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Case report

Rhabdomyosarcoma with Bone Marrow Infiltration Mimicking Hematologic Neoplasia

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A B S T R A C T

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children younger than 15 years. According to the World Health Organization, there are embryonal, alveolar and pleomorphic types of RMS. Most RMS patients present with a tumor mass in the head and neck region, urogenital tract or lower extremities. Unusual clinical presentation of the disease with massive bone marrow infiltration at the disease onset and mimicking hematologic neoplasm is rarely seen. A case is presented of a 14-year-old, previously healthy girl hospitalized for outpatiently detected leukocyte elevation. For the last two weeks, she had complained of fatigue, myalgia and frequent bruising. On admission, clinical examination revealed numerous petechiae and hematomas, enlarged left inguinal lymph node and palpable spleen 2 cm below left costal arch. Laboratory findings showed leukocytosis, anemia and thrombocytopenia. Bone marrow fine needle aspiration (FNA) produced a hypercellular bone marrow sample with suppression of all three hemocytopoiesis lines and bone marrow infiltration with numerous undifferentiated tumor cells. Considering the morphological, cytochemical and phenotypic characteristics, the cytologic diagnosis was: bone marrow infiltration with RMS cells. Abdominal computerized tomography revealed a primary tumor occupying the entire retropeoritoneal space. Tumor biopsy confirmed alveolar subtype of RMS. In conclusion, in cases of bone marrow infiltration with primitive, immature cells, RMS should be considered as differential diagnostic possibility. Adjuvant technologies (cytochemistry, immunocytochemistry, cytogenetic analysis, flow cytometry, and molecular analysis) can be very helpful in diagnostic work-up, and may lead to definitive diagnosis in some cases.

Key words: *rhabdomyosarcoma, bone marrow infiltration*

Introduction

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children younger than 15 years[1,2]. RMS accounts for 4–5% of all tumors in pediatric patients[3] and is one of the most common soft tissue sarcomas in adolescents and adults[1]. RMS rarely occurs after age of 45[1]. According to World Health Organization classification, there are three RMS subtypes: embryonal, alveolar and pleomorphic RMS[2]. Embryonal RMS is the most common subtype[1,2,4], accounting for 50–60% of all RMS cases[1]. It most frequently develops in children under age 15[1,2], with as many as 46% of cases recorded in children younger than 5 years[2]. It is mostly localized in the head and neck region[1,2] (about 47% of cases)[2], followed by the genitourinary system and retroperitoneum, and extremities[1,2]. This tumor typically develops in the orbit, nasal cavity, middle ear, prostate and paratesticular region[4]. Unlike embryonal RMS, the second most common alveolar RMS occurs predominantly in adolescents and young adults, less frequently in pediatric patients[1,2,4]. Extremities are the most common seat of alveolar RMS[2,4] (about 39%), followed by the paraspinal and perineal region and paranasal sinuses[2]. Pleomorphic RMS is the rarest form[1,2] which almost exclusively occurs in adults[2], predominantly in the region of large muscles of extremities[1,2,4].

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Rhabdomyoblast is a diagnostic cell in all RMS subtypes. Rhabdomyoblast has eccentrically positioned eosinophilic granular cytoplasm. Rhabdomyoblast can be rounded or elongated, known as tadpole or strap cells, and can show striated pattern under light microscope[4]. RMS phenotype depends on the degree of rhabdomyoblast differentiation. On immunocytochemistry, the most primitive tumor cells are only positive for vimentin, however, further differentiation renders them positive for desmin and muscle-specific actin, whereas the cells with the highest degree of differentiation are positive for myoglobin and myosin[2,5]. Antibodies to MyoD1[1,2,5] and myogenin show high specificity and sensitivity for RMS[2]. Embryonal and alveolar RMS shows various gene alterations. A characteristic t(2,13)(q35;q14) translocation is detected in most cases of alveolar RMS[2–4], while t(1;13)(p36;q14) translocation is less common[2,4]. In embryonal RMS, the loss of heterozygosity (LOS) is found on 11p15 locus, with the loss of maternal and duplication of paternal genetic material[2,3].

