




TEST OF THE MONTH

The clinical relevance of detectable plasma iron species in iron overload states and subsequent to intravenous iron-carbohydrate administration

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Abstract

Many disorders of iron homeostasis (e.g., iron overload) are associated with the dynamic kinetic profiles of multiple non-transferrin bound iron (NTBI) species, chronic exposure to which is associated with deleterious end-organ effects. Here we discuss the chemical nature of NTBI species, challenges with measuring NTBI in plasma, and the clinical relevance of NTBI exposure based on source (iron overload disorder vs. intravenous iron-carbohydrate complex administration). NTBI is not a single entity but consists of multiple, often poorly characterized species, some of which are kinetically non-exchangeable while others are relatively exchangeable. Prolonged presence of plasma NTBI is associated with excessive tissue iron accumulation in susceptible tissues, with consequences, such as endocrinopathy and heart failure. In contrast, intravenous iron-carbohydrate nanomedicines administration leads only to transient NTBI appearance and lacks evidence for association with adverse clinical outcomes. Assays to measure plasma NTBI are typically technically complex and remain chiefly a research tool. There have been two general approaches to estimating NTBI: capture assays and redox-activity assays. Early assays could not avoid capturing some iron from transferrin, thus overestimating NTBI. By contrast, some later assays may have promoted the donation of NTBI species to transferrin during the assay procedure, potentially underestimating NTBI levels. The levels of transferrin saturation at which NTBI species have been detectable have varied between different methodologies and between patient populations studied.

1 | INTRODUCTION

The biological importance of iron is well-described and the negative impact of iron deficiency on clinical outcomes is widely recognized, being typically associated with low transferrin saturations.¹ In contrast, iron overload conditions are typically associated with increased transferrin saturations, which when exceeding 70% to 75%,² lead to

the appearance of loosely bound, kinetically dynamic, heterogeneous plasma iron species, that have been generally classified as non-transferrin bound iron (NTBI). This term typically refers to those plasma iron species that appear when sufficient iron binding sites on transferrin are not available kinetically, and which may be partially removed by excess iron-free transferrin or exogenous chelators. Therefore, operationally, as explained later, NTBI excludes plasma

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heme and ferritin iron, IV iron-carbohydrate complexes used for the treatment of iron deficiency, and iron complexes of therapeutic chelators. Indeed, the original use of the NTBI term referred operationally to iron extracted from pathological sera, found in thalassemic patients, subjected to chelation and size filtration, whereby only low molecular weight iron that was not protein-bound fulfilled that term. NTBI species are typically detectable in capture assays, some of which species are also redox-active and detectable in labile plasma iron (LPI) assays.³⁻⁶

2 | NTBI PREVALENCE

Pathological (*vide infra*) plasma NTBI is found in primary iron overload disorders with high TSAT, such as hemochromatosis and thalassemia syndromes, or secondary iron overload resulting from repeated blood transfusions. Plasma NTBI may also appear in disease states where iron is not cleared efficiently by transferrin in the erythron, such as during myeloablative chemotherapy.^{7,8} NTBI species have also been identified in plasma in the absence of raised TSAT (e.g., in diabetes^{9,10}), where their presence may relate to the presence of biochemically altered (e.g., glycosylated, oxidized) proteins acquiring thereby a high affinity for iron.¹¹

3 | NTBI RELATIONSHIP WITH TSAT

The relationship between TSAT and plasma NTBI depends, broadly speaking, on the method used for NTBI estimation and the patient population under study. The presence of plasma NTBI is typically linked to raised TSAT; in most reports linking NTBI to raised TSAT authors observe this at TSAT >70%.¹²⁻¹⁷ This apparent threshold has been observed in patients with established NTBI, that is, where NTBI has been present for a long time and is typically at dynamic equilibrium in iron overloaded patients, such as with untreated hereditary hemochromatosis, thalassemia, or red cell aplasia. In these patients, we think that high iron entry into plasma through cellular (especially macrophage) ferroportin, finite hepatic clearance of NTBI, and finite erythroid clearance of transferrin iron together contribute to the persistence of NTBI at TSAT between 70% and 100%. Similar permissive TSAT thresholds are observed when NTBI is generated for the first time, as in myeloablation^{7,8} or after transfusion of old red cells.¹⁸

