Iron overload in MDS

Supportive care and chelation therapy:

ARE WE SAVING LIVES OR JUST LOWERING IRON?

Leitch HA and Vickars LM. ASH education-2009:664-672
TOTAL BODY IRON CONTENT

40-50mg/kg

Hemoglobin --- 30mg/kg
Myoglobin --- 4mg/kg
Enzymes --- 2mg/kg
Moderate transfusion requirement:
  2 units / month
  24 units / year
  \(~ 100 \text{ units} / 4 \text{ years}~

High transfusion requirement:
  4 units / month
  48 units / year
  \(~ 100 \text{ units} / 2 \text{ years}~

100 units: \( \geq 20 \text{ g iron} \)

Normal body iron: 3-4 g
When physiological iron ligands (transferrin and ferritin) are saturated

FREE IRON SPECIES ARE GENERATED
Non-Specific Serum Iron in Thalassaemia: an Abnormal Serum Iron Fraction of Potential Toxicity

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(Received 9 December 1977; accepted for publication 24 April 1978)

Summary. Iron binding in the sera of 35 patients with β thalassaemia major and intermedia was studied. In patients receiving regular blood transfusions since infancy transferrin was completely saturated and about 2.7–7.1 μmol/l of the serum iron could be removed by dialysis or ultrafiltration in the presence of a chelating agent or by filtration on DEAE–Sephadex–catecholdisulphonic acid columns. In contrast, less than 1.0 μmol/l of transferrin bound iron was removed when subjected to the same procedures. The non-specific iron of thalassaemic sera could no longer be demonstrated after incubation with normal serum. These findings indicate that non-specific iron is a chelatable compound which is readily available for transferrin binding. In view of the known toxicity of unbound iron, its identification in thalassaemic sera might be of relevance to the pathogenesis of tissue damage and the protective effect of iron chelating therapy in this disease.
Free Iron Species in the Plasma For Assessment of Iron Loading

• Non Transferrin Bound Iron (NTBI) found when transferrin approaches saturation\(^1\)
• Labile Plasma Iron (LPI): a chelatable redox-active component of NTBI\(^2\)
• In TM, NTBI and LPI values correlate approximately with
  – serum ferritin\(^3-5\) and LIC\(^6\)
• Values also reflect erythropoietic rate
  – increased by suspension of erythropoiesis\(^7\)
  – increased by ineffective erythropoiesis\(^8\)
• Values are increased by transfusional iron loading rate\(^9\)
• NTBI partially removed with DFO\(^4,10\)
• LPI is removed in presence of chelators\(^2\)

LPI and elevated ferritin are detected when transferrin saturation >80%

LPI correlates with ferritin and labile iron deposited on RBC membranes
Variable LPI levels in different diseases with similar serum ferritin

Baseline LPI values in EPIC Trial

<table>
<thead>
<tr>
<th></th>
<th>Median serum ferritin (µg/L)</th>
<th>Labile plasma iron (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM</td>
<td>472</td>
<td>2.25</td>
</tr>
<tr>
<td>MDS</td>
<td>225</td>
<td>0.27</td>
</tr>
<tr>
<td>AA</td>
<td>27</td>
<td>0.02</td>
</tr>
<tr>
<td>SCD</td>
<td>55</td>
<td>0.05</td>
</tr>
<tr>
<td>Rare anaemias</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AA = aplastic anaemia; MDS = myelodysplastic syndromes.

Excessive ingress of LPI into cells leads to a rise in labile iron pool (LIP), attaining toxic levels.

LIP can engage in the formation of production of reactive O species (ROS) by catalyzing the formation of noxious OH• radicals.

LIP (Fe³⁺ and Fe²⁺) reacts with reactive O intermediates (O₂⁻ and H₂O₂ produced by respiration and other incomplete reductions of O₂) forming OH• radicals (Haber Weiss cycle)

\[
\begin{align*}
\text{Fe}^3+ + \text{O}_2^- & \rightarrow \text{Fe}^2+ + \text{O}_2 \\
\text{Fe}^2+ + \text{H}_2\text{O}_2 & \rightarrow \text{Fe}^3+ + \text{OH}^- + \text{OH}^-(\text{Fenton reaction})
\end{align*}
\]

Sustained levels of LIP impose a persistent oxidative stress.
Intracellular Iron Homeostasis: Ferritin functions as a ferroxidase, converting Fe$^{2+}$ to Fe$^{3+}$ as iron is internalized and sequestered in the ferritin mineral core. Reactive species (shown as yellow spheres) can directly damage DNA and proteins. DMT1 = divalent metal ion transporter 1, Tf = Transferrin, TfR = Transferrin receptor.
Where and how does labile iron cause cell damage?

