Characterisation of Hepatic and Cardiac Iron Deposition During Standard Treatment of Anaemia in Haemodialysis

(Running Title: Hepatic and cardiac iron in dialysis patients)

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Abstract

Background Parenteral iron is integral in the treatment of anaemia of chronic kidney disease (CKD) patients on haemodialysis (HD). However, increased liver iron concentration (LIC) can result from such treatment and this correlates poorly with serum ferritin or transferrin saturation values. It is unclear whether increased cardiac iron concentration also occurs in this setting. We aimed to evaluate the relationship of intravenous iron supplementation to hepatic and cardiac iron deposition in chronic HD subjects.

Methods A cohort of 10 patients on chronic HD for at least 1 year underwent MRI-based quantitation of hepatic and cardiac iron content to evaluate the relationship between intravenous iron supplements and hepatic and cardiac iron deposition. The results were compared against the cumulative parenteral iron dose and serum iron markers.

Results The median age was 61 years (95% confidence interval (CI) 50 - 71), HD time 2.5 years (95%CI 2.0-5.3) and cumulative iron dose 4300mg (95%CI 2110-9045). Hepatic iron concentration was elevated in 8 of 10 subjects (median 46mmol/kg, range 31-76). Cardiac iron levels were within the reference range in all subjects. There was poor correlation between conventional haematinic values and either LIC or cardiac iron levels. None of the study subjects exhibited elevated cardiac iron concentration.

Conclusion Whilst HD patients receiving standard parenteral iron therapy have elevated LICs, this is not associated with cardiac iron deposition. Transferrin saturation and serum ferritin levels are poor markers of either liver or cardiac iron deposition in HD subjects.

Word count = 240

Introduction

The growing number of patients with diabetes mellitus and hypertension has been accompanied by an increased incidence of chronic kidney disease (CKD)[1], including end-stage kidney disease (ESKD). The 2015 ANZDATA report identified 12,091 ESKD patients treated with peritoneal dialysis or haemodialysis (HD) in Australia[2]. CKD is an inflammatory condition associated with high levels of circulating IL-6. This stimulates hepcidin production disrupting enteric iron absorption and tissue iron release[3-5]. This causes both absolute and functional iron deficiency and is one of the principle causes of anaemia in this population. Because anaemia is associated with increased mortality and morbidity [6-8] HD patients are routinely prescribed erythropoetic stimulating agents (ESA) and parenteral iron to increase haemoglobin levels[9, 10]. The KHA-CARI (Kidney Health Australia - Caring for Australians with Renal Impairment) guidelines on the use of iron in CKD patients recommend adjustments on iron dosage based on serum ferritin and transferrin saturation (TSAT) values [10]. This approach is inherently inaccurate, as these parameters are influenced by the systemic inflammation associated with CKD[11, 12]. The uncertainty is reflected in the varied recommendations of national-guidelines worldwide. Japanese recommendations in particular are more conservative than their Australian counterparts[13, 14]. Excess reabsorption or administration of iron leads to toxic accumulation, as there is no physiological mechanism for excretion. Surplus iron accumulates within metabolically active tissues, where it is transiently stored as ferritin. If the cell's storage capability is overwhelmed, free ferric ions may accumulate in the cytosol causing oxidative stress and eventually cell dysfunction or death [15].

Innovative magnetic resonance imaging (MRI) techniques such as FerriScan and T2*/R2* cardiac MRI provide a well-validated, non-invasive means to measure hepatic and cardiac iron deposition that is independent of systemic inflammatory states[16, 17]. Using R2* Ferriscan to measure liver iron concentrations (LIC) Ferrari et al demonstrated elevated LIC in some HD patients receiving standard iron dosages [12] and these findings were confirmed by Rostoker et al.[18]. Although ESKD is associated with dysfunction of many organs, the liver is rarely a cause of significant morbidity in this cohort. The leading cause of death in HD patients is sudden cardiac death (SCD)[19]. In these patients coronary artery disease and heart failure are often not the underlying cause of SCD and other factors such as electrolyte shift or vascular calcification are presumed to play a greater in SCD[19]. An alternative explanation for the high incidence of SCD in HD patients could be cardiac iron overload. Whether the observed increased LIC in dialysis patients [12] is accompanied by evidence of increased cardiac iron deposition has not been extensively investigated. Only one recent study reported on a lack of evidence for cardiac iron overload despite

moderate liver iron overload both measured as T2* MRI in long-term HD patients[20]. However, there is poor agreement between T2*-LIC with R2-LIC (Ferriscan)[21], the latter being the method used in previous studies that assessed liver iron overload in CKD patient [12, 18]. Therefore this pilot study was designed to evaluate the relationship of intravenous iron supplementation to hepatic measured by R2-Ferriscan and cardiac iron deposition measured by cardiac T2* in chronic HD patients.

