Original Article

Monitoring Iron Overload: Relationship between R2* Relaxometry of the Liver and Serum Ferritin under Different Therapies

Michaela Plaikner, Christian Kremser, Heinz Zoller[1], Werner Jaschke, Benjamin Henninger

Departments of Radiology and [1]Internal Medicine, Medical University of Innsbruck, 6020 Innsbruck, Austria

Received : 26-04-2018
Accepted : 09-07-2018
Published : 18-09-2018

ABSTRACT

Objective: The objective of this study was to evaluate the relationship between hepatic magnetic resonance imaging (MRI) with R2* relaxometry and serum ferritin in therapy monitoring of patients with iron overload. Further, a possible influence of the chosen therapy (phlebotomy or chelation) was assessed. **Materials and Methods:** We retrospectively evaluated 42 patients with baseline and follow-up R2* relaxometry and determination of serum ferritin before and during therapeutic phlebotomy or iron chelation therapy or watchful waiting, respectively. Linear regression analysis was used to analyze the correlation between changes of R2* and serum ferritin. Regression lines for different groups were compared with analysis of covariance. **Results:** We found a moderate positive statistical correlation (r = 0.509) between serum ferritin and R2*, a moderate positive correlation between absolute R2* changes and serum ferritin changes (r = 0.497), and a strong correlation for percentage changes (r = 0.712). The correlation analysis between relative changes of R2* and serum ferritin for the different therapies resulted in a strong correlation between phlebotomy and chelation (r = 0.855/0.727) and a moderate for no applied therapy (r = 0.536). In 22/92 paired examinations, a discordance of R2* and ferritin was found, particularly involving patients under chelation. **Conclusions:** Despite the good correlation between serum ferritin and R2* relaxometry in monitoring iron overload, treatment response may be misinterpreted when only serum ferritin is considered. Although ferritin is an acceptable and far cheaper tool for monitoring, MRI should be performed for confirmation, especially in case of unexpected ferritin changes, particularly under chelation therapy.

Keywords: *Iron, liver, relaxometry, serum ferritin, therapy monitoring*

Introduction

Different methods are available for the assessment of a patient's iron status,.[1] Serum ferritin is one of the most important clinical parameters[2] and its determination can be seen as a routine laboratory assay.[3] Despite the previously described positive correlation between serum ferritin and total iron stores,[4] there are known confounding factors so that serum[5,6] can also be elevated in other contexts.[4,7] Consequently, serum ferritin values only provide a rough indication of total body iron burden[8] and may not always correlate with its changes.[9]

The most accurate method to define iron overload is the estimation of the liver iron concentration (LIC) with its linear relation to the total body iron.[10] LIC is increasingly assessed by magnetic resonance imaging (MRI) providing a fast, noninvasive, and previously well-investigated and validated diagnostic method.[11]

To prevent possible severe clinical consequences in patients with iron overload, an adequate therapeutic management is required, thereby all the available

Access this article online

Quick Response Code:

Website: www.clinicalimagingscience.org

DOI: 10.4103/jcis.JCIS_30_18

Address for correspondence:
PD Dr. Benjamin Henninger,
Department of Radiology, Medical University of Innsbruck,
Anichstrasse 35, 6020 Innsbruck, Austria.
E-mail: benjamin.henninger@i-med.ac.at

How to cite this article: Plaikner M, Kremser C, Zoller H, Jaschke W, Henninger B. Monitoring Iron Overload: Relationship between R2* Relaxometry of the Liver and Serum Ferritin under Different Therapies. J Clin Imaging Sci 2018;8:40.
Available FREE in open access from: http://www.clinicalimagingscience.org/text.asp?2018/8/1/40/241524

therapies need accurate monitoring, in which serum ferritin and LIC are the actual parameters of interest.[12] Some studies have already pointed out that serum ferritin is a suboptimal parameter to measure the total iron at any time;[13] nevertheless, its trends are persistently used to estimate changes in the LIC.[4]

We evaluated the relationship between MRI-based R2* relaxometry of the liver and serum ferritin in therapy monitoring of patients with iron overload. Further, we investigated a possible influence of different therapies (phlebotomy or chelation) and other factors, such as hepatic fat or even the underlying disease.

Materials and Methods

Patients and study design

We retrospectively enrolled 42 patients with iron overload who were referred to our department between March 2007 and September 2015 and met the following inclusion criteria: (1) at least two MRI examinations of the liver accomplished with the sequences listed below under therapeutic surveillance and (2) blood testing including the determination of blood serum iron, ferritin, transferrin, and transferrin saturation within 60 days of the MRI examination.

