Iron overload of hematological origin: Validation of a screening procedure for cardiac overload by MRI in routine clinical practice


a Radiology Department, Hôpital Huriez, INSERM U703, CHRU de Lille, 1, rue Polonovski, 59037 Lille, France
b Pediatric Hematology Department, Centre de référence des Thalassémies [Thalassemia reference center], CHU Timone, boulevard Jean-Moulin, 13005 Marseille, France
c Radiology Department, CHU Timone, boulevard Jean-Moulin, 13005 Marseille, France
d Radiology Department, Hôpital Huriez, CHRU de Lille, 1, rue Polonovski, 59037 Lille, France
e Methodological assistance unit for clinical research, DRRC/AP—HM, Laboratoire de santé publique, Faculté de médecine, 27, boulevard Jean-Moulin, 13385 Marseille, France
f Radiology Department, Hôpital Saint-Louis (AP—HP), Université Paris 7, 1 avenue Claude-Vellefaux, 75475 Paris, France
g North Cardiology Center, 32-36, rue des Moulins-Gémeaux, 93200 Saint-Denis, France
h R4M, CNRS UMR8081, Université Paris-Sud, bâtiment 220, 91405 Orsay, France
i Hematology Department, Hôpital Saint-Vincent, Université Catholique de Lille, University Nord-de-France, boulevard de Belfort, 59000 Lille, France
j Medical Imaging Department, Centre Hospitalier Intercommunal, 40, avenue de Verdun, 94010 Créteil, France
k Inserm, U703, University Lille-Nord-de-France, CHRU, Institut Hippocrate, 152, rue du Dr-Yersin, 59120 LoosLille, France
l Clinical Hematology Department, Hôpital Avicenne (AP—HP), Université Paris 13, 125, rue de Stalingrad, 93009 Bobigny, France

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Abstract
Purpose: Screening for cardiac iron overload is generally done by magnetic resonance imaging (MRI) and demonstrated by a shortening of the myocardial T2* below 20 ms at 1.5 Tesla. This measurement was validated with a specific sequence and the CMRTools® calculation software
Many diseases can cause iron overload. The liver is always the main storage organ. However, in case of post-transfusion overload (such as constitutional anemia or low risk myelodysplasia), the main cause of death induced by excess iron is myocardial overload [1]. For all of these diseases treated in the long-term by transfusions, the implementation of oral or injectable chelating agents is necessary. Hepatic iron concentrations estimated by biopsy or MRI as well as serum ferritin concentrations do not directly reflect hepatic iron overload [2]. It is therefore critical to be able to screen for a possible myocardial iron overload, which requires the chelating agent to be intensified and adjusted before irreversible heart failure sets in, which is often irreversible.

Excess iron in the tissues induces a significant collapse of the signal due to changes in their magnetic susceptibility, causing a shortening of the T2* time constant. The measurement of hepatic iron concentrations by MRI is commonly carried out in routine clinical practice [3,4].

Currently, there is no MRI technique that allows the precise measurement of myocardial iron concentrations. However, it has been established that the minimum nominal value of the myocardial T2* is 20 ms at 1.5 T for about a dozen years [5]. Iron overload induces a shortening of the T2* below this value. The short-term risk of heart failure secondary to iron overload appears for T2* values of less than 10 ms [6]. Several teams have validated the reproducibility of this measurement by using strictly identical sequences [7] and the same specific post-treatment software program (CMRTool®; Cardio-vascular Imaging Solutions, London, UK, http://www.cmrtools.com) [8]. Under these conditions, cardiac MRI is an important detection technique for myocardial iron overload in at risk patients. The French National Authority for Health recommends an MRI every 1 or 2 years for patients with thalassemia [9].

The existence of various MRI machines makes it difficult to use an identical sequence at all of the sites. In addition, most of the centers do not have the specific commercial calculation software for myocardial T2*.

