❋ CHAPTER 25

Evaluation and treatment of secondary iron overload

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1. Conditions associated with secondary iron overload

The term secondary iron overload defines a heterogeneous group of chronic conditions, genetic or acquired, where iron overload is not due to a primary defect of the iron regulation system. Table 1 contains a partial list of these conditions, most of which are characterised by anaemia.

Table 1: **A partial list of conditions potentially associated with secondary iron overload**
A. Hereditary disorders
Thalassaemias
Glucose-6-phosphate dehydrogenase (G6PD) deficiency
Pyruvate kinase deficiency
Congenital dyserythropoietic anaemias
Severe haemolytic anaemias (Hereditary spherocytosis, etc.)
Sideroblastic anaemias
Porphyrias
Aplasia
B. Acquired disorders
Aplasia
Sideroblastic anaemia
Dyserythropoietic anaemias
Myelodysplastic syndromes
Liver diseases
Dietary iron overload
Inappropriate iron treatment (iron deficiency, dialysis patients, athletes, etc.)
Off-therapy leukaemias, bone marrow transplant recipients
Post-portacaval shunting
Dysmetabolic iron-overload syndrome

The source of iron may be parenteral, from transfusions or iron compounds, or from increased oral intake (diet, iron compounds, or enhanced iron absorption due to ineffective erythropoiesis or liver disease). The common pathophysiological mechanism is often the down-regulation of hepcidin expression (1). More than one factor may be present in the same patient. The coexistence of mutations involved in genetic haemochromatosis may or not aggravate a secondary iron overload (2). In the past the clinical relevance of iron overload and its treatment for the most severe of the above conditions was limited by the poor prognosis of the underlying disease, which overshadowed the long term risk of iron-related complications.

Today several factors facilitate a more comprehensive approach: advances in diagnostics, progress in the management of the underlying diseases, especially the improved long-term results obtained in thalassaemia, and the availability of new oral chelators. There is growing interest in a variety of conditions where iron overload may be less obvious. These include patients with low risk myelodysplastic syndromes (3, 4) and patients who have been treated for leukaemias (5) and lymphomas or have received stem cell transplantation (6).

2. Evaluation of iron overload

An accurate assessment of iron status is initially required, to evaluate its clinical relevance, the need for treatment, and the timing and monitoring of therapy. Different diagnostic tools may be useful in evaluating different aspects of iron overload (Table 2). The diagnostic methods may also be distinguished on the basis of being direct approaches (atomic absorption spectrometry and SQUID magnetic susceptometry) or indirect (all the others).

Table 2: **Methods for iron overload assessment according to the type of evaluation**
A. Clinical features
• Signs and symptoms
• Iron-related complications
B. Serum markers
• Serum iron
• Serum transferrin
• Transferrin saturation
• Serum ferritin
C. Tissue iron concentration/Distribution
• Liver iron concentration (LIC) by:
- Liver biopsy
- SQUID magnetic susceptometry
- Quantitative MRI
• Cardiac MRI
• MRI of other tissues/organs
D. Iron toxicity markers
• Non transferrin bound iron (NTBI), labile plasma iron (LPI)
• Markers of oxidative damage
• Liver fibrosis
E. Iron balance calculations
• Iron load with transfusions
• Iron removal by phlebotomy
• Iron excretion by chelators

2.1 Clinical features

The timing of appearance of signs or symptoms depends on the rate of iron overload. In severe conditions such as thalassaemia major, they may appear in childhood, with skin hyperpigmentation, growth impairment, delayed puberty, cardiac arrhythmias and the onset of overt organ dysfunction, including congestive heart failure or diabetes. Otherwise, as in genetic haemochromatosis, signs and symptoms may be very late and non-specific: weakness, fatigue, loss of libido, and/or arthralgia. If the iron burden progresses and is not treated, all the clinical features may become manifest, with heart disease, diabetes, hypothyroidism, hypoparathyroidism, hypogonadism, and cirrhosis. A clear association with the risk of developing hepatocellular carcinoma has been established, at least for thalassaemias (7). In acquired conditions requiring regular blood transfusions, in particular MDS patients, the impact of iron overload is less clearly established, but seems to be more prone in low-risk patients where non-leukaemia related complications are the main causes of death (4).

2.2 Serum markers

Serum ferritin is the most widely used and the least expensive parameter for assessing iron status. A positive correlation exists between serum ferritin concentration and iron stores (8), but independent conditions may falsely elevate ferritin levels (cancer, hepatitis, inflammation, haemolysis, vitamin C deficiency) (9). Furthermore, the accuracy diminishes at high ferritin values and the correlation may have different slopes in different haematological conditions such as thalassaemia intermedia where serum ferritin values notably underestimate iron overload (10). Therefore serial assessments are recommended.

Transferrin saturation (TS) is usually extremely high in regularly transfused patients and its level may suggest the site of iron accumulation (reticuloendothelial iron overload alone is associated with normal TS, whereas parenchymal iron overload leads to a high TS value).

2.3 Iron toxicity markers

A high transferrin saturation value and other markers may be useful in the assessment of iron toxicity. When serum transferrin is almost fully saturated (above 70%) a toxic fraction of plasma iron appears. This is called non-transferrin-bound (NTBI) or labile plasma iron (LPI), according to the method used to measure it (11, 12). NTBI promotes the formation of free hydroxyl radicals and the peroxidation of membrane lipids. It possibly represents the fraction of iron directly involved in iron-induced tissue toxicity and is related with the intracellular labile iron pool (LIP).

Preliminary results, showed a positive correlation between the presence of serum NTBI in thalassaemic patients with transfusional iron overload and an increased risk of siderotic heart disease (13). However these assessments are limited to few laboratories and clinical correlations are up to now limited (13).

