

Acute lymphoblastic leukemia arising after treatment of Ewing sarcoma was misdiagnosed as bone marrow metastasis of Ewing sarcoma

A case report

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Abstract

Rationale: Both acute lymphoblastic leukemia (ALL) and Ewing sarcoma (ES) are small round cell tumors, and it is difficult to differential diagnose them because of overlapping clinical, radiographic, histologic, and immunophenotypic features.

Patient's concerns: A 5-year-old boy was admitted to our hospital because of pains in his left leg without obvious inducement and lameness worsening with walking over a two 2-month period.

Diagnoses: Based on the comprehensive analysis of radiography, magnetic resonance imaging (MRI), pathology biopsy and immunohistochemistry, the lesion was confirmed to be ES.

Interventions: The patient received neoadjuvant chemotherapy with 2 cycles of VAC (vincristine 1 mg/m², adriamycin 50 mg/m², cyclophosphamide 800 mg/m²) and 2 cycles of IE (ifosfamide 1.2 g/m², etoposide 70 mg/m², mesna 1.2 g/m²) regimens.

Outcomes: After 16 months, the results of routine blood tests showed reduced hemoglobin levels and decreased platelet counts. In addition, blast-like cells were found in a peripheral blood smear. All of the results suggested that the patient should undergo bone marrow aspiration and biopsy, which showed blast-like cells similar to that observed in cases of ES. Thus, a diagnosis of bone marrow metastasis of ES was established. However, when combined with immunohistochemistry data and medical history, the patient was eventually diagnosed as ALL arising after treatment of ES.

Lessons: When there was an abnormality in peripheral blood, it was easily misdiagnosed as bone marrow metastasis of ES after ES patient received neoadjuvant chemotherapy. We should jointly analyze bone marrow aspiration smear, bone marrow biopsy, immunohistochemistry, analysis of the medical history, even cytogenetic and molecular analysis for differential diagnosis.

Abbreviations: ALL = acute lymphoblastic leukemia, ES = Ewing sarcoma, IE = (ifosfamide, etoposide), MRI = magnetic resonance imaging, RT-PCR = reverse transcription polymerase chain reaction, VAC = (vincristine, adriamycin, cyclophosphamide).

Keywords: acute lymphoblastic leukemia, bone marrow metastasis, Ewing sarcoma, immunohistochemistry, secondary malignant tumor

1. Introduction

Some rare malignant tumors present clinical characteristics and bone marrow aspirate or biopsy morphology similar to that of acute leukemia. Ewing sarcoma (ES),^[1,2] medulloblastoma,^[3] neuroblastoma,^[4] anaplastic oligodendroglioma,^[5] rhabdomyosarcoma,^[6] and neuroendocrine tumors have been reported to exhibit acute leukemia-like morphology in bone marrow aspirate

samples after metastasizing to the bone marrow. Among these tumors, ES is the second most common primary malignant tumor of the bone, which mainly affects young patients of 5 to 20 years of age.^[7] ES is a highly aggressive small round blue cell tumor that generally has extremely poor prognosis; the most common primary tumor sites are the pelvis, femur, and rib, yet 25% of tumors occur in soft tissue rather than bone.^[8] It is characterized by the proliferation of small round cells, and the most common symptoms of ES are tumor mass formation, induration, pain, swelling, venous dilation, and hyperemia. Radiographic signs of ES are permeative and infiltrative destruction of the affected bone, accompanied by periosteal reaction, such as onion skin-like appearance and Codman triangle, or calcified spicule.^[9]

Clinically, approximately 25% of patients show evidence of metastasis at diagnosis, whereby the ES has usually migrated and disseminated to other organs predominantly via the blood, and where the most common sites of metastases are lungs, bones, and bone marrow.^[8,10] However, advances in management techniques have resulted in significantly improved rates of survival in patients with ES. Meanwhile, second malignant tumor arising after bone treated at chemotherapy in patients with ES has been paid more attention.^[11] Herein, we report an unusual case of a child diagnosed with acute lymphoblastic leukemia (ALL) arising after treatment of ES. In this case, abnormal blast-like cells were observed in a bone marrow sample and peripheral blood by ordinary optical

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Figure 1. MRI showing bone marrow cavity and soft tissue lesions on both sides of the middle and lower part of the tibia and the lower end of the femur, in which the middle and lower front edge of the left tibia demonstrates periosteal reaction.

microscopy. Because the morphology of blast-like tumor cells is similar to the small round tumor cells with little cytoplasm, it is easily misdiagnosed as bone marrow metastasis of ES.

