ORIGINAL ARTICLES

Clinical and methodological factors affecting non-transferrin-bound iron values using a novel fluorescent bead assay

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Nontransferrin-bound iron (NTBI) is a heterogeneously speciated plasma iron, typically detectable when transferrin saturation (TfSat) exceeds 75%. Here, we examine factors affecting NTBI levels by a recently discovered direct chelator-based (CP851) fluorescent bead-linked flow-cytometric assay (bead-NTBI), compared with the established indirect nitrilotriacetate (NTA) assay in 122 iron-overloaded patients, including 64 on recent iron chelation therapy and 13 healthy volunteers. Both methods correlated ($r = 0.57$, $P < 0.0001$) but with low agreement, attributable to 2 major factors: (1) the NTA method, unlike the bead method, is highly dependent on TfSat, with NTBI under-estimation at low TfSat and over-estimation once Tf is saturated, (2) the bead method detects <3-fold higher values than the NTA assay in patients on recent deferiprone-containing chelation due to greater detection of chelate complexes but lower values for patients on deferasirox. The optimal timing of sample collection relative to chelation dosing requires further study. Patients with splenectomy, high-storage iron, and increased erythropoiesis had greater discrepancy between assays, consistent with differential access by both methods to the NTBI pools associated with these clinical variables. The bead-NTBI assay has advantages over the NTA assay, being less dependent on TfSat, hence of less tendency for false-negative or false-positive values at low and high TfSat, respectively. (Translational Research 2016;177:19–30)

Abbreviations: AAS = atomic absorption standard; AlAT = alanine-aminotransferase; CFBs = control fluorescent beads; CI = confidence interval; CIC = cardiac iron content; CSA = congenital sideroblastic anemia; DBA = Diamodn-Blackfan anemia; DCI = directly chelatable iron; DFO = deferoxamine; DFP = deferiprone; DFX = deferasirox; ELISA = enzyme-linked immunosorbent assay; f.c. = final concentration; FBC = full blood count; IQR = interquartile range; LIC = liver iron content; LPI = labile plasma iron; MDS = myelodysplastic syndrome;

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MOPS = 3-(N-morpholino)propanesulfonic acid; NRBC = nucleated red blood cells; NTA = nitrilotriacetic acid; NTBI = nontransferrin-bound iron; SD = standard deviation; SF = serum ferritin; sTfR = soluble transferrin receptors; Tf = transferrin; TfSat = transferrin saturation; ULN = upper limit of normal; UV = ultraviolet

AT A GLANCE COMMENTARY

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Background

Non-transferrin-bound iron (NTBI) is increasingly understood as a multispeciated plasma iron pool, regulated separately from transferrin-bound iron and implicated in the complications of iron overload. The established nitrilotriacetate (NTA)-NTBI method is not optimal for distinguishing transferrin bound iron from NTBI.

Translational Significance

Here, we compared a novel fluorescent bead method with the NTA method, across clinical diagnoses. The NTA assay underestimates or overestimates NTBI at low- or high-transferrin saturations respectively, which the bead assay being robust to effects of transferrin does not. The greater specificity of the bead assay should clarify links between raised NTBI levels and their clinical consequences.

INTRODUCTION

Plasma non-transferrin-bound iron (NTBI), first described in 1978,[1] is a pathological iron pool detectable when Tf saturation exceeds 75%.[2-4] NTBI appears when iron influx into the plasma compartment exceeds iron efflux, for example, with iron overload, ineffective erythropoiesis, or decreased transferrin iron clearance in erythroid hypoplasia.[5] NTBI is considered the main conduit of hepatic[6-8] and extra-hepatic[9-12] iron loading of tissues, under hemosiderotic conditions. Quantitating NTBI is of value in understanding NTBI generation under different pathophysiological settings[5] but can also be potentially useful in the management of iron-overloaded patients. However, using established methods for NTBI quantitation, clear consensus, and guidelines on how to use NTBI measurement in patient management have yet to emerge. This is partly because NTBI is multispeciated, consisting of a range of iron-citrate,[13,14] albumin-bound complexes,[13] glycated protein-iron complexes,[15,16] or iron-chelate complexes in recently chelated patients.[17] Consequently, it is unlikely that NTBI assays relying on different principles will measure different NTBI species to the same extent. Hence, a consistent pattern of association between NTBI values and clinical outcomes has yet to emerge.

There is, therefore, a need to identify a robust and well-characterized NTBI assay that can be applied in a standardized manner in the management of iron-overloaded patients. A range of NTBI methods used previously differ considerably in their detection principles and reported reference ranges.[18] The most long-standing and frequently reported NTBI method[19] involves iron capture from NTBI by a high concentration of a low affinity/specificity iron chelator, nitrilotriacetic acid (NTA, 80 mM), followed by ultrafiltration and detection of NTA-iron by high-performance liquid chromatography[2,19] or spectrophotometrically.[3] Another approach is measuring NTBI indirectly by quantifying the redox-active subset of NTBI, which has been termed the 'labile plasma iron' assay.[20] A further approach is measuring the directly chelatable iron with a fluorophore-labeled high-affinity chelator,[18,21,22] but background fluorescence in plasma may interfere with data interpretation. Most recently, an adaptation of this approach was described, using a high-affinity fluorescent chelator CP851, covalently linked to magnetic beads with fluorescence signal separated flow-cytometrically from plasma autofluorescence.[23] This potentially circumvents the auto-fluorescence problem in the plasma sample and problems related to the indirect capture of NTBI by NTA.

The initial paper describing the bead method[23] examined only 30 patients and did not, therefore, explore the variables affecting the agreement between the NTA and the bead method systematically. In particular, the effects of TfSat, chelators, splenectomy status, and underlying diagnosis were not explored. A recent round robin[18] comparing various NTBI and labile plasma iron methods on 60 patients reported their overall lack of agreement in absolute values despite similar correlations but did not specifically look at the agreement between these 2 methods and could not, therefore, comment on the possible reasons for their poor agreement. Here, we compare levels of NTBI detected by this assay with the NTA method in various clinical conditions, including 122 iron-overloaded patients with approximately half (n = 64) receiving regular chelation therapy. Part of this work was presented as Abstract no. 241 at BioIron Conference Sep 6-10, 2015 in Zhejiang University, China.

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