All patients gave their informed consent for any imaging as well as blood analysis and therapeutic decisions. Institutional review board approval was granted by means of a general waiver for studies with retrospective data analysis (Ethics commission of the Medical University of Innsbruck, 2009-02-20).

The respective therapy (phlebotomy or chelation) applied between the two examinations was documented. If the therapy was interrupted between two MRI examinations due to an already sufficient adjustment, not tolerable adverse effects, or even an insufficient collaboration of the patient, "no therapy" was documented ("watchful waiting").

For evaluating changes of hepatic R2* values and correlation with changes of the laboratory parameters, only patients with pathological R2* values (>70 1/s) at the time of their first examination were included, always considering pairs of consecutive examinations. Furthermore, the different applied therapies, hepatic fat fractions (FFs), as well as the underlying disease (hemochromatosis in terms of a positive human hemochromatosis protein (gene HFE) positive) or secondary iron overload) were included into the analysis. Since ferritin levels are variable, we applied a threshold of 14%, based on the publication of Pilon et al.,[14] to classify a change of ferritin values as a real (effective) change. In addition, we have chosen a threshold of 20 1/s, based on our experience, to classify a change of R2* as a real change.

Magnetic resonance imaging

MRI was performed using a 1.5T MR scanner (Magnetom Avanto, Siemens Healthcare Sector, Erlangen, Germany) with an 8-channel body phased-array surface coil. R2* values were obtained using a fat-saturated (frequency-selective fat saturation as provided by the manufacturer), multigradient echo (GRE) sequence with 12 echoes (TR=200 ms; TE-initial = 0.99 ms; Delta-TE = 1.41 ms; 12 echoes; flip-angle: 20°). During one breath-hold, a 10-mm single-transversal slice (acquisition matrix: 128 × 128; field of view (FOV): 380 mm × 380 mm) was acquired; the acquisition was repeated for five different slice positions. The fatty degeneration of the liver parenchyma was evaluated by performing a chemical shift-based imaging method composed of a two-dimensional T1-weighted transverse-spoiled GRE sequence (fast low-angle shot-FLASH) in opposed-phase (OP) and in-phase (IP) (TE1: 2.37 ms, TE2: 5.05 ms, TR: 103, flip angle: 70°, matrix: 256 × 192, FOV: 300 mm × 400 mm, slice thickness: 5 mm) as provided by the manufacturer of the MRI. Image analysis was performed independently by a radiologist (region of interest [ROI] placement) and a physicist (calculation of R2* and FF maps). Offline postprocessing included quantitative image analysis using ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). R2* maps were calculated from the magnitude images by pixel-wise fitting with a truncation model[15] using a custom-written ImageJ plugin. Later, echo times were manually excluded from the fit when the signal in the respective image dropped below the noise level and stayed approximately constant for further echo times.[16] Three ROIs were placed in the subcapsular liver parenchyma of one transverse section on a level with the portal vein (two in the right lobe and one in the left lobe) for both sequences at identical positions. ROIs had a diameter in the order of 12 mm with an area of around 1.1 cm^2 and were carefully placed to avoid major vessels and movement artifacts or possible focal lesions. The mean R2* was calculated using the three ROI measurements. Furthermore, the FF was calculated by correcting the IP and OP signals for R2*decay using a global mean R2* value as obtained from the above R2* maps. The FF was then calculated using the following formula: FF = (IP − OP)/(2*IP) with subsequent correction for T1 bias. A FF higher than 5.6% was determined to be abnormal and to indicate hepatic steatosis.[17] For abnormal iron deposition, R2* was defined as pathologic using a threshold of 70 1/s according to the reference values of the literature.[18,19]

Serum parameters

The laboratory parameters (serum ferritin, serum transferrin, and transferrin saturation) were determined

in peripheral venous blood. Normal values for serum ferritin lie between 15 and 150 ng/ml, for serum transferrin between 200 and 360 mg/dl, and for transferrin saturation between 16% and 45%.[20]

Statistical analysis

Statistical calculations were performed using the R Project for Statistical Computing (R Development Core Team [2006], Vienna, Austria, URL: http://www.R-project.org, Version 3.2.5). To analyze the relationship between changes of R2* and different parameters, linear regression analysis was performed by fitting a simple linear model to the data. In this case, the obtained coefficient of determination R^2 corresponds to the square of Pearson's correlation coefficient r, which is subsequently specified for our data. In addition, also the Spearman's correlation coefficient rho was calculated for data, which showed marked outliers. To compare regression lines for different groups, analysis of covariance was used. The results were considered statistically significant when $P < 0.05$.