In contrast, some studies report NTBI at lower TSAT levels >35%¹⁹ or ≥50%.²⁰ For example, in HH patients undergoing sequential depletive phlebotomy,¹⁹ or where modified proteins with high affinity for iron¹¹ are present in plasma, such as in diabetes.^{9,10} In HH patients on iron depletion, whilst two of these factors probably remain relatively unaltered (plasma iron entry and hepatic clearance of NTBI, although plasma iron entry should increase as phlebotomy reduces hepcidin further²¹), the now increased erythroid clearance of transferrin iron²² lowers TSAT (to <35%),^{2,19} which effectively eliminates NTBI when monoferric transferrins and apotransferrin predominate.¹⁹ TSAT is

significantly lower in HH patients before ($87 \pm 16\%$, $n = 23$) and after ($40\% \pm 14\%$, $n = 14$) therapeutic phlebotomy.²

In the case of modified proteins (by glycation or oxidation), these can function as iron binding sites that do not exchange iron with transferrin (and actually compete with transferrin for iron), but their iron content is detected as NTBI.^{11,23,24} These latter forms of NTBI are then **not** a consequence of high iron entry into plasma or erythroid transferrin shuttling but a consequence of the presence of modified proteins, which form due to exposure to, for example, hyperglycemia, uremia, and inflammation.

The second major difference between studies is how the NTBI assays were performed. For example, whereas some studies use NTBI blocking to stop the shuttling of NTBI from NTA onto transferrin (e.g., with cobalt^{9,19,25}), others do not.^{20,26} In addition, some studies use a lower concentration of NTA than the standard technique (Zhang et al²⁷ vs. Singh et al²⁸), and some of them do not report or appear to control for (e.g., by standardization through spiking^{29,30}) iron contamination in the NTA used,^{26,27} which is highly variable.²⁹ Some of these assays report reference ranges of NTBI in normal controls up to 2 μM,²⁶ which is at variance with previously published data and at variance with the concept of NTBI as a pathological entity. For all these reasons, it is difficult to be sure what is the true level of TSAT at which NTBI is likely to be detected.

Considering the above, we would like to note that elevated morbidity and mortality in the general population have been linked with TSAT being increased³¹⁻³⁵ to levels which would not be typically associated with the presence of NTBI or indeed excess iron accumulation. It is not clear at this point to what extent these increased risks relate directly to iron deposition in some tissues, such as periportal hepatocytes, and/or other peripheral tissues via transferrin bound iron uptake, or to what extent such risks relate directly to chronic exposure to low levels of NTBI.

4 | MECHANISMS OF NTBI TOXICITY

The prolonged presence of plasma NTBI in chronic iron overload conditions leads to excessive tissue iron accumulation via unregulated pathways independent of transferrin receptors.^{36,37} The ingress of NTBI species into different tissues proceeds via unregulated membrane routes (channels or carriers) that typically admit other cations such as zinc,³⁸ manganese,³⁹ or calcium.³⁶ This perturbs physiological iron distribution, leading to non-physiological accumulations of iron in the parenchymal cells of the liver, heart, and endocrine systems.^{40,41} The major toxic potential of NTBI, rather than by catalyzing free radical formation in plasma, originates from iron maldistribution in tissues that do not typically handle large fluxes of iron, such as the heart or endocrine glands, and/or are not equipped with “sufficient” anti-oxidant capacity to counteract the potential toxicity of the redox-active iron, particularly in intracellular compartments. It is possible that there are NTBI species that are taken up by tissues, but which are not detected in the LPI assay.

In non-iron overloaded patients, there is a low concentration (nanomolar⁶) of transient NTBI species, possibly including iron citrate,

and these are involved in the cell-to-transferrin iron flux. At what level such complexes take on a pathological role is not clear. Thus, while strictly speaking, NTBI is present in normal plasma, it is not at levels, which are pathological. In this overview, when we use the term NTBI, we refer to NTBI at an elevated level that is consequently pathological.

