The Usefulness of MRI R2* Value In Diagnosing And Staging of Rat Liver Fibrosis

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Abstract

Background: Liver fibrosis involves the increase of iron deposition. however, whether R2* measurement can be used as a noninvasive method to characterize processes of fibrogenesis with iron deposition is not clear. This study aims at assessing the usefulness of magnetic resonance imaging (MRI) R2* value in diagnosing and staging of rat liver fibrosis.

Methods: Male Sprague-Dawley rats were injected intraperitoneally with a mixture of 1.0 ml/kg carbon tetrachloride (CCl_4) and oil (1:1 v/v) twice a week for 12 weeks. Liver R2* value was quantitatively determined by multi - echo fast gradient echo sequence. Liver iron content (LIC) was evaluated by an atomic absorption spectrophotometer. The stage of liver fibrosis was assessed by pathological METAVIR scores. The performances of R2* values for each fibrosis stage were evaluated. The receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff values for fibrosis stage.

Results: R2* values and LIC gradually increased during the progression of the liver fibrosis, the correlation between the R2* values and the LIC was high-positive. There were significant differences in R2* values among the stages of liver fibrosis ($F= 30.84$, $P < 0.001$). There was a significant positive correlation between R2* values and LIC ($r= 0.984$, $P < 0.001$). The most discriminating cutoff values of R2* were 46.84 Hz for ≥ F1, 55.30 Hz for ≥ F2, 68.06 Hz for ≥ F3, and 78.79 Hz for F4.

Conclusion: R2* values can be used for detecting and staging liver fibrosis. The degree of liver fibrosis was related to the degree of increase in R2* measurements.

Keywords: Liver fibrosis, Magnetic resonance imaging, R2* value, Diagnosis, Stage

Background

Liver fibrosis is a consequence of chronic injury from a variety of causes and represents a common feature of almost all chronic liver diseases, which can be the development of cirrhosis, portal hypertension and hepatocellular carcinoma [1-2]. Progression of early fibrosis can be reversed by the effective intervention, such as specific antifibrotic therapy or elimination of the cause [3-4]. Therefore, the early identification of liver fibrosis has crucial clinical implications in the determination of treatment options and prognosis. Liver biopsy is a good standard for detecting and staging liver fibrosis. However, it is an invasive procedure with inherent risks [5], and is subject to sampling error [6]. Serum markers are noninvasive alternatives to biopsy for staging liver fibrosis, which include measurement of the doses of specific markers of fibrosis, such as N-terminal collagen III propeptide and hyaluronic acid. However, because fibrosis is not specific to the liver, the role of these markers is limited [2,3,5].

Iron-induced oxidant stress is involved in fibrogenesis, and causes hepatocytes necrosis and activates hepatic stellate cells (HSCs) and Kupffer cells [7,8], which ultimately cause liver fibrosis and other diseases.

Magnetic resonance imaging (MRI) is considered as a noninvasive and reliable method for detecting LIC [9-11]. The local field inhomogeneity caused by the paramagnetic effect of tissue iron is the base of detecting iron with MRI [12]. This causes more rapid signal decay resulting in increased the transverse relaxation rates of R2 and R2* (the reciprocal of T2 and T2* transverse relaxation times, respectively).

In particular, multi-echo gradient (mGRE) sequences are used to measure R2* of liver tissue [9-11]. Liver biopsy-proven LIC and measured R2* have a good correlation [11, 12]. Because liver fibrosis involves the increase of iron deposition [7], we hypothesize that R2* measurement may be used as a noninvasive method to characterize processes of fibrogenesis with iron deposition. The aim of our study was to investigate the correlation between R2* measurements and the degree of liver fibrosis in a rat model.

Methods

Animal model

Our study was approved by our Animal Experimentation Ethics Committee (approval number: K2015122) and was carried out in accordance with the guidelines of north sichuan medical college and ARRIVE guidelines. Thirty-five male Sprague-Dawley rats (200 -250 g, Laboratory Animal Center of North Sichuan Medical College, Nanchong, China) were randomly divided into fibrosis and control groups. Thirty rats in the fibrosis group were injected intraperitoneally with a mixture of 1.0 ml/kg of carbon tetrachloride (CCl_4) and oil (1:1 v/v) twice a week for 12 weeks. The other five healthy rats were used as the control animals. The animals were fed on a standard diet and subjected to a 12-hour light/dark cycle in an air-conditioned room at 25 °C.