Methods

The study included patients on chronic maintenance HD that satisfied the following inclusion criteria: *(1)* maintenance HD for at least 12 months, *(2)* standard parenteral iron therapy for at least 12 months, *(3)* full history of all parenteral iron infusions and blood transfusions since commencing HD, *(3)* no contraindications to MR imaging, *(4)* alcohol consumption of less than two standard drinks per day, *(5)* absence of liver disease, active malignancy, pregnancy or other confounding illnesses. Exclusion criteria were *(1)* absolute or functional iron deficiency (ferritin <100µg/L and/or TSAT <20%), *(2)* anaemia requiring transfusion, *(3)* Vitamin B12 or folate deficiency, *(4)* parathyroid hormone level >100pmol/L, *(5)* urea reduction ratio <65%, *(6)* presence of systemic haematological disease (including antibody-mediated pure red cell aplasia) or known haemoglobinopathy and *(7)* major surgery, infection, acute myocardial infarction or malignancy within the last 3 months. The study was approved by the institutional Human Research Ethics Committee and all patients gave their written informed consent to participate in the study.

All subjects received iron polymaltose as a parenteral iron-replacement agent according to KHA-CARI guidelines. The erythropoetic-stimulating agent (ESA) was Epoetin alfa in nine patients and Darbopoetin alfa in one, in the latter the darbopoetin dose was converted to an erythropoietin-equivalent value using the recommended conversion factor of 200:1. Data on cumulative iron and ESA dose were obtained from review of dialysis unit and hospital medical records.

Biochemical and haematological parameters were collected as standard of care in the management of HD subjects, with the only non-standard of care intervention being the performance of MR for measurement of liver and cardiac iron concentrations. All scans and blood tests were acquired at least 14 days after the most recent iron infusion to avoid confounding results.

Liver R2 and cardiac T2* MRI*

MRI using a 1.5 Tesla Siemens Magnetom Vision Plus machine and standardised T2*/R2* relaxometry techniques was performed to assess the extent of hepatic and cardiac iron

deposition[22]. The liver iron concentration (LIC) was measured by R2-Ferriscan as previously described by our group[12,16]. Contiguous ferritin and TSAT levels were obtained to enable correlation between blood-based measures of body iron content and MR techniques.

The reference range for LIC was 3-33mmol/kg dry weight[17]. A LIC >130mmol/kg has been associated with liver injury and a LIC >270mmol/l has been associated with cardiac toxicity[23]. The cardiac T2* reference range was adapted from the work of Wood et al[24]. Cardiac T2* greater than 20ms is not associated with apparent cardiac iron deposition. T2* readings of 10-20ms are consistent with some cardiac iron deposition but little immediate risk of iron-induced cardiac decompensation. Values less than 10ms represent significantly increased risk of iron induced cardiac decompensation.

Statistical analysis

Statistical analysis was performed with STATA 13.1 (StataCorp. 2013. STATA statistical software. College Station, TX: StataCorp LP) using standard parametric and nonparametric methods. Correlation coefficients were assessed using Pearsons method. All P-values are 2-sided and a P-value ≤ 0.05 was considered to be statistically significant.

Results

Baseline data

Ten patients (3 males, 7 females) participated in this study. The median age was 61 years (95% confidence interval (CI) 50 - 71), time on HD 2.5 years (95%CI 2.0-5.3), cumulative iron dose 4300mg (95%CI 2110-9045) and ESA weekly dose 7625U (95%CI 2623-13126) (Table 1). Median TSAT was 26% (95%CI 19-38) and serum ferritin was 371µg/l (95%CI 175-1025). None of the patients had TSAT >50%, but 4 patients had serum ferritin >500µg/l.

Liver R2 and cardiac T2 MRI*

Median LIC was 46mmol/kg (95%C 31-76), 8 patients had LICs above the upper limit of the reference range (33mmol/kg) and 2 patients had LICs >60mmol/kg. Median cardiac T2*was 27.4ms (95%C 24.4-32.9) and all patients were within the reference range. There was no correlation between LIC and cardiac T2* iron concentration (R^2 0.27, p=0.12) (Figure 1).

Correlations between MRI and other key variables

There was a significant correlation between LIC and serum ferritin levels (R^2 0.63, P<0.01). However, there was no significant correlation between LIC and either TSAT (R^2 0.004), cumulative iron dose (R^2 0.03) or time on dialysis (R^2 0.09). There was no correlation

between T2* and TSAT, serum ferritin, cumulative iron dose and number of days on dialysis.