Iron toxicity may be assessed also looking at levels of lipid peroxidation products as malondialdehyde, and physiological antioxidants as vitamin E, A and C. The clinical significance of these changes and the benefits of antioxidant supplementation have not been fully explored by controlled studies.

2.4 Tissue iron concentration/distribution

The liver contains most of the body iron stores (70-80%) and is the main crossroads of iron trafficking (storage from intestinal absorption and from red-cell catabolism, chelation by iron chelating drugs, excretion through the bile). Where liver biopsy is performed, the histology may provide a semi-quantitative evaluation of iron load and its distribution, the degree of fibrosis or cirrhosis, and possible independent factors as viral hepatitis, alcohol and steatosis.

Liver iron concentration (LIC) determined chemically after a liver biopsy has been till recently the gold standard (14) and was largely used in prospective studies on iron chelators (15-17). LIC is well correlated with total body iron stores in thalassaemia and haemochromatosis (14, 18).

High LIC level predict cardiac disease and early death in thalassaemia (19). Recent advances in non-invasive techniques for the assessment of LIC (20, 21) and liver fibrosis (22) have drastically reduced the indications for performing a liver biopsy in iron overload conditions.

A comparison of these techniques with tissue biopsy is summarised in Table 3.

Table 3: **Characteristics of method for assessment of iron overload**			
	Biopsy (AAS)	**SQUID Susceptometry**	**MRI**
Invasive	Yes	No	Poorly
Direct method	Yes	Yes	No
Sampling error	Yes	No	No
Histology	Yes	No	No
Availability	Yes	Poor	Yes
Validation (Liver)	Yes	Yes	Yes
Heart assessment	Not useful	No	Yes

AAS: atomic absorption spectrometry

2.5 SQUID magnetic susceptometry

Superconducting quantum interference device (SQUID) susceptometry is potentially the most accurate non-invasive method for quantitative estimation of the LIC (19). The peculiar paramagnetic response of iron in ferritin and haemosiderin in a constant magnetic field is detected by a very sensitive SQUID. The system has been validated with chemically measured LIC, demonstrating direct linearity up to 12 mg/g dry weight (23). It has been applied to the evaluation of the long-term efficacy of iron chelators (24, 25-27) and the relationship between serum ferritin and LIC (28, 29). Availability is however poor, with only four systems working in the world. Current research on room temperature susceptometers may make these precise instruments less expensive and more widely available.

2.6 Magnetic resonance imaging (MRI)

The iron concentration is indirectly assessed by the effect of ferritin and haemosiderin iron in shortening proton relaxation times and in decreasing the signal intensity and getting the tissue darker (30). If specific methods in strict conditions are applied, MRI may have low variability (31), good transferability (32, 33), inter-scan reproducibility (34, 35) and the ability to assess iron loading in various organs. MRI assessment of liver iron load is largely validated, and was found to be well correlated with chemically measured LIC values in many studies (20, 31, 36-38). However, methods remain numerous and not standardised, mainly because several technical parameters are able to influence the level of precision: magnetic field strength, imaging sequences (time echo (TE), repetition time), type of proton relaxation time studied (T2 or R2,1/T2,T2*, signal intensity ratio liver/muscle), mathematical method used to analyse the relaxation curve) (37).

The pros and cons of the commonly used methods are summarised in Table 4.

Table 4: **: Pros and cons of main MRI methods used**			
Technique	**References**	**Advantages**	**Drawbacks**
Gradient-recalled-echo imaging. Liver/muscle intensity ratio	Gandon Y Rose C	Routine MR scanners Free methods for signal analysis*	Less accurate
R2 (1/T2) relaxometry	St Pierre	Accuracy	Requires phantoms and each scan requires a centralised data analysis
R2* (1/T2*) relaxometry	Anderson LJ Virtanen JM	Transferability between different scanners	

*http://www.radio.univ-rennes1.fr/Sources/EN/HemoCalc15.html
http://oernst.f5lvg.free.fr/liver/iron.html

2.6.1 Cardiac MRI

As the primary cause of death in severe iron overload and low risk MDS is heart failure, an affordable cardiac assessment is extremely useful for clinical management. In the past many limitations have led to difficulting achieving this aim: the uneven distribution of iron in the heart (39), its relatively low concentration (10 times lower than in the liver), blood flow and motion artifacts and the limited possibility of validating MRI measurements against tissue samples. Nevertheless cardiac MRI (myocardial T2*) is today able to accurately assess the magnitude of cardiac iron overload (40). A retrospective study on thalassaemic patients on deferoxamine (DFO) treatment found a significant correlation between myocardial T2* and left ventricular function (21). Many patients with a T2* below 20 ms had ventricular dysfunction (40). Cardiac T2* did not correlate with serum ferritin value and LIC (21). This is true only in patients on long term chelation, but led to the important finding that both iron loading and iron clearance follow different patterns in the liver and the heart (41). Up to now data on cardiac iron in conditions other than thalassaemia, such as MDS, are limited and conflicting (42-44). As cardiac iron loading appears late in thalassaemic patients on regular transfusion and chelation (45), an MRI assessment is not needed before 6-8 years of age, when it requires anaesthesia.

2.6.2 Iron load in other tissues

Appropriate MRI acquisition techniques allow an estimation of iron in target tissues (pancreas, thyroid, pituitary, hypothalamus (46, 47)) and may be of help in preventing the other clinical complications of iron overload.

