

Case Report

Extramedullary blast crisis of CD34⁺ CD38⁻ leukemia stem cells in chronic myeloid leukemia resistant to tyrosine kinase inhibitors: a report of two cases

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Abstract: We report two cases of chronic-phase chronic myeloid leukemia (CML), who presented extramedullary blast crisis; biopsy revealed proliferation of uniform blast cells, and immunophenotype was suggestive of CD34⁺ primitive hematopoietic cells. The BCR/ABL fusion gene detected in a paraffin-embedded section of the lesion by double-color fluorescence in situ hybridization (FISH), indicating an extramedullary tumor composed of CML leukemia stem cells in both cases. Although treated with dasatinib, systemic chemotherapy, and local radiotherapy, both patients died of infection-related complications. Therefore, in our cases, CD34⁺ CD38⁻ leukemia stem cells of CML appear to have migrated to and proliferated in extramedullary sites. Once blast crisis develops, the prognosis is very poor, more potent tyrosine kinase inhibitors such as dasatinib will be ineffective, and a transplant of human stem cells may be needed.

Keywords: Extramedullary blast crisis, leukemia stem cells, chronic myeloid leukemia, tyrosine kinase inhibitors

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative clonal disorder of stem cells, and its typical clinical course includes a chronic phase, accelerated phase, and blast crisis phase. Blast crisis is the end stage of the disease and usually indicates a poor prognosis. Certain patients will develop extramedullary blast crisis (EBC) caused by the extramedullary infiltration of blast cells. EBC occurs primarily in lymph nodes, and secondarily in bone and certain other soft tissues [1]. Blast Crisis (BC) cells are mostly of myeloid lineage but may also be of lymphoid lineage [2] and rarely of megakaryocytic-type, mixed-type, or biphenotypic lineage [3-5]. However, extramedullary tumors of CD34⁺ CD38⁻ stem cells have not been reported, and herein we present two cases of CML in patients with CD34⁺ CD38⁻ cells in EBC recently admitted to our department.

Case 1

A 31-year-old man presented to our department with severe pain and swelling in the left

knee for two months with no history of trauma. In 2008, he was diagnosed with CML in the chronic phase, and was treated with hydroxyurea. In September 2014, a bone marrow examination revealed remarkable myeloid hyperplasia with 8% blasts. The karyotype t(9;22)(q34;q11) was identified in all cells examined, but no additional chromosomal abnormality, major BCR/ABL fusion gene, and E255K mutation were detected, suggesting the disease had progressed to an accelerated phase. Imatinib mesylate (400 mg) was given daily, achieving bone marrow (BM) complete remission (CR). However, since January 2015, he developed left knee pain and swelling progressively. On referral to our department, a physical examination revealed a red, swollen left knee, fever, but no hepatosplenomegaly. His blood counts were normal, however, a heterogeneous low signal intensity mass was detected on T1 and T2 images in the lower part of the femur as well as the upper part of the tibia (**Figure 1A**), and then left femoral tumor incision biopsy was performed. Histological analysis revealed some necrotic areas and the proliferation of round

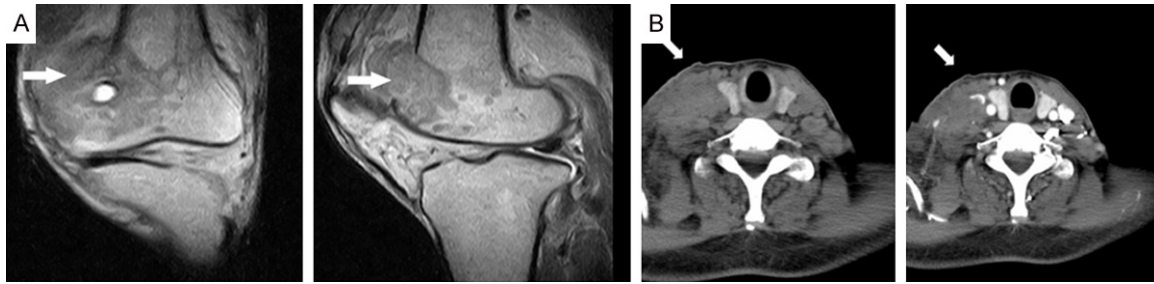


Figure 1. A: Magnetic resonance image of the lower part of the left femur. Coronal T2 enhanced (the left one) and sagittal T2-enhanced (the right one) image of the lesion (indicated by arrow). B: Computed tomography scans show the presence of a large soft-tissue mass on the right side of the neck (arrow).

