European Radiology (2019) 29:411–421
https://doi.org/10.1007/s00330-018-5602-0

GASTROINTESTINAL

Apparent diffusion coefficient as a potential marker for tumour differentiation, staging and long-term clinical outcomes in gallbladder cancer

Ji Hye Min[1,2] · Tae Wook Kang[1] · Dong Ik Cha[1] · Seong Hyun Kim[1] · Kyung Sook Shin[2] · Jeong Eun Lee[2] · Kee-Taek Jang[3] · Soo Hyun Ahn[4]

Received: 2 April 2018 / Revised: 19 May 2018 / Accepted: 7 June 2018 / Published online: 25 June 2018

Abstract

Objectives To evaluate the correlation between tumour differentiation or stage of gallbladder cancer (GBC) and the apparent diffusion coefficient (ADC), as well as to assess whether ADC value can predict long-term disease-free survival (DFS) after surgery.

Methods This retrospective study was approved by the institutional review board and the requirement for informed consent was waived. Between March 2008 and June 2016, 79 patients who underwent magnetic resonance (MR) imaging with diffusion-weighted image and subsequent surgery for GBC were included in this study. Correlations between quantitative ADC values and tumour differentiation or stage based on the American Joint Committee on Cancer (AJCC) were assessed using Spearman's correlation analysis. Prognostic factors for DFS were identified with multivariate Cox regression analysis using imaging and clinical characteristics.

Results All patients were classified as having well- ($n = 18$), moderately ($n = 35$) or poorly differentiated GBCs ($n = 26$). The ADC value of GBCs was significantly correlated with tumour differentiation and AJCC stage ($p < 0.001$ and $p < 0.001$, respectively). Sixty-nine patients were followed up for 2.0–92.4 months (median, 23.5 months). On multivariate analysis, the significant prognostic factor for DFS was not tumour differentiation or AJCC stage but a binary tumour ADC value (hazard ratio, 4.29; $p = 0.009$). DFS rates were significantly different according to the classification of tumour ADC value (cut-off value = 1.04×10^{-3} mm^2/s; $p = 0.004$).

Conclusion The ADC value of GBCs was significantly correlated with tumour differentiation as well as AJCC stage. In addition, it predicted long-term outcomes after surgery in patients with GBC.

Key points

- *ADC values of GBC and tumour differentiation were negatively correlated.*
- *Lower ADC values of GBC were significantly correlated with higher tumour stage.*
- *Tumour ADC value could be useful for risk stratification of GBC patients.*

Keywords Gallbladder neoplasms · Diffusion · Magnetic resonance imaging · Treatment outcome

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00330-018-5602-0) contains supplementary material, which is available to authorized users.

✉ Tae Wook Kang
kaienes.kang@samsung.com; garamond@hanmail.net

[1] Department of Radiology and Center for Imaging Science, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-Ro, Gangnam-gu, Seoul 06351, Republic of Korea

[2] Department of Radiology, Chungnam National University Hospital, Chungnam National University College of Medicine, Daejeon, Republic of Korea

[3] Department of Pathology and Translational Genomics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

[4] Department of Mathematics, Ajou University, Suwon, Republic of Korea

Abbreviations

ADC	Apparent diffusion coefficient
AJCC	American Joint Committee on Cancer
CI	Confidence interval
DFS	Disease-free survival
DWI	Diffusion-weighted imaging
GBC	Gallbladder cancer
HR	Hazard ratio
MR	Magnetic resonance
NCCN	National Comprehensive Cancer Network
PACS	Picture archiving and communication system
ROI	Region-of-interest
TNM	Tumour, node and metastasis

Introduction

Gallbladder cancer (GBC) is the most prevalent cancer of the biliary tract worldwide [1]. It remains a highly aggressive tumour with limited therapeutic options and poor prognosis showing a 5-year overall survival rate of 5–10% and a median survival of 3–6 months [2, 3]. Although various treatments of the GBC are available depending on their clinical stage and the characteristics of the tumour cells, complete surgical resection is the only curative treatment option for GBC currently [1]. There are several prognostic factors for GBC including histologic type, tumour differentiation, vascular invasion and tumour, node and metastasis (TNM) stage [1, 3–5]. Among these, the TNM tumour stage has been the most reliable predictor in patients with GBC [3, 6]. However, patients diagnosed at the same tumour stage often have markedly different clinical prognoses due to tumour heterogeneity [7].

