Liver Iron Load Influences Hepatic Fat Fraction in End-Stage Renal Disease Patients on Dialysis: A Proof of Concept Study

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is a spectrum of diseases including steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis, and end-stage liver failure. Hepatic iron accumulation has been linked to hepatic fibrosis severity in NASH and NAFLD. Iron overload induced by parenteral (IV) iron therapy is a potential clinical problem in dialysis patients. We analyzed the hypothetical triggering and aggravating role of iron on NAFLD in patients on dialysis.

Methods: Liver iron concentration (LIC) and hepatic proton density fat fraction (PDFF) were analyzed prospectively in 68 dialysis patients by magnetic resonance imaging (MRI). Follow up of LIC and PDFF was performed in 17 dialysis patients during iron therapy.

Findings: PDFF differed significantly among dialysis patients classified according to LIC: patients with moderate or severe iron overload had increased fat fraction (PDFF: 7.9% (0.5–14.8%)) when compared to those with normal LIC (PDFF: 5% (0.27–11%)) or mild iron overload (PDFF: 5% (0.30–11.6%); P = 0.0049). PDFF correlated with LIC, and ferritin and body mass index. In seven patients monitored during IV iron therapy, LIC and PDFF increased concomitantly (PDFF: initial 2.5%, final 8%; LIC: initial 195 μmol/g, final 160 μmol/g; P = 0.0156), whereas in ten patients with iron overload, PDFF decreased after IV iron withdrawal or major dose reduction (initial: 8%; final: 4%; P = 0.0098) in parallel with LIC (initial: 195 μmol/g, final: 45 μmol/g; P = 0.002).

Interpretation: Liver iron overload influences hepatic fat fraction in dialysis patients. Iron overload induced by iron therapy may aggravate or trigger NAFLD in dialysis patients.

Trial registration number (ISRCTN): 80100088.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of diseases including isolated steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis, and end-stage liver failure [1,2]. Patients with NAFLD, especially those with NASH, can develop cirrhosis [1,2]. Liver steatosis related to excessive and pathologic intra-hepatocellular accumulation of fat (mainly as triglycerides) is the histologic hallmark of this syndrome [1,2]. NAFLD is associated with obesity and type 2 diabetes mellitus, and is now becoming epidemic in Western countries where it affects about 10% of children and around 20–30% of adults. NAFLD may become the leading cause of liver transplantation within the next 20 years [1,2]. Noninvasive alternatives to liver biopsy for the assessment of hepatic steatosis have been developed in the past decade including magnetic resonance spectroscopy, which accurately measures the hepatic proton density fat fraction (PDFF; defined as the fraction of mobile protons belonging to triglycerides in relation to those of water), a valuable imaging surrogate biomarker of liver fat content, and magnetic resonance imaging (MRI), using multi-peak fat spectral modeling, which allows reliable liver fat and iron quantification [3–5].

Mild or moderate liver iron deposits are seen in liver biopsies in about 40% of patients with chronic hepatitis B and C, and have been linked to the severity of these diseases [6]. Similarly, mild to moderate increases in liver tissue iron are encountered in about half of patients with NASH and NAFLD [1,2]. Mesenchymal iron deposition is more common than hepatocellular iron accumulation in NASH and NAFLD, and...
Nonalcoholic fatty liver disease (NAFLD) is a spectrum of diseases including isolated steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis, and end-stage liver failure. Hepatic iron accumulation has been linked to the severity of hepatic fibrosis in NASH and NAFLD. Iron overload induced by parenteral (IV) iron therapy (required for anaemia treatment) is now recognized as a potential clinical problem in dialysis patients.

We measured Pascal, Embase, and Medline data-bases with the terms “dialysis”, “intrahepatic iron”, “iron overload”, “liver iron concentration (LIC)”, “liver proton density fat fraction (liver-PDFF)”, “NAFLD” and “NASH” and found no study analyzing the potential relationship between NASH and NAFLD and iron therapy in end-stage renal disease (ESRD).

We analyzed the hypothetical triggering and aggravating role of iron on NAFLD in dialysis patients. We studied 68 dialysis patients receiving erythropoiesis-stimulating agents (ESA) and iron therapy by hepatic MRI (signal intensity ratio and T2* IDEAL IQ), and simultaneously measured liver iron concentration (LIC) and hepatic proton density fat fraction (PDFF).

We found that 57.4% of the dialysis patients in our cross-sectional study had hepatic iron overload (of differing degrees of severity), and that abnormal PDFF was mainly observed in patients with moderate and severe iron overload. In this cohort of dialysis patients, fat fraction was found to correlate with body mass index, and LIC, and ferritin. The similar evolution of LIC and hepatic PDFF, with increasing values in patients on IV iron therapy (iron-sucrose), was observed in seven patients in a longitudinal study, as was a simultaneous decrease after iron withdrawal or a major reduction of iron dosage in ten other patients. These observations strongly support the influence of liver iron load on hepatic fat fraction in dialysis patients.

The detrimental effect of iron overload on liver steatosis shown in this study adds new knowledge on the adverse structural consequences of iron deposits on the liver in addition to the perturbation of hepcidin synthesis shown in cases of iron overload and hypothesized to be one of the factors responsible for the development of cardiovascular complications in ESRD.

In addition to the general dialysis population, overweight patients (who now represent about 20-30% of haemodialysis patients in Western countries and even more in the USA) and patients with known NAFLD and dysmetabolic iron overload syndrome (DIOS) (an increasing contingent of new dialysis patients) may be at increased risk of developing or aggravating fatty liver disease with the indiscriminate and unmonitored use of IV iron therapy.