MRI techniques

Anesthesia was induced by intraperitoneal injection of 3.0 % pentobarbital sodium (1.0 ml/kg). After anesthesia, animals were scanned in a prone position. Images were obtained with a 3-T MR system (Discovery 750, GE Healthcare, Wisconsin, USA) by

using a 3-inch-diameter circular surface coil. For T2*-weighted imaging, an axial multi-echo fast gradient echo sequence with six echoes was used. The main parameters were as follows: TR/TE range, TR/TE160/2.7-22.3 ms; slice thickness, 2.5 mm; interslice gap, 0mm; FOV, 10 cm×10 cm; number of slices, 12; pixel size, 0.625mm×0.625mm; matrix size, 160×160; flip angle 30°).

Image processing

The software of R2Star (Function tool 4.4, GE Healthcare, Wisconsin, USA) was employed for calculating the R2* values on R2* map. For R2* values measurement, two radiologists with five years' experience in abdominal MRI were blinded to histopathologic results and the same two consecutive R2* sections at mid liver were analyzed. Three regions of interest (ROIs) of approximately 2-3mm^2 were placed on each slice avoiding major blood vessels and artifacts (Fig.1a, b). R2* values for all six ROIs were recorded and the mean values for all six ROIs were calculated in each liver.

Histopathological examinations and detection of liver iron content

After the MR examinations, the animals were sacrificed by intraperitoneal injection of 3% pentobarbital sodium (3.0 ml/kg). The livers, which were harvested from the rats were divided into two parts for histopathological examinations and detection of liver iron content, respectively. A small amount of liver tissue (approximately 10×10×10mm^3) was used for masson trichrome staining. The residual liver tissue was used for detecting LIC by an atomic absorption spectrophotometer (Hitachi Z-5000, Tokyo, Japan) as previously described [13].

The fibrosis stage was assessed by the METAVIR scoring system: F0 indicates no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis [14].

Statistical Analysis

All the data were expressed as mean ± standard deviation (SD). SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. A one-way ANOVA test was used to determine the significance of the differences in R2*values between different stages of fibrosis. Spearman correlation coefficients were used to evaluate the correlations between the R2* value and the degree of fibrosis and between the R2* value and LIC. The receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff values of the R2* for fibrosis stage. A $P<0.05$ was considered to be significant.

Results

Fibrosis stages and R2* value

Twenty-three rats in the fibrosis group survived and seven died after 12 weeks. The mortality was 23.3%. Five rats in the control group were at stage F0; four rats in the treatment group were at stage F1; eight rats, at stage F2; seven rats, at stage F3 (Fig.1c); and four rats, at stage F4.

According to the stage of fibrosis, the distribution of R2* values is shown in Table 1. The R2* values were 42.28Hz, 50.11Hz, 56.46Hz, 75.38Hz, and 85.62Hz in stages F0, F1, F2, F3 and F4 respectively. With the increase of fibrosis degree, R2* value had a trend toward an increase (Table 1). There were significant differences in R2*

values among the stages of liver fibrosis (F=30.84，P<0.001). The R2* values were significantly positively correlated with the stage of fibrosis (r=0.902, P<0.001) (Fig. 2). The most discriminating cutoff values of R2* were 46.84 Hz for ≥ F1, 55.30 Hz for ≥ F2, 68.06 Hz for ≥ F3, and 78.79 Hz for F4 (Fig. 3, Table 2). Corresponding sensitivities and specificities values are given in Table 2.

R2* value and LIC

LIC gradually increased during the progression of the liver fibrosis (Table 1). LIC values ranged from 111.48 to 248.01 μg Fe/g. There was a significant positive correlation between R2* values and LIC (r= 0.984, P<0.001) (Fig.4).

Discussion

Our initial findings indicate that R2* values are a noninvasive method of detecting and staging liver fibrosis.