Results

A total of 42 patients (28 males and 14 females; age range: 10–80 years, mean age: 48.10 years) were enrolled. About 19/42 had HFE-associated hemochromatosis, 3/42 aceruloplasminemia, and 20/42 were affected from secondary iron overload due to various reasons including dysmetabolic iron overload syndrome, repeated transfusion therapies due to various underlying diseases, as well as repeated iron infusions in the context of hemodialysis.

Twenty-seven patients had two and 15 patients had more than two follow-up examinations including MRI and blood analysis, respectively. Of the 15 patients with more than two consecutive examinations, five patients had three, four patients had four, two patients had five, one patient had nine and three patients had 10 follow-up examinations. This resulted in a total of 92 paired examinations, whereby 27 paired examinations were from 27 patients, 10 from five patients, 12 from four patients, 8 from two patients, 8 from 1 patient, and 27 paired exams from three patients. The maximum time interval between hepatic R2* determination and laboratory analysis was 60 days (average time interval: 7.3 days), whereby 27 patients had imaging and the laboratory examination on the same day.

The mean liver R2* value was 284.65 1/s (median: 219.03, range: 74–948). All patients had significant iron overload (R2* >70 1/s) at the time of their first examination. About 13/42 patients had concomitant hepatic steatosis. Detailed information for patients with hepatic iron and concomitant fat is provided in Table 1. Altogether, 21 patients were treated with phlebotomy, 12 patients received a chelation therapy, and nine patients were handled with no therapy ("watchful waiting"). Among 18/42 patients, R2* values normalized (R2* <=70 1/s) during the course of therapy.

The relation between liver R2* (1/s) and serum ferritin values (ng/ml) for all patients and examinations is shown in Figure 1. A moderate positive, statistically significant correlation of $r = 0.509$ ($P < 0.001$) and rho = 0.624 ($P < 0.001$) was found.

The relation between absolute changes as well as percentage changes of liver R2* values and ferritin for each pair of consecutive examinations ($n = 92$) is shown in Figures 2 and 3, respectively. Thereby, an almost moderate correlation between absolute R2* changes and absolute ferritin changes ($r = 0.497$, $P < 0.001$; rho = 0.626, $P < 0.001$) and a strong correlation ($r = 0.712$, $P < 0.001$; rho = 0.759, $P < 0.001$) for percentage changes were found.

As described in materials and methods section, a threshold of 14% was used to classify a change of ferritin values as a real (effective) change and a threshold of 20 1/s to classify a change of R2* as a real change. With this, in 41/92 consecutive examinations, a concurrent decrease of R2* and serum ferritin was observed; hepatic steatosis was found for 12 of these 41 examinations. About 29/92 consecutive examinations showed stable or increased R2* and serum ferritin values. In none of these patients, hepatic steatosis was found. For 12 consecutive examinations (associated with 8/42 patients), R2* decreased and ferritin either stayed stable or increased and, in three of these cases, a pathological fat content was found. Finally, in ten consecutive examinations (associated with 9/42 patients), R2* was stable or increased and ferritin decreased, whereby three patients had hepatic steatosis. In total, thus for 22 paired examinations (23.9%), a discordance between ferritin and R2* was found.

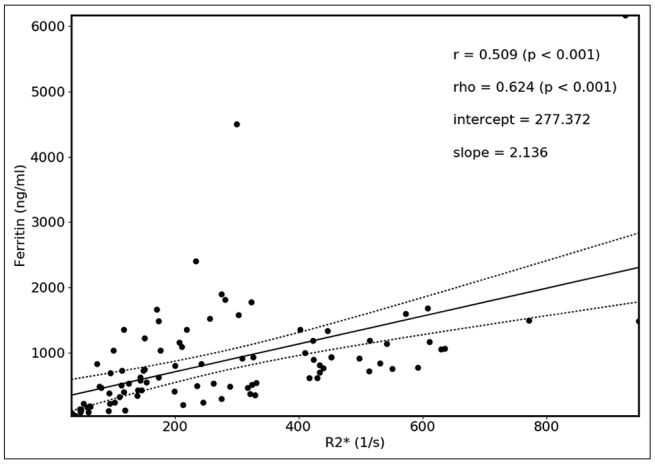

Figure 1: Correlation between liver R2* values and serum ferritin values of all patients and examinations. The solid line represents the best linear fit to the data; the dotted lines correspond to the 95% confidence interval of the linear regression.

