“PATIENTS AND HEALTH PROFESSIONALS TOGETHER FOR OPTIMAL CARE”

Clinical Management of Thalassaemia

“IRON OVERLOAD”

Aurelio Maggio
IRON OVERLOAD

MAIN ISSUES

• WHICH ARE THE KEY MECHANISMS CAUSING IRON OVERLOAD?
• HOW IS IT POSSIBLE TO DEFINE AND DETECT IRON OVERLOAD?
• ARE THERE ANY EVIDENCE OF GENETIC MODIFIERS INFLUENCING SEVERITY OF IRON OVERLOAD?
• WHICH IS THE CORRELATION BETWEEN ORGAN COMPLICATION AND IRON OVERLOAD?
• WHICH IS THE CORRELATION BETWEEN IRON OVERLOAD AND SURVIVAL?
• WHICH IS THE FUTURE IN IRON OVERLOAD MANAGEMENT?
A: Iron is reduced to the ferrous state by duodenal ferric reductase (Dcytb). B: Iron is transported into the cell by divalent metal transporter 1 (DMT1) and released by way of ferroportin. C: Hepatocytes take up iron from the circulation either as free iron or transferrin-bound iron. D: Transferrin receptor 2 may serve as a sensor of circulating transferrin-bound iron. E: Hepcidin is secreted into the circulation, where it downregulates the ferroportin-mediated release of iron from enterocytes, macrophages, and hepatocytes.
TRANSFUSIONAL IRON OVERLOAD

- One unit of transfused blood contains around 200–250 mg of iron\(^1\)
- Iron accumulates with repeated infusions
  - Chronic transfusion-dependent patients have an iron excess of \(~0.4–0.5 \text{ mg/kg/day}\^2\) (1 g/month)
  - Signs of iron overload can be seen after 10–20 transfusions\(^1\)
- Iron overload can have a significant impact on morbidity and mortality\(^3,4\)

3. Ballas SK. *Semin Hematol* 2001;38
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DEFINITIONS OF IRON OVERLOAD

• SERUM FERRITIN LEVEL >1000 ng/ml OR WORST >2500 ng/ml.


• LIVER IRON CONCENTRATION > 3.2 mg/gr/ dried weight >57,14 micro M/g / dried weight

THIS STATEMENT IS BASED ON HEREDITARY HEMOCHROMATOSIS CLINICAL STUDIES (Olivieri NF and Britthenam GM, Blood 1997)
DIRECT AND INDIRECT METHODS TO DETECT IRON OVERLOAD IN PATIENTS AFFECTED BY SECONDARY HEMOCHROMATOSIS

<table>
<thead>
<tr>
<th>METHODS</th>
<th>EVALUATION</th>
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<tbody>
<tr>
<td><strong>INDIRECT</strong></td>
<td></td>
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<tr>
<td><strong>FERRITIN</strong></td>
<td>• Easily, available</td>
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<tr>
<td></td>
<td>• Useful as an index of compliance with therapy</td>
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<tr>
<td></td>
<td>• Does not correlate with LIC in single cases</td>
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<td><strong>TRANSFERRIN SATURATION</strong></td>
<td>• Useful in genetic hemochromatosis and thalassemia intermedia</td>
</tr>
<tr>
<td></td>
<td>• Not useful in thalassemia major</td>
</tr>
<tr>
<td><strong>DIRECT</strong></td>
<td></td>
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<tr>
<td><strong>LIVER BIOPSY</strong></td>
<td>• “Gold standard” for the evaluation of liver damage (International Guidelines)</td>
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<td></td>
<td>• It is invasive, accuracy for LIC determination in presence of fibrosis and cirrhosis could be lower</td>
</tr>
<tr>
<td><strong>NMR (MRI)</strong></td>
<td>• Good correlation in several studies, particularly MRI using R2*</td>
</tr>
<tr>
<td></td>
<td>• Only available tool to evaluate heart iron</td>
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<tr>
<td><strong>SQUID</strong></td>
<td>• Reproducibility in very specialized centers</td>
</tr>
<tr>
<td></td>
<td>• Available only in few centers</td>
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</tbody>
</table>
SERUM FERRITIN CONCENTRATIONS ARE CORRELATED TO HEPATIC IRON CONCENTRATION IN THALASSEMIA MAJOR

LIVER IRON CONCENTRATION REFLECTS BODY IRON STORES

Regression line and 95% confidence limits (upper and lower) are shown
LIC = liver iron content
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• Twenty-five patients with iron overload, cirrhosis, and liver samples ≥1 mg dry weight, were assessed

