Diffuse osteolytic lesions in leukemic transformation of myelofibrosis

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SUMMARY

Myelofibrosis is a clonal myeloproliferative disorder characterized by splenomegaly, abnormal deposition of reticulin and collagen in the bone marrow, extramedullary hematopoiesis, dacryocytosis and leukoerythroblastic blood smear. Development and sustainment of fibrosis are mediated by complex network of several cytokines. Osteosclerosis is the most frequently observed bone change in myelofibrosis. We present an atypical case of leukemic transformation in myelofibrosis associated with diffuse osteolytic lesions and extremely elevated lactate dehydrogenase in serum, which indicates high bone turnover during leukemic infiltration and bone destruction.

Key words: *Myelofibrosis; Osteolysis; Lactate dehydrogenases; Parathyroid Hormone; Cell Transformation, Neoplastic; Leukemia, Myeloid*

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INTRODUCTION

Primary myelofibrosis is a chronic clonal myeloproliferative disorder characterized by splenomegaly, bone marrow fibrosis and extramedullary hematopoiesis, dacryocytosis, and leukoblastic blood smear (1-4). Between 5% and 20% of patients with myelofibrosis terminate with acute leukemia that can display certain morphological or immunophenotypic subtype. Secondary leukemia in myelofibrosis complicates natural course of disease due to chemotherapy or irradiation treatment (4).

The precise mechanism leading to bone marrow fibrosis remains unclear, but there are suggestions that reactive proliferation of fibroblasts and clonal process exists. Development and sustainment of fibrosis are mediated by complex network of several cytokines. These cytokines mainly include tumor necrosis factor-α (TNF-α) as well as other: transforming growth factor β (TGF-β), basic fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet factor 4, and calmodulin (5,6).

TNF-α has the influence on proliferation of both normal and malignant cells, stimulates fibroblastic proliferation and it is a key mediator of fever and cachexia (6-9). For better explanation of the role of TNF-α in pathogenesis of leukemic transformation in myelofibrosis we determined TNF-α values in sera of the patients with acute myeloid leukemia (AML) developed from myelofibrosis. To exclude other reasons for diffuse osteolytic lesions, we additionally analyzed serum level of parathormone and its production from cultured bone marrow cell and peripheral blood cells.

CASE REPORT

A 49-year-old female developed malaise and abdominal pain in 1991. Physical examination disclosed splenomegaly. Laboratory analyses showed hemoglobin (Hb) 102 g/l, platelets 158 x10^9/l, white blood cell (WBC) count of 8.7 x10^9/l, erythroblast in peripheral blood of 12/100 WBC, and dacryocytes. In differential leukocyte formula there was 3% myeloblasts, 6% myelocytes, 10% metamyelocytes, 7% bands, 32% segmented neutrophils, 13% monocytes, and 29% lymphocytes. Cytogenetic analyses showed normal female karyotype (46, XX). The first bone marrow biopsy disclosed hypocellularity, presence of all cell lineages with relatively normal maturation, and marked reticulin fibrosis (grade 3). The prominent proliferation, clustering and pleomorphism of megakaryocytes suggested the diagnosis of cellular phase of primary myelofibrosis.

Immunohistohemical staining with CD34 did not review any increase of blast cells. *In vitro* culture studies of peripheral and bone marrow progenitor cells showed spontaneous growth of erythroid and granulocyte cells colonies. Based on the presence of splenomegaly, leukoerythroblastosis and dacryocytosis in peripheral blood, bone marrow fibrosis and cytogenetic finding a diagnosis of myelofibrosis was established. The patient was treated symptomatically.