1. 33 g of ROI = reactive oxygen intermediates produced per day*

2. LPI present in systemic iron overload leads to accumulation of labile iron pool (LIP)

3. ROI react with LPI producing noxious ROS, e.g. OH· radicals

4. OH· radicals are highly reactive and they can modify DNA, proteins and lipid components of cells

ROI are normally converted to water by resident enzymes SOD and GPX

* Up to 3Kg ROI /d in inflammation!
Sources and targets of systemic iron overload. Iron absorbed by the organism or recycled by the reticuloendothelial system is normally sequestered by plasma transferrin (referred as transferrin bound iron-TBI). Following blood transfusions (generally thalassaemia major-TM and some myelodysplastic-MDS patients) there is a massive influx of iron into plasma, surpassing TBI and generating non-TBI (referred as NTBI). An analogous rise in NTBI occurs in hereditary hemochromatosis (HH) and Thalassaemia intermedia (TI) where iron is hyperabsorbed by the gut. Physiological TBI is taken up by tissues by regulated pathways, whereas the pathological NTBI, especially its labile component LPI (labile plasma iron) access cells, including red and white blood cells (RBC and WBC) via opportunistic routes, raising the labile cell iron (LCI) levels and in extreme conditions overriding the cellular antioxidant capacities. Chelators (Ch) can act by suppressing LPI and/or by permeating into cells and chelating LCI and thereby gradually reducing the tissue iron loads, most of which are associated with ferritin and hemosiderin.
Excess iron is deposited in multiple organs, resulting in organ damage

- Cardiac failure
- Liver cirrhosis/fibrosis/cancer
- Diabetes mellitus
- Infertility
- Endocrine disturbances → growth failure
NTBI levels in MDS and healthy controls

Santini et al, Plos one 2011; 6: e23109
Distribution of NTBI levels in WHO subtypes of MDS

Santini et al, Plos one 2011; 6: e23109
Reactive oxygen species are tightly controlled

Biological oxidants are important in physiology and pathology
- Involved in normal cell function (e.g. growth regulation, host defense)

Physiological balance between production and elimination
Oxidant – Antioxidant Balance

**Antioxidants**

*Enzymes*
- Superoxide dismutase (SOD),
- Catalase, Peroxiredoxins
- Glutathione peroxidase (GPX)

*Non-enzymes*
- Vitamins E & A, ascorbate,
- *Glutathione* (GSH) & thiols, metal chelators, bilirubin, phenols, uric acid

*Blood components*
- Albumin, Caeruloplasmin,
- Transferrin

**Oxidants**

*O**₂⁻
- Phagocyte activation, Arachidonic acid metabolism, Mitochondrial respiration,
- Xanthine oxidase, Cyt P450

*H**₂O**₂
- Metabolic enzymes, phagocyte activation, O₂⁻-dismutation

**Reactive nitrogen species**

NO⁻, ONOO⁻ - nitric oxide synthase, phagocytes

**Reactive chlorine species**

HOCl – myeloperoxidase neutrophils

Low level oxidative damage is inevitable – measured in healthy volunteers
ROS generation by peripheral blood cells in MDS

Peripheral blood cells were stained with DCF

(A) Forward scatter (FCS) vs side scatter (SSC) dot plot. The gates indicate platelets (R1), RBC (R2), monocytes (R3), and PMN (R4)

(B–D) Histograms showing DCF fluorescence of platelets, RBC, and PMN of a normal donor (white) and of a patient with MDS (grey)

The mean fluorescent channel (MFC) of each population is indicated


DCF = 2’-7’-dichlorofluorescin diacetate; PMN = polymorphonuclear leukocytes.
Evidence for oxidative stress in MDS blood cells

(Ghoti H et al, European J of Hematol. 79;463-7, 2007)

**ROS (% of control)**

- **Platelets**
- **RBC**
- **PMN**

**GSH (% of control)**

- **Platelets**
- **RBC**
- **PMN**
Oxidative Stress Mechanisms in MDS

Farquhar and Bowen, Int J Hematology 77:342, 2003
Control of hepcidin production in different types of MDS

Anemia, hypoxia

- EPO
- Inflammatory cytokines (IL-6...)

- Prevailing in CMML and RAEB

- Iron stores (transfusional overload)

- Ineffective erythroid differentiation (by unknown factors)

Response tend to be conserved in RA, 5q- syndrome, and in RARS, but relatively blunted as indicated by the hepcidin/ferritin ratio

Tend to prevail in RA, 5q- syndrome, and particularly in RARS

Santini et al, Plos one 2011; 6: e23109
# Hepcidin levels in WHO subtypes of MDS

Hepcidin levels in healthy subjects 4.20 (3.53-5.00) nmol/L

<table>
<thead>
<tr>
<th></th>
<th>RA (n=31)</th>
<th>RARS (n=9)</th>
<th>RCMD (n=19)</th>
<th>RAEB (n=32)</th>
<th>5q-syndrome (n=7)</th>
<th>CMML (n=7)</th>
<th>Unclass (n=8)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin * (nmol/l)</td>
<td>3.46 (2.06-5.81)</td>
<td>1.43 (0.51-4.03)</td>
<td>3.83 (1.85-7.96)</td>
<td>11.31 (7.38-17.32)</td>
<td>6.62 (1.26-34.84)</td>
<td>10.04 (2.10-48.00)</td>
<td>6.06 (1.18-31.27)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Santini et al, Plos one 2011; 6: e23109
Non-leukaemic causes of death linked to iron overload?