5 | REDOX ACTIVITY OF NTBI SPECIES

The exact chemical make-up of the heterogeneous plasma NTBI species still remains to be defined in the different pathological states of systemic iron overload and after IV iron-carbohydrate complex administration. Advances made in the understanding of NTBI speciation indicated that a range of iron citrate species with varying kinetic abilities to exchange with transferrin are important components of plasma NTBI.⁴² Some NTBI species will exhibit strong redox catalytic activity, and indeed, an abundance of ferric iron in the presence of reducing compounds in plasma can generate reactive oxygen species via the Fenton reaction, inducing oxidative stress and cytokine activation.⁴³ This redox activity also depends on the ligand type and the so-called coordination sphere of iron, which controls the accessibility of both water molecules and reducing compounds; these phenomena also play a role in iron transport across membranes. It should be noted that not all forms of plasma NTBI species are necessarily redox-active and/or chelatable. Those that are, have been traditionally detected using the LPI assay⁴⁴ (or the bleomycin assay⁴⁵) and therefore are referred to as labile (i.e., redox-active and susceptible to chelation). The chemical term “labile” refers to metal–ligand complexes that are able to rapidly exchange ligands and/or rapidly change the metal redox state. Nevertheless, the dichotomy between LPI and NTBI species is inappropriate because many of these species remain in mutual equilibrium.

6 | NTBI EXCEPTIONS

There are a number of iron species in plasma that are not considered to be part of the NTBI pool. Thus, the natural plasma components, such as heme and ferritin iron have never been considered as being NTBI components; their iron is not exchangeable with transferrin in plasma (but it is when mediated by cells) and is not exchangeable with therapeutic iron chelators. In similar fashion, we consider that the IV iron nanomedicine prodrugs fall into this category and are therefore not components of NTBI, but may become NTBI sources in plasma. The iatrogenic nature of the latter as well as of the therapeutic chelator-iron complexes excludes both types from NTBI.

7 | RESEARCH GAPS

Research gaps remain in the scientific understanding of the *in vivo* behavior of plasma NTBI and its fluxes between the plasma and tissue

compartments across disease states and after IV iron-carbohydrate complex administration. The notion that chronic tissue iron overload could be prevented by controlling NTBI levels in plasma has led to the development of chelator regimes that aim to minimize tissue exposure to plasma NTBI components. Those chelation regimes have aimed at continuously maintaining sufficient chelator plasma levels to prevent a rise in plasma NTBI, a process that can occur within a few minutes of the removal of an exogenous chelator from the plasma compartment.^{3,29} This remains an important concept in the clinical management of systemic iron overload because of tissue iron accumulation; and hence much of the morbidity and mortality in systemic iron overload can be attributed to massive exposure to NTBI. Although the concept of plasma NTBI has been associated with the pathology of systemic iron overload, its quantitative and qualitative (i.e., regarding speciation) assessment has remained problematic, and thus its clinical applicability has not been fully established, let alone universally accepted. These challenges are even more apparent when evaluating the plasma NTBI appearance after IV iron-carbohydrate complex administration.⁴⁶ The challenges of clinical assessment of NTBI are to a large extent due to the variable levels of NTBI reported by different analytical assays and the lack of a bona fide standard for NTBI.^{14,47}

8 | CHEMICAL IRON SPECIES IN IRON OVERLOADED PLASMA

The heterogeneous forms of iron in biological fluids can broadly be classified into two categories: tightly bound and therefore kinetically non-exchangeable, (e.g., to transferrin and to ferritin) or weakly bound and exchangeable and potentially chelatable, (e.g., bound to citrate or adsorbed to plasma proteins such as albumin).^{3,5,6} Kinetically labile iron species exist in a “weakly” bound state and therefore they can potentially be transported into cells³⁶ and damage lipids, proteins, and/or DNA components when antioxidant defenses are surpassed.³⁷ In biological fluids, weakly bound iron such as ferric mononitrate will be in dynamic transition between different chemical structures.^{3,42} Among the potential non-protein ligands found in plasma, such as phosphate, acetate, amino-acids, polyphosphates (e.g., $\text{PPi} + \text{ATP}$), and citrate, citrate is reported to be the predominant coordinating iron ligand. This is because polyphosphates preferentially interact with divalent cations that are more abundant than iron, such as calcium and magnesium, under physiological conditions, binding these divalent cations >100 times more tightly. On the other hand, phosphate and carboxylate groups, present in both acetate and amino acids, cannot compete with the hydroxyl anion for iron, at pH 7.4. Since the plasma citrate concentration tends to remain stable at about 100 μM , the citrate-to-iron ratio and hence, the chemical nature of the iron-citrate complex will be determined by the relative increase in NTBI. More chelatable ferric citrate species dominate at a 100:1 ratio and become less abundant as the ratio approaches 10:1.⁵ An individual patient's health status and comorbidities (e.g., inflammation) can also seriously affect the plasma redox environment by reducing the levels of plasma antioxidant capacity.⁴⁸

