THE Hematologist
ASH NEWS AND REPORTS®
MARCH/APRIL 2015 VOLUME 12 ISSUE 2

4 **ASK THE HEMATOLOGISTS –**
Drs. Frits Van Rhee and David Fajgenbaum discuss approaches to Castleman disease.

6 **MINI REVIEW: LABILE IRON –**
Drs. Ioav Cabantchik and Eliezer Rachmilewitz cover toxicity in iron overload.

Labile iron: Potential Toxicity in iron overload Disorders

Hematologist: ASH News and Reports

Zvi ioav Cabantchik[1] and Eliezer Rachmilewitz[2]

1. Professor emeritus, A&M Dellapergola Chair in Life Sciences, Hebrew University of Jerusalem, Edmond and Lilly Safra Campus Givat Ram, Jerusalem, Israel
2. Professor emeritus, Hadassah Medical School of the Hebrew University of Jerusalem; Hematology Department, Edith Wolfson Medical Center, Holon, Israel

Iron homeostasis relies on a regulated network of systemic and cellular mechanisms for the acquisition, transportation and cellular utilization of the metal.[1] once in inner body fluids, iron is swiftly captured by a chemically shielded vehicle (transferrin) that circulates and safely delivers it to cells commensurate with their metabolic needs. In the cellular milieu, most of the iron is also protein associated, either directly or via heme or iron-sulfur-cluster moieties. However, the biosynthesis of these groups depends to a large extent on the availability of a basal level of redox-active and mobilizable iron, which we define as labile cell iron (LCI), often also referred to as the labile iron pool.[2] The obligatory maintenance of a physiological level of labile iron is not devoid of potential liabilities, as labile iron has the capacity to catalyze the conversion of natural reactive oxygen intermediates (ROIs) of the respiratory chain (e.g., $O_2^{\cdot-}$ and H_2O_2) to noxious reactive oxygen species (ROS; e.g., $OH^{\cdot}$) that can damage proteins, lipids, and nucleic acids. This creates a continuous burden on cells to swiftly eliminate ROIs by enzymatic reactions (superoxide dismutase, peroxidases, and catalases) aided by reducing/antioxidant agents such as glutathione and the cellular reductants NADPH and NADH. Moreover, being devoid of extrusion tools to relieve themselves of iron, cells must cope with fluctuations in labile iron levels by balancing iron intake according to utilization, and also by producing the requisite amount of ferritin units to absorb "surplus" labile iron (Figure).

It is widely accepted that a disruption of links in systemic or cellular iron networks can lead to an aberrant buildup of cell iron, either due to excessive amounts of circulating iron in the plasma and/or by a mismatch in cell iron distribution. Cells endowed with limited iron shielding capacities and/ or antioxidant power are the most susceptible to damage generated by iron accumulated in mitochondria, the primary site of ROI/ROS formation. This is classically demonstrated in cell and animal models of systemic iron overload disorders (IODs) that recapitulate the clinical scenario of patients with primary or transfusional hemosiderosis. In that scenario, an outpouring of iron from gut or reticulo-endothelial cells that is not matched locally by sufficient circulating apotransferrin not only leads to an upsurge in plasma iron, but also to the accumulation of forms of iron not bound to transferrin (NTBI†) .[3-5] Such forms can infiltrate cells opportunistically by resident transporters or channels, resulting in tissue iron overload and end-organ failure. Cellular siderosis with pathologic outcomes in single or multiple organs (brain, heart, endocrine glands), or hematopoietic cells are also found in the absence of plasma IO (e.g., Freidreich Ataxia; Figure). This regional type of siderosis generally results from abnormalities (genetic or acquired) in cell iron utilization[6] that perpetuate metal and oxidative damage to the cell.[2] However, in either systemic or regional siderosis, it is the labile iron pool that builds up in mitochondria and results in siderotic damage that, in some cases, can be prevented or significantly reduced by chelators that can gain access to the organelle.[2]