37/38 patients who died from cardiac/hepatic failure = serum ferritin ≥ 1,000 μg/L

Cardiac failure was more frequent in transfused patients (p = 0.01)

Total number of pRBC units transfused

- Death caused by cardiac/hepatic failure
- Death from other causes

Probability of non-leukaemic death in patients with low-risk MDS

- Cardiac
- Hepatic

N = 75

p = 0.0033


Prevalence of comorbidities in transfusion-dependent MDS

Transfused MDS patients have a higher prevalence of cardiac events, diabetes mellitus, dyspnoea, and hepatic and infectious diseases than non-transfused MDS patients.

According to the MDS US registry, chelated patients had significantly longer OS and time to AML transformation, as well as significantly fewer deaths.

Iron chelation delays AML transformation
(Multi-center analysis from US registry)$^2$

<table>
<thead>
<tr>
<th></th>
<th>Non-chelated n=337</th>
<th>All Chelated n=263</th>
<th>Chelated ≥6 months n=191</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML transformation n (%)</td>
<td>30 (8.9)</td>
<td>12 (4.6)</td>
<td>10 (5.2)</td>
</tr>
</tbody>
</table>

Lyons et al., ASH 2012, abstract 3800
Key considerations

- Transfusion-requiring patients are at risk for iron overload.
  - Magnitude of risk varies from patient to patient.
  - "Iron burden" is not straightforward to measure with available tools.
  - Current biomarkers (ferritin, T2* signal, etc.) are inadequate measures of risk.

- Chelation therapy is of clear benefit in thalassemia major and other severe congenital anemias...
  - But MDS is not thalassemia (clonal, older pts, more AEs etc).

- Retrospective studies suggest chelation benefit in MDS.
  - But are subject to confounding.
  - Currently no prospective morbidity/mortality benefit.
  - Prospective Novartis study accruing slowly.

"There are bigger fish to fry."
What about higher risk MDS and AML progression?

- Iron is mutagenic in hemopoietic cells and can promote progression to AML in mice\(^1\)

- NTBI $\rightarrow$ LPI $\rightarrow$ ROS*  
  - ROS damage
    - membranes
    - proteins
    - nucleic acids

- Chelation induced apoptosis, differentiation & repressed signalling in AML cells & cell lines in vitro & in vivo\(^2-5\)

\(^1\) Chan LSA, et al. Blood 2010;116:[abstract 122]  

ROS = reactive oxygen species, NTBI = non-transferrin bound iron
Iron overload and progression from MDS to AML in irradiated mice

- Does iron loading accelerate leukaemogenesis?

**Diagram:**
- B6D2F1 mice
- Radiation: 3Gy
- Dexamethasone: 0.5 mg
- 0/5 mg Iron dextran
- 2 Weeks
- 3 Months
- > 3 Months

- Analysis (early effects) (n = 3 each)
- Continual observation (late effects) (n = 3 each)

Observations from this mouse model suggest that iron is mutagenic in haemopoietic cells and can promote progression of a pre-leukaemic state to frank AML.

AML = acute myeloid leukaemia.

The effect of iron and Deferasirox on ROS, LIP and 8-oxoguanine in normal and MDS culture-derived myelo-monocytic cells
The effect of iron and Deferasirox on DNA damage in culture-derived MDS cells

- Untreated
- Treated with FAS
- Treated with FAS + DEF
Chelation Therapy of Iron Overload

1. To induce iron excretion in order to reduce excess tissue iron levels
2. To detoxify the deleterious effects of “Free iron”
3. To correct DNA damage
4. To prevent? Delay? transformation to Leukemia
### Diagnostic tools for the evaluation of body iron status in MDS patients