Finally, our results also suggest the potential adverse effects of iron products on the pathophysiology of fatty liver disease in non-renal patients either as a first hit, favoring its occurrence, or as a second hit, worsening this epidemic disease.
the study; it followed the European Renal Best Practice (ERBP)-2013 anaemia statement and comprised, when required, ESA in haemodialysis and peritoneal dialysis patients, and IV iron in haemodialysis patients (iron-sucrose, Mylan) [18]; oral iron was used as first-line therapy in peritoneal dialysis patients and IV iron was used in these patients as second-line treatment in cases of intolerance or severe iron deficiency [18].

2.3. Longitudinal Study

Changes in liver iron stores and hepatic fat fraction were closely monitored during iron therapy by repeated hepatic MRI in seven patients with low ferritin levels and iron deficiency. Ten other patients with radiologic hepatic iron overload were also closely monitored by repeated hepatic MRI to follow changes in liver iron stores and hepatic fat fraction after iron withdrawal (n = 6) or a major iron dose reduction (n = 4).

2.4. Quantitative MRI of Hepatic Iron Stores and Fat Fraction

A MRI signal-intensity ratio (SIR) method was used for measurement of LIC, based on T1 and T2* contrast imaging without gadolinium (on an Optima MR450W MRI unit; GE Medical Systems, Milwaukee, Wisconsin, USA, operating at a field strength of 1.5 Tesla), as established by Gandon et al. at Rennes University [19], and recently shown to accurately identify iron load in haemodialysis patients by comparison with liver histology (Perls staining) [20]. Wherever possible, patients on iron therapy (IV or oral) received their last iron dose at least one week before MRI [21].

MRI measurements were performed centrally at the Division of Radiology, Claude Galien hospital by the same senior radiologist (YC), who was unaware of the patients' medical history (with the exception of the diagnosis technique), and biochemical results. LIC is expressed in μmol/g of dry liver. Normal LIC values of ≤50 μmol/g were set in dialysis patients [15]. 50 ≤ LIC values ≤ 100 μmol/g represent mild iron overload, 100 ≤ LIC values ≤ 200 μmol/g moderate iron overload, and >200 μmol/g severe iron overload [15,19].

During the same session, PDFF was determined by MRI multi-peak fat spectral modeling using the IDEAL IQ algorithm, Food and Drug Administration (FDA) validated software of GE Healthcare, which is a three-dimensional volumetric imaging sequence used to create T2* and triglyceride fat fraction maps from a single breath-hold acquisition [22,23]. This technique was used to estimate R2* (T2*) and PDFF in the liver in a single simultaneous acquisition. The resulting PDFF maps were then corrected for T2* effects [22,23]. Normality of hepatic PDFF was set at ≤5% [3]. In the same session, we also assessed liver iron load by T2* MRI and splenic iron load by T2* MRI (normal value ≥15 ms) [16]. Liver iron load determined at classical R2* and by R2* IDEAL IQ were used for validation of LIC determined by signal intensity ratio as advocated by Puisant et al. [16].

It should be noted that, in France, hepatic MRI is fully reimbursed by the national health insurance system for the diagnosis and monitoring of iron overload diseases.

2.5. Biological Markers

The efficacy of anaemia treatment was determined using a haemoglobin assay and reticulocyte counts every two weeks in patients on haemodialysis and monthly in those treated by peritoneal dialysis. Monthly measurements were also performed of iron biomarkers (ferritin, transferrin, serum iron, and transferrin saturation (TSAT)), and C-reactive protein. Metabolic biomarkers were analyzed every three months and comprised glycemia, HbA1C, total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), and serum levels of liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and y-glutamyl transpeptidase (GGT)).

2.6. Search for HFE Gene Mutation

Patients with abnormal iron load by MRI were screened for the C282Y, H63D, and S65C HFE gene mutations (Laboratory CERBA, Saint-Ouen-l’Aumône, France).

2.7. Alcohol Use Disorder Identification Test (AUDIT) and Charbon’s Comorbidity Index

A validated French translation of the AUDIT questionnaire was used to quantify alcohol consumption and to detect alcohol addiction [24]. The age modified Charlson’s comorbidity index, validated in dialysis patients, was calculated as described by Di Iorio et al. [25].

2.8. Statistical Analyses

As values did not conform to a Gaussian distribution (Shapiro-Wilk normality test), according to Sheskin, all data are expressed as medians and range [26]. Percentages are given either crude or with their 95% confidence intervals (CI; modified Wald method) [26]. Patients with moderate iron overload and those with severe iron overload were pooled together for all statistical analyses.

The different groups of patients were compared using non-parametric analysis of variance (Kruskal-Wallis test) for continuous variables, followed by Dunn’s post-test, and with the Chi² test for categorical variables [26]. Correlations between the different variables and LIC by MRI, or hepatic PDFF by MRI, were identified with the Spearman’s rank-order correlation coefficient. Spearman’s rank-order coefficient was also used to analyze correlations between the three methods of determination of LIC, namely SIR according to Rennes University, T2* and T2* IDEAL IQ. Bland and Altman analysis of LIC measurements by T2* IDEAL IQ as compared to T2* measurements of LIC was also used in the evaluation of the influence of fat fraction on liver iron concentration. Prism 7 software (Graphpad, San Diego, USA) was used for all statistical analyses, and P < 0.05 was considered to denote statistical significance [26].

Finally, binary logistic regression analysis was used to determine the capacity of several clinically and biologically relevant variables to classify patients as having normal (<5%) or elevated (≥5%) hepatic PDFF by MRI, and also to classify patients as having normal (<50 μmol/g) or increased LIC (>50 μmol/g) by MRI (SPSS software; IBM Bois-Colombes, France) [26].

