Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Comparative Study on the Measurement of Liver LICdw between Ferriscan and T2* Based LICdw Obtained by Different Software's

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Competing interests: The authors declare no conflict of Interest.

Abstract. ***Objective*: To explore the relationship between the liver iron concentration (LIC_F) from FerriScan and T2* based LIC obtained by Circle Cardiovascular Imaging CVI42 (CVI42), CMRtools / Thalassemia Tools (CMRtools), and Excel spreadsheet (Excel).**
***Methods*: Liver T2* values in 78 thalassemia patients were measured using CVI42, CMRtools, and Excel. Then the Garbowski formula was used to obtain LIC from T2*. Finally, the relationship of the LIC measured by the above three software and the LIC_F were compared.**
***Results*: There was no statistical difference between the T2* values measured by CVI42, CMRtools, and Excel ($P>0.05$), but there was a high degree of consistency between them ($P<0.001$), and there was a high linear positive correlation between them ($P<0.001$). There was no statistical difference between the LIC clinical grading results of CVI42, CMRtools, and Excel and LIC_F grading results ($P>0.05$), and they were highly consistent ($P<0.001$).**
***Conclusion*: The liver T2* values measured by CVI42, CMRtools, and Excel are equivalent. The LIC measured by CVI42, CMRtools, and Excel is equivalent to the LIC_F.**

Keywords: Thalassemia; Liver iron concentration; Comparative study; Iron overload.

Citation: Xu F., Yi J., Liang B., Tang C., Feng Q., Peng P. Comparative study on the measurement of liver LICdw between Ferriscan and T2* based LICdw obtained by different software's. Mediterr J Hematol Infect Dis 2022,14(1): e2022072, DOI: http://dx.doi.org/10.4084/MJHID.2022.072

Published: November 1, 2022 **Received: May 5, 2022** **Accepted: October 8, 2022**

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Introduction. The liver is the central conductor of systemic iron balance. Liver Iron Concentration (LIC) can reflect the total iron load of the body and is an important reference index for clinical monitoring and treatment of iron overload.[1,3] Magnetic resonance (MR) techniques, based on gradient echo T2* sequences, have been identified as a non-invasive gold standard for quantifying tissue iron levels.[4,5] After obtaining MR scanning images of the liver of patients with iron overload, this technique requires the measurement of the corresponding relaxation parameters. Currently, many methods and software have been developed and applied to measure the values of T2* and R2* (1000/T2*) to obtain an estimate of LIC. Some of these methods or

software are based on the researcher's correction formula,[6] some are built-in software of the MR operating system, and some are third-party commercial software. Software used to calculate T2*/R2* values of organs on the market includes FuncTool, Matlab, Quanta Hematology, CMRtools, CVI42, Excel, etc. Some of these pieces of software, certified by the U.S. Food and Drug Administration (FDA), have high accuracy, but their operation and maintenance are expensive. Furthermore, there are some uncertified measurement methods, such as Excel-based methods.

Due to different economic and medical levels in different regions, many developing countries and regions still use uncertified Excel for cardiac and liver T2* / R2* measurements in patients with iron overload. Some studies have proved that the T2*/R2* values of organs, measured by Excel, are correlated and consistent with the results of FDA-certified software such as CMRtools and CVI42.[7,9] However, most studies only conducted comparative investigations between T2*/R2* values measured by software. However, there was a lack of a comparison taking the corresponding iron concentration as a standard for clinical grading. Therefore, the author aims to evaluate the relationship between the three measurement results by comparing the liver T2* values of thalassemia patients measured by CVI42, CMRtools, and Excel. Furthermore, the author used the LIC_F provided by the FDA-certified FerriScan as a reference to evaluate the three software's accuracy for clinical grading of liver iron deposition.

Material and Methods.