<table>
<thead>
<tr>
<th>Diagnostic tool</th>
<th>Characteristics</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculation of transfusion iron burden</td>
<td>▪ Provide a direct quantitative estimate of the iron body burden</td>
<td>▪ Easy to calculate; inexpensive</td>
<td>▪ Unreliable in patients with bleeding or chelation therapy</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>▪ Indirect serological estimation of iron body burden</td>
<td>▪ Widely available; easy to perform; low-cost; repeatable</td>
<td>▪ Unreliable in patients with inflammation, liver function deficiency, and ascorbate deficiency</td>
</tr>
<tr>
<td>Serum transferrin saturation</td>
<td>▪ High sensitivity and specificity in non-transfused patients</td>
<td>▪ Widely available; easy to perform; low-cost; repeatable</td>
<td>▪ No quantitative correlation to iron burden</td>
</tr>
<tr>
<td>SQUID</td>
<td>▪ Direct instrumental estimation of hepatic iron concentration</td>
<td>▪ Non-invasive; repeatable</td>
<td>▪ Expensive; not widely available; not validated; significant underestimation; not applicable for cardiac assessment</td>
</tr>
<tr>
<td>MRI R2</td>
<td>▪ Indirect instrumental estimation of iron tissue concentration</td>
<td>▪ Non-invasive; repeatable; validated on the liver</td>
<td>▪ Expensive, not widely available; reliable up to LIC of 15 mg/g dry wt; not applicable for cardiac assessment</td>
</tr>
<tr>
<td>MRI T2*</td>
<td>▪ Indirect instrumental estimation of iron tissue concentration</td>
<td>▪ Non-invasive; repeatable; validated on the heart; providing information on cardiac function</td>
<td>▪ Expensive; not widely available; complex, requiring a skilled radiologist; not validated on the liver</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>▪ Provides a direct estimation of iron overload</td>
<td>▪ Validated and quantitative method to estimate hepatic iron concentration (gold standard)</td>
<td>▪ Invasive (cannot be employed in many patients with MDS)</td>
</tr>
<tr>
<td>NTBI/LPI/LIP</td>
<td>▪ Research tool at present</td>
<td>▪ Non-invasive method; estimates generation of toxic iron species</td>
<td>▪ Not widely available</td>
</tr>
<tr>
<td>Serum hepcidin</td>
<td>▪ Research tool at present</td>
<td>▪ Non-invasive method; identifies patients at high-risk of iron loading</td>
<td>▪ Not widely available</td>
</tr>
</tbody>
</table>

LIC, liver iron concentration; MRI, magnetic resonance imaging.
<table>
<thead>
<tr>
<th>Countries</th>
<th>Transfusion status</th>
<th>Serum ferritin (ng/mL)</th>
<th>Patient profile</th>
<th>Chelator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian</td>
<td>≥ 50 RBC units</td>
<td></td>
<td>• Life expectancy &gt; 6 months</td>
<td>DFO</td>
</tr>
<tr>
<td>UK</td>
<td>~ 25 RBC units (5 g iron)</td>
<td></td>
<td>• Pure sideroblastic anemia • del 5q</td>
<td>DFO</td>
</tr>
<tr>
<td>US (NCCN)</td>
<td>20-30 RBC units (≥5-10 g iron)</td>
<td>&gt; 2500</td>
<td>• IPSS Low or Int-1</td>
<td>DFO Deferasirox</td>
</tr>
<tr>
<td>International (Nagasaki)</td>
<td>transfusion-dependent</td>
<td>&gt;1000-2000</td>
<td>• RA, RARS, del 5q • IPSS Low or Int-1</td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td>&gt; 40 Japanese units</td>
<td>&gt;1000</td>
<td>• Life expectancy &gt; 1 year</td>
<td></td>
</tr>
<tr>
<td>Canadian</td>
<td>transfusion-dependent</td>
<td>&gt;1000</td>
<td>• RA, RARS, del 5q • IPSS Low or Int-1 • IPSS Int-2 or High (if SF &gt;1000 and • SCT candidates/life expectancy &gt;1yr)</td>
<td>DFO Deferasirox</td>
</tr>
<tr>
<td>Spanish</td>
<td>transfusion-dependent</td>
<td>&gt;1000</td>
<td>• IPSS Low or Int-1 • WPSS Very low, Low, or Int • Spanish prognostic index Low risk</td>
<td>DFO Deferasirox</td>
</tr>
<tr>
<td>Austrian</td>
<td>transfusion-dependent</td>
<td>&gt;2000</td>
<td>• Life expectancy &gt; 2 years</td>
<td>DFO, DFP, Deferasirox</td>
</tr>
<tr>
<td>MDS Foundation</td>
<td>2 RBC units/month for ≥1 year</td>
<td>&gt;1000</td>
<td>• Life expectancy &gt; 1 year</td>
<td>Physician discretion</td>
</tr>
</tbody>
</table>

Steensma D, Gatterman N.  *Best Practices Hematol Oncol* 2014
An R2 image of an iron-overloaded human liver superimposed on a T-2 weighted image. Bright areas represent high iron concentration; dark areas represent low iron concentration.

Molecular level: ROS concentration in hepatocytes increases with LIC.

Iron overload and ROS stress result in mitochondrial damage.
Does MRI evidence for myocardial iron loading exist in MDS?

