

# Diffusion-weighted imaging of the liver in assessing chronic liver disease: effects of fat and iron deposition on ADC values

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**Abstract. – OBJECTIVE:** This study was designed to evaluate whether fat and iron affect the apparent diffusion coefficient (ADC) values of the liver parenchyma in the settings of fibrosis and inflammation.

**PATIENTS AND METHODS:** We evaluated the diffusion-weighted images (DWIs) of 58 patients with chronic liver disease and 48 control subjects. Liver specimens of patients were assessed for fibrosis, necroinflammation, iron, and steatosis. Liver ADCs, spleen ADCs, and normalized liver ADCs (defined as the ratio of the liver ADC to spleen ADC) values were analyzed after stratifying patients with either fibrosis stages or histology activity index (HAI) scores. The relationship between ADC values and histopathological findings was studied using multiple linear regression analysis.

**RESULTS:** The median liver and normalized liver ADC values were significantly lower in higher stages of fibrosis and HAI scores. Compared to the control group, patients with the highest stages of fibrosis and inflammation had significantly higher spleen ADCs. The effect of the fibrosis stage on liver ADC and normalized liver ADC values was significant in the setting of inflammation, whereas the degree of steatosis and iron grade did not affect these ADC values.

**CONCLUSIONS:** ADC values can distinguish both later stages of liver fibrosis and inflammation. There is no significant effect of fat and iron on ADC values. Therefore, DWI may be reliable in evaluating liver fibrosis and inflammation.

*Key Words:*

ADC measurements, Chronic liver disease, Diffusion-weighted MRI, Fat, Iron, Magnetic resonance imaging.

## Abbreviations

ADC: Apparent diffusion coefficient; DWI: Diffusion-weighted image; HAI: Histology activity index.

## Introduction

It is necessary to determine the exact stage of liver fibrosis and follow up the disease course because the proper treatment helps to preserve and prolong efficient liver function<sup>1</sup>. In the early stages, the advancement of liver fibrosis can be impeded or reversed by eliminating the cause or by optimum treatment<sup>2-7</sup>. An increase in the progression of liver fibrosis results in decreasing prognosis and increasing the risk of end-stage liver disease and upcoming hepatocellular carcinoma<sup>8</sup>. The assessment of inflammation is also critical to the clinical management of chronic liver disease patients, which leads to tissue damage and accompanies the development of liver fibrosis. Liver histology is still the reference standard to determine the definite stage of liver fibrosis and inflammation. However, liver biopsy is associated with sampling error, interobserver variability, and potential complications, such as pain and bleeding<sup>9-11</sup>. A supreme interest of current literature is to search for a more reliable, simple, reproducible, and non-invasive technique for diagnosing, quantifying, and monitoring liver fibrosis and inflammation.

With the recent advances in technology, functional and advanced imaging methods including MR elastography, MR spectroscopy, diffusion-weighted images (DWIs), and perfu-

sion-weighted imaging have been widely introduced in abdominal imaging to detect and characterize focal liver lesions and evaluate diffuse liver diseases<sup>12</sup>. DWI is a preferred consulting MRI technique for the imaging of the liver because it usually provides additional information to conventional imaging sequences, it is fast and can perform repeatedly, and there is no need for a contrast agent; thus, it is beneficial in patients with severe renal dysfunction at risk for nephrogenic systemic fibrosis<sup>13</sup>. It is essentially the best imaging technique for the *in vivo* evaluation of the combined effects of capillary microcirculation (perfusion) and diffusion using the quantitative parameter called the apparent diffusion coefficient (ADC)<sup>14</sup>. Theoretically, the diffusion restriction in the cirrhotic liver is because of the accumulation of extracellular collagen fibers, proteoglycans, and glycosaminoglycans<sup>2</sup>. A decrease in perfusion in the cirrhotic liver has also been found to be responsible for reducing ADC values. The reduction in the ADC of liver parenchyma in chronic liver disease has made diffusion-weighted MRI progressively at a premium for the detection and staging of liver fibrosis. Although initial findings are inconsistent, DWI is a promising tool for detecting and grading liver fibrosis. In addition to measurements of ADCs, the normalized liver ADC has also been defined using the spleen as a reference organ to improve reproducibility and reduce variability in the ADC measurements<sup>12,15</sup>.