Clinically, most tumors present as a solid tumor mass in the head and neck region, genitourinary tract or extremities[6]. In 20% of cases, metastases are present at the time of diagnosis. The most common sites of metastases are the lungs (involved in 2/3 of cases with metastases), lymph nodes and bone marrow[1]. RMS is one of the rare sarcomas that metastasize to local lymph nodes.

Case Report

A 14-year-old female patient was admitted to the hospital for suspected hemoblastosis. There were no serious diseases in her medical history. The patient presented with severe myalgia that had persisted for a week, poor appetite, fatigue and frequent bruising. Upon admission, the patient's clinical status revealed numerous petechiae and hematomas on the skin, left inguinal lymph node enlargement of 5 cm in diameter, palpable liver margin and palpable spleen 2 cm below the left costal arch. Laboratory findings: leukocytosis (white blood cell count of 28.5×10^9/L), anemia (red blood cell count of 3.02×10^{12}/L; hemoglobin of 85 g/L), thrombocytopenia (platelet count of 15×10^9/L), elevated lactate dehydrogenase (6620 U/L) and elevated C-reactive protein (27.1 mg/L). Bone marrow FNA produced a hypercellular bone marrow sample with suppression of all three hematopoetic cell lines and bone marrow infiltration with primitive, immature, medium-sized cells. The cells were mostly single, partly in small clusters and 'rosettes', with reticular chromatin structure, and moderate to abundant, frequently finely

