MRI-Based Liver Iron Content Determination at 3T in Regularly Transfused Patients by Signal Intensity Ratio Using an Alternative Analysis Approach Based on R_2^* Theory

MRI-basierte Leber-Eisen-Quantifizierung bei 3 T in regelmäßige transfundierten Patienten mittels Signalintensitätsverhältnissen unter Verwendung eines alternativen Ansatzes zur Datenanalyse basierend auf der R_2^*-Theorie

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Key words

- abdomen
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Zusammenfassung

Ziel: Evaluierung der Möglichkeit, den Leber-Eisengehalt (LIC, von liver iron conentration) regulär transfundierter Patienten mittels MRI-Signalintensitätsverhältnissen (SIR, kurz nach dem engl. Signal Intensity Ratio) bei 3 T zu bestimmen. Hierzu wurde ein neuartiger Ansatz der Datenanalyse erarbeitet.

Methoden: 47 konsekutive Untersuchungen regulär transfundierter Patienten wurden in die Studie eingeschlossen. Bei ihnen wurde ein hoher Lebereisengehalt erwartet. Die Untersuchung erfolgte an einem 3T-MRI-Scanner mit Multi-Echo-Gradienten-Echo-(GRE)-Sequenzen mit vier verschiedenen Flipwinkeln zwischen 20° und 90° mit Echozeiten (TE) von 0,9 bis 9,8 ms. Eine MRI-Methode basierend auf Spin-Echo-Protokollen bei 1,5 T diente als Referenz zur Bestimmung des Leber-Eisengehalts. Für die Analyse der 3T-GRE-Daten wurde das Signal-Intensitätsverhältnis (SIR) zwischen Leber- und Muskelgewebe ermittelt. Da zu erwarten war, dass sich die für 1,5 T bekannte Methode nicht für die Auswertung von 3T-Daten eignete, wurde zur Ermittlung einer passenden Umrechnungsvorschrift der theoretische Zusammenhang zwischen SIR und LIC aus der Formel für den R_2^*-Signalzerfall abgeleitet. Aus dem Zusammenhang der SIR-Werte mit den LIC-Referenzwerten wurde eine Relation ermittelt, um den LIC und dessen Unsicherheit aus den GRE-Daten zu bestimmen. Diese Ergebnisse wurden mit den LIC-Referenzwerten korreliert und für zwei LIC-Schwellwerte die diagnostische Genauigkeit berechnet.

Ergebnisse: Der LIC konnte in unserem Patientenkollektiv auch für hohe Eisenüberladung verlässlich aus den SIR-Werten bestimmt werden. Der Median der Unsicherheit der ermittelten LIC-Werte lag bei 10 %, die diagnostische Genauigkeit bei 0,92 bzw. 0,91.

Abstract

Objectives: To evaluate the feasibility of addressing liver iron content (LIC) in regularly transfused patients by MR imaging at 3 T based on the signal intensity ratio (SIR). An innovative data analysis approach was developed for this purpose.

Methods: 47 consecutive examinations of regularly transfused patients were included. In all cases, we expected high LIC levels. Patients were scanned with MRI at 3 T with multi-echo gradient echo sequences (GRE) at four different flip angles between 20° and 90° with echo times (TE) ranging from 0.9 to 9.8 ms. Spin-echo protocols were acquired to determine the LIC with a reference MRI method working at 1.5 T. 3 T GRE data were analyzed using the liver-to-muscle SIR. Since the method known for 1.5 T was not expected to be applicable for analyzing 3 T data, theoretic dependence of the SIR on the LIC was derived from the equation describing R_2^* signal decay. Obtained SIR values were correlated to reference LIC to get a relation for calculating LIC from SIR quantities. LIC values and their uncertainties were determined from GRE data and correlated to LIC reference values. For two LIC thresholds, the diagnostic accuracy was determined.

Results: LIC was reliably determined from SIR in our patient cohort even for large LIC values. Median of LIC uncertainties was 10 %, and the diagnostic accuracy was 0.92 and 0.91, respectively.

Conclusion: Determination of even high LIC, resulting in small SIR values, is feasible at 3 T using appropriate SIR analysis.

Key Points:

- Determination of Liver Iron Concentration (LIC) based on GRE MRI at 3T is feasible even for high LIC levels using Signal Intensiy Ratios.
- Relative uncertainty of LIC determined with 3T GRE MRI was below 13 % in most cases.
- The patient-management relevant threshold (LIC = 80 µmol/g (4.5 mg/g)) yielded an accuracy of .92 in our cohort.

Schlussfolgerung: Selbst hohe LIC-Werte, bei denen kleine SIR auftreten, können bei 3 T verlässlich bestimmt werden.