Research materials. The clinical data and MRI of 150 thalassemia patients in the First Affiliated Hospital of Guangxi Medical University were collected from January 2011 to December 2015. The inclusion criteria were: (1) Patients were genetically diagnosed with thalassemia and had a regular history of blood transfusion. (2) 9 years old ≤ age ≤ 50 years old. (3) Patients had both the T2* sequence MRI with intact liver 12 echoes and the Ferriscan LIC report (which is R2 based) in the corresponding period (the T2* images were acquired in the same MRI session after the Ferriscan procedure). The exclusion criteria were: (1) MRI artifacts were too large to meet the measurement requirements. (2) Patients had other chronic liver diseases or tumor diseases. Finally, 78 patients were included. There were 51 males and 27 females, ranging in age from 9 to 44 years old, with an average of (15.54±7.693) years old.

This study was performed in line with the principles of the Declaration of Helsinki. Moreover, the study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Jan 18.2022/No: KY-E-029).

MR scanning method. MRI was performed on a 1.5T scanner (MAGNETOM Avanto Fit, Siemens Healthcare, Erlangen, Germany).

The FerriScan acquisition consisted of a free-breathing 2D multislice spin-echo pulse sequence. Relevant pulse sequence parameters include: flip angle =90°, echo time (TE)=6, 9, 12, 15, 18 ms, repetition time (TR)=1000 ms, FOV read=400 mm×400 mm, matrix =256 mm×256 mm, and 11 slices of 5 mm thickness.

T2* data were acquired using a breath hold multiecho GRE scanning sequence at the same liver level as FerriScan acquisition at free breathing. Relevant pulse sequence parameters include: flip angle=20°, echo time (TE)=1.29, 2.35, 3.43, 4.6, 5.68, 6.85, 7.93, 9.1, 10.18, 11.35, 12.43, 13.6 ms, repetition time (TR)=200.00 ms, FOV read=400 mm×400 mm, matrix=256 mm×256 mm, Slice thickness=10 mm. Scan time was 15s.

Data processing. The T2 image data was sent to FerriScan for processing. As mentioned before, the required T2 image scan time for FerriScan is in the same MRI session as the corresponding T2* image scan time, and the difference between the FerriScan LIC reporting time and the corresponding T2* measurement time does not exceed 48 hours.

The T2* image data were all post-processed by the three software to measure the T2* value, namely the CVI42 (Circle Cardiovascular Imaging Inc., Calgary, Canada), the CMRtools (CMRtools/Thalassemia Tools, Cardiovascular Imaging Solutions, London, UK) and the Excel (Microsoft Corp., Redmond, WA). Measurement process (**Figure 1**): for CMRtools and CVI42, the image was imported into the software. Avoiding the intrahepatic blood vessels and bile ducts seen by naked eyes at the same level of the liver, the roughly same ROI was drawn according to the area measured by FerriScan. The drawn ROI and matching T2* values appeared in the post-processing software, and the cutoff method was used to discard the interference signal value deviated from the fitted curve and record the T2* value at the determination coefficient value (R^2=0.98). For Excel, the SI corresponding to the 12 TE time was derived from the original MR scan device. The SI and TE values were entered manually into Excel. The T2* values were calculated using the embedded formula SI=S0e-TE/T2*+C (S0 represents the signal intensity when TE=0 and C represent the background noise). As MRI filtering noise had little influence on the T2* value and was often ignored, constant C=0 was selected.[7,8] Furthermore, the cutoff method is used to discard the signal value interference deviating from the fitted curve and record the T2* value at the determination coefficient value (R^2=0.98). The Garbowski formula[10] was used to obtain LIC from the T2* values obtained by the different pieces of software. According to the LIC, patients were divided into a normal group (<1.8 mg/g dry weight), mild