Recently, diffusion-weighted imaging (DWI) with measurement of apparent diffusion coefficient (ADC), a functional magnetic resonance (MR) imaging technique, which uses the diffusion properties of water within the targeted tissue, has been shown to have value in predicting the tumour grade and prognosis of various abdominal neoplasms. These include pancreatic neuroendocrine tumour [8, 9], gastric gastrointestinal tumour [10], rectal cancer [11] and hepatocellular carcinoma [12]. With regard to the gallbladder, this imaging technique demonstrates a gamut of clinical applications ranging from improvement of diagnostic accuracy for differentiating benign from malignant gallbladder lesions to assessment of tumour differentiation between adenoma and poorly differentiated GBC [13–17].

Therefore, we hypothesise that the ADC value may be one of the prognostic factors for disease-free survival (DFS) in patients with GBC after curative resection. However, there have been no studies on the ADC value as a prognostic factor for the prediction of long-term outcomes. Thus, we aimed to investigate the tumour ADC value as a prognostic factor for patients with GBC, as well as the relationship between the ADC value and other known prognostic parameters, including tumour differentiation and clinical tumour staging based on the 7th American Joint Committee on Cancer (AJCC) system.

Materials and methods

Patient selection

We conducted a retrospective study at a single tertiary academic centre. Our institutional review board approved this study. The requirement for informed consent was waived. Patients were identified through a search of our pathology department's registry database at the Samsung Medical Center, Sungkyunkwan University, Seoul, Korea. A total of 2157 patients who underwent any type of abdominal surgery with cholecystectomy between March 2008 and June 2016 were selected for this study. Among them, we identified 244 consecutive patients who were diagnosed with pathologically proven GBC using a database search by index keywords: the search terms "*specimen*: gallbladder" and "*pathology*: cancer or carcinoma". Then, 126 patients without preoperative MR imaging within 1 month prior to histopathologic confirmation of GBC, 24 without DWI or poor quality ADC map, and 15 with rare histologic subtypes (7 adenosquamous carcinomas, 6 neuroendocrine carcinomas and 2 undifferentiated carcinomas) were sequentially excluded. Finally, 79 patients (36 men, 43 women; median age, 63 years; range, 37–86 years) with pathologically proven gallbladder adenocarcinoma who underwent preoperative MR imaging with DWI were included in this study. The median time interval between pretreatment MR imaging and pathologic confirmation was 6 days (range, 1–29 days). The workflow of patient selection for this study is detailed in Fig. 1.

MR examination

All MR images were acquired using a 3.0-Tesla system (Intera Achieva; Philips Healthcare) with a 32-channel torso phased-array receiver coil. The baseline MR imaging was composed of a T1-weighted three-dimensional dual gradient-echo sequence, a breath-hold multi-shot T2-weighted sequence and a respiratory-triggered single-shot T2- and heavily T2-weighted sequence [18]. For contrast material-enhanced imaging, the arterial phase (20–35 s), portal phase (60 s), delayed phase (3 min) and hepatobiliary phase (20 min) were obtained using a T1-weighted three-dimensional gradient-echo sequence (T1 high-resolution isotropic volume examination, THRIVE, Philips Healthcare) with a spectral attenuated inversion recovery fat suppression technique. Gadoxetic acid (Primovist or Eovist; Bayer Schering Pharma) was intravenously administered at a rate of 1 mL/s for a dose of 0.025 mmol/kg using an injector, followed by a 20-mL saline flush [19]. The time for the arterial phase was determined using the MR fluoroscopic bolus detection technique [20]. DW images were acquired before the administration of