2.7 Iron balance calculations

2.7.1 Iron load with transfusions

Knowing that each gram of haemoglobin contains 3.4 mg of iron, the precise amount of transfusional iron can be easily and accurately estimated. Depending on data given by the blood bank (weight, volume, haematocrit or total haemoglobin), simple calculations may be applied (Table 3). Transfusion-dependent conditions such as aplasias or severe thalassaemias have a blood consumption of 100-200 mL/kg/year of red blood cells, corresponding to 0.32-0.64 mg/kg/day. Differences among patients may be significant (varying from 0.15 to 0.80 mg/kg/day), depending on the underlying condition, transfusional scheme, spleen status and the presence of red cell immunisation. In transfusion dependent patients the accurate recording of transfused iron must be part of high quality monitoring and is relevant for efficient iron chelation (48).

2.7.2 Quantitative phlebotomy

The amount of iron removed by repeated phlebotomies gives a retrospective, accurate, direct measure of the total body iron stores. This is important in patients with genetic haemochromatosis, as survival and morbidity are related to the peak iron load. The same approach is applicable to patients with transfusion-dependent anaemias, such as when a successful bone marrow transplantation is followed by phlebotomy (5, 49). In thalassaemia patients a close correlation has been demonstrated between the iron removed and the liver iron concentration at the start of phlebotomies (14). This means that it is possible to estimate the total iron stores from a single liver iron concentration measurement, applying a simple formula (Table 5).

Table 5: **Iron balance calculations**
A. Transfused iron (mg)
• Weight (grams) x haematocrit (ratio) x 1.16
• Volume (mL) x 1.056 x haematocrit (ratio) x 1.16
• Haemoglobin (total grams) x 3.4
B. Iron removed by phlebotomy (mg)
• Patient's Hb (g%) x blood removed (mL) x 0.034
C. Total body iron stores (mg/kg)
• Liver iron concentration (mg/kg dry weight x 10.6)

2.7.3 Iron excretion by chelators

Iron excretion induced by chelators is the sum of urinary and faecal fractions. For deferoxamine urinary iron excretion (UIE) represents around 50% of total excretion (50), for deferiprone 80-98% (51) and for deferasirox less than 5% (52). The evaluation of faecal iron excretion is laborious and limited to iron balance trials. The 24 hour UIE after deferoxamine or deferiprone may be useful in clinical practice in monitoring the iron chelation efficacy (53). UIE depends only in part on drug dose and degree of iron overload. For deferoxamine, the administration route and delivery time, the degree of erythropoietic expansion, the ascorbate status and liver disease influence the response, with important individual variations in the fraction of faecal iron excretion. For deferiprone, the glucuronidation efficiency appears to depend almost only on UGT1A6, especially in the liver. Genetic variations and differences in the expression of splice variants represent a potential source of variation in deferiprone metabolism (54).

3. Treatment of iron overload

3.1 Phlebotomy
Phlebotomy is the reference treatment for iron overload in genetic haemochromatosis. It may be applied also to patients after successful bone marrow transplantation for thalassaemia and other conditions, as indicated previously (49, 55).

3.2 Iron chelation therapy
The aims of iron chelation are the prevention of iron-related complications, the maintenance of safe tissue iron levels and the reversal of iron-related complications (56). More than 30 years of research have led to important advances in iron chelation (Figure 1).

Figure 1: **Overview of iron chelation**
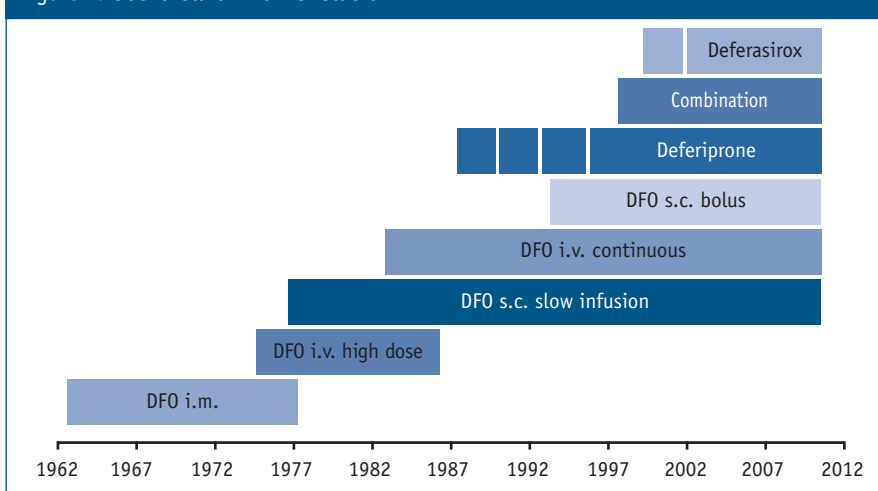
DFO: deferoxamine. Hatched lines in deferasirox and deferiprone represent development period.

3.2.1 Deferoxamine
A single excellent drug, deferoxamine (DFO) has been available for many years. DFO displays a strong and specific affinity for iron with a theoretically capability of binding 8.5 mg ferric iron every 100 mg of DFO. The subcutaneous slow infusion has been the standard iron chelation choice since the end of the seventies. In thalassaemia major the regular use of DFO resulted in an impressive improvement in life expectancy and a reduction in the prevalence and severity of iron-related clinical

complications. With experience and skill it is possible to limit side effects and optimise compliance in most patients (57).

The standard prescription is a slow subcutaneous infusion over 8-12 hours of a 10% DFO solution by an infusion pump at a standard dose of 20-40 mg/kg for children, and up to 50-60 mg/kg for adults. Alternatives in treatment modalities, such as s.c. bolus injection or i.v. continuous infusion enable DFO to be used in a wide range of conditions and special needs. Symptomatic heart disease can be reversed by high dose intravenous treatment.

Recent data show that a significant proportion of patients on long-term s.c. DFO still have evidence of cardiac iron load despite low serum ferritin levels (58). A DFO-starch polymer combination, 40SD02 regained attention and is now in phase II development. With a single i.v. infusion it is possible to obtain a significant iron excretion lasting up to several days, with limited side effects (59, 60).