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>patients with cardiac IO</th>
<th>%</th>
<th>Units transfused</th>
<th>serum ferritin (ng/ml)</th>
<th>N° of chelated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jensen et al. 2003¹</td>
<td>12</td>
<td>9</td>
<td>75</td>
<td>44–254 (mean 115)</td>
<td>1740–8715</td>
<td>0</td>
</tr>
<tr>
<td>Chacko et al. 2007²</td>
<td>11</td>
<td>1</td>
<td>9</td>
<td>23–257 (median 112)</td>
<td>1109–6651</td>
<td>6</td>
</tr>
<tr>
<td>Konen et al. 2007³</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>50–140 (mean 89)</td>
<td>1260–4450</td>
<td>7</td>
</tr>
<tr>
<td>Di Tucci et al. 2008⁴</td>
<td>27</td>
<td>2</td>
<td>7</td>
<td>16–225 (median 64)</td>
<td>1300–6214</td>
<td>2</td>
</tr>
<tr>
<td>Roy et al. 2011⁵</td>
<td>53</td>
<td>8</td>
<td>15</td>
<td>2–602 (median 64)</td>
<td>16–10218</td>
<td>0</td>
</tr>
</tbody>
</table>

¹Jensen PD et al. Blood 2003;101:4632–4639;
⁵Roy NBA et al, . Br J Haematol 2011; 154, 521–524
Relation of Cardiac Iron Deposits (CID) to Amount of Blood Transfused

- 131 transfused adult patients
  - 101 leukemias
  - 30 other anemias

Cardiomyocytes before and after iron loading

Hearts of iron-loaded mice had irregularly shaped mitochondria, electron dense material

CTRL                                       iron loaded

M: mitochondria
MF: myofibrils
H: electron dense iron stores

Organ damage

Cardiac complications in MDS

- Cardiac events are increased in MDS, especially in transfusion-dependent patients
- Cardiac imaging
  - 19% moderate IO ($T2^* \leq 20\text{ms}$)
  - 4% severe IO ($T2^* \leq 10\text{ms}$)
- What is the mechanism of cardiac complications in MDS?

Preclinical: Uptake of LPI into cardiac cells leads to arrhythmia and CHF

CHF = congestive heart failure; IO = iron overload; LPI = labile plasma iron.

Direct toxicity of ROS in the heart

Mitocondrial production of ROS

ROS production

Tissue damage
STRUCTURE OF CHELATOR-IRON COMPLEXES

Deferoxamine  Hexadentate

Deferiprone  Bidentate

Deferasirox  Tridentate
Membrane-permeable iron chelators, such as deferiprone, can shuttle iron within the cell between endosomes (e), mitochondria (m), the nucleus, and the cytoplasm. Chelator-mediated mobilization of the metal from iron-overloaded organelles or cellular deposits reduces the local formation of toxic hydroxyl-radical and results in transport of the iron across membranes, delivery of the metal to transferrin, and subsequent transferrin receptor (TfR)—mediated acquisition of iron by erythroid progenitor (ep) cells to be used for heme biosynthesis.
Effects of monotherapy and combined therapy on LPI

Each colour represents LPI values of individual patients starting at 8AM and followed for the next 24 hours.

Effect of iron chelation on LPI in MDS

US03 study\(^1\)

<table>
<thead>
<tr>
<th>Time, months</th>
<th>Baseline</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>55</td>
<td>38</td>
<td>39</td>
<td>37</td>
<td>34</td>
</tr>
</tbody>
</table>

Mean LPI ± SD, \(\mu\text{mol/L}\)

Threshold of normal LPI (\(\leq 0.5\ \mu\text{mol/L}\))

P \(\leq 0.00001\)

Patients with baseline LPI \(\geq 0.5\ \mu\text{mol/L}: 41\%

EPIC study – MDS cohort\(^2\)

<table>
<thead>
<tr>
<th>Time, weeks</th>
<th>Baseline</th>
<th>12</th>
<th>28</th>
<th>52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-administration</td>
<td></td>
<td></td>
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</tbody>
</table>

Threshold of normal LPI

Normal threshold

Oxidation Parameters in MDS Following Treatment with Deferasirox

- 19 patients with 5q- (4) RA (7) RARS (3) RCMD (3) REMD (1) completed 3 months of treatment

- The mean time of documented disease was 4±2 years (range 1-9 years)

- The number of transfusions was 20-238 pc units (mean 63±61)

- 37% of the patients were male and 63% female (n=19)

- Their age varied between 40 to 87 years, (mean 69±13 years)

- Mean treatment duration was 94±11 days (n=18)

- 16 patients received Deferasirox 20mg/kg
Changes in mean levels +SD of (A) LIP in RBC and platelets (n=18), and (B) LPI (n=16)
Effect of deferasirox on LPI in MDS

