Received:
05 June 2017

Revised:
19 December 2017

Accepted:
03 January 2018

<https://doi.org/10.1259/bjr.20170421>

Cite this article as:

Huang Y-cheng, Chen T-wu, Zhang X-ming, Zeng N-lin, Li R, Tang Y-lian, et al. Intravoxel incoherent motion diffusion-weighted imaging of resectable oesophageal squamous cell carcinoma: association with tumour stage. *Br J Radiol* 2018; **91**: 20170421.

FULL PAPER

Intravoxel incoherent motion diffusion-weighted imaging of resectable oesophageal squamous cell carcinoma: association with tumour stage

^{1,2}YU-CHENG HUANG, MM, ¹TIAN-WU CHEN, MD, ¹XIAO-MING ZHANG, MD, ¹NAN-LIN ZENG, MM, ¹RUI LI, MD, ¹YU-LIAN TANG, MM, ¹FAN CHEN, MM and ¹YAN-LI CHEN, MM

¹Department of Radiology, Sichuan Key Laboratory of Medical Imaging, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, China

²Department of Radiology, Dazhou Central Hospital, Dazhou, Sichuan, China

Address correspondence to: Prof Tian-wu Chen

E-mail: chentw@aliyun.com

Objective: To determine whether intravoxel incoherent motion (IVIM) diffusion-weighted imaging (DWI) derived parameters can be associated with tumour stage of oesophageal squamous cell carcinoma (SCC).

Methods: 60 patients with resectable oesophageal SCC and 20 healthy individuals underwent oesophageal DWI-using multi b -values with a 3.0T MR system. Pure diffusion coefficient (D), perfusion-related incoherent microcirculation (D^*), microvascular volume fraction (f) and apparent diffusion coefficient (ADC) were measured on DWI. Statistical analyses were performed to determine associations of DWI-derived parameters with T-stage.

Results: ADC ($r = -0.842$), D ($r = -0.729$), D^* ($r = -0.301$) and f ($r = -0.817$) were negatively correlated with T-stage of oesophageal SCC (all $p < 0.01$), and the multinomial regression analyses revealed that IVIM-derived parameters including D ($p = 0.038$; odds ratio <1) and f ($p < 0.001$; odds ratio <1) were associated with T-stage.

The Mann-Whitney U tests with Bonferroni correction showed that D , f and ADC could discriminate oesophageal SCC, especially T_1 -staged tumour, from normal oesophagus (all $p < 0.05$) while D^* could not ($p > 0.05$). By receiver operating characteristic analyses, f could be the best indicator for detecting oesophageal SCC with an area under receiver operating characteristic (AUC) of 0.964, especially T_1 -staged cancer with an AUC of 0.984, and for discriminating T_1 -stages between T_{0-1} and T_{2-3} with an AUC of 0.957, and between T_{0-2} and T_3 with an AUC of 0.945 in comparison with any other DWI-derived parameter.

Conclusions: IVIM derived parameters can be associated with T-stage of oesophageal SCC.

Advances in knowledge (1) IVIM-derived parameters are negatively correlated with stage of oesophageal SCC. (2) Among IVIM-derived parameters, microvascular volume fraction helps detect and stage oesophageal SCC.

INTRODUCTION

Oesophageal squamous cell carcinoma (SCC) is one of the most frequent causes of death from digestive systemic malignant neoplasms, but this tumour can be curatively resected before spreading through the bloodstream to distant tissues or before the aortic and primary bronchi invasion.¹⁻⁷ Pre-operative chemotherapy or radiation therapy is not necessary before T_1 - or T_2 -staged oesophageal SCC is treated by endoscopic therapy or by oesophagectomy.⁸ As a gold standard procedure, endoscopic biopsy has been universally adopted to determine oesophageal tumour as early as possible for treatment decision-making. This invasive method is limited by intraobserver variability and sampling errors, and oesophagus biopsy cannot be performed when the oesophagus is completely obstructed by oesophageal cancer.

