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Response to Cabantchik & Hershko Commentary “Plasma nontransferrin bound iron – nontransferrin bound iron revisited: Implications for systemic iron overload and in iv iron supplementation”

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Dear Editor,

We note with interest the commentary by Cabantchik and Hershko, two authors who have made fundamental contributions in this field, in your journal. However this article represents a significant departure from the previous consensus about the nature of non-transferrin-bound iron (NTBI), its measurement, and the meaning of the word labile. These issues have been recently brought into acute focus following measurements of NTBI and labile plasma iron (LPI) after intravenous iron infusions¹. While there are parts of this commentary that differ significantly from our perspective, we see an opportunity for a consensus.

We believe that the proposed re-categorization of plasma iron species into labile and non-labile, while helpful when nuanced, might confuse important concepts and overgeneralizes the distinctions between different NTBI species. In particular, chelateable iron species are not necessarily the same entities as labile iron species. Furthermore species taken into tissues may not be confined to redox-active species.

We wish to make the following specific points:

The authors use the reference of *Dziuba, Hardy & Lindahl*² about normal plasma NTBI not containing citrate to offer an explanation for the detection of basal LPI levels in normal plasma (and NTBI presence in normal plasma). However the authors fail to mention that in *Dziuba et al* the concentrations of iron species in normal plasma are extremely small if and when present (i.e. 10-100nM). Submicromolar levels are not detectable by any of the clinical assays including the most sensitive bead-NTBI assay¹ in normal plasma. Furthermore, in the absence of iron overload, iron-free transferrin predominates, and iron citrate complexes are not predicted to exist at such concentrations.

A key concept in the commentary is the distinction between labile and non-labile iron but this is an unrealistic dichotomy. In reality, plasma iron species are in equilibrium between redox-active and -inactive forms, as well as between chelateable and non-chelateable species. In the commentary, labile NTBI is used to refer to “iron that is transferable/chelateable and redox-active”. However, non-redox-active iron can also be toxic if it leads to inappropriate uptake into cells that have not evolved to utilise high iron fluxes (cardiac and endocrine tissue).

The term labile plasma iron was originally coined by Cabantchik when describing the “LPI assay” in *Esposito et al*³ which identifies activity of iron species that are redox-active. However this should not be conflated with the wider, common use of the term labile. The term lability, in principle, can refer to a changeable redox state or to how stably and completely any given ligand binds to a metal ion, with more stable binding indicating less labile species. It is important not to confuse the transient sojourn of any chemical species in an open system compartment with lability, because stable complexes (non-labile) can also have a short sojourn in compartments such as plasma (transferrin iron).

Detectable species in the LPI assay are heterogeneous both structurally and kinetically, for instance partial (i.e. not complete 3:1) iron complexes of deferiprone are detected in the LPI assay⁴, as are plasma iron species derived from IV iron prodrugs¹.

It is important therefore not to conflate the concept of labile plasma iron (as detectable by the LPI assay) with toxic iron species in plasma or elsewhere in the body. LPI may be absent even in the presence of severe iron overload both in non-transfusion-dependent thalassemia (NTDT)⁵ and transfusion-dependent thalassaemia^{4,6}; furthermore not all forms of NTBI require reduction prior to tissue uptake, although reduction may enhance such uptake. Far more NTDT patients have high liver iron content than have high LPI, and more patients have high NTBI than high LPI⁵. Therefore conflating/equating LPI with either toxic iron species or tissue uptake iron species stretches the meaning of the concept of lability.

It is important to understand that neither NTBI nor LPI assays directly relate to tissue iron concentration. While NTBI assays are predominantly ‘capture assays’, the LPI assays are redox activity assays of species present in plasma. In the context of the IV iron infusions, transiently generated iron species will be detectable in plasma using both LPI and NTBI assays. These are likely to disappear after 2-4 days and conventional measurements of transferrin saturation (TSAT) should suffice, provided they are performed after the transient TSAT elevation post-dose has resolved (5 days to 1 week). It is therefore unnecessary to measure NTBI or LPI *in order to* identify overtreatment effect of IV iron preparations and conventional TSAT measurements should be sufficient.

We also believe it is unhelpful to equate IV iron nanomedicines, which are pro-drugs, with NTBI (and to label them as NTBI), although these pro-drugs may become both direct and indirect sources of NTBI. The source of the generated NTBI is not the same as NTBI itself.

Conceptually there are forms of iron preparations, which are designed to load parenchymal cells, whereas colloidal preparations are designed to replenish macrophage iron stores. Therefore while the former could be referred to as NTBI in a tissue culture setting, the latter are pro-drugs that release iron in macrophages by design but may also generate NTBI unintentionally.

Ultimately all forms of NTBI are toxic in the sense that they produce toxicity either directly (by being redox-active) or indirectly (pathological tissue uptake/distribution and its sequelae), therefore the distinction between toxic and non-toxic forms of NTBI (e.g. by means of LPI assay) is problematic and likely to be unjustified.

We suggest plasma iron species are not usefully divided into ‘labile’ and ‘non-labile’ species as they overlap.

References

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