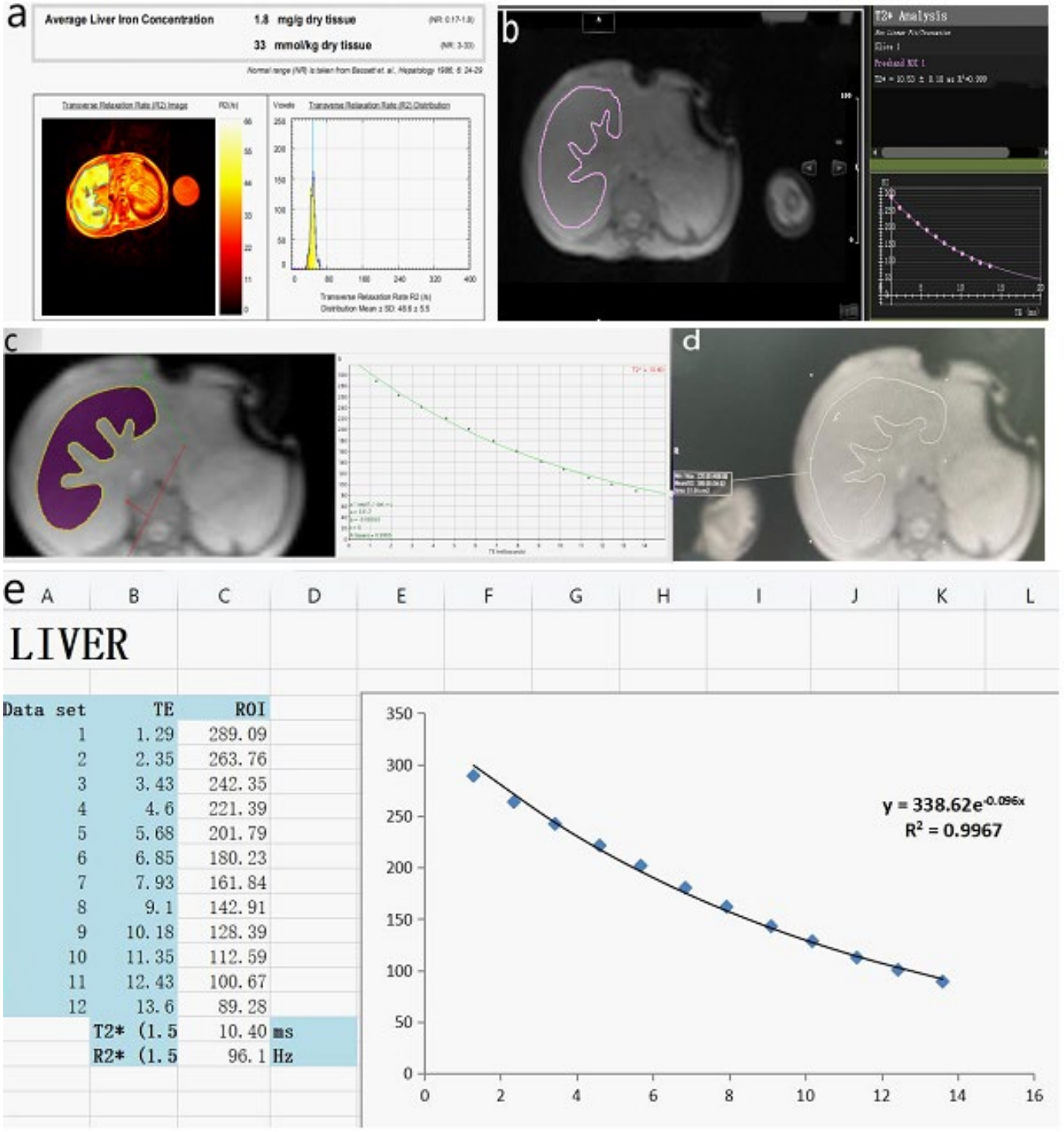

Figure 1. Male, 9 years old, patient with iron overload beta(β)-thalassemia in the mild liver group. LIC_F is 1.8mg/g dry weight (**a**); CVI42 shows that the mean value of T2* is 10.53ms, R^2 (a measure of the goodness of fit of a model) is 0.999, and LIC is 2.987 mg/g dry weight (**b**); The liver T2* value calculated by CMRtools is 10.80ms, R^2 is 0.9965, and LIC is 2.861mg/g dry weight (**c**); The mean value of TE=1.29 on 1.5T MR scanner is 289.09 (**d**); The T2* value of liver calculated by Excel is 10.40ms, R^2 is 0.9967, and LIC is 2.972mg/g dry weight (**e**). All of Valid readings: 12 | Manual truncation to 12 readings.

group (1.8~7.0 mg/g dry weight), moderate group (7.0~14.0 mg/g dry weight), and severe group (>14.0 mg/g dry weight).