In order to overcome the limitations of oesophagus biopsy, imaging examination is indispensable. MRI is famous for its excellent ability such as characterizing diseased tissues, lacking of ionizing radiation, and possibility of performing multiparametric and functional imaging. Nevertheless, Quint et al reported that relaxation time (T_1 and T_2) varied widely for imaging the normal oesophageal wall and oesophageal tumours, suggesting that T_1 and T_2 weighted imaging could not be useful for predicting the stage of oesophageal tumour.⁹ However, diffusion-weighted imaging (DWI) has been extensively used in oncological imaging for tumour detection, tumour characterization or monitoring, and predicting treatment response.¹⁰⁻¹⁵ Based on the Brownian motion of water molecules within tissues, the apparent diffusion coefficient (ADC), which was calculated by b -value of 0 s mm⁻² and another b -value using

a monoexponential model, is the most common quantitative method in DWI.^{16–18} Considering the dependence of ADC on the *b*-value, variability in ADC measurements may confuse the results.¹⁹

Initially developed by Le Bihan *et al*²⁰ intravoxel incoherent motion (IVIM) imaging has been used to quantitatively assess the microscopic translational motion that occurs in each image voxel on MRI. Unlike ADC, which is known to reflect variable combinations of diffusion and perfusion effects within tumour depending on the acquisition parameters, perfusion and diffusion factors can be separated by IVIM.^{15,21–25} The major advantage of IVIM DWI is that it allows the acquisition of diffusion and perfusion parameters at the same time, and therefore, can provide both measurements within corresponding solid lesion without the requirement for a further coregistration processing step.²⁶ To our knowledge, IVIM MRI has not been used to evaluate the tumour stage of oesophageal cancer. Therefore, the purpose of this study was to determine whether IVIM DWI derived parameters including ADC can be associated with the tumour stage of oesophageal SCC.

METHODS AND MATERIALS

Participant selection

The institutional ethics committee of the Affiliated Hospital of North Sichuan Medical College approved this study with a retrospective review of prospectively acquired data. Written informed consent was obtained from each participant before this study.

From July 2014 to June 2016, 65 consecutive patients with primary oesophageal SCC confirmed initially by endoscopic pathology were enrolled into this study according to the following inclusion criteria: (a) the patient did not receive any tumour-related treatment such as radiation therapy, chemotherapy and biotherapy before undergoing IVIM DWI; (b) the tumour was considered resectable by CT; and (c) patients desired to undergo surgical resection and there were no contraindications to surgery. The exclusion criteria were as follows: (a) the quality of the MR images was poor ($n = 3$), or (b) the tumour was too small to draw the region of interest (ROI) when we reviewed the IVIM data ($n = 2$). Finally, 60 patients (41 males and 19 females, age ranged 49–78 years, and mean age of 62.2 years) were ultimately enrolled into our study.

Additionally, 20 consecutive healthy individuals were randomly enrolled into this study, and served as the reference group from which benchmarks of IVIM-derived parameters were obtained to explore the distinction between oesophageal SCC and the healthy oesophagus. The inclusion criteria were as follows: (a) the participants had no oesophageal diseases, acute infection, or history of disease such as malignant tumour; and (b) the healthy individuals could cooperate in the MRI examination during IVIM scans.

All subjects including the patients with oesophageal SCC and the healthy individuals underwent oesophageal IVIM DWI. After DWI, the oesophageal SCC patients received radical oesophagectomy with three-field lymphadenectomy. The interval

between IVIM DWI and surgery was less than 1 week, and these patients did not receive any tumour-related treatment during this interval. After the surgical treatment, all resected specimens were fixed in formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin, and subsequently examined by light microscopy for pathological examination. In each patient, the postoperative pathology revealed that the cut edge of resected specimen was not involved by this tumour. According to the American Joint Committee on Cancer (AJCC) criteria,²⁷ the patients based on the TNM stage are listed in Table 1. In addition, the oesophageal SCC patients with lymphatic metastasis received adjuvant radiation therapy or chemotherapy after the surgery.