Liver fibrosis is initiated by the accumulation of collagen and other materials within the extracellular matrix, including type I collagen which are formed by HSCs and hepatic myofibroblasts (MFB) [15, 16]. Assessment of liver fibrosis in chronic liver disease is beneficial for determining disease progression and assessing complications, such as esophageal varices and hepatocellular carcinoma. The development of noninvasive markers of liver fibrosis would reduce biopsy-related complications and facilitate early diagnosis and improve monitoring of progression of chronic liver disease.

Medical imaging is of great significance for the diagnosis and staging of liver fibrosis. Many imaging based techniques are used including ultrasonography-based

155 elastography, such as 2D-Shear wave elastography, transient elastography, and diverse
156 MRI-based techniques, such as MR elastography, diffusion-weighted imaging (DWI),
157 T1 ρ MR imaging, MR perfusion imaging and dynamic contrast–enhanced MR
158 imaging [15, 17, 18]. The development of larger gradients, higher field strengths,
159 improved surface coils, and parallel imaging techniques have considerably improved
160 the speed and quality of MRI examinations [19]. R2* has been proposed to be
161 sensitive to tissue iron. R2* is used for noninvasive quantitative evaluation of LIC and
162 plays an important role in the management of patients undergoing chelation therapy
163 [12]. Measured liver R2* does not require the intravenous injection of potentially
164 nephrotoxic contrast agents or the use of an additional transducer hardware, such as in
165 the case of MR elastography. Due to the paramagnetic nature of iron, R2* increases
166 with iron deposits in the liver. Measured liver R2* has been shown to have a strong
167 correlation with biopsy-proven LIC [11, 12]. Excess accumulation of Fe^{2+} generates a
168 radical OH, which leads to apoptosis of hepatocytes, activates HSCs, and promotes
169 the process of fibrosis [16]. The R2* is also affected by some confounding factors,
170 including collagen contents, fat and necroinflammation. Results from this study
171 suggest that liver fibrosis is associated with a R2* value increase, and this R2* value
172 increase is correlated with LIC. There were significant differences in R2* values
173 among the stages of liver fibrosis ($F=30.84$，$P<0.001$). With the use of ROC analysis,
174 the most discriminating cutoff values of R2* were 46.84 Hz for ≥ F1, 55.30 Hz for ≥
175 F2, 68.06 Hz for ≥ F3, and 78.79 Hz for F4. In our study, the R2* measurements can
176 clearly separate the different fibrosis stages. For example, the cutoff value of 46.84

177 Hz for stage 1 or greater fibrosis had a sensitivity was 91.3 % and a specificity of
178 100%. This high accuracy for the diagnosis of intermediate fibrosis stages is clinically
179 important, because patients with hepatitis C genotype 1 infection should be treated
180 only when substantial fibrosis is observed. [19] Furthermore, with an optimized cutoff
181 value of 68.06 Hz for stage 3 or greater fibrosis, the sensitivity of R2* value was
182 91.2% at a specificity of 94.1%. This high accuracy for the diagnosis of advanced
183 fibrosis is also important because portal hypertension and hepatocellular carcinoma
184 should be alert in patients with advanced fibrosis [20]. We believe a clear association
185 between increasing R2* values and fibrosis stage has been shown ($r=0.902$, $P<0.001$),
186 R2* values can potentially be used noninvasively for detecting and staging liver
187 fibrosis.

188 The variability of biopsy LIC depends on the size of the specimen. Biopsy LIC
189 measurement also varies widely among laboratories because of differences in the
190 actual ratios of wet-to-dry weights of tissue samples [11]. In our study, the vast
191 majority of liver tissue was used for detecting of LIC by atomic absorption
192 spectrophotometer. Thus, this method can better reflect the LIC compared with
193 biopsy.

194 There was one limitation to our study. Because this is a report of our initial
195 experience, the results were limited by the sample size; more specifically the small
196 number of rats with stage F1 and stage F4 of liver fibrosis.

197 **Conclusions**

198 In conclusion, this study shows that measured liver R2* is a noninvasive and

quantitative method to evaluate liver fibrosis. Liver R2* values and LIC increased when liver fibrosis progressed.