3.2.2 Oral iron chelators

During the past few years the body of advances on iron chelation research has been impressive, leading to the development of new oral chelators (61). Deferiprone (DFP) (Apotex, Toronto, ON, Canada) also known as L1, CP20, Ferriprox and Kelfer, is a 1,2 dimethyl-3hydroxypyrid-4-one, was initially synthesised in 1982, but its development did not follow a systematic design. DFP is a bidentate molecule that forms 1:3 iron chelator complexes, absorbed with a mean half-life of 160 and 91 minutes in two different studies (62, 63). More than 90% of free drug is eliminated from plasma within 3-6 hours of ingestion and excreted in urine. The drug is inactivated by glucuronidation (64). From pharmacokinetic and metabolic studies it was shown that 75 mg/kg/day of DFP on 7 days/week overall produces a similar level of iron excretion to DFO 40 mg/kg given subcutaneously on 5 days/week (64). Because of the short half-life, the recommended dosage is 75 mg/kg/day divided in three doses. Several aspects of its safety and efficacy led to serious discussions and controversies (64). The safety profile requires close monitoring. Nowadays many independent studies indicate an overall efficacy similar to DFO (26, 65, 66). A systematic review based on the Cochrane database (67) showed no consistent difference in reduction of iron overload between DFO and DFP treatment. Due to its membrane crossing ability, DFP has been shown to shuttle tissue iron into circulation; studies in iron-loaded rat heart cells and in gerbils had shown its efficacy in removing iron from myocardial cells at concentrations that can be achieved at a therapeutic dosage (68). A retrospective study compared 54 thalassaemia major patients treated with DFP with 75 treated with DFO for an average of 6 years. Survival and heart disease rates were significantly better in the DFP group (69). A further

study on a larger series confirmed this type of difference between the two drugs (70). A randomised controlled on 61 thalassaemia major patients over 1 year of treatment of DFO or DFP, showed changes in myocardial T2* and LVEF significantly greater for DFP than DFO (71). Several studies suggest that doses up to 100 mg/kg/day may be safely utilised where indicated (64).

Deferasirox (ICL670, Exjade™) (DFX), a tridentate-bis-hydroxypheny-triazole chelator has been recently approved for treating transfusional iron overload. The drug is highly lipophilic and 99% protein-bound. Two molecules of DFX are required to bind one Fe^{3+}, forming a stable 1:2 iron-chelator complex (72). Due to its long half-life (8-16 hours), it can be taken once a day, 30 minutes before a meal, as the type of food, caloric and fat content influence DFX availability (73). Rapidly absorbed, DFX can efficiently and selectively mobilise iron from hepatocytes and cardiomyocytes, and can promote iron excretion. The DFX-iron complex is excreted in the faeces and not redistributed (71, 74). No significant differences in the pharmacokinetic, safety and efficacy profile of DFX were observed in paediatric and adolescent patients compared with adults (75). The results of pivotal data from a large-scale, randomised Phase III trial in transfusion-dependent beta-thalassaemia patients, showed that iron balance is achieved at 20 mg/kg/day, and significantly reduction in iron burden was observed at 30 mg/kg/day (76). The response to DFX depends both on dose transfusional iron intake (48). In other conditions including sickle cell disease and Diamond-Blackfan anaemia (DBA) efficacy results are similar to those observed in thalassaemia (77, 78).

Recent data on patients with beta-thalassaemia demonstrate that daily trough levels of DFX suppress LPI for 24 hours (79). In regularly transfused MDS patients, DFX is also efficient in reducing LIC (78). In this condition there is a higher rate of adverse events and more frequent discontinuation of therapy (80). Indications are only based on an expert consensus (81), additional data are required for a better definition of patients with the best risk-benefit ratio (82). In a recent study on MDS patients DFX may reduce transfusion requirement (83). The hypothesis that there is a direct effect of DFX on the neoplastic clone is under investigation (84). At high doses DFX shows a positive effect on heart iron (85-87). A systematic review on DFX analyses also its economic aspects (88).

Table 6 shows the properties of available iron chelators.

3.2.3 Side effects of iron chelation

Most of the side effects of iron chelation are caused by the iron subtraction from iron-dependent physiological pathways. Age, high doses of chelator and low level of iron overload are the main risk factors, whereas certain side effects are

Table 6: **Comparison of iron chelators**			
Property	**Deferoxamine**	**Deferiprone**	**ICL670**
Chelator: iron binding	1:1	3:1	2:1
Usual dosage	25-40 mg/kg/d	75 mg/kg/d	20-30 mg/kg/d
Route of administration	Subcutaneous, intravenous	Oral, 3 times daily	Oral, once daily
Half-life	20-30 minutes	3-4 hours	12-16 hours
Excretion	Urinary, faecal	Urinary	Faecal
Effect in lowering liver iron	+++	From – to ++++	+++
Effect in lowering heart iron	>40 mg/kg and i.v. use	75-100 mg/kg	30-40 mg/kg
Difficulty in compliance	+++++	++	+

characteristic of each drug. For detecting early iron chelation toxicity and minimising
its consequences, a close monitoring schedule should be individually tailored. This
may include: auxological assessment (weight, body fat, standing and sitting height,
pubertal stages, radiological assessment of bone age and the main metaphyses),
bone densitometry, liver function tests, ophthalmological examination, audiometry,
plasma zinc, rheumatological assessment are recommended. For DFO specific
attention must be paid to early signs of infection for diagnosis and treatment of
iron related complications as Yersinia enterocolitica septicaemia. For DFP weekly
check of absolute neutrophil count is required. For DFX regular renal function
monitoring is recommended. Table 7 shows a comparison of side effects of available
iron chelators.

