Quantification of Hepatic Iron Concentration in Chronic Viral Hepatitis: Usefulness of T2-weighted Single-Shot Spin-Echo Echo-Planar MR Imaging

Tatsuyuki Tonan1,*, Kiminori Fujimoto1,2,*, Aliya Qayyum3, Takumi Kawaguchi4, Atsushi Kawaguchi5, Osamu Nakashima6, Koji Okuda7, Naofumi Hayabuchi1, Michio Sata8

1 Department of Radiology, Kurume University School of Medicine, Kurume University Hospital, Kurume, Fukuoka, Japan, 2 Center for Diagnostic Imaging, Kurume University Hospital, Kurume, Fukuoka, Japan, 3 Department of Radiology and Biomedical Imaging, University of California San Francisco, San Francisco, California, United States of America, 4 Department of Digestive Disease Information & Research and Department of Internal Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan, 5 Biostatistics Center, Kurume University School of Medicine, Kurume, Fukuoka, Japan, 6 Department of Clinical Laboratory Medicine, Kurume University Hospital, Kurume, Fukuoka, Japan, 7 Department of Surgery, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan, 8 Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan

Abstract

Objective: To investigate the usefulness of single-shot spin-echo echo-planar imaging (SSEPI) sequence for quantifying mild degree of hepatic iron stores in patients with viral hepatitis.

Methods: This retrospective study included 34 patients with chronic viral hepatitis/cirrhosis who had undergone histological investigation and magnetic resonance imaging with T2-weighted gradient-recalled echo sequence (T2-GRE) and diffusion-weighted SSEPI sequence with b-factors of 0 s/mm2 (T2-EPI), 500 s/mm2 (DW-EPI-500), and 1000 s/mm2 (DW-EPI-1000). The correlation between the liver-to-muscle signal intensity ratio, which was generated by regions of interest placed in the liver and paraspinous muscles of each sequence image, and the hepatic iron concentration (μmol/g dry liver), which was assessed by spectrophotometry, was analyzed by linear regression using a spline model. Akaike information criterion (AIC) was used to select the optimal model.

Results: Mean ± standard deviation of the hepatic iron concentration quantified by spectrophotometry was 24.6±16.4 (range, 5.5 to 83.2) μmol/g dry liver. DW-EPI correlated more closely with hepatic iron concentration than T2-GRE (R square values: 0.75 for T2-EPI, 0.69 for DW-EPI-500, 0.62 for DW-EPI-1000, and 0.61 for T2-GRE, respectively, all P<0.0001). Using the AIC, the regression model for T2-EPI generated by spline model was optimal because of lowest cross validation error.

Conclusion: T2-EPI was sensitive to hepatic iron, and might be a more useful sequence for quantifying mild degree of hepatic iron stores in patients with chronic viral hepatitis.

Introduction

Abnormalities of iron metabolism are frequently observed in patients with chronic liver diseases such as viral hepatitis, nonalcoholic fatty liver disease, and cirrhosis [1,2]. Iron excess, which increases oxidative stress via the formation of hydroxyl radicals and other highly reactive oxidizing molecules, leads to hepatotoxicity; it is related to the fibrogenesis and hepatocarcinogenesis associated with chronic viral hepatitis [1,3].

In recent years, several research groups have reported on the efficacy of iron reduction therapies by phlebotomy [4–10]. Yano et al. [6] reported that phlebotomy therapy contributed to improvement of biochemical markers in patients with hepatitis C virus infection. Kato et al. [10] stated that phlebotomy therapy may potentially lower the risk of progression to hepatocellular carcinoma (HCC) in patients with hepatitis C virus infection. Therefore, precise quantification of hepatic iron overload might be beneficial for managing iron reduction therapy in patients with chronic viral hepatitis.

Assessment of body iron stores by measurement of serum ferritin concentration has poor specificity [11]. Liver biopsy, the most reliable method to measure hepatic iron stores, is an invasive procedure. Magnetic resonance imaging (MRI) is sensitive to hepatic iron because iron leads to a decline of MR signal due to T2-shortening effect related to paramagnetic properties. MRI has recently been recognized as a suitable noninvasive technique for...
quantifying hepatic iron overload [12]. Quantification of hepatic iron overload by MRI is useful in that it obviates the need for invasive liver biopsy and allows for repeat performance.