Statistical methods. Statistical analysis was performed using SPSS 26.0 statistical software package.

The LIC and T2* values measured by the different methods did not conform to normal distribution. Friedman's M test was used to explore the differences. If $P>0.05$, there is no statistically significant difference. Intraclass correlation coefficient (ICC) was used to evaluate the consistency level. If ICC>0.75, and $P<0.05$, it was considered to have a high degree of consistency. Spearman rank correlation analysis was used to explore the degree of correlation. A high degree of correlation was indicated if the correlation coefficient was $|r_s|>0.75$

and $P<0.05$.

To further evaluate the accuracy of the CVI42, CMRtools, and Excel for the clinical grading of liver iron deposition, Fisher's exact probability test was used to analyze the difference between the LIC_F clinical grading results and the three post-processing software grading results. If $P>0.05$, there was no statistically significant difference. Agreement analysis of categorical variables was performed using the Kappa test. If Kappa>0.75 and $P<0.05$, it was considered to have a high degree of consistency.

Results. The results of liver T2* values and LIC of 78 thalassemia patients measured by the different methods are reported in **Tables 1** and **2**.

Among the number of cases, two patients were classified as having moderate liver iron overload by FerriScan (LIC_F=13.90, 13.70 mg/g dry weight) but were classified as severe by CMRtools (LIC=16.48, 14.43 mg/g dry weight), CVI42 (LIC=16.93, 14.49 mg/g dry weight) and Excel (LIC=16.84, 14.43 mg/g dry weight). Four patients were classified as a mild liver iron overload by FerriScan (LIC_F=6.80, 6.40, 5.60, 5.20 mg/g dry weight) but were classified as moderate by CMRtools (LIC=10.21, 9.07, 8.92, 9.61 mg/g dry weight), CVI42 (LIC=10.34, 8.36, 8.84, 8.94 mg/g dry weight) and Excel (LIC=10.48, 8.50, 8.79, 8.67 mg/g dry weight). One patient was classified as having mild liver iron overload by FerriScan (LIC_F=4.90 mg/g dry weight), CVI42 (LIC=6.78 mg/g dry weight, and Excel (LIC=6.78 mg/g dry weight) but was classified as moderate by CMRtools (LIC=7.11 mg/g dry weight).

Through the scatter plot (**Figure 2**), it is initially understood that there is a close correlation between either the T2* values measured by the three software measurements and between the LIC and the LIC_F.

By statistical test, there was no statistical difference between the T2* values measured by CVI42, CMRtools, and Excel (M=4.507, P=0.105), and they were highly consistent {ICC=0.998 (95%CI=0.997 ~ 0.999), $P<0.001$}. Furthermore, the three pairs of liver T2* values measured by CVI42 and CMRtools, CVI42 and Excel, and CMRtools and Excel were all highly linearly positively correlated (r_s=0.959, 0.911, 0.883, $P<0.001$).

The LIC_F and LIC measured by CVI42, CMRtools, and Excel were highly consistent {ICC=0.853 (95%CI=0.687~0.922), $P<0.001$}. On the other hand, the LIC_F and LIC measured by CVI42, CMRtools, and Excel were highly positively correlated (r_s=0.857, 0.851, 0.862, $P<0.001$).

There was no statistical difference between the LIC clinical grading results of CVI42, CMRtools, and Excel (as shown in **Figure 3**) and LIC_F grading results (χ^2=1.230, P=0.814; χ^2=2.013, P=0.581; χ^2=1.230, P=0.814). And they were highly consistent (Kappa=0.809, 0.778, 0.809, $P<0.001$).

It is suggested that the liver T2* values measured by CVI42, CMRtools, and Excel are equivalent. Likewise, the LIC_F and LIC measured by CVI42, CMRtools, and Excel are equivalent.

