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Assessment of Labile Plasma Iron in Patients Who Undergo Hematopoietic Stem Cell Transplantation

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Key Words
Labile plasma iron · Hematopoietic stem cell transplantation · Iron overload · Toxicity

Abstract
Body iron disorders have been reported after myeloablative conditioning in patients undergoing hematopoietic stem cell transplantation (HSCT). There is a concern that labile plasma iron (LPI), the redox-active form of iron, can be involved in the occurrence of toxicity and other complications commonly observed in the early post-HSCT period. In order to better understand the LPI kinetics and its determinants and implications, we undertook sequential LPI determinations before and after conditioning until engraftment in 25 auto-HSCT patients. Increased LPI was present in only 5 patients before starting conditioning. Shortly after conditioning, LPI levels were increased in 23 patients, with peak at day 0, returning to normal range upon engraftment in 21 patients. Overall, LPI levels correlated weakly with serum ferritin and more strongly with transferrin saturation; however, both parameters were apparently not applicable as surrogate markers for increased LPI. Although this was a small cohort, logistic regression suggested that baseline LPI levels could predict occurrence of grade III or IV toxicity. In conclusion, LPI kinetics is influenced by aplasia following conditioning and engraftment. Measuring LPI before starting conditioning can offer an opportunity to predict toxicity and, perhaps, the need for chelation therapy.

Introduction

Iron overload, whether transfusion-related or not, is a well-documented complication of patients undergoing hematopoietic stem cell transplantation (HSCT) [1]. Non-transferrin-bound iron (NTBI) has been reported after myeloablative chemotherapy in HSCT patients, and it is assumed that the appearance of unbound iron in this non-transfusion setting possibly reflects a major disturbance in body iron distribution which is related to its underutilization due to erythropoiesis suppression, release of iron from dying cells in bone marrow and other tissues and liver toxicity with decreased transferrin production [2, 3].

Labile plasma iron (LPI) represents the most deleterious, organ-penetrating and toxic fraction of NTBI components and includes the redox-active forms that are

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amenable to chelation. Detection of LPI, rather than NTBI, provides a direct measure of iron that is both labile and chelatable, fulfilling a diagnostic need that is not answered by other tests of plasma iron such as transferrin saturation and ferritin [4–6]. Increased LPI levels have been associated with clinical complications such as neurological damage following ischemic stroke [7], increased mortality after myocardial infarction in diabetic patients [8], endothelial dysfunction in thalassemia children [9] and increased morbidity in transfused patients [10]. In addition, sequential LPI determinations have also been used to monitor the efficacy of different chelation protocols including apotransferrin in thalassemic mice [11], deferasirox in patients with myelodysplastic syndrome [12] and thalassemia major [13] and deferiprone in thalassemia major patients [14].

To our knowledge, sequential LPI determinations have not yet been reported for HSCT patients. The measurement of LPI in patients undergoing HSCT is of relevance for both diagnosis and therapy for the following reasons: (1) it could serve as a strong and reliable indicator of iron status in individuals at risk for iron overload, (2) it could confirm the association between free iron and early HSCT toxicity and complications and (3) it is a potential target for iron chelators.

The purpose of this study was to evaluate the kinetics of LPI levels in HSCT patients by obtaining sequential LPI determinations before and after conditioning until engraftment, and correlating them with standard iron parameters and early posttransplant complications.

Patients and Methods

Study Population and Sampling

Twenty-five consecutive adult patients undergoing first autologous HSCT following myeloablative conditioning were enrolled. All patients gave informed consent and none received iron chelation therapy at the time of transplant. The study protocol received approval from the local ethics committee.

Fasting serum samples for LPI measurements were obtained at the following times: two baseline samples within 7 days before conditioning, after 48 h of starting conditioning, on day 0 (before stem cell infusion) and thereafter, at least 3 times per week until documented engraftment. Serum was separated by centrifugation and frozen at –80°C until analysis. Standard iron parameters were also measured on all collected samples: serum ferritin was measured by immunoassay, serum iron and total iron binding capacity were analyzed colorimetrically on a Merck autoanalyser (Micro Lab, Merck, Germany) and the percentage of transferrin saturation was calculated from serum iron and total iron binding capacity (serum iron × 100/total iron binding capacity). Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of at least 0.5×10^9/l. Early clinical complications were recorded until day +100 and included toxicity and fever. Toxicity was graded according to published criteria [15].

