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# Intravoxel incoherent motion diffusion-weighted imaging for assessment of histologic grade of hepatocellular carcinoma: comparison of three methods for positioning region of interest

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## Abstract

**Objectives** To prospectively compare the diagnostic performances of three methods of region of interest (ROI) placement for the measurements of intravoxel incoherent motion (IVIM) diffusion-weighted MR imaging in differentiating the histologic grade of hepatocellular carcinoma (HCC).

**Methods** Eighty-seven patients with 91 newly diagnosed HCCs were studied using IVIM imaging. Two attending radiologists separately identified the selection of tumour tissue for ROI positioning. Three different ROI positioning methods, namely the whole tumour volume (WTV) method, three-ROI method and one-section method, were used for the measurement. Kruskal–Wallis rank test or one-way ANOVA was used to compare the difference in IVIM parameters and ADC across the three different ROI positioning methods. Spearman correlation analysis was used to determine the correlation between each parameter and Edmondson–Steiner (E–S) grade. Receiver operating characteristics (ROC) curve analyses were performed to evaluate the diagnostic performance.

**Results** For the ADC and $ADC_{slow}$, the mean value measured by using the WTV method was significant higher than the one-section and three-ROI methods (all $p < 0.01$). For the $ADC_{slow}$, the highest area under curve (AUC) with a value of 0.969 was obtained by using the WTV method, followed by the one-section method (AUC = 0.938) and three-ROI method (AUC = 0.873). Additionally, for the ADC, AUC values were 0.861 for WTV method, 0.840 for one-section method and 0.806 for three-ROI method.

**Conclusions** Different ROI positioning methods used significantly affect the IVIM parameters and ADC measurements. Measurements of $ADC_{slow}$ value derived from WTV method entailed the highest diagnostic performance in grading HCC.

**Key Points**

- *Diffusion MRI is useful for non-invasively differentiating the histologic grade of hepatocellular carcinoma.*
- *Different ROI positioning methods used significantly affect the IVIM parameters and ADC measurements.*
- *IVIM model is advantageous over mono-exponential model for assessing the histologic grade of hepatocellular carcinoma.*

**Keywords** Magnetic resonance imaging · Hepatocellular carcinoma · Diagnostic imaging

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**Abbreviations**

| | |
|---|---|
| ADC | Apparent diffusion coefficient |
| $ADC_{fast}$ | Pseudo ADC |
| $ADC_{slow}$ | True ADC |
| AUC | Area under curve |
| DWI | Diffusion-weighted imaging |
| E–S | Edmondson–steiner grade |
| *f* | Perfusion fraction |
| HCC | Hepatocellular carcinoma |
| IVIM | Intravoxel incoherent motion |
| ROC | Receiver operating characteristics |

ROI Region of interest
WTV Whole tumour volume

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second leading cause of cancer-related deaths [1]. Hepatic resection and transplantation have been regarded as the most effective treatment of HCC [2]; however, the overall prognosis still remains poor because of diagnosis at a late stage and high recurrence [3]. Pathological differentiation grade is one of the most important factors for the recurrence and prognosis of HCC, as poorly differentiated HCC is often associated with worse survival compared with well- differentiated and moderately differentiated HCC [4]. Thus, accurate preoperative grading of HCC is of great importance in predicting prognosis.

Intravoxel incoherent motion (IVIM) diffusion-weighted MR imaging is a non-invasive approach to probe molecular diffusion of water on the microscopic scale without contrast administration, and the diffusion of water can be quantitatively described by apparent diffusion coefficient (ADC) values [5–7]. Furthermore, with multiple *b* values calculated by using a bi-exponential model, IVIM enables one to determine true molecular diffusion and perfusion from the blood microcirculation in the capillary networks compared with diffusion-weighted imaging (DWI) [8, 9]. Recently, investigators have demonstrated that IVIM can be useful in the grading of HCC and thus improve the prediction of survival after resection [10–16]. However, literature on the use of IVIM for the grading of HCC is inconsistent. While some investigators demonstrated strong correlation between ADC values and pathological differentiation grade [10–14], others reported that no correlation was obtained [15]. Several studies demonstrated significantly higher ADC values in well-differentiated HCC compared with moderately differentiated HCC [10, 11, 13], whereas others reported no difference [12]. These conflicting results may be attributed to several factors, including field strength and the heterogeneity of the tumour tissue as whether the representative part of tumour tissue or the whole tumour volume should be included in the region of interest (ROI). To our knowledge, few studies have compared the diagnostic performance of IVIM parameters and DWI in terms of their capability to evaluate the pathological differentiation grade of HCC by using different methods of ROI positioning.

The purpose of this study was to prospectively determine the diagnostic performance of IVIM parameter and ADC in differentiating the pathological differentiation grade of HCC, and to subsequently evaluate the effect of different ROI positioning methods on the IVIM parameters and ADC measurements.

## Materials and methods

### Participants

This study was approved by the institutional review board, and written informed consent was obtained from all patients. This study was conducted in accordance with the Declaration of Helsinki. Between January 2016 and April 2017, 245 consecutive patients suspected of having HCC or identified with malignant hepatic lesions on the basis of previous CT or ultrasonography examinations underwent a preoperative MR examination with IVIM pulse sequence. Among them, 158 subjects were excluded for the following reasons: (1) histopathological findings were not available because operations were not performed ($n$ = 26); (2) history of preoperative treatment (radiofrequency ablation ($n$ = 16), transarterial chemoembolisation ($n$ = 85) or a combination of these ($n$ = 12)); (3) lesions histopathologically diagnosed as other tumours rather than as HCC ($n$ = 14); (4) the image quality was unsatisfactory ($n$ = 5).