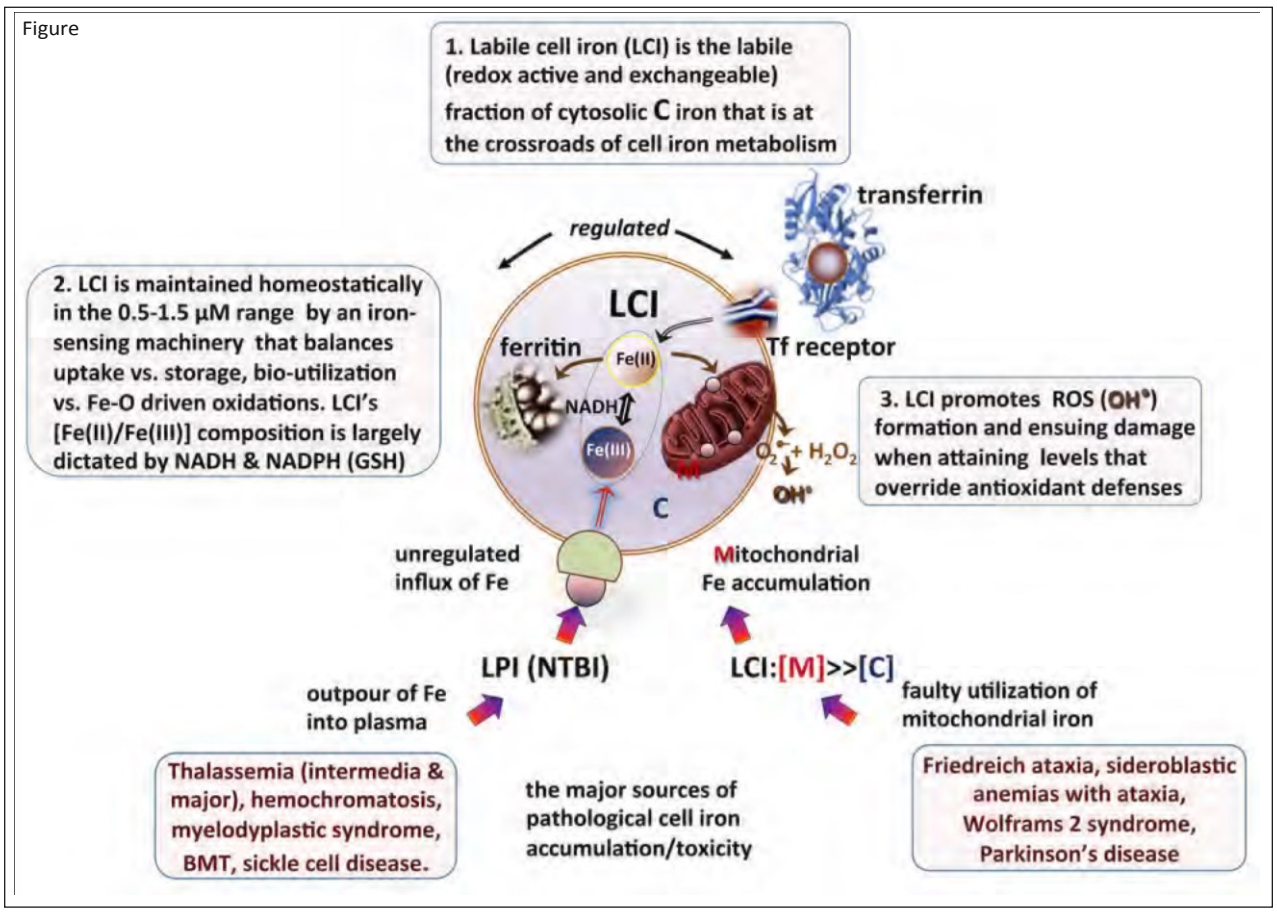
The etiopathologic features of siderosis described herein are of clinical importance because they determine to a large extent the guidelines for clinical assessment of hemosiderosis. Those guidelines rely presently on surrogate markers of liver iron storage such as serum ferritin (when not confounded by inflammation) and increasingly on measurements of organ-accumulated metal assessed by noninvasive multiorgan spectroscopy (T2 and T2* MRI) that detects signals associated with clusters of iron composed of ferritin and/or hemosiderin units.[7,8] However, as these markers are essentially end-organ indicators of iron accumulation, but not the actual perpetrators of siderotic cell damage or the direct targets of chelators that neutralize labile iron, their detection might be out of phase with natural disease progression or with functional response to treatment. Could markers associated with upstream factors of impending iron overload, such as plasma NTBI or labile plasma iron (LPI), or with downstream factors such as LCI changes in particular cell types, provide information of clinical value? Such information could potentially be useful for early detection of iron overload and also for assessing the adequacy of a chelation regimen in continuously maintaining LPI-free plasma. until recently, plasma NTBI and its chemically labile component LPI have been measured extensively in healthy individuals and in patients with congenital or acquired IODs (e.g., thalassemia major and intermedia, sickle cell disease, myelodysplastic syndrome, bone marrow transplantation, chemotherapy, hemochromatosis, etc.),[5,9-11] particularly during treatment. Briefly, those studies indicated that: 1) either NTBI or LPI is virtually undetectable in normal individuals and detectable in greater than 90 percent of patients with transferrin saturation (TSAT) greater than 70 to 75 percent; 2) administration of chelators such as deferrioxamine (DFO; intravenously), deferiprone (DFP; orally), or deferasirox (DFR; orally) can virtually eliminate LPI within minutes (DFO) or within one to two hours (DFO or DFR) and maintain it at basal levels (< 0.2 μM) for different time periods, depending on the dose and frequency of chelator administration (*vis-á-vis* its pharmacokinetics and the rate at which NTBI resurges in the plasma of a given patient); and 3) chelation regimens can attain daily coverage from LPI resurgence in most thalassemia intermedia patients treated with either DFP (25 mg/kg twice daily) or DFR (20 mg/ kg once daily) and in 40 percent of thalassemia major patients treated thrice daily with DFP (total 75 mg/kg) or with DFR (40 mg/kg once daily), whereas combined treatment of daily DFP and nightly DFO can attain full-day coverage in greater than 95 percent of patients.[9] A decisional algorithm to start iron chelation in thalassemia major patients has been proposed on the basis of threshold TSAT values which in poly-transfused patients are invariably accompanied by the presence of LPI.[12]