3. Results

3.1. Characteristics of the Study Population

The study cohort comprised 68 adult ESRD patients: 62 treated by intermittent haemodialysis at Claude Galien Hospital (Quincy-sous-Sénart), three treated by intermittent haemodialysis at Clinique du Landy (Saint-Ouen), and three by peritoneal dialysis (two at Hospital Marc Jacquet, Melun, and one at Pitié-Salpêtrière Hospital, Paris). MRI was performed at least seven days after IV iron infusion or iron pill consumption in 66 patients and five days after in two other patients. For these latter patients, this delay was considered sufficient to avoid any influence of iron consumption on MRI results [21]. A total of 77 patients were initially screened and studied by MRI for simultaneous iron and fat liver determination; nine patients were excluded from the study for the following reasons: pre-existing liver disease (n = 4), refusal to sign the informed consent form (n = 1), blood transfusions (n = 1), IV iron infusion 48 h before MRI (n = 1), chronic severe inflammatory process (n = 1), lack of any biological markers available (n = 1). The demographic, clinical, and biological characteristics of these patients are summarized in Tables 1 and 2.
Table 1
Characteristics and findings in 68 dialysis patients monitored simultaneously for liver iron and fat content (classified according to hepatic non-heme iron stores measured by MRI).

| Variables | Normal iron load (LiC ≤ 50 μmol/g) (Group A; N = 29) | Mild iron overload (50 < LiC ≤ 100 μmol/g) (Group B; N = 23) | Moderate and severe iron overload (LiC > 100 μmol/g) (Group C; N = 16) | P-value* | Comparison of groups A, B, C** |
|-----------|------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------|-----------------------------|
| Age (years) | 70 (41–88) | 66 (26–92) | 63.5 (28–96) | 0.0700 | A/B, P = 0.0032 |
| Female, n (%) | 11 (37.9) | 5 (21.7) | 9 (56.3) | 0.0879 | A/C, P = 0.0041 |
| Dialysis vintage before MRI (months) | 11 (2–60) | 30 (5–147) | 20 (5–138) | 0.0016 | B/C, P = 0.9999 |
| ESA therapy, n (%) | 0 (0–61) | 5 (22.7) | 2 (12.5) | 0.1589 | A/B, P = 0.0084 |
| Iron in RBC packs transfused per month of dialysis (mg/month) | 11 (37.9) | 19/22 (86.4) | 12 (75) | 0.0251 | A/C, P = 0.0078 |
| Liver iron concentration by MRI-SIR (μmol/g dry weight) | 15 (5–50) | 70 (55–100) | 195 (150–260) | 0.0001 | B/C, P = 0.4373 |
| Liver T2* (ms) | 21.6 (12.8–33.3) | 9.3 (7.1–29.7) | 5.96 (3.9–11.3) | 0.0001 | A/B, P = 0.0001 |
| Liver T2* IDEAL IQ (ms) | 24.0 (13.6–34.0) | 10.3 (4.7–37.0) | 7.05 (1.8–12.3) | 0.0001 | A/C, P = 0.0001 |
| Liver fat fraction by MRI (%) | 5 (0.27–11.0) | 5 (0.3–11.6) | 7.9 (0.5–14.8) | 0.0049 | A/C, P = 0.0001 |
| Splenic T2* (ms) | 27.35 (8.5–72.3) | 13 (3.9–36.1) | 6.2 (3.5–34.2) | 0.0001 | B/C, P = 0.0056 |
| Splenic T2*; n (%) abnormality (>15 ms) | 3/28 (10.7) | 13 (56.5) | 11 (68.8) | 0.0001 | A/B, P = 0.0021 |
| Modified Charlson’s comorbidity index | 6 (2–11) | 5.5 (2–12) | 6 (2–10) | 0.8715 | A/C, P = 0.3886 |
| AUDIT alcohol index | 3 (0–9) | 1 (0–12) | 2 (0–8) | 0.0766 | B/C, P = 0.0001 |
| Weight (kg) | 72.25 (41.5–130) | 71 (46.5–103) | 70.25 (47–96) | 0.7824 | A/B, P = 0.0001 |
| Body mass index (kg/m²) | 26.05 (18.2–40.1) | 23.7 (18.2–36.5) | 24.25 (18.6–37.5) | 0.4862 | A/C, P = 0.0001 |
| Diabetes, n (%) | 6 (20.7) | 4 (17.4) | 5 (31.3) | 0.5743 | B/C, P = 0.3886 |

All values shown are median (range) unless stated otherwise.

LIC: liver iron concentration; ESA: erythropoiesis stimulating agents; MRI: magnetic resonance imaging; SIR: Signal Intensity Ratio; IV: intravenous.

Iron in RBC packs transfused: iron in red blood cell packs transfused (we calculate 200 mg of iron per red blood cell packs transfused and we report per month of dialysis).

P value determined using either:
* Kruskal-Wallis or X² test.
** Dunn’s post test.

