Logarithmic quantitation model using serum ferritin to estimate iron overload in secondary haemochromatosis

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Abstract

Nineteen children and adolescents receiving repeated transfusions and subcutaneous desferrioxamine treatment were investigated in an attempt to quantitate iron overload non-invasively. Before patients were started on desferrioxamine individual relationships were correlated for 12 to 36 months between transfused iron, absorbed iron estimated gastro-intestinally, and increasing serum ferritin concentrations. Patients with inflammation, increased liver enzymes, or haemolysis were excluded from analysis. The relationship between the variables could be described by a logarithmic regression curve ($y = \text{transfused iron [plus eventually gastrointestinally absorbed iron]} = \text{iron overload} = a + b \log [x = \text{serum ferritin}]$) for each individual patient. All patients showed close correlation (R^2) between x and y (median R^2 of 0.909, 0.98, and 0.92 in thalassaemia, aplastic anaemia, and sickle cell anaemia patients, respectively). When started on desferrioxamine, current serum ferritin concentrations were used to derive the iron overload from each individual regression curve. The derived estimated iron overload ranged from 0.6 g to 31 g. Left ventricular dilatation was observed in three patients with β thalassaemia and in one patient with aplastic anaemia with median iron overload of 20.7 (14.1–31.3) g and 24.0 g respectively. Hypothyroidism was found in four patients with β thalassaemia and one patient with aplastic anaemia with iron overload between 14.7 (6.8 and 26.1) g and 15.1 g respectively. Human growth hormone deficiency was detected in three patients with β thalassaemia with an iron overload of 4.2 (3.5–6.8) g; all three patients had excellent desferrioxamine compliance.

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Secondary haemochromatosis with cardiomyopathy, liver cirrhosis, hypogonadism, delayed puberty, and diabetes are the most common complications of high transfusion regimens in children with β thalassaemia, aplastic anaemia, and other refractory anaemias.¹

After the introduction of chelation treatment with desferrioxamine, estimating iron

overload became extremely important for therapeutic monitoring. One of the best and most reliable ways to estimate iron overload non-invasively is to determine the serum ferritin concentration, which correlates with rising parenchymal and non-parenchymal iron stores.^{2,3} One problem of this method is the wide range in the relationship between stored iron and ferritin (between 5 and 10 mg iron/ng ferritin/ml⁴). Other problems such as liver enzyme elevations, haemolysis, tumours, and inflammation may also influence this relationship.

In patients with severe haemochromatosis, other non-invasive techniques have been introduced such as SQUID (superconducting quantum interference device), dual energy computed tomography, and magnetic resonance imaging.^{5–7} All are expensive and time consuming and children require sedation or even anaesthesia. Other techniques, such as liver biopsy with measurement of liver iron content, are invasive with a risk of intra-abdominal bleeding.^{8,9}

The aim of this study was to improve the individual reliability of serum ferritin monitoring in patients with secondary iron overload using a simple logarithmic quantitation model that individualises the relationship of serum ferritin and iron stores.

Subjects and methods

Nineteen patients with refractory anaemia (14 with β thalassaemia major, aged 3–18 years; four with aplastic anaemia, aged 16–18 years; and one with sickle cell anaemia, aged 5 years) were investigated between 1975 and 1992. All patients received regular leucocyte-poor red blood cell preparations every 2–4 weeks to maintain haemoglobin concentrations >100 g/l. Since 1979, subcutaneous desferrioxamine (5–7 times/week, 30–100 mg/kg body weight) was given in those with imminent iron overload (ferritin concentrations >1500 μ g/l). A transfusion requirement exceeding 240 ml/kg/year was considered an indication for splenectomy ($n=3$). Measurements of urinary iron excretion (phenanthroline and tripyridiltriazine method) were regularly done to determine the most effective desferrioxamine dosage (faecal iron excretion was estimated to be 50% of total iron excretion).¹⁰ Amounts of transfused blood were noted and summated in each patient (200 ml leucocyte-poor blood = 200 mg iron).