### MR imaging

For all examinations, studies were carried out by using a 3.0-T MR system (Discovery MR750, GE Healthcare). An eight-channel phased-array torso coil (GE Medical System) was used for all measurements. IVIM was performed by using an echo-planner imaging in the axial plane with respiratory gating. The parallel imaging was used to shorten the scanning time and reduce image distortion. Thirteen *b* values from 0 to 1200 s/mm$^2$ (0, 10, 20, 40, 80, 100, 150, 200, 400, 600, 800, 1000, 1200) were used and the NEX for each *b* was 1, 6, 4, 2, 2, 2, 1, 1, 2, 4, 6, 6 and 8, respectively. The routine MR imaging sequences included in the standardised scanning protocol were respiratory-triggered axial T2-weighted fast recovery fast spin echo sequence with fat suppression, in and out of phase T1-weighted imaging acquired with fast spoiled gradient recalled dual echo sequence, pre- and postcontrast liver acceleration volume acquisition (LAVA) were acquired with gradient recalled echo sequence at arterial phase (20 s), portal venous phase (60 s) and delayed phase (180 s) after injection of 0.1 mmol/kg gadopentetate dimeglumine (Magnevist; Bayer Schering Pharma AG) at a rate of 2 mL/s. The detailed parameters of each acquisition sequence are shown in Table 1.

### Imaging analysis

All the MR images were obtained and transferred to the workstation (Advantage workstation 4.6; GE Medical System). The ADC value was calculated by using the following mono-exponential model:

**Table 1** Parameters of IVIM, T1-weighted imaging, T2-weighted imaging and LAVA sequence

| Parameter | IVIM | In/out of phase T1-weighted imaging | T2-weighted imaging | LAVA |
|---|---|---|---|---|
| Repetition time (ms) | 6500–7500 | 150 | 6500–7200 | 4.3 |
| Echo time (ms) | 84.7 | 2.4/5.8 | 83 | 1.7 |
| Field of view ($cm^2$) | 40 × 30 | 42 × 42 | 40 × 40 | 40 × 32 |
| Scan matrix | 80 × 128 | 288 × 192 | 320 × 320 | 260 × 180 |
| Slice thickness (mm) | 6 | 6 | 6 | 4 |
| Slice gap (mm) | 2 | 2 | 2 | – |
| Motion compensation | Respiratory trigger | Breath hold | Respiratory trigger | Breath hold |
| Slice per station | 24 | 24 | 24 | 52 |
| Parallel imaging factor | 2 | 2 | 2 | 2 |
| Partial Fourier factor | 1 | 1 | 1 | 1 |
| Pixel size (mm) | 5 × 3.1 | 1.5 × 2.2 | 1.25 × 1.25 | 1.5 × 2.2 |
| H/F coverage (mm) | 192 | 192 | 192 | 208 |
| Fat suppression | Fat saturation | None | Fat saturation | Dixon |
| Number of excitations | – | 1 | 2 | 1 |

Repetition time of IVIM and T2WI is automatically calculated on the basis of the respiratory rate and respiratory intervals number

*H/F* head to feet

$$\frac{S(b)}{S(0)} = \exp(-b \times ADC)$$

where S($b$) is the signal intensity in the pixel with diffusion gradient $b$, and $S(0)$ is the signal intensity in the pixel without diffusion gradient. For the parameter of true ADC ($ADC_{slow}$), pseudo ADC ($ADC_{fast}$) and perfusion fraction ($f$), the following bi-exponential IVIM model was used:

$$\frac{S(b)}{S(0)} = f\exp(-b \times ADC_{fast}) + (1-f)\exp(-b \times ADC_{slow})$$

All the MR images were analysed separately by two radiologists (M.W., Z.H., with 13 and 7 years of experience in reading MR images, respectively) who were blinded to the histopathological results. Identification of selection of the representative tumour tissue for ROI placement was performed on the DW images ($b$ = 400). T2-weighted and dynamic contrast-enhanced MR images were also used to avoid the haemorrhagic, calcified and necrotic areas. Three different kinds of ROI positioning methods were used for the measurement (Fig. 1): (a) placement of three ROIs on the solid tumour components (three-ROI method); (b) placement of single freehand ROI to outline the tumour on one section (one-section method); (c) placement of ROIs outlining the tumour on the whole tumour volume (WTV). The shape, size and position of ROIs were the same on ADC, $ADC_{slow}$, $ADC_{fast}$ and $f$ map.

## Histopathological examinations

All the surgically resected hepatic specimens were used for the pathological evaluation. Identification of the pathological differentiation grade of HCC was blindly evaluated. According to the Edmondson–Steiner (E–S) grade classification [17], the pathological differentiation grade was subcategorised as Edmondson–Steiner grade 1 to 4, and the determined pathological differentiation grade was used to analyse the radiological-pathological correlation.

## Statistical analysis

All statistical analyses were performed by using a statistical software package (SPSS19.0; SPSS Inc, Chicago, IL, USA). Numerical variance is indicated as the mean and standard deviation. Numerical values were tested for normal distribution. Intraclass correlation coefficient (ICC) was firstly used to determine the reliability between the two independent radiologists in relation to each parameter; ICC values less than 0.5 are indicative of poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability and values greater than 0.90 indicate excellent reliability. Kruskal–Wallis rank test or one-way ANOVA