3.2.4 New approaches to treatment

The availability of more than one drug stimulated the search for benefits from
combination therapy. Some *in vitro* data suggested the potential of an additive and
even synergistic effect (89). These effects have been demonstrated *in vivo*. In a
randomised trial in thalassaemia patients, the results of combined treatment were
superior to DFO alone in removing myocardial iron and improving cardiac and
endothelial function (90). Other studies confirmed these findings (91, 92). Many
independent papers on the reversal of heart failure by combination therapy are
available, even if most are single case or small series reports (93). A single
uncontrolled study suggests that combination therapy may reverse endocrinological
complications such as glucose intolerance in thalassaemia patients (94).
The term "combination" is presently used for a wide range of treatment schemes
that need to be distinguished, including the daily taking of both drugs at full doses

Table 7: **Comparison of iron chelators side effects**			
Side effects	**Deferoxamine**	**Deferiprone**	**ICL670**
Local side effects	Yes	No	No
G.I. symptoms	Rare	Yes	Yes
Skin rashes	No	No	Yes
Growth arrest	Yes	No	No
Bone changes	Yes	No	No
Osteoporosis	Yes ?	No	No
Liver enzymes changes	No	Yes	Yes
Yersinia infection	Yes	?	No
Arthropathy	Rare	Yes	No
Retinal toxicity	Yes	No	No
Lens opacity	Rare	No	Rare
Hearing loss	Yes	No	No
Renal changes	High doses	No	Yes
Weight gain	No	Yes	No
Agranulocytosis	No	Yes	No
Neutropenia	Rare	Yes	Rare

and simultaneously, or alternating the two drugs during the week. The following is a suggested approach to a common terminology (95):

- *Monotherapy*: a single chelator is prescribed and taken for more than three months.
- *Alternate therapy*: in any single day only a single chelator is taken; the two chelators are alternated on a weekly, monthly or quarterly basis (e.g., DFP five days a week and DFO two days a week)
- *Combination therapy*: prescription of more than one chelator, to be taken on the same day for a significant part of the treatment period. Treatment may be:
  - *Sequential:* in a single day two chelators are taken in sequence; no substantial overlapping of the two drugs in the plasma (e.g., DFP three times a day and DFO night time).
  - *Simultaneous or concomitant:* in a single day two chelators are taken at the same time with substantial overlapping of the two drugs in the plasma.

Combination treatment may be considered every time there is a need to look for an additive or synergistic effect such as for reversing heart disease (90, 96). At the onset of clinical heart disease, it is important to minimise cardiotoxicity, suppressing NTBI by continuous treatment. Intensive DFO chelation with continuous intravenous infusion has been demonstrated to be effective. Subcutaneous 24 hours a day DFO infusions may be an alternative when i.v. treatment is not feasible. The addition

of DFP greatly enhances the efficacy and reduces the time needed to normalise tissue iron levels. Individually tailored combination may maintain efficacy and tolerability in patients with dose-related side effects to DFO or DFP.

Finally, different types of combination may be considered in the near future, looking at the different characteristics of the available chelators. Unpublished data on combined DFO/DFX seem to be promising.

4. Conclusions

The evaluation and monitoring of iron overload require an integrated use of several indices: clinical signs, genetic markers, iron toxicity markers, quantification of iron overload, evaluation of iron-related complications, indeces of chelation therapy efficacy. None of them alone is sufficiently informative to guide the doctor in making the necessary clinical decisions. Iron overload conditions can today be diagnosed early and accurately, thanks to the recent progress in molecular medicine. Advances in biochemical and instrumental diagnostics allow an accurate quantification of iron overload and iron-related toxicity. The new treatment options greatly enhance the efficacy of iron chelation for preventing and treating the iron overload complications, even in conditions once considered irreversible.

References

1. Nemeth E. Iron regulation and erythropoiesis. Curr Opin Hematol 2008; 15: 169-175.
2. Longo F, Zecchina G, Sbaiz L et al. The influence of hemochromatosis mutations on iron overload of thalassemia major. Haematologica 1999; 84: 799-803.
3. Rose C, Cambier N, Mahieu M et al. [Iron overload and myelodysplastic syndromes]. Transfus Clin Biol 2001; 8: 422-432.
4. Cazzola M, Della Porta M, Malcovati L. Clinical relevance of anemia and transfusion iron overload in myelodysplastic syndromes. Hematology Am Soc Hematol Educ Program 2008; 166-175.
5. Rose C, Ernst O, Hecquet B et al. Quantification by magnetic resonance imaging and liver consequences of post-transfusional iron overload alone in long term survivors after allogeneic hematopoietic stem cell transplantation (HSCT). Haematologica 2007; 92: 850-853.
6. Armand P, Kim H, Cutler C et al. Prognostic impact of elevated pretransplantation serum ferritin in patients undergoing myeloablative stem cell transplantation. Blood 2007; 109: 4586-4588.
7. Borgna-Pignatti C, Vergine G, Lombardo T et al. Hepatocellular carcinoma in the thalassaemia syndromes. Br J Haematol 2004; 124: 114-117.
8. Finch C, Bellotti V, Stray S et al. Plasma ferritin determination as a diagnostic tool. West J Med 1986; 145: 657-663.
9. Lee D, Jacobs DJ. Serum markers of stored body iron are not appropriate markers of health

effects of iron: a focus on serum ferritin. Med Hypotheses 2004; 62: 442-445.

10. Pakbaz Z, Fischer R, Fung E et al. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. Pediatr Blood Cancer 2007; 49: 329-332.

11. Cabantchik ZI, Breuer W, Zanninelli G, Cianciulli P. LPI-labile plasma iron in iron overload. Best Pract Res Clin Haematol 2005; 18: 277-287.

