostat
BRAF – proto-oncogenic kinase (BRAF-v-raf murine sarcoma viral oncogene homologue B1)	Vemurafenib
PD-1 – programmed cell death-1 receptor	Pembrolizumab

system does not apply in the case of RMS, where the most important prognostic factor is the histological type [37]. The present pathologic description of RMS is based on the CWS 2002, 2006, and RMS guidance 2014 European Paediatric Soft Tissue Sarcoma Study Group (EpSSG) programs and includes the so-called polymorphous RMS in children and adolescents within the ERMS type. ERMS and its variants together with spindle-cell RMS are accepted as a favourable pathology, while ARMS with its solid variant is accepted as an unfavourable pathology. This classification is significant for the evaluation of the clinical stage.

The clinical stage of rhabdomyosarcomas

The most important aspect to determine the risk of tumour recurrence is clinical staging. For this purpose, the stage of disease defined before initiation of treatment (TNM Pretreatment Staging Classification), the surgical-pathologic group (IRS Clinical Group Classification), and histologic type of the neoplasm are taken into account. The neoplasm stage before treatment depends on tumour localisation, the extent of infiltration, lymph node involvement, and the presence of distant metastases. The surgical-pathologic group results from the level of the tumour resection completeness. Current prognostic classification of RMS in children (according to CWS-guidance *Version 1.6.1. from 24.05.2014)* combining all the classifications mentioned above is presented in Table V.

In summary, the current RMS classifications are based not only on the morphological but also on the immunohistochemical image. Further molecular classification should be considered in the future.

Table V. Current prognostic classification of RMS in children (according to CWS-guidance for risk-adapted treatment of soft tissue sarcoma and soft tissue tumours in children, adolescents, and young adults Version 1.6.1. from 24.05.2014)

Risk group	Subgroup	Pathology	IRS	Localisation	Involvement of the lymph nodes	Size of the tumour and age of the patient
Low-risk	A	Favourable	I	Any	N0	≤ 5.0 cm and ≤ 10 years of age
Standard	B	Favourable	I	Any	N0	> 5.0 cm or > 10 years of age
	C	Favourable	II, III	Favourable	N0	any
	D	Favourable	II, III	Unfavourable	N0	≤ 5.0 cm and ≤ 10 years of age
High-risk	E	Favourable	II, III	Unfavourable	N0	> 5.0 cm or > 10 years of age
	F	Favourable	II, III	Any	N1	Any
	G	Unfavourable	I, II, III	Any	N0	Any
Very high-risk	H	Unfavourable	II, III	Any	N1	Any

Histology
favourable = ERMS, spindle cell and botryoid variant; unfavourable = ARMS, solid variant
IRS – clinical stage according to Intergroup Rhabdomyosarcoma Study
Group I – primary complete resection (R0); group II – microscopically non-radical resection (R1) or primary radical resection but with N1 stage; group III – macroscopically non-radical resection (R2)

Localisation:
Favourable – orbital, urogenital, excluding urinary bladder and prostate (peritesticular or vagina/uterus)
Unfavourable – all others (periosteum, extremities, urogenital – urinary bladder and prostate)
Lymph node involvement according to the TNM classification
N0 – no clinical and pathologic involvement characteristics; N1 – clinical or pathologic involvement of the lymph nodes

The development of molecular diagnostics gives the opportunity not only to confirm the RMS diagnosis, but also to monitor residual disease during treatment and, more importantly, offers the possibility for the application of targeted therapy.

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