The deposition of both iron and fat is a common situation in chronic liver disease, and it has been suggested to play a role in the progression of hepatic fibrosis and the development of cirrhosis and even hepatocellular carcinoma<sup>16</sup>. In addition, the deposition of fat may result in impairments of iron metabolism<sup>17</sup>. Contradictory findings have been found in previous studies on whether or not iron and fat confound liver ADC values<sup>18-20</sup>. Some studies have found that the deposition of fat and iron does not affect the ADC values<sup>18</sup>. On the contrary, Poyraz et al<sup>21</sup> found a significant inverse correlation between the liver fat content and ADC values. Chandarana et al<sup>22</sup> reported that hepatic iron deposition lowers liver ADCs. In this regard, our current understanding of the varying effects of fat and iron on ADC values is poor in chronic liver disease. According to the present literature, DWI is a valuable tool in diagnosing or staging liver fibrosis<sup>23</sup>. The reliability of DWI for staging fibrosis has been studied in the settings of iron and fat<sup>24</sup>. It is still not well known whether the presence of iron or fat affects the reliability and

diagnostic performance of DWI in patients with chronic liver disease. The present study aimed to evaluate the effects of iron and fat on the ADC values in the setting of both fibrosis and inflammation.

## Patients and Methods

### Patients

We retrospectively assessed 58 successive adult patients with chronic liver disease who had a routine MRI of the upper abdomen, including echoplanar DWI, and who underwent concomitant liver biopsy or liver transplantation. For all of the included study groups, upper abdominal MR imaging that contained DWI had been obtained within three months of liver biopsy or transplantation. The study population involved 35 men and 23 women with a mean age of  $47.4 \pm 14.4$  years. The patients had different causes of liver disease, with chronic viral hepatitis [hepatitis B virus ( $n = 34$ ), hepatitis C virus ( $n = 3$ )] being present in more than 63% of the total patient group. The other underlying liver diseases of the patients were autoimmune hepatitis ( $n = 2$ ), toxic hepatitis ( $n = 3$ ), Wilson's disease ( $n = 2$ ), Budd-Chiari ( $n = 2$ ), and unknown ( $n = 12$ ). We also included 48 healthy subjects (19 men and 29 women) with a mean age of  $43.4 \pm 16.3$  years who underwent upper abdominal MRI, including echoplanar DWI, as a control group through a database search. Clinical indications for MRI were as follows: small liver cyst or hemangiomas ( $n = 18$ ), donor candidates in living donor liver transplantation ( $n = 15$ ), adrenal adenoma ( $n = 7$ ), renal cyst ( $n = 6$ ), and focal nodular hyperplasia ( $n = 2$ ). These selected subjects had normal liver function tests, had no history of viral diseases or diffuse liver disease, and had no liver imaging findings of fat and iron deposition. These MR-DWIs of the control patients were selected over the same period to ensure consistency of MRI protocol.

This retrospective study was performed in a single center and approved by the Institutional Ethics Board. All of the clinical and radiological data acquired during the study were under the control of two particular radiologists.

### MR Imaging

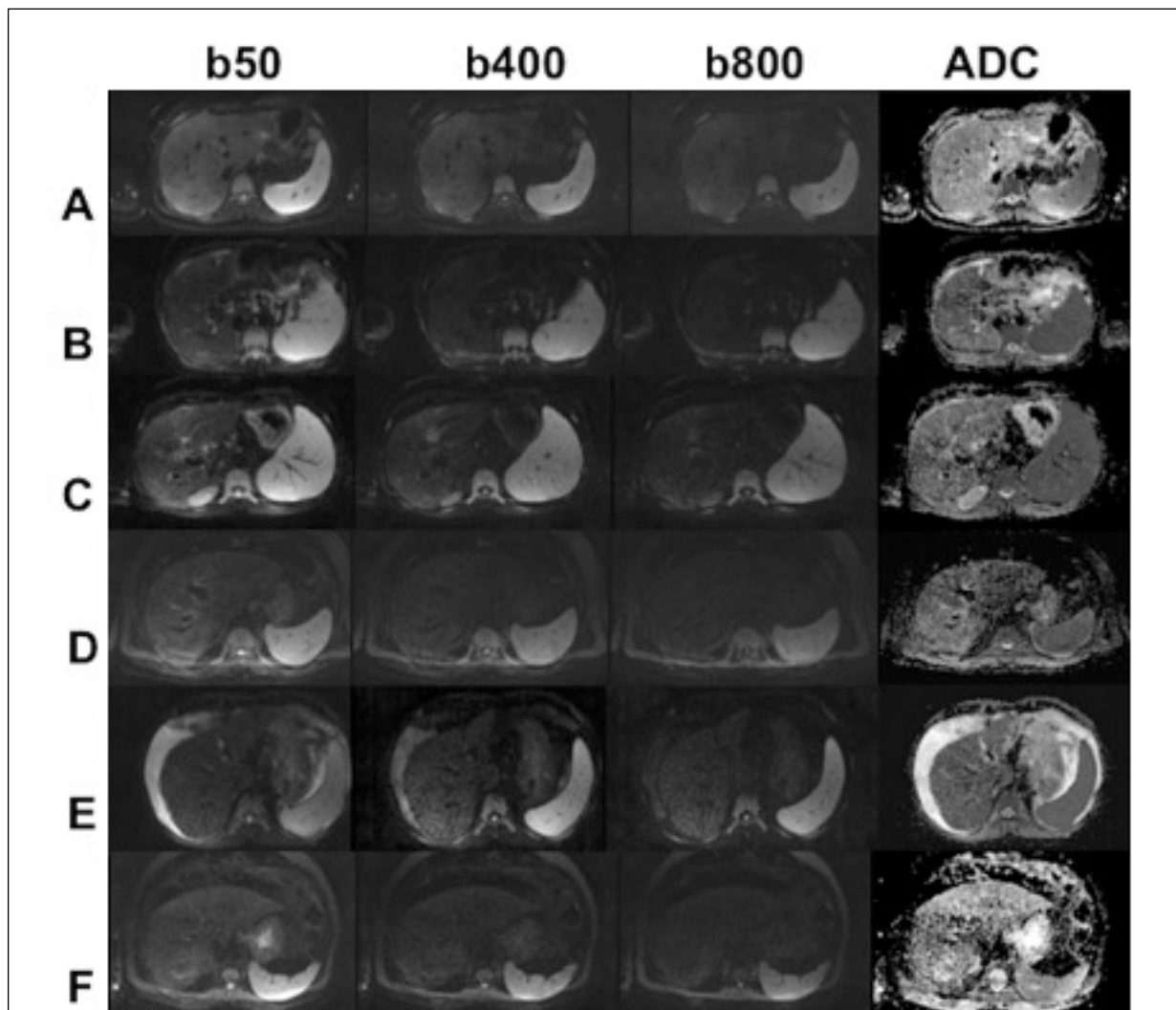
All patients underwent MRI using a 1.5 T scanner (Magnetom Avanto; Siemens Healthcare, Erlangen, Germany) with ten receiver channels, using one 6-channel phased-array body coil ante-