Generally, it is accepted that gradient-recalled echo (GRE) sequences are the most sensitive sequence to quantify mild degree of hepatic iron overload [13–20]. However, many studies evaluating GRE sequence with different echo-time and flip angle report variable results in the quantification of hepatic iron overload. Although the reproducibility of the technique and the quantification algorithm has been validated in various centers, these results are complicated.

Diffusion-weighted (DW) single-shot spin-echo echo-planar imaging (DW-EPI) has become a sequence used routinely in many institutions since the image quality was improved by recent technical progress such as parallel imaging and respiratory triggering [21–23]. In previous studies, it was reported that single-shot spin-echo EPI (SSEPI) sequence also had a high susceptibility effect [24,25].

We postulate that DW-EPI sequence might be superior to GRE sequence for quantifying mild degree of hepatic iron stores. To our knowledge, the investigation of hepatic iron overload by DW-EPI sequence has not been examined. The aim of this study was to investigate the usefulness of SSEPI sequence for quantifying mild degree of hepatic iron stores in patients with viral hepatitis.

Materials and Methods

Patients

The institutional review board (the Ethics Committee of Kurume University) approved this retrospective study (Approval No. 09112), which complied with the principles of the Declaration of Helsinki (2008 version). All included patients gave written informed consent to participate.

Our study was targeted at patients with viral chronic hepatitis/ cirrhosis and HCC because such patients with chronic liver impairment may have increased liver iron and would have undergone both liver MR imaging and hepatic surgery.

We reviewed the patients who admitted use of both liver specimens and MR images before hepatic surgery at our institution between January 2007 and April 2008 and identified patients who met the following inclusion criteria: (a) patients had both chronic viral hepatitis/cirrhosis and HCC; (b) patients underwent abdominal MR imaging with T2-weighted GRE sequence and DW-EPI sequence with b-factors of 0 s/mm², 500 s/mm², and 1000 s/mm² (these sequences were part of our standard abdominal MR imaging protocol during this period); and (c) patients underwent an operation for HCC and received a histopathologic diagnosis of either chronic hepatitis or cirrhosis that was based on findings at surgical resection, performed within a month after MR imaging.

Forty-six patients fulfilled these criteria. Twelve of these 46 patients were excluded on the basis of the following reasons: (a) Available imaging data did not correspond to available histopathologic data because of interval surgery (n = 5); (b) MR studies were incomplete (n = 3); (c) an artifact was observed on MR images and precluded accurate measurement of signal intensity (n = 1); and (d) other causes of chronic liver disease such as alcoholic hepatitis (n = 2) and non-alcoholic steatohepatitis (a = n = 1). Thirty-four patients formed the final study group (21 men and thirteen women; median age, 65 years; range, 52–83 years). Histopathologic sampling of all patients included in the study was performed after MR imaging (median, 3 days; range, 1–30 days). The cause of chronic liver disease was hepatitis C virus infection (n = 26) or hepatitis B virus infection (n = 8). None of the patients had a clinical diagnosis of hemochromatosis that was based on review of medical records.

Hepatic iron concentration and histological analysis

A partial hepatic resection was performed in all patients with HCC. For each patient, 50 mg of wet liver tissue was extracted from the surgically removed specimen by a MLS1200 MEGA microwave digestion system (Milestone General Co. Ltd., Kawasaki, Japan) for 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min 400 W, and 5 min at 500 W. For determination of hepatic iron concentration (µmol/g dry liver), the resulting extracts were analyzed by spectrophotometry with a graphite atomic absorption camera (Polarized Zeeman Atomic Absorption Spectrophotometer, Hitachi, Ltd., Tokyo, Japan) and were converted to the units shown above [26].