MR imaging protocol

Oesophagus IVIM DWI was performed with a GE Signa 3.0 T scanner (Discovery MR 750, GE Medical Systems, Milwaukee, WI) in each patient with oesophageal SCC and healthy participant. Each subject was required fasting for 6 h before MRI. The participants were intramuscularly injected 12 ml scopolamine butyl bromide before the examination to weaken the inherent peristalsis of oesophagus. When the cardiac and respiratory signals were satisfied, every participant was positioned supinely in a 32-channel array body coil. The MR sequences included sagittal T_2 weighted fat-suppressed sequence, axial T_1 weighted LAVA-Flex mask, and axial DWI with multiple *b*-values. In the oesophageal SCC patients, 15 ml gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA Magnevist, Schering, Berlin, Germany) was intravenously injected at a rate of 2.5 ml s⁻¹ for a total dose of 0.2 mmol per kg of body weight via the pressure injector (Spectris MR Injection System, Medrad, Warrendale, PA) followed by a 20-ml saline solution flush for the sagittal T_1 WI triphasic enhanced scans. Scanning parameters for each sequence are shown in Table 2. In particular, DWI was carried out by using 11 *b*-values of 0, 20, 50, 80, 100, 150, 200, 400, 600, 800 and 1000 s mm⁻² with either monopolar or bipolar diffusion encoding schemes. The acquisition time for DWI was approximately 3 min and 40 s. Both DWI and sagittal T_2 weighted fat-suppressed sequence were performed with regular breathing, and either the axial T_1 weighted LAVA-Flex mask or the sagittal T_1 weighted triphasic enhanced scanning was acquired in a breath-hold. In addition, the sagittal T_2 weighted fat-suppressed imaging and the axial T_1 weighted imaging were performed for the localization of oesophageal SCC so as to plan the IVIM scans of this tumour, and the sagittal T_1 weighted triphasic enhanced scanning was carried out for identifying the necrosis region within oesophageal SCC.

Image analysis

All original IVIM DWI data were loaded to the workstation (GE Advantage Workstation v. 4.4–09, Sun Microsystems, Palo Alto, CA) for image analysis. The MR images were reviewed by two radiologists (Tian-wu Chen who had 19 years of experience in abdominal radiology, and Yu-cheng Huang with 4 years of experience in radiology) working in consensus to guarantee accurate drawing of ROI within the neoplasm, but the measurements of IVIM-derived parameters were performed separately by the previous two radiologists.

Table 1. Summary of patients with oesophageal squamous cell carcinoma ($n = 60$)

T-staging (primary tumour)	Patients (percentage)
T_1 (tumour is limited to mucosa or submucosa)	11 (18.33%)
T_2 (tumour invades the muscularis propria)	12 (20.00%)
T_3 (tumour invades the subserosa)	37 (61.67%)
T_4 (tumour invasion is contiguous to or exposed beyond the seorsa, or invades the adjacent structures)	0
N-staging (lymph node metastasis)	
N_0 (no regional lymph node metastasis)	40 (66.67%)
N_1 (metastases in 1 to 2 positive regional lymph nodes)	13 (21.67%)
N_2 (metastases in 3 to 6 positive regional lymph nodes)	6 (10.00%)
N_3 (metastases in more than 6 positive regional lymph nodes)	1 (1.66%)
M staging (distant metastasis)	
M_0 (no distant metastasis)	60 (100%)
M_1 (distant metastasis)	0
G (histological grade)	
G_1 (well-differentiation)	22 (36.67%)
G_2 (moderately differentiation)	33 (55.00%)
G_3 (poorly differentiation)	5 (8.33%)
Anatomical distributions (superior margin or proximal of tumour)	
Lower thoracic portion	5 (8.33%)
Mid-thoracic portion	44 (73.33%)
Upper thoracic portion	11 (18.34%)

In oesophageal SCC patients, the tumour volume was measured on MRI to determine how to draw an ROI for IVIM-derived parameters' measurement. Subsequently, an ROI was drawn within the tumour with an area of more than 60% of the area of the entire tumour randomly on one maximal slice of the oesophageal tumour to perform the biexponential IVIM-derived parameters' measurement, avoiding areas of artefact, periesophageal fat, oesophageal lumen, and necrotic and haemorrhage areas (Figure 1). The pure diffusion coefficient (D), perfusion-related incoherent microcirculation (D^*), and microvascular volume fraction (f) maps as well as the corresponding IVIM-derived parameters were all generated automatically by the software. The ADC of the tumour for b -value of 800 s mm^{-2} was derived on the basis of a monoexponential function. The

previous measurements were performed until three consecutive maximal slices of the oesophageal tumour were covered. An ultimate estimate of D , D^* , f and ADC values of each tumour were obtained by averaging the corresponding parameters across the three representative slices. For the previous DWI-derived parameters' measurement, the tumour was located on the sagittal fat-suppressed T_2 WI, and the contrast-enhanced T_1 WI was reviewed to identify the necrosis region within oesophageal SCC. The axial T_1 weighted LAVA-Flex mask sequence and fat-suppressed T_2 WI were conducive to discriminate the haemorrhage and periesophageal fat. For the previous tumour volumetry and DWI-derived parameters' measurement of oesophageal SCC, the abnormal oesophageal wall was determined if the thickness of oesophageal wall was more than 5 mm on transverse T_1 WI, and

