Impact of labile plasma iron and iron chelation on the viability of cultured mononuclear cells from patients undergoing autologous hematopoietic stem cell transplantation

TO THE EDITOR: Disturbances in iron metabolism are often observed after myeloablative conditioning in patients undergoing hematopoietic stem cell transplantation (HSCT) [1, 2]. A major determinant of this effect is a reduction in iron utilization resulting from erythropoiesis suppression by conditioning, leading to an increase in non-transferrin bound iron in plasma, including the labile plasma iron (LPI) pool, which is the redox-active, cell-penetrating, and toxic form of iron. In fact, there is evidence to suggest that about half of the amount of iron normally transported for erythropoiesis is released into plasma after initiation of conditioning [3, 4].

In most patients who undergo HSCT, LPI kinetics show a pattern in which conditioning promptly, as early as 48 h after its initiation, causes a substantial increase in LPI levels that persists until erythropoiesis is restored at engraftment [5, 6]. It has been suggested that increased levels of LPI may cause cell damage and ultimately lead to tissue toxicity, susceptibility to infections, and other complications commonly observed in the early post-HSCT period. Recently, we demonstrated an association between baseline LPI levels and regimen-related toxicity in patients who undergo HSCT; however, given the small size of the study, this observation should be confirmed in larger studies [6].

From a practical perspective, obtaining tissues from patients undergoing HSCT to evaluate iron toxicity is not feasible. However, as nearly all patients with planned autologous HSCT are subjected to mobilization with granulocyte-stimulating factors and harvesting of peripheral blood mononuclear cells (MNC) by leukapheresis, we postulated that MNC represent a valuable, culturable, and easily obtainable material to examine iron-mediated cell toxicity.

To determine whether increased LPI levels can cause cell toxicity, we evaluated their impact on the viability of MNC obtained from the peripheral blood of eight patients undergoing autologous HSCT for multiple myeloma (N=4) or non-Hodgkin’s lymphoma (N=4). An environment with normal and increased LPI concentrations was created by collecting plasma samples before (baseline) and after conditioning (on day 0). LPI concentrations were normal (<0.5 μM) in all baseline samples (mean, 0.2 μM; range, 0.0-0.4 μM) and increased in all day 0 samples (mean, 2.0 μM; range, 1.1-4.0 μM). An aliquot of the leukapheresis product from each patient was obtained on day 0, just before infusion, and MNC were isolated by Ficoll gradient and placed in DMEM in duplicate for five days in four experiments: i) without the addition of plasma (control), ii) with the addition of autologous plasma with normal LPI levels (baseline sample), iii) with the addition of autologous plasma with increased LPI levels (day 0 sample), and iv) with autologous plasma with increased LPI levels (day 0 sample) in the presence of an iron chelator (50 μM deferiprone). MNC were cultured with autologous plasma at a 1:1 volume ratio. Viability of cultured MNC was determined by the trypan blue method at 3, 24, 48, 72, 96, and 120 hours.

Informed consent was obtained from all individual participants included in this study, and all the procedures were performed in accordance with the ethical standards of the local institutional research committee.

As expected, MNC viability decreased over time in all experiments (P<0.001; Fig. 1). There was no difference in viability between cells cultured without plasma (control) and cells cultured with plasma and normal LPI levels. Cells cultured with plasma with increased LPI levels showed the lowest viability of cells in all culture conditions at all time points (P<0.001). Interestingly, the viability of cells cultured with plasma, increased LPI, and an iron chelator was similar to that of cells cultured with plasma and normal LPI levels at all time points (P>0.05).

The reduced viability of MNC cultured with day 0 plasma samples could be attributed to factors other than increased LPI that promote reactive oxygen species (ROS) generation. Actually, a pro-oxidant state associated with increased expression of plasma oxidative stress biomarkers and reduced activity of plasma antioxidant enzymes, is usually observed...
Changes in the viability of mononuclear cells cultured without the addition of plasma (control), with the addition of autologous plasma with normal LPI levels (LPI normal), with the addition of autologous plasma with increased LPI levels (LPI increased), and with the addition of autologous plasma with increased LPI levels and 50 μM deferiprone (LPI increased + chelator).