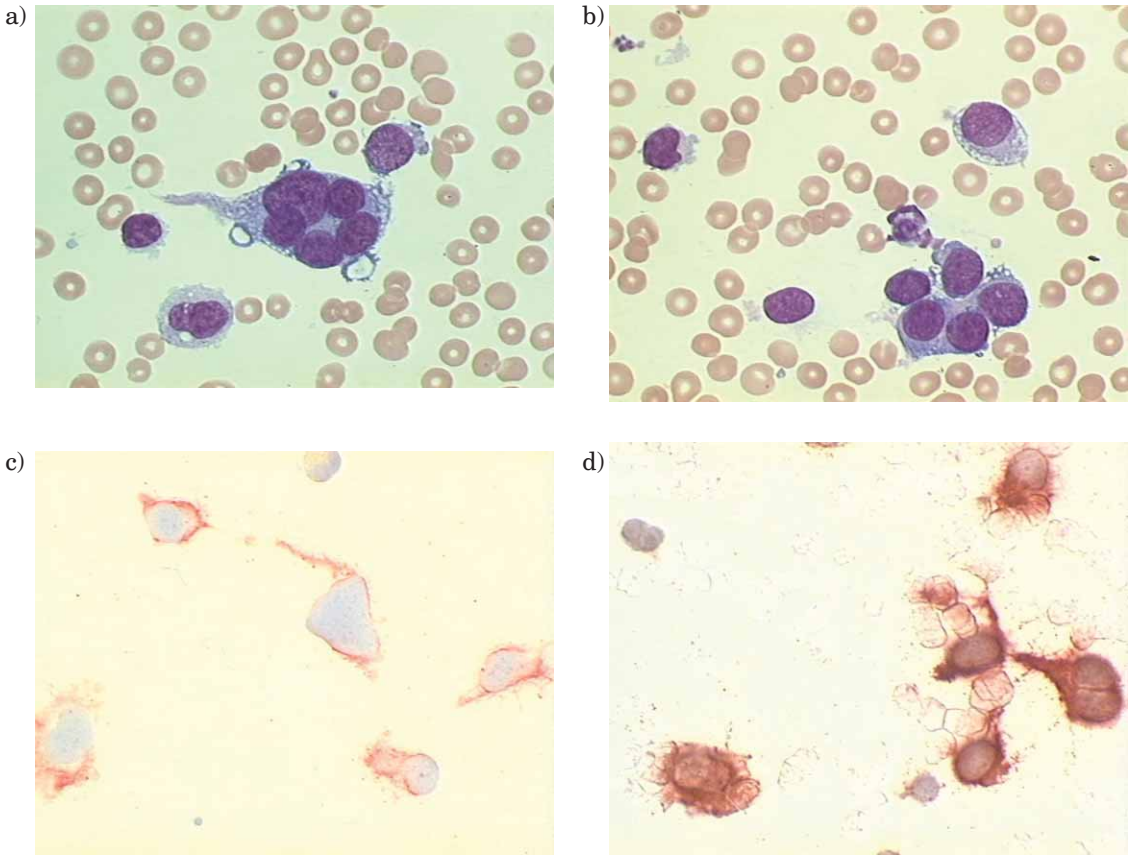

Fig. 1. a and b) tumor cells in bone marrow aspirate (May-Grünwald-Giemsa, x1000), c) vimentin pozitive tumor cells in bone marrow aspirate (immunocytochemistry, LSAB x1000), d) desmin pozitive tumor cells in bone marrow aspirate (immunocytochemistry, LSAB x1000).

vacuolated and in part elongated cytoplasm. Immunocytochemistry and cytochemistry staining revealed the cells to be negative for LCA, CD20, CD19, CD3, CD10, NSE, Ber EP 4, oil red, peroxidase and alpha-naphthyl acetate esterase, and strongly positive for PAS, vimentin and in part for desmin and CD68 (Figure 1). The cytologist believed it was a case of bone marrow infiltration with malignant small cell tumor that corresponded best to RMS according to its morphology, cytochemistry and immunocytochemistry characteristics. Tumor cells of the same type were also found in peripheral blood smears (3%) and enlarged inguinal lymph node FNA sample (Figure 2). Cytogenetic analysis of the bone marrow aspirate showed the following karyotype: 46~47,XX,der(2), del(2p), add(2q),–13,+r(1–2)[15]/[15]. Bone marrow immunophenotyping by flow cytometry showed the blast/monocyte sized cells not to express the pan-leukocyte marker CD45 or any of the lymphoid or myeloid line markers, with the exception of the adhesion molecules CD56 and CD138. PCR analysis of bone marrow for IgH and TCR gamma gene rearrangement failed to demonstrate clonal rearrangement.

Primary tumor was found by abdominal CT, which showed a large tumor mass that occupied the entire retroperitoneal space, descending to the pelvis. Enlarged spleen and enlarged retroperitoneal and inguinal lymph nodes were also observed. Thoracic CT revealed enlarged bronchopulmonary lymph nodes. Retroperitoneal tumor biopsy confirmed it to be RMS of alveolar subtype. Histologically, tumor tissue was composed of solid rounded cell clusters with occasional fibrovascular septa between them, in part showing the pattern of alveolar formations. Immunohistochemically, tumor cells were negative for LCA, CD10, CD20, CD43, TDT, MIC2, NSE, NF and cytokeratin, and positive for vimentin, actin and desmin.

Discussion

The case presented showed an unusual and very rare clinical picture of RMS, mimicking clinical picture of acute leukemia with typical clinical symptoms and signs such as pancytopenia and presence of primitive immature cells in the bone marrow and peripheral blood. According to the Intergroup Rhabdomyosarcoma Study report, bone marrow infiltration found at the time of diagnosis was recorded in 29% of RMS patients in clinical stage IV (disease dissemination with metastases)[8]. Alveolar RMS was diagnosed in as many as half of the patients[8], which is consistent with the hypothesis on alveolar RMS to have a higher tendency to disseminate than embryonal RMS[8,9]. Although RMS can metastasize to bone marrow, it very rarely presents with the symptoms and signs associated with bone marrow infiltration by foreign cells as the initial and sole disease manifestation,