Abbreviations

MRI: magnetic resonance imaging; CCl_4: carbon tetrachloride; LIC: liver iron content; ROC: receiver operating characteristic; HSCs: hepatic stellate cells; mGRE: multi-echo gradient; MFB: myofibroblasts; DWI: diffusion-weighted imaging; SD: standard deviation.

Declarations

Ethics approval and consent to participate

Our study was approved by our Animal Experimentation Ethics Committee (approval number: K2015122) and was carried out in accordance with the guidelines of north sichuan medical college and ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

YJ and LY conceived of the present idea and designed the study. Data acquisition and statistical analysis was performed by YC, ZH and SK. YJ and LY contributed to the data analysis and interpretation. YJ and LY were major contributors and contributed equally to writing the manuscript. All the authors read and approved the final manuscript.

Acknowledgments

Not applicable.

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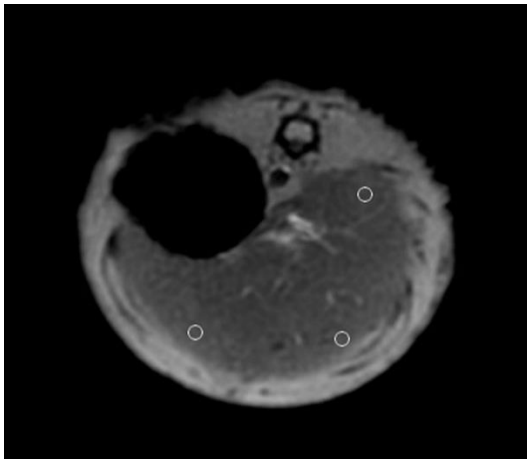

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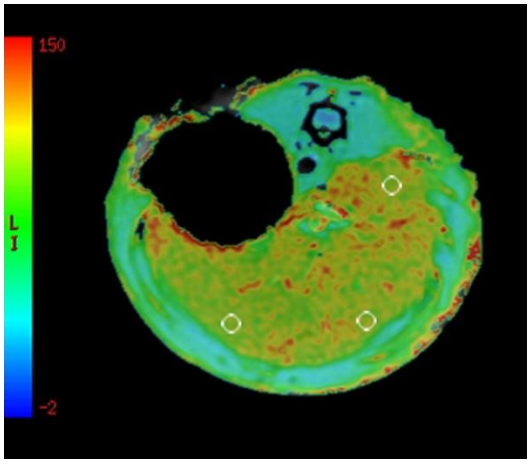

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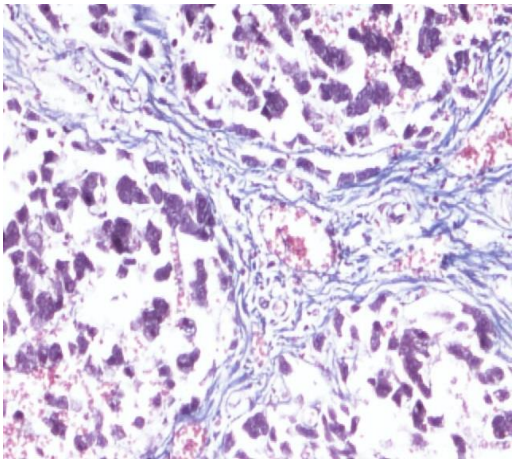


Fig.1. Rat liver with stage F3 fibrosis. (a) source image of T2*-weighted imaging; (b) R2* map. Color bar from blue to red represented the increasing of R2* value; (c) Masson

trichrome-stained histologic section (original magnification, ×200).