Discussion. After years of research, MRI has become the de-facto gold standard for tracking iron levels in the body because it is accurate, reproducible, well tolerated by patients, and can track iron levels in different body organs.[11] In addition, the T2*/R2* relaxation method has become reliable for constructing a linear relationship with LIC.[12] Many medical centers have used the T2*/R2* relaxation method, self-made sequences, and post-processing software with specific LIC calibration formulas to quantitatively examine the viscera's iron concentration.[6] With the T2*/R2* values measured by different post-processing software, each center can perform a more accurate non-invasive assessment of organs for patients with iron overload.[13]

In this study, we first compared the liver T2* values measured by CVI42, CMRtools, and Excel; and found that the results of the three measurements were highly relevant and consistent, which is consistent with the results of Ouederni[7] and Fernandes.[8,9] Then, by

Table 1. Comparison of the results of measuring the liver T2*(ms) value of 78 thalassemia patients with different software.

Group	n	T2* value range	P_{25}	P_{75}	M
CMRtools	78	0.86～28.10	1.1000	2.1975	1.2250
CVI42	78	0.87～27.06	0.9750	2.1625	1.1500
Excel	78	0.86～27.31	1.0225	2.1850	1.2500

Note. n is the number of cases. P_{25} and P_{75} are inter quartile range (IQR). M is the median.

Table 2. Comparison of the results of measuring the LIC (mg/g dry weight) of 78 thalassemia patients by different methods.

Group	n	LIC range	P_{25}	P_{75}	M
CMRtools	78	1.12～37.22	14.5275	24.7700	30.1150
CVI42	78	1.13～36.78	14.8575	26.7800	32.0225
Excel	78	1.12～37.28	14.5275	24.7700	30.1150
FerriScan	78	1.00～43.00	13.8500	37.9500	43.0000

Note. n is the number of cases. P_{25} and P_{75} are inter quartile range (IQR). M is the median.