All of the MRI machines currently have multi-echo gradient echo sequences. With these sequences, it is therefore theoretically possible to measure the T2* time constant, either using a software program integrated into the machine or by simple calculation.

The objective of this study was therefore to study the reproducibility of myocardial T2* measurements obtained in routine clinical practice for the detection of cardiac iron overload (T2* < 20 ms) in standard machines at 1.5 T, without an additional commercial software program. The first phase consisted of the phantom study of the correlation of the measurements obtained using imaging machines of different brands at 1.5 T, with the standard software programs and sequences available. The second phase consisted of comparing the T2* values obtained using two methods in patients at risk for myocardial iron overload. The first method used the reference sequence described by Anderson et al. [5], specially implanted in the machines, followed by the calculation with the CMRTool® software program. The second method used the acquisition sequences available in standard practice in the machines followed by calculation with a spreadsheet. Hepatic iron concentrations were measured during the same MRI examination.

Material and methods

Phantom study

To compare the T2* measurements between several imaging machines, it was necessary to carry out a phantom study including several tubes with different T2* values, close to the heart values observed in clinical practice. Eleven tubes were thus prepared containing an agarose gel with a variable concentration of ferucarbotran (Cliaivist, Bayer-Shering Pharma, Loos, France). The different concentration values were calculated using the transverse relaxivity of the agarose estimated to be 30 s⁻¹ and of the ferucarbotran estimated to be 82 mmol/s, to obtain the calculated T2* values between 4 and 33 ms, respectively.

The T2* value of each tube was then measured at three clinical sites using the sequences used in routine clinical practice, on three 1.5 T machines of different brands (MRI1:...
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Intera Philips, Best, Netherlands/MRI2: Discovery MR450 General Electric, Milwaukee, WI, USA/MRI3: Magnetom Symphony Tim Siemens, Erlangen, Germany). The sequences are described in Table 1. On the MRI1, three sequences, including two echoes were used. The first versions of this machine only allowed two echoes to be used. For the three sequences, the value of the first echo time was unchanged (2.5 ms) and the signal of each echo was standardized on the signal of the first echo by calculating the ratio of each value (considered echo signal/first echo signal). Thanks to this standardization, the value of the signal of the first echo (2.5 ms) was 1 for each acquisition and the value of the signal of the second echo was the percentage of the signal compared to the first echo. This standardization thus made it possible to obtain the equivalent of one sequence with four echoes, by using three sequences, which only had two echoes.

In the three machines, the signal of each tube was noted individually for each of the acquired echoes.

At the site of the MRI1, the T2* values were calculated using the formula \( y = K e^{-\frac{T2*}{T1}}\),

where \( y \) is the intensity of the signal, \( K \) is a constant and \( T \) is the echo time. The T2* in ms is then obtained by adjusting an exponentially decreasing curve using the spreadsheet function of the free software program Openoffice.org (http://www.openoffice.org) using the LOGREG function. This function calculates the adjustment of the data entered in the form of an exponential regression curve \( y = b + m^x \)

At the MRI2 and MRI3 sites, the T2* was calculated directly by the software program included in the machine.

Study in the patients

The data of 75 patients who had a cardiac MRI to screen for post-transfusion iron overload between May 2009 and May 2011 were included in this study retrospectively.

The median age of the patients was 36 years (11–88 years). All of the patients were transfused regularly and received more than 20 units of red blood cell concentrates.

These patients had major thalassemia (n=36), sickle-cell anemia (n=10), intermediate thalassemia (n=6), myelodysplastic syndrome (n=9), acute leukemia (n=7), aplastic anemia (n=3), pyruvate kinase deficiency (n=2), Blackfan–Diamond disease (n=1) and congenital dyserythropoietic anemia (n=1).

The examinations were carried out in two different centers by two different medical teams, but on the same model of machine, Intera Philips (Best, Netherlands). For each patient, the cardiac T2* was measured according to two procedures.