Table 2. Scanning parameters corresponding to each MR sequence

	Sagittal T_2 WI	Axial T_1 WI	Axial DWI	Contrast-enhanced T_1 WI
TR (ms)	3158	4	10,000	4.5
TE (ms)	100	1.8	92.9	2.1
Field of view (mm)	340 × 340	300 × 270	300 × 300	300 × 270
Matrix	352 × 352	260 × 192	80 × 128	320 × 224
Section thickness (mm)	4.0	4.0	5.0	4.0
Intersection gap (mm)	0.8	0	1.5	0
Flp angle (°)	0	12	0	12

DWI, diffusion-weighted imaging; T_1 WI, T_1 weighted imaging; T_2 WI, T_2 weighted imaging

Figure 1. The representative intravoxel incoherent motion diffusion-weighted imaging (a, b, c) of T_1 -, T_2 - and T_3 -staged oesophageal squamous cell carcinoma in a 56-year-old female, a 62-year-old male and a 63-year-old male, respectively. Region of interest is drawn on diffusion-weighted imaging within each tumour to obtain the corresponding intravoxel incoherent motion derived parameters.

the signal of oesophageal wall increased on T_2 WI and DWI.²⁸ In the healthy individuals, the MRI data were similarly analysed on magnified images for the DWI-derived parameters' measurement except that more than 50% of the area of normal oesophageal wall was drawn as an ROI randomly on one section.

Additionally, the ADC value was proposed for use in a monoexponential model as an alternative to the pure diffusion coefficient, and it has been widely applied for the quantification of diffusion in biological tissues.²⁹ The calculation of ADC value is performed using the simple equation $S_b/S_0 = \exp(-bADC)$, where S_b and S_0 denote the diffusion-weighted signal intensity obtained with the diffusion sensitivity coefficient of b and 0, respectively. However, ADC measurement depends on the diffusion sensitivity coefficient (b -value), which lacks consensus.³⁰ Since the ADC value is a non-specific parameter for both diffusion and perfusion, the findings for numerous lesions based on the ADC measurement can be quite different with poor comparability between studies. According to the IVIM theory, a biexponential model is used in this study to describe the behaviour of the diffusion-weighted signals from the tumours through the following equation $S_b/S_0 = f \times \exp[-b(D + D^*)] + (1 - f) \times \exp(-bD)$, where S_0 is the signal acquired without diffusion weighting, S_b is the signal intensity at a given b -value, the microvascular volume fraction is represented by f , D is the pure diffusion coefficient of a water molecule, and D^* represents the perfusion-related incoherent microcirculation. The effects of D^* on the signal decay at a large b -value ($>200 \text{ s mm}^{-2}$) can be neglected for considering that D^* is

significantly greater than D .^{20,23} The equation for the biexponential model can be simplified, and the estimation of D can be obtained by using only b -values greater than 200 s mm^{-2} with the simple linear fit equation $S_b/S_0 = \exp(-bD)$.

Statistical analysis

Statistical analysis was performed by using the Statistical Packages for Social Sciences v. 13.0 (SPSS, Chicago, IL) and MedCalc (MedCalc Software, Acaciaaan 22, B-8400 Ostend, Belgium). Quantitative data were expressed as mean $\pm$ standard deviation. Statistical difference was considered when the p -value was less than 0.05.

The IVIM-derived parameters of oesophageal SCC were randomly chosen to test the interobserver agreement of the previous two observer' measurements by using the Bland-Altman statistics. Good agreement between the replicated measurements could be obtained when the interclass correlation coefficient (ICC) was more than 0.99, and 95% confident interval of the mean difference between replicated measurements and 95% limits of agreement were close to 0.³¹ Values of the first set of measurement were regarded as the parameters for the lesions when the ICC was more than 0.75.³² When the ICC was less than 0.75, the measurement of the corresponding DWI derived parameter was repeated by the previous reviewers, and an average of the four measurements was used as the final result for the subsequent analysis.