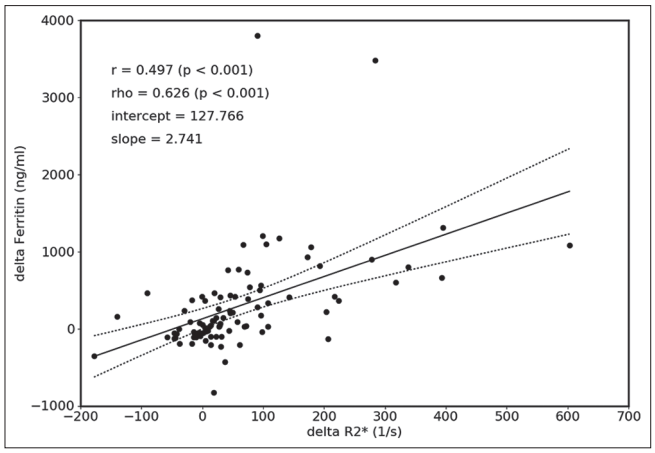

Figure 2: Correlation between absolute changes of pathological liver R2* values and ferritin changes. Only pairs of consecutive examinations and only patients with R2* >70 1/s at their first examination were considered. The solid line represents the best linear fit to the data; the dotted lines correspond to the 95% confidence interval of the linear regression. Negative values represent an increase of the respective value between examinations.

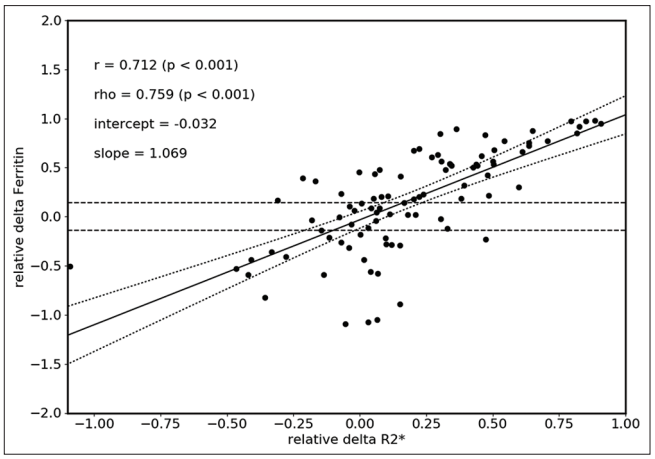

Figure 3: Correlation between relative changes of hepatic R2* measurements and serum ferritin levels. Only pairs of consecutive examinations and only patients with R2* >70 1/s at their first examination were taken into account. The solid line represents the best linear fit to the data. The dotted lines correspond to the 95% confidence interval of the linear regression and the dashed lines represent the variability of ferritin (±14%) which was used to accept a real change of this parameter. Negative values represent an increase of the respective value between examinations.

Table 1: R2* and fat fraction values for patients with a pathological R2* (>70 1/s) and an increased hepatic fat fraction (>5.6%)

Patient	Age	R2* (1/s)	FF (%)
1	57	771.33	19
2	50	100.67	28.56
3	70	117.7	7.71
4	54	171	7.38
5	46	151.57	15.59
6	33	948.63	20.83
7	43	115.23	6.27
8	71	113.9	6.83
9	63	299.87	10.55
10	51	81.37	18.6
11	24	402.53	8.65
12	32	927.57	21.44
13	53	223.6	12.51

R2* (1/s): R2* relaxation rate, FF: Fat fraction

By defining cases with both a decrease in R2* and serum ferritin as true positive, cases with a stable or increasing R2* and serum ferritin as true negative, cases with stable or increasing ferritin, but decreasing R2* as false positive, and cases with decreasing ferritin but stable or increasing R2* as false negative, we obtain a specificity of 0.707, a sensitivity of 0.804, a positive predictive value of 0.774, and an accuracy of 76%.

Table 2 summarizes all individual true- and false-positive results as well as true- and false-negative results (number of examinations) regarding the three therapeutic possibilities based on the above definitions (e.g., a stable or increasing R2* and serum ferritin were defined as true negative).

Figure 4a and b shows examples of liver R2* and ferritin time courses for two different patients during therapy.