2. Case report

A 5-year-old boy was admitted to our hospital in December 2014 because of pains in his left leg without obvious inducement and lameness worsening with walking over a 2-month period. General examination was undertaken and magnetic resonance imaging (MRI) scan revealed chronic suppurative osteomyelitis in both legs (Fig. 1). Subsequently, the patient underwent chronic osteomyelitis debridement and catheter drainage of his left tibia in January 2015. Pathological examination of the debridement sample indicated left tibia ash black tissue at a size of $1.0 \times 0.8 \times 0.2$ cm, and microscopic examination showed uniform small round cells with scanty cytoplasm (Fig. 2). Furthermore, Ki-67 expression was approximately 80%. Immunohistochemical examination revealed that tumor cells were positive for Fli-1, CD99 and CD34, and were negative for CD1a, CD3, CD10, CD15, CD19, CD20, CD56, CD57, CD79a, CD117, D2, MPO,

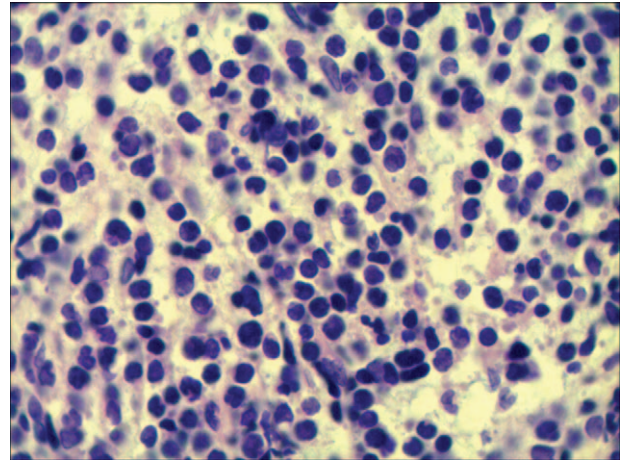


Figure 2. Histological findings from debridement sample showing the classic histological features of uniform small round cells with scanty cytoplasm (hematoxylin and eosin, $\times 400$).

CgA, and Syn, which allowed us to determine that his condition was primarily ES. Subsequently, this patient underwent neoadjuvant chemotherapy with 2 cycles of VAC (vincristine 1 mg/m^2 , adriamycin 50 mg/m^2 , cyclophosphamide 800 mg/m^2) and 2 cycles of IE (ifosfamide 1.2 g/m^2 , etoposide 70 mg/m^2 , mesna 1.2 g/m^2) regimens up to April 2015. After chemotherapy, the pain in the left leg disappeared and the patient was discharged from the hospital without further treatment.

When the patient came back to the hospital 1 year later for re-examination, he seemed to be in good spirits, eating regularly, and without significant changes in body weight other than normal growth for a child. Complete blood count results were as follows: white blood cell counts of $4.11 \times 10^9/\text{L}$, red blood cell counts of $4.18 \times 10^{12}/\text{L}$, hemoglobin decreased to 92.3 g/L , and platelet counts decreased to $78.10 \times 10^9/\text{L}$ with 24% blast-like cells found in a peripheral blood smear (Fig. 3A). This suggested that the patient should undergo bone marrow aspiration and biopsy. The bone marrow aspirate results showed 97.5% blast-like cells, which were small in size with 1 to 3 nucleoli in the nucleus. Minimal cytoplasm, vacuolation, irregular microspikes, or pseudopodia at the edge of the cytoplasm were observed, similar to that observed in cases of ES (Fig. 3B). These cells were negative for peroxidase. Periodic acid-Schiff staining showed strongly positive granular (Fig. 3C). The bone marrow biopsy (easily aspirated) revealed nucleated cells with marked proliferation and activity, and 90%