riorly and one 4-channel spine cluster posteriorly. The DWI sequence was a non-breath holds sequence with respiratory gating. Respiratory-trigger was placed around the abdomen and used to synchronize patients' breath. The following parameters were used for our routine protocol for echoplanar DWI in an axial plane: repetition time = 6,000-6,200 ms, echo time = 85 ms, bandwidth = 1,736 Hz/pixel, matrix size = 192X153, slice thickness = 7 mm, gap = 20%, field of view = 380 mm, three averages, and b values of 50, 400, and 800 s/mm<sup>2</sup>. The DWI images were acquired before intravenous administration of the contrast medium. The algorithms implemented within the

Siemens Magnetom scanner software were used to constitute quantitative ADC maps using b values of 50, 400, and 800 s/mm<sup>2</sup>.

#### Image Analysis

Images were analyzed by an experienced radiologist with at least ten years of experience in hepatobiliary imaging, using Osirix™ software (v.3.6, 64-bit, Pixmeo, Bernex, Switzerland) for ADC measurements of the liver and spleen. The observer was blind to both clinical and histopathological results. Following visual assessment, the diffusion images were interpreted concurrently with the ADC map to avoid misreading (Figure 1).



**Figure 1.** Axial echo planar diffusion-weighted images for b-values of 50 s/mm<sup>2</sup>, 400 s/mm<sup>2</sup> and 800 s/mm<sup>2</sup> and apparent diffusion coefficient (ADC) maps in a healthy subject (A) and in patients with chronic liver diseases (B-F). Histopathological findings were as follows: with no fibrosis, steatosis and iron (B); with fibrosis stage 6, no steatosis and no iron (C); with fibrosis stage 6, steatosis degree 70% and no iron (D); with fibrosis stage 6, no steatosis and iron grade 3 (E); with fibrosis stage 6, steatosis degree 80% and iron grade 1 (F).

Quantitative ADC values of the liver parenchyma were obtained by placing three freehand regions of interest (ROIs), measuring 10-12 mm<sup>2</sup>, to the middle-inferior portion of the right lobe of the liver. We did not choose the left lobe of the liver to avoid potential alterations in the measurements of the ADC values related to cardiac motion artifacts<sup>25</sup>. Supreme care was taken to avoid visible biliary and vascular structures, focal lesions, and artifacts, such as chemical shifts and magnetic susceptibility. The spleen was preferred as a reference organ. The ADC measurements of the spleen were also acquired via placing three ROIs, measuring 10-12 mm<sup>2</sup>, to the spleen on the same slice with the liver ADCs measured. Mean ADC values were recorded for each ROI. The average of the mean ADC values of the three ROIs were documented as the final ADCs of the liver and spleen. The normalized liver ADC was estimated as the ratio of liver ADCs to spleen ADCs.