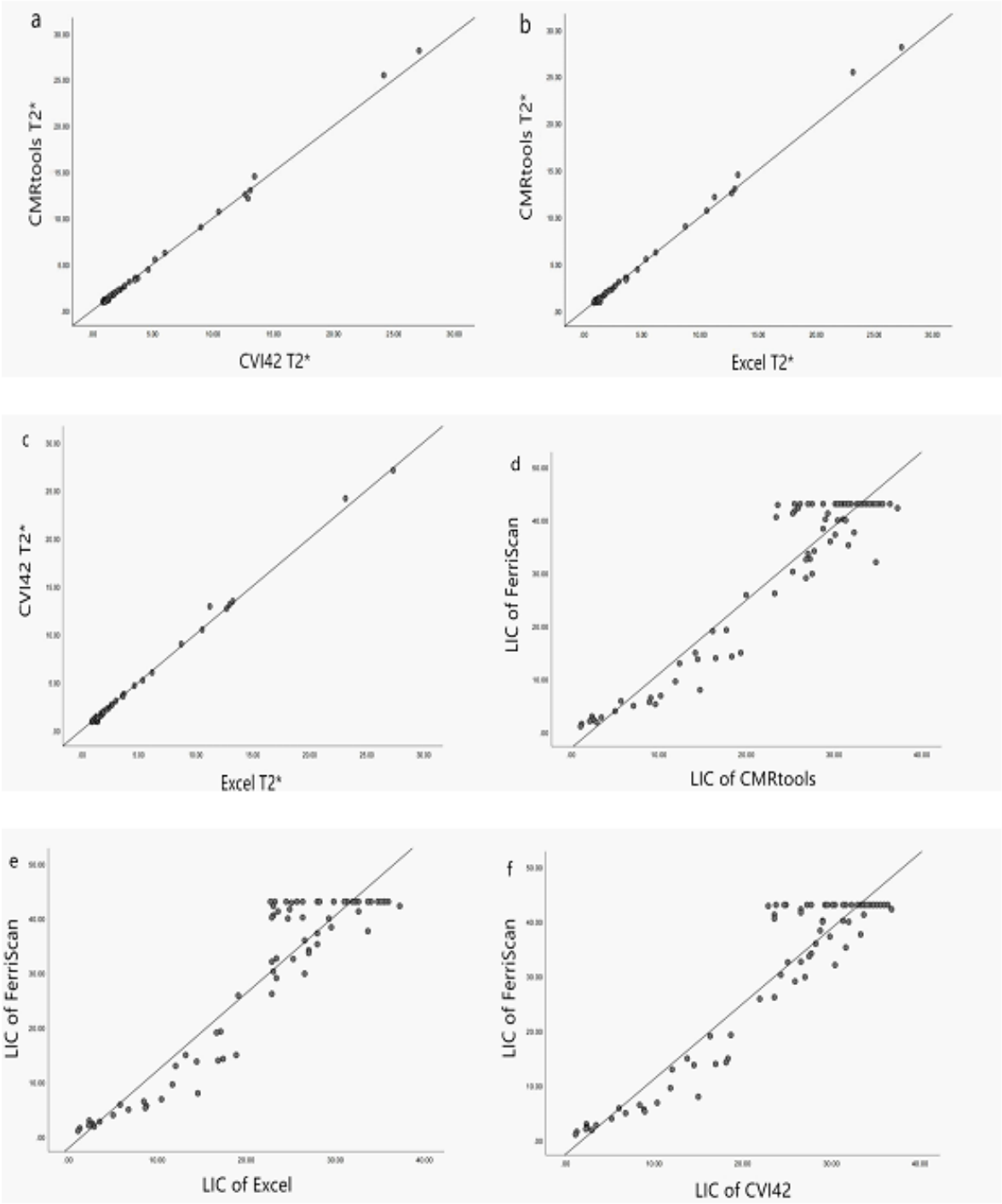

Figure 2. Bivariate scatter plots (**a, b, c**) between the liver T2* values measured by CMRtools, CVI42 and Excel; Bivariate scatter plot (**d, e, f**) between the LIC measured by the three post-processing software and the LIC measured by the FerriScan.

comparing the relationship between the LIC obtained by different methods and the LIC_F provided by FerriScan. We found that the LIC obtained by the different methods were not statistically different from the LIC_F, and they were highly correlated and consistent (including raw measurement data analysis and categorical variables data after clinical classification analysis).

Among the cases, two patients, graded as moderate iron overload in the liver by FerriScan, were graded as severe by CVI42, CMRtools, and Excel. Two patients, graded as mild iron overload in the liver by FerriScan, were graded as moderate by CVI42, CMRtools, and Excel. These cases' non-overlapping clinical grading results of FerriScan and three post-processing software may be caused by the technical difference between the LIC obtained by R2 and R2* technology.[14] Studies by Jhaveri,[14] Chan,[15] Sussman,[16] and others showed that, under the premise of using the LIC_F provided by FerriScan as the reference standard, there is a certain degree of difference in the specificity and sensitivity of

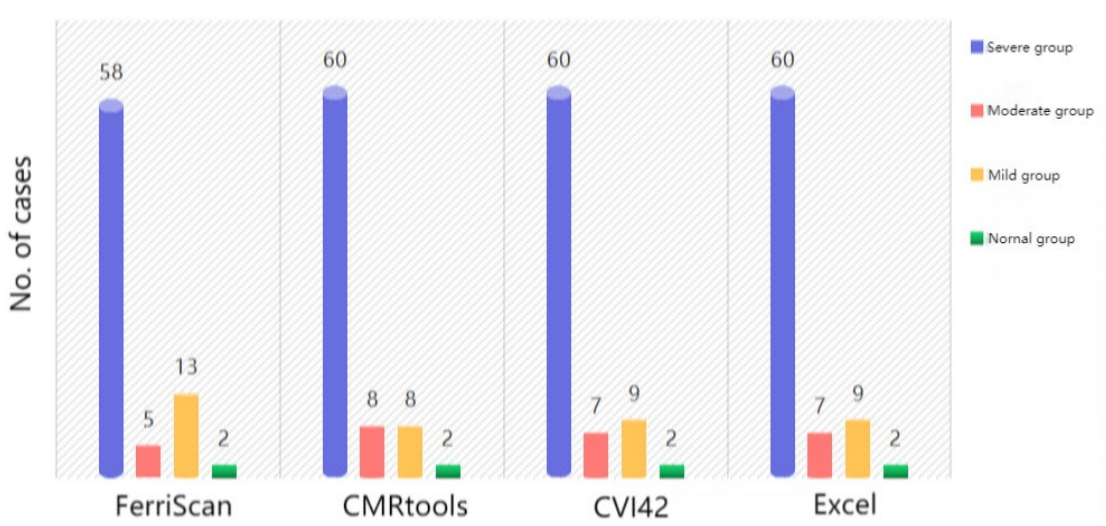

Figure 3. Bar graph of the distribution of clinical classification according to the LIC measured by the four different methods. The four methods showed minimal difference in clinical grade of LIC for the same group of patients.

