Creation of an MRI phantom based on super-paramagnetic nanoparticles of iron oxide (SPIO) for standardization of the conversion of T2* values in the concentration of iron in the liver

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Abstract. In this study the standardization method for T2* maps acquisition on various MR scanners (3T and 1.5T) is proposed. The reproducibility of the obtained T2* values is realized through the MR-compatible phantom containing paramagnetic complex iron oxide nanoparticles. The repeatability of measurements results has shown that the created phantom retains all the required characteristics (homogeneity, stability of concentrations and manifested paramagnetic properties) over a long period of time. The application of standardized T2* values allows to use previously received T2*, [ms] to iron concentrations in the dry substance of the liver (LIC), [mg/ml] conversion formulas for accurate, fast and non-invasive MRI diagnostics of liver iron overload.

1. Introduction
Iron overload is a condition in which extra iron builds up in organs and tissues causing toxic damage and consequently, organ dysfunction. Iron overload may be due to hereditary hemochromatosis or result from anemia requiring regular transfusions of donor red blood cells [1]. The classic method for determining the concentration of iron in the liver is the atomic absorption study of a biopsy sample [2]. However, biopsy, as an invasive procedure, has a number of negative factors, such as risks of infection and complications, as well as pain, which is especially relevant when working with children.

An alternative to biopsy can be the technique of noninvasive determination of iron concentration in the liver by MRI methods [3]. This method is based on the construction of T2* maps, because T2* values depend on the magnetic susceptibility of the tissue, which changes with iron overload. Then, these values are compared with biopsy data and experimental dependence between the values of iron concentrations in the dry substance of the liver (LIC), mg/ml and T2* values, ms is constructed. We
obtained a similar formula earlier at the Dmitri Rogachev Pediatric Hematology Center [4]. This allowed us to introduce into clinical practice a noninvasive assessment of liver iron overload. However, the obtained formulas of T2* to LIC conversion can be reliably used only on those MRIs on which it was obtained. For the reason that T2* values are sensitive to different sequence parameters, which may not coincide in different software releases or in different MRI vendors.

For this purpose, we have created an MR-compatible phantom containing various concentrations of paramagnetic nanoparticles of complex iron oxide (SPIO).

2. Materials and methods

2.1. Phantom creation

The phantom consisted of 28 test tubes (50 ml), with various concentrations of a colloidal solution (Fig. 1) containing paramagnetic nanoparticles of complex iron oxide (Fe3O4). These nanoparticles were obtained as a result of the Elmore chemical reaction [5]:

$$\text{FeSO}_4 \cdot 7\text{H}_2\text{O} + 2\text{FeCl}_3 \cdot 6\text{H}_2\text{O} + 8\text{NH}_3\cdot\text{H}_2\text{O} = \text{Fe}_3\text{O}_4 + 6\text{NH}_4\text{Cl} + (\text{NH}_4)_2\text{SO}_4 + 23\text{H}_2\text{O},$$

where 6-water iron (III) chloride and 7-water iron (II) sulfate were used as carriers of ions of two and three valence iron, ammonium hydrate was used to start the process of precipitation and formation of nanoparticles, and a surface active agent was used to control the growth and stabilization of particles. Substance - citric acid. The resulting reaction solution was centrifuged at 1500 rpm for 5 minutes to precipitate large particles. The upper fraction of the solution was filtered through standard filter paper with an average pore diameter of 3-5 μm. The resulting solution was the desired colloidal suspension of iron oxide nanoparticles.

The resulting solutions with high iron concentration were diluted in order to achieve T2* values similar to those seen in a healthy liver and in the four grades of iron overload (Grade 1: >7 ms; Grade 2: 2.5 – 7 ms; Grade 3: 1 – 2.5 ms; Grade4: 0 – 1 ms) [4].

![Figure 1. View of the MR-compatible phantom consist of 28 tubes with different T2* values from diapason (0.2 – 15, ms) (Image)
2.2. *Atomic adsorption spectroscopy*

After the creation of solutions of nanoparticles for measuring the concentration of iron in NUST MISIS, atomic adsorption spectroscopy of the samples was carried out on an Agilent 4200 apparatus (Agilent Technologies, USA). For this purpose, the method of atomic adsorption spectroscopy with inductively coupled plasma was used [6].