### ***Histological Assessment***

A majority of the study population (n = 42) underwent liver transplantation. Liver biopsy was performed on 14 patients, and segmental resection was done on two patients with chronic liver disease. A representative section was sampled from the right lobe of the explanted or resected liver for histopathologic analysis. No biopsy was performed on the control group. All specimens were scored by an experienced histopathologist for fibrosis and necroinflammation according to the modified Knodell histology activity index (HAI)<sup>26</sup>. The combined scores of portal inflammation, piecemeal necrosis, spotty necrosis, and confluent necrosis formed the HAI scores (Ishak score, 0-18). These histopathological samples were also retrospectively examined for iron overload and degree of steatosis. Liver iron overload was semi-quantitatively evaluated to grade on a 5-point scale. Hepatic fat content was evaluated as a percentage, and >5% was considered a significant degree of steatosis.

### ***Statistical Analysis***

The distributions were analyzed to test normality via the Kolmogorov-Smirnov test. The data were presented as median values (25%-75%). The Kruskal Wallis H test was employed for comparing the groups, and the Mann Whitney U test, with Bonferroni correction as a post-hoc test, was used to compare the pairs of groups. The mathematical function of the relationship be-

tween dependent and independent variables was analyzed with multiple linear regression analysis. *p*-values of less than 0.05 were considered as investigative of statistical significance. Statistical analysis was made using IBM Statistical Package for Social Sciences (SPSS) software, version 22.0 (SPSS Inc., Armonk, NY, USA).

## **Results**

### ***Histopathological Findings***

The majority of patients [42 of 58 patients (72%)] had fibrosis stages 5 and 6. Of 16 (28%) patients with lower stages of fibrosis, 6 (11%) patients had stage 0. Accordingly, all patients with chronic liver disease were firstly classified as group 1 (n = 6), group 2 (n = 10), and group 3 (n = 42) according to the fibrosis stage 0, stages 1-4, and stages 5-6, respectively. The incidence of established significant hepatic inflammation (total histology activity index > 6) was 72% (n = 42). Subsequently, HAI scores were scattered as follows: 16 patients (28%) with HAI 1-6, 24 patients (41%) with HAI 7-12, and 18 patients (31%) with HAI 13-18. Thus, participants were secondly allocated to three groups of HAI scores. A significant amount of steatosis, defined as a fat deposition of >5%, was seen in 20% of patients (in 12 of 58 patients). Of 58 patients who underwent iron grading, 75% had no grade of iron deposition; iron overload accepted as a grade of 1-4 was seen in 15 patients (25%). Furthermore, in 8 patients (14%), there were significant degrees of steatosis and iron overload together.

Our analysis consists of two main steps: initially, we compared the ADC values between groups stratified either by fibrosis stage or inflammation score. In the final step, we used the measured histopathological findings to understand if these parameters affect ADC values.

### ***Distribution of ADC Values Within the Liver and Spleen***

In the control group, median values of liver ADC, spleen ADC, and normalized liver ADC were  $1039.0 \times 10^{-6}$  mm<sup>2</sup>/s (IR: 982.5-1129.0  $\times 10^{-6}$  mm<sup>2</sup>/s),  $794.5 \times 10^{-6}$  mm<sup>2</sup>/s (IR: 743.0-872.0  $\times 10^{-6}$  mm<sup>2</sup>/s), and 1.28 (IR: 1.19-1.44), respectively. The liver and normalized liver ADCs of all patients with chronic liver disease were significantly lower than those of the control group. Firstly, findings of groups stratified by fibrosis stage showed a reduction in median values of liver ADCs and

normalized liver ADCs, in the progression from the control group towards group 3. Afterward, median values of liver ADCs and normalized liver ADCs decreased in patient groups classified according to the HAI score compared to those of the control group.

In the overall patient population and control subjects, the spleen ADC measurements were significantly lower than the liver measurements. Compared to the control group, patients with a higher stage of fibrosis (FS = 5-6) had significantly higher spleen ADCs ( $p = 0.003$ ). In addition, there were no significant differences in median spleen ADC values between the control group, group 1, and group 2. Of all groups stratified by HAI scores, spleen ADC values in groups 2 and 3 were significantly higher than that of the control group ( $p = 0.006$ ).

#### **ADC Values vs. Stage of Fibrosis**

The distribution of liver ADC, spleen ADC, and normalized liver ADC values in patients stratified by fibrosis stage is shown in Table I. Although there were some overlaps in the liver ADC values, the median liver ADC value of the control group was higher than that of the patient groups. The median liver ADC and normalized liver ADC values of the patient group with high fibrosis stage (FS 5-6; group 3) were significantly lower than the other groups ( $p < 0.001$ ). The difference in normalized liver ADC values by fibrosis stages group 2 (FS = 1-4) and group 3 (FS = 5-6) was also significant ( $p < 0.001$ ). No significant difference was investigated between other groups in the setting of liver ADC and normalized liver ADC values. Normalized liver ADC values were inversely correlated with fibrosis stages. There was only a significant difference between spleen ADC values of the control group and group 3 ( $p = 0.003$ ).