Table 2
Biochemical markers of iron, glucose, and lipid metabolism, and liver enzymes in 68 dialysis patients monitored simultaneously for liver iron and fat content (classified according to hepatic non-heme iron stores measured by MRI).

| Variables | Normal iron load (LiC ≤ 50 μmol/g) (Group A; N = 29) | Mild iron overload (50 < LiC ≤ 100 μmol/g) (Group B; N = 23) | Moderate and severe iron overload (LiC > 100 μmol/g) (Group C; N = 16) | P-value* | Comparison of groups A, B, C** |
|-----------|------------------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|---------|-----------------------------|
| Haemoglobin (g/dL) | 11.2 (7–17.1) | 11.7 (10.1–14.5) | 11.4 (7.6–13.8) | 0.1078 | A/B, P = 0.0021 |
| Serum ferritin (ng/mL) | 70 (9–326) | 185 (22–562) | 378 (16–1229) | 0.0003 | A/C, P = 0.0003 |
| Serum iron (μmol/L) | 11.35 (3.5–29.7) | 10.1 (4.5–18.3) | 10.9 (6–21.5) | 0.5682 | A/B, P = 0.0003 |
| Serum transferrin (g/L) | 2.25 (1.3–3.1) | 1.9 (1.5–2.6) | 1.8 (1.3–2.8) | 0.0021 | A/C, P = 0.0014 |
| Transferrin saturation (%) | 17.02 (6.67–54) | 20.8 (9.47–43.06) | 25 (12.63–47.78) | 0.0452 | A/B, P = 0.0014 |
| CRP (mg/L) | 3.1 (1–15.8) | 1.9 (1–10.7) | 4.1 (1–14.2) | 0.3914 | A/C, P = 0.0006 |
| Glycated haemoglobin (HbA1c) (%) | 5.6 (4.6–6.5) | 5.3 (4.6–7.1) | 5.5 (4–7.7) | 0.2234 | B/C, P = 0.0037 |
| Total cholesterol (mmol/L) | 4.37 (2.8–8.13) | 4.16 (2.08–7.87) | 4.6 (3.23–5.98) | 0.8621 | A/B, P = 0.0014 |
| HDL cholesterol (mmol/L) | 1.2 (0.43–2.31) | 1.2 (0.47–2.76) | 1.56 (0.88–3.47) | 0.0961 | A/C, P = 0.0037 |
| LDL cholesterol (mmol/L) | 2.28 (0.76–5.21) | 2.25 (0.9–5.3) | 2.28 (0.59–3.88) | 0.9699 | B/C, P = 0.0037 |
| Triglycerides (mmol/L) | 1.91 (0.58–4.56) | 1.25 (0.58–3.32) | 1.4 (0.5–4.23) | 0.3734 | A/B, P = 0.0014 |
| ASAT (U/L) | 14.5 (6–40) | 13 (6–23) | 13 (6–22) | 0.3207 | A/C, P = 0.0014 |
| ALT (U/L) | 14 (5–34) | 14 (5–27) | 11 (7–21) | 0.6078 | B/C, P = 0.0037 |
| GGT (U/L) | 26 (9–143) | 25 (9–262) | 23 (11–53) | 0.5972 | A/C, P = 0.0037 |

All values shown are median (range).

MRI: magnetic resonance imaging; LIC: liver iron concentration; CRP: C-reactive protein; HDL: high density lipoprotein; LDL: low density lipoprotein; ASAT: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: γ-glutamyl transferase.

P value determined using “Kruskal-Wallis test; ** Dunn’s post test.”
Fig. 1. Correlations analyzed by Spearman’s rank-order coefficient between three methods of determination of liver iron concentration in 68 dialysis patients: Signal Intensity Ratio according to Rennes University, T2* and T2* IDEAL IQ. 
(a) Spearman coefficient between liver T2* and T2* IDEAL IQ. 
(b) Spearman coefficient between LIC-SIR and liver T2*. 
(c) Spearman coefficient between LIC-SIR and liver T2* IDEAL IQ.

Fig. 2. Bland-Altman plots of liver T2* IDEAL IQ and liver T2* in 68 dialysis patients: difference versus average.
3.2. Hepatic iron Load by MRI

LIC was normal (≤50 μmol/g) in 29/68 patients (42.7% [95%CI: 31.59–54.49]) (Table 1). Iron overload by MRI was mild (50 < LIC ≤ 100 μmol/g) in 23/68 patients (33.8% [95%CI: 23.68–45.69]) (Table 1). A total of 16 dialysis patients (23.5% [95%CI: 14.94–34.95]) had either moderate (n = 9; 100 < LIC ≤ 200 μmol/g) or severe (n = 7; LIC > 200 μmol/g) iron overload by MRI (Table 1). Values of LIC at signal intensity ratio according to Rennes University were highly correlated with both values at T2* (rho = −0.8932, P < 0.0001, Spearman’s correlation test) and those at T2* IDEAL IQ (rho = −0.8823, P < 0.0001, Spearman’s correlation test) (Fig. 1). Values of LIC at T2* were closely correlated with those at T2* IDEAL IQ (rho = 0.9636, P < 0.0001, Spearman’s correlation test (Fig. 1). Bland and Altman analysis of LIC measured by T2* IDEAL IQ and by T2* showed a good agreement between the two methods (bias = 1.80 ms; standard deviation of bias = 2.76 ms and 95% limits of agreement from −3.61 to 7.22 ms) (Fig. 2).

Most patients with liver iron overload had decreased splenic T2* indicating concomitant excess iron in the spleen (Table 1). Iron overload by MRI was not associated with either homozygous or heterozygous C282Y, H63D, or S65C HFE gene mutations. Hepatic iron stores correlated with dialysis vintage, and serum ferritin, and iron dose infused per month in both the Spearman’s correlation test and binary logistic regression (P < 0.05; Table 3).