Comparison of R2* and transferrin values resulted in a weak negative correlation ($r = -0.342$, $P < 0{,}001$). Analysis of the relative changes of transferrin values with changes of pathological R2* showed a moderate negative correlation (-0.513, $P < 0.001$). A weak correlation was found between liver R2* and transferrin saturation ($r = 0.163$, $P = 0.0457$). Considering the relative changes of these two parameters, no improvement was observed ($r = 0.164$, $P = 0.119$).

Figure 5 depicts the particular therapy which was applied between the respective consecutive examinations for each data point of Figure 3.

In 26/92 data points, the therapy consisted of phlebotomy, in 44/92 of chelation, and, in 22/92 cases, no therapy (watchful waiting) was applied. Analyzing the relation between relative changes of pathological liver R2* values and relative ferritin changes taking into account the different applied therapies, we found a strong correlation for phlebotomy ($r = 0.855$, $P < 0.001$), a strong correlation for chelation ($r = 0.727$, $P < 0.001$), and a moderate correlation for no applied therapy (watchful waiting) ($r = 0.536$, $P = 0.0102$). No significant difference with respect to slope and intercept was found between the linear regression models of phlebotomy and chelation; however, there was a significant difference for the intercept between phlebotomy and no therapy ($P = 0.0043$) as well as chelation and no therapy ($P = 0.0016$).

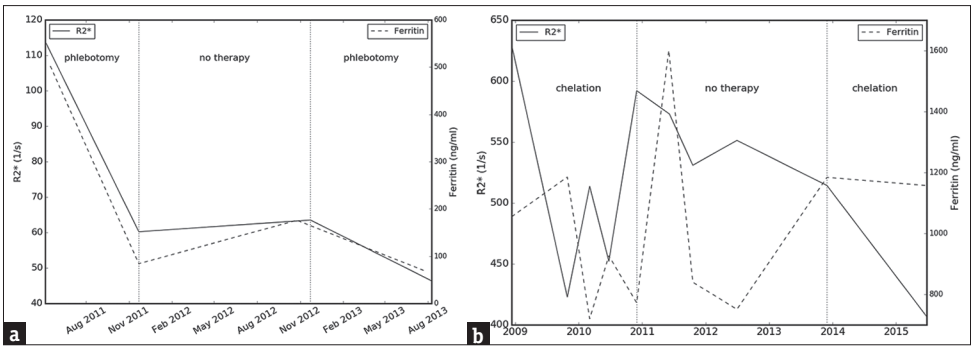

Figure 4: (a) Ferritin and R2* values over time in one patient with HFE-associated hemochromatosis treated with phlebotomy – no relevant differences in the time course between ferritin and R2* values are found.(b) Ferritin and R2* values over time in another patient with iron overload due to aceruloplasminemia treated with chelation. Discrepancies with opposite gradients between ferritin and R2* are seen in this patient.

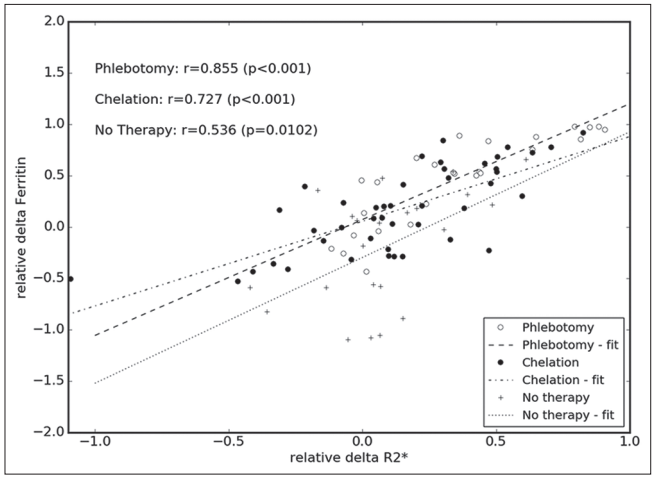

Figure 5: Correlation between relative changes of hepatic R2* measurements and serum ferritin levels. In this figure, the same data as in Figure 3 are shown; however, taking into account the different applied therapies, the shown lines represent the best linear fit to the respective data: dashed line – phlebotomy data; dash-dotted line – chelation; and dotted line – no therapy.

Calculating the linear regression between relative ferritin change and relative R2* change in patients with and without a hepatic steatosis did not result in a significant difference (no fat: $r = 0.718$ [$P < 0.001$], fat: $r = 0.503$ [$P = 0.0796$]; significance of difference in the slope: $P = 0.698$ and significance of difference in the intercept: $P = 0.078$). Between patients with and without HFE-associated hemochromatosis, the linear regression between relative ferritin change and relative R2* change also did not show a significant difference (HFE negative $r = 0.631$ ($P < 0.001$), HFE positive $r = 0.795$ ($P < 0.001$), significance of difference in the slope: $P = 0.139$, and significance of difference in the intercept: $P = 0.35$).

