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LETTER TO THE EDITOR

Extensive bone marrow necrosis resolved by allogeneic umbilical cord blood mesenchymal stem cell transplantation in a chronic myeloid leukemia patient

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Extensive bone marrow necrosis (BMN) is a relatively rare clinical pathologic entity. Usually, 90% of BMN cases are associated with malignancies (mainly hematological). The morphological features of BMN are disruption of normal marrow architecture, and necrosis of myeloid tissue and medullary stroma.[1] The pathogenesis of BMN is still unclear.[2] It has been suggested that BMN could be mediated by reactive $CD8^+$ cytotoxic T cells[3] or the release of either toxins or soluble mediators.[4] BMN might correlate with elevated TNF-α levels.[5,6] Traditionally, there is a high mortality rate in BMN.[7,8] The prognosis of patients with BMN that is secondary to neoplastic disease is extremely poor, with survival not exceeding 6 months from the date of marrow necrosis diagnosis.[9] There is no effective treatment for BMN; therefore, it is particularly important to seek an effective treatment.

Umbilical cord-derived MSCs (UC-MSCs) are precursor cells that can differentiate into BM stromal cells, which have a critical role in providing the essential microenvironment for hematopoiesis. On the basis of the hematopoietic support, immunosuppressive properties and low immuno-phenotype of UC-MSCs, we postulated that BMN may be corrected by infusion of allogeneic MSCs. Here, for the first time, we present a BMN case with CML patient that was successfully resolved by UC-MSCs.

A 10-year-old boy was transferred to our hospital on 3 February 2010, because of marked leukocytosis and splenomegaly. The BM was hypercellular with the manifestations of chronic-phase CML. The ratio of BCR-ABL/ABL determined by real-time RT–PCR of peripheral blood metaphases was 64.8%. He was diagnosed with CML chronic phase, and received hydroxyurea 2 g/daily peros for a week, then switched to imatinib mesylate 200 mg/daily peros in March 2010. The BCR-ABL/ABL ratio in the peripheral blood was monitored by real-time RT–PCR every 3 months, and declined gradually to 0.03% 1 year later.

In May 2011, the patient developed increasing bone pain in the shoulders, knees and ankles of both sides, together with fever. He was readmitted to our hospital on 7 June 2011. He had moderate pallor. There was no lymph node enlargement or organomegaly. Hematologic examination revealed 7.6 g/dL hemoglobin, 228×10^9/L platelets, 2.11×10^9/L WBC and 225×10^9/L reticulocytes; in the peripheral blood smear, normoblasts and immature granulocytes were seen; LDH level was increased to 2383 IU/L and alkaline phosphatase level to1013 IU/L. All microbiological tests