Table 3
Correlations between demographic, clinical variables, and biochemical markers, and hepatic iron stores in 68 dialysis patients monitored by MRI.

| Parameters                                                | Correlation of LIC | Binary logistic regression: Model of LIC in two groups |
|-----------------------------------------------------------|--------------------|--------------------------------------------------------|
|                                                           | Spearman’s correlation test | Univariate analysis | Multivariate analysis |
|                                                           | Spearman rho (r) [95%CI] | Odds Ratio [95%CI] | P-value | Odds Ratio [95%CI] | P-value |
| Age (years)                                               | −0.1735 [−0.4016; 0.0749]; P = 0.1570 | 0.984 [0.953; 1.016] | 0.318 | Not studied |
| Dialysis vintage before MRI (months) before MRI           | 0.3914 [0.1599; 0.5822]; P = 0.0011 | 1.037 [1.010; 1.066] | 0.008 | 1.065 [1.012; 1.122] | 0.016 |
| Parenteral iron dose/month (IV) (mg/month) before MRI     | 0.6013 [0.3834; 0.7559]; P = 0.0001 | 1.012 [1.005; 1.020] | 0.001 | 1.011 [1.000; 1.023] | 0.045 |
| Darbepoetin dose/month (μg/month)                         | 0.2233 [−0.0643; 0.4763]; P = 0.1156 | Not studied | Not studied |
| Liver fat fraction by MRI (%)                             | 0.2763 [0.0336; 0.4868]; P = 0.0224 | 1.084 [0.922; 1.274] | 0.331 | Not studied |
| Body mass index (kg/m²)                                   | −0.1449 [−0.3818; 0.11]; P = 0.2496 | 0.962 [0.873; 1.061] | 0.438 | Not studied |
| C-reactive protein (mg/L)                                 | 0.0748 [−0.1834; 0.3234]; P = 0.5600 | Not studied | Not studied |
| Total cholesterol (mmol/L)                                | 0.0345 [−0.2265; 0.2908]; P = 0.7920 | Not studied | Not studied |
| Triglycerides (mmol/L)                                    | −0.2807 [−0.5031; −0.0235]; P = 0.0284 | 0.503 [0.267; 0.947] | 0.033 | Not studied |
| Glycated haemoglobin (HbA1c %)                            | −0.1203 [−0.3677; 0.1431]; P = 0.3559 | 0.609 [0.283; 1.308] | 0.203 | Not studied |
| AUDIT alcohol index                                       | −0.1386 [−0.3857; 0.1271]; P = 0.2911 | 0.859 [0.685; 1.076] | 0.186 | Not studied |
| Modified Charlton’s comorbidity index                     | −0.0178 [−0.2637; 0.2302]; P = 0.8863 | Not studied | Not studied |
| Liver T2* (ms) by MRI                                     | −0.08932 [−0.9338; −0.8297]; P < 0.0001 | Not studied | Not studied |
| Liver T2* IDEAL IQ (ms) by MRI                            | −0.8823 [−0.9727; −0.8124]; P < 0.0001 | Not studied | Not studied |
| Iron in RBC packs transfused per month of dialysis (mg/month) | −0.1182 [−0.3884; 0.1709]; P = 0.4089 | Not studied | Not studied |
| Serum ferritin (ng/mL)                                    | 0.4757 [0.2493; 0.6528]; P < 0.0001 | 1.009 [1.003; 1.014] | 0.003 | 1.029 [1.004; 1.054] | 0.021 |
| Transferrin saturation (%)                                | 0.2790 [0.0147; 0.493]; P = 0.0335 | 1.054 [0.994; 1.118] | 0.080 | 0.952 [0.781; 1.161] | 0.629 |

CI: confidence interval; LIC: Liver iron concentration; MRI: magnetic resonance imaging; IV: intravenous.

Iron in RBC packs transfused: Iron in red blood cell packs transfused (we calculate 200 mg of iron per red blood cell pack transfused and we report per month of dialysis).

Fig. 3. Scattergrams of liver fat fraction in 68 dialysis patients classified according to liver non-heme iron stores measured by magnetic resonance imaging.
Table 4

Correlations between demographic, clinical variables, and biochemical markers and liver fat fraction in 68 dialysis patients.