**US03 study¹**

<table>
<thead>
<tr>
<th>Patients, n</th>
<th>55</th>
<th>38</th>
<th>39</th>
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Patients with baseline LPI ≥ 0.5 μmol/L = 41%

**EPIC study – MDS cohort²**

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<th>28</th>
<th>52</th>
</tr>
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</table>

Threshold of normal LPI (≤ 0.5 μmol/L)

p ≤ 0.00001 *

Hematological responses in MDS patients treated with deferasirox: an EPIC post-hoc analysis using IWG 2006 criteria

Percentage of patients experiencing hematological responses

<table>
<thead>
<tr>
<th></th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb/trans</td>
<td>22.6%</td>
</tr>
<tr>
<td>Trans</td>
<td>14.0%</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>19.6%</td>
</tr>
</tbody>
</table>

Median time to hematological response

<table>
<thead>
<tr>
<th></th>
<th>Median time to response (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytosis</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Transfusion</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11 (5)</td>
</tr>
<tr>
<td>Platelet</td>
<td>16 (9)</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>22 (6)</td>
</tr>
</tbody>
</table>

Hematological response according to median decrease in serum ferritin from baseline to end of study: EPIC

Gattermann N, et al.
Hematologic response in first year of treatment

Median time to hematologic response was 169 days (84 to 382)

List et al., J Clin Oncol. 2012 Apr 30. [Epub ahead of print]
Changes in mean levels of GSH in RBC, platelets and PMN, and ROS and lipid peroxidation in RBC

Cell fluorescence (MFC) is proportional to ROS and GSH but inversely proportional to lipid peroxidation
Deferasirox inhibits NF-κB activity

Messa E et al. Haematologica 2010;95:1308 - 1316
Deferasirox inhibits m-TOR signaling

Deferasirox represses signaling through the mTOR and reduces tumor volume in myeloid leukemia cells in mice.

Ohyashiki et al. Cancer Science 2009;100: 970-977
The effect of Deferasirox on myelo-monocytic differentiation of a human leukemic cell line
The effect of Deferasirox on serum hepcidin levels in 19 patients with MDS

a) Mean changes in serum hepcidin during 12 weeks treatment
b) Serum hepcidin levels of individual patients before and after treatment
INITIAL SERUM HEPCIDIN LEVELS WERE RELATED TO THE INITIAL NUMBER OF TRANSFUSIONS AND INCREASED FOLLOWING DEFERASIROX, PROBABLY DUE TO AMELIORATION OF OXIDATIVE STRESS
Overall survival of low and intermediate-1 IPSS risk in 97 MDS patients according to intensity of iron chelation therapy. Median survival was 124 months in the adequately chelated patients – 85 months in those receiving weak chelation and 51 months in non-chelated patients – p< 0.01

(Rose C. et al, Leukemia Research 2010; 34(7):864-870)
US22: relation between chelation and outcomes in lower-risk MDS

By log-rank test for non-chelated vs both chelated groups.


a
Düsseldorf: ICT improves survival in patients with MDS

Survival benefit restricted to patients with a decrease in SF levels

Moffitt Cancer Center: impact of ICT on overall survival and AML transformation in lower risk MDS patients

Retrospective assessment of IPSS low/int-1 risk MDS patients with SF ≥ 1000 μg/L

<table>
<thead>
<tr>
<th></th>
<th>ICT (n = 45)</th>
<th>No ICT (n = 52)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean SF, μg/L</td>
<td>2680</td>
<td>3038</td>
<td>0.77</td>
</tr>
<tr>
<td>WHO subtype, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RARS</td>
<td>11 (24.4)</td>
<td>10 (19.2)</td>
<td></td>
</tr>
<tr>
<td>non-RARS</td>
<td>34 (75.5)</td>
<td>42 (80.8)</td>
<td></td>
</tr>
<tr>
<td>IPSS risk group, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>15 (33)</td>
<td>9 (17.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Int-1</td>
<td>30 (66.7)</td>
<td>43 (82.7)</td>
<td></td>
</tr>
<tr>
<td>Median OS, months</td>
<td>59</td>
<td>34</td>
<td>0.013</td>
</tr>
<tr>
<td>AML transformation rate (%)*</td>
<td>15.6</td>
<td>21.2</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*following adjustment for age >60 and MDS Anderson risk Score.