Kernaussagen:

- Bestimmung des Leber-Eisengehalts (LIC, kurz für Liver Iron Conentration) basierend auf GRE MRT bei 3T mittels Signal-Intensitäts-Verhältnissen ist möglich, auch für hohe Leber-Eisen-Werte.
- Die realtive Unsicherheit des mit der vorgeschlagenen Methode bestimmten Leber-Eisengehalts war in den meisten Fällen kleiner als 13 %.
- Bei der für das Patienten-Management relevanten LIC-Schwelle von 80 µmol/g (4,5 mg/g) ergab sich für unser Patientenkollektiv eine Genauigkeit von 0,92.
- Die dargestellte Methode ist schnell und einfach, sowohl die Daten-Akquisition als auch die Auswertung.
- The proposed method is quick and simple, both in terms of data acquisition and analysis.

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Introduction

The periodic assessment of liver iron content (LIC) is important for the management of secondary hemochromatosis in regularly transfused patients with thalassemia and other chronic forms of anemia. MRI has the advantage of widespread availability, is non-invasive, and the MR liver signal depends on the LIC. Therefore, the development of reliable standardized MRI-based methods to determine LIC has been of clinical and scientific interest for a long time (e. g. [1 – 6]). As there are few recent studies using 3 T in animals [7, 8] and humans [9, 10], established broadly accessible methods work at 1.5 T. A commercially available method based on T_2 analysis of spin-echo (SE) data [3] is FDA approved and meets the quality requirements for clinical use. It requires scanner calibration and long measurement times, and data has to be transferred for data analysis. The upper limit for this method is an LIC value of 769 µmol/g (43 mg Fe per g dry liver tissue).

Gradient echo (GRE)-based approaches are of particular interest since they have the advantage of shorter measurement times, requiring only a few breath-holds. Several attempts (e. g. [2, 11 – 13]) have been made to determine LIC from transverse relaxation time T_2^*, however, with inconclusive results. A synopsis is given in [14]. Also, there are indications that in animals at 4.7 T the liver-to-muscle signal intensity ratio (SIR) applied to spin-echo data shows superior correlation to LIC than T_2 [15].

Involving the signal of reference tissue by SIR as proposed, e. g., by [1, 4 – 6, 16], demands less data than needed for relaxometry and allows quick and easy LIC determination. Previous attempts using SIR were based on the empirically revealed assumption that there is a linear relationship between SIR and LIC [1, 4]. Alustiza et al. proposed a different, nonlinear approach [5]. To understand the underlying mechanisms, we present a theoretic background for the dependence of SIR values on LIC in the appendix.

One of the approaches involving the SIR of gradient echo (GRE) data has been made publicly available for LIC determination by Gandon [1]. However, certain deviations from SE [6, 17] and GRE [18] relaxometry have been observed. The inconsistency between GRE SIR and SE relaxometry is less pronounced ([17]) when working with the SIR approach proposed by Alustiza [5]. The upper LIC limit for Gandon's method is 350 µmol/g (= 20 mg/g) which is lower than for the above-mentioned SE method, mainly due to the reduced signal-to-noise ratio in GRE compared to SE. Since a significant number of affected patients actually present an LIC above the threshold for Gandon's method, an approach was presented in [4] for highly transfused patients scanned at 1.5 T for an LIC between 350 – 900 µmol/g (20 – 50 mg/g). However, this is prone to LIC overestimation in patients with liver steatosis due to the short TE of 1.8 ms where the water and fat signals are dephased.

Recently, a meta-analysis [19] revealed limited diagnostic accuracy for 1.5 T MRI-based liver iron determination, esp. for the convenient GRE techniques. Therefore, we see a need for methodological improvement.

MRI at 3 T is supposed to overcome shortcomings observed at 1.5 T due to an improved signal-to-noise ratio (SNR). The feasibility of 3 T MRI in liver iron overload has been proved, however, without an LIC reference [9, 10]. The reduced first in-phase echo time compared to 1.5 T is expected to compensate for larger R_2^* values observed at 3 T [8]. Since Gandon's and Alustiza's methods were not anticipated to work at 3 T, an alternative relation had to be found.

As stated in the appendix, theory yields, under certain assumptions, the following dependence:

$$\ln(\mathrm{SIR}) = m \cdot \mathrm{LIC} + c \quad (1)$$

with

$$m = -T_E \cdot R_{Fe} \quad (2)$$

where R_{Fe} is the relaxivity of iron in liver tissue, and

$$c = \ln\left(\frac{S_{0\,liver}}{S_{0\,muscle}}\right) + T_E\,(R_2^*{}_{muscle} - R_2^*{}_{iflt}) \quad (3)$$

with $R_2^*{}_{iflt}$: theoretical R_2^* value of iron-free liver tissue, $R_2^*{}_{muscle}$: R_2^* of muscle tissue, and $S_{0\,liver}$, $S_{0\,muscle}$: MR scaling constants for liver signal and muscle signal, respectively. Testing of the assumptions this relation is based on is beyond the scope of this study. Here, we concentrate on the applicability in daily routine.