| Parameters                                      | Correlation of liver fat fraction | Binary logistic regression: Model of liver fat fraction in two groups |
|-------------------------------------------------|-----------------------------------|---------------------------------------------------------------|
|                                                 | Spearman’s correlation test       | Univariate analysis                                           |
|                                                 | [95%CI]                           | Odds Ratio [95%CI] P-value                                    |
|                                                 | [95%CI]                           | Multivariate analysis                                         |
|                                                 | [95%CI]                           | Odds Ratio [95%CI] P-value                                    |
| Age (years)                                     | −0.0149 [−0.2591; 0.2312]; P = 0.9043 | 1.010 [0.978; 1.042] 0.547 Not studied                       |
| Gender                                          | Not studied                        | 1.851 [0.640; 5.358] 0.256 6.222 [0.944; 41.024] 0.057       |
| Dialysis vintage before MRI (months)            | 0.0606 [−0.1892; 0.3031]; P = 0.6260 | 1.003 [0.988; 1.017] 0.705 Not studied                       |
| Parenteral iron dose per month (mg/month) before MRI | 0.1293 [−0.1598; 0.398]; P = 0.3658 | 1.002 [0.996; 1.007] 0.503 Not studied                       |
| Darbeopectin dose per month (µg/month)          | −0.1826 [−0.443; 0.1062]; P = 0.1997 | Not studied                                                 |
| Liver iron concentration by MRI (µmol/g)        | 0.2765 [0.0336; 0.4886]; P = 0.0024 | 1.010 [1.002; 1.019] 0.020 1.015 [1.002; 1.028] 0.019        |
| Liver T2* (ms)                                   | −0.3179 [−0.5224; −0.0788]; P = 0.0002 | Not studied                                               |
| Liver T2* (IDEAL IQ) by MRI (ms)                | −0.2818 [−0.4944; −0.0374]; P = 0.0020 | Not studied                                               |
| Body mass index (kg/m²)                         | 0.4537 [0.2289; 0.6325]; P = 0.0001 | 1.190 [1.042; 1.358] 0.010 1.574 [1.142; 2.168] 0.006        |
| C-reactive protein (mg/L)                       | 0.276 [0.0229; 0.4959]; P = 0.0285 | 1.115 [0.942; 1.320] 0.207 Not studied                       |
| Total cholesterol (mmol/L)                      | −0.1788 [−0.4184; 0.0840]; P = 0.1679 | Not studied                                               |
| Triglycerides (mmol/L)                          | 0.1255 [−0.1379; 0.3723]; P = 0.3352 | 1.232 [0.674; 2.251] 0.497 Not studied                       |
| Glycated haemoglobin (HbA1c) (%)                | −0.0113 [−0.2695; 0.2483]; P = 0.9309 | Not studied                                               |
| AUDIT alcohol index                             | 0.0988 [−0.1666; 0.3508]; P = 0.4529 | 1.183 [0.918; 1.526] 0.194 Not studied                       |
| Modified Charlson’s comorbidity index           | 0.0338 [−0.215; 0.2785]; P = 0.7861 | Not studied                                               |
| Iron in RBC packs transfused per month of dialysis (mg/month) | −0.2361 [−0.4686; 0.0506]; P = 0.0954 | Not studied                                               |
| Diabetes                                        | Not studied                        | 2.839 [0.716; 11.262] 0.138 1.574 [1.142; 2.168] 0.006        |
| Serum ferritin (ng/mL)                          | 0.3131 [0.0814; 0.542]; P = 0.0085 | 1.005 [1.001; 1.009] 0.025 1.008 [0.996; 1.015] 0.030        |
| Transferrin saturation (%)                      | 0.2112 [−0.0482; 0.444]; P = 0.0994 | 1.040 [0.982; 1.102] 0.181 0.960 [0.878; 1.049] 0.362        |

MRI: magnetic resonance imaging; IV: intravenous; CI: confidence interval.

Table 5

Follow up of hepatic iron stores and liver fat fraction determined by MRI and biochemical parameters during iron therapy in 17 dialysis patients.

| Changes in 7 patients with increasing liver iron stores | Changes in 10 patients with decreasing liver iron stores |
|--------------------------------------------------------|--------------------------------------------------------|
| Initial  | Final  | Difference [95%CI] | P-value* | Initial  | Final  | Difference [95%CI] | P-value* |
| Liver iron concentration by MRI (µmol/g)               | 20 (5–45) | 160 (45–210) | 125 [65.52; 165.9] | 0.0156 | 195 (90–260) | 45 (17–180) | −131.5 [−164.20; −90.84] | 0.0020 |
| Liver fat fraction by MRI (%)                          | 2.53 (1.5–5) | 8 (6.67–11.6) | 4.6 [3.32; 8.19] | 0.0156 | 8 (5.45–13.73) | 4 (1–10.3) | −4.45 [−8.06; −0.84] | 0.0098 |
| Mean parenteral iron dose (mg/month)                   | 92 (0–201) | 283 (177–352) | 244 [97.41; 294.3] | 0.0156 | 213.5 (0–308) | 0 (0–156) | −177.5 [−289; −66.72] | 0.0625 |
| Mean ESA dose (µg/month)                               | 110 (46–237) | 96 (0–240) | 5 [−83.76; 102] | 0.0375 | 215.5 (111–371) | 196.5 (0–630) | −19.0 [−26.1; 10.5] | 0.6875 |
| Haemoglobin (g/dL)                                    | 10.6 (8.5–12.2) | 9.8 (8–12.5) | −0.5 [−2.15; 1.01] | 0.4688 | 11.4 (7.6–12.5) | 10.95 | −0.05 [−1.32; 1.12] | 0.9102 |
| CH₄ (µg)                                              | 29.7 (27.3–31.1) | 31.9 (25.7–33.3) | 2.1 [−3.04; 4.47] | 0.8125 | 31.8 (27.3–36.3) | 30.85 | −0.25 [−1.66; 3.2] | 0.6797 |
| Serum ferritin (ng/mL)                                | 32 (14–69) | 199 (16–728) | 162 [20.8; 491.4] | 0.0781 | 463 (16–1170) | 166 (22–460) | −262.5 [−565.9; −106.5] | 0.0039 |
| Liver iron concentration by MRI (µmol/L)              | 8 (5.6–10.5) | 11.3 (6.73–17.3) | 4.2 [−0.2; 8.97] | 0.1094 | 10.45 (6–21.5) | 8.2 (5–18) | −2.25 [−4.35; 0.17] | 0.0820 |
| Liver transferrin (g/L)                               | 2.1 (1.7–2.6) | 1.9 (1.7–2.3) | 0 [−0.59; 0.19] | 0.4062 | 1.85 (1.3–2.4) | 1.9 (1.7–3.5) | −0.05 [−1.13; 1] | 0.0078 |
| Transferrin saturation (%)                            | 15.84 (8.6–20) | 26.59 (12.83–34.44) | 12.33 [0.59; 19.77] | 0.0781 | 25.33 | 18.33 | −6.6 [−12.25; −1.07] | 0.0039 |
| C-reactive protein (mg/L)                             | 4.2 (1.136) | 3.5 (1.7–2) | 0 [−4.16; 0.76] | 0.25 | 4.75 (1.7–19.9) | 7 (1–20.2) | −7.5 [−17.17; 2.15] | 0.1641 |

Data shown are median (range) unless stated otherwise.