Serum ferritin concentrations were determined simultaneously and were excluded in

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patients with inflammation, increases in liver enzymes, or haemolysis.

As the majority of thalassaemic patients were on a high transfusion regimen (inhibiting erythropoiesis and excessive gastrointestinal iron absorption) from their first year of life their gastrointestinal iron absorption was estimated to be about 15% of food iron (table 1).

In those thalassaemic patients, who first received transfusions after infancy or had chronically low haemoglobin concentrations of ≤ 80 g/l (no transfusions, after splenectomy) iron absorption was estimated to be about 30% of food iron (table 1).¹¹

The correlation between serum ferritin concentrations and iron overload is described as a logarithmic regression curve or exponential curve.⁴ First, we determined the relationship between serum ferritin and iron overload in individual patients before desferrioxamine treatment was started. For this purpose each patient had serial serum ferritin measurements made during a period of 12–36 months. The level of iron stores was taken as 'transfused iron' (1 mg iron/ml blood transfused). For this period of time, gastrointestinal iron absorption was ignored in those patients who received their first blood transfusion in the first 12 months of life ($y = \text{transfused iron} = \text{iron overload} = a + b \log [x = \text{serum ferritin}]$). In others, who received their first blood transfusion after infancy, we estimated gastrointestinally absorbed iron and calculated iron stores as transfused iron plus absorbed iron ($y = \text{transfused iron} [+ \text{gastrointestinally resorbed iron}] = \text{iron overload} = a + b \log [x = \text{serum ferritin}]$). We plotted serum ferritin concentrations against iron load, producing regression curves for each patient (figs 1 and 2).

After desferrioxamine had been started, we measured serum ferritin concentrations and, using the regression curves previously determined for individual patients, derived a value for iron overload after desferrioxamine for each child. Next, we subtracted from this value the total amount of iron transfused and absorbed through the gastrointestinal tract to give a value for total faecal and urinary excretion of iron (table 2).

At the same time as measuring ferritin and iron overload, all patients had a regular (every 6–12 months) cardiovascular check up with echocardiography and/or radionuclide ventriculography. Endocrinological evaluation included thyrotrophin releasing hormone response and growth hormone values subse-

Table 1 Gastrointestinal absorption of iron based on an average iron content of food of 5.5 mg iron/1000 kcal. Patients with β thalassaemia with haemoglobin concentrations > 90 g/l have an average iron absorption of 15%, patients with β thalassaemia with haemoglobin concentrations ≤ 80 g/l were estimated to have an iron absorption of 30%. Modified according to Heinrich et al¹¹

Age (years)	Energy intake (kcal/day)	Food iron (mg/day)	Physiological loss of iron (mg/day)	Net iron intake (mg/day) (15% intestinal absorption)	Net iron intake (mg/day) (30% intestinal absorption)
1–3	1300	7.15	0.5	0.5	1.5
4–6	1600	8.8	0.5	0.8	2.1
7–9	2100	11.6	1.0	0.75	2.5
10–12	2400	13.2	1.0	1.0	3.0
13–15	3000	16.5	1.5	1.0	3.5
16–18	3400	18.7	1.5	1.35	4.15
19–28	2900	16.0	1.5	0.95	3.3

Figure 1 Patient 12 with β thalassaemia had his first transfusion and ferritin determination within the first year of life. In this case the transfused iron was taken to be equivalent to 'iron overload' (the estimated gastrointestinally absorbed iron was ignored).

quent to arginine, clonidine, and growth hormone releasing factor. Haemoglobin, reticulocyte counts, and hepatitis A, B, HIV-1 and HIV-2 serology were done routinely before transfusion. In those with negative hepatitis B virus serology, hepatitis B vaccination was given.

STATISTICAL ANALYSIS

Median, mean, regression (logarithmic), R^2 values were obtained with Statview and Cricketgraph programs using a Macintosh Plus PC (Apple).

Results

In all patients, we found close correlation between individual ferritin determinations and the amount of transfused and gastrointestinally

Figure 2 Patient 4 with β thalassaemia had his first transfusion and ferritin determination at the age of 108 months. In this exceptional case with a long anaemic period, estimated gastrointestinally absorbed iron (about 6 g) was added to the amount of transfused iron to give a measure of 'iron overload'.