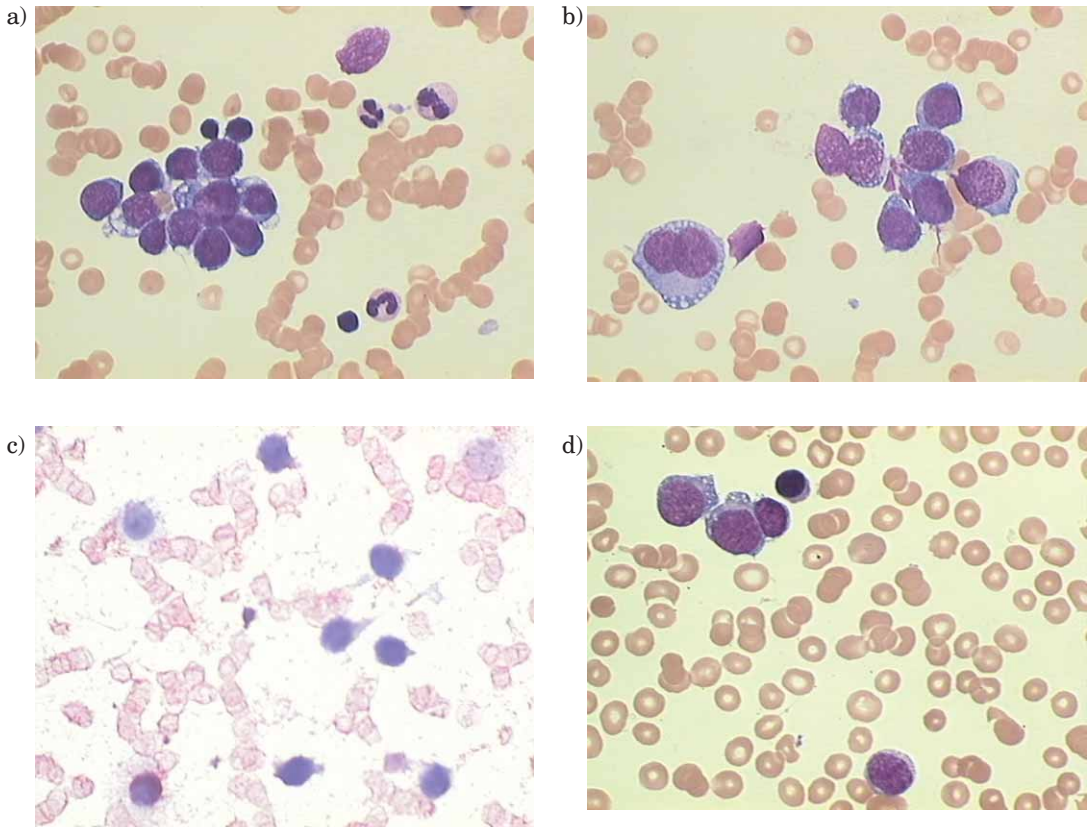

Fig. 2. a and b) tumor cells in lymph node aspirate (May-Grünwald-Giemsa, x1000), c) LCA negative tumor cells in lymph node aspirate (immunocytochemistry, LSAB x1000), d) tumor cells in peripheral blood smear (May-Grünwald-Giemsa, x1000).