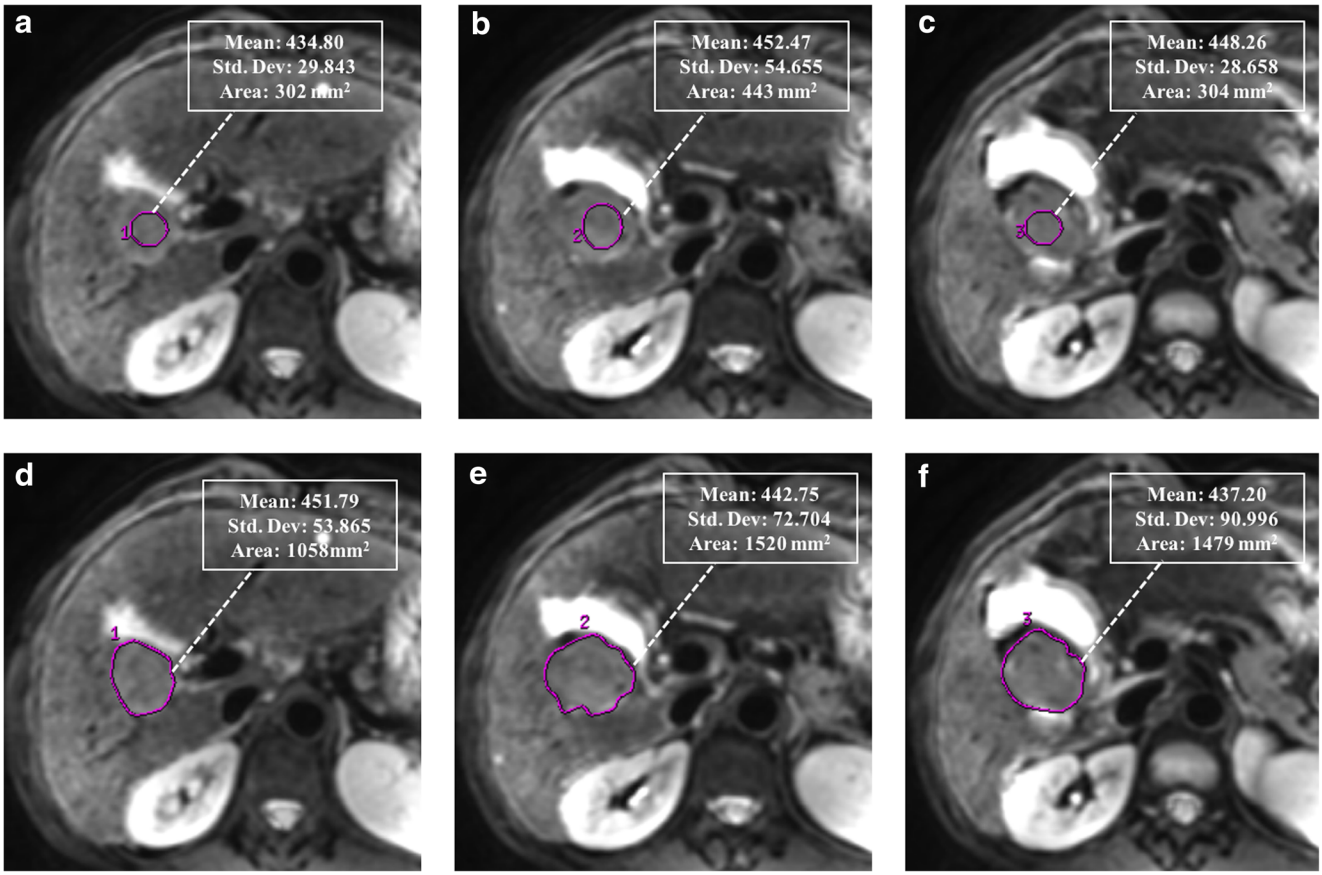


**Fig. 1** Three-ROI method (top row) and WTV method (bottom row) in a 65-year-old man; ADC measurement obtained with the circular ROI and freehand ROI

was used to compare the difference in IVIM parameters and ADC across the three different ROI positioning methods and the E–S grade, and the difference of multiple comparisons between two varieties were compared by using LSD-t or the Games–Howell test. Mean values of the diffusion parameters from the two radiologists were used for the diagnostic performance analysis. Spearman correlation analysis was used to determine the correlation between the IVIM and DWI parameters and E–S grade; a correlation coefficient rho ($r$) less than 0.25 indicates a poor relationship, values between 0.25 and 0.50 indicate a moderate relationship, values between 0.5 and 0.75 indicate a good relationship and values greater than 0.75 indicate an excellent relationship. Receiver operating characteristics (ROC) curve analyses were performed to evaluate the diagnostic performance of each IVIM parameter and DWI in distinguishing the E–S (1–2) from the E–S (3–4) groups, and to determine the optimal parameter for the grading of HCC. The cut-off point was selected by using the maximised values of Youden indexes, and the sensitivity, specificity at the threshold value for each parameter were determined. $Z$ test was used to compare the area under ROC curves (AUC) in different ROI positioning methods.

## Results

### Patient characteristics

The final population included for analysis consisted of 87 patients (63 men and 24 women; mean age, 53.20 ± 10.66 years; range, 30–74 years) with 91 newly diagnosed HCCs. The tumour diameter ranged from 21 to 152 mm (81.38 ± 26.10 mm). For the study group, there were 81 patients with Child–Pugh A, 4 patients with Child–Pugh B and 2 with Child–Pugh C. Histologically, there were 15 HCCs with E–S grade 1, 41 HCCs with E–S grade 2, 23 HCCs with E–S grade 3 and 12 HCCs with E–S grade 4.

### Interobserver reproducibility

For the three-ROI method, ICC values were 0.916 for ADC, 0.935 for $ADC_{slow}$, 0.914 for $ADC_{fast}$ and 0.862 for $f$. For the one-section method, ICC values were 0.925 for ADC, 0.940 for $ADC_{slow}$, 0.888 for $ADC_{fast}$ and 0.832 for $f$. For the WTV method, ICC values were 0.959 for ADC, 0.957 for $ADC_{slow}$, 0.904 for $ADC_{fast}$ and 0.893 for $f$. The Bland–Altman analysis results for

interobserver agreement of the IVIM and ADC parameters across three different ROI positioning methods are shown in Fig. 2.

## Comparison of three different ROI positioning methods in terms of IVIM parameters and ADC measurement

For the ADC, the mean value measured by using the WTV method was significant higher than the one-section method (R1, $p < 0.001$; R2, $p < 0.001$) and three-ROI method (R1, $p < 0.001$; R2, $p < 0.001$); statistical significance was also obtained between the one-section and three-ROI method (R1, $p = 0.017$; R2, $p < 0.001$). For the $ADC_{slow}$, the mean valued measured by using the WTV method was significantly higher than the one-section method (R1, $p < 0.001$; R2, $p = 0.001$) and three-ROI method (R1, $p < 0.001$; R2, $p < 0.001$), and the value between one-section and three-ROI methods also showed a difference (R1, $p = 0.001$; R2, $p < 0.001$). However, both the $ADC_{fast}$ value (R1, $p = 0.947$; R2, $p = 0.688$) and $f$ value (R1, $p = 0.673$; R2, $p = 0.905$) did not show a statistically significant difference in the three different ROI positioning methods. The mean values of IVIM parameters and ADC are listed in Table 2.