The current study had four objectives:

1. To study the feasibility of determining LIC with 3 T MRI using multi-echo GRE sequences in regularly transfused patients presenting high liver iron levels.
2. To evaluate the flip angle (FA) best suited for LIC calculation from SIR data at 3 T.
3. To address result uncertainties of SIR-based LIC determination.
4. To determine sensitivity and specificity with respect to certain LIC thresholds in our patient group.

Methods

47 consecutive examinations of 41 regularly transfused patients (18f, 23m; age: 13 – 72 years, mean age: 28.6y; 6 patients scanned twice at an interval of one year, in one case 15 month) referred to

Table 1 Underlying disorders of enrolled patients.

Tab. 1 Krankheitsbilder der eingeschlossenen Patienten.

thalassemia major	17
other thalassemia syndromes	4
sickle cell disease	6
hereditary hemochromatosis	2
acute lymphoblastic leukemia (ALL)	5
diamond-blackfan anemia (DBA)	3
congenital dyserythropoietic anemia (CDA)	3
other	7

the radiology department for determination of liver iron content were evaluated. The study was approved by our local ethics committee. Written informed consent of the patients and/or parents was obtained. The majority of patients were affected by thalassemia syndromes. For other diagnoses cf. **Table 1**. 5 transverse 5 mm slices covering part of the liver were acquired using a multi-echo RF spoiled 2 D gradient echo (GRE) sequence at 3 T (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany). Further parameters were T_R/T_E 250/0.9 – 1.7 – 2.5 – 3.3 – 4.1 – 4.9 – 6.1 – 7.3 – 8.6 – 9.8 ms, FoV 338 × 450 mm and matrix size 62 × 128, yielding a resolution of 3.5 mm^2 reconstructed voxel size. The acquisition was performed at 4 excitation flip angles (FA) of 20°, 30°, 50° and 90° requiring 4 breath-holds of 15 seconds each. To improve image signal homogeneity, a body coil was used as the receiver.

To obtain Ferriscan® LIC reference, all patients were scanned at 1.5 T (Avanto, Siemens Healthcare) immediately before the 3 T exam. Spin-echo images were acquired at T_R/T_E 2500/6 – 9-12 - 15- 18 ms in free breathing at a matrix size of 256 × 256, FoV of 420 mm and resolution of 1.6 mm^2. Each T_E required an acquisition time of 3 minutes and 15 seconds. Investigations exceeding the upper limit of 769 µmol/g (43 mg/g) were excluded from the correlation analysis. However, since clear evidence is given concerning LIC thresholds even in those cases, all examinations were included in the sensitivity/specificity analysis.

GRE data was analyzed with a custom-made tool named "MUltiEcho data Analysis Tool for Liver Iron Verification by Embracing signal intensity Ratio" (MUCAT-LIVER) written in IDL (Excelis Vis Inc., McLean, VA). For each patient, three slices best suited for analysis, i. e. with sufficient vessel-free liver parenchyma and good visibility of paraspinal muscles, were chosen for analysis. Three ROIs of fixed size were drawn manually by an investigator blinded to the LIC reference values in vessel-free liver tissue, preferably the right liver lobe, and one each in the left and right paraspinal muscle. Liver-to-muscle SIR values based on both the mean and median of ROI voxel values were calculated for T_Es 2.5, 4.9 and 9.8 ms, for all flip angles. Uncertainties of the SIR values were calculated via error propagation by the MUCAT-LIVER tool and were exported together with the SIR as text files for further analysis.

SIR Using Logarithm of ROI Median (SIR-ULM) values were determined. As cross-check, the ROI mean values were also investigated. The average value of all slices for each patient at three different T_E values was correlated to the LIC and tested for linear correlation theoretically expected as outlined in the 'Signal Intensity Ratio' section of the appendix, cf. eq. (7). Statistical analysis including linear regression was performed in SPSS (IBM corp., Armonk, NY), yielding coefficient of determination R^2, as well as slope and intercept values for regression lines. For LIC determination from SIR-ULM values, the appropriate relation was derived from these results. According to this, LIC values (referred to below as GRE LIC) and their uncertainties were determined from the SIR-ULM values. Linear correlation of GRE-LIC to reference LIC was studied with SPSS as outlined above. Finally, both methods were compared using a Bland-Altman plot and determination of sensitivity and specificity. For the latter, we considered two thresholds: 125 µmol/g (7 mg/g) dry weight as used in the meta-analysis of Sarigianni et al. [19] as well as 80 µmol/g (4.5 mg/g) as proposed in the guidelines of the German Society of Pediatric Oncology and Hematology [20] (for the latest version, cf. http://www.awmf.org/leitlinien/detail/ll/025 – 029.html).