Table 3. The interobserver agreements of intravoxel incoherent motion-derived parameters' measurement

Parameter	Mean differences of replicated measurements			95% Interclass correlation coefficient
	Differences between two measurements	95% CI of the difference	95% limit of agreement	
ADC ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	0.22 ± 0.10	0.0243 to 0.4252	0.0550 to 0.4293	0.967 (0.945 to 0.980)
D ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	0.20 ± 0.18	-0.1522 to 0.5682	-0.1595 to 0.5755	0.851 (0.751 to 0.911)
D^* ($\times 10^{-1} \text{ mm}^2 \text{ s}^{-1}$)	0.24 ± 0.33	-0.3988 to 0.8820	-0.4119 to 0.8951	0.748 (0.577 to 0.849)
f (%)	0.18 ± 0.25	-0.3048 to 0.6613	-0.3147 to 0.6712	0.938 (0.897 to 0.963)

Data are mean $\pm$ SD. ADC, apparent diffusion coefficient; CI, confidence interval; D , pure diffusion coefficient; D^* , perfusion-related incoherent microcirculation; f , microvascular vol fraction; SD, standard.

Figure 2. Bland-Altman plots show bias of (a) ADC, (b) pure diffusion coefficient (D), (c) perfusion-related incoherent microcirculation (D^*) and (d) microvascular volume fraction (f) of oesophageal squamous cell carcinoma. ADC, apparent diffusion coefficient.

Spearman's rank correlation analysis was used to evaluate the correlation of D , D^* , f and ADC with tumour stage. On the basis of the correlation analysis, the possible interrelationship of the main explanatory variables including IVIM-derived parameters, tumour volume, age and sex with T-stage were further tested with ordinal regression analysis to determine the IVIM-derived parameters as useful variables for differentiating T-stages independent of other indicators. Because the IVIM-derived parameters were of skewed distribution, the Kruskal-Wallis test was performed for multigroup comparison among normal oesophagus and different stages of oesophageal SCC. When significant difference was found in any IVIM-derived parameter by the Kruskal-Wallis test, the Mann-Whitney U tests with Bonferroni correction for multigroup

comparisons were further applied as *post-hoc* comparisons of D , D^* , f and ADC between oesophageal SCC and normal oesophagus, or between tumour stages.³³ Receiver operating characteristic (ROC) analysis was performed to determine if any IVIM derived parameter could help identify the stage of oesophageal SCC with an area under ROC curve (AUC).

RESULTS

Interobserver agreements of DWI derived parameters' measurements of oesophageal SCC
 In all the enrolled participants, the volume of oesophageal SCC ranged from 5.98 to 22.78 cm³ with mean volume of 11.25 cm³.

Table 4. Intravoxel incoherent motion-derived parameters of normal oesophageal wall and staged oesophageal squamous cell carcinoma

	ADC ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	D ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	D^* ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	f (%)
Normal oesophagus ($n = 20$)	1.97 ± 0.24	1.67 ± 0.53	30.85 ± 33.91	31.94 ± 1.82
Oesophageal tumour ($n = 60$)	1.28 ± 0.30	1.32 ± 1.29	24.59 ± 31.25	22.51 ± 5.64
T_1 -stage ($n = 11$)	1.78 ± 0.11	1.65 ± 0.10	40.39 ± 33.40	27.96 ± 0.94
T_2 -stage ($n = 12$)	1.37 ± 0.12	1.28 ± 0.27	19.60 ± 25.20	24.86 ± 1.28
T_3 -stage ($n = 37$)	1.11 ± 0.19	1.24 ± 1.64	21.51 ± 31.66	20.12 ± 5.89

Notes. Data are means $\pm$ standard deviation. ADC, apparent diffusion coefficient; D , pure diffusion coefficient; D^* , perfusion-related incoherent microcirculation; f , microvascular vol fraction.