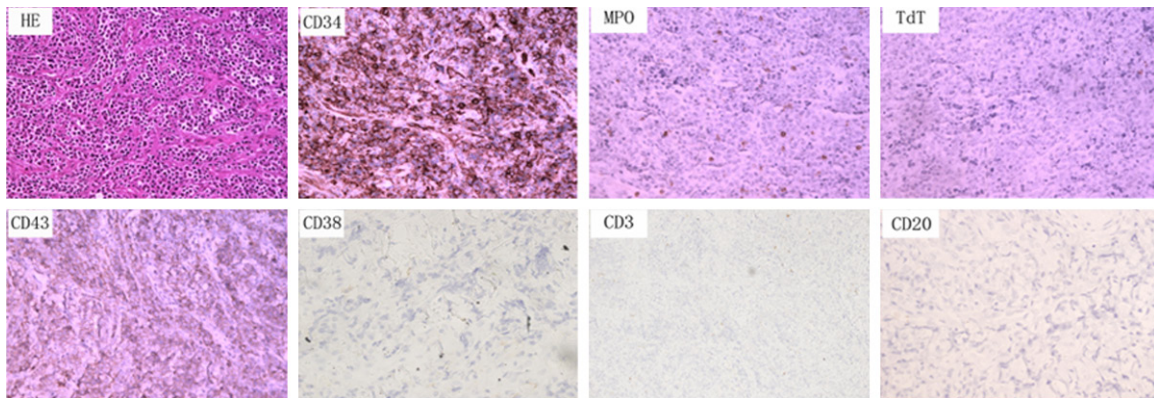


Figure 2. Biopsy of the lower part of the femur of the first person shows the proliferation of round blast cells (hematoxylin and eosin stain, original magnification $\times 200$) diffusely positive for CD34 and CD43 but negative for CD38, MPO, CD3, CD20, and TdT (original magnification $\times 200$).

blast cells. Immunohistochemistry showed that these cells were LCA, CD43, and CD34 positive, and CD20, CD3, MPO, TdT, CD38, Auer negative (**Figure 2**), and the proliferative activity (i.e., Ki67) was 50%. A screening of the biopsy specimen for cytogenetic abnormalities by fluorescence in situ hybridization (FISH) found that 70% of blast cells had nuclei containing the Bcr/Abl fusion gene (**Figure 3**), which confirmed that the blasts were derived from Ph⁺ leukemia cells.

Examination of the bone marrow suggested chronic-phase CML. A t(9;22)(q34;q11.2) abnormality was revealed by chromosomal analysis of the bone marrow cells, with the Bcr/Abl fusion gene revealed in 70% of interphase nuclei by FISH analysis, high Bcr/Abl mRNA expression detected by reverse transcriptase polymerase chain reaction (RT-PCR) in 42.3% of cells, and E255K mutation occurring in 55% of cells.

After diagnosis, 140 mg of dasatinib daily and a course of high dose methotrexate (5 g/m²)

were given. As pain persisted, he was started on local radiotherapy 20 Gy in five fractions; however, his pain was not relieved and a few days later, he died of pneumonia and septic shock.

Case 2

A 60-year-old man was admitted in February 2015 because of a gradually enlarging mass in his neck for 2 months. He had been diagnosed with chronic-phase CML in 2009 and was treated with 400 mg of imatinib mesylate every day, which resulted in complete cytogenetic remission 4 months later. In May 2014, the patient was admitted to our department because of headache. A computed tomography scan of his head demonstrated no specific signs of meningeal and cerebral involvement. And lumbar puncture revealed blasts in the cerebrospinal fluid (CSF), and several doses of intrathecal chemotherapy with cytarabine, methotrexate and dexamethasone given once a week, as well as 140 mg of dasatinib given every day, led to

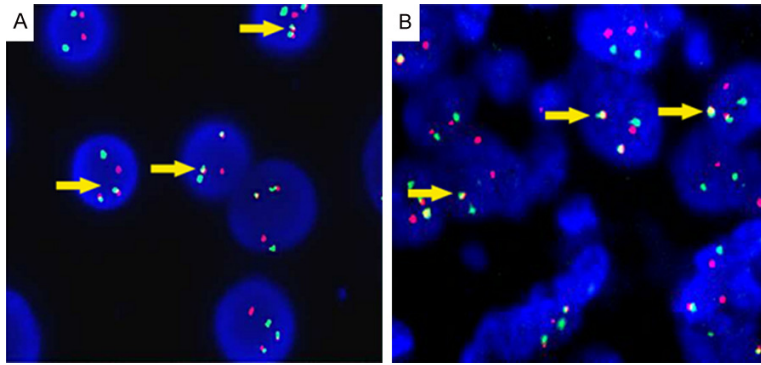


Figure 3. Detection of Bcr-Abl fusion genes by FISH in a paraffin-embedded tissue section. Bcr-Abl fusion genes are indicated by co-localized signals (arrows) for Bcr genes (green signals) and Abl genes (red signals). The counter-stain is 4,6-diamino-2-phenyl-indole (A: Patient 1, B: Patient 2).

the disappearance of the headache and the blasts in the CSF. After 7 months with complete hematologic and cytogenetic remission, the patient developed a subcutaneous tumor on the right side of his neck.