For histological analysis, fibrosis stage and necroinflammation grade were evaluated semiquantitatively using the METAVIR scoring system [27]. Fibrosis stage graded on a scale of 0 to 4, as follows: F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis and few septa; F3 = numerous septa without cirrhosis; and F4 = cirrhosis. The necroinflammatory activity score was graded on a scale of 0 to 3, as follows: A0 = none; A1 = mild; A2 = moderate; A3 = severe. Distribution of steatosis was also retrospectively evaluated as the overall impression of the percentage of fat-containing hepatocytes on hematoxylin and cosin–stained specimens [28,29]. Steatosis grade was scored on a scale of 0 to 2, as follows: grade 0 = absence of steatosis; grade 1 = steatosis <5%; and grade 2 = steatosis ≥5%.

MRI technique and analysis

Within one month prior to surgery, MR imaging was performed at field strength of 1.5 T (Magnetom Symphony Advanced; Siemens, Erlangen, Germany) with use of a body phased-array surface coil. A series of DWIs and T2-weighted GRE sequence were obtained using parallel imaging with generalized auto calibrating partially parallel acquisition (GRAPPA) of acceleration factor 2 in all patients. DWI was performed in the transverse plane by respiratory-triggered combining SSEPI sequence with a chemical shift–selective pulse (CHESS). Any antiperistalsis drug was not used.

The imaging parameters for DW-EPI were as follows: repetition time (TR), 2000 msec; echo time (TE), 81 msec; directions of the motion-probing gradient, three orthogonal axes; gradient factor b values of 0 sec/mm² (T2-weighted SSEPI, hereafter T2-EPI), 500 sec/mm² (DW-EPI-500), and 1000 sec/mm² (DW-EPI-1000); 2170-Hz per pixel bandwidth; 350-mm field of view; 128×80 rectangular matrices; 9-mm-thick sections; 1-mm intersection gap; six signals acquired; and acquisition time of approximately 1 minute 30 seconds.

T2-weighted GRE sequence (hereafter, T2-GRE) was performed in the transverse plane by fast low angle shot (FLASH) with one signal acquired during a 22-second breath hold. The imaging parameters for T2-GRE were as follows: TR, 246 msec; TE, 9.5 msec; flip angle (FA), 30°; 350-mm field of view; 9-mm-thick sections; 1-mm intersection gap; 16-number of sections; 256×192 matrix; and 130-Hz per pixel bandwidth.

Quantitative image analysis was conducted by measuring the signal intensities of the liver parenchyma and paraspinal muscles. Image analysis was performed by two independent radiologists using plug-in software developed in-house by one of the authors [30,31] (Figure 1). Five separate regions of interest (ROIs) were carefully placed manually in the anterior and posterior segments of the right hepatic lobe at the level of the porta hepatitis (whenever possible) on each sequence; care was taken to avoid focal lesions,
major vascular structures, and artifacts such as chemical shifts, magnetic susceptibility, and cardiac motion. Liver signal intensities were recorded as the mean values generated from the five measurements (total liver ROI area sampled, 500 mm²). The procedure was repeated to measure muscle signal intensity by placing two separate ROIs on the right and left paraspinous muscles in the same slice section used to measure liver signal intensity; care was taken to avoid artifacts such as chemical shifts, magnetic susceptibility, and motion on each sequence.

Muscle signal intensities were recorded as the mean values generated from the two measurements (total muscle ROI area sampled, 200 mm²). We calculated the liver-to-muscle signal intensity ratio (LMR) by dividing mean liver signal intensity by mean muscle signal intensity for each sequence [15].

Statistical analysis

A Bland-Altman plot was used to analyze the 95% limits of interobserver agreement for the LMR on each sequence [32]. The correlation of the LMR obtained by the two observers on each sequence was determined using the Pearson correlation coefficient (r).

The relationship between the LMR on each sequence and hepatic iron concentration was analyzed by means of scatter plots. These results were inspected for linearity and goodness of fit. The relationship between the LMR on each sequence and hepatic iron concentration was modeled by regression techniques using a spline model. Details of spline models are given in the next section.