1c

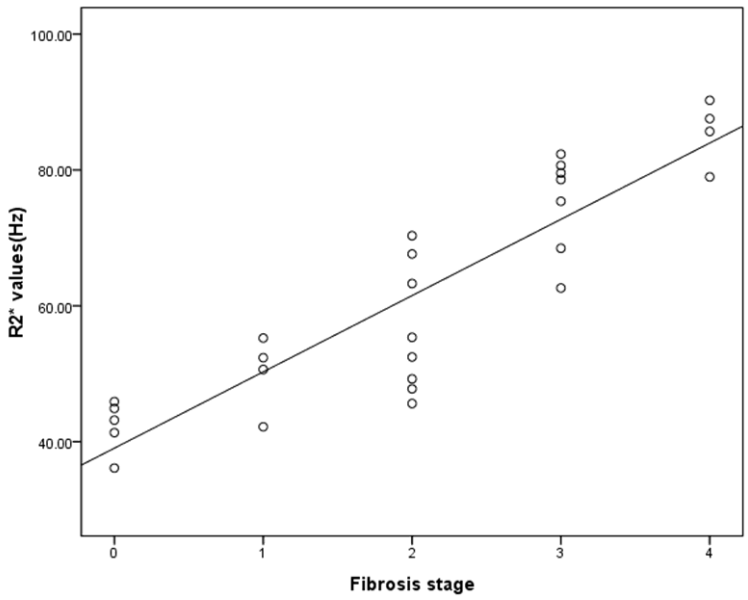
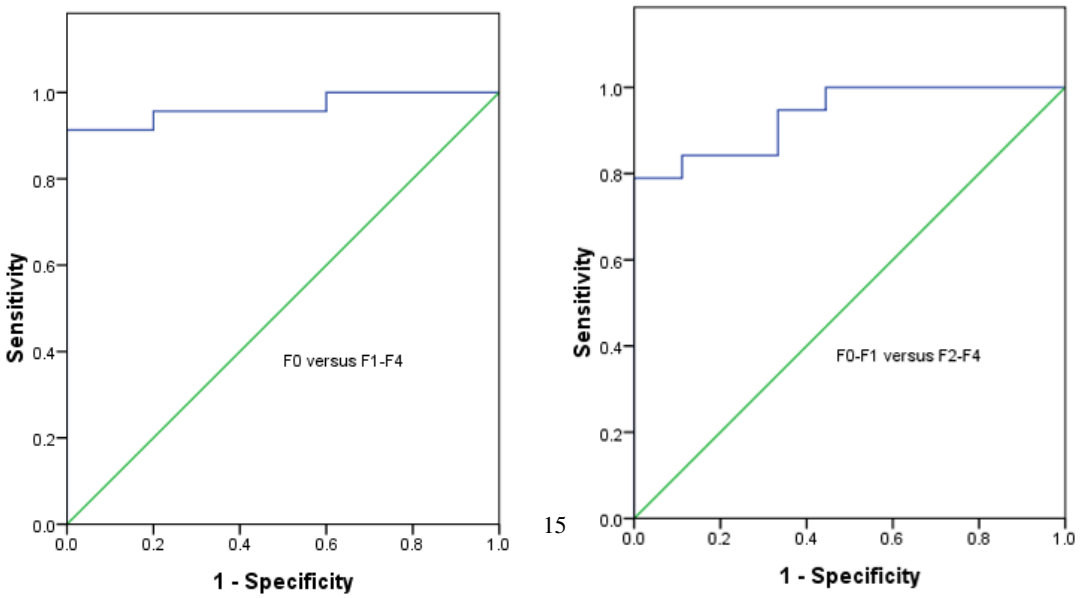


Fig.2. Scatterplot shows the relationship between the R2* values and fibrosis stage. A high positive correlation was found between the R2* values and fibrosis stage (r=0.902, P<0.001) with Spearman correlation rank test.