Discussion

Quantifying hepatic iron by means of MRI is proposed as the method of choice for treatment monitoring of iron overload due to the known linear correlation of LIC with total body iron.[10] Even though hepatic iron quantification with MRI has already been proven to be a very reliable method, serum ferritin is still frequently used for monitoring therapy in a clinical setting.[21] The purpose of our study was to compare the relationship of R2* relaxometry with clinical biochemical parameters as used in our clinical setting in treated patients under monitoring (including watchful waiting).

Table 2: Number of true-positive and false-positive classifications as well as true- and false-negative classifications regarding ferritin and R2* changes for the three therapeutic possibilities

	Phlebotomy	Chelation	No therapy
TP	15	21	5
TN	6	12	11
FP	1	8	3
FN	4	3	3

To count any change of ferritin or R2* as effective change, a threshold of 14% was used for ferritin and 20 1/s for R2*, respectively. TP: True positive, ferritin and R2* decrease, TN: Ferritin and R2* increase or stable, FP: Ferritin increase or stable and R2* decrease, FN: Ferritin decrease und R2* increase or stable

We found a strong correlation between relative R2* and ferritin changes for patients receiving phlebotomy and chelation and a moderate correlation when no therapy (watchful waiting) was applied. However, in 22 of 92 paired examinations, a discordance of R2* and ferritin was found. in 12 examinations, R2* decreased and ferritin either stayed stable or increased and, in 10 examinations, R2* was stable or increased and ferritin decreased. Only relying on ferritin changes alone, this could have led to an unnecessary increase in treatment (overtreatment) in 21% of the associated patients or to a reduction in treatment (undertreatment) in 19% of the patients during some point of therapy. This has a crucial impact on clinical management and should be considered as an even more important result than the direct correlation between R2* and ferritin

changes. Especially in patients with unexpected ferritin changes under therapy, that might be interpreted as a failure, and hence MRI with R2* should be considered for confirmation. Puliyel et al.,[22] used a very similar approach comparing R2* values with laboratory parameters. Their study concluded that neither ferritin nor the change in ferritin showed accurate concordance with LIC or changes of LIC and should therefore be interpreted with caution when used to draw clinical decisions such as adjusting chelation management in chronically transfused patients. Porter et al.,[23] concluded in a recent study that instead of using serum ferritin trends alone, the use of liver MRI is preferable to differentiate true from apparent nonresponders to deferasirox.

Rombout-Sestrienkova et al.,[24] postulated that depending on the treatment procedure, blood iron parameters can change differently. To the best of our knowledge, no study evaluated different therapies (phlebotomy vs. chelation) with respect to monitoring by ferritin or R2* relaxometry. We could show a strong correlation of relative serum ferritin and hepatic R2* changes for phlebotomy and chelation, but only a moderate correlation when no therapy was conducted (watchful waiting). No significant difference between phlebotomy and chelation but a significant difference between phlebotomy/chelation and no therapy (watchful waiting) was found. Interestingly, we found an increase or stable ferritin with a decrease of R2* in eight examinations under chelation but in only two examinations under phlebotomy. This implies that especially when dealing with chelation, R2* of the liver should be performed in cases with unexpected increase of ferritin. Beutler et al.,[25] pointed out that when effective chelation therapy is started, the serum ferritin falls more rapidly than body iron. This may be in some extent due to the improvement in liver function and on the other hand because ferritin may predominantly reflect reticular endothelial iron rather than parenchymal iron in the liver and other organs.[26]

Moreover, the underlying disease should also be considered. The study by Puliyel et al.,[22] did only include patients with secondary iron overload (chronically transfused patients, predominantly with thalassemia and sickle syndromes), while our study also considered patients with primary iron overload (hereditary hemochromatosis). Both patient groups may differ from each other in severity, course of disease, and even therapeutic possibilities as well as therapeutic response. Nevertheless, we found no significant difference between patients with primary and secondary iron overload, which implies that the underlying disease does not determine the choice of the therapy monitoring tool.