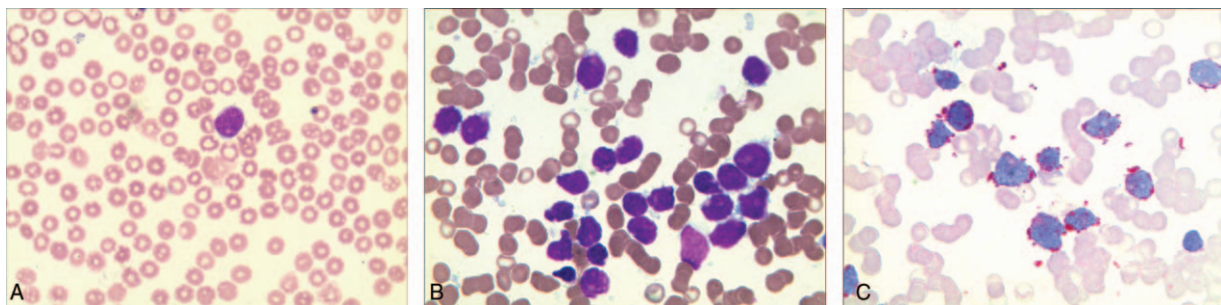


Figure 3. (A) Peripheral blood smear showing suspected tumor cells (Wright-Giemsa staining, $\times 400$). (B) Bone marrow aspirate smear showing diffuse infiltration with a large number of blast-like cells (Wright-Giemsa staining, $\times 400$). (C) Periodic acid-Schiff staining detected coarse granules, which were considered to be glycogen molecules ($\times 400$).

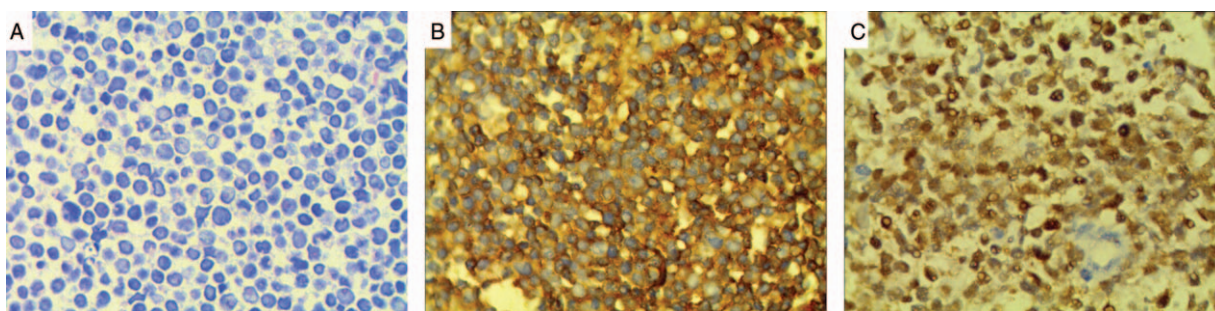


Figure 4. (A) Bone marrow biopsy smear showing diffuse infiltration with a large number of blast-like cells (hematoxylin and eosin, $\times 400$). (B) The membranes of the blast-like cells showing strong staining for CD99 ($\times 400$). (C) The nuclei of the blast-like cells were positive staining for TdT ($\times 400$).

blast-like cells (Fig. 4A). Ki-67 expression was approximately 50%. Immunohistochemistry of the bone marrow biopsy revealed that the blast-like cells were positive for CD10, CD34, and CD99 (Fig. 4B), and were negative for CD2, CD3, CD20, CD38, CD117, and CD138. In our case, the initial diagnosis was bone marrow metastasis of ES; however, further immunohistochemistry revealed positive staining for TdT (Fig. 4C) and PAX5 and negative for NSE. Therefore, we could exclude the possibility of bone marrow metastasis of ES, and a diagnosis of ALL arising after treatment of ES was established. The patient's mother signed informed consent and this study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