Results

2 patients, 1f, 1 m, both with thalassemia major, exceeded the limit for our reference method and were excluded from the correlation analysis. For the remaining patients (age range and mean as above), examinations showed reference LIC values in the range of 10 – 552 mmol Fe/kg dry liver tissue (corresponds to 0.6 – 30.8 mg Fe/g dry liver tissue).

The correlation values of different protocols and ROI averaging methods are given in **Table 2**. The best correlation of SIR-ULM vs. ref. LIC was found at FA = 30° and T_E = 2.5 ms (**Fig. 1**), yielding for the dependence of GRE-LIC on SIR-ULM

GRE-LIC = -166.8 µmol/g * SIR-ULM + 33.4 µmol/g

with the LIC value in µmol/g, where the uncertainty of slope was 7.3 µmol/g and the uncertainty of intercept was 8.2 µmol/g. If the SIR for T_E = 2.5 ms exceeded 1.1, GRE-LIC was calculated from the SIR-ULM values acquired with T_E = 9.8 ms using the appropriate relation. This affected only the range of normal LIC (below 30 µmol/g) where correction was required, concerning 8 patients (18 %). While FA 20° gives the best correlation for T_E = 9.8 ms, we used SIR values at FA 30° with minimal deviations.

In the high LIC range, 2 patients exceeded the limit of the reference method used. With GRE, their LIC was determined as 542 and 636 µmol/g, respectively, which means an underestimation by GRE-LIC.

The results of this combined approach are shown as a function of LIC reference values in **Fig. 2**. The correlation of the GRE-LIC to the reference LIC was excellent with R^2 = 0.925. The slope of the regression line was 0.975 ± 0.042 and its intercept was 6.7 ± 7.7, indicating that identity was within the confidence range. All patients with a normal reference LIC (below 33 µmol/g) also showed a normal GRE-LIC, and vice versa. **Table 3, 4** show 2 × 2 tables for thresholds of 80 µmol/g and 125 µmol/g. The sensitivity, specificity and diagnostic accuracy values were 0.91, 0.93 and 0.92, respectively, with accuracy of at least 0.82 for the threshold of 80 µmol/g (4.5 mg/g) and 0.9, 0.93 and 0.91, respectively, with a lower bound for accuracy of 0.81 for the larger threshold of 125 µmol/g (7 mg/g). The upper bound for accuracy was 1.0 for both thresholds.

Relative uncertainties of results are displayed in **Fig. 3**, indicating best performance for patients with an excessive iron overload, showing uncertainties below 13 % for an LIC > 90 µmol/g. **Fig. 4** shows the Bland-Altman plot comparing both methods. The three data points outside the 1.96 SD confidence interval correspond to patients with beta thalassemia, ALL and DBA, respectively.

Table 2 R^2 as measure for the correlation of SIR-ULM, acquired with different parameters, to LIC. For all acquisition parameters, ROI median and mean values were analyzed.

Tab. 2 Bestimmtheitsmaß R^2 als Maß der Korrelation von SIR-ULM mit LIC, bei verschiedenen Aufnahmeparametern. Bei allen Parameterkombinationen wurden jeweils Mittelwert und Median der ROIs analysiert.

	FA	20°	30°	50°	90°
ROI averaging					
median	TE = 2.5 ms	0.915	0.923	0.915	0.922
mean	TE = 2.5 ms	0.916	0.922	0.915	0.922
median	TE = 4.9 ms	0.795	0.810	0.815	0.822
mean	TE = 4.9 ms	0.786	0.804	0.809	0.815