Afterward, histopathological findings, including HAI scores, iron grades, and degree of steatosis, were analyzed between patient groups stratified by fibrosis stages (Table II). The HAI scores were significantly different in all groups ( $p < 0.001$ ), while degrees of steatosis were not. The iron grades in group 2 were also significantly different from group 3 ( $p = 0.025$ ).

#### **ADC Values vs. HAI Scores**

The distribution of all ADC values in patients stratified by histological HAI score was investigated in Table III. In comparison, a significant difference in the liver ADC and normalized liv-

er ADC values was present between all groups, except for the control group vs. group 1 and group 2 vs. group 3 ( $p < 0.001$ ). There were only significant differences between spleen ADC values of the control group and groups 2 and 3 ( $p = 0.006$ ).

The histopathological findings, including fibrosis grades, iron grades, and degree of steatosis, have been analyzed between patient groups stratified by HAI scores (Table IV). The fibrosis grades in group 1 were significantly different from groups 2 and 3 ( $p < 0.001$ ), while degrees of steatosis were not. The iron grades in group 1 were also significantly different from group 2 ( $p = 0.016$ ).

#### **The Effects of Histopathological Findings on ADC Values**

The results of multiple regression analysis are shown in Table V and Table VI. The effects of the fibrosis stage on liver ADC and normalized liver ADC values were significant in group 1 stratified by HAI scores ( $p < 0.05$ ). On the other hand, steatosis and iron grade did not affect the liver or normalized liver ADC values ( $p > 0.05$ ).

## **Discussion**

The accurate and precise detection of the fibrosis stage is critical to improving follow-up and treatment of chronic liver diseases. Liver biopsy remains a standard reference in evaluating diffuse liver diseases involving fibrosis, inflammation, fat, and iron<sup>27</sup>. Advanced MRI techniques for assessing liver fibrosis and quantifying liver fat and iron have been used in recent years as an alternative to biopsy. With novel advances in technology, diffusion-weighted imaging has been widely introduced in abdominal imaging to either detect and characterize focal liver lesions or evaluate and monitor diffuse liver diseases<sup>12,28</sup>. DWI, which utilizes the alterations in water diffusion between tissues, has exhibited potential for improving detection of stages of liver fibrosis, as the increased fibrosis of liver parenchyma is associated with reduced tissue water diffusion. However, variable technical parameters and imaging systems inhibit the comparison of DWI findings from study to study. Subsequently, the revealed ADC values were quite variable in similar disease groups and even in the healthy liver<sup>23</sup>. By all means, future standardization of DWI protocols of abdominal MRI, involving both ac-

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**Table I.** Comparison of ADC values (expressed in  $\times 10^{-6}$  mm<sup>2</sup>/s) between control group and patient groups stratified by fibrosis stages.

	Liver ADC values			Spleen ADC values			Normalized Liver ADC values		
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75
Control Group (n=48)	1039.0 <sup>a</sup>	982.5	1129.0	794.5 <sup>a</sup>	743.0	872.0	1.29 <sup>a,b</sup>	1.20	1.45
Group 1 (FS=0) (n=6)	1021.5 <sup>a</sup>	984.0	1137.0	851.5	718.0	880.0	1.31 <sup>a</sup>	1.16	1.37
Group 2 (FS=1-4) (n=10)	995.5 <sup>a</sup>	857.0	1070.0	838.5	800.0	894.0	1.18 <sup>a</sup>	1.09	1.29
Group 3 (FS=5-6) (n=42)	844.0	793.0	919.0	882.0	845.0	956.0	0.93	0.84	1.04
<i>p</i> -value		< 0.001			0.003			< 0.001	

\*<sup>a</sup>: vs. Group 3 (FS = 5-6), ( $p < 0.05$ ); <sup>b</sup>: vs. Group 2 (FS = 1-4) ( $p < 0.05$ ); FS: Fibrosis stage.

**Table II.** The comparison of histopathological findings among patient groups stratified by fibrosis stages.

	Group 1 (FS = 0) (n = 6)			Group 2 (FS = 1-4) (n = 10)			Group 3 (FS = 5-6) (n = 42)			<i>p</i> -value
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
HAI score	4 <sup>a,b</sup>	3	5	6 <sup>b</sup>	5	6	11	8	14	< 0.001
Iron grade	0	0	0	0 <sup>b</sup>	0	0	0	0	2	0.025
Steatosis (%)	0	0	5	5	0	5	1	0	5	0.516
Fibrosis stage	0 <sup>a,b</sup>	0	0	2 <sup>b</sup>	1	3	6	6	6	< 0.001

\*<sup>a</sup>: vs. Group 2 (FS = 1-4), ( $p < 0.05$ ); <sup>b</sup>: vs. Group 3 (FS=5-6), ( $p < 0.05$ ); FS: Fibrosis stage.