For the first procedure, three gradient-echo sequences at two echoes identical to the one described for MRI1 for the phantom study were used. The angle was 20° for the three sequences with the following repetition time (TR) and echo time (TE): TR = 23 ms, TE = 2.5–20 ms/TR = 13 ms, TE = 2.5–10 ms/TR = 8 ms, TE = 2.5–5 ms. The three sequences allowed the equivalent of a sequence with four echo times (2.5, 5, 10 and 20 ms) to be obtained. The second procedure, the reference technique, used the sequence described by Anderson et al. [5]. The parameters of this sequence, installed at each site by a Philips study engineer, were as follows: nine echoes of 5.6 to 17 ms, TR = 19.3 ms, angle 35°. The signal was acquired in the minor axis of the heart with synchronization by a pulse sensor. The examinations were performed with patients who were holding their breath.

The technique with the three bi-echo sequences in combination with the calculation of the T2* using a spreadsheet, which is called the tested technique. The reference sequence combined with the calculation by the CMRTools® software program is called the reference technique.

The T2* calculations were carried out on a blind basis at the end of the study in a different way for each sequence.

For the first procedure, the signal of the inter-ventricular septum was picked up in the same oval region of interest for each echo. The signal of each echo was standardized based on that of the first echo and afterwards the T2* was then calculated with the openoffice.org software program. For the second procedure, the T2* was calculated with the CMRTools® software program.

Hepatic iron concentrations measured during the same MRI examination and serum ferritin values were noted for each patient.

Statistic study

All of the data were analyzed with the PASW Statistics software program (Version 17.0, SPSS Inc, Chicago, IL, USA).

For the phantom study, the coefficient of variation between each measurement and the mean value was calculated using the standard deviation of the half-square of the mean value of the differences. The coefficient of variation is expressed as a percentage. The intraclass correlation coefficient (ICC) was also calculated. ICC values, greater than 0.90, were considered to be excellent.

For the study in patients, the specificity, sensitivity and positive and negative predictive values for the detection of a shortening of the myocardial T2* were calculated compared to the results obtained with the reference technique. The agreement between the two methods was obtained by Bland–Altman analysis. The agreement between the myocardial T2* values depending on the two techniques and

| Table 1 | Parameters of the sequences used for the study of the phantom (1.5 T). |
|-------------------------|-------------------------|-------------------------|-------------------------|
|                         | MR13 sequences 2 echoes | MR21 sequence 8 echoes  | MR31 sequence 12 echoes |
| TE (ms)                 | 2.5–5 / 2.5–10 / 2.5–20 | 2.1–21.3                | 12 echoes: 2.1–25.3     |
| TR (ms)                 | 8 / 13 / 23             | 23.7                    | 29.7                    |
| Angle (degree)          | 20                     | 20                      | 20                      |

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hepatic iron concentrations and serum ferritin levels was studied using the Pearson correlation coefficient.

Results

Phantom study

The T2* values measured in the 11 tubes are between a minimum of 3.28 ms and a maximum of 33.15 ms (Fig. 1). The results obtained with the different ones were very close. The coefficients of variation between the three machines were 5.3%, 6.4% and 6.7%, respectively. The mean value of the differences between each machine was 0.6 ms. The ICC value was excellent (ICC > 0.99).

Patient study

The mean myocardial T2* was measured at 28 ms (5 to 57 ms) for the tested technique and 32 ms (10 to 59 ms) for the reference technique (Figs. 2 and 3). The T2* values calculated for each method are presented in Fig. 4. For the detection of the shortening of less than 20 ms of the myocardial T2*, the tested technique had a sensitivity of 100% (0.95 CI = 71.7—100), a specificity of 94.6% (0.95 CI = 85.6—98.7), a positive predictive value of 81.2% (0.95 CI = 53.7—95) and a negative predictive value of 100% (0.95 CI = 92.4—100) compared to the reference technique.