Discussion

Our study extends the observations of previous investigators[12, 18] by demonstrating that the majority (80%) of long-term HD patients receiving iron therapy exhibit significantly elevated LICs, but with no increase in cardiac iron concentration. In fact, cardiac T2* results for all 10 study patients fell within normal limits, excluding cardiac iron overload in our cohort. This is consistent with the findings of Ghoti et al.[20], who performed MR examination of both liver and heart using MRI T2* studies in 21 HD patients in Israel. The lack of statistical significance is likely to be explained by the small sample size in the current study thus lacking the power to demonstarte the relationship of iron accumulation between two known target organs of iron overload[15]. Furthermore, only 20% of patients exceeded the LIC threshold for iron chelation in other settings[23], compared to 60% in our previous study[12]. In the latter the cumulative Fe dose was the strongest determinant of LIC and on average it was 25% higher in the previous cohort[12]. It is therefore possible that ESKD patients with evidence of more pronounced liver iron overload may demonstarte increase cardiac iron deposition. Thus, a high index of suspicion should be maintained for measurement of LIC using noninvasive MR-based methods in patients receiving large dosages of parenteral iron/ESA over a protracted period and who exhibit persistent hyperferitinaemia as this is the only method that is accurate for diagnosis of tissue iron overload in this setting.

The clinical relevance of the LIC above the threshold for iron chelation[23] in dialysis patients is uncertain, as none had apparent hepatic decompensation on the basis of blood tests or physical examination. None of them had severely elevated LICs >130mmol/kg, a level associated with increased risks of liver injury and fibrosis in hemochromatosis[25]. Severely elevated LIC is used as to identify subjects at risk of iron induced cardiomyopathy in thalassaemia major and other transfusion dependent disorders[24]. No study patient approached this LIC threshold and this may reflect the much lower levels of iron loading incurred during iron and ESA treatment of HD subjects. The estimated average cumulative iron dose of a transfusion dependent thalassaemia major patient is 0.4 mg/kg/day[26, 27], whereas our study patients received a mean 0.055 mg/kg/day. Post-mortem studies of HD patients in the 1970s-1980s demonstrated cardiac siderosis[7, 28, 29]. However, these took place at a time when anaemia was routinely treated with blood transfusions, iron replacement regimes were less conservative and ESA were not routinely used. It is likely that such patients also received substantially larger cumulative iron dosages than would

be typical today thus accounting for the discrepancy. As ESA can induce production of erythroferrone, a known inhibitor of hepcidin production[30], it is also possible that it may promote unloading of iron stored in various tissue repositories, including the liver and myocardium, potentially reducing the severity of iron loading.

The poor correlation between LIC and TSAT levels in HD patients confirms previous observations by multiple groups[12, 23, 31]. It has been attributed to highly variable serum iron levels and TSAT in the context of intercurrent inflammation together with the effects of intemittent iron dosing and normal diurnal variation. Interestingly we identified a moderate correlation with serum ferritin (R^2 0.63, $p<0.01$) but not with either dialysis time or cumulative iron dose. Whilst at variance with an earlier observation by our group[12] it demonstrates that ferritin levels may also be unreliable in the population of HD subjects as a guide of iron status. A possible explanation resides in differences between the two study cohorts as patients in this study received smaller cumulative iron dosages, had lower ferritin levels, a shorter HD duration and higher mean CRP.

We conclude that adherence to current guidelines for ESA/parenteral iron replacement can result in LIC's above the upper limits of the reference range. However, cardiac siderosis does not accompany this increase in liver iron concentration. Therefore, these data suggest that current dosing of iron in haemodialysis is safe form a cardiac perspective with no sign of cardiac iron excess. Serum iron markers are poor predictors of either liver or cardiac iron deposition in HD subjects.

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Bibliography

1 Chadban SJ, Briganti EM, Kerr PG, Dunstan DW, Welborn TA, Zimmet PZ, *et al*. Prevalence of kidney damage in Australian adults: The AusDiab kidney study. *J Am Soc Nephrol*. 2003; **14**: S131-8.

2 ANZDATA., Registry. Prevalence of End Stage Kidney Disease. *ANZDATA Registry 2014 Report*. 2015; **2**: 1-11.

3 Chua AC, Graham RM, Trinder D, Olynyk JK. The regulation of cellular iron metabolism. *Crit Rev Clin Lab Sci*. 2007; **44**: 413-59.

4 Graham RM, Chua AC, Herbison CE, Olynyk JK, Trinder D. Liver iron transport. *World J Gastroenterol*. 2007; **13**: 4725-36.