Table 2 Estimated gastrointestinally absorbed, excreted iron, and remaining iron overload

Patient No	Age (years)	Age at first transfusion (months)	Units of blood transfused	Transfused iron (g)	Gastrointestinally absorbed iron (g)	Total iron load (g)	Excreted iron in urine and faeces (g)	Iron overload (g)	Actual ferritin ($\mu\text{g/l}$)	Iron overload ferritin ratio	R ²
<i>β Thalassaemia major</i>											
1	18	12	382	80.45	5.58	86.03	59.93	26.1	5200	5.02	0.981
2	18	122	337	69.15	7.97	77.12	45.82	31.3	5000	6.26	0.988
3	16	31	198	39.58	5.87	45.44	31.34	14.1	4600	3.07	0.976
4	17	108	191	39.95	7.05	47.00	31.70	15.3	4478	3.42	0.862
5	11	19	176	34.80	2.88	37.68	27.88	9.8	1930	5.08	0.951
6	10	6	159	31.60	1.70	33.30	26.50	6.8	4000	1.70	0.919
7	14	36	133	27.50	4.21	31.71	17.61	14.1	4760	1.61	0.854
8	7.5	6	103	19.60	2.20	21.62	18.22	3.4	1250	2.72	0.864
9	8	7.5	97	19.16	2.02	21.24	17.84	3.5	1750	2.00	0.647
10	7.5	24	92	18.70	2.60	21.30	11.22	10.1	2740	3.69	0.927
11	6	3	84	15.80	1.38	17.18	14.88	2.3	700	3.29	0.893
12	7	5	71	14.00	1.72	15.72	11.62	4.1	1700	2.41	0.905
13	4	9	43	8.40	0.85	9.25	2.25	7.0	4300	1.63	0.903
14	9	80	21	4.15	2.05	6.20	5.60	0.6	500	1.20	0.913
<i>Aplastic anaemia</i>											
15	18	133	203	43.70	6.77	50.47	26.47	24.0	4000	6.00	0.980
16	18	126	201	38.05	2.14	40.19	23.49	16.7	3300	5.06	0.980
17	18	126	155	30.50	3.10	33.60	26.80	6.8	1080	6.30	1.000
18	17.5	98	140	30.45	3.86	34.25	19.15	15.1	1345	11.23	0.810
<i>Sickle cell anaemia</i>											
19	5.5	4	99	19.90	1.74	21.63	18.31	3.3	3000	1.11	0.925

*R² values for logarithmic ferritin transfused iron regression curves.

absorbed iron. The curves shown in figs 3–5 represent the extrapolated 'idealised' logarithmic ferritin-iron overload regression curves of each individual patient, determined before desferrioxamine was started.

Using contemporaneously determined ferritin concentrations during desferrioxamine treatment, the individual iron overload could be derived from each individual regression curve. The median iron overload detected by this method was estimated as 8.8 (0.6–31.3) g in β thalassaemia, 15.9 (6.8–24) g in those with aplastic anaemia, and 3.25 g in the patient with sickle cell anaemia.

The median of estimated excreted iron was 18.05 (2.25–59.93) g in β thalassaemia, 24.98 (19.15–26.8) g in those with aplastic anaemia, and 18.31 g in the patient with sickle cell anaemia (table 2).

Left ventricular dilatation proved by

echocardiography was observed in three patients with β thalassaemia with a median iron overload of 20.7 (14.1–31.3) g and in one patient with aplastic anaemia with an iron overload of 24.0 g. One patient in each group had a reduction in ejection fraction proven by radionuclide ventriculography (table 3).

Hypothyroidism was found in four patients with β thalassaemia with a median iron overload of 14.7 (6.8–26.1) g and in one patient with aplastic anaemia and an iron overload of 15.1 g (table 3).

Human growth hormone deficiency was detected in three patients with β thalassaemia with a median iron overload of 4.2 (3.5–6.8) g; all three patients had excellent desferrioxamine compliance (table 3).