**Table III.** Comparison of ADC values (expressed in  $\times 10^{-6}$  mm<sup>2</sup>/s) between control group and patient groups stratified by HAI scores.

	Liver ADC values			Spleen ADC values			Normalized Liver ADC values		
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75
Control Group	1039.0 <sup>a</sup>	982.5	1129.0	794.5 <sup>a</sup>	743.0	872.0	1.28 <sup>a</sup>	1.19	1.44
Group 1 (HAI=1-6) (n=16)	1000.0 <sup>a</sup>	980.5	1084.5	857.0	809.5	897.0	1.21 <sup>a</sup>	1.10	1.34
Group 2 (HAI=7-12) (n=24)	814.5 <sup>b</sup>	794.0	896.5	876.0 <sup>b</sup>	851.0	923.0	0.93 <sup>b</sup>	0.84	1.00
Group 3 (HAI=13-18) (n=18)	866.0 <sup>b</sup>	703.0	935.0	882.0 <sup>b</sup>	823.0	956.0	0.96 <sup>b</sup>	0.83	1.10
<i>p</i> value		< 0.001			0.006			< 0.001	

\*<sup>a</sup>: vs. Group 2 (HAI = 7-12) and Group 3 (HAI = 13-18), (*p* < 0.05).

**Table IV.** The comparison of histopathological findings among patient groups stratified by HAI scores.

	Group 1 (HAI = 1-6) (n = 16)			Group 2 (HAI = 7-12) (n = 24)			Group 3 (HAI = 13-18) (n = 18)			<i>p</i> -value
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
Fibrosis stage	1 <sup>a</sup>	0	3	6	5	6	6	6	6	< 0.001
Iron grade	0 <sup>b</sup>	0	0	0	0	2	0	0	1	0.016
Steatosis (%)	3	0	5	1	0	5	5	0	10	0.667
HAI score	5 <sup>a</sup>	4	6	9	8	11	15 <sup>b</sup>	14	15	< 0.001

\*<sup>a</sup>: vs. Group 2 (HAI = 7-12) and Group 3 (HAI = 13-18) (*p* < 0.05); <sup>b</sup>: vs. Group 2 (HAI = 7-12) (*p* < 0.05).

**Table V.** The results of multiple linear regression analysis for liver ADC values in Group 1 stratified by HAI scores.

	Unstandardized coefficients		p-value	95.0% confidence interval for B	
	B	Std. Error		Lower bound	Upper bound
Constant	1082.7	30.8	0.000	1016.6	1148.8
Fibrosis stage	-40.3	13.4	0.010	-69.2	-11.5

quisition and analysis of images, is required to facilitate the early non-invasive detection of fibrosis and inflammation and to enable the preoperative assessment of patients considered for liver transplantation.

In this study, we initially found that the liver ADC and normalized liver ADC values were correlated with either the higher fibrosis stage or the HAI scores, supporting the results found in previous studies<sup>12,14,28-34</sup>. We acquired a significant relation in ADC values, showing a reduction in median liver ADC values in progression from the control group towards higher stages of fibrosis or HAI scores. For example, the median ADC value decreased from  $1021 \times 10^{-6}$  mm<sup>2</sup>/s at fibrosis stage 0 to  $844 \times 10^{-6}$  mm<sup>2</sup>/s at fibrosis stages 5-6 ( $p = 0.000$ ) and from  $1000 \times 10^{-6}$  mm<sup>2</sup>/s at HAI scores 0-6 to  $866 \times 10^{-6}$  mm<sup>2</sup>/s at HAI scores 13-18 ( $p < 0.001$ ). These decreased values seem proportional to the severity of both fibrosis and inflammation. The correlations of the normalized liver ADC values were similar to that of liver ADC values. The probable causes for the finding that the liver ADC values were significantly associated with the fibrosis stage and HAI scores are as follows. The chronic liver disease was depicted by a number of changes in liver parenchymal morphology because of progressive fibrosis and inflammation. The collagen fibers, which are the main constituents of the extracellular matrix in hepatic fibrosis, are proton-poor, and their protons are strictly bound. There is a reverse correlation between the degree of restriction to water diffusion and tissue cellularity and integrity of the cell membrane<sup>35</sup>.