To investigate effects of each LMR on hepatic iron concentration, we applied the linear models containing not only a main term but also knot terms which play a role as an inflection point. The Akaike information criterion (AIC) was used to evaluate these alternate models [33]. The number and location of knots were determined objectively with the minimum AIC among their prespecified candidates, which were 20, 40, 60, and 80 percentiles of each LMR. To evaluate the predictive accuracy, a leave one out cross validation (CV) error [34] was computed.

The Kruskal–Wallis test was used to determine significant differences in the LMR on each sequence among category classification in each histological finding (i.e. necroinflammation grade, fibrosis stage, and steatosis grade). All analyses were performed using SPSS statistical software (version 12.0 J; SPSS, Inc., Chicago, IL, USA). P<0.05 was considered statistically significant.

Details of the spline models used in statistical analysis

Response and predictor variables are denoted by y and x, respectively. The general form of the univariate (first order) spline model is

$$y = a + bx + \sum_{j=1}^{m} \gamma_j (x - r_j) + \epsilon$$

where a, b, and \(\gamma_j\) (j = 1, 2, ..., m) are parameters to be estimated, (z) = max(0, z), \(r_1, r_2, \ldots, r_m\) are called knots which play a role as an inflection point, and \(\epsilon\) is an error following a normal distribution with mean 0 and a constant variance. Note that in the case of \(\gamma_1 = \gamma_2 = \ldots = \gamma_m = 0\) the model can be identified as a simple linear regression model. The parameters in the model (1) are estimated by an ordinary least squares method to minimize squared residuals \(Q\) in (2) from samples \((x_i, y_i)\) (i = 1, 2, ..., n) from n patients.

$$Q = \sum_{i=1}^{n} \left( y_i - a - bx_i - \sum_{j=1}^{m} \gamma_j (x_i - r_j) \right)^2$$

To illustrate the interpretation of parameters in the spline model, we consider the model as with only one knot as in (3). This model contains two lines whose slope and intercept are changed at \(x = r\).

$$y = a + bx + \gamma (x - r) + \epsilon$$

In the range \(x \leq r\), the slope is \(b\) and the intercept is \(a\). In the other range \(x > r\), the slope is \(b + \gamma\) and the intercept is \(a - \gamma r\). This modeling can easily be implemented by standard software such as SAS, SPSS, and R. Supposing that the data set has two columns corresponding to response \(y\) and predictor \(x\) variables, one can add the computed \((x - r)\) as the third column. Then, the multiple regression model can be applied with the response \(y\) and two predictors, \(x\) and \((x - r)\). If you want more knots, you can add the corresponding columns and predictors in the regression model.

The essential point in the use of this spline model is to select the number and location of knots. As used in this paper, one choice for candidates for knots is the quantiles for continuous variables taking into account the sample size. Once one specifies the candidates, the problem turns to the variable selection for predictor variables used in the multiple regression model, which can also be implemented by standard software. One effective method is to use information criteria such as AIC. This kind of modeling [35] is useful to investigate the flexible relationship between the response and predictor.
Results

Hepatic iron concentration and histological findings

Mean ± SD of the hepatic iron concentration quantified by spectrophotometry was 24.6 ± 16.4 (range, 5.5 to 83.2) μmol/g dry liver. Histological necroinflammation grade was A1 in 21 patients and A2 in 13 patients. Fibrosis stage was F1 in 13 patients, F2 in 4 patients, F3 in 5 patients, and F4 (i.e. cirrhosis) in 12 patients. Steatosis grade was 0 in 14 patients, grade 1 in 11 patients, and grade 2 in 9 patients.