After 4 years, patient's condition deteriorated with malaise and bone pains. The physical examination at that time showed pale skin and mucous membranes with enlarged spleen that packed the entire abdominal cavity, 270 mm in diameter. The laboratory analyses showed Hb of 54 g/l, WBC of 8.0 x 10^9/l, platelets of 122 x 10^9/l, with myeloblasts 39%, myelocytes 7%, metamyelocytes 1%, bands 6%, segmented neutrophils 18%, eosinophils 1%, lymphocytes 22%, monocytes 6%, and 13 erythroblasts/100 leukocytes. Concentrations of immunoglobulines were IgA of 1.88 g/l, IgM of 1.23 g/l, and IgG of 11.27 g/l. The biochemical analyses were normal except extremely elevated sera LDH activity (1339 U/l). The parathormone determined in sera and from *in vitro* cultured leukemic cells were in reference values. Serum calcium is without changes in comparison to reference values. The serum TNF-α determined by bioassay, previously reported (7,8) was extremely increased (1421 pg/ml) in comparison to healthy controls (700 pg/ml).

Bone marrow aspirate was hypocellular with 72% of blasts mostly with characteristics of myeloblasts and more than 20% of monoblastic type. Cytochemical staining with myeloperoxidase showed that 30% of blasts were positive, and 25% of blasts were alpha-naphthol-esterase positive. The cytological finding was in accordance with FAB-M4 type of acute leukemia. Bone marrow biopsy, showed hypocellular bone marrow with collagen fibrosis and increasing numbers of blasts. The blast cells were CD34+, CD13+ and MPO+. The diagnosis of leukemic transformation of myelofibrosis was established.

The immunophenotyping of the peripheral blood cells obtained after cell separation on gradient were analyzed by Flow cytometry (Becton Dickinson, San Jose, USA). These analyses revealed clone which expressed HLA-DR (74.96%), CD34 (77.99%), CD13 (60.36%), CD33 (42.60%), CD14 (39.89%), CD4 (42.40%). Other immunophenotypic lymphoid and myeloid markers have been negative. The diagnosis of AML, FAB type M4 was confirmed.

Cytogenetic examination of bone marrow cells showed inversion of chromosome 16 [46,XX, inv(16)(p13q22)]. Molecular analyses studied by