## IVIM parameters and ADC in the differentiation of the grade

Both ADC and $ADC_{slow}$ values showed statistical significance in grading HCC; however, $ADC_{fast}$ and $f$ can not be used to evaluate the grade of HCC (Table 3) (Figs. 3 and 4). For the ADC, the E–S grade 1 value determined by using the three different ROI positioning methods was significantly higher when compared with E–S grade 2, 3 and 4 (all $p < 0.01$); and a significant difference between E–S grade 3 and 4 was also obtained by using three-ROI ($p = 0.004$), one-section ($p = 0.001$) and WTV ($p = 0.022$) methods. However, no difference between E–S grade 2 and 3 was obtained by using the three-ROI ($p = 0.319$) or one-section method ($p = 0.322$), in contrast to the WTV ($p = 0.001$) method. For the $ADC_{slow}$, multiple comparisons between each pair of different grades showed statistical significance by using three-ROI, one-section and WTV methods (all $p < 0.001$). Figure 5 shows the quantitative comparison of difference in different ROI positioning methods among E–S grades 1 to 4 of HCC.

## Diagnostic performance of IVIM parameters and DWI

The receiver operating characteristics curves (ROC) in distinguishing the E–S 1–2 group from the E–S 3–4 group are shown in Fig. 6. For the $ADC_{slow}$, the highest area under

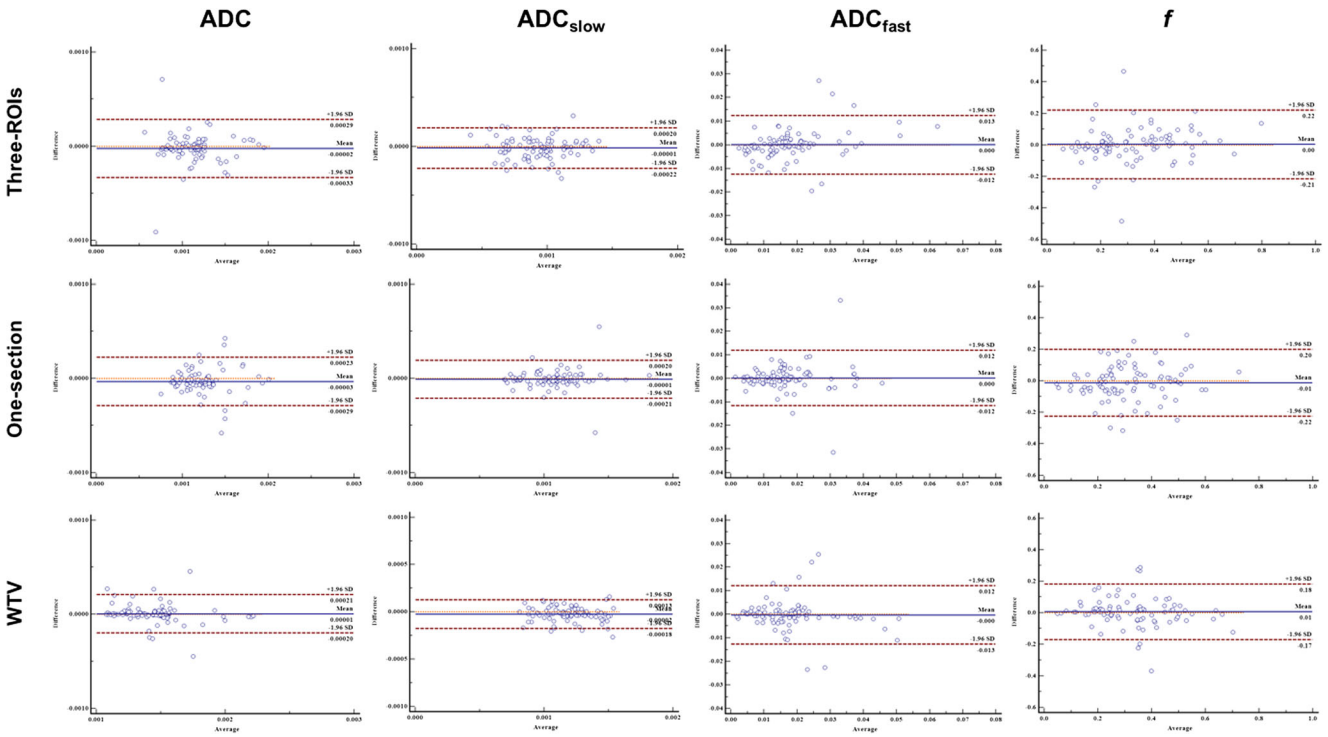


**Fig. 2** Bland–Altman analysis for IVIM parameters and ADC between two radiologists. The differences were relatively small

**Table 2** IVIM and DWI parameters of the different ROI positioning methods in HCC

| Parameters | Observer | Three-ROI | One-section | WTV | $x^2(F)$ | *p* value |
|---|---|---|---|---|---|---|
| ADC[†] | R1 | 1.11 ± 0.28 | 1.22 ± 0.25 | 1.45 ± 0.25 | 77.773 | < 0.001 |
| | R2 | 1.13 ± 0.28 | 1.25 ± 0.25 | 1.44 ± 0.27 | 61.643 | < 0.001 |
| $ADC_{slow}$ | R1 | 0.92 ± 0.22 | 1.06 ± 0.22 | 1.18 ± 0.18 | 33.996 | < 0.001 |
| | R2 | 0.94 ± 0.22 | 1.07 ± 0.21 | 1.20 ± 0.19 | 36.217 | < 0.001 |
| $ADC_{fast}$[a] | R1 | 17.79 ± 12.23 | 16.67 ± 9.6 | 17.30 ± 10.32 | 0.109 | 0.947 |
| | R2 | 17.65 ± 10.19 | 16.44 ± 9.40 | 17.56 ± 11.12 | 0.794 | 0.688 |
| *f*[a] | R1 | 0.32 ± 0.17 | 0.31 ± 0.15 | 0.32 ± 0.14 | 0.791 | 0.673 |
| | R2 | 0.31 ± 0.15 | 0.32 ± 0.14 | 0.32 ± 0.15 | 0.200 | 0.905 |