Although NTBI and LPI methodologies still need to be clinically validated and standardized for different IODs, they have reached a stage where they can not only provide insights into the pathobiology of hemosiderosis, but can also serve as diagnostic tools for identifying the presence of potentially toxic species in plasma whose selective elimination is not only attainable but also recommended.

†**Note**: The three-decades-old term "plasma NTBI"[3] has lately been amply recognized in the pathophysiology of systemic iron overload as a pathological component of plasma iron composed of chemical forms (not bound to transferrin) that can infiltrate cells and overload them with iron. The capturing of plasma NTBI by iron chelators and their elimination from circulation provides the basis for "iron overload" prevention in various IODs. However, the intrinsically apophatic "NTBI" term should not be used for iatrogenic NTBI agents such as iron polymers used clinically for parenteral iron supplementation or iron chelates that are formed *in vivo* following chelation treatment.

Dr. Cabantchik and Dr. Rachmilewitz indicated no relevant conflicts of interest.

Figure



1. Ganz T. systemic iron homeostasis. Physiol Rev. 2014;93:1721-1741.
2. Cabantchik ZI. Labile iron in cells and body fluids: physiology, pathology, and pharmacology. Front Pharmacol. 2014;5:45.
3. Hershko C, Graham G, Bates GW, et al. Non-specific serum iron in thalassaemia: an abnormal serum iron fraction of potential toxicity. Br J Haematol. 1978;40:255-263.
4. Breuer W, Hershko C, Cabantchik ZI. The importance of nontransferrin bound iron in disorders of iron metabolism. Transfus sci. 2000;23:185-192.
5. Brissot P, Ropert M, Le Lan C, et al. Non-transferrin bound iron: a key role in iron overload and iron toxicity. Biochim Biophys Acta. 2012;1820:403-410.
6. Rouault TA. Iron metabolism in the CNs: implications for neurodegenerative diseases. Nat Rev Neurosci. 2013;14:551–564.
7. Detterich J, Noetzli L, Dorey F, et al. electrocardiographic consequences of cardiac iron overload in thalassemia major. Am J Hematol. 2012;87:139-144.
8. Baksi AJ, Pennell DJ. Randomised controlled trials of iron chelators for the treatment of cardiac siderosis in thalassaemia major. Pharmacology. 2014;5:2014.
9. Zanninelli G, Breuer W, Cabantchik ZI. Daily labile plasma iron as an indicator of chelator activity in thalassaemia major patients. Br J Haematol. 2009;147:744-751.
10. Hider RC, silva AM, Podinovskaia M, et al. Monitoring the efficiency of iron chelation therapy: the potential of nontransferrin-bound iron. Ann N Y Acad sci. 2010;1202:94-99.
11. Aydinok Y, evans P, Manz CY, et al. Timed non-transferrin bound iron determinations probe the origin of chelatable iron pools during deferiprone regimens and predict chelation response. Haematologica. 2012;97:835-841.
12. Danjou F, Cabantchik ZI, origa R, et al. A decisional algorithm to start iron chelation in patients with beta thalassemia. Haematologica. 2014;99:e38-e40.