5 Kuragano T, Shimonaka Y, Kida A, Furuta M, Nanami M, Otaki Y, *et al*. Determinants of hepcidin in patients on maintenance hemodialysis: role of inflammation. *Am J Nephrol*. 2010; **31**: 534-40.

6 Carter RA, Hawkins JB, Robinson BH. Iron metabolism in the anaemia of chronic renal failure. Effects of dialysis and of parenteral iron. *Br Med J*. 1969; **3**: 206-10.

7 Gokal R, Millard PR, Weatherall DJ, Callender ST, Ledingham JG, Oliver DO. Iron metabolism in haemodialysis patients. A study of the management of iron therapy and overload. *Q J Med*. 1979; **48**: 369-91.

8 Benz RL, Pressman MR, Hovick ET, Peterson DD. A preliminary study of the effects of correction of anemia with recombinant human erythropoietin therapy on sleep, sleep disorders, and daytime sleepiness in hemodialysis patients (The SLEEPO study). *Am J Kidney Dis*. 1999; **34**: 1089-95.

9 KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Anemia in Chronic Kidney Disease. *Am J Kidney Dis*. 2006; **47**: S11-145.

10 Roger S. The CARI guidelines. Haematological targets. Iron. *Nephrology (Carlton)*. 2006; **11 Suppl 1**: S217-29.

11 Kalantar-Zadeh K, Lee GH. The fascinating but deceptive ferritin: to measure it or not to measure it in chronic kidney disease? *Clin J Am Soc Nephrol*. 2006; **1 Suppl 1**: S9-18.

12 Ferrari P, Kulkarni H, Dheda S, Betti S, Harrison C, St Pierre TG, *et al*. Serum Iron Markers Are Inadequate for Guiding Iron Repletion in Chronic Kidney Disease. *Clin J Am Soc Nephrol*. 2011; **6**: 77-83.

13 Yamamoto H, Tsubakihara Y. Limiting iron supplementation for anemia in dialysis patients--the Basis for Japan's conservative guidelines. *Semin Dial*. 2011; **24**: 269-71.

14 McMahon L. The CARI guidelines. Biochemical and haematological targets. Haemoglobin. *Nephrology (Carlton)*. 2008; **13 Suppl 2**: S44-56.

15 Olynyk JK, Trinder D, Ramm GA, Britton RS, Bacon BR. Hereditary hemochromatosis in the post-HFE era. *Hepatology (Baltimore, Md)*. 2008; **48**: 991-1001.

16 St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, *et al*. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*. 2005; **105**: 855-61.

17 Wood JC. Impact of iron assessment by MRI. *Hematology Am Soc Hematol Educ Program*. 2011; **2011**: 443-50.

18 Rostoker G, Griuncelli M, Loridon C, Couprie R, Benmaadi A, Bounhiol C, *et al*. Hemodialysis-associated hemosiderosis in the era of erythropoiesis-stimulating agents: a MRI study. *Am J Med*. 2012; **125**: 991-99.e1.

19 Green D, Roberts PR, New DI, Kalra PA. Sudden cardiac death in hemodialysis patients: an in-depth review. *Am J Kidney Dis*. 2011; **57**: 921-9.

20 Ghoti H, Rachmilewitz EA, Simon-Lopez R, Gaber R, Katzir Z, Konen E, *et al*. Evidence for tissue iron overload in long-term hemodialysis patients and the impact of withdrawing parenteral iron. *Eur J Haematol*. 2012; **89**: 87-93.

21 Garbowski MW, Carpenter JP, Smith G, Roughton M, Alam MH, He T, *et al*. Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. *J Cardiovasc Magnetic Res*. 2014; **16**: 40.

22 St Pierre TG, Clark PR, Chua-Anusorn W. Single spin-echo proton transverse relaxometry of iron-loaded liver. *NMR Biomed*. 2004; **17**: 446-58.

23 Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. *Blood*. 1997; **89**: 739-61.

24 Wood JC. Magnetic resonance imaging measurement of iron overload. *Curr Opin Hematol*. 2007; **14**: 183-90.

25 Brittenham GM, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, *et al*. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. *N Engl J Med*. 1994; **331**: 567-73.

26 McLaren GD, Muir WA, Kellermeyer RW. Iron overload disorders: natural history, pathogenesis, diagnosis, and therapy. *Crit Rev Clin Lab Sci*. 1983; **19**: 205-66.

27 Gordeuk VR, Bacon BR, Brittenham GM. Iron overload: causes and consequences. *Annual Rev Nutr*. 1987; **7**: 485-508.

28 Pitts TO, Barbour GL. Hemosiderosis secondary to chronic parenteral iron therapy in maintenance hemodialysis patients. *Nephron*. 1978; **22**: 316-21.