Interobserver agreement for the LMR on each sequence

There was no significant difference between measurements made by the two observers for the two parameters; the interclass Pearson correlation coefficients were 0.96 (95% confidence interval [CI]: 0.86, 1.00) for T2-GRE, 0.99 (95% CI: 0.92, 1.00) for T2-EPI, 0.97 (95% CI: 0.85, 1.00) for DW-EPI-500, and 0.98 (95% CI: 0.97, 1.00) for DW-EPI-1000; the mean difference (± standard deviation) was −0.0027 ± 0.054 for T2-GRE, −0.0069 ± 0.052 for T2-EPI, 0.017 ± 0.11 for DW-EPI-500, and 0.013 ± 0.16 for DW-EPI-1000; and the coefficients of repeatability were 0.108 for T2-GRE, 0.105 for T2-EPI, 0.213 for DW-EPI-500, and 0.316 for DW-EPI-1000. Bland-Altman plots with 95% limits of agreement for each sequence are shown in Figure 2. There was no proportional bias or fixed bias in each Bland-Altman plot for the two parameters.

Correlation between the LMR on each sequence and hepatic iron concentration

Figure 3 shows results for the line fit by the selected regression model. Created simple regression models to estimate the hepatic iron concentration in each sequence are as follows:

T2−GRE:  y = 103.7 − 85.7 × LMR + 58.2 × (LMR − 1.05)
T2−EPI:  y = 131.0 − 139.7 × LMR + 106.5 × (LMR − 0.73) + 27.4 × (LMR − 1.24)
DW−EPI−500:  y = 80.2 − 51.8 × LMR + 43.0 × (LMR − 1.24)
DW−EPI−1000:  y = 66.7 − 29.3 × LMR + 25.7 × (LMR − 1.76)

Figure 2. Bland-Altman plots for measurements of T2-GRE (A), T2-EPI (B), DW-EPI-500 (C), and DW-EPI-1000 (D) in liver parenchyma. Each Bland-Altman plots demonstrates good interobserver agreement and lack of proportional bias or fixed bias. The average of the measurements made by the two observers is plotted against the difference between the measurements made by the two observers. The thin lines represent the mean value of all differences between the two observers, and the thick lines represent the 95% limits of agreement. SD = standard deviation.

doi:10.1371/journal.pone.0033868.g002
where LMR is the measurement value on each sequence (appendix).

The regression analyses showed an excellent overall negative correlation on each sequence. Particularly, T2-EPI correlated most closely with hepatic iron concentration. R square values on each sequence were as follows: 0.75 for T2-EPI, 0.69 for DW-EPI-500, 0.62 for DW-EPI-1000, and 0.61 for T2-GRE (F-test, $P<0.0001$, respectively).

Using the AIC, the linear regression model on T2-EPI \[ y = 131.0 - 139.7 \times \text{LMR} + 106.5 \times (\text{LMR} - 0.73) + 27.4 \times (\text{LMR} - 1.24) + \ldots \] was chosen as having the best fit, since it had the lowest CV error. The corresponding CV errors were as follows: 14161.3 for T2-GRE, 11357.4 for T2-EPI, 12220.0 for DW-EPI-500, and 14376.2 for DW-EPI-1000.

**Correlation between the LMR on each sequence and histological findings**

No significant differences were found for the LMR on each sequence among category classification of histological findings (i.e. necroinflammation grade, fibrosis stage, and steatosis grade). $P$ values (Kruskal-Wallis test) were as follows: (a) necroinflammation grade: $P=0.4$ for T2-GRE, $P=0.89$ for T2-EPI, $P=0.68$ for DW-EPI-500, and $P=0.6$ for DW-EPI-1000; (b) fibrosis stage: $P=0.39$.

---

Figure 3. Scatter plots of LMR and hepatic iron concentration ($\mu$mol/g dry liver) on T2-GRE (A), T2-EPI (B), DW-EPI-500 (C), and DW-EPI-1000 (D). Correlation between LMR and hepatic iron concentration for linear regression with spline models are shown as solid lines on each sequence. The linear regression model \[ y = 131.0 - 139.7 \times \text{LMR} + 106.5 \times (\text{LMR} - 0.73) + 27.4 \times (\text{LMR} - 1.24) + \ldots \] on T2-EPI was optimal.

doi:10.1371/journal.pone.0033868.g003
In patients with chronic viral hepatitis, steatosis is a common secondary phenomenon. Westphalen et al. [41] reported that iron stores in background liver complicated measurement of steatosis by opposed-phase MR imaging. Alternatively, a recent study reported that concomitant steatosis lowers the diagnostic performance of T2-GRE sequence and chemical shift imaging for quantifying mild degree of hepatic iron stores because intravoxel constructive and destructive interference between fat and water spins due to chemical shift effect of the second kind potentially affect the signal intensity measurements for T2-GRE sequence [36]. Therefore, it might be important to consider the influence of each factor in background liver tissue in the quantification of steatosis and iron stores using MR imaging.