Transferrin saturation is highly valuable for the diagnosis of hereditary iron overload.[27] Wood et al.,[11] described transferrin and transferrin saturation as valuable in tracking the therapeutic response to iron removal therapies. However, due to the many shortcomings, their use as a sole marker for chelator efficacy is not recommended. Our correlation analysis between hepatic R2* values and transferrin showed a weak negative correlation based on the absolute changes, improving to a moderate negative correlation regarding the relative changes. Transferrin saturation revealed a weak positive correlation for absolute as well as relative changes. This may be because transferrin can also be influenced by confounding factors and many chronically transfused patients have fully saturated transferrin, making it unsuitable for adequate measurement in the follow-up.

Steatosis is a common co-factor in liver injury in patients with iron overload.[28,29] Powell et al.,[30] stated that these patients go along with higher serum ferritin levels and experience an acceleration of liver injury. Recently, Idilman et al.,[31] indicated that in conventional MRI, the detection and quantification of iron and fat becomes difficult because, for example, IP and out-of-phase images can cause diagnostic confusion. We systematically analyzed the simultaneous incidence of steatosis in patients with manifest iron overload and found 30% (13/42) concomitant steatohepatitis (SH). We found no significant differences regarding relative ferritin change versus relative R2* change in patients with and without concomitant steatosis. Therefore, hepatic fat does not seem to influence ferritin measurements for monitoring iron overload. This study did not focus on a possible inflammatory component as in nonalcoholic SH (NASH). We are aware of this limitation, but the diagnosis of NASH is based on criteria that were not available for our study.

Another limitation that should be addressed is the fact that the MRI measurement of LIC and the laboratory test was not on the same day in all patients. Although 27/50 patients had MRI and the laboratory analysis on the same day, the maximum tolerated interval was 60 days. Furthermore, no liver biopsies have been performed due to their invasive characteristic with known limitations such as hemorrhage risk and sampling errors.[32] Therefore, no histological verification/correlation of LIC measurements was carried out. Last but not the least, as described in detail, we included 42 patients and evaluated the change from one examination to the subsequent examination so that altogether 92 paired examinations could be analyzed. This means that as presented in the results' part some

of the paired examinations were derived from follow-up examinations of the same patients. Thereby, for example, the three patients with aceruloplasminemia treated with chelation who underwent ten follow-up examinations accounted for 27 of all the 92 paired examinations. However, as treatment decisions are based on changes of ferritin or R2* between consecutive examinations, this fact should not influence our results.

