Comparison of Bleomycin-Detectable Iron and Labile Plasma Iron Assays

To the Editor:

Non–transferrin-bound iron (NTBI) is detected in the plasma of patients with diseases in which the transferrin-binding capacity for iron is exceeded by a massive iron overload, such as in certain forms of thalassemia and hemochromatosis, repeated blood transfusions, or bone marrow failure. NTBI may occur in plasma as insoluble polynuclear ferrhydrate species, as Fe(III) citrate or acetate complexes, or bound to certain flavonoids, amino acids, albumin, or modified albumin. Excess iron is harmful because it causes oxidative stress, accumulates in and damages tissues, and enhances the growth of pathogens. Therefore, the measurement of NTBI is of clinical importance, because NTBI may be used as an indicator of systemic iron overload and iron toxicity (1, 2).

We compared 2 NTBI assays: a labile plasma iron (LPI) assay that uses desferrioxamine as the iron chelator (3) and a microwell modification of a bleomycin-detectable iron (BDI) assay (2). The BDI assay is an indirect method based on the formation redox-active complexes between NTBI in the sample and the bleomycin reagent. The Fe(II)–bleomycin complex then degrades DNA in the sample mixture via a free-radical reaction, which can be measured photometrically. The detection limit of our BDI assay was 0.05 μmol/L, and the interassay CV was 18% and 9.8% at 0.2 μmol/L and 1.5 μmol/L, respectively (2). The LPI assay is based on the measurement of the redox-active and readily chelatable fraction of serum NTBI. The assay measures iron-catalyzed radical generation in the presence of a low ascorbate concentration. Radical generation is measured with the fluorogenic redox-sensitive probe dihydrorhodamine 123 (Sigma-Aldrich), and iron-catalyzed radical generation is calculated by subtracting the radical generation in the presence of 100 μmol/L of the iron chelator desferrioxamine (Novartis International) (3). In our laboratory, the detection limit of this assay was 0.07 μmol/L, which is calculated as 3 SDs above the mean of a zero sample (n = 8). The interassay CV was 11% and 12% at 0.3 μmol/L (n = 8) and 2.1 μmol/L (n = 8), respectively. The transferrin concentration was measured, and the percentage of transferrin saturation (TS) was calculated as previously described (2).

We measured NTBI and TS in 78 serum samples from 5 leukemia patients undergoing allogeneic hematopoietic stem cell transplantation, as described in a previous report (4). The median serum NTBI concentration was 0.12 μg/L (95% CI, 0.06–0.21 μg/L) and 0.10 μg/L (95% CI, 0.050-0.16 μg/L) as measured with the BDI and LPI assays, respectively. The results were correlated according to the equation: y = 1.33x + 0.06 μg/L, where x and y are the NTBI concentrations obtained with the BDI and LPI assays, respectively (Sxy = 0.17, Deming regression). There was no statistically significant difference between the NTBI results obtained by the 2 methods (P = 0.329, paired t-test). The median TS in the samples was 89.9% (95% CI, 83.1%–92.4%). The NTBI concentration measured by both methods was correlated with TS (P ≤ 0.0001). The NTBI concentrations in samples with TS <87% were low: For the BDI assay, all NTBI results except one (NTBI, 0.21 μg/L; TS, 13.6%) were <0.1 μmol/L. For the LPI assay, all NTBI results were <0.17 μmol/L (Fig. 1).

The exact biochemical nature of NTBI is unknown, and analytical approaches to measure NTBI vary with respect to testing principle and practical application. In 2005, an international round-robin for the quantification of serum NTBI compared 8 methods in 6 different laboratories (5). Seven of the methods were chelating assays, based on 5 different chelators,
and one was our BDI assay (2). The methods differed considerably in their NTBI results, and the BDI assay measured the lowest NTBI concentrations (5). The mobilization of NTBI by chelators from complexes in which NTBI is not available for the bleomycin reagent may explain this discrepancy. The LPI and BDI methods produced concordant results in the present study, suggesting that both methods measure the same form of NBTI in plasma (r = 0.87).

We compared NTBI assays with serum samples from patients undergoing stem cell transplantation for hematologic malignancies (2). Therapies related to iron chelation are of special interest for this patient group. Avoiding NTBI formation is also an important therapeutic goal in other diseases in which NTBI has been observed, i.e., hemochromatosis, hereditary or ferroportin deficiency, β-thalassemia, liver diseases, end-stage renal disease, diabetes, and myelodysplastic syndrome (1). The LPI assay detects the redox-active NTBI, i.e., the potentially toxic species of plasma NTBI, and may therefore be more relevant for clinical use than the BDI assay.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References


Outi Itkonen2,3*
Lauri Vaahtera2,4
Jaakko Parkkinen2,5

2 Department of Clinical Chemistry
University of Helsinki
Helsinki, Finland
3 HUSLAB
Helsinki University Central Hospital
Helsinki, Finland
4 Department of Biosciences and
5 Institute of Clinical Medicine
University of Helsinki
Helsinki, Finland

*Address correspondence to this author at:
HUSLAB
P.O. Box 140, 00029 HUS
Helsinki, Finland
E-mail outi.itkonen@hus.fi

Previously published online at DOI: 10.1373/clinchem.2013.205302