• The results demonstrated the linear relationship between total body iron stores and LIC levels (r = 0.98, p<0.001)
MAGNETIC RESONANCE IMAGING-R2 METHODOLOGY SHOWS HIGHEST CORRELATION VERSUS BIOPSY LIVER IRON CONCENTRATION

$r=0.98$

$CV=2.1\%$

STAINABLE IRON CAN BE DETECTED IN THE HEART

Figure 1. Stainable iron in the left ventricular myocardium of two patients. **Top**, Severe iron deposition. **Bottom**, Mild iron deposition. (Iron stain; **Top**, ×360, reduced by 15%; **Bottom**, ×180, reduced by 15%.)

Olson LJ et al. JACC 1987 Dec;10:1239
Olson LJ et al. Cardiac iron deposition in idiopathic hemochromatosis: histologic and analytic assessment of 14 hearts from autopsy. JACC 1987 Dec; 10(6):1239-43

HEART IRON IS HETEROGENEOUSLY DISTRIBUTED WITH PREVALENCE IN THE SUBEPICARDIUM
NORMAL VALUES OF HEART IRON

Jensen P.D. et al., MAGMA, (2001)
Population: 5 Normal Autopsy Controls
Results: $224 \pm 59$ (range 165 to 312) µg/gr/dw

Olson L.J. et al., JACC, (1987)
Population: 14 Normal Autopsy Controls
Results: $399$ (range 183 to 674) µg/gr/dw

Consideration:
• Small iron burden variations in sites as the heart could have very dangerous effects without any influence on the overall body iron burden;
NO CORRELATION WAS SHOWN BETWEEN GLOBAL HEART T2* VALUES AND LIVER T2*

$R = -0.2; P = 0.3$
T2* MAGNETIC RESONANCE IMAGING IS ABLE TO DETECT SMALL VARIATIONS OF HEART IRON CONCENTRATION

![Graph showing normal and pathological cardiac iron concentration levels](image-url)
HEART T2* MRI MULTIECHO MULTISLICE TO BETTER STUDY HETEROGENEITY PATTERN OF HEART IRON

apical

medium

basal
MAGNETIC RESONANCE IMAGING OF HYPOPHYSIS

A) NORMAL PICTURE

B) HYPOINTENSITY SUGGESTING ORGAN IRON OVERLOAD

From “Clinica e Terapia della Talassemia”, G. Fiorelli, S. Zatelli, SEE, 2000, Firenze
T2* MRI IMAGING OF PANCREAS

NORMAL

IRON OVERLOAD
Validation in 21 healthy subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Normal Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head pancreas T2*(ms)</td>
<td>37</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Body pancreas T2*(ms)</td>
<td>36</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Tail pancreas T2*(ms)</td>
<td>36</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Global pancreas T2*(ms)</td>
<td>36</td>
<td>6</td>
<td>24</td>
</tr>
</tbody>
</table>

$p = NS$
Pancreatic Iron Overload is correlated with Myocardial Iron Overload.
NEW SCENARIO IN THE DEFINITION OF IRON OVERLOAD

• SERUM FERRITIN LEVEL >1000 ng/ml OR WORST >2500 ng/ml.


• LIC (LIVER IRON CONCENTRATION) > 3.2 mg/gr/ DRIED TISSUE OR >57,14 micro M/g

THIS STATEMENT IS BASED ON HEREDITARY HEMOCHROMATOSIS CLINICAL STUDIES (Olivieri NF and Britthenam GM, Blood 1997)

• MAGNETIC RESONANCE IMAGING ALLOWS TO DEFINE IRON OVERLOAD AS SINGLE ORGAN IRON DEPOSITION. IN THIS SCENARIO, IT COULD BE MOST CRUCIAL THE SYTE WITH IRON OVERLOAD (I.E. HEART IRON OVERLOAD) RATHER THAN THE OVERALL BODY IRON BURDEN
IRON OVERLOAD

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IRON OVERLOAD

GENETIC MODIFIERS INFLUENCING SEVERITY OF IRON OVERLOAD

- CO-EXISTENCE OF BETA-THALASSEMIA AND C282Y HFE HOMOZYGOTES WAS REPORTED TO BE ASSOCIATED WITH MORE SEVERE IRON LOADING (Rees et al., 1997; Piperno et al., 2000);

- STUDIES SUGGESTED AS CARRIERS HOMOZYGOTE FOR H63D HAVE HIGHER SERUM FERRITIN LEVELS (Melis et al., 2002);