thus imitating the clinical picture of hemoblastosis[7,8,10,11]. Therefore, cytomorphologist should consider RMS on differential diagnosis in patients with primitive immature cells found in the bone marrow and peripheral blood. The poorly differentiated forms of RMS composed of small, uniform, poorly differentiated cells are difficult to distinguish morphologically from hemoblastosis and other small blue cell tumors of childhood such as neuroblastoma, Ewing sarcoma, peripheral neuroectodermal tumor, non-Hodgkin's lymphoma, Wilms' tumor, small cell hepatoblastoma, pancreaticoblastoma, small cell desmoblastic tumor, and seminoma[1,12,13]. Additional cytochemistry and immunocytochemistry staining is crucial to reach an accurate diagnosis[6,7,10]. In the case presented, cytochemistry staining of bone marrow FNA smears revealed the tumor cells to be negative for myeloid markers (peroxidase, alpha-naphthyl acetate esterase) and positive for PAS, which is seen in leukemia and lymphoma, but also in RMS[6]. The RMS phenotype depends on the degree of differentiation. On immunocytochemistry, the most primitive tumor cells are only positive to vimentin, to be rendered positive for desmin and muscle-specific actin by further differentiation, whereas the best differentiated cells are also positive for myoglobin and myosin[2,5]. In our patient, tumor cells in the bone marrow FNA sample were positive for vimentin and desmin on immunocytochemistry, while immunohistochemistry staining of the retroperitoneal tumor biopsy sample revealed positivity for vimentin, muscle-specific actin and desmin. Although cytogenetic studies have demonstrated the t(2,13)(q35;q14) translocation and less commonly the t(1,13)(p36;q14) translocation to be characteristic of alveolar RMS[2–4], others have reported the presence of additional chromosomal material, also involving chromosome 2; add(2)(q37), in RMS cases[6,14,15]. In our patient, the characteristic t(2,13)(q35;q14) translocation was not found either, but the mentioned add(2q) aberration, already recorded in RMS cases[6,14,15], was present, along with other chromosomal aberrations involving chromosomes 2 and 13: 46~47,XX,der(2),del(2p),add(2q), –13,+r(1–2)[15]/[15].

In case of bone marrow infiltration with primitive immature cells, RMS should be taken in consideration on differential diagnosis, whereby adjuvant technologies such as cytochemistry, immunocytochemistry, cytogenetic analysis, flow cytometry[16] and molecular analysis can prove very useful and in some cases may lead to definitive diagnosis.

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INFILTRACIJA KOŠTANE SRŽI STANICAMA RABDOMIOSARKOMA

SAŽETAK

Rabdomiosarkom (RMS) je najčešći sarkom mekog tkiva u djece mlađe od 15 godina. Prema SZO razlikujemo: embrionalni, alveolarni te pleomorfni oblik RMS-a. Većina pacijenata s RMS-om prezentira se tumorskom masom u području glave i vrata, genitourinarnog trakta ili donjih ekstremiteta. Rijetko se vidi neobična klinička prezentacija bolesti s opsežnom infiltracijom koštane srži na početku bolesti i imitiranjem hematološke neoplazme. Prikazujemo slučaj četrnaestogodišnje, prethodno zdrave djevojčice, koja je hospitalizirana na klinici zbog ambulantno nađenih povišenih vrijednosti leukocita. Unazad dva tjedna žalila se na umor, bolove u mišićima, te učestalu pojavu modrica na koži. Kod prijema u kliničkom statusu nađe se dosta petehija te hematoma na koži, povećan limfni čvor ingvinalno lijevo, te

palpabilna slezena 2 cm ispod lijevog rebrenog luka. U laboratorijskim nalazima prisutna leukocitoza, anemija i trombocitopenija. Punkcijom koštane srži dobije se hipercelularan uzorak koštane srži uz potisnute sve tri loze hemocitopoeze i infiltraciju koštane srži brojnim nezrelim tumorskim stanicama koje su citokemijski i imunocitokemijski pozitivne na PAS, vimentin i dezmin, a negativne na LCA, CD20, CD19, CD3, CD10, NSE, Ber EP 4, OIL RED i ANAE. Uzevši u obzir morfološke, citokemijske te fenotipske karakteristike mišljenje citologa je govorilo da se radi o infiltraciji koštane srži stanicama RMS-a. CT-om abdomena nađen je primarni tumor koji ispunjava čitav retroperitonealni prostor. Učinjena biopsija tumora je potvrdila da se radi o rabdomiosarkomu alveolarnog suptipa. Kod infiltracije koštane srži primitivnim, nezrelim stanicama diferencijalno dijagnostički treba razmišljati i o RMS-u pri čemu dodatne tehnologije (citokemija, imunocitokemija, citogenetska analiza, protočna citometrija, molekularna analiza) ne samo da mogu biti od pomoći već u nekim slučajevima dovode do definitvne dijagnoze.