Sixteen patients had a T2* of less than 20 ms with the tested technique vs thirteen with the reference technique. For the three discordant patients, the T2* values were measured with the multi-echoes sequence and the sequence of the Brompton Hospital at 18 vs 20 ms, 18 vs 21.2 ms and 19.84 vs 30.2 ms, respectively.

For all of the patients, the mean of the T2* differences and the standard deviation of the T2* differences between the two techniques were −4.07 ms and 10.45 ms, respectively, as per the Bland—Altman method. For T2* values of less than 20 ms, these values were 0.28 ms and 3.14 ms, respectively.

The mean serum ferritin levels were 866 ng/mL (35—5574) and the mean hepatic iron concentrations were 215 μmol/g (10—830). There was no correlation between the myocardial T2* value and hepatic iron concentrations (R = 0.13, P = 0.26) or serum ferritin levels (R = 0.11, P = 0.36).

Discussion

The vital prognosis of the patients with post-transfusion iron overload is mainly related to cardiac overload [1]. Therefore, MRI monitoring of myocardial overload via the T2* has become an essential screening and monitoring tool for chelating treatments. It has been established that these treatments significantly reduce the morbidity and mortality of these patients by improving their cardiac prognosis [10].

In these patients, the regular dosage of serum ferritin levels is still the most commonly used piece of information for the evaluation of the degree of iron overload [11]. A significant correlation between the assay of serum ferritin concentrations and intrahepatic iron concentrations has been reported in several studies [11,12]. On the other hand,
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Figure 2. Patient with significant hepatic iron overload at 15 times the normal value and an absence of myocardial overload ($T2^* = 44$ ms). The images to the left correspond to an acquisition with an echo time of 2.5 ms, and the images to the right of 5/10/20 ms, from the top to the bottom. While the signal of the liver and spleen decrease considerably starting at 5 ms of TE, the signal of the heart only shows a very small decrease.
Figure 3. Patient with a significant hepatic iron overload at seven times the norm and a clear myocardial overload ($T2^* = 10.2\text{ ms}$). The images to the left correspond to an acquisition with an echo time of 2.5 ms, and the images to the right of 5/10/20 ms, from the top to the bottom. While the signal of the liver and spleen decrease considerably starting at 5 ms of TE, the signal of the heart only shows a very small decrease. The signal of the liver and spleen decrease considerably starting at 5 ms of TE, and the signal of the inter-ventricular septum of the heart disappears almost completely at 20 ms, which demonstrates a clear shortening of the myocardial $T2^*$ characteristic of an iron overload.
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Figure 4. Graph comparing the myocardial T2* values of 75 patients obtained using the reference method and the technique available in routine clinical practice.

as it has been described in the literature [13,14], our study did not demonstrate a significant correlation between the myocardial T2* and serum ferritin levels as with intrahepatic iron concentrations. Serum ferritin levels and intrahepatic iron concentrations are therefore not very predictive of a cardiac iron overload [2].

Cardiac MRI with T2* measurement has been shown to be a sensitive, non-invasive and reproducible method in the evaluation and monitoring of myocardial iron overload, then for the prevention of cardiac complications related to this overload [1]. The carrying out of myocardial biopsies in routine clinical practice is not very realistic. There is only one study of 12 patients at 1.5T the myocardial T2* value to cardiac iron concentrations [15]. Therefore, only a T2* value can be given, contrary to the case of the liver, where a veritable iron concentration can be measured thanks to calibration curves established using liver biopsies [3,4].

Since the first publication of the importance of the measurement of T2* in thalassemia [5], the technique has been validated in routine clinical practice. It has been shown that the method is indeed reproducible, by using the same sequence and the same calculation software program [8]. The objective of our study was therefore to validate those similar results could be obtained with different machines, different sequences and different calculation software programs.

Our phantom study allowed us to demonstrate an excellent correlation between the measurements of T2* on different machines and according to different sequences and calculation methods used in routine clinical practice. The calculation of T2* values is therefore indeed reproducible, in vitro, on standard clinical machines of the same magnetic field intensity by using the sequences and the calculation software programs available. A recent study also showed that the T2* calculation results were not dependent on the software program used [16].