On arrival at our hospital, physical examination showed that the 5*6 cm lump was hard, ill-defined, and not obviously tender. Moreover, no enlarged superficial lymph nodes were detected. A CT scan showed the subcutaneous tumor (**Figure 1**), and a biopsy with immunochemical staining of the tumor demonstrated the proliferation of blast cells (Ki67 proliferation index, 75%), of LCA⁺, CD43⁺, CD34⁺, CD20⁺, CD3⁺, MPO⁺, TdT⁺, and CD38⁺, which were similar to the first case. A FISH analysis revealed the presence of a Bcr-Abl gene rearrangement in the biopsy specimen; bone marrow was still in chronic phase disease; cytogenetic analysis showed normal karyotype; RT-PCR revealed Bcr-Abl mRNA expression in only 0.2% of the cells, and genetic analysis failed to detect any mutations.

Two courses of combination chemotherapy and local radiotherapy, as well as 140 mg/day of dasatinib, failed to shrink the neck mass. And the patient gave up treatment and soon died of pneumonia.

Discussion

CML in EBC is defined as the development of extramedullary disease caused by the infiltration of blasts independent of the proliferation of blasts in the bone marrow [6]. CML in EBC is rare and it often occurs simultaneously with (or

a few months prior to) blast crisis in the marrow [7]. In our report, both patients were still in the chronic phase of their disease when EBC appeared and both died of infection-related complications not more than 5 months after developing EBC.

Most EBC cells are of myeloid lineage, and some are of lymphoid lineage. Determining the cell lineage of the blasts is clinically important for predicting the response to chemotherapy and survival in

blast-crisis CML. Immunohistochemical staining makes it possible to identify tumor cell markers. Tumor cells in these two patients were CD34⁺ CD38⁺, and positive for Bcr-Abl rearrangement, so we believed that these tumor cells originated from bone marrow leukemia stem cells.

Researchers have detected the Bcr-Abl fusion gene not only in different BM hematopoietic myeloid cell lineages but also in CD34⁺ precursors from CML patients [8]. Since TKI became widely used to treat CML patients, the incidence of EBC has increased, and the reason is not clear. Some researchers believe that blast cells changed in the early stage can evade TKI killing and selectively proliferate in extramedullary sites [2]. Other researchers hold that Bcr-Abl kinase mutation occurs in leukemia stem cells before TKI therapy because of the instability of the BCR-ABL fusion gene in CML stem cells [9]. Our two patients had a history of pre-TKI use, and the first patient had the E255K mutation. We suppose that BM leukemia stem cells migrate to the extramedullary sites.

As we all know, persistence of leukemia stem cells is the main reason for the relapse and refractoriness in CML patients. According to Su Chu et al., CD34⁺ CD38⁺ stem cells function as a major reservoir of residual BCR-ABL⁺ cells in patients in remission on imatinib mesylate treatment and can generate BCR-ABL⁺ cells after transplantation to immunodeficient mice [10]. Consequently, we hypothesize that leukemia stem cells can migrate to and proliferate in

extramedullary sites to form extramedullary tumors, and our two cases provide further evidence to support this hypothesis.

There is no standard treatment for CML-EBC. Naito et al [11] confirmed that imatinib can penetrate lymph nodes in animal models, so imatinib for CML blast crisis in lymph nodes may be effective. In recent years, treatment of CML-EBC with imatinib was successful in some cases [12], and because dasatinib can penetrate extramedullary tissue or the CNS, patients receiving it may achieve a good response [13]. Though given a standard dose of dasatinib, our two patients failed to respond. Effectiveness may depend on tumor cell type. According to the literature, CML hematopoietic stem cells are not sensitive to TKIs; even dasatinib could not induce a pro-apoptotic response in CML progenitors [14]. Thus, dasatinib cannot be expected to inhibit the proliferation of leukemia stem cells in extramedullary sites.

We are the first to report that CML patients can progress to EBC of CD34⁺ CD38⁻ leukemia stem cells during TKI treatment, which provides further evidence that leukemia stem cells can migrate in extramedullary sites and proliferate. At this point, the prognosis is very poor, even more potent ABL kinase inhibitors is ineffective. So other pathways of CML stem cell survival need to be identified and targeted to enhance their elimination, HSCT may be needed.

Disclosure of conflict of interest

None.

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