12. Cabantchik ZI, Kakhlon O, Epsztejn S et al. Intracellular and extracellular labile iron pools. Adv Exp Med Biol 2002; 509: 55-75.

13. Piga A, Longo F, Duca L et al. High nontransferrin bound iron levels and heart disease in thalassemia major. Am J Hematol 2009; 84: 29-33.

14. Angelucci E, Brittenham G, McLaren C et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000; 343: 327-331.

15. Piga A, Galanello R, Forni GL et al. Randomized phase II trial of deferasirox (Exjade, ICL670), a once-daily, orally-administered iron chelator, in comparison to deferoxamine in thalassemia patients with transfusional iron overload. Haematologica 2006; 91: 873-880.

16. Galanello R, Piga A, Forni GL et al. Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in pediatric patients with beta-thalassemia major. Haematologica 2006; 91: 1343-1351.

17. Cappellini MD, Cohen A, Piga A et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 2006; 107: 3455-3462.

18. Angelucci E, Barosi G, Camaschella C et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica 2008; 93: 741-752.

19. Brittenham GM, Badman DG. Noninvasive measurement of iron: Report of an NIDDK workshop. Blood 2003; 101: 15-19.

20. Gandon Y, Olivie D, Guyader D et al. Non-invasive assessment of hepatic iron stores by MRI. Lancet 2004; 363: 357-362.

21. Pennell DJ. T2* magnetic resonance and myocardial iron in thalassemia. Ann N Y Acad Sci 2005; 1054: 373-378.

22. Mirault T, Lucidarme D, Turlin B et al. Non-invasive assessment of liver fibrosis by transient elastography in post transfusional iron overload. Eur J Haematol 2008; 80: 337-340.

23. Brittenham G, Farrell D, Harris J et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med 1982; 307: 1671-1675.

24. Brittenham G, Griffith P, Nienhuis A et al. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med 1994; 331: 567-573.

25. Adamkiewicz T, Berkovitch M, Krishnan C et al. Infection due to Yersinia enterocolitica in a series of patients with beta-thalassemia: Incidence and predisposing factors. Clin Infect Dis 1998; 27: 1362-1366.

26. Fischer R, Longo F, Nielsen P et al. Monitoring long-term efficacy of iron chelation therapy by deferiprone and desferrioxamine in patients with beta-thalassaemia major: Application of SQUID biomagnetic liver susceptometry. Br J Haematol 2003; 12: 938-948.

27. Fischer R, Piga A, Harmatz P, Nielsen P. Monitoring long-term efficacy of iron chelation

treatment with biomagnetic liver susceptometry. Ann N Y Acad Sci 2005; 1054: 350-357.

28. Fischer R, Tiemann C, Engelhardt R et al. Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptometry. Am J Hematol 1999; 60: 289-299.

29. Pakbaz Z, Fischer R, Fung E et al. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. Pediatr Blood Cancer 2007; 49: 329-332.

30. Wood JC. Diagnosis and management of transfusion iron overload: The role of imaging. Am J Hematol 2007; 82(12 Suppl): 1132-1135.

31. Wood JC, Enriquez C, Ghugre N et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. Blood 2005; 106: 1460-1465.

32. Virtanen JM, Komu ME, Parkkola RK. Quantitative liver iron measurement by magnetic resonance imaging: in vitro and in vivo assessment of the liver to muscle signal intensity and the R2* methods. Magn Reson Imaging 2008; 26: 1175-1182.

33. He T, Kirk P, Firmin DN et al. Multi-center transferability of a breath-hold T2 technique for myocardial iron assessment. J Cardiovasc Magn Reson 2008; 10: 11.

34. Westwood MA, Anderson LJ, Firmin DN et al. Interscanner reproducibility of cardiovascular magnetic resonance T2* measurements of tissue iron in thalassemia. J Magn Reson Imaging 2003; 18: 616-620.

35. Westwood MA, Firmin DN, Gildo M et al. Intercentre reproducibility of magnetic resonance T2* measurements of myocardial iron in thalassaemia. Int J Cardiovasc Imaging 2005; 21: 531-538.

36. Rose C, Vandevenne P, Bourgeois E et al. Liver iron content assessment by routine and simple magnetic resonance imaging procedure in highly transfused patients. Eur J Haematol 2006; 77: 145-149.

37. Jensen PD. Evaluation of iron overload. Br J Haematol 2004; 124: 697-711.

38. St Pierre TG, Clark PR, Chua-anusorn W et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood 2005; 105: 855-861.

39. Barosi G, Arbustini E, Gavazzi A et al. Myocardial iron grading by endomyocardial biopsy. A clinico-pathologic study on iron overloaded patients. Eur J Haematol 1989; 42: 382-388.

40. Anderson LJ, Holden S, Davis B et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001; 22: 2171-2179.

41. Noetzli LJ, Carson SM, Nord AS et al. Longitudinal analysis of heart and liver iron in thalassemia major. Blood 2008; 112: 2973-2978.

42. Chacko J, Pennell DJ, Tanner MA et al. Myocardial iron loading by magnetic resonance imaging T2* in good prognostic myelodysplastic syndrome patients on long-term blood transfusions. Br J Haematol 2007; 138: 587-593.

43. Di Tucci AA, Matta G, Deplano S et al. Myocardial iron overload assessment by T2* magnetic resonance imaging in adult transfusion dependent patients with acquired anemias. Haematologica 2008; 93: 1385-1388.

44. Jensen PD, Jensen FT, Christensen T et al. Evaluation of myocardial iron by magnetic

resonance imaging during iron chelation therapy with deferrioxamine: Indication of close relation between myocardial iron content and chelatable iron pool. Blood 2003; 101: 4632-4639.