Accordingly, chronic liver disease has been concomitant with low ADC values because of increased cellularity and decreased proton density. Consequently, the median ADC values of the liver tend to reduce in the advancement from lower degrees of fibrosis towards higher degrees. The influence of inflammation on ADC values was mainly due to the increase in fibrosis as described in the literature. The present study indicated that the quantitative ADC assessment is potentially valuable in the evaluation of chronic liver disease concerning both the fibrosis stage and HAI score. The ADC values of the liver, added to the routine abdominal MRI, could be considered as a valuable indicator of patient monitoring as a radiation-free and more sensible alternative to biopsy.

Our results, as also previously reported<sup>12,14,28-34</sup>, indicate that DWI is inefficient in differentiating between healthy liver and earlier stages of fibrosis and HAI scores. Still, there was a pleasant correlation in distinguishing the later stages of fibrosis and HAI scores from the normal liver and earlier stages of fibrosis and HAI scores. It would be better to detect each separate stage of fibrosis or HAI score to plan proper treatment and prevent disease progression. In our study population, stages of fibrosis in 42 patients who underwent liver transplantation are as follows: fibrosis stage 0 ( $n = 1$ ), stages 1-4 ( $n = 2$ ), and stages 5-6 ( $n = 39$ ). Nevertheless, this finding shows us that differentiating the later stages (stages 5-6) of fibrosis from the earlier ones may still be robust enough to manage the treatment of patients, and detection of later stages of fibrosis provides an important

**Table VI.** The results of multiple linear regression analysis for normalized liver ADC values in Group 1 stratified by HAI scores.

	Unstandardized coefficients		p-value	95.0% confidence interval for B	
	B	Std. Error		Lower bound	Upper bound
Constant	1.340	0.057	0.000	1.219	1.462
Fibrosis stage	-0.079	0.025	0.007	-0.132	-0.026



and key clinical factor in deciding on liver transplantation. However, the effects of the potential technical confounders on ADC values, such as liver fat and iron overload, are not comprehensible till now, and the question of DWI reliability remains undetermined.

The deposition of fat and iron may present simultaneously in chronic liver disease. Although the relationship between those deposits and degree of either fibrosis or inflammation is not well documented, in our study population, both hepatic steatosis and iron overload were found to be more prevalent in patients with higher stages of fibrosis but randomly scattered in groups with low, intermediate, or high HAI scores. This finding highlights the probable mechanisms of the deposition of fat and iron in chronic liver diseases.

In the current study, histopathological findings were further examined between patient groups stratified by either stage of fibrosis or HAI scores. In all groups stratified by fibrosis stages, the degree of steatosis was not significantly different. Iron grades in group 2 (FS = 1-4) were significantly different from group 3 (FS = 5-6), and the HAI scores were significantly different between all groups. According to HAI score stratification, iron grades in group 1 (HAI = 1-6) were significantly different from group 2 (HAI = 7-12), whereas fibrosis stages in group 1 (HAI = 1-6) were significantly different from groups 2 (HAI = 7-12) and 3 (HAI = 13-18). Therefore, although there was a slight overlap in the median ADC values, we revealed that the iron grades, fibrosis stages, and HAI scores showed significant differences between all or some patient groups with chronic liver disease.

In our study, the reliability of DW-MRI was mainly analyzed for the staging of liver fibrosis and inflammation in the presence of liver fat and iron accumulation. Reliability is essential and should be kept in mind while evaluating the ADC values as a surrogate marker of fibrosis or inflammation. Theoretically, and in our daily experience, liver fat and iron could be possible confounders in the measurements of ADCs. Hepatic steatosis has been shown to influence liver ADC values in an animal model of liver fibrosis<sup>36</sup>. A probable explanation for this increase in ADC values in groups without fatty liver is that increased fat content of hepatocytes and extracellular area could restrict the diffusion of water and lower ADC values<sup>21</sup>. The iron overload can decrease the ADC values in patients with cirrho-

sis<sup>22</sup>. The probable explanations for the decrease in ADC measurements in iron overload may relate to its ability to shorten T2 and T2\* values or through other mechanisms in which iron-related magnetic field inhomogeneity results in shifts on b values<sup>22</sup>. In our analysis, a multiple linear regression model in group 1 stratified by HAI score showed that iron grade and degree of steatosis did not significantly contribute to the liver ADC and normalized liver ADC values. Therefore, should not be taken into account in the assessment of liver ADCs in the staging of fibrosis or scoring of inflammation. However, the effects of fibrosis stages on liver ADC and normalized liver ADC values were only significant, confirming the literature. Further comprehensive studies, including those performed in more homogeneous patient groups with different scores of fibrosis and HAI, are required to confirm these results and shed light on the definite effect of fat and iron on ADC values observed in chronic liver disease.