ADC, $ADC_{slow}$, $ADC_{fast}$ are in units of $\times 10^{-3}$ $mm^2/s$, *f* is in units of 100%

*ADC* apparent diffusion coefficient, $ADC_{slow}$ true ADC, $ADC_{fast}$ pseudo ADC, *CV* coefficient of variation, *R1* radiologist 1, *R2* radiologist 2

[a] Data are compared with Kruskal–Wallis test

curve (AUC) with a value of 0.969 (95% confidence interval [CI] 0.909–0.994) was obtained by using the WTV method, followed by the one-section method with the value of 0.938 (CI 0.867–0.978) and 0.873 (CI 0.787–0.943) for the three-ROI method. The AUC values for the WTV method were statistically greater than three-ROI method ($Z$ = 2.895, $p$ = 0.003); however, no statistically significant difference was obtained when comparing the AUC values between the one-section and three-ROI method ($Z$ = 1.587, $p$ = 0.112) or the one-section and WTV method ($Z$ = 1.264, $p$ = 0.206). Additionally, for the ADC, AUC values were 0.861 (CI 0.773–0.925) for the WTV method, 0.806 (CI 0.710–0.882) for the three-ROI method and 0.840 (CI 0.748–0.90) for the one-section method; however, no statistically significant differences were obtained from the three-ROI and one-section method ($p$ = 0.287), three-ROI and WTV method ($p$ = 0.129) or one-section and WTV method ($p$ = 0.392). Table 4 shows the sensitivity and specificity of IVIM-DWI and conventional DWI parameters at optimal cut-off values in differentiating E–S 1–2 from E–S 3–4 groups.

## Correlations between IVIM and DWI parameters and histopathological results

For the ADC, Spearman correlation analysis demonstrated that there was good inverse correlation between the pathologic differentiated grade and ADC value measured by using the

**Table 3** IVIM and DWI parameters of the different pathological grade in HCC

| Parameter | Edmondson–Steiner grade | | | | | |
|---|---|---|---|---|---|---|
| | 1 ($n$ = 15) | 2 ($n$ = 41) | 3 ($n$ = 23) | 4 ($n$ = 12) | $x^2(F)$ | *p* value |
| ADC[a] | | | | | | |
| Three-ROI | 1.46 ± 0.28 | 1.12 ± 0.21 | 1.03 ± 0.20 | 0.85 ± 0.08 | 42.480 | < 0.001 |
| One-section | 1.53 ± 0.26 | 1.24 ± 0.15 | 1.16 ± 0.20 | 0.96 ± 0.08 | 44.767 | < 0.001 |
| WTV | 1.77 ± 0.29 | 1.47 ± 0.18 | 1.31 ± 0.14 | 1.19 ± 0.10 | 46.296 | < 0.001 |
| $ADC_{slow}$ | | | | | | |
| Three-ROI | 1.20 ± 0.17 | 0.96 ± 0.16 | 0.83 ± 0.11 | 0.67 ± 0.04 | 35.365 | < 0.001 |
| One-section[a] | 1.33 ± 0.17 | 1.13 ± 0.13 | 0.96 ± 0.10 | 0.76 ± 0.04 | 63.625 | < 0.001 |
| WTV | 1.47 ± 0.04 | 1.23 ± 0.09 | 1.07 ± 0.06 | 0.90 ± 0.05 | 174.277 | < 0.001 |
| $ADC_{fast}$[a] | | | | | | |
| Three-ROI | 22.21 ± 10.98 | 17.99 ± 10.23 | 16.50 ± 12.73 | 13.49 ± 6.88 | 5.552 | 0.136 |
| One-section | 18.21 ± 9.21 | 18.54 ± 9.62 | 14.39 ± 7.73 | 11.864 ± 6.96 | 6.358 | 0.095 |
| WTV | 20.75 ± 12.74 | 18.62 ± 8.86 | 16.19 ± 11.66 | 11.60 ± 5.89 | 7.446 | 0.059 |
| *f* value[a] | | | | | | |
| Three-ROI | 0.33 ± 0.12 | 0.30 ± 0.13 | 0.31 ± 0.13 | 0.37 ± 0.25 | 0.603 | 0.896 |
| One-section | 0.33 ± 0.10 | 0.29 ± 0.10 | 0.32 ± 0.15 | 0.36 ± 0.21 | 0.635 | 0.888 |
| WTV | 0.33 ± 0.12 | 0.30 ± 0.11 | 0.33 ± 0.14 | 0.37 ± 0.22 | 2.064 | 0.559 |