On DW-EPI, we found no significant differences in LMR among histological steatosis grades. Use of fat saturation pulse (i.e., CHESS) on DW-EPIs could eliminate the influence of steatosis, which might support the better utility of this sequence for quantifying mild degree of hepatic iron stores. On the other hand, although previous studies reported that liver fibrosis decreased the diffusion signal [30,42,43], no significant differences were found in LMR on DW-EPIs among histological fibrosis stages, which suggest that influence of liver fibrosis to the signal of DW-EPIs was low as a result. The quantification of iron stores by DW-EPIs may have suffered potential influence by fibrosis, which might be one of the reasons that T2-EPI was most accurate sequence for quantifying mild degree of iron stores. Therefore, we recommend the T2-EPI with b values of 0 sec/mm², which is not affected to the diffusion signal, for quantifying mild degree of iron stores.

Several limitations of the present study warrant mention. First, the study was conducted retrospectively and sample size was small. Although a major effort was made to exclude sample bias, there was limited sample size for examination of liver iron concentration using spectrophotometry because of its retrospective nature. Second, all measurements for the LMR were obtained in the right lobe of the liver to avoid motion-related artifact. Because the pathologic specimens were obtained at surgery for an HCC, histologically sampled areas did not completely correspond to radiologically sampled areas. A prospective study with a substantially larger sample is needed to further validate our findings.

In conclusion, DW-EPI (especially, T2-weighted SSEPI) was sensitive to hepatic iron, and might be a more useful sequence for quantifying mild degree of hepatic iron stores in patients with chronic viral hepatitis.

**Author Contributions**

Conceived and designed the experiments: TT KF TK MS. Performed the experiments: TT KF TK MS. Analyzed the data: TT KF AK. Contributed reagents/materials/analysis tools: KF TK ON KO NH MS. Wrote the paper: TT KF AQ AK. Designed the software used in experiments: TT KF TK ON. Analyzed the data: TT KF AK. Designed the experiments: TT KF TK MS. Performed the experiments: TT KF AK ON KO NH MS.

References

1. Bonkovsky HL, Banner BF, Rothman AL. (1997) Iron and chronic viral hepatitis. Hepatology 25: 759–768.
2. Younossi ZM, Granlich T, Bacorn BR, Matteoni CA, Boparai N, et al. (1999) Hepatic iron and nonalcoholic fatty liver disease. Hepatology 30: 847–850.
3. Niederha C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, et al. (1985) Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med 313: 1256–1262.
4. Hayashi H, Tsubakawa T, Nishimura N, Yano M, Isomura T, et al. (1994) Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. Am J Gastroenterol 89: 986–988.
5. Di Biaseglie AM, Bonkovsky HL, Chopra S, Chopra S, Flaim M, et al. (2000) Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who have previously not responded to interferon: a multicenter, prospective, randomized, controlled trial. Hepatology 32: 135–138.
6. Yano M, Hayashi H, Yoshiba K, Koda K, Saito H, et al. (2004) A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction
Hepatic Iron Overload in Chronic Viral Hepatitis

therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. J Gastroenterol 39: 570–574.

7. Kawamura Y, Akita N, Sasaki H, Hosaka T, Somuya T, et al. (2005) Determinants of serum ALT normalization after phlebotomy in patients with chronic hepatitis C infection. J Gastroenterol 40: 901–906.

8. Sumida Y, Kanemasa K, Fujimoto K, Yoshida N, Sakai K (2007) Effects of dietary iron reduction versus phlebotomy in patients with chronic hepatitis C: results from a randomized, controlled trial on 40 Japanese patients. Intern Med 46: 837–842.