- TRANSFERRING RECEPTOR 2 (TFR2), FERROPORTIN (FPN), HEPCIDIN (HAMP) AND HEMOJUVELIN (HJV) COULD BE, THEORETICALLY, INVOLVED AS GENETIC MODIFIERS (Hentze et al., 2004);

- GSTM1, ELF5A, SULF2, NTS, AND HO –1 GENES WERE IDENTIFIED BY MICROARRAY ANALYSIS AS GENES THAT MIGHT AFFECT THE DEGREE OF IRON ACCUMULATION (Flanagan et al., 2009).
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LOWER HEART T2* VALUES ARE ASSOCIATED WITH INCREASED RISK FOR HEART FAILURE

“In comparison with cardiac T2* values >20 ms, there was a significantly increased risk of heart failure associated with cardiac T2* values < 10 ms (Relative Risk 159, P < 0.001) and T2*<6 (RR 268, P < 0.001)”

(Kirk P et al SCMR 2009)
PARTIAL CORRELATION BETWEEN MYOCARDIAL T2* AND CARDIAC FUNCTION

Left Ventricular ejection fraction (%)

Global Heart T2* (ms)

526 TM pts

Fibrosis

Myocarditis

$P < 0.0001$

$r = 0.2$

$P < 0.0001$

$r = 0.1$

$P < 0.003$
COMPLICATION AND IRON OVERLOAD
HYPOGONADISM
(630 Thalassemia Major pts)

Iron overload was measured as heart and liver T2* MRI signal. Hatched lines show normal values for heart and liver.

P<0.01

P=0.092
COMPLICATION AND IRON OVERLOAD
CIRRHOSIS
(512 Thalassemia Major patients)

IRON OVERLOAD WAS MEASURED AS HEART AND LIVER T2* MRI SIGNAL. HATCHED LINES SHOW NORMAL VALUES FOR HEART AND LIVER

\[ P=0.555 \]

\[ P=0.525 \]
COMPLICATION AND IRON OVERLOAD ARRHYTHMIAS (582 Thalassemia Major patients)

Iron overload was measured as heart and liver T2* MRI signal. Hatched lines show normal values for heart and liver.

*Global Heart T2* (ms) P=0.559

*Liver T2* (ms) P=0.622
IRON OVERLOAD WAS MEASURED AS HEART AND LIVER $T_2^*$ MRI SIGNAL. HATCHED LINES SHOW NORMAL VALUES FOR HEART AND LIVER.
AGE, HEART T2* AND COMPLICATIONS IN 430 THALASSEMIA MAJOR > 30 YRS

<table>
<thead>
<tr>
<th>COMPLICATION</th>
<th>AGE</th>
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<tbody>
<tr>
<td>Hypogonadism:</td>
<td>33.1 ± 5.9</td>
</tr>
<tr>
<td>Heart Failure:</td>
<td>33.5 ± 6.3</td>
</tr>
<tr>
<td>Arrhythmias:</td>
<td>34.9 ± 5.8</td>
</tr>
<tr>
<td>Diabetes:</td>
<td>35.7 ± 6.4</td>
</tr>
<tr>
<td>Cirrhosis:</td>
<td>36.3 ± 6.7</td>
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</tbody>
</table>
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THE CONTROL OF IRON OVERLOAD USING DESFERRIOXAMINE TREATMENT WAS ASSOCIATED WITH SURVIVAL IMPROVING

Figure 2. Kaplan-Meier survival curves, after the first decade of life, by birth cohort (A) and by sex (B).

C. Borgna-Pignatti et al. Haematologica, 2004
FURTHER IMPROVEMENT OF SURVIVAL CURVES WERE OBTAINED DURING DEFERIPRONE RELATED TREATMENTS


$X^2 = 19.55, P < 0.0001$
IMPROVEMENT IN SURVIVAL CURVE OBTAINED WITH DEFERIPRONE TREATMENT WAS NOT RELATED TO SERUM FERRITIN LEVELS

Borgna-Pignatti et al., 2006
PAST-HEART FAILURE, CIRRHOSIS, ARRHYTHMIA ALONE ARE ASSOCIATED WITH HIGHER RISK FOR DEATH THAN SERUM FERRITIN CONCENTRATION ALONE