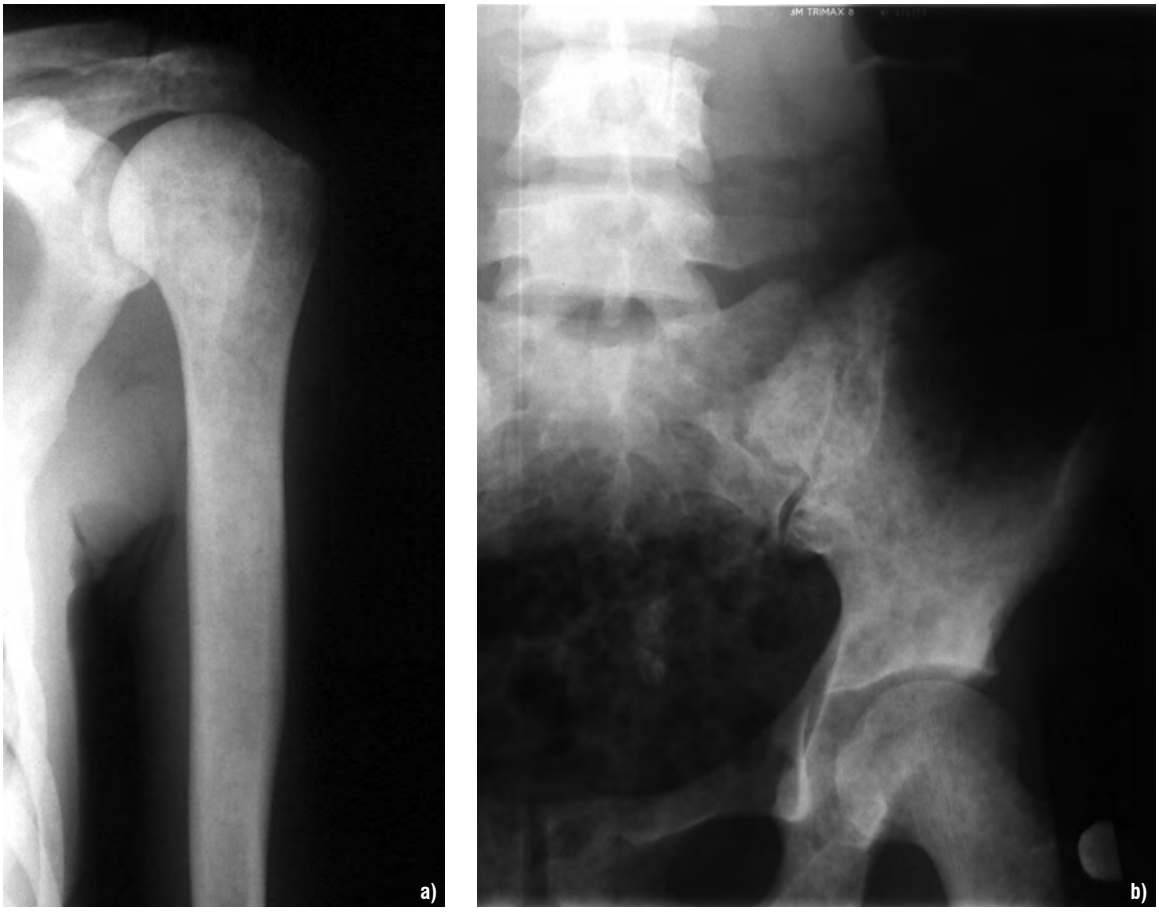

Figure 1 a, b. X-ray showing multiple osteolytic lesions in humerus and pelvis.

RT-PCR confirmed cytogenetic finding and revealed the *CBF*β/*MYH11* fusion gene transcript. PCR disclosed the presence of *FLT3* Asp835 mutation. Retrospective analyses of extracted DNA from bone marrow histological specimen at the time of diagnosis, showed that there no presence of FLT3 mutations before leukemic transformation.

X-ray showed the presence of diffuse osteolytic lesions in the pelvis and long bones (Figure 1). Multiple osteolyses were also present in the bodies of vertebra. The global skeletal scintigraphy documented diffuse increase of accumulation of the radiopharmaceuticals.

The patient was treated with cytosine-arabinoside, IV. She developed pancytopenia with high fever and hemorrhagic syndrome for which she received pooled platelets and antibiotics. After chemotherapy, leukemic infiltration of the bone marrow was again documented. She is not in remission, but she is alive and on supportive therapy with blood transfusion ever since.

DISCUSSION

We report an unusual case of spontaneously developed acute myeloid leukemia FAB M4 type in a patient with myelofibrosis associated with diffuse osteolytic lesions. These osteolytic lesions were accompanied with extremely elevated TNF-α and LDH but no disturbance in parathormone determined in sera and in the supernates of cultured leukemic cells were evident.

Osteosclerosis is the most frequently observed bone change in myelofibrosis mostly mediated by elevated TGF in irradiated patients or experimental animal models (6,7). In this case we found diffuse osteolytic bone lesions that are rarely reported in literature (10,11).

In our patient the presence of diffuse osteolytic lesions can be related to the leukemic transformation *per se* by means of enhanced secretion of cytokines, or ectopic secretion of the parathormone, parathormone-like mediators or vitamin D3 (10-13). Ectopic secretion of parathormone is usually associated with hypercalcemia, which is not case in this patient. We did not find elevated parathormone in sera or supernates from separated and cultured leukemic cells.

We postulated that extremely elevated TNF-α could be reason for lytic bone lesions in this patient, accompanied with high sera LDH activity indicating high bone turnover.

Osteolytic bone lesions could be also a consequence of leukemic bone infiltration or focal bone destruction by TNF-α locally released by leukemic cells (7,8). We previously reported that TNF-α can induce apoptosis in leukemic cell lines *in vitro* (9,14) and can stimulate osteoclast activation with subsequent development of bone degradation. Osteolytic lesions in myelofibrosis have been described but rarely and only in irradiated patients (1,11). Association of bone marrow necrosis and elevated TNF-α was described in leukemoid reaction in patients with metastatic prostate cancer (15). Proliferation of the stromal cells, which produce marrow fibrosis may also induce TNF-mediated bone destruction.

Based on significant and permanently increased concentration of serum TNF-α and LDH in myelofibrosis in our patient we postulated that this cytokine might have important role in bone destructions.

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Conflict of interest

We declare no conflicts of interest.

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