[a] Data are compared with Kruskal–Wallis test

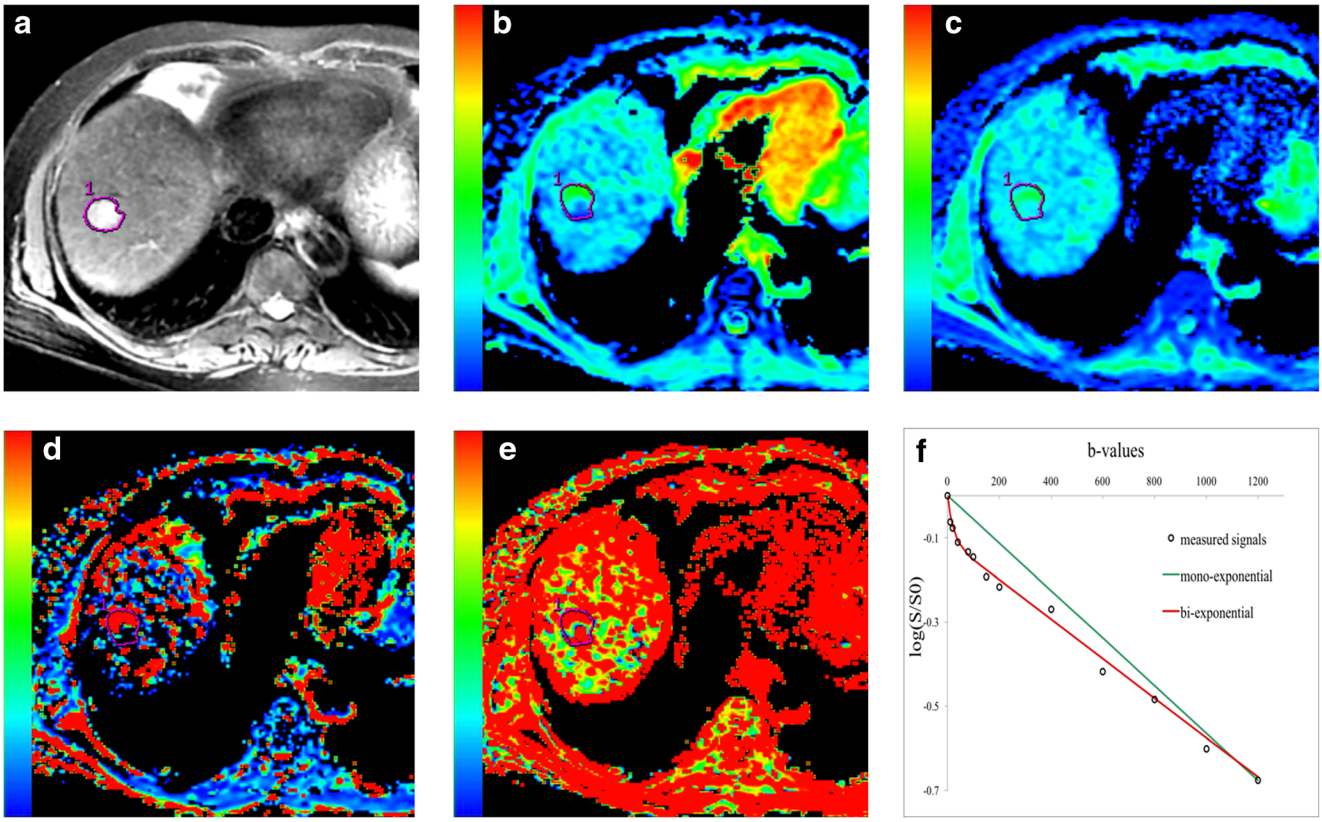


**Fig. 3** MR images in a 63-year-old man with surgically proven HCC of E–S grade 2. **a** T2-weighted image. **b** ADC map. **c** $ADC_{slow}$ map. **d** $ADC_{fast}$ map. **e** *f* map. **f** IVIM and DWI fitting of the diffusion signal decay. The tumour demonstrates high signal intensity on T2-weighted image and slightly high signal on ADC and $ADC_{slow}$ map, which indicated a moderately differentiated HCC; the IVIM model achieved significant better fitting of the moderately differentiated HCC than the DWI

WTV method ($r = -0.71$, $p < 0.001$), as well as one-section method ($r = -0.69$, $p < 0.001$) and three-ROI method ($r = -0.66$, $p < 0.001$). Moreover, for the $ADC_{slow}$, excellent inverse correlation was depicted by using WTV ($r = -0.90$, $p < 0.001$), one-section method ($r = -0.84$, $p < 0.001$) and three-ROI method ($r = -0.75$, $p < 0.001$).

## Discussion

In the present study, the mean ADC and $ADC_{slow}$ values derived from the WTV method were significantly higher than the one-section method and three-ROI method, and the value also differed between the one-section and three-ROI method. These results were in accordance with those of Blazic et al [18], who also demonstrated higher ADC values measured by using the WTV method in evaluating rectal cancer. Our results demonstrated that different ROI positioning methods substantially influence the ADC and $ADC_{slow}$ value measurement; however, regarding the $ADC_{fast}$ and *f*, no differences were obtained from these three different ROI positioning methods which could be explained by the large variation in perfusion component of the tumour, so the effect of the ROI positioning methods could be neglected.

Our results indicated that both ADC and $ADC_{slow}$ showed statistical significance in assessing tumour cell differentiated grade, and the numeric values of the poorly differentiated HCC were lower than the moderately differentiated and well-differentiated groups. Histologically, as the tumour become more poorly differentiated during hepatocarcinogenesis, the cellularity and nuclear to cytoplasmic ratio increased, which could decrease the extracellular space and limit water diffusion [14, 19]; eventually, the increased cellularity could decrease the ADC and $ADC_{slow}$ values in the poorly differentiated group. Furthermore, the numerical values of the ADC were greater than the $ADC_{slow}$ in each group and that was because the contribution of the perfusion component could be removed by $ADC_{slow}$ derived from the IVIM model. Previous studies on IVIM and DWI in the evaluation of pathological differentiation by Woo et al [10] and Granata et al [16] also demonstrated higher ADC and $ADC_{slow}$ values in well-differentiated HCC compared with moderately and poorly differentiated HCC, observations which were consistent with our results.

It is noteworthy that the IVIM-derived $ADC_{slow}$ showed significantly higher diagnostic performance compared with ADC in differentiating the E–S 1–2 from E–S 3–4 group. The better diagnostic performance of $ADC_{slow}$ can be