9 | NTBI ASSAYS

Assays for NTBI tend to be technically complex so that their clinical application, particularly in syndromes with transient LPI species, has mainly remained as a research tool for chelator drug development and optimization of chelation strategies.¹⁴ Regulatory agencies do not require measurement of NTBI for iron-carbohydrate nanomedicines *in vivo*. Although an ideal NTBI assay for assessing systemic iron overload in plasma should aim to measure all of the plasma NTBI species, it is not a priori clear whether all species are potentially toxic per se in plasma or as sources of tissue iron overload. In fact, the majority of assays detect most, but not necessarily all, of the redox-active and/or chelatable iron components. The assays for quantifying plasma NTBI species work broadly on two detection principles based on: (1) redox activity that is abrogated in the presence of selective iron chelators,⁴⁴ or (2) using a selective chelator capture assay for the iron that is not tightly bound to transferrin.^{28,49,50} The capture-based methods are dependent on the action of a relatively high concentration of a small (for steric access), relatively low affinity chelator acting as an iron mobilizer (such as nitrilotriacetic acid, NTA) to displace the metal from the NTBI ligands and donate the displaced iron to a high affinity chromophore chelator (for assay readout).²⁸ With redox-activity assays, the mechanism is to generate an oxido-reductive coupling between the labile iron species of NTBI and a reducing agent (such

as ascorbate) in the presence of a ROS-sensitive probe (such as dihydrorhodamine) in order to measure the redox-activity of the labile species.^{4,14} Table 1 describes various approaches used to estimate NTBI species in plasma. Notably, there is still no single standardized assay for these species. Although LPI assays have been used clinically to assess drug regimen efficacy in terms of maintaining individuals LPI-free for 24 h, as for other clinical utility, such as prediction of or correlation with disease states, such as iron related toxicity, definitive studies have not been undertaken. However, there are some correlative data suggesting links between LPI and disease states, such as MDS survival.^{26,51}

Historically, assays developed to measure NTBI could not avoid the problem of capturing some iron from transferrin and presenting it as NTBI, leading to an overestimation of NTBI. This, in principle, can also occur with intravenously administered iron-carbohydrate nanoparticle preparations used therapeutically. Similarly, certain iron complexes of therapeutic chelators are detected under the conditions of some NTBI assays. The inclusion of iron-chelate complexes and intravenous iron prodrugs as NTBI is not consistent with the definition presented in the introduction. A converse problem occurs when NTBI is present without transferrin being 100% saturated, and the chemical conditions of the NTBI assay lead to inadvertent shuttling of the iron from the scavenging assay chelator to apotransferrin. This leads to an underestimation of NTBI (and even negative values, given that the

TABLE 1 Assay methodologies to measure NTBI

Method	Principal species detected	Examples	Challenges
<i>Chelatable, indirect capture</i> ^{28,30} Uses an iron chelator or ICP-AES to assess iron after sample treatment with an iron mobilizing agent	Total circulating NTBI	Mobilization of iron by NTA, followed by: a. Ultrafiltration and HPLC with on-column chelation with 3-hydroxy-1-propyl-3-methyl-pyridin-4-one ²⁸ b. Colorimetric assay with bathophenanthroline sulfonate ³⁰ c. Quantification by ICP-AES ³⁰ Mobilization of iron by oxalate, followed by binding to desferrioxamine or read-out using calcein-iron complex ⁷⁷	Mobilizing agents bind free iron that is weakly bound to substrates to a varying degree Quantitative assay values cannot be compared between assay methods
<i>Chelatable, direct capture</i>	DCI (directly chelatable iron)	Desferrioxamine chelatable iron ⁷⁸ Pyridinone chelator attached to a fluorescent probe, bound to beads, separated by flow cytometry ^{15,54}	Some species may not be available to these chelating agents Some species may not be available to these chelating agents
<i>Redox</i> Capitalizes on redox potential and measures end products of oxidation by colorimetric or fluorescence detection	LPI	Bleomycin detectable iron (BDI) ⁴⁵ Detection by an oxidation-sensitive probe of reactive oxygen species induced by a reducing (ascorbate) and an oxidizing (O ₂) agent ⁴⁴	BDI assay is technically complex and challenging to reproduce Activity assays performed with non-physiological standard Quantitative assay values cannot be compared between assay methods Activity assays performed with non-physiological standard Quantitative assay values cannot be compared between assay methods