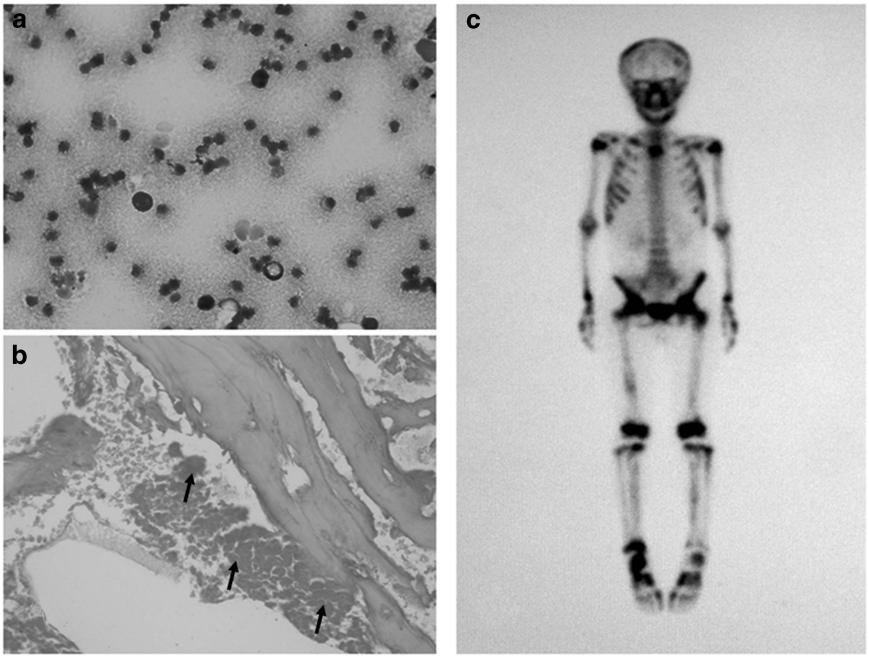

Figure 1. (**a**) BM smear was stained with Giemsa, which revealed a differential, irregular staining with the presence of an amorphous eosinophilic proteinaceous material, enmeshed within which were seen 'ghost-like' hematopoietic cells with irregular or indistinct cell membrane and nuclei showing the nuclear features; (**b**) BM biopsies revealed extensive foci of gelatinous transformation (black arrows) with necrosis; (**c**) Technetium-99 m sulfur colloid BM imaging showed extensive radioactive anomalies in the skull and chest, ribs, humerus, double side shoulder blades, bilateral iliac crest, femur and tibia and right ankle. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

were negative. The right aspirate from BM was of an altered serosanguinous type. A watery, dark red to clear fluid was aspirated from the left side of the iliac crest. The smears showed a differential, irregular staining with the presence of an amorphous eosinophilic proteinaceous material enmeshed within which were seen 'ghost-like' hematopoietic cells with irregular or indistinct cell membranes, and with nuclei showing the nuclear features. BM biopsies revealed extensive foci of gelatinous transformation with necrosis (the necrosis area accounted for 75% of the biopsy). Technetium-99 m sulfur colloid BM imaging showed extensive radioactive anomalies in the skull and chest, ribs, humerus, double side shoulder blades, bilateral iliac crest, femur and tibia and right ankle. Extensive BM necrosis (>50% of BM biopsy showing necrosis) was diagnosed (Figures 1a and c).The ratio of BCR-ABL/ABL was 1.19%. We suspected that the patient might be on the clonal evolution to blast crisis.

Due to the reports about BMN secondary to imatinib usage,[6,10] we stopped imatinib administration to the patient, and gave him allopurinol tablets 100 mg/m^2/dose and antibiotics. Supportive therapy and pain control were applied and hematopoietic support was provided to him, when required. Three weeks later, the patient's symptoms, including fever and bone pain subsequently aggravated, and hematologic examination revealed 8.1 g/dL hemoglobin, 48×10^9/L platelets, 1.78×10^9/L WBC and 285×10^9/L reticulocytes; BM aspiration of both sides posterior superior iliac spine (PSIS) still indicated BMN. After approval of the study by our ethics committee and written informed consent by the patient`s guardian, we decided to investigate whether allogeneic UC-MSCs had therapeutic effects on the patient.

Human umbilical cord samples were obtained from a healthy woman with a written informed consent. UC-MSC was prepared as described previously.[11]

On 6 July 2011, BM aspiration was done from the right and left PSIS, respectively (Figures 2a and b; Table 1); We aspirated the patient's BM from multi-sites, purified and cultured the MSCs, with no success (meanwhile, by the same culture methods, MSCs were harvested successfully from the healthy volunteer). This was followed by administration of a total of 2×10^7 allogeneic UC-MSCs in 20 mL of solution (normal saline) by intra-BM injection via the left PSIS. In all, 20 mL normal saline by intra-BM injection via the right PSIS was used as a control. No UC-MSC infusion-related side effects were noted. Two weeks later, BM aspiration was done again from both side PSIS; besides BM smear, 5 mL of BM specimen was aspirated to perform BM-MSC incubation as described, and sex chromosome detected by FISH for identifying MSC engraftment. The BM smear of the left PSIS showed BMN disappearance and active proliferation of BM cells (Figure 2d, Table 1); however, BMN on the right side was still present