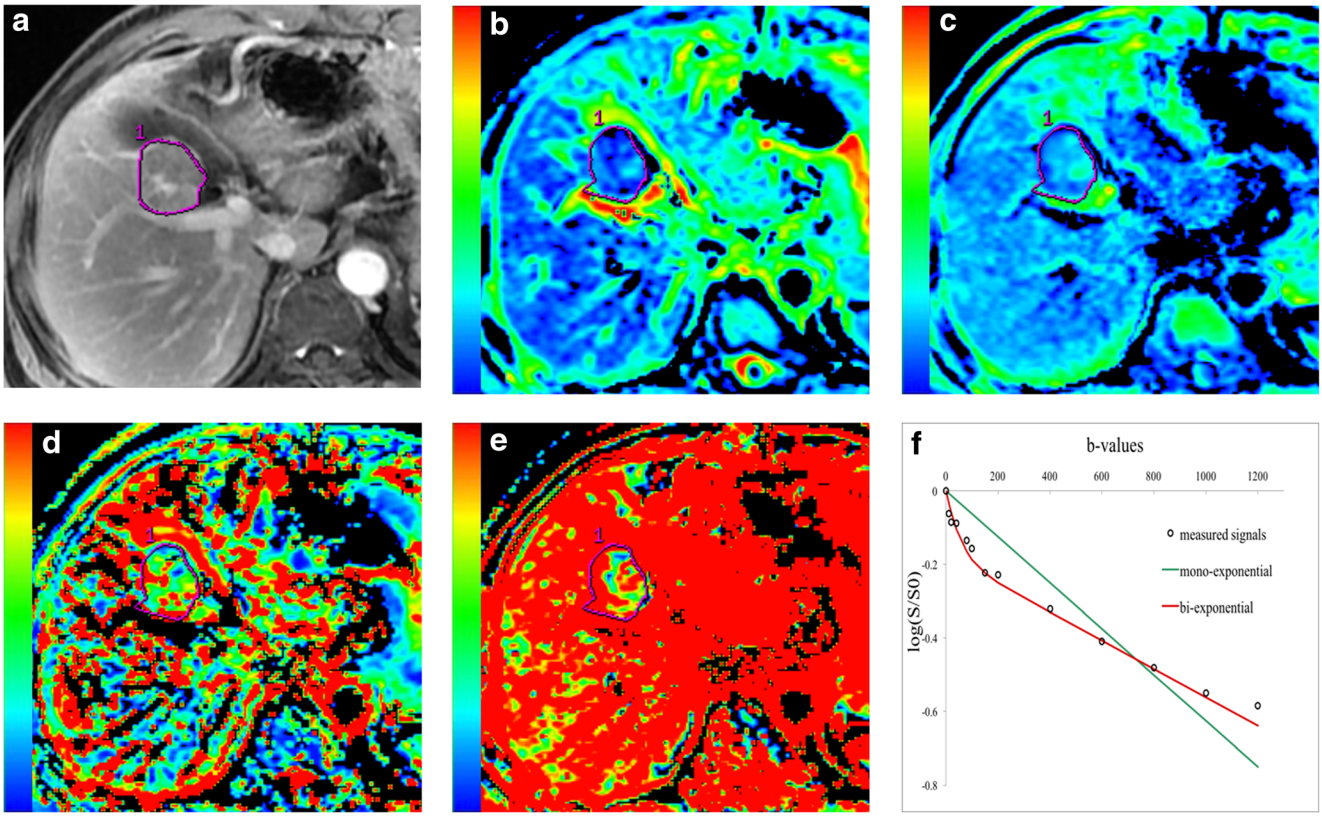


**Fig. 4** MR images in a 58-year-old woman with surgically proven HCC of E–S grade 3. **a** Portal venous phase image. **b** ADC map. **c** $ADC_{slow}$ map. **d** $ADC_{fast}$ map. **e** *f* map. **f** IVIM and DWI fitting of the diffusion signal decay. The tumour demonstrates a slightly high signal intensity on T2-weighted image and blue areas were observed on the ADC and $ADC_{slow}$ map, which indicate a poorly differentiated hepatocellular carcinoma. IVIM-DWI model achieved significant better fitting than the DWI

explained by the fact that ADC is a non-specific parameter and which could not only be influenced by the microcirculation-related perfusion but also by the cellularity-related diffusion. In our study, the WTV method is superior to three-ROI method and one-section method for assessing the pathological differentiation grade of HCC. Compared with the three-ROI method and one-section method, the use of WTV method achieved the highest AUC values and Youden index for grading HCC, and that was because the WTV method involves the sampling of the whole tumour which can

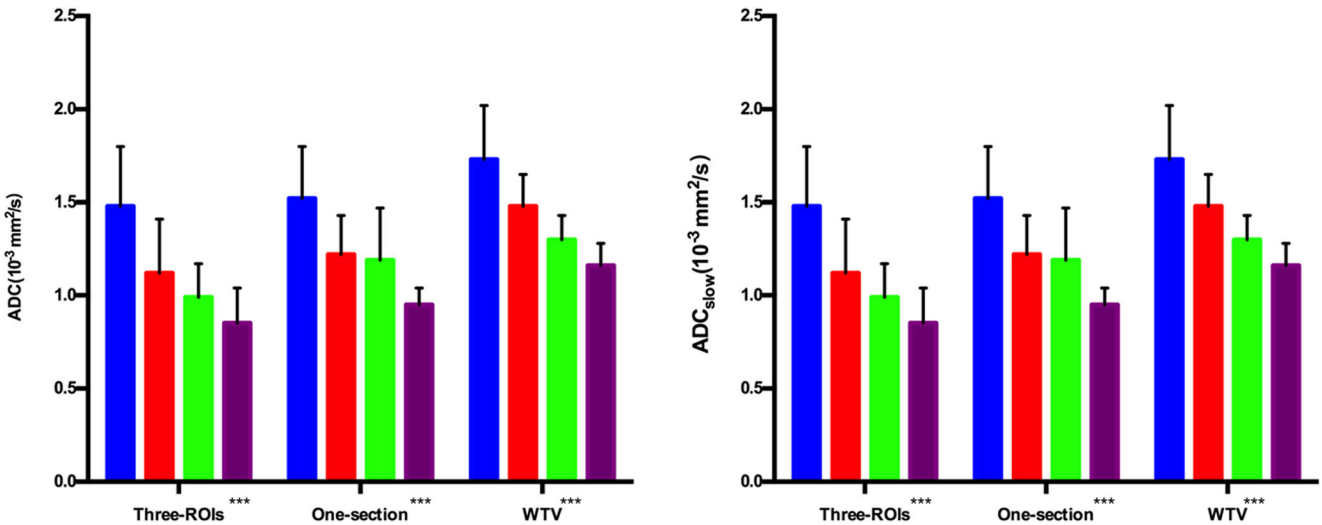


**Fig. 5** ADC and $ADC_{slow}$ value of E–S grade 1–4 measured by using three-ROI, one-section and WTV method. The ADC and $ADC_{slow}$ of the E–S grade 1 were significant higher than E–S grade 2, E–S grade 3 and E–S grade 4. Differences of the ADC and $ADC_{slow}$ in grading HCC across the three ROI positioning were obtained ($^{***}p < 0.001$)