R2* technology in detecting LIC>7 mg/g liver weight. Moreover, repeatability and consistency across multiple platforms cannot achieve very good results.

On the other hand, in case of significant iron overload, since the liver signal is already lower than that of the muscle in the shortest TE and collapses rapidly with TE elongation, the R2* technique is likely to cause some error in measuring the LIC of patients with high liver iron overload.[16] Studies by d'Assignies[17] and Gandon[18] showed that it is probably better to use the calculation of R2* for low or moderate overloads and to switch to the signal intensity ratio between the liver and the paravertebral muscles (SIR) method for heavy overloads. There are already pieces of software capable of both T2* technology and SIR method LIC, such as MRQuantif, which allows doctors to choose the optimal measure based on the severity of iron overload.

We think that although there was good consistency of clinical measurement data, it could not prove that there was no difference in their diagnostic efficacy or clinical grade composition ratio. The specific explanations are as follows: (1) The difference test between the T2* values measured by the software in some studies generally classifies the data as normality measurement data and uses the paired t-test, which is only a test and analysis of the average level of the data set. (2) Correlation analysis tests the closeness and direction of the correlation between the two variables. The LIC_F, LIC, and T2* value data in this study did not obey the normality distribution; the Spearman rank correlation analysis was used to evaluate the overall monotonic relationship between the two variables. (3) Using the LIC_F clinical grading as the reference standard, the chi-square test was performed by converting the LIC of continuous measurement data into count data of categorical variables through a clear medical reference value. Although some information was lost and the test power was reduced, the composition and distribution of clinical data could be explored to clarify the accuracy of clinical grading of LIC measured by different software. (4) The range of medical reference value should be treated rationally. That is, when the numerical variable of an indicator is within the normal reference range, it can only mean that the indicator has a high probability of being normal. Similarly, when the numerical variable of an indicator is outside the normal reference range, it can only indicate a large probability of problems with the indicator.

The deficiencies of this experiment are as follows: (1) In the setting of ROI, we need to delineate the ROI on three different post-processing pieces of software and try to keep it as consistent as possible with the ROI delineated in the FerriScan image report. However, artificial ROI delineation is susceptible to various subjective and objective factors, and measurement error is inevitable. (2) Due to the characteristics of the etiology received by our clinical center, the clinical grading of liver iron deposition in the subjects included in this study was biased towards moderate and severe, and there was a certain "selection bias". Nevertheless, this does not affect the lateral comparison of the measured results between the software.

Conclusions. The liver T2* values, measured by the CVI42, CMRtools, and Excel methods, were equivalent. The LIC measured by three methods of CVI42, CMRtools, and Excel was equivalent to the LIC_F reported by FerriScan. The cost of different software or measurement methods varies. Different research centers can choose different measurement methods to test patients' LIC according to their own needs and economic level.

Author Contributions. Peng Peng contributed to the study's conception and design. Material preparation and data collection were performed by Fengming Xu, Jixing Yi, Cheng Tang, and Qing Feng. Data analysis was

performed by Fengming Xu and Jixing Yi. The first draft of the manuscript was written by Fengming Xu, Bumin Liang, and all authors commented on previous versions. Finally, all authors read and approved the final manuscript.

Data Availability. The dataset used in support of the findings of this study are available from the corresponding author at email address upon request.

Ethics Approval. This study was performed in line with the principles of the Declaration of Helsinki. Furthermore, the study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Jan 18.2022/No: KY-E-029).

Consent to Participate. All patients (or parents/guardians) gave written informed consent to participate in the study.

Funding. This work was supported by grants from the Natural Science Foundation of China (81760305), Natural Science Foundation of China (81641066), and Innovation Project of Guangxi Graduate Education (YCSW2021135).

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