Table 1. Patient characteristics (n = 25)

Mean age, years (range)	46 (21–68)
Gender (M/F)	10/15
Underlying disease	
Multiple myeloma	13
Malignant lymphoma	8
Acute leukemia	3
Seminoma	1
Disease status	
Complete remission	12
Partial remission	10
Relapse; refractory	3

LPI Assay

LPI was determined with the probe dihydrorhodamine hydrochloride (DHR) after reactive species formed by ascorbate and iron (but blocked by iron chelators) convert the non-fluorescent DHR to its fluorescent derivative in a manner dependent on iron concentration [4]. Briefly, in the LPI assay, each serum sample is tested under two conditions: with 40 μM ascorbate alone and with 40 μM ascorbate together with 50 μM iron chelator (deferiprone, Apotex). The difference in the rate of oxidation of DHR in the presence and absence of the chelator represents LPI, the component of plasma NTBI that is redox-active.

Statistical Analysis

The paired t test was applied to detect differences in LPI levels at established time points until engraftment. LPI levels <0.5 μM were considered normal. Pearson correlation was used to correlate LPI with serum ferritin and transferrin saturation levels. Logistic regression was performed to predict the influence of baseline LPI levels on the occurrence of early complications related to HSCT. All statistics were performed using SPSS software (version 11.5).

Results

The characteristics of the study population are summarized in table 1. There was a predominance of patients in complete remission and with low transfusional burdens prior to HSCT. All 25 patients engrafted (mean 12 days, range 9–16) and received RBC transfusion during the study period (mean 3 units, range 1–7). All patients survived during follow-up (100 days).

Sequential LPI determinations demonstrated that mean baseline levels of LPI were normal in the majority of patients, but increased significantly 48 h after the start of conditioning, with a peak at day 0 and remained increased until day 6, returning to the normal range

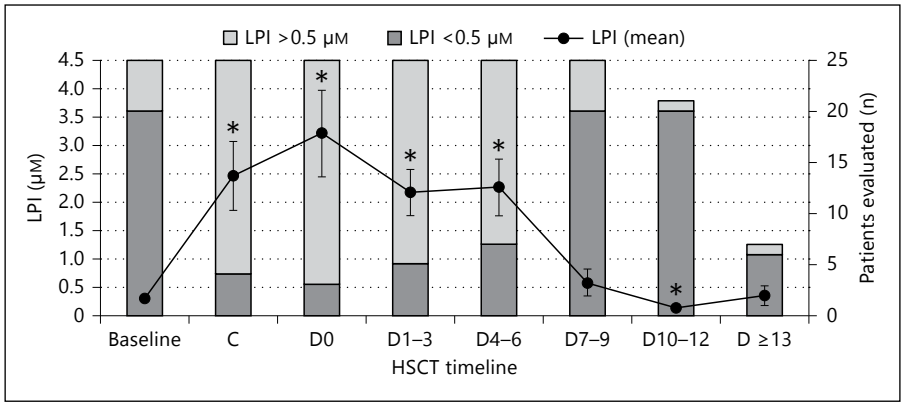

Fig. 1. LPI levels (mean ± SEM) and proportion (increased vs. normal) in 25 HSCT patients. LPI levels <0.5 μM are considered normal. Baseline levels represent mean LPI levels of two baseline samples and C represents levels 48 h after starting conditioning. * $p < 0.05$ in relation to mean LPI baseline levels. D = Day.