2.3. *MRI Scanning and Post-processing*

The resulting phantom was scanned (Figure 2) using 4 MR scanners from different vendors with different field strengths (Table 1). Research protocols included obtaining T2* maps using multi-phase fast gradient echo (mGRE) techniques. On all scanners, the main parameters of the sequences used corresponded to those used in in vivo studies: FA, flip angle- 45 °, TE, echo time - 1.2 ms, the number of echoes - 20 with a step ΔTE = 1.5 ms, TR, repetition time - 350 ms; resolution - 1.5 × 1.5 mm. For MR scanners with field strengths of 1.5 and 3 T, the slice thickness was 10 and 7 mm, respectively. Relaxometric phantom maps were calculated automatically using the T2* mapping software integrated in the Philips tomograph and the commercial software package ReportCARD Functool (GE Healthcare). For each ROI, an average T2* value (ms) was automatically obtained by automatic approximation of the attenuation curve using the maximum likelihood estimation.

| The name of the scanner / Magnetic field strength | Medicine center |
|-------------------------------------------------|-----------------|
| Philips Achieva (Best, The Netherlands) / 3T     | Dmitry Rogachev National Medical Research Center of Pediatric Hematology |
| Philips Achieva dStream(Best, The Netherlands) / 3T | Research Institute of Emergency Pediatric Surgery and Traumatology |
| Signa GE (Chicago, Illinois, USA) / 1.5T         | Dmitry Rogachev National Medical Research Center of Pediatric Hematology |
| Philips Ingenia (Best, The Netherlands) / 1.5T   | N.N. Priorov Scientific Research Institute of Traumatology and Orthopedics |

**Table 1. MRI scanners used in the research**
2.4. Statistical Analysis

We calculated mean T2* values and standard deviations for each tube. The statistical analysis was conducted using GraphPad Prism 8.0.1 software. The repeatability and reproducibility of T2* measurements were assessed. Repeatability was defined as the stability of data obtained during a number of scanning sessions on the same (reference) MRI scanner, over a long period of time. In order to assess repeatability, we also calculated mean coefficients of variation with the following formula:

\[ \frac{\sigma}{\bar{x}} \times 100\% \]

Reproducibility was defined as the degree of agreement between T2* values obtained on the control MRI scanners and those obtained on the reference scanners. To test the reproducibility of the results across different MRI scanners, we estimated the correlation between measurements obtained on the reference MRI scanners and those tested for reproducibility.

Additionally, Bland-Altman plots were created to visually assess the repeatability and reproducibility of the results.

3. Results and discussion

The iron concentrations in the phantoms were measured by atomic adsorption spectroscopy. However, the structure of these nanoparticles [7] does not coincide with the structure of iron in the liver, bound to the ferritin protein and contained in the pigment hemosiderin, which have not yet been fully studied [8,9]. Therefore, the range of iron concentrations in test tubes obtained by atomic adsorption spectroscopy (0.1 - 1.5 mg / ml) did not coincide with those obtained by applying the formula for converting T2* to LIC (1-30 mg / ml). Thus, we used the paramagnetic properties of the material to achieve the required range precisely for T2*, ms. In addition, the study of this material can also be interesting from the point of view of contrast materials and other studies where it is used [10–12].

An example of a T2* card, as well as signal attenuation curves for two tubes with different iron concentrations are shown in the figure 3.

As a result of statistical analysis, the T2* values showed good repeatability on both our scanners, which is confirmed by the graphs of Bland Altman (Figure 4). The phantom was stable throughout, indicating that it can be used for long time.
On the other scanners tested we found statistical correspondence of values, so the \( T2^* \) values obtained on them can be recalculated in LIC with the help of our formulas, which is confirmed by the graphs of Bland Altman and correlations (Figure 5). In case of inconsistency of values next steps must be carried out:

1) calibration of scanning parameters and \( T2^* \) mapping;
2) introducing additional calibration coefficients into the recalculation formula.

Thus, to be able to use formulas, it is enough to check the correspondence of \( T2^* \) values using the phantom we created. If there are discrepancies, it is necessary to refine the scanning protocol up.

**Figure 3.** An example of a \( T2^* \) map (on the left) obtained with commercial processing software on the Philips 3T scanner. For two tubes, the examples of \( T2^* \) signal decay and the dependence of signal
intensity (relative units) on TE, ms (TE₁ = 1.2 ms, ΔTE = 1.5 ms) are given. The upper plot was constructed for the tube with the real iron concentration of 0.21 mg/mL and the T2* time = 1.2 ± 0.2 ms, the lower plot was constructed for the tube with the real iron concentration of 0.77 mg/mL and the T2* time = 9 ± 0.2 ms.

Figure 4. Testing the repeatability of the results: Bland-Altman plots for the repeatability of the results obtained on the reference scanners with field strengths of 1.5T (A) and 3T (B), showing the mean deviations of the T2* values (ms) from the average during 6 observations.

Figure 5. Testing the reproducibility of the results: A, B - correlations between the T2* values obtained on reference and other devices with field strengths of 3T and 1.5T, respectively. C, D - Bland-Altman plots for similar T2* values.
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