Figure 3 Course of extrapolated (idealised) logarithmic ferritin-iron overload regression curves in five patients with β thalassaemia and moderate to severe secondary haemochromatosis.

Figure 4 Course of extrapolated (idealised) logarithmic ferritin-iron overload regression curves in seven patients with β thalassaemia and slight secondary haemochromatosis.

Figure 5 Course of extrapolated (idealised) logarithmic ferritin-iron overload regression curves in three patients with aplastic anaemia and one patient (19) with sickle cell anaemia.

Discussion

Non-invasive estimation of iron overload in secondary haemochromatosis is one of the main problems in monitoring desferrioxamine treatment. Although desferrioxamine is able to prevent and partially reverse tissue damage due to iron overload,¹²⁻¹⁵ to some degree, damage may be irreversible.

We have used a method to 'individualise' the relationship between iron overload and serum ferritin. The major part of iron load in chronic anaemia is the amount of transfused iron, which is easily calculated. Gastrointestinally absorbed iron can be a source of considerable iron loading in patients with β thalassaemia^{10 11} without regular transfusions. Consequently, in cases of β thalassaemia with longer periods of anaemia

≤ 80 g/l (for example, no transfusion due to splenectomy, β thalassaemia intermedia, or caused by poverty) an estimate of gastrointestinally absorbed iron must be added to the amount of transfused iron before forming the ferritin-iron overload regression curve.

In patients with aplastic anaemia, sickle cell anaemia, and β thalassaemia who are on high transfusion regimens, gastrointestinal iron absorption can be ignored in forming the ferritin-iron overload regression curve.¹⁰ When exclusion criteria for ferritin are considered, the relationship between transfused iron (plus gastrointestinally absorbed iron), liver iron content, and ferritin is close.^{3 4 16}

As myocardial siderosis is a significant contributing factor in the early death of patients with refractory anaemias^{13 17} we tried to confirm our iron studies by evaluating the cardiovascular status of our patients. According to our data, an iron overload of ≥ 14 g (ferritin about 4000–5000 μ g/l) during desferrioxamine treatment can cause left ventricular dilatation due to cardiomyopathy (table 3).

Other investigations showed early cardiomyopathy after transfusion with 100 units of blood (about 20 g iron) in patients without desferrioxamine.¹⁸ This discrepancy might be explained by the fact that those patients with cardiomyopathy had received a total amount of iron greater than 30 g which might have damaged cardiac tissue before being partially removed by intensive desferrioxamine treatment. The same threshold of 14 g iron overload was also observed in hypothyroidism. All patients with hypothyroidism had an iron overload of ≥ 14 g except for one with an estimated iron overload of about 7 g (total received iron 33 g, current serum ferritin 4000 μ g/l). This child had unexplained high ferritin concentrations even though the estimated iron overload was quite low and the desferrioxamine compliance was good. This patient will need a liver biopsy.

We discovered that all children with abnormal growth hormone values had a low iron burden and excellent desferrioxamine compliance (table 3). Whether neurotoxic adverse effects of desferrioxamine^{14 15} are responsible for this phenomenon is unclear and will require further investigation.

According to our experience, where liver enzymes are chronically raised or ferritin concentrations exceed 4000 μ g/l, desferrioxamine treatment has to be intensified to decrease ferritin and transaminases. For this purpose, high dose intravenous desferrioxamine via a Broviac catheter or Portsystems may be an alternative to subcutaneous desferrioxamine administration if compliance is poor. If it is possible to normalise transaminases and to reduce ferritin concentrations ≤ 4000 μ g/l the logarithmic regression curve can be used to quantitate iron overload. If this is not possible, or rapid evaluation of iron status is necessary, magnetic resonance imaging (T2 relaxation time determination⁷), SQUID (if available), and/or liver biopsy and measurement of liver iron content is necessary.