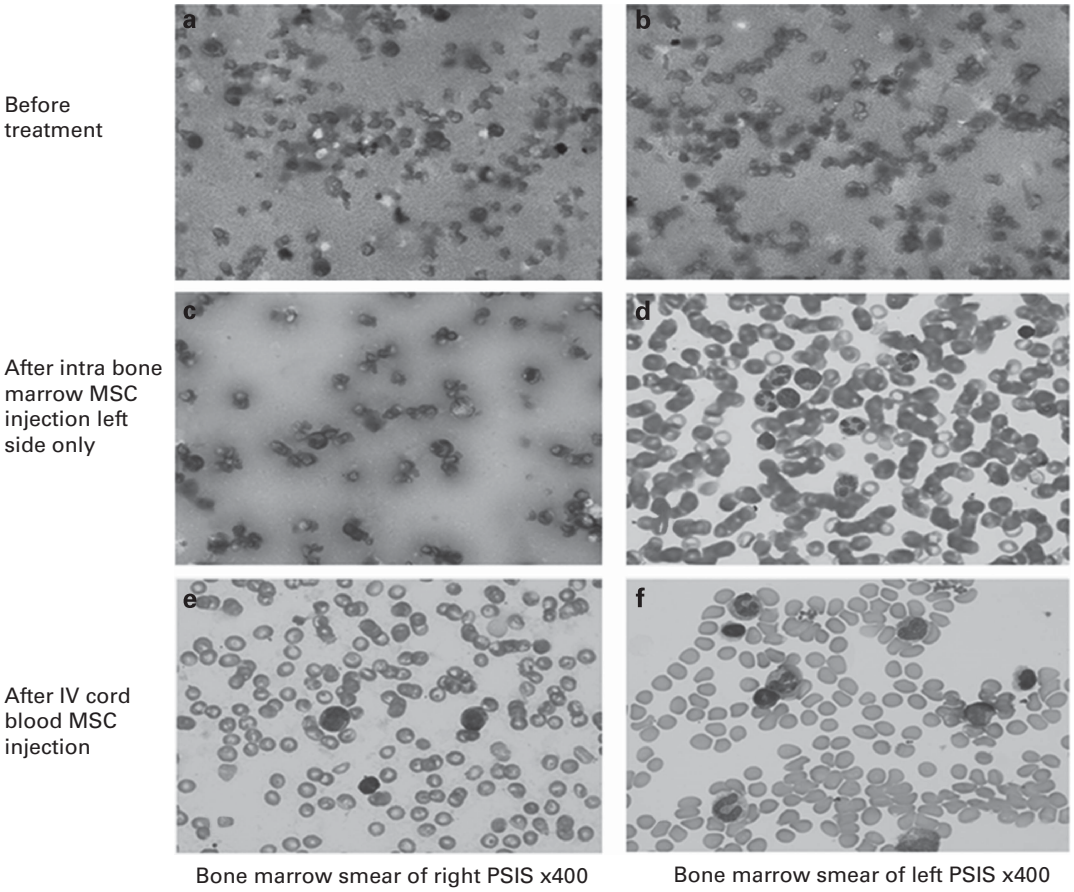

Figure 2. (**a, b**) BM smears from the right (**a**) or left (**b**) PSIS before treatment (2011.7.6) showed a differential, irregular staining with the presence of an amorphous eosinophilic proteinaceous material; enmeshed within which were seen 'ghost-like' hematopoietic cells with irregular or indistinct cell membranes and nuclei showing the nuclear features. Then, 2×10^7 allogeneic MSCs were administered by intra-BM injection via the left PSIS. (**c**, **d**) Two weeks (2011.7.21) after allogeneic MSC administration by intra-BM injection via the left PSIS, BM smears from right (**c**) showed just as before, numerous degenerated BM cells with indistinct cellular structure, necrotic cells displayed karyopynosis, karyorrhexis and karyolysis; however, BM smears from the left side PSIS (**d**) showed active proliferation of BM cells and BM necrosis disappeared; so 2×10^6 /kg UC-MSCs were delivered by i.v. infusion. (**e**, **f**) Two weeks later after MSCs were delivered by i.v. infusion, BM smear of two sides recovered to normal. The Wright stain was used.