**ICT was associated with improved overall survival and a trend to lower AML transformation in patients with low/int-1 risk MDS and SF ≥ 1000 μg/L**
EUMDS: mortality increases as serum ferritin increases

Significantly greater mortality was noted in MDS patients who
- were transfusion-dependent (p < 0.0001)
- had a baseline SF of ≥ 1,000 µg/L (p < 0.0001)
- required transfusions of > 20 units (p < 0.0001)

Overall survival of transfusion-dependent patients by baseline SF status

<table>
<thead>
<tr>
<th>SF (µg/L)</th>
<th>Transfusion-independent</th>
<th>Transfusion-dependent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 300</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>300–1,000</td>
<td>13</td>
<td>56</td>
</tr>
<tr>
<td>≥ 1,000</td>
<td>13</td>
<td>61</td>
</tr>
</tbody>
</table>

Mortality rates and risk ratios for transfusion-independent and dependent MDS patients.

HR, hazard ratio.

Impact of iron chelation therapy prior to hematopoietic stem cell transplant (SCT) outcome. Improved survival in 74 patients was superior compared with 26 unchelated patients with SF>1000ng/ml prior to SCT.

Lee JW, et al. BMT 2009;44:793-797
Outcome of patients by pre-transplantation serum ferritin value

A pretransplantation serum ferritin level >2515 ng/mL was associated with:

- Significantly decreased overall and disease-free survival
- Significantly increased treatment-related mortality

Prognostic impact of pre-transplantation transfusion history and secondary iron overload in patients with MDS undergoing allogeneic stem cell transplantation: a GITMO study

According to transfusion burden

- ≤ 20 pRBC units, n = 64
- 21–40 pRBC units, n = 45
- > 40 pRBC units, n = 26

HR = 1.34
p = 0.04

According to serum ferritin

- Serum ferritin < 1,000 µg/L
- Serum ferritin 1,000–1,999 µg/L
- Serum ferritin 2,000–3,000 µg/L
- Serum ferritin > 3,000 µg/L

HR = 1.40
p = 0.01

HR = 1.42; p = 0.03

Post-Transplant Survival by RBC transfusion dependency

Overall survival

Leukemia-free survival

(n = 2,241)

No RBC transfusion dependency

RBC transfusion dependency

No RBC transfusion dependency

RBC transfusion dependency

P < 0.0001

P < 0.0001

Sanz et al., Blood 2008; 112(11) ASH abstract #640
NTBI during allogeneic HSCT

C = onset of conditioning regimen.

Current monitoring of iron overload and iron chelation are mainly evaluated by changes in serum ferritin, % transferrin saturation, and more recently by T2* MRI and serum NTBI, and LPI

Several retrospective studies document a better survival in chelated vs unchelated patients with transfusion dependent RA and RARS, and a better outcome after SCT

It is possible that there might be an indication for treating patients with advanced forms of MDS in order to: 1) Lowering infection risks; 2) Delaying leukemic transformation (Pullarkat, Blood 2009)

Until results of prospective studies will be available it is recommended to treat at least patients with documented increased levels of NTBI and/or LPI in order to ameliorate oxidative stress

NTBI is increased during conditioning for BMT

Iron overload plays a role in the survival of patients after BMT
Conclusion

I.O. is a problem in multitransfused patients with **LOW RISK MDS (RA, RARS)** due to the presence of free iron species: **NTBI, LPI and LIP** which catalyze formation of **ROS**.

Therefore, there is an indication that free iron species should be chelated (even if there are still insufficient data that iron chelation prolongs survival).
COLLABORATORS

• Y. Cabanchik  LPI
• A. Winder  MDS
• S. Rivella
• T. Ganz  Hepcidin
• M. Westerman
• G. Rechavi
• E. Konen  MRI
• O. Goitein
• E. Fibach
• J. Amer  ROS - GSH
• H. Ghoti
• A Ackerstein  Novartis
• R. Merilius
• Colleagues from Hadassah, Patients Tel-Hashomer, Ichilov and Soroka Hospitals
I would like to thank my colleagues:

* Valeria Santini, University of Firenze, (Italy)

* Daniela Cilloni, University of Turin, (Italy)

* David Steensma, Dana-Farber Cancer Institute, Boston, MA. (U.S.A)

for their permission to use some of their slides
Studies examining the impact of iron overload on outcome of intensive chemotherapy with or without hematopoietic stem cell transplantation for hematologic malignancies

