

Massive Presentation of Circulating Tumor Cells in Localized Ewing Sarcoma with no Sign of Metastatic Spreading: A Case Report

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Abstract

Ewing's sarcoma (ES) is an uncommon malignancy of childhood and adults that constitutes 6%-8% of all primary malignant tumors and the third-most common tumor after osteosarcoma and chondrosarcoma. This article presents a case of localized ES iliac wing in a 12-year-old male patient, treated in accord to the ISG-EW1 protocol, showing extensive percentage of circulating tumor cells if compared with other non-metastatic patients, which is not a frequent presentation. Histopathological needle biopsy from the soft tissue extension of the tumor revealed small round-cell tumor with strong CD99 positivity, supporting the diagnosis of Ewing's sarcoma. The diagnosis was confirmed by reciprocal translocation of chromosomes 11 and 22. Analysis of tumor circulating cells revealed a massive percentage of tumor cell in peripheral blood, comparable to the levels that usually were found in metastatic patient.

Keywords: CD99; Ewing's sarcoma; Circulating tumor cells; Soft tissue; Metastatic spreading

Introduction

Ewing's sarcoma (ES) is a rare malignant round cell tumor occurring as a primary neoplasm of bone sarcoma which was named after James Ewing first described it in the year 1921 [1]. It belongs to the ES family of tumors (ESFT) which is an aggressive form of childhood malignancy. The other malignancies in ESFT include peripheral primitive neuroectodermal tumor (PNET), neuroepithelioma and Askin's tumor (neoplasm involving thoracopulmonary region). ES is considered as the second-most common tumor in children and adolescents [2]. Although it may involve any bone, diaphysis of long bones and pelvic girdle are involved most commonly [3]. Until now combined chemotherapy regimen, radiotherapy and surgery, remain the only strategy to overcome the disease. At diagnosis, approximately 25-30% of patients with ES have metastatic disease [4]. It presents high incidence of local or distant relapse, up to 40% in metastatic setting. Unfortunately, approximately 20-30% of patients with metastatic or recurrent disease have a poor prognosis even undergoing intensive multi-drug chemoterapic regimen with long-term [5]. For patients with localized disease, intensive systemic chemotherapy regimen, combined with surgery and/or radiotherapy, present a survival rates of approximately 70%. The 10year survival rate for patients with metastatic disease increased from 16 to 30% after the introduction of multi chemotherapy. New methods for an earlier detection of recurrence and metastasis could change the clinical approach to Ewing Sarcoma surveillance and treatment.

Case Presentation

A 12-year-old male reported with an important, not responsive to paracetamolo pain and very large swelling in the region left iliac wing for 9-12 months. The swelling was initially small in size measuring around 3 cm, which then gradually increased to the present size. Pain started 12 months back, was dull and intermittent in nature, aggravated while sleeping on left side and relieved spontaneously.

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Blood tests showed that the concentrations of lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were very high, but he was negative for C-reactive protein (CRP). X-rays. Magnetic resonance imaging (MRI) and PET-TC scan Figure 1A-E showed high accumulation in early phase and no wash out appearance in delayed phase, with no metastatic lesions (Figure 1C and D). Histological examination of a needle biopsy sample showed diffuse proliferation of cytoplasmically poor heterotypic cells (Figure 2A) positive for EWS-FLI1 translocation (Figure 2B), resulting in a diagnosis of Ewing's sarcoma. Bone marrow aspirated was morphologically negative (Figure 2C) and confirmed by molecular analysis. Interestingly, peripheral blood examination revealed a massive fraction of CD99 positive cells in CD45 negative population (51% of CD99+ on CD45- cells) if compared to other patients with localized disease (Figure 3). The percentage of CTCs was even higher if considered the amount of CTCs usually evidenced in patients with metastatic disease and bone marrow infiltration.

The patient was treated according to ISG/EW1 protocol and was administered 4 cycles of neoadjuvant chemotherapy, consisting of vincristine (2 mg/m^2) for 1 day, doxorubicin (37.5 mg/m^2) for 2 days, and cyclophosphamide (1.2 g/m^2) (VDC) for 1 day, alternating with ifosfamide (1.8 g/m^2) and etoposide (100 mg/m^2) (IE) for 5 days. Because MRI after preoperative chemotherapy showed a significant reduction in the size of the extra-skeletal mass, but not disappearance (Figure 4), surgery was not performed and the patient was considered

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Figure 1: Magnetic resonance imaging (MRI) and PET-TC scan. (A-B) T2 axial and STIR coronal MRI images demonstrate an aggressive heterogeneous hyperintense lesion centered at the left iliac bone with associated great soft tissue mass; (C-D) Axial and coronal FDG PET/CT show a heterogeneous destructive mass at the left iliac bone with central hypermetabolism; (E) CT scan, performed for biopsy, confirms the presence of the left iliac lesion with cortical breakthrough and associated soft tissue mass.