9. Yano M, Hayashi H, Wakuwasa S, Sanae F, Taktawa T, et al. (2002) Long term effects of phlebotomy on biochemical and histological parameters of chronic hepatitis C. Am J Gastroenterol 97: 135–137.

10. Kato J, Kobune M, Nakamura T, Kurota G, Nakada K, et al. (2001) Normalization of elevated hepatic 8-hydroxy-2-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 61: 6967–6972.

11. Brisset P, Deugnier Y, Haemochromatosis In: McIntyre N, Benhamou JP, Bircher J, Rizzetto M, Rodes J, eds. Oxford textbook of clinical hepatology Oxford: Oxford University Press, pp 1379–1391.

12. Olthof AW, Sijens PE, Kreeftenberg HG, Hapert P, Iwan R, et al. (2007) Correlation between serum ferritin levels and liver iron concentration determined by MR imaging: impact of hematologic disease and inflammation. Magn Reson Imaging 25: 228–231.

13. Alustiza JM, Castiella A, De Juan MD, Empananza JI, Artero J, et al. (2007) Iron overload in the liver diagnostic and quantification. Eur J Radiol 61: 499–506.

14. Alustiza JM, Artero J, Castiella A, Agirre C, Empananza JI, et al. (2004) Gipuzkoa Hepatic Iron Concentration by MRI Study Group. MR quantification of hepatic iron concentration. Radiology 230: 479–484.

15. Gandon Y, Olive D, Guyader D, Aubé C, Oberli F, et al. (2003) Non-invasive assessment of hepatic iron stores by MRI. Lancet 363: 357–362.

16. St Pierre TG, Clark PR, Chua-anusorn W, Fleming AJ, Jeffrey GP, et al. (2005) Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood 105: 855–861.

17. Olthof AW, Sijens PE, Kreeftenberg HG, Kappert P, van der Jagt EJ, et al. (2008) Non-invasive liver iron concentration measurement by MRI. Comparison of two validated protocols. Eur J Radiol 71: 116–121.

18. Bonkovsky HL, Rubin RB, Cable EE, Daviddoff A, Rijhen MH, et al. (1999) Hepatic iron concentration: noninvasive estimation by means of MR imaging techniques. Radiology 212: 227–234.

19. Gandon Y, Guyader D, Heauot JF, Reda MI, Yawoanq J, et al. (1994) Hemochromatosis: diagnosis and quantification of liver iron with gradient-echo MR imaging. Radiology 195: 533–538.

20. Kreeftenberg HG, Jr., Mooyvaert EL, Huijzerne JR, Schier WJ (2000) Quantification of liver iron concentration with magnetic resonance imaging by combining T1-, T2-weighted spin echo sequences and a gradient echo sequence. Neth J Med 56: 133–137.

21. Bannmer R, Keising SL, Augustin M, Pruessmann KP, Wolf R, et al. (2001) Improved diffusion weighted single-shot echo-planar imaging (EPI) in stroke using sensitivity encoding (SENSE). Magn Reson Med 46: 548–554.

22. Taouli B, Martin AJ, Qayyum A, Merriman RB, Vigneron D, et al. (2004) Parallel imaging and diffusion tensor imaging for diffusion weighted MRI of the liver: preliminary experience in healthy volunteers. AJR Am J Roentgenol 183: 677–680.

23. Murtz P, Flack S, Treber F, van den Brink JS, Gieseke J, et al. (2002) Abdomen: diffusion-weighted MR imaging with pulse-triggered single-shot sequences. Radiology 224: 258–264.

24. Tanimoto A, Kuribayashi S (2006) Application of superparamagnetic iron oxide to imaging of hepatocellular carcinoma. Eur J Radiol 58: 200–216.

25. Coenegechts K, Mats C, Ter Beck I, Mertens T, Hageda H, et al. (2009) Focal liver lesion detection and characterization: Comparison of non-contrast enhanced and SPIO-enhanced diffusion-weighted single-shot spin echo echo planar and turbo spin echo T2-weighted imaging. Eur J Radiol 72: 432–439.