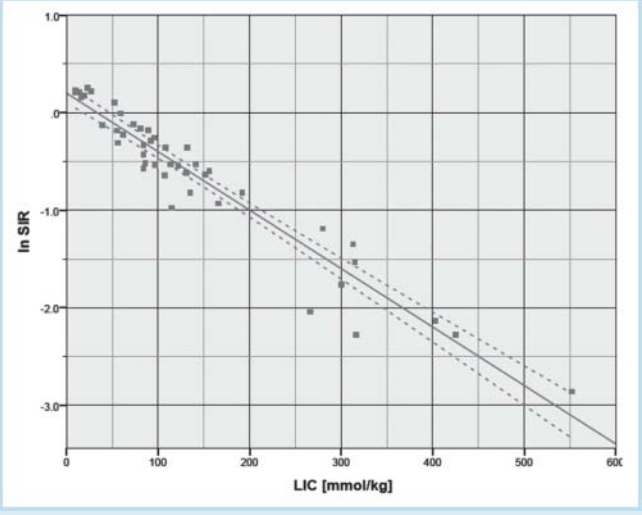

Fig. 1 Natural logarithm of measured SIR vs. reference LIC. Linear regression as well as confidence intervals are indicated. This correlation was used to calculate GRE LIC from ln (SIR) values. Data from patients exceeding the upper threshold for the reference method are excluded from the regression analysis and are not shown in this diagram.

Abb. 1 Natürlicher Logarithmus der gemessenen Signal-Intensitäts-Verhältnisse in Abhängigkeit von den Referenz-Lebereisen-Werten. Die lineare Regression sowie deren Konfidenz-Intervall ist dargestellt. Diese Korrelation wurde herangezogen, um aus den ln (SIR)-Werten den Leber-Eisengehalt zu bestimmen. Daten, deren LIC den Grenzwert für die Referenzmethode überschreitet, wurden von der Regressionsanalyse ausgeschlossen, und sie erscheinen nicht in diesem Diagramm.

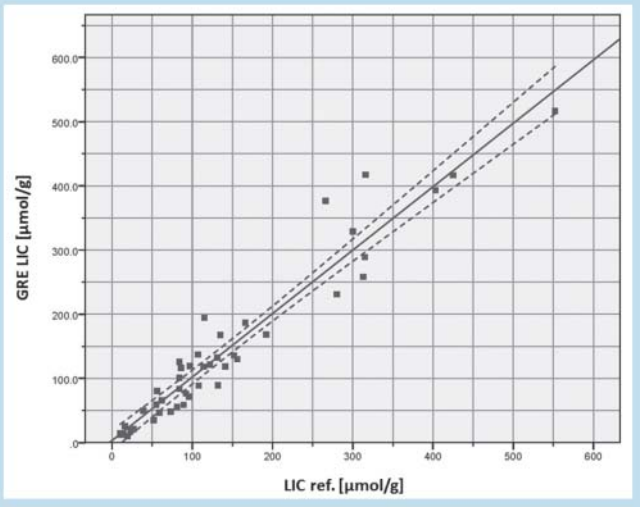

Fig. 2 LIC determined from GRE SIR vs. reference LIC. The solid line is the regression line. As in **Fig. 1**, data from patients exceeding the upper threshold for the reference method were excluded from the regression analysis and are not shown in this diagram.

Abb. 2 LIC, bestimmt aus GRE SIR, in Abhängigkeit vom Referenz-LIC. Wie in **Abb. 1** wurden Daten, deren LIC den Grenzwert der Referenzmethode überschreitet, von der Regressionsanalyse ausgeschlossen, und sie erscheinen nicht in diesem Diagramm.

Table 3 2 × 2 table for LIC threshold of 80 µmol/g as proposed in the latest version of the guidelines [18], yielding a sensitivity of 0.91, specificity of 0.93 and diagnostic accuracy of 0.92 (confidence interval 0.82 – 1.0).

Tab. 3 2 × 2-Tabelle für LIC-Grenzwert von 80 µmol/g, wie er in der aktuellen Version der Leitlinie [18] vorgeschlagen wird. Es ergab sich eine Sensitivität von 0,91, eine Spezifität von 0,93 und eine diagnostische Genauigkeit von 0,92 mit einem Konfidenzindervall von 0,82 ... 1,0.

	LIC GRE ≤ 80 µmol/g	LIC GRE > 80 µmol/g	sum
LIC ref ≤ 80 µmol/g	13	1	14
LIC ref > 80 µmol/g	3	30	33
sum	16	31	47

Table 4 2 × 2 table for LIC threshold of 125 µmol/g as used by Sarigianni et al. [17]. This yielded a sensitivity of 0.9, specificity of 0.93 and diagnostic accuracy of 0.91 (confidence interval 0.81 – 1.0).

Tab. 4 2 × 2-Tabelle für LIC-Grenzwert von 125 µmol/g, wie er in der Metaanalyse von Sarigianni [17] verwendet wurde. Es zeigte sich eine Sensitivität von 0,9, eine Spezifität von 0,93 und eine diagnostische Genauigkeit von 0,91 mit einem Konfidenzindervall von 0,81 ... 1,0.