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M vs. F)</td>
<td>3.04 (0.83; 11.2)</td>
<td>0.096</td>
</tr>
<tr>
<td>Age (≥25 vs. &lt;25)</td>
<td>1.98 (0.45; 9.07)</td>
<td>0.375</td>
</tr>
<tr>
<td>Treatment (DFO vs. others)</td>
<td>29.4 (3.6; 240)</td>
<td>0.0016</td>
</tr>
<tr>
<td>Past heart failure (yes vs. no)</td>
<td>14.6 (2.81; 75.6)</td>
<td>0.0014</td>
</tr>
<tr>
<td>Arrhythmia (yes vs. no)</td>
<td>20 (5.24; 76.5)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Cirrhosis (yes vs. no)</td>
<td>41.71 (0.8; 161)</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Diabetes (yes vs. no)</td>
<td>8.57 (2.14; 34.3)</td>
<td>0.0024</td>
</tr>
<tr>
<td>Hypogonadism (yes vs. no)</td>
<td>4.96 (1.03; 23.9)</td>
<td>0.046</td>
</tr>
<tr>
<td>Hypothyroidism (yes vs. no)</td>
<td>4.47 (2.43; 36.9)</td>
<td>0.0012</td>
</tr>
<tr>
<td>Splenectomy (yes vs. no)</td>
<td>3.32 (0.834; 13.2)</td>
<td>0.089</td>
</tr>
<tr>
<td>Ferritin (≥1000 and &lt;2500 vs. &lt;1000)</td>
<td>0.68 (0.09; 5.55)</td>
<td>0.72</td>
</tr>
<tr>
<td>Ferritin (≥2500 vs. &lt;1000)</td>
<td>1.9 (0.22; 17.02)</td>
<td>0.57</td>
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The hazard ratio shows the relative risk of death for patients in the first category relative to the second category; CI = Confidence Interval.

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MRI METHODOLOGY AIMED AT TARGETING ORGAN IRON OVERLOADING

- HYPOPHYSIS
  - Normal
  - Iron overloading

- PANCREAS
  - Iron overloading
  - Normal

- HEART
  - Iron overloading
  - Normal

- LIVER
  - Iron overloading
  - Normal

TO TAILOR CHELATION TREATMENT ON ORGAN DAMAGE
SIGNIFICANT IMPROVING OF T2* HEART MRI SIGNAL DURING A PROSPECTIVE SURVEY USING DEFERASIROX, DEFERIPRONE AND DESFERRIOXAMINE

Patients with myocardial iron overload at baseline (global heart T2* < 20 ms)
IMPROVEMENT IN THE GLOBAL HEART T2* IS SIGNIFICANTLY GREATER FOR DEFERIPRONE IN COMPARISON WITH DESFERRIOXAMINE AND DEFERASIROX GROUP

\[ P = 0.009 \]

- **DFP**: 10.7 ± 7.1 ms
- **DFO**: 3.6 ± 5.4 ms
- **DFX**: 4.6 ± 4.8 ms
CONCLUSIONS

• WHICH ARE THE KEY MECHANISMS CAUSING IRON OVERLOAD?
  -HEPCIDIN CONTROL OF IRON HOMEOSTASIS: MINOR ROLE IN THALASSEMIA MAJOR
  -TRANSFUSIONAL IRON OVERLOAD

• HOW IS IT POSSIBLE TO DEFINE AND DETECT IRON OVERLOAD?
  -TODAY, THERE IS THE POSSIBILITY OF DEFINING BOTH BODY THAT SINGLE ORGAN IRON BURDEN (i.e. HEART IRON)
  -USING INDIRECT AND DIRECT METHODS

• ARE THERE ANY EVIDENCE OF GENETIC MODIFIERS INFLUENCING SEVERITY OF IRON OVERLOAD?
  AT PRESENT, THERE IS LITTLE UNDERSTANDING ABOUT THE GENETIC MODIFIERS THAT MAY INFLUENCE SEVERITY OF SECONDARY HEMOCROMATOSIS

• WHICH IS THE CORRELATION BETWEEN ORGAN COMPLICATION AND IRON OVELOAD?
  THIS RELATION IS NOT LINEAR. TIMING OF DAMAGE, COMPLIANCE, SURVIVAL SELECTION, CHELATION MAY EXPLAIN THIS FINDING
CONCLUSIONS

• WHICH IS THE CORRELATION BETWEEN IRON OVERLOAD AND SURVIVAL?
  The control of iron overload improves survival. Some chelation treatments have more effect on survival independently from body iron overload control. However, some complications have more influence on the impairment of survival.

• WHICH IS THE FUTURE IN IRON OVERLOAD MANAGEMENT?
  Targeting chelation treatment according to the organ damage