Table 5. Comparisons of intravoxel incoherent motion-derived parameters between normal oesophagus and T-staged oesophageal squamous cell carcinomas

	ADC ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	D ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	D^* ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	f (%)
Normal vs T_1	0.004 ^a	0.005 ^a	0.307	<0.001 ^a
Normal vs T_2	<0.001 ^a	<0.001 ^a	0.083	<0.001 ^a
Normal vs T_3	<0.001 ^a	<0.001 ^a	0.052	<0.001 ^a
T_1 vs T_2	<0.001 ^a	<0.001 ^a	0.059	<0.001 ^a
T_2 vs T_3	<0.001 ^a	<0.001 ^a	0.898	<0.001 ^a
T_1 vs T_3	<0.001 ^a	<0.001 ^a	0.007 ^a	<0.001 ^a
T_0 vs T_{1-3}	<0.001 ^a	<0.001 ^a	0.066	<0.001 ^a
T_{0-1} vs T_{2-3}	<0.001 ^a	<0.001 ^a	0.006 ^a	<0.001 ^a
T_{0-2} vs T_3	<0.001 ^a	<0.001 ^a	0.013 ^a	<0.001 ^a

Notes, ADC, apparent diffusion coefficient; D , pure diffusion coefficient; D^* , perfusion-related incoherent microcirculation; f , microvascular vol fraction. The normal oesophageal wall is defined as T_0 -staged tumour. Normal, and T_1 , T_2 , T_3 , T_{0-1} , T_{0-2} , T_{1-3} , T_{2-3} represent normal oesophageal wall, and the T_1 , T_2 , T_3 , T_{0-1} , T_{0-2} , T_{1-3} , T_{2-3} stages of oesophageal squamous cell carcinoma, respectively.

^adenotes significant difference in the parameters between stages after Bonferroni correction ($p < 0.05$).

The volume of oesophageal SCC was big enough to hand-draw the ROI for the measurements of IVIM derived parameters of oesophageal SCC. The interobserver agreements of ADC, D , D^* and f of oesophageal SCC are shown in Table 3 and Figure 2. When the ICC was less than 0.75, the measurement of the corresponding IVIM derived parameter was repeated by the previous reviewers, and an average of the four measurements was used as the final results for the further analysis.

Comparison of bi-exponential and mono-exponential DWI derived parameters between oesophageal SCC patients and healthy individuals or between T-stages

The mean D , D^* , f and ADC values of oesophageal SCC and normal oesophagus are shown in Table 4.

The correlation analysis showed that ADC ($r = -0.842$, $p < 0.01$), D ($r = -0.729$, $p < 0.01$), D^* ($r = -0.301$, $p < 0.01$), and f ($r = -0.817$, $p < 0.01$) decreased from normal oesophagus to tumour Stage 1, to Stage 2, and to Stage 3 of oesophageal SCC. Satisfactory negative correlation of ADC, D and f with T-stage could be found.

Because the correlation analysis showed that the correlation coefficient between D^* and T-stage was just -0.301 , D^* did not enter the subsequent multinomial regression analysis. Due to the serious collinearity between ADC and D , IVIM-derived parameter (D and f) together with tumour volume, age and sex entered the multinomial regression analysis. The multinomial regression analysis revealed that D ($p = 0.038$; odds ratio <1), f ($p < 0.001$; odds ratio <1) and tumour volume [$p = 0.003$; odds

Table 6. Receiver operating analyses of ADC, D and f for the detection and stage of oesophageal squamous cell carcinoma

	Cut-off value	Differentiations	AUC	Sensitivity	Specificity
ADC ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	1.73	T_0 vs T_1	0.818	0.900	0.636
	1.71	T_0 vs T_{1-3}	0.956	0.950	0.900
	1.59	T_{0-1} vs T_{2-3}	0.983	0.968	0.980
	1.27	T_{0-2} vs T_3	0.969	0.953	0.946
D ($\times 10^{-3} \text{ mm}^2 \text{ s}^{-1}$)	1.68	T_0 vs T_1	0.811	0.800	0.818
	1.60	T_0 vs T_{1-3}	0.869	0.900	0.883
	1.52	T_{0-1} vs T_{2-3}	0.916	0.903	0.980
	1.13	T_{0-2} vs T_3	0.923	0.953	0.892
f (%)	29.35	T_0 vs T_1	0.984	0.900	0.919
	29.15	T_0 vs T_{1-3}	0.964	0.950	0.933
	27.00	T_{0-1} vs T_{2-3}	0.957	0.968	0.959
	23.55	T_{0-2} vs T_3	0.945	0.953	0.946