	LIC GRE ≤ 125 µmol/g	LIC GRE > 125 µmol/g	sum
LIC ref ≤ 125 µmol/g	26	2	28
LIC ref > 125 µmol/g	2	17	19
sum	28	19	47

Discussion

We studied the feasibility of addressing LIC in regularly transfused patients by MRI at 3 T. Some of our patients showed severe iron overload above the threshold of a standardized method using MRI at 1.5 T. However, it is important for these patients to monitor treatment efficiency in follow-up investigations.

Using the SIR approach, we were able to successfully differentiate between extreme levels of iron overload, minimizing the influence of liver fat fraction by using in-phase T_E. This was not feasible using previously proposed methods, as discussed e. g. in [4, 21]. By utilizing the fact that SIR-ULM correlates linearly to LIC for the smallest T_E used, we were able to derive a relationship for the whole LIC range covered by our patient cohort, with the only exception being small, i. e. normal, LIC values. In most cases, this makes it unnecessary to decide which protocol is appropriate to determine LIC from signal values observed in a specific patient. Stepwise linearizing the dependence of SIR on LIC, as proposed in

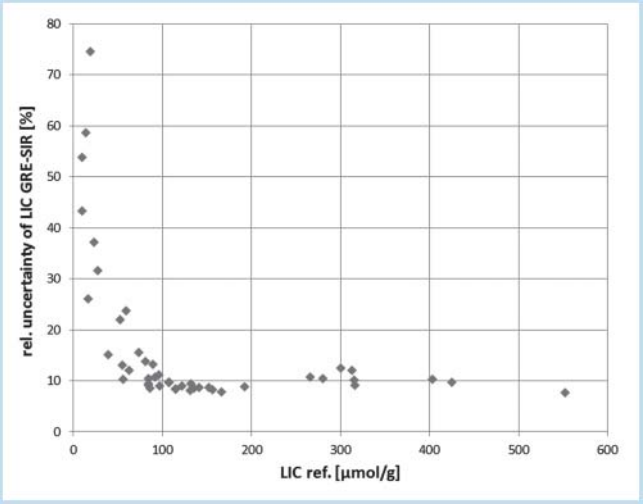

Fig. 3 Relative uncertainties of LIC determined by GRE based on SIR-ULM vs. reference LIC.

Abb. 3 Relaltive Unsicherheiten des aus den GRE-Daten mittels SIR-ULM bestimmten LIC in Abhängigkeit vom Referenz-LIC.

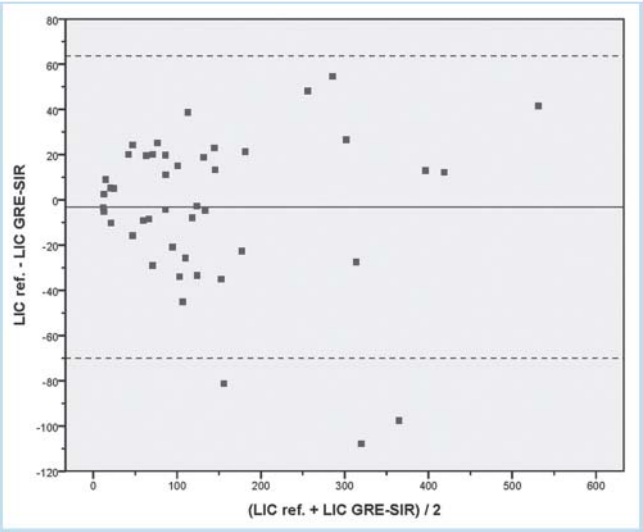

Fig. 4 Bland-Altman plot comparing GRE LIC results and the reference method.

Abb. 4 Bland-Altman-Plot zum Vergleich von GRE-LIC und der Referenzmethode.

[1], may be a reason for the deviations reported in [6] between the results of Gandon's method and Ferriscan®. A linear correlation of SIR to LIC was reported at 3 T in an animal study [7]. The limited LIC range obtained in their animal model of up to 10 mg/g, corresponding to 179 µmol/g and indicating mild iron overload, may account for that finding. Deviations from linearity, however, were observed in humans for the first in-phase T_E at LIC levels above 350 µmol/g ([1]). Rose et al. proposed a protocol for highly transfused patients [4]. Within the coverage given for their method, a linear dependence between SIR and LIC is observed, however, for a T_E shorter than the first in-phase T_E. This causes LIC overestimation in the presence of fatty liver.

The number of required breath-holds for multiple echoes as well as the measurement time was reduced by the use of multi-echo GRE sequences. The feasibility of these for liver relaxometry has been proven previously [22]. In this study, we used it successfully as the basis for SIR calculation.