45. Wood JC, Origa R, Agus A et al. Onset of cardiac iron loading in pediatric patients with thalassemia major. Haematologica 2008; 93: 917-920.

46. Au W, Lam W, Chu W et al. A cross-sectional magnetic resonance imaging assessment of organ specific hemosiderosis in 180 thalassemia major patients in Hong Kong. Haematologica 2008; 93: 784-786.

47. Sparacia G, Midiri M, D'Angelo P, Lagalla R. Magnetic resonance imaging of the pituitary gland in patients with secondary hypogonadism due to transfusional hemochromatosis. MAGMA 1999; 8: 87-90.

48. Cohen A, Glimm E, Porter J. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 2008; 111: 583-587.

49. Piga A, Longo F, Voi V et al. Late effects of bone marrow transplantation for thalassemia. Ann N Y Acad Sci 1998; 850: 294-299.

50. Pippard M, Callender S, Finch C. Ferrioxamine excretion in iron-loaded man. Blood 1982; 60: 288-294.

51. Kontoghiorghes G, Goddard J, Bartlett A, Sheppard L. Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. Clin Pharmacol Ther 1990; 48: 255-261.

52. Galanello R, Piga A, Alberti D et al. Safety, tolerability, and pharmacokinetics of ICL670, a new orally active iron-chelating agent in patients with transfusion-dependent iron overload due to beta-thalassemia. J Clin Pharmacol 2003; 43: 565-572.

53. Porter J, Davis B. Monitoring chelation therapy to achieve optimal outcome in the treatment of thalassaemia. Best Pract Res Clin Haematol 2002; 15: 329-368.

54. Benoit-Biancamano M, Connelly J, Villeneuve L et al. Deferiprone glucuronidation by human tissues and recombinant UGT1A6: An in vitro investigation of genetic and splice variants. Drug Metab Dispos 2009, 37: 322-329.

55. Rose C, Ernst O, Hecquet B et al. Quantification by magnetic resonance imaging and liver consequences of post-transfusional iron overload alone in long term survivors after allogeneic hematopoietic stem cell transplantation (HSCT). Haematologica 2007; 92: 850-853.

56. Angelucci E, Barosi G, Camaschella C et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. Haematologica 2008; 93: 741-752.

57. Gabutti V, Piga A. Results of long-term iron-chelating therapy. Acta Haematol 1996; 95: 26-36.

58. Tanner M, Galanello R, Dessi C et al. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. J Cardiovasc Magn Reson 2008; 10: 12.

59. Mousa S, Ritger R, Smith R. Efficacy and safety of deferoxamine conjugated to hydroxyethyl starch. J Cardiovasc Pharmacol 1992; 19: 425-429.

60. Harmatz P, Grady R, Dragsten P et al. Phase Ib clinical trial of starch-conjugated

deferoxamine (40SD02): A novel long-acting iron chelator. Br J Haematol 2007; 138: 374-381.

61. Cappellini M, Piga A. Current status in iron chelation in hemoglobinopathies. Curr Mol Med 2008; 8: 663-674.

62. Matsui D, Klein J, Hermann C et al. Relationship between the pharmacokinetics and iron excretion pharmacodynamics of the new oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one in patients with thalassemia. Clin Pharmacol Ther 1991; 50: 294-298.

63. al-Refaie F, Hershko C, Hoffbrand A et al. Results of long-term deferiprone (L1) therapy: A report by the International Study Group on Oral Iron Chelators. Br J Haematol 1995; 91: 224-229.

64. Hoffbrand VA. Deferiprone therapy for transfusional iron overload. Best Pract Res Clin Haematol 2005; 18: 299-317.

65. Olivieri N, Brittenham G, Matsui D et al. Iron-chelation therapy with oral deferipronein patients with thalassemia major. N Engl J Med 1995; 332: 918-922.

66. Olivieri N, Brittenham G, McLaren C et al. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. N Engl J Med 1998; 339: 417-423.

67. Roberts D, Brunskill S, Doree C et al. Oral deferiprone for iron chelation in people with thalassaemia. Cochrane Database Syst Rev 2007: CD004839.

68. Hershko C, Link G, Pinson A et al. Iron mobilization from myocardial cells by 3-hydroxypyridin-4-one chelators: Studies in rat heart cells in culture. Blood 1991; 77: 2049-2053.

69. Piga A, Gaglioti C, Fogliacco E, Tricta F. Comparative effects of deferiprone and deferoxamine on survival and cardiac disease in patients with thalassemia major: A retrospective analysis. Haematologica 2003; 88: 489-496.

70. Borgna-Pignatti C, Cappellini M, De Stefano P et al. Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. Blood 2006; 107: 3733-3737.

71. Pennell D, Berdoukas V, Karagiorga M et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Blood 2006; 107: 3738-3744.

72. Nick H, Acklin P, Lattmann R et al. Development of tridentate iron chelators: From desferrithiocin to ICL670. Curr Med Chem 2003; 10: 1065-1076.

73. Galanello R, Piga A, Cappellini M, Forni G, Zappu A, Origa R et al. Effect of food, type of food, and time of food intake on deferasirox bioavailability: Recommendations for an optimal deferasirox administration regimen. J Clin Pharmacol 2008; 48: 428-435.

74. Nick H, Wong A, Acklin P et al. ICL670A: Preclinical profile. Adv Exp Med Biol 2002; 509: 185-203.

75. Galanello R, Piga A, Forni G et al. Phase II clinical evaluation of deferasirox, a once-daily oral chelating agent, in pediatric patients with beta-thalassemia major. Haematologica 2006; 91: 1343-1351.

76. Cappellini M, Cohen A, Piga A et al. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 2006; 107: 3455-3462.

77. Vichinsky E, Onyekwere O, Porter J et al. A randomised comparison of deferasirox versus

deferoxamine for the treatment of transfusional iron overload in sickle cell disease. Br J Haematol 2007; 136: 501-508.