MRI: magnetic resonance imaging; ESA: erythropoiesis stimulating agents; CH₄: reticuloocyte haemoglobin content; CI: confidence interval.

* Wilcoxon paired test.
3.3. Hepatic Fat Fraction by MRI

Liver PDFF differed significantly between dialysis patients classified according to hepatic non-heme iron stores: those with moderate or severe iron overload by MRI (LIC > 100 μmol/g) had increased fat fraction (PDFF: 7.9% (0.5–14.8)) when compared to those with either normal iron load (LIC ≤ 50 μmol/g) (PDFF: 5% (0.27–11)) or mild iron overload (50 < LIC ≤ 100 μmol/g) (PDFF: 5% (0.30–11.6); P = 0.0049 Kruskal-Wallis test) (Table 1 and Fig. 3). Similarly, the percentage of dialysis patients with abnormal PDFF (≥5%) was significantly higher in
the group with moderate and severe iron overload (93.8% [95% CI: 69.69 – 99.99]) when compared to the groups of patients with normal iron load (55.2% [95% CI: 37.54 – 71.6]) and mild iron overload (52.2% [95% CI: 32.96 – 70.77]) (P = 0.0148, Chi² test).

Among the clinical variables, liver PDFF correlated with LIC (at signal intensity ratio MRI rho = 0.2765, P = 0.0224, at T2* rho = –0.3179, P = 0.0082, and at T2* IDEAL IQ rho = –0.2818, P = 0.0209), with body mass index (BMI) (rho = 0.4537, P = 0.0001), C-reactive protein (rho = 0.276, P = 0.0285) and serum ferritin (rho = 0.3313, P = 0.0085, Spearman’s correlation test) (Table 4), whereas none of the following demographic, clinical, and biological parameters correlated with PDFF: dialysis vintage, age, modified Charlson’s comorbidity index score, AUDIT score, TSAT, total cholesterol, triglyceride levels, or HbA1C (P > 0.05, Spearman’s correlation test) (Table 4).

Binary logistic regression analyses, three variables correctly classified the dialysis patients into those with normal liver fat content and those with increased fat fraction (PDFF ≥ 5%), namely LIC (Odds ratio (OR): 1.015 [95%CI: 1.002 – 1.028]; P = 0.019), BMI (OR: 1.574 [95%CI: 1.142 – 2.168]; P = 0.006) and ferritin (OR = 1.008 [95%CI: 1.001 – 1.015]; P = 0.030) (Table 4).

3.4. Longitudinal Study

In the seven patients monitored closely during parenteral therapy, both LIC and PDFF increased significantly during follow up (P = 0.0156, Wilcoxon’s paired test) (Table 5 and Fig. 4).

In the ten iron-overloaded patients, both LIC and PDFF decreased significantly after iron withdrawal (n = 6), or after a major reduction in the infused dose (n = 4) (P < 0.01, Wilcoxon’s paired test) (Table 5 and Fig. 4).

4. Discussion

Our study investigated dialysis patients routinely receiving ESA and iron therapy by means of a non-invasive tool, namely hepatic MRI, and focused simultaneously on LIC and PDFF. We found that 57.4% of dialysis patients in our cross-sectional study had hepatic iron overload (of differing degrees of severity) and that abnormal liver fat fraction was mainly observed in patients with moderate and severe iron overload. In this cohort of dialysis patients, PDFF was found to correlate with BMI and LIC, and ferritin. Serum ferritin was indicative of liver iron stores in these non-inflected dialysis patients, in line with a previous study specifically devoted to the diagnostic value of iron biomarkers for estimating LIC performed in 212 fit haemodialysis patients free of overt inflammation and malnutrition [27]. Interestingly, serum ferritin was also indicative of PDFF in these dialysis patients in line with data in NAFLD and also suggesting a direct pathway between liver iron load and hepatic steatosis in ESRD patients.

The similar evolution of LIC and PDFF, with increasing values observed in seven patients on IV iron therapy, as well as their simultaneous decrease after iron withdrawal or a major reduction in iron dosage (in eight out ten patients), strongly supports the influence of LIC on PDFF in dialysis patients.

The main limitation of our pilot study relates to its design, mainly cross-sectional, with most of the patients (91%) coming from one single haemodialysis center. Moreover, as a proof of concept study, the number of patients was limited; we did not have the possibility of studying the influence of non-HFE iron regulating genes and to analyze concomitant fibrosis by Fibroscan. All these important issues remain to be explored by a confirmatory clinical study with a larger sample size.

However, our results are unlikely to be a technique artefact since iron and fat exert opposite effects on T2* MRI and MRI fat spectral modeling, and IDEAL IQ software takes this phenomenon into account as well as the influence of other confounders [22,23].

The high correlation and linear relationship between LIC measured by signal intensity ratio and T2* measured by IDEAL IQ suggest that the PDFF method has not been adversely influenced by the liver iron content in our study. Similarly, the high correlation and linear relationship between LIC measured by T2* and T2* IDEAL IQ, together with the low bias and good agreement at the Bland-Altman analysis between these two methods, also suggest that liver iron concentration has not been influenced by hepatic fat content in this study.