Notes, ADC, apparent diffusion coefficient; AUC, area under the receiver operating curve; D , pure diffusion coefficient; f , microvascular vol fraction. The normal oesophageal wall is defined as T_0 -stage tumour. Normal, T_1 , T_{1-3} , T_{0-1} , T_{2-3} , T_{0-2} and T_3 represent normal oesophageal wall, T_1 , T_{1-3} , T_{0-1} , T_{2-3} , T_{0-2} and T_3 stages of oesophageal squamous cell carcinoma, respectively.

Figure 3. The receiver operating curves show the utility of ADC, pure diffusion coefficient (D) and microvascular volume fraction (f) to detect oesophageal squamous cell carcinoma (a), and discriminate grouped stage T_{0-1} of this tumour from T_{2-3} (b) and stage T_{0-2} from T_3 (c), and stage T_1 from normal oesophageal wall (d). ADC, apparent diffusion coefficient.

ratio = 1.228; 95% confidence interval for odds ratio (1.074–1.404)] were associated with T-stage, whereas the age ($p = 0.996$) and sex ($p = 0.119$) were not.

The Mann–Whitney U tests with Bonferroni correction for multigroup comparisons (Table 5) demonstrated that statistical differences in D , f and ADC were found between oesophageal SCC patients and healthy volunteers (all $p < 0.05$), whereas no difference could be found in D^* ($p = 0.066$). In detail, D , f and ADC could distinguish T_1 -staged tumour (all $p < 0.05$) from normal oesophageal wall. In addition, no differences in all IVIM-derived parameters could be found between oesophageal SCC patients with and without node metastasis (all $p > 0.05$).

ROC analyses of biexponential and monoexponential DWI derived parameters to stage oesophageal SCC

Based on the previous results of Mann–Whitney U tests with Bonferroni correction for multigroup comparisons, we performed ROC analysis of the IVIM-derived parameters with significant difference for detecting and staging oesophageal SCC. In detail, the ROC analyses of these parameters were performed between oesophageal SCC and normal oesophageal wall for detecting this tumour, and between stages T_{0-1} and T_{2-3} and between stages T_{0-2} and T_3 for determining the grouped stages, and between T_1 -stage oesophageal SCC and normal oesophagus

(i.e. stage T_0) for early detecting this tumour. The cut-off values, AUC, sensitivity and specificity of D , f and ADC are illustrated in Table 6 for detecting and staging oesophageal SCC. As shown in Figure 3 and Table 6, f might be the best indicator for detecting this tumour, and could help determine tumour stage because f had larger AUC than any other IVIM-derived parameter. In addition, the ROC analysis was not performed in D^* because of its large variability as shown in Table 3.

DISCUSSION

In this initial study, we explored the association of IVIM derived parameter of resectable oesophageal SCC with T-stage. We found that ADC and f decreased with T-stage of oesophageal SCC. Histologically, the nests of squamous epithelial cells penetrate beyond the epithelial basement membrane with little desmoplastic response, and the tumour contains extensive keratinization with cells that have eosinophilic cytoplasm and intercellular bridges.³⁴ The amount of keratinization decreases and the proportion of basaloid cells increases as oesophageal SCC becomes more advanced. The increased cellularity in the tumours could result in an even more restricted diffusion,³⁵ thus a marked decrease of ADC could be found with the stage of oesophageal SCC.

The decrease of f -value with the tumour stage can be explained as follows. Vascular endothelial cells may be proliferated

to either generate or not the functional vessels with lumen in the process of tumourigenesis.³⁶ The intratumoral interstitial hypertension increases with the development of the tumour vasculature, and partial or total collapse of vessels may contribute to a low perfusion.³⁷ In addition, the irregular morphology of tumour vasculature and tortuous vessels also contributes to low perfusion, despite the abundant presence of angiogenic tumour vessels.³⁸ The previous pathologic mechanisms may result in the decrease of f -value, which represents microcapillary perfusion.