In general, MRI LIC assessment based on SIR, as first proposed by Anderson et al. [16] and adapted for routine application by Gandon et al. [1] using MRI at 1.5 T, is a technique without demand for extensive mathematics. Fenzi et al. found that LIC determination based on liver signal-to-noise ratio (SNR) calculation is superior to liver-to-muscle SIR in animals [15]. However, we expected difficulties in reliably determining noise, especially in uncooperative patients. Furthermore, the SNR may vary between systems of different vendors. Therefore, we preferred using the SIR in the presented approach.

Use of SIR, however, is limited by some essential prerequisites:

1. Homogenous signal throughout the imaging plane.
2. Signal noise (SNR).
3. Assumptions concerning liver and muscle signal.
4. Dephasing between water and fat MRI signal.

1. To improve signal homogeneity, use of a body volume coil is mandatory [1]. We are aware of the fact that body coil isn't the best choice with respect to SNR. Also, a lack of homogeneity is sometimes observed, probably due to inhomogeneous B_1, which may be a reason for deviation of results.

2. Signal noise causes problems if not negligible compared to tissue signal, which is the case even at the shortest T_E for high LIC levels. Correlation of SIR-ULM was shown to be good at $T_E = 2.5$ ms also for large LIC. For patients excluded from correlation analysis due to exceeding reference LIC threshold, GRE-LIC was underestimated, most probably due to signal noise artificially elevating the SIR and diminishing GRE-LIC. However, since GRE-LIC values are clearly above 350 µmol/g (20 mg/g), the LIC threshold considered as a risk for severe organ siderosis, there is no effect on diagnostic accuracy, thus indicating that noise has no impact on clinical performance. Follow-up studies should be meaningful even in that LIC range since noise effects are monotonic, implying that changing LIC causes GRE-LIC to change the same way, i. e., rising LIC causes rising GRE-LIC.

3. Assumptions concerning muscle R_2^* and liver-to-muscle S_0 ratio effects on LIC were made to get an analytical equation for the dependence of LIC on SIR. Stark et al. [23] report liver T_1 dependence on LIC at 1.5 T, however, with only small percentage changes. Influence of T_1 on S_0 is minimal for small flip angles. Our results, however, indicate a good correlation independent of FA. From this, we conclude only minimal T_1 effects on SIR at 3 T.

4. Using T_Es where the main fat component is in-phase with the water signal should reduce the effect of liver fat content. However, going into detail, conditions are more complex since there are several fat components, cf. [24]. Hernando et al. demonstrate the influence of this factor for R_2^* determination [25, 26]. We expect also a certain influence on the SIR. However, this was minimized by employing the first and fourth 'in-phase' T_E, thus minimizing the effects of fat. The remaining influence of liver steatosis, probably present in some of our patients, may account for the lower correlation between the LIC determined by SIR-ULM and our reference method based on SE, where dephasing effects are reversed by refocusing pulse. Details could be evaluated with simulations, but this was beyond the scope of this study.

Despite all of these shortcomings and limitations, we were able to demonstrate that an appropriate analysis of SIR data at 3 T results in reliable LIC determination. Sensitivity and specificity prove

that the proposed technique is quite robust. Compared to other GRE approaches considered by Sarigianni et al. [19], our method shows superior performance and the largest patient cohort. With a single breath-hold approach, we were able to cover a larger LIC range compared to other studies at 3 T [9]. Storey et al. had to adapt their MRI acquisition protocol if low liver signals were observed, i. e. for an LIC larger than 25 mg/g [10].

Theoretical considerations are independent of field strength. Therefore, equation (7) is expected to be valid also for 1.5 T data, but at different values for slope and intercept of LIC vs. SIR-ULM. Smaller R_2^* values expected at 1.5 T are compensated for by the fact that in-phase T_E are elongated by approx. the same factor. Therefore, only a low influence of R_2^* on the measured signal is expected. We anticipated a lower SNR at 1.5 T as the dominant observable effect which may cause deviations from the expected correlation at high LIC levels. However, preliminary results with SIR-ULM at 1.5 T showed similar performance compared to 3 T in terms of R^2 [27].

Analysing the median value was originally intended to reduce disturbing influence of liver vessels sometimes barely seen due to partial volume effects, but also has a positive impact by decreasing noise effects. For comparison with previous studies [17, 28] working with mean values of ROI voxel signals, these were also evaluated. Our results show that the effect of different types of ROI averaging is only minimal, except for the second in-phase T_E with a slight benefit for median values, probably due to the reduced influence of signal noise.

The reason for choosing FA = 30° also in the normal LIC range is the advantage that LIC can be determined in a single breath-hold with multi-echo GRE sequences. Results differ only marginally, and only 8 (18 %) of our examinations were affected by this choice.