One other important issue relates to the potential limitation in the validation of the IDEAL IQ PDFF method for fat determination in the range of liver iron concentrations encountered in this study. IDEAL, the ancestor of IDEAL IQ, was shown in an experimental model using ob/ob NAFLD mice not to be influenced by liver iron overload induced experimentally by injection of supermagnetic iron oxide (SPIO) [28], whereas SPIO infused in 14 patients with liver disease and investigated with IDEAL IQ significantly increased R2* in liver (and spine) without modifying hepatic (and spine) PDFF [29]. IDEAL IQ has been shown to correctly analyze liver steatosis in iron overload patients of various etiologies in Taiwan [30], and to correctly diagnose focal sparing of iron and fat in iron overloaded liver of thalassemia and haemotologic diseases [31]. Conversely, extensive patchy artifacts on PDFF maps have recently been described in heavily iron overloaded thalassemic patients in Turkey, with liver R2* > 670 Hz/liver T2* < 1.5 ms studied by IDEAL IQ together with artifactual high MRI-PDFF values in these patchy areas [32]. No patchy artifactual PDFF map was observed during this study and none of our patients had T2* < 1.5 ms; in the group with severe iron overload, the lowest observed T2* IDEAL IQ value was 1.8 ms.

Interestingly, the potential relationship between LIC and PDFF in dialysis patients has already been pointed out by Ali et al. in an autopsy study of 36 iron overloaded haemodialysis patients performed in the pre-ESA era. In addition to heavy iron deposits, these authors also noticed other notable histologic features in the liver including fatty changes, central venous congestion, and an increase in the fibroconnective framework [33].

The detrimental effect of iron overload on liver steatosis shown in this study adds new perspectives on the adverse structural consequences of iron deposits on the liver in the setting of dialysis, in addition to perturbations of the iron hormone-regulating role of the liver with an abnormal increase in hepcidin synthesis shown in cases of iron overload and hypothesized to be one of the factors involved in the development of cardiovascular complications of ESRD [11,14,15,34].

Most of our patients had mesenchymal liver iron deposition; both hepatocellular and reticuloendothelial iron accumulation have been associated with the severity of hepatic histology in NAFLD [1,2], but mesenchymal iron deposition has recently been linked to more severe fibrosis and NASH, oxidative stress, and apoptosis [35,36].

The pathophysiology of iron overload predisposing to NAFLD may involve a direct role of iron in the activation of liver macrophages and hepatic stellate cells [37]. Beside this role in inflammatory signaling, recent studies in Caenorhabditis Elegans have highlighted that iron overload induces the expression of sgk-1, encoding serum and tissue glucocorticoid-inducible kinase, to simultaneously promote the synthesis of ferritin, the storage protein of iron with fat accumulation [38]. Sgk-1 positively regulates the expression of the genes acs-20 and vit-2/3, which are the homologs of mammalian FATP 1/4 fatty acid transport proteins and yolk lipoprotein genes, thus favoring cellular lipid uptake and translocation of lipids into lipid droplets [38].

The frequency of cirrhosis in dialysis patients is an overlooked topic which was confounded in the 1980s and 1990s by the spread of hepatitis B in dialysis units. This was successfully counteracted in Western countries by drastic hygiene measures, vaccination and serotherapy of patients, and thereafter by hepatitis C virus infection, which has only very recently been managed in ESRD patients with new direct antiviral agents leading to viral eradication [39–41]. Moreover, all forms of cirrhosis (related to haemosiderosis, alcoholic liver disease, viral hepatitis, NASH, and genetic haemochromatosis) generally take many years to
fully develop: therefore, the short lifespan of many dialysis patients may also account for the scarcity of diagnosed cirrhosis in ESRD patients on dialysis.

Conversely, NAFLD is now increasingly observed in ESRD patients on dialysis with a prevalence as high as 74% in peritoneal dialysis in Croatia and 56% in haemodialysis in Japan; this fatty liver disease is also studied for its adverse influence on dialysis morbidity and mortality [42,43].

Thus, beside the general dialysis population, overweight patients (who now represent about 20–30% of haemodialysis patients in Western countries and even more in the USA) and patients with known NAFLD and DIOS (an increasing contingent of new dialysis patients) may be at increased risk of developing or aggravating their fatty liver disease with the indiscriminate and unmonitored use of IV iron therapy [11,42–44].

In conclusion, indiscriminate iron therapy may trigger or worsen NAFLD in the setting of dialysis. Our results also suggest the potential adverse effects of iron products on the pathophysiology of NAFLD in non-renal patients, either as a first hit, favoring its occurrence, or as a second hit, worsening this epidemic disease.

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Author Contributions

Guy Rostoker contributed to the conception, design and supervision of the study, data acquisition, and planning and conduct of the study in the dialysis unit of the Hôpital Privé Claude Galien, Division of Nephrology and Dialysis. He supervised the statistical analysis, data interpretation and reporting of the work, and wrote the article.

Christelle Loridon contributed to data acquisition, analysis and interpretation, and to the statistical analysis, and prepared the tables and figures.

Mireille Grincacci contributed to data acquisition, analysis and interpretation, and to the statistical analysis, and prepared the tables and figures.

Clémentine Rabaté contributed to data acquisition, and to the planning and conduct of the study in the dialysis unit of the Hôpital Privé Claude Galien, Division of Nephrology and Dialysis.

Fanny Lepeytre contributed to data acquisition, and to the planning and conduct of the study in the dialysis unit of the Hôpital Privé Claude Galien, Division of Nephrology and Dialysis.

Pablo Ureña-Torres contributed to data acquisition, and planning and conduct of the study in the Peritoneal Dialysis Unit of the Division of Nephrology and Dialysis, Département d’Urorlogie et de Nérophologie, Groupe Hospitalier Pité-Salpêtrière.

Nasredine Ghali contributed to data acquisition, and to the planning and conduct of the study in the Dialysis Unit of the Centre Hospitalier Marc Jacquet, Division of Nephrology and Dialysis.

Yves Cohen contributed to the conception and design of the study, to the acquisition and analysis of centralized MRI exams, and to data interpretation in the Radiology Unit of the Hôpital Privé Claude Galien.

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