In conclusion, our results indicate that increased LPI levels can substantially decrease the viability of MNC and that this effect can be prevented or at least attenuated by using iron chelators.

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Acute megakaryoblastic blast crisis as a presentation manifestation of chronic myelogenous leukemia

TO THE EDITOR: Blast crisis may be encountered during the course of chronic myelogenous leukemia, commonly myeloid and less frequently lymphoid or megakaryoblastic. Why or how a patient will progress to myeloid, lymphoid, or megakaryoblastic crisis is still unknown. However, this transition has been attributed to the accumulation of various mutations. The prognosis in such cases varies depending on the type of blast crisis and the molecular signature of the patient. We present a case of CML presenting initially in the chronic phase and progressing to megakaryoblastic crisis in a short period despite receiving treatment.

Chronic myelogenous leukemia (CML) is a clonal disorder of pluripotent hematopoietic stem cells characterized by a balanced, reciprocal translocation involving chromosomes 9 and 22. Most cases present in the chronic phase (CP) of the disease. The progression from CP to the accelerated phase (AP) or blast crisis (BC) may be seen in patients on maintenance therapy due to the accumulation of various mutations. The most common BC type is myeloid, followed by lymphoid, and rarely megakaryoblastic. We report a case of megakaryoblastic crisis in CML, which is extremely rare and has been documented in very few cases to date.

**Case**

A 42-year-old man presented with complaints of abdominal distension accompanied by fatigue, weakness, and weight loss for the past three months. His medical history did not include any preexisting hematological or medical disorders. Upon examination, he had mild pallor, and a local abdominal examination revealed massive splenomegaly (6 cm below the costal margin). Complete blood counts showed leukocytosis (White blood cells (WBC), \(35 \times 10^9/L\)), severe thrombocytosis (platelet count, \(10 \times 10^9/L\)), and mild anemia (hemoglobin (Hb), 9.5 g/dL). A peripheral blood smear revealed a normocytic red blood cells along with the presence of immature myeloid precursors (differential count: 5% blasts, 17% promyelocytes, 15% myelocytes, 10% metamyelocytes, 2% basophil, 1% eosinophil, and 50% segmented neutrophils). Based on the above findings, a diagnosis of CML was suggested, which was confirmed by PCR for the bcr-abl transcript (Fig. 1A). The patient was orally administered 400 mg of imatinib (Gleevec) and 20 mg/kg of hydroxyurea once daily and was followed up regularly. Upon re-examination after three months of therapy, the patient showed no response to therapy as his cell counts remained unaltered, so he was switched to oral administration of 100 mg of dasatinib once daily. After this, the patient was lost to follow-up and presented after one year with rashes, pain in the upper abdomen and limbs, and general ill health. His counts revealed extreme leukocytosis (WBC: \(90 \times 10^9/L\)) with anemia and marked thrombocytosis (Hb: 8 g/dL, platelet count: \(19.10 \times 10^9/L\)). A peripheral blood smear examination revealed large blasts constituting 65% of total leukocytes along with platelet aggregates, marked platelet anisocytosis, and giant platelets. Bone marrow aspiration was markedly hypercellular with the presence of 80% of blasts having a round nucleus, open-structured nuclear chromatin, prominent nucleoli, and an abundant granular cytoplasm (Fig. 2A). A flow cytometric evaluation was performed on peripheral blood, which identified two populations of blasts. One population (constituting around 40%) was negative for CD45, all B-cell (CD19, CD10, CD20), T-cell (CD4, CD8, CD3, CD7), and myeloid markers (CD13, CD14, CD33, CD117, CD64), as well as CD34, HLA-DR, CD56. The other population of blasts (constituting around