Additionally, the slight decrease of D -value with the tumour stage of oesophageal SCC might be probably related to the smooth muscle proliferation and extracellular stroma expansion that blocks the free water diffusion in the progress of oesophageal SCC.³⁹ As shown in our study, the interobserver agreement of D^* measurement can not be as good as that of any other IVIM-derived parameter, resulting in the poor correlation of D^* with the tumour stage, which might be due to this IVIM-derived parameter's dependence on signal-to-noise ratio levels, data instability and its huge standard deviation.⁴⁰

Clinically, we found that D , f and ADC can be used to detect oesophageal SCC, and especially can help early detect this tumour. In comparison with any other IVIM-derived parameter, f can be a suitable parameter to detect oesophageal SCC because the largest AUC of more than 0.95 could be obtained in our study. In particular, the T_1 -staged tumour can be found by using the cut-off f -value of 29.35% with an AUC of 0.984, which could be higher than by using any other IVIM-derived parameter.

Moreover, we found that some IVIM-derived parameters could help stage oesophageal SCC, and f can be a suitable marker. In comparison with D and ADC, a higher AUC and specificity could be achieved by using the f cut-off value of 27% for differentiating T-stages between T_{0-1} and T_{2-3} , and the f cut-off of 23.55% for the differentiation of T-stages between T_{0-2} and T_3 based on the present data with the ROC analyses. Therefore, the f -value from biexponential model could be a suitable index to identify the stage of oesophageal SCC.

However, oesophageal SCC with and without node metastasis could not be distinguished by any IVIM-derived parameter of oesophageal SCC. As a matter of fact, the incidence of lymph node metastasis are not only affected by diffusion and perfusion, but also influenced by adhesion molecule and angiolymphatic invasion.⁴¹ Our finding was consistent with the research of Aoyagi et al.³⁹ According to Daniel et al, no significant differences of perfusion characteristics could be found between metastatic and non-metastatic lymph nodes.⁴²

There were several limitations in this study. Firstly, the sample sizes of T_1 - and T_2 -staged oesophageal SCC are not large because the symptoms of these patients are not obvious and referral to hospital at early stage. Further study involving a larger number of samples will be required to reconfirm the association of

IVIM-derived parameter with the stage of oesophageal SCC. Secondly, DWI is not good at spatial resolution, and the tiny lesion usually shows partial volume averaging which can lead to inaccurate measurements of the IVIM-derived parameters.⁴³ Further researches on the effect of different DWI acquisition methods on IVIM-derived parameters will be warranted to provide consensus on this issue. Thirdly, the ADC value had a large mean difference as shown in this study. This parameter cannot be a suitable index for detecting oesophageal SCC and differentiating its T-stage, suggesting a very small negative effect on our results. Fourthly, because the resectable oesophageal SCC can be staged pathologically, whereas the stage of unresectable lesions cannot be confirmed by this golden procedure, we only assessed the resectable lesions by using IVIM-derived parameters. We will carry out relevant study for the unresectable lesions to confirm the findings in this study in the future. Fifthly, our study demonstrated that tumour volume might be correlated to stage of oesophageal cancer, but we did not stratify the analysis by the size. We will perform the relevant study in the future. Sixthly, our study has the limitation of possible *post-hoc* derivation of optimal parameters for tumour stage because the IVIM data were prospectively acquired but retrospectively reviewed. We will perform the external validation of our findings in a prospective cohort.

CONCLUSION

IVIM-derived parameter from biexponential model can be negatively correlated with the stage of oesophageal SCC. The biexponential model-derived parameter (f) can be better than any other parameter for detecting oesophageal SCC, and differentiating the grouped T-stages. We hope that our findings could be helpful for early detection of oesophageal SCC and staging this cancer for treatment decision-making.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (grant no. 81571645), the Sichuan Province Special Project for Youth Team of Science and Technology Innovation (grant no. 2015TD0029), and the Construction Plan for Scientific Research Team of Sichuan Provincial Colleges and Universities (grant no. 2015TD0023).

FUNDING

This study was supported by the National Natural Science Foundation of China (grant no. 81571645), the Sichuan Province Special Project for Youth Team of Science and Technology Innovation (grant no. 2015TD0029), and the Construction Plan for Scientific Research Team of Sichuan Provincial Colleges and Universities (grant no. 2015TD0023).

ETHICS APPROVAL

The institutional ethics committee of the Affiliated Hospital of North Sichuan Medical College approved this study.

CONSENT

All authors approved the submission of this study.

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