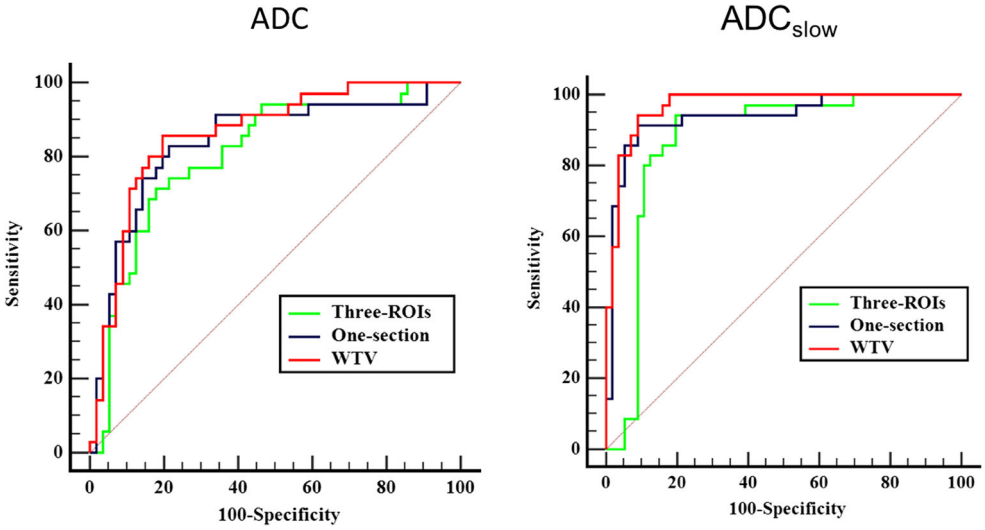


**Fig. 6** ROC curves of IVIM parameters and ADC measured by using three different kinds of ROI positioning methods. AUC value of ADC with one-section, three-ROI and WTV method were 0.840, 0.806 and 0.861, respectively. AUC value of $ADC_{slow}$ with one-section, three-ROI and WTV method were 0.938, 0.873 and 0.969, respectively

effectively reduce the bias caused by sampling errors. Furthermore, the intratumour heterogeneity can be better captured by using the whole tumour volume analysis and is better for determining the dominant grade of tumour tissue. This result corresponded well that of Nougaret et al [20] and Lambregts et al [21], who investigated the diagnostic performance of different ROI positioning methods in rectal cancer. The $ADC_{slow}$ value determined using the WTV method may have great clinical importance as the pathological differentiation grade is one of the most important factors to predict the recurrence and survival of HCC patients with hepatic resection and transplantation.

Our data demonstrated that the perfusion parameters $ADC_{fast}$ and *f* were not statistical significant in differentiating the pathological differentiation grade of HCC. In theory, the $ADC_{fast}$ is correlated with the average blood flow rate and *f* is associated with the fraction volume of capillary blood flow, which could be used to reflect the vascularity in tissue, and has been shown to be helpful in evaluating the pathological differentiation grade of the carcinoma [22]. Paradoxically, $ADC_{fast}$ and *f* values of the E–S grade 1, 2, 3 and 4 groups showed no statistical difference in our study. The current result may be attributed to the relative small proportion of the E–S grade 1 and grade 4 patients. Furthermore, the respiratory artefact, relaxation effects and the T2 contribution can also influence results.

We acknowledge some limitations of our study. First, the limited number of E–S grade 1 and E–S grade 4 samples leaves the question about reproducibility open; therefore, we should continue to collect more samples. Second, this is a single-centre study with a single MR unit which leaves the question about reproducibility open. Third, five patients with unsatisfactory image quality were excluded because of the severe motion artefacts and signal-to-noise ratio that challenged the calculation of high-quality IVIM images. Finally, the hepatic specimens that underwent IVIM examinations were not included in our study, as this process could entirely eliminate the effect of perfusion.

In conclusion, the results of the preliminary study have demonstrated that the different ROI positioning methods used

**Table 4** Sensitivity and specificity of ADC and $ADC_{slow}$ at optimal cut-off value in differentiating the E–S (1–2) from the E–S (3–4)

| Group | Optimal cut-off value | Sensitivity (100%) | Specificity (100%) | Youden index |
|---|---|---|---|---|
| ADC[a] | | | | |
| Three-ROI | 1.03 | 71.43% (25/35) | 82.14% (46/56) | 0.536 |
| One-section | 1.20 | 82.86% (29/35) | 78.57% (44/56) | 0.614 |
| WTV | 1.41 | 85.71% (30/35) | 80.36% (45/56) | 0.661 |
| $ADC_{slow}$[a] | | | | |
| Three-ROI | 0.94 | 94.29% (33/35) | 80.36% (45/56) | 0.745 |
| One-section | 1.01 | 91.43% (32/35) | 91.07% (51/56) | 0.825 |
| WTV | 1.15 | 94.29% (33/35) | 91.07% (51/56) | 0.854 |

*ADC* apparent diffusion coefficient, $ADC_{slow}$ true ADC, *WTV* whole tumour volume

[a] Values are in units of $\times 10^{-3}$ $mm^2/s$

significantly affect the IVIM parameters and ADC measurements. Measurements of $ADC_{slow}$ value derived from the WTV method entailed the highest diagnostic performance in grading HCC.

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## Compliance with ethical standards

**Guarantor** The scientific guarantor of this publication is Bin Song.

**Conflict of interest** The authors of this manuscript declare no relationships with any companies whose products or services may be related to the subject matter of the article.

**Statistics and biometry** One of the authors (Yi Wei) has significant statistical expertise.

**Informed consent** Written informed consent was obtained from all subjects (patients) in this study.

**Ethical approval** Institutional review board approval was obtained.

**Methodology**
- prospective
- diagnostic study
- performed at one institution

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