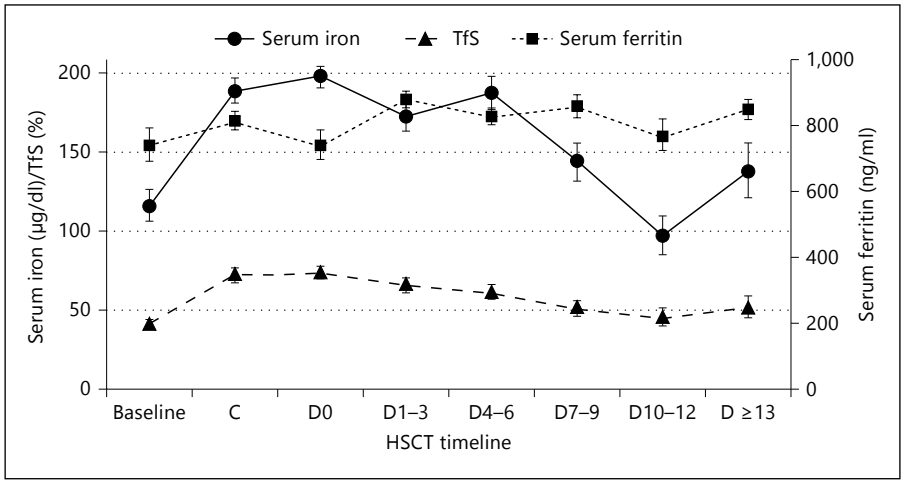

Fig. 2. Serum iron, transferrin saturation (TfS) and serum ferritin in 25 HSCT patients. Values represent mean ± SEM. Baseline levels represent mean levels of two baseline samples and C represents levels 48 h after starting conditioning. D = Day.

(<0.5 μM) around days 7 and 9 and thereafter (fig. 1). Increased LPI levels were found in 23 patients during the study and, upon engraftment, LPI levels returned to a normal range in 21 patients (91%).

Serum iron, transferrin saturation and serum ferritin levels observed during the study period are shown in figure 2. The Pearson correlation test showed that, considering all samples collected, LPI levels were positively correlated with serum ferritin and transferrin saturation levels throughout the study (fig. 3).

Mean baseline LPI levels (mean obtained from the two baseline samples) were increased in 5 patients, who did not differ from others in relation to baseline serum ferritin and transferrin saturation levels ($p = 0.338$ and $p = 0.833$; respectively), the amount of previous red blood cell transfusion (more than or less than 10 units; $p = 0.45$) or disease status at time of HSCT (complete remission vs. active disease; $p = 0.50$). Six patients presented with grade III or IV toxicity during follow-up (gastrointestinal n = 5 and hepatic n = 1). Logistic regression analysis showed that mean baseline levels of LPI could predict occurrence of grade III/IV toxicity ($p = 0.049$) with an odds ratio of 1,034, meaning that for each increment of 0.01 μM on LPI levels, there would be an increase of 3.5% in the risk of developing this complication, so that an LPI level of 0.5 μM (the threshold of normality) nearly doubled (175%) the risk of grade III or IV toxicity evolving. There was no influence of baseline LPI levels regarding onset of fever ($p = 0.118$).

Discussion

The kinetics of LPI in this study show a pattern in which, as early as 48 h after its initiation, conditioning causes a prompt and substantial increase in LPI levels that persists until engraftment in most patients. The pattern observed with LPI levels in our study matches patterns reported for NTBI levels in similar populations undergo-

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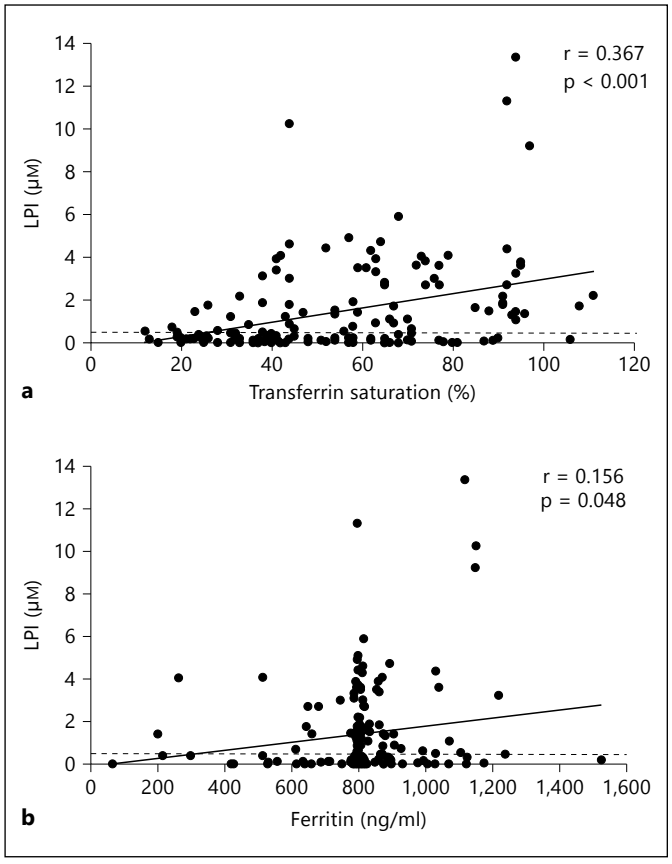