Figure 2: Analysis of a needle biopsy. (A) Histological examination shows diffuse proliferation of cytoplasmically poor heterotypic cells; (B) FISH analysis shows positive signals for EWS-FL11 translocation; (C) Bone marrow aspirated analysis shows absence of tumor cells.

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Figure 3: Cytoflowrimetric analysis of peripheral blood cells. Representative FACS plots show: (A) Gate selection of CD45⁻ cells; (B) Percetage of CD45⁻ CD99⁺ cells subpopulation; (C-D) Percentage of \Box 3 positive cells in control sample without Anti- \Box 3 staining and with Anti- \Box 3-PE staining.



Figure 4: Magnetic resonance imaging (MRI) after preoperative chemotherapy. Axial and coronal MRI images show significant reduction in the size of the mass, but not disappearance.

a poor responder and treated by adjuvant chemotherapy associated to local radiotherapy 45 Gy and high-dose chemotherapy and autologous stem cell transplantation. The surgery will be evaluated at the end of treatment, also based on the clearance of the circulating tumor cells and necrosis area.

Detection of CD45⁻ CD99⁺ tumor cells was performed with flow cytometry. Briefly, peripheral blood red cells were lysed and mononuclear cells washed with PBS, then stained with commercially available CD45 microbeads for CD45⁺ cells depletion following the manufacturer's protocol (Miltenyi Biotec). The CD45- cells obtained were stained with commercially available monoclonal antibodies, CD45-APC and CD99-FITC, for 15 minutes at 4°C. Cells were then washed and collected on a MACSQuant Analyser (Miltenyi Biotec).

Discussion

Ewing's sarcoma is an essentially aggressive bone and soft tissue tumor defined high aggressive systemic disease for the elevate incidence of relapse and metastasis ranging from isolated pulmonary metastasis to widely disseminated multi organ disease with varied outcomes.

Patients usually have heterogeneous disease with different outcomes that varied between 40%-<10%. Current methods to detect recurrence or metastasis depend largely on clinical exam, radiographic imaging and positron emission tomography (PET) to identify areas of tumor growth or increased metabolic activity and give evidences of disease when the relapse is already present or advanced. CTCs can provide valuable information about tumor composition, invasiveness, drug susceptibility and therapeutic strategy by modulation of different drug administration [6]. Accumulating evidences suggested that the presence of CTCs can predict cancer spread and tumor relapse. This is the reason why the analysis of CTC is considered a minimally invasive method for marker detection [7]. CTCs have been identified in PB of patients affected by the most disparate types of tumors and have been found to have significant clinical utility in many tumor type but most common in epithelial malignancies, such as carcinoma of the colon, breast and prostate. In these tumors CTCs' presence and number were correlated with disease stage and recurrence. The utility of CTCs analysis in pediatric cancer is controversial. In particular, in ES patients CTCs in peripheral blood have been often found but their presence has not been associated with metastasis and outcome, since until now there are not evidences that tumor cells detected in patients PB own the ability to give rise to metastasis [8]. At the present time, the true biological meaning or clinical relevance of detecting CTCs in the blood of ES patients is unknown. On the other hand, it was observed that detectable CTCs

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in bone marrow samples have been identified most often in patients with known metastatic disease. For example, CTCs were detected in 46 % of patients with metastatic disease and in only 19% of patients without metastatic disease [9] and are associated with poor prognosis. In peripheral blood, CTCs were detected in 22 and 20% of patients with or without known metastatic disease. The majority of the studies were performed on ES by reverse transcription-polymerase chain reaction (RT-PCR) analysis for the search of the fusion gene products EWSR1/ FLI1 and EWSR1/ERG as disease markers. Particularly in patients with prognostic unfavourable disseminated disease, ctDNA is a valuable addition for assessment of therapy response, another potential method uses flow cytometry to detect CD99 positive cells within the CD45- cell population and it has been shown that this method is able to evidence CTCs in PB of ES patients regardless of the presence and type of the molecular rearrangement [10]. The identification and capturing of CTCs requires extremely sensitive and specific methods, which usually consist of a combination of enrichment and detection procedures.

Conclusion

In this case report we reported a patient who presented a localized tumor mass without evidences of bone marrow infiltration. The clinical evaluation after imaging techniques analysis and the localization of the tumor mass in the left iliac wing led to the inclusion of the patient among the poor responder cases. With the cytofluorimetric analyses and the detection of the specific ES marker CD99 in CD45 negative cells isolated from PB, we observed a massive fraction of CD99+ CTCs. These data confirm that, despite the absence of tumoral cells infiltrating the bone marrow, the presence of a high percentage of CD99+ CTCs in PB together with the particular localization site of the tumor mass in the pelvis should make consider the patient as at high risk of relapse for the primary tumor site.

Conflict of Interest

There was no conflict of interest among authors.

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