3. Discussion

Advances in treatment have resulted in more and more long-time survivor of ES during the decades. Recently, large cohort studies of treated patients with ES have shown an increased risk of second malignancies.^[11,12] Paulussen et al^[12] reported that 6 of 690 patients with ES have developed second cancer, including solid tumours and haematopoietic neoplasms. Bacci et al^[11] also expressed that 14 of 597 patients with ES developed second malignancies. These second malignancies comprised 8 radiation-induced osteosarcomas, 3 acute leukemias, and 3 carcinomas.^[11] It has been demonstrated that secondary haematopoietic neoplasms were closely related to chemotherapy, especially the use of alkylating agents, topoisomerase II inhibitors.^[13] In our case, the patient underwent neoadjuvant chemotherapy with 2 cycles of VAC and 2 cycles of IE regimens after the initial diagnosis of ES. Sixteen months later, when bone marrow is invaded, not only bone marrow metastasis of ES, but second malignancy arising after treatment of ES should be also considered.

Both ALL and ES are small round cell tumors; it is difficult to differential diagnose them because of overlapping clinical, radiographic, histologic, and immunophenotypic features. Currently, flow cytometry is used to detect tumor cells for bone marrow involvement of ES patients' blood and bone marrow because of the CD99⁺ CD45⁻ profile. Other tumors, such as ALL and lymphoma, also express high levels of surface CD99,^[14,15] which express CD45 at the same time, thereby contributing to differentiate ES from other malignancies.^[16] Reverse transcription polymerase chain reaction (RT-PCR) has also been used to detect tumor cells for bone marrow involvement of ES patients' blood and bone marrow. This often reveals specific chromosomal translocations including t(11;22)(q24;q12) translocation resulting in the *EWS-FLI1* fusion gene, which is not expressed in normal cells.^[17,18] While these methods have contributed to expedited diagnoses, they may actually delay diagnosis because of inconclu-

sive results, even in experienced medical centers. Because of this, an open biopsy is still considered the gold standard for diagnosis of sarcoma in many specialized sarcoma centers.

In the present case, the morphology of abnormal blast-like cells of ALL in peripheral blood and bone marrow is similar to that observed in cases of bone marrow metastasis of ES, as previous literature by Worcester and Vasef.^[19] ALL arising after the treatment of ES is easily misdiagnosed as bone marrow metastasis of ES, thus it is critical to differentiate ALL from ES. Furthermore, both ALL and ES tumor cells contain abundant glycogen levels, and periodic acid-Schiff-positive granules are observed in their cytoplasm. Immunohistochemistry is a reliable method to differentiate the 2 diseases, but incomplete immunohistochemical markers can induce pathological doctors to misdiagnose. Preliminary immunohistochemistry of the bone marrow biopsy revealed that the blast-like cells were positive for CD10, CD34, and CD99, and were negative for CD2, CD3, CD20, CD38, CD117, and CD138. Lucas et al^[20] have reported that all lymphomas and leukemias were positive for TdT compared with none of the ES; and none of the ES expressed other lymphocytic markers, such as PAX5. In our case, the initial diagnosis was bone marrow metastasis of ES, whereas additional immunohistochemistry revealed positive staining for PAX5 and TdT and negative for NSE. Consequently, the definite diagnosis of the disease is ALL arising after treatment of ES instead of bone marrow metastasis of ES.

In summary, our case presented with ALL arising after treatment of ES. Accordingly, in clinical practice, we should pay attentions to discovering abnormal cells in peripheral blood smears when there is an abnormality in peripheral blood. If blast-like cells are observed in bone marrow samples, we cannot immediately diagnose bone marrow metastasis of ES. The final diagnosis should depend on bone marrow aspiration smear, bone marrow biopsy, immunohistochemistry, and analysis of the medical history. When necessary, it is needed to do cytogenetic and molecular analysis for differential diagnosis.

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