Jensen et al. point out that SE and GRE address complementary aspects of liver iron storage mechanisms [29]. Therefore, we have to keep in mind that differences observed between GRE-LIC and the reference method Ferriscan® represent general deviations between GRE and SE. Nevertheless, we had to judge them as a lack of accuracy of the proposed SIR-ULM method. Hence, in terms of accuracy, performance of LIC determination based on SIR-ULM may be better in principle than our results, but is impaired by incompatibility between the compared methods. This shortcoming could be addressed by choosing another reference method, e. g. SQUID or biopsy. Nevertheless, Ferriscan® was used due to its good availability and noninvasiveness.

Precision of our method substantiates its suitability for routine application. For low LIC in the normal and slightly iron overloaded ranges, uncertainties are relatively large, mainly due to the high incertitude of linear regression results based on only 23 patients. An uncertainty of over 40 % in these cases may appear high at first glance, but these large relative uncertainties correspond to small absolute values. Since normal LIC values range from 3 – 33 µmol/g (0.17 – 1.8 mg/g), thus spanning an interval of ± 83 % with regard to the mean normal value, the observed uncertainties near this LIC value are regarded as acceptable. Yet, for LIC values from moderate to severe iron overload, the SIR-ULM method performed at 3 T yields correct results in contrast to established, popular SIR approaches at 1.5 T. Whereas existing methods at 1.5 T show impaired performance at high LIC levels [1, 4], the 3 T approach described here demonstrates superior performance in terms of precision for that LIC range compared to normal LIC.

In summary, we were able to demonstrate that 1. MRI-based LIC determination in regularly transfused patients is feasible at 3 T with multi-echo GRE sequences using the quick and easy SIR analysis method, 2. best performance is observed at 30° FA, 3. precision of this method is suitable for routine application, and 4. sensitivity and specificity with regard to our reference method are superior to other GRE techniques as reported in [19].

Conclusion

We confirmed the theoretically expected relation between liver iron concentration (LIC) and liver-to-muscle signal intensity ratio (SIR) at 3 T for LIC levels covered by our patient cohort. The easy operability of this method allowing instant and precise LIC determination makes it suitable for daily routine.

Appendix

Tissue relaxation

Transverse relaxation of tissue is influenced by the concentration of incorporated agents – in our case, iron components. Linear dependence on liver iron concentration (LIC) is expected for relaxation rate $R_2^*_{liver}$ of the liver, cf. e. g. [2, 12]:

$$R_2^*_{liver} = R_2^*_{iflt} + LIC \cdot R_{Fe} \quad (4)$$

where $R_2^*_{iflt}$ means the theoretic R_2^* value of iron-free liver tissue (iflt), and R_{Fe} is the relaxivity of iron stored in the liver.

GRE signal

Signal decay of a gradient echo image due to transverse relaxation can be described by

$$S = S_0 \cdot \exp(-T_E R_2^*) \quad (5)$$

with S_0 being the theoretical value of the signal intensity for echo time $T_E = 0$, which depends on the measurement parameters repetition time T_R, flip angle FA and tissue longitudinal relaxation time T_1; T_E: echo time, and R_2^*: tissue transverse relaxation rate.

For a complete description of the MR signal in tissue containing fat and water, its complex behavior has to be considered, cf. refs. [24 – 26, 30]. To minimize the influence of fat fraction, we restricted SIR analysis to echoes where water and main fat component show coherence, so-called 'in-phase' echoes.

Signal intensity ratio

For in-phase T_E, and disregarding signal noise, we can use (2) to derive an equation for the signal intensity ratio (SIR) of two different tissues (e. g., liver and muscle) with signals S_{liver} for the liver and S_{muscle} for muscle tissue:

$$SIR = \frac{S_{liver}}{S_{muscle}} = \frac{S_{0\,liver} \cdot \exp(-T_E R_2^*_{liver})}{S_{0\,muscle} \cdot \exp(-T_E R_2^*_{muscle})} \quad (6)$$

Taking the natural logarithm of both sides yields the following

$$\ln(SIR) = \ln \frac{S_{0\,liver}}{S_{0\,muscle}} - T_E R_2^*_{liver} + T_E R_2^*_{muscle} \quad (7)$$

This can be interpreted as a linear equation with the logarithm of the SIR as a function of liver R_2^* with T_E as the slope and T_E times muscle R_2^* plus the logarithm of the S_0 ratio as the intercept. Since liver R_2^* depends linearly on the LIC (eq. 4), we expect the relation above, eq. (1), if a) muscle R_2^* is less influenced by liver iron overload than liver R_2^* and b) the logarithm of the S_0 ratio for muscle and liver is constant or changes linearly with liver iron overload.

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