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>SCT</th>
<th>n</th>
<th>IOL</th>
<th>OS</th>
<th>P</th>
<th>TRM</th>
<th>Relapse</th>
<th>SOS</th>
<th>GVH</th>
<th>Infection</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Armand</td>
<td>Retro</td>
<td>Allo</td>
<td>543</td>
<td>Ferritin quartile</td>
<td>54% 27%a</td>
<td>&lt;0.001</td>
<td>HR 3.2, P = 0.002</td>
<td>NS</td>
<td>OR 1.7, P = 0.054</td>
<td>NS</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pullarkat</td>
<td>Pro</td>
<td>Allo</td>
<td>190</td>
<td>Ferritin ≥ 1000</td>
<td>HR 2.28</td>
<td>0.03</td>
<td>HR 3.82, P = 0.003</td>
<td>–</td>
<td>–</td>
<td>HR 1.99, P = 0.032</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Platzbecker</td>
<td>Retro</td>
<td>Allo</td>
<td>172</td>
<td>Ferritin &gt; 1000</td>
<td>↓ 1.48</td>
<td>0.017</td>
<td>HR 1.42, P = 0.03</td>
<td>HR 1.4, P = 0.02</td>
<td>↓ P = 0.03</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Alessandrino</td>
<td>Retro</td>
<td>Allo</td>
<td>357, 217 MA</td>
<td>Ferritin, transfusion dependence</td>
<td>HR 1.40</td>
<td>0.017</td>
<td>HR 1.42, P = 0.03</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lee</td>
<td>Retro</td>
<td>Allo</td>
<td>101</td>
<td>Ferritin &gt; 1000</td>
<td>↓ 0.001</td>
<td>↑ P = 0.003</td>
<td>NS</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>G ≥ 3 HB, P = 0.01; ARDS, P = 0.055</td>
<td>–</td>
</tr>
<tr>
<td>Altes</td>
<td>Pro</td>
<td>Allo &amp; AU</td>
<td>15 + 10</td>
<td>Ferritin ≥ 3000</td>
<td>HR 4.4</td>
<td>≤ 0.03</td>
<td>HR 6.7, P = 0.01f</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>↑ P ≤ 0.05</td>
<td>–</td>
</tr>
<tr>
<td>Altes</td>
<td>Pro</td>
<td>Allo &amp; AU</td>
<td>8 + 23</td>
<td>Ferritin ≥ 100%</td>
<td>HR 3.5</td>
<td>0.01f</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>↑ P ≤ 0.006b</td>
<td>–</td>
</tr>
<tr>
<td>Storey</td>
<td>Pro</td>
<td>Allo &amp; AU</td>
<td>46 + 31</td>
<td>Iron score ≥ 2</td>
<td>↓ 0.01</td>
<td>↑ P = 0.02</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>OR 1.78 (95% CI 1.02–8.08)</td>
<td>–</td>
</tr>
<tr>
<td>Maradei</td>
<td>Retro</td>
<td>Allo &amp; AU</td>
<td>248 + 179</td>
<td>Ferritin &gt; 1000</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kataoka</td>
<td>Retro</td>
<td>Allo &amp; NMA</td>
<td>230 + 34</td>
<td>Ferritin ≥ 599</td>
<td>RR 1.68</td>
<td>≤ 0.005</td>
<td>RR 2.47, P = 0.01</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>↑ P &lt; 0.011</td>
<td>P &lt; 0.019</td>
</tr>
<tr>
<td>Sorror</td>
<td>Retro</td>
<td>Allo &amp; NMA</td>
<td>1014 + 434</td>
<td>Ferritin &gt; 1000</td>
<td>↓ 0.005</td>
<td>–</td>
<td>HR 1.4, P = 0.03k</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mahindra</td>
<td>Retro</td>
<td>NMA</td>
<td>64</td>
<td>Ferritin &gt; 1000</td>
<td>HR 2.74</td>
<td>0.001</td>
<td>↑ P = 0.051</td>
<td>NS</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kim</td>
<td>Retro</td>
<td>RIC</td>
<td>38</td>
<td>Ferritin</td>
<td>54.6% 27%</td>
<td>0.03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>OR 5.09, P = 0.008</td>
<td>–</td>
</tr>
<tr>
<td>Mahindra</td>
<td>Retro</td>
<td>AU</td>
<td>315</td>
<td>Ferritin &gt; 685</td>
<td>↓ 0.002</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>OR 3.42, P &lt; 0.011</td>
<td>–</td>
</tr>
<tr>
<td>Ozyilmaz</td>
<td>Retro</td>
<td>NR</td>
<td>148</td>
<td>Ferritin &gt; 1000</td>
<td>–</td>
<td>0.045</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>P = 0.018</td>
<td>–</td>
</tr>
<tr>
<td>Mattiuzi</td>
<td>Pro</td>
<td>AML CT</td>
<td>112</td>
<td>Ferritin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>IFI P = 0.039</td>
<td>–</td>
</tr>
</tbody>
</table>
Protection from LPI appearance with standard chelation regimens

Deferasirox
20 mg/kg/day

Deferiprone
25 mg/kg/day

Deferoxamine
40 mg/kg/day

Mean LPI (µmol/L)

Post-dose (hours)

LPI <0.2 µM
>90% of patients

<60% of patients

<50% of patients

Relevant LPI values are trough values, obtained from blood samples withdrawn following the longest drug-washout period (24 hours for deferasirox, 10 hours for deferiprone, and 12 hours for deferoxamine)

1Zanninelli G et al. 2008 submitted