26. Griesmann GE, Hartmann AC, Farris FF (2009) Concentrations and correlations for eight metals in human liver. Int J Environ Health Res 19: 231–238.

27. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, et al. (1995) Histological grading and staging of chronic hepatitis. J Hepatol 22: 696–699.

28. Kleiner DE, Brunt EM, Van Natta M, Belling G, Comos MJ, et al. (2005) : Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41: 1315–1321.

29. Brun IM, January CG, Di Biagolim AM, Neusichlanser-Tieti BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 94: 2467–2474.

30. Fujimoto K, Toman T, Azuma S, Kage M, Nakashima O, et al. (2011) Evaluation of the mean and entropy of apparent diffusion coefficient values in chronic hepatitis C: Correlation with pathologic fibrosis stage and inflammatory activity grade. Radiology 258: 739–748.

31. Toman T, Fujimoto K, Qayyum A, Azuma S, Ishihashi M, et al. (2011) Correlation of Kupffer cell function and hepatocyte function in chronic viral hepatitis evaluated with superparamagnetic iron oxide-enhanced magnetic resonance imaging and scintigraphy using technetium-99m-labelled galactosyl human serum albumin. Exp Ther Med 2: 607–613.

32. Blend JM, Ahman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1: 307–310.

33. Akafe H (1974) A new look at the statistical model identification. IEEE Trans Automatic Control 19: 716–723.

34. Stone M (1974) Cross-validatory choice and assessment of statistical predictions. Journal of Royal Statistical Society B 36: 111–147.

35. Kawaguchi A, Yonemoto K, Tanizaki Y, Kiyohara Y, Yanagawa T, et al. (2008) Application of functional ANOVA models for hazard regression to the Hisayama data. Stat Med 27: 3515–3527.

36. Lim RP, Tupia K, Hajihi CH, Losada M, Gupta R, et al. (2010) Quantification of hepatic iron deposition in patients with liver disease: comparison of chemical shift imaging with single-echo T2*-weighted imaging. AJR Am J Roentgenol 194: 1208–1295.

37. Taouli B, Sandberg A, Stemmer A, Parikh T, Wong S, et al. (2009) Diffusion-weighted imaging of the liver: comparison of navigator triggered and breathhold acquisitions. J Magn Reson Imaging 30: 561–568.

38. Nasa K, Kurolj I, Sekiuchu R, Nasuno S (2006) The effect of simultaneous use of respiratory triggering in diffusion weighted imaging of the liver. Magn Reson Med Sci 5: 129–136.

39. Gourtsoyianni S, Papakonulas N, Yannoneu S, Maris T, Karantanas A, et al. (2008) Respiratory gated diffusion weighted imaging of the liver: value of apparent diffusion coefficient measurements in the differentiation between most commonly encountered benign and malignant focal liver lesions. Eur Radiol 18: 489–492.

40. Ashach P, Klessen C, Krouneke TJ, Klunerc S, Stemmer A, et al. (2005) Magnetic resonance cholangiopancreatography using a free-breathing T2*-weighted turbo spin echo sequence with navigator-triggered prospective acquisition correction. Magn Reson Imaging 23: 953–945.

41. Wespahlum AC, Qayyum A, Yeh BM, Merriman RB, Lee JA, et al. (2007) Liver fat: effect of hepatic iron deposition on evaluation with opposed-phase MR imaging. Radiology 242: 450–455.

42. Taouli B, Tola AJ, Losada M, Babb JS, Chan ES, et al. (2007) Diffusion-weighted MRI for quantification of liver fibrosis: preliminary experience. AJR Am J Roentgenol 189(4): 799–806.

43. Taouli B, Choudi M, Martin AJ, Qayyum A, Coakley FV, et al. (2008) Chronic hepatitis C: role of diffusion-weighted imaging and diffusion tensor imaging for the diagnosis of liver fibrosis and inflammation. J Magn Reson Imaging 29(1): 89–95.

PloS ONE | www.plosone.org 7 March 2012 | Volume 7 | Issue 3 | e33868