Conclusions

A good correlation was observed between serum ferritin and MRI R2* relaxometry in monitoring iron overload. Nevertheless, in 22/92 (23.9%) examinations, taking only serum ferritin into account would have let to a misinterpretation of the used therapy. Further, neither hepatic fat nor the underlying disease had an influence on the correlation between ferritin changes under therapy and R2*. In general, ferritin is an acceptable and far cheaper tool for monitoring iron overload, but should be used with caution. MRI should be performed for confirmation, especially in the case of unexpected ferritin changes under therapy, particularly in patients treated with chelation.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Brissot P. Optimizing the diagnosis and the treatment of iron overload diseases. Expert Rev Gastroenterol Hepatol 2016;10:359-70.
2. Wood JC. Diagnosis and management of transfusion iron overload: The role of imaging. Am J Hematol 2007;82:1132-5.
3. Brissot P, Bourel M, Herry D, Verger JP, Messner M, Beaumont C, et al. Assessment of liver iron content in 271 patients: A reevaluation of direct and indirect methods. Gastroenterology 1981;80:557-65.
4. Cappellini MD, Porter J, El-Beshlawy A, Li CK, Seymour JF, Elalfy M, et al. Tailoring iron chelation by iron intake and serum ferritin: The prospective EPIC study of deferasirox in 1744 patients with transfusion-dependent anemias. Haematologica 2010;95:557-66.
5. Goot K, Hazeldine S, Bentley P, Olynyk J, Crawford D. Elevated serum ferritin – What should GPs know? Aust Fam Physician 2012;41:945-9.
6. Borgna-Pignatti C, Castriota-Scanderbeg A. Methods for evaluating iron stores and efficacy of chelation in transfusional hemosiderosis. Haematologica 1991;76:409-13.
7. Fischer R, Harmatz PR. Non-invasive assessment of tissue iron overload. Hematology Am Soc Hematol Educ Program 2009;1:215-21.
8. Beutler E, Felitti V, Ho NJ, Gelbart T. Relationship of body iron stores to levels of serum ferritin, serum iron, unsaturated iron binding capacity and transferrin saturation in patients with iron storage disease. Acta Haematol 2002;107:145-9.
9. Lorcerie B, Audia S, Samson M, Millière A, Falvo N, Leguy-Seguin V, et al. Diagnosis of an increased serum level of ferritin. Rev Med Interne 2015;36:522-9.
10. Angelucci E, Brittenham GM, McLaren CE, Ripalti M, Baronciani D, Giardini C, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000;343:327-31.
11. Wood JC. Use of magnetic resonance imaging to monitor iron overload. Hematol Oncol Clin North Am 2014;28:747-64, vii.
12. 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.
13. Brittenham GM, Cohen AR, McLaren CE, Martin MB, Griffith PM, Nienhuis AW, et al. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. Am J Hematol 1993;42:81-5.
14. Pilon VA, Howanitz PJ, Howanitz JH, Domres N. Day-to-day variation in serum ferritin concentration in healthy subjects. Clin Chem 1981;27:78-82.
15. He T, Gatehouse PD, Smith GC, Mohiaddin RH, Pennell DJ, Firmin DN, et al. Myocardial T2* measurements in iron-overloaded thalassemia: An *in vivo* study to investigate optimal methods of quantification. Magn Reson Med 2008;60:1082-9.
16. Meloni A, Rienhoff HY Jr., Jones A, Pepe A, Lombardi M, Wood JC, et al. The use of appropriate calibration curves corrects for systematic differences in liver R2* values measured using different software packages. Br J Haematol 2013;161:888-91.
17. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, et al. Measurement of intracellular triglyceride stores by H spectroscopy: Validation *in vivo*. Am J Physiol 1999;276:E977-89.
18. Hankins JS, McCarville MB, Loeffler RB, Smeltzer MP, Onciu M, Hoffer FA, et al. R2* magnetic resonance imaging of the liver in patients with iron overload. Blood 2009;113:4853-5.
19. Henninger B, Kremser C, Rauch S, Eder R, Zoller H, Finkenstedt A, et al. Evaluation of MR imaging with T1 and T2* mapping for the determination of hepatic iron overload. Eur Radiol 2012;22:2478-86.
20. Fleming RE, Ponka P. Iron overload in human disease. N Engl J Med 2012;366:348-59.
21. Inati A, Khoriaty E, Musallam KM. Iron in sickle-cell disease: What have we learned over the years? Pediatr Blood Cancer 2011;56:182-90.
22. Puliyel M, Sposto R, Berdoukas VA, Hofstra TC, Nord A, Carson S, et al. Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. Am J Hematol 2014;89:391-4.
23. Porter JB, Elalfy M, Taher A, Aydinok Y, Lee SH, Sutcharitchan P, et al. Limitations of serum ferritin to predict liver iron concentration responses to deferasirox therapy in patients with transfusion-dependent thalassaemia. Eur J Haematol 2017;98:280-8.
24. Rombout-Sestrienkova E, Koek GH, Neslo R, van Kraaij M, Menheere PP, Masclee A, et al. Course of iron parameters in HFE-hemochromatosis patients during initial treatment with erythrocytapheresis compared to phlebotomy. J Clin Apher 2016;31:564-70.
25. Beutler E, Hoffbrand AV, Cook JD. Iron deficiency and overload. Hematology Am Soc Hematol Educ Program 2003;1:40-61.
26. Zanella A, Berzuini A, Colombo MB, Guffanti A, Lecchi L, Poli F, et al. Iron status in red cell pyruvate kinase deficiency: Study of Italian cases. Br J Haematol 1993;83:485-90.
27. Adams PC. Epidemiology and diagnostic testing for

hemochromatosis and iron overload. Int J Lab Hematol 2015;37 Suppl 1:25-30.
28. George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, et al. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 1998;114:311-8.
29. Moirand R, Mortaji AM, Loréal O, Paillard F, Brissot P, Deugnier Y, et al. A new syndrome of liver iron overload with normal transferrin saturation. Lancet 1997;349:95-7.
30. Powell EE, Ali A, Clouston AD, Dixon JL, Lincoln DJ, Purdie DM, et al. Steatosis is a cofactor in liver injury in hemochromatosis. Gastroenterology 2005;129:1937-43.
31. İdilman İS, Akata D, Özmen MN, Karçaaltıncaba M. Different forms of iron accumulation in the liver on MRI. Diagn Interv Radiol 2016;22:22-8.
32. Villeneuve JP, Bilodeau M, Lepage R, Côté J, Lefebvre M. Variability in hepatic iron concentration measurement from needle-biopsy specimens. J Hepatol 1996;25:172-7.