29 Ali M, Fayemi AO, Rigolosi R, Frascino J, Marsden T, Malcolm D. Hemosiderosis in hemodialysis patients. An autopsy study of 50 cases. *JAMA*. 1980; **244**: 343-5.

30 Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nature Genetics*. 2014; **46**: 678-84.

31 Canavese C, Bergamo D, Ciccone G, Longo F, Fop F, Thea A, *et al*. Validation of serum ferritin values by magnetic susceptometry in predicting iron overload in dialysis patients. *Kidney Int*. 2004; **65**: 1091-8.

Table 1 Patient demographics and biochemical parameters and MRI iron concentration results in 10 chronic maintenance haemodialysis patients. Results are median and 95% confidence interval (CI)

	Median	95% CI
Age	61	50–71
BMI (kg/m^2)	35.1	30.2-40.4
Dialysis (years)	2.5	2.0-5.3
ESA dose (U/week)	7625	2623-13126
Cumulative Fe (mg)	4300	2110-9045
Cumulative Fe (mg/kg)	60	17-140
Monthly Fe dose (mg)	144	92-189
TSAT (%)	26	19-38
Ferritin (µg/l)	371	175-1025
Hb (g/l)	110	103-114
LIC (mmol/kg)	37	31-76
Cardiac T2* (ms)	27.4	24.4-32.9

ESA: Erythropoetic stimulating agents, LIC: liver iron concentration,

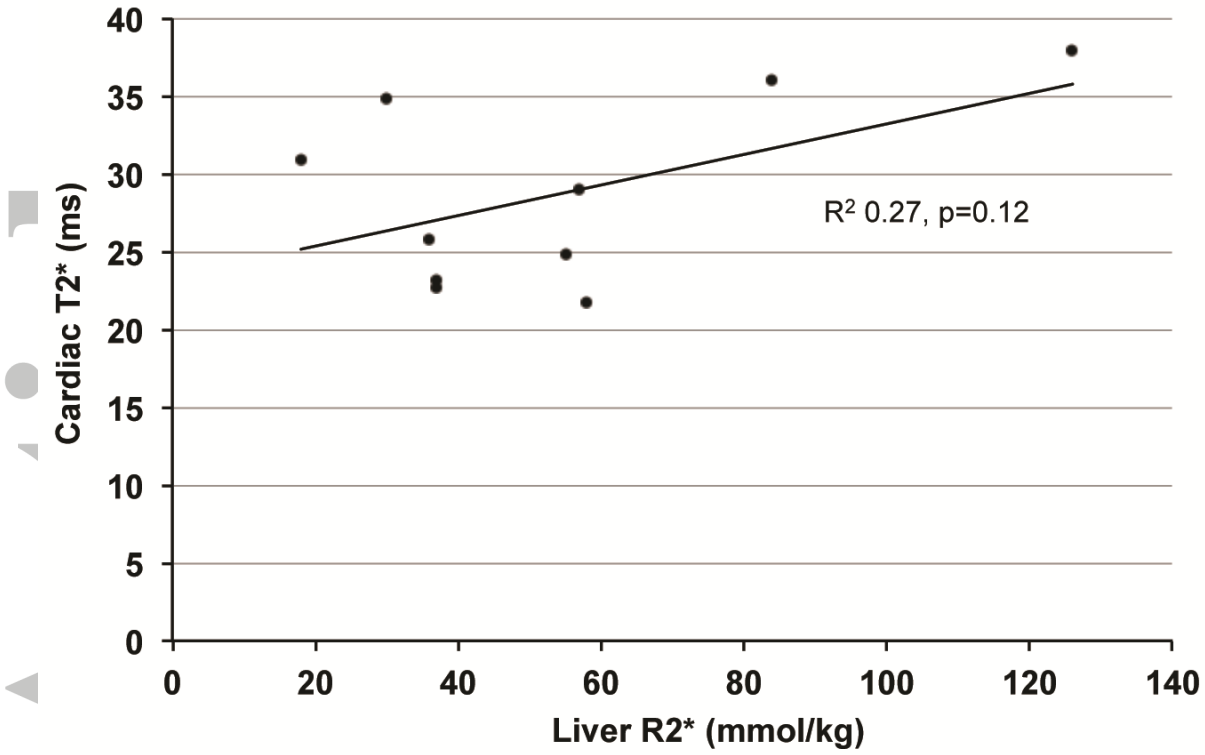

Figure 1 Correlation between iron concentration in the liver, measured by R2*-Ferriscan and heart, measured by cardiac T2* MRI.