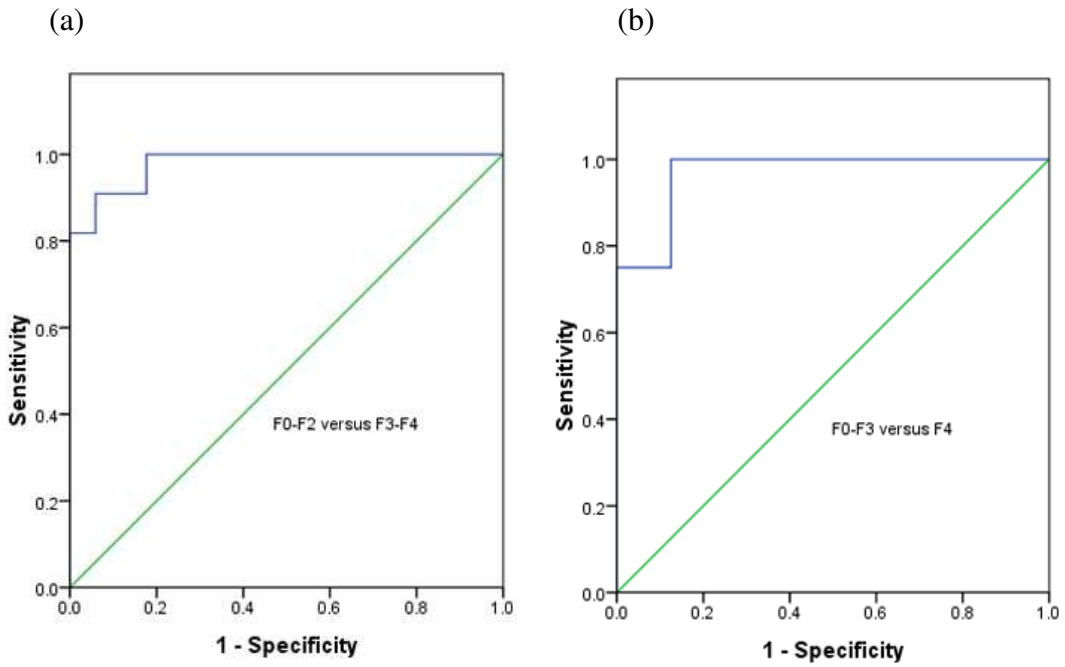


Fig.3. ROC curves for R2* values at METAVIR fibrosis score of (a) ≥F1, (b) ≥F2, (c) ≥F3, and (d)F4.

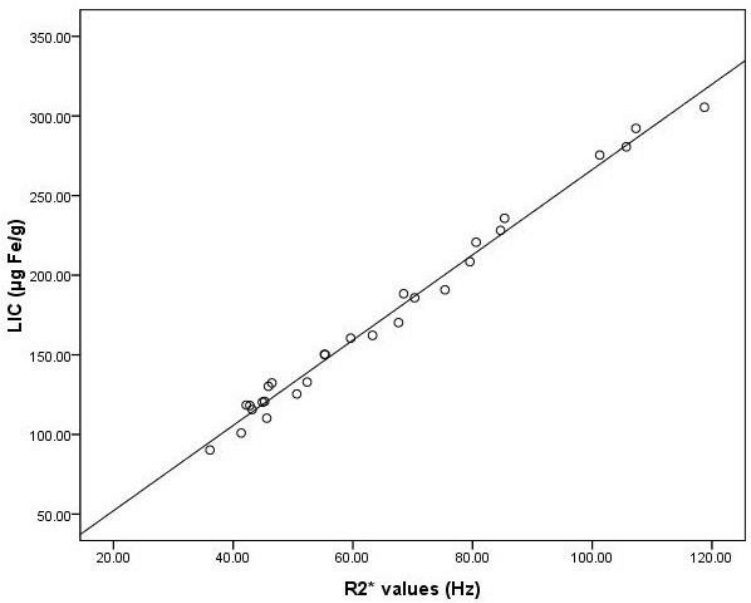


Fig 4. Scatterplots of R2* values and LIC. The spearman correlation coefficient between R2* values and LIC was 0.984.

Table 1. R2* (Hz) values and LIC (μg Fe/g) at different fibrosis stages

Fibrosis stage	No. of Rats	R2* value	LIC
0	5	42.28±3.86	111.48±15.89
1	4	50.11±5.61	131.79±13.71
2	8	56.46±9.45	143.74±27.48
3	7	75.38±7.24	204.67±26.46
4	4	85.62±4.84	248.01±24.03

Table 2. R2* Cutoff Values at different fibrosis score with corresponding sensitivities and specificities

Parameter	≥F1	≥F2	≥F3	F4
R2* Cutoff Values (Hz)	46.84	55.30	68.06	78.79
Sensitivity (%)	91.3	78.9	91.2	100
Specificity (%)	100	100	94.1	87.5

Supplementary Files

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