matrix of the standards is not serum-based and contains no transferrin). To counter these biases, assay development has relied on various blocking strategies.^{30,52} Another aspect of assays using a capturing methodology is that the capture may be incomplete; for example, if desferrioxamine is used as the capture agent, only about 40% of total NTBI is captured without the addition of a shuttle ligand.⁵³ On the other hand, the CP851 chelator-bead NTBI assay is a capture method that is robust to transferrin shuttling (in either direction), does not require the presence of a scavenging small molecular weight chelator such as NTA, and furthermore does not shuttle iron to other therapeutic chelators but can accept iron from deferasirox and deferiprone.⁵⁴

10 | CLINICAL RELEVANCE OF WEAKLY BOUND IRON SPECIES FOLLOWING IV IRON ADMINISTRATION

For IV iron-carbohydrate nanomedicines, the problem of capturing some iron from the therapeutic iron-carbohydrate nanoparticles during NTBI assays has implications for regulatory evaluation of nanomedicines recommending labile iron assessment under physiologically relevant conditions.⁵⁵ Although these assays have recently been recommended for regulatory evaluation of generic intravenous iron-carbohydrate bioequivalence evaluation they have not been validated with all IV iron products, and their use in the clinical setting has not been evaluated.^{56–58} It is also worth noting that there are methodological caveats with commonly used clinical iron indices in the presence of therapeutic iron nanoparticle preparations that lead to spurious elevations of these indices. These elevations may persist for a relatively long time, predominantly after intravenous administration of iron-carbohydrate complexes, which are cleared more slowly from plasma (Table 2).

During iron chelation therapy for iron overload, iron-chelator complexes may be captured during the NTBI assay and hence be indistinguishable from pre-existing NTBI. In general, the iron complexes of desferrioxamine are sufficiently stable to avoid this problem, but the complexes of deferiprone, for example, can be detected in several NTBI or LPI assays for several days after drug administration.⁵⁹ Intravenously administered iron-carbohydrate nanomedicines act as a source of iron and therefore of NTBI in some kinetic contexts. They may thus transiently contribute to measured NTBI.⁶⁰ Although some might generate minor amounts of labile and/or loosely bound forms in plasma, those forms are relatively short lived and cause only minor, if any, interference with physiological processes.^{61,62}

Intravenous iron-carbohydrate nanomedicines are designed as relatively stable iron complexes that are susceptible to uptake by blood monocytes, peripheral macrophages, Kupffer cells, or endothelium.^{63,64} Subsequently, the complexes are broken down, and the generated iron is either incorporated into cellular ferritin or directly released into the plasma. The replete iron stores can then provide iron for hemoglobinization via homeostatic signaling to release iron to transferrin. Pharmacokinetic profiles demonstrate that total serum iron is elevated for a relatively short time after IV infusion before

TABLE 2 Caveats in interpreting commonly available clinical iron indices after intravenous iron administration

Clinical laboratory measurement	Challenges	Recommendations for clinical practice
Serum iron	Indiscriminately elevated after IV iron administration (including the injected iron) Direct interference with ferrozine-generated signal	Wait 2 days after administration with small MW IV iron, 1 week after large doses, large MW IV iron
TIBC	Multiple methods to calculate which yield different results depending on iron status. ⁷⁹ Chromogen will detect injected iron both before and after acidification	Be aware of local laboratory methods Avoid testing at multiple different laboratories Wait 2 days after administration with small MW IV iron, 1 week after large doses, large MW IV iron
TSAT	Derived from serum iron and TIBC, see above Or derived from transferrin concentration ⁸⁰	TSAT >70%–75% increases predictability of the presence of NTBI ^{5,14} Wait 2 days after administration with small MW IV iron, 1 week after large doses, large MW IV iron
Ferritin	Measurement of ferritin typically unaffected by the presence of intravenous iron preparation in the sample Indiscriminately elevated in diseases associated with pro-inflammatory states Increases in ferritin may be higher in inflamed patients	May take more than 2 weeks to reach steady state especially after large doses of IV iron. Serum concentrations are a poor predictor of NTBI/LPI ⁶⁰

Abbreviation: MW, molecular weight.