The sequences used for the T2* calculation in patients of course had to be modified in relation to the sequences used in the phantom. A different geometry and the need to use cardiac synchronization must be taken into account. A clinical study was therefore absolutely necessary to confirm the experimental results.

The purpose of our clinical study was therefore to validate the possibility of using MRI sequences and the software programs that are available in routine clinical practice to screen for the shortening of the myocardial T2*, which proves that there is iron overload. In our series, we obtained excellent sensitivity values, specificity values, positive predictive value and negative predictive value for the detection of T2* of less than 20 ms compared to the reference technique. Three patients out of 75, however, had a result that was different between the two techniques. Two patients had a T2* measured at 18 ms with the tested technique for 20 and 21.2 ms, respectively, with the reference technique. The third patient had a T2* measured at 19.8 ms for a measurement of 30.2 ms with the reference method. These errors therefore only concern results close to 20 ms.

The Bland–Altman analysis shows that the T2* measurement error, compared to the reference technique, is clearly higher for values above 20 ms (standard deviation of 10.45 ms) than for values lower than 20 ms (standard deviation of 3.14 ms). This is explained by the value of 20 ms used for the longest echo. This value corresponds to a 63% reduction in the free precession signal for a T2* of 20 ms, which is sufficiently significant to minimize the effect of the noise. For longer T2* values, the drop in the signal is lower and the relative effect of the noise increases. The measurement therefore becomes very imprecise. It is not possible to reliably estimate T2* values that are clearly greater than the longest TE [17]. Regardless of whether it is for the reference technique or the tested technique, the sequences were not optimized to measure T2* values greater than 20 ms, as the objective was to detect an abnormal shortening below 20 ms, which remains the clinical objective.

The study in the phantom and in the patients shows that the measurement of the shortening of the cardiac T2* is a measurement that can be reproduced using different multiple gradient echo sequences and different T2* calculation software programs. However, the value of the magnetic field must be identical, as the T2* values depend on the intensity of the magnetic field. To appreciate a shortening of less than 20 ms of the T2*, the longest echo must be at least 20 ms.

Currently, multiple gradient echo acquisitions are routinely available from all manufacturers. As all of the echoes are acquired during the same sequence, these multiple echo acquisitions are preferable to multiple sequences that only have two echoes.

The main limitation of the tested method is the imprecision of the measurement, which, in our study, led to a diagnostic error in three out of 75 patients. This imprecision, which is clearly greater than in the phantom study, is probably due to the multiple artefacts encountered in clinical practice, particularly, artefacts due to respiratory movements. However, all the T2* values of less than 18 ms with the tested technique corresponded to a shortening of less than 20 ms with the reference technique. Therefore, the errors only concerned small reductions in T2* that do not have a direct clinical impact in the short-term, as the risk of heart failure generally occurs below the threshold of 10 ms [6]. In case of routine use in clinical practice of the
tested technique, small shortening of the T2* therefore do not make it possible to diagnose iron overload with certainty. It can therefore be useful to carry out a remote control examination.

Conclusion

In conclusion, the screening for a myocardial T2* shortening can be carried out in clinical practice with the standard sequences available on MRI machines. The T2* can be calculated with the software programs of the manufacturer of the machine or on a simple spreadsheet. As serum ferritin levels, hepatic iron concentrations and myocardial iron concentrations reflect different storage compartments, it is important to monitor these three parameters [11]. During an MRI exam to monitor iron overload of hematological origin, we are therefore conducting at the same time the calculation of hepatic iron concentrations and the calculation of the myocardial T2*, as these 2 measurements were recommended by the French National Authority for Health in patients with thalassemia [9]. We also associate the measurement of the ventricular ejection fraction. In one single examination, the MRI thus makes it possible to measure several absolutely necessary parameters for the monitoring of chronic post-transfusion iron overload.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References