Although DWI in chronic liver disease was extensively studied, the effects of fat and iron on ADC values were not clear until now, and the question of DWI reliability remains undetermined. Bulow et al<sup>24</sup> analyzed DWI for the staging of liver fibrosis in the presence of fat and iron, and these biological factors were found to be effective in the evaluation of the quantitative ADCs. The discordant findings of our study compared with that of Bulow et al (24) may be explained partly by variant patient populations. The majority of the patient population (n = 60) in their study consisted of patients with no fibrosis (fibrosis stage 0), whereas we included the patients with fibrosis stages 5 and 6 (n = 42) by a majority. The probable reason for the discrepancy between our results and theirs is unknown. Our study confirms a previous study that indicated the liver ADC values were not significantly influenced by the deposition of fat and iron in chronic liver disease<sup>18</sup>. This fact enables the radiologist to confidently utilize DWI even in the presence of fat and iron.

In addition to examining the relationship between ADC values and fibrosis scores, we also investigated the relationship between ADC values and HAI scores. To our knowledge, few studies have evaluated the reliability of ADC values to distinguish HAI scores in the presence of fat and iron. The influence of HAI scores on ADC values has been reported previously<sup>18</sup>, and our results confirmed that inflammation correlates with a decrease in liver ADCs and normalized liver ADCs.

The spleen was used as a reliable standard reference organ to normalize liver ADC values<sup>12,28,37</sup>. The normalized liver ADC values increased the diagnostic accuracy in detecting liver fibrosis and cirrhosis<sup>12</sup>. Likewise, in our study, normalized liver ADC values were significantly different between the control group and group 2 (FS = 1-4), while liver ADC values were not. Previous reports found that spleen ADC values did not differ between healthy subjects and patients with chronic liver disease or between different stages of liver fibrosis, which suggested that the spleen ADC values remain unaffected by chronic liver disease<sup>18,38</sup>. However, in our study, the ADC measurements of the spleen were higher in-patient groups than in the control group, which was consistent with another prior report<sup>39</sup>. The mechanism responsible for increased spleen ADC values is probably related to increased portal venous pressure, leading to vasogenic edema induced by sinusoidal congestion and dilatation.

There were some limitations of our study that result from its retrospective projection. Firstly, the study included a nonhomogeneous study population consisting of patients with chronic liver disease with different etiological factors. Although this limitation may have generated bias during the study, we believe the results are acceptable. Secondly, small number of patients with earlier stages of fibrosis was the weakness of this study. A controlled prospective study including a sufficient number of patients with similar diseases and stages may be necessary to validate our results. Another limitation of this study is that the liver biopsy was the reference standard. The possibility of sampling error in the examined portion of the biopsied or explanted liver tissue may limit our study. Our study also has additional limitations related to the technique that was used. We acquired the DWI with a minimum *b* value of 50. Therefore, diffusion-weighted data that carried over low *b*-values (<50 s/mm<sup>2</sup>), which is susceptible to micro perfusion, was not included in our findings. Girometti et al<sup>23</sup> described that the lower *b*-values, depending on the effects of micro perfusion, procure better fibrosis detection. If a wider range of low *b*-values (<50 s/mm<sup>2</sup>) were used, effects of perfusion on ADC values could also be included; this idea should be used in further studies. Finally, ADC measurements are known to be subject to inter- and intraobserver variability, which is why three ROIs were measured and only the median values were recorded.

## Conclusions

The data from our study, combined with findings of previous studies, indicate that the ADC values of the liver and spleen are potentially reliable and valuable for the diagnosis of liver fibrosis and inflammation. We also essentially conclude that these ADC values were significantly affected by fibrosis stages but not by liver fat and iron. Therefore, hepatic deposition of fat or iron may not be taken into account while using ADC values as an alternate marker of liver fibrosis and inflammation. Further longitudinal studies are needed to investigate the relationship between ADC values and fat and iron deposition in chronic liver disease, as well as to explore the ability of this noninvasive technique to distinguish the exact degree of fibrosis stage and HAI score.

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### Conflict of Interest

The Authors declare that they have no conflict of interests.

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None.

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### Ethical Approval

All patients or their relatives provided written informed consent for their clinical records to be used for research purposes. Approval has been obtained from the Local Ethics Committee of Inonu University.

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### Availability of Data

The data used in the present study are available from the corresponding author upon request.

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### Authors' Contribution

A.S.K. is the principal author of this study. A.S.K. and B.K. developed the theory and identified the cases to be included in the study. A.S.K. analyzed and collected all data from cross-sectional images. N.S. assessed specimens and collected all histopathological results. S.Y. encouraged and supervised the findings of this work. A.S.K., Z.Y.M., L.K., and B.K. carried out the statistical analysis. All authors discussed the results and contributed to the final manuscript.

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