being cleared by uptake into the reticuloendothelial system.⁶⁰ However, a transient amount of NTBI may be present before or shortly after uptake into macrophages, but the proportion of NTBI relative to stable iron within the core is unclear.^{60,65} This is because some iron within the iron-carbohydrate complex may be reported as "total serum iron" using regular clinical assays for serum iron. To better understand this, it is therefore important to be able to distinguish NTBI species from iron that is bound within the complex, even when such iron is present at very high concentrations. In a recent study, comprehensively evaluating the pharmacokinetics

and pharmacodynamics of three intravenous iron nanomedicine preparations, maximal NTBI concentrations ranged from 0.13 to 1.25 μM with the relatively less stable iron-carbohydrate complexes (e.g., iron sucrose) producing higher concentrations.⁶⁰ The study also measured LPI concentrations and demonstrated that these were also transiently raised but at lower levels.^{44,60}

The clinical significance of plasma NTBI species relates to the levels, the duration of exposure, and possibly the speciation of the NTBI in question. To take an extreme example with acute oral iron poisoning in children, plasma iron levels in excess of 10 mg/L (>180 μM) have been described⁶⁶ clearly exceeding the iron binding capacity (40–80 μM) of transferrin by over 100 μM . Massive and prolonged exposure to free iron concentrations (a proportion of which will be labile) leads to myocardial stunning and can be rapidly fatal if untreated.^{66,67} On the other hand, chronic exposure to low levels of NTBI in transfusional iron overload (<10 μM , established from later studies) typically caused death from heart failure in the setting of severe cardiac hemosiderosis before the age of 20 years in the era before the introduction of modern chelation regimens.^{68,69} In our opinion, it is currently difficult, in the absence of relevant prospective clinical trials, to link the transient low NTBI appearance after IV iron-carbohydrate nanomedicine injections to any relevant clinical sequelae. Furthermore, even though NTBI appearance after both oral and IV iron administration has been associated with acute increases in biomarkers of oxidative stress in clinical studies, no biomarker has been established that has specificity for iron-induced redox activity, nor is there a standard by which to assess potential IV-iron-induced oxidative stress.⁷⁰ A large epidemiological study evaluating different dosing strategies for intravenous iron in hemodialysis patients did not observe any increased risk of cardiovascular events associated with larger, bolus doses of IV iron.⁷¹ Recently, a large, prospective clinical trial conducted to compare low and high doses of iron sucrose over 4 years in hemodialysis patients did not show that adverse cardiovascular outcomes were worse with increasing iron exposure.⁷² Additional published analyses of large dialysis patient datasets have shown modest associations with increased infections at higher doses of intravenous iron, however, this was not observed in a more recent prospective clinical trial in hemodialysis patients.^{73–75} Notably, patients with advanced chronic kidney disease have profound background inflammation and oxidative stress attributable to their co-morbidities.⁷⁶ The commonly measured biomarkers (malondialdehyde, TBARS, etc.) react non-specifically to iron-induced oxidative stress and may be higher or lower than the magnitude described in clinical trials.⁷⁰ Thus, although there currently remains a limited amount of evidence linking intravenous iron-carbohydrate nanomedicines to oxidative stress and resultant clinical outcomes, the inherent caveats of the established NTBI/LPI assays, as well as the lack of a definitive biomarker for iron-induced oxidative stress still need to be addressed to adequately study this issue. In summary, the clinical relevance of measuring NTBI and/or labile iron in plasma in disease states with chronic exposure is established, while linkage to any clinical outcomes in acute exposure scenarios (e.g., after IV iron administration) has not been established.

CONFLICT OF INTEREST

MG declares consultancy agreement with BMS and Vifor and advisory committee with Vifor. JBP declares consultancy agreement (Celgene BMS, bluebird bio, Agios), honoraria (Silence Therapeutics, Vifor, Celgene BMS, bluebird bio, La Jolla Pharmaceuticals, Protagonist), advisory committee (Silence Therapeutics, Vifor, Celgene BMS, bluebird bio). IC, CH and RH declare no conflict.

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How to cite this article: Garbowski MW, Cabantchik I, Hershko C, Hider R, Porter JB. The clinical relevance of detectable plasma iron species in iron overload states and subsequent to intravenous iron-carbohydrate administration. *Am J Hematol*. 2023;98(3):533-540. doi:[10.1002/ajh.26819](https://doi.org/10.1002/ajh.26819)