78. Porter J, Galanello R, Saglio G et al. Relative response of patients with myelodysplastic syndromes and other transfusion-dependent anaemias to deferasirox (ICL670): A 1-yr prospective study. Eur J Haematol 2008; 80: 168-176.

79. Daar S, Pathare A, Jain R et al. T2* cardiovascular magnetic resonance in the management of thalassemia patients in Oman. Haematologica 2009; 94: 140-141.

80. Gattermann N, Schmid M, Porta MD et al. Efficacy and Safety of Deferasirox (Exjade(R)) during 1 Year of Treatment in Transfusion-Dependent Patients with Myelodysplastic Syndromes: Results from EPIC Trial. ASH Annual Meeting Abstracts 2008; 112: 633.

81. Bennett JM. Consensus statement on iron overload in myelodysplastic syndromes. Am J Hematol 2008; 83: 858-861.

82. Cazzola M, Della Porta MG, Malcovati L. Clinical Relevance of Anemia and Transfusion Iron Overload in Myelodysplastic Syndromes. Hematology 101182/ASH Education-20081166. 2008; 166-175.

83. Capalbo S, Spinosa G, Franzese M, Palumbo G. Early Deferasirox Treatment in a Patient with Myelodysplastic Syndrome Results in a Long-Term Reduction in Transfusion Requirements. Acta Haematol 2009; 121: 19-20.

84. Messa E, Cilloni D, Messa F et al. Deferasirox treatment improved the hemoglobin level and decreased transfusion requirements in four patients with the myelodysplastic syndrome and primary myelofibrosis. Acta Haematol 2008; 120: 70-74.

85. Otto-Duessel M, Brewer C, Gonzalez I et al. Safety and efficacy of combined chelation therapy with deferasirox and deferoxamine in a gerbil model of iron overload. Acta Haematol 2008; 120: 123-128.

86. Wood JC, Thompson AA, Paley C et al. Deferasirox (Exjade(R)) Monotherapy Significantly Reduces Cardiac Iron Burden in Chronically Transfused {beta}-Thalassemia Patients: An MRI T2* Study. ASH Annual Meeting Abstracts 2008; 112: 3882.

87. Pennell D, Porter JB, Cappellini MD et al. Efficacy and Safety of Deferasirox (Exjade(R)) in Reducing Cardiac Iron in Patients with {beta}-Thalassemia Major: Results from the Cardiac Substudy of the EPIC Trial. ASH Annual Meeting Abstracts 2008; 112: 3873.

88. McLeod C, Fleeman N, Kirkham J et al. Deferasirox for the treatment of iron overload associated with regular blood transfusions (transfusional haemosiderosis) in patients suffering with chronic anaemia: A systematic review and economic evaluation. Health Technol Assess 2009; 13: iii-iv, ix-xi, 1-121.

89. Link G, Konijn A, Breuer W et al. Exploring the "iron shuttle" hypothesis in chelation therapy: Effects of combined deferoxamine and deferiprone treatment in hypertransfused rats with labeled iron stores and in iron-loaded rat heart cells in culture. J Lab Clin Med 2001; 138: 130-138.

90. Tanner M, Galanello R, Dessi C et al. A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. Circulation 2007; 115: 1876-1884.

91. Maggio A, Vitrano A, Capra M et al. Long-term sequential deferiprone-deferoxamine versus

deferiprone alone for thalassaemia major patients: A randomized clinical trial. Br J Haematol 2009 Feb 18. [Epub ahead of print].

92. Aydinok Y, Ulger Z, Nart D et al. A randomized controlled 1-year study of daily deferiprone plus twice weekly desferrioxamine compared with daily deferiprone monotherapy in patients with thalassemia major. Haematologica 2007; 92: 1599-1606.

93. Mamtani M, Kulkarni H. Influence of iron chelators on myocardial iron and cardiac function in transfusion-dependent thalassaemia: A systematic review and meta-analysis. Br J Haematol 2008; 141: 882-890.

94. Farmaki K, Angelopoulos N, Anagnostopoulos G et al. Effect of enhanced iron chelation therapy on glucose metabolism in patients with beta-thalassaemia major. Br J Haematol 2006; 134: 438-444.

95. Piga A, Roggero S, Marletto F et al. Combined use of oral chelators and desferrioxamine in thalassemia. Hematology 2005; 10 Suppl 1: 89-91.

96. Hershko C, Cappellini M, Galanello R et al. Purging iron from the heart. Br J Haematol 2004; 125: 545-551.

Multiple Choice Questionnaire

To find the correct answer, go to http://www.esh.org/iron-handbook2009answers.htm

1. **The appearance of NTBI or labile plasma iron (LPI) occurs when transferrin saturation level is about:**
   a) 30% .... □
   b) 70% .... □
   c) 50% .... □
   d) 99% .... □

2. **Ferritin levels can be influenced by all these conditions *except* one:**
   a) Liver disease .... □
   b) Vitamin C deficiency .... □
   c) Inflammation .... □
   d) Hyperglycaemia .... □

3. **Which is the most accurate MRI technique for measuring cardiac iron?**
   a) Signal Intensity Ratio (SIR) .... □
   b) R2 .... □
   c) T2* .... □

d) None of the above .... ☐

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4. Which iron chelator has the longest half-life:
a) Deferoxamine .... ☐
b) Deferiprone .... ☐
c) Deferasirox .... ☐
d) Deferitrin .... ☐

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5.   In the management of iron chelation all the following are important
***except* one:**
a) Cardiac iron .... ☐
b) Transfusional iron input .... ☐
c) Liver iron concentration .... ☐
d) Serum iron .... ☐

NOTES