Fig. 3. Correlations between LPI levels and transferrin saturation (**a**) and serum ferritin (**b**) levels throughout the study.

ing autologous HSCT [16, 17]. The fact that the majority of the patients presented with increased LPI levels shortly after conditioning and during the aplasia phase and returned to having normal LPI levels upon engraftment is consistent with the hypothesis that inhibition and reconstitution of erythropoietic activity following myeloablative chemotherapy play a leading role in the kinetics of the appearance and disappearance of LPI [16].

Unbound iron is thought to catalyze the generation of reactive oxygen species that can ultimately cause tissue injury in HSCT patients whereas disruption of antioxidant defense caused by cytotoxic therapy can boost the risk of iron mediated toxicity [18–20]. It has been assumed that NTBI becomes detectable when transferrin saturation exceeds 80% [16, 17, 21]. However, measuring NTBI in plasma does not necessarily reflect the amount of the essentially redox-active forms of iron, as these may not be detected in samples with increased NTBI levels [4]. LPI levels, in turn, represent this fraction and the sequential LPI measurements obtained in this study can provide a more reliable assessment of how frequently HSCT patients are being exposed to pathologically relevant and toxic forms of iron. In agreement with previous reports on NTBI following cytotoxic chemotherapy [16, 22], LPI levels were weakly correlated with ferritin but strongly correlated with transferrin saturation. Interestingly, increased LPI levels were found in samples with mildly increased or even normal levels of transferrin saturation, whereas normal LPI levels were observed in some patients whose transferrin was highly saturated. These results strengthen the observation that standard iron parameters are not appropriate surrogate markers for increased LPI levels [23]. Also, the rise in LPI levels above normal in patients without suspected iron overload as measured by serum ferritin indicates that adverse effects due to an increased LPI level can occur even in patients generally not considered as having an iron overload.

Few reports have attempted to verify a connection between the presence of unbound iron and clinical complications in patients undergoing cytotoxic chemotherapy. Some authors speculated on the impact of unbound iron in relation to toxicity in HSCT patients, based mainly on the fact that the time of appearance of NTBI concurred with the onset of toxicity [17, 24]. In our study, it was shown that baseline LPI levels could predict the occurrence of grade III or IV toxicity. Although it is tempting to hypothesize a causal relationship between baseline LPI and early severe toxicity based on these results, it should be noted that they were based on a small cohort and without controlling for other variables such as conditioning and comorbidities, and should be confirmed in a larger and more homogeneous group of patients.

In conclusion, our study shows that LPI kinetics after myeloablative conditioning are closely related to bone marrow ablation and clinical parameters of engraftment. Serum ferritin and transferrin saturation, even though they show positive correlations with LPI, cannot be used as surrogate markers for increased LPI levels. Further studies are needed to evaluate the clinical benefits of iron chelators on temporarily lowering LPI levels until engraftment occurs in HSCT patients. In relation to future strategies for risk assessment and chelation therapy, our findings reveal the opportunity of measuring LPI before starting conditioning as a means of predicting toxicity and, possibly, the need for chelation therapy.

Disclosure Statement

Dr. Naoum has received research support from Novartis Oncology. The other authors declare no potential conflict of interest.

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