Table 3 Endocrine and cardiac involvement in patients with secondary haemochromatosis taking desferrioxamine

Patient No	Date of splenectomy	Cardiovascular status (by RNV* and/or ultrasound)	Endocrine status†
β Thalassaemia major			
1	—	—	Hypothyroidism
2	—	Left ventricular dilatation	—
3	May 1977	Left ventricular dilatation	Latent hypothyroidism
4	—	Left ventricular dilatation	Latent hypothyroidism
5	February 1979	—	—
6	—	—	Hypothyroidism, decreased growth hormone
7	—	—	—
8	—	—	—
9	—	—	Decreased growth hormone
10	—	—	—
11	—	—	—
12	—	—	Decreased growth hormone
13	—	—	—
14	—	—	—
Aplastic anaemia			
15	—	Left ventricular dilatation	—
16	—	—	—
17	—	—	—
18	November 1977	—	Hypothyroidism
Sickle cell anaemia			
19	—	—	—

*RNV=Radionuclide ventriculography.

†Assessed by thyrotrophin releasing hormone test, growth hormone after clonidine, arginine, and growth hormone releasing factor.

- 1 Finch CA. *Hemochromatosis in hematology*. 2nd Ed. New York: Mc-Graw Hill, 1972: 413-7.
- 2 Addison GM, Beamish MR, Hales CN, *et al*. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *J Clin Pathol* 1972; 25: 326-9.
- 3 Cazzola M, Borgna-Pignatti C, Stefano PD, *et al*. Internal distribution of excess iron and sources of serum ferritin in patients with thalassaemia. *Scandinavian Journal of Haematology* 1983; 30: 289-96.
- 4 Kaltwasser JP, Werner E. Diagnosis and clinical evaluation of iron overload. Iron chelating therapy. *Baillieres Clin Haematol* 1989; 3: 363-89.
- 5 Brittenham GM, Farrell D, Harris JW, *et al*. Magnetic-susceptibility measurement of human iron stores. *N Engl J Med* 1981; 27: 1671-5.
- 6 Houang MTW, Arozana X, Skalicka A, *et al*. Correlation between computer tomographic values and liver iron content in thalassaemia major with iron overload. *Lancet* 1979; i: 1322-3.
- 7 Kaltwasser JP, Gottschalk R, Schalk KP, Hartl W. Noninvasive quantitation of liver iron overload by magnetic resonance imaging. *Br J Haematol* 1990; 74: 360-3.
- 8 Barry M, Sherlock S. Measurement of liver-iron concentration in needle biopsy specimens. *Lancet* 1971; i: 100-3.
- 9 Bassett MI, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 1986; 6: 24-9.
- 10 Pippard MJ. Iron overload and iron chelation therapy in thalassaemia and sickle cell haemoglobinopathies. *Acta Haematol* 1987; 78: 206-11.
- 11 Heinrich HC, Gabbe EE, Oppitz KH, *et al*. Absorption of inorganic and food iron in children heterozygous and homozygous β -thalassaemia. *Zeitschrift fur Kinderheilkunde* 1973; 115: 1-22.
- 12 Grisaru D, Goldfarb AW, Gotsman MS, *et al*. Deferoxamine improves left ventricular function in β -thalassaemia. *Arch Intern Med* 1986; 146: 2345-9.
- 13 Wolfe L, Olivieri N, Sallan D, *et al*. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassaemia major. *N Engl J Med* 1985; 312: 1600.
- 14 Olivieri NF, Buncic JR, Chew E, *et al*. Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. *N Engl J Med* 1986; 314: 869-73.
- 15 Schiliro G, Russo A. Reappraisal of high-dose desferrioxamine therapy. *Acta Haematol* 1986; 76: 63-4.
- 16 Prieto J, Barry M, Sherlock S. Serum ferritin in patients with iron overload and with acute and chronic liver disease. *Gastroenterology* 1975; 68: 525-33.
- 17 Ehlers RH, Giardina PJ, Lesser ML, *et al*. Prolonged survival in patients with beta-thalassaemia major treated with deferoxamine. *J Pediatr* 1990; 118: 540.
- 18 Buja LM, Roberts WC. Iron in the heart. Etiology and clinical significance. *Am J Med* 1971; 51: 209-21.