Table 1. Bone marrow aspiration results (%)

Cell type	*Before treatment*		*After intrabone injection*		*After i.v. injection*	
	Left side	*Right side*	*Left (treatment)*	*Right (control)*	*Left side*	*Right side*
Myeloblasts			3.0		2.6	2.9
Promyelocytes			6.8		5.5	5.2
Myelocytes						
Neutrophilic	3.0	1.0	21.5	2.0	19.7	18.2
Eosinophilic	1.2		2.0		1.8	1.6
Basophilic	0.8		0.4		0.6	0.8
Metalyolocytes	1.0	2.0	15.6	2.0	16.4	17.7
Polymorphonuclear						
Neutrophilic	75.0	89.0	34.2	82.0	33.8	32.8
Eosinophilic			0.5		0.7	0.6
Basophilic			3.5		3.1	3.3
Lymphocytes	18.0	7.0	7.6	12.0	7.9	8.3
Plasma cells						
Monocytes			1.2		1.6	1.9
Reticulum cells			0.5		0.7	0.8
Mitotic figures						
Abnormal cells						
Megakaryocytes						
Megaloblasts						
Pronormoblasts						
Normoblasts	1.0	1.0	3.2	2.0	5.6	5.9
Myeloid/erythroid ratio	81.0:1	92.0:1	27.3:1	43.0:1	15.0:1	14.1:1

(Figure 2c, Table 1). Due to extensive BMN, on 20 July, 2×10^6/kg MSCs were delivered by i.v. infusion without premedication. After 2 weeks, hematologic examination revealed 9.6 g/dL hemoglobin, 167×10^9/L platelets, 12.05×10^9/L WBC and 278×10^9/L reticulocytes; BM smear of both sides recovered to normal (Figures 2e and f, Table 1), and bone pain and fever were relieved.

FISH results showed the patient displayed evidence of mostly donor origin at +14 days after UC-MSC transplantation. This patient was male, and the UC-MSC sample was obtained from a female infant. At +14 days, 197/200 (98.5%) of the MSCs were XX, which represents the most donor origin. The dynamic observation continued at +60 days and 124/200 (62.0%) of the UC-MSCs were XX. At +180 days, the chimerization status disappeared, with 200/200 (100.0%) XY MSCs.

The patient developed B-cell blastic transformation after 2 months, and was treated with chemotherapy with the tyrosine kinase inhibitor imatinib at 400 mg/day; to date, he has been in stable condition for 38 months.

More than 300 cases of BMN have been reported.[2,9] The current coverage-related BMN treatment is limited to the primary disease and support treatment, and there is no treatment targeting BMN itself.[9]

Allogeneic or autologous BM transplantation has been suggested to be a lifesaving treatment strategy.[12,13] But allotransplantation is limited by the availability of histocompatible donors and a variety of potentially lethal complications. Auto-SCT is not suitable for the treatment of acute or chronic leukemia.

We used allogeneic UC-MSC to successfully correct BMN, for the first time. Before the cellular therapy, the BM MSCs were cultivated *in vitro*, without success. We postulated that this outcome might be due to the patient's MSC damage, and infusion of allogeneic MSCs might correct the defect. The BMN patient was treated successfully by i.v. injection of allogeneic UC-MSCs, and completed by i.v. infusion of an additional dose of MSCs. This case indicates that BMN might be caused by the damage of BM MSCs, although further research about potential mechanisms involved is still needed. The therapeutic effects of MSC treatment may also be attributed to their immuno-regulatory activities. MSCs have been reported to suppress cytotoxic T cell-mediated cytotoxicity, increase the proportion of $CD4^+CD25^+$ $FoxP3^+$ regulatory T cells[14] and inhibit cytokine secretion (IL-12, TNF-α and IFN-γ) in activated T cells.[15] Allogeneic UC-MSCs might be a better option for BMN treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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