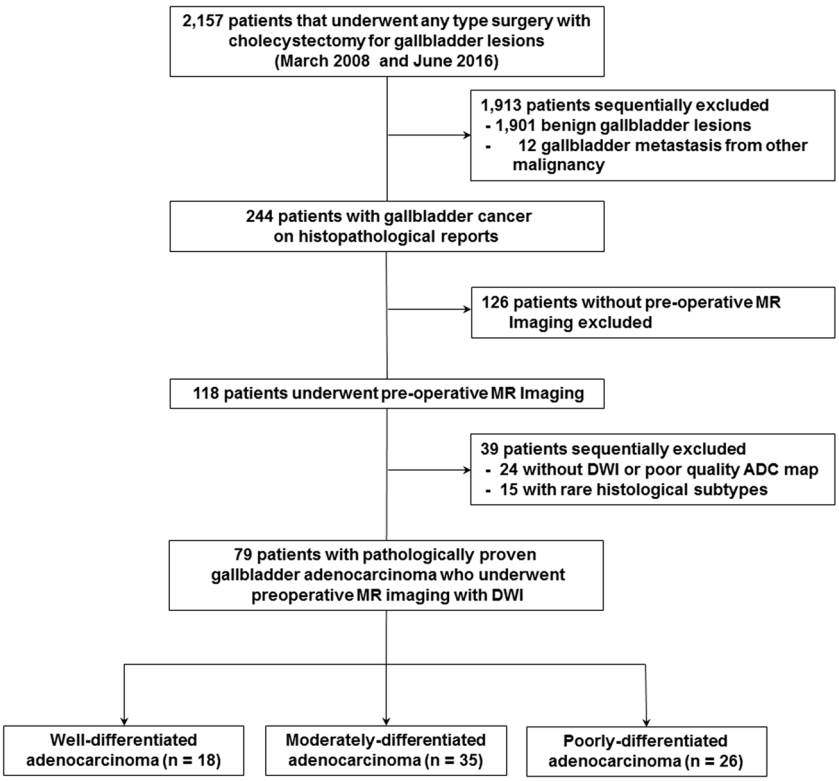

Fig. 1 Flow diagram of patient selection for the study. MR magnetic resonance, DWI diffusion-weighted imaging, ADC apparent diffusion coefficient

contrast agent by using respiratory-triggered single-shot echo-planar imaging with *b* values of 0, 100 and 800 mm^2/s. The ADC was calculated by a mono-exponential function using *b* values of 0 and 800 mm^2/s. The detailed parameters of the MR sequences used are shown in Table 1.

MR image analysis

Qualitative and quantitative MR imaging analyses were assessed by two abdominal radiologists (S.H.K. and J.H.M., with 20 and 11 years of experience in abdominal CT and MR imaging, respectively) who were blinded to the clinical-surgical-pathologic results, except for the information that all patients were confirmed to have GBC. All images were assessed using a picture archiving and communication system (PACS) (Centricity RA 1000; GE Healthcare).

Qualitative analysis

MR images were independently reviewed and a consensus on imaging features was reached afterwards. The imaging features evaluated were as follows: (a) morphologic type of GBC (wall-thickening type, polypoid or mass-forming type, and gallbladder replacement type) (representative figures in supplementary material Fig. E1); (b) tumour location (fundus, body, neck and diffuse); (c) presence of gallbladder stones; (d) pericholecystic infiltration; (e) adjacent hepatic invasion on images; (f) biliary dilatation; (g) regional lymph nodes enlargement (> 1 cm in short

Table 1 MR imaging sequences and parameters

Sequence	TR/TE (ms)	Flip angle (degree)	Section thickness (mm)	Matrix size	Bandwidth (Hx/pixel)	Field of view (cm)	Acquisition time (s)
T1-weighted 2D dual GRE	3.5/1.15–2.3	10	6	256 × 194	1918.6/0.226	32–38	14
T1-weighted 3D GRE	3.1/1.5	10	2	256 × 256	723.4/0.601	32–38	16.6
Breath-hold multi-shot T2-weighted imaging	1623/70	90	5	324 × 235	235.3/1.702	32–38	55
Respiratory-triggered single-shot heavily T2-weighted imaging	1156/160	90	5	376 × 270	388.9/1.117	32–38	120
Diffusion-weighted images	1600/70	90	5	112 × 112	79.5/5.467	32–38	126

2D two-dimensional, *3D* three-dimensional, *GRE* gradient echo, *TR* repetition time, *TE* echo time

axial diameter) and (h) signal intensity of tumour on T1- and T2-weighted images. The presence of pericholecystic infiltration was defined as circumferential infiltration that covered an area of the pericholecystic space [21]. The adjacent hepatic invasion was considered as fat plane between the gallbladder and adjacent liver and was disrupted on MR imaging [22]. The presence of biliary dilatation by GBC was defined when infiltrative tumour growth with spread along bile duct, lymph node enlargement, and intraductal spread of tumour results in biliary dilatation and obstruction [23]. The signal intensity of the lesion on T1- and T2-weighted images was classified as hypointense, isointense or hyperintense, according to a comparison with the adjacent normal hepatic parenchyma in each phase.

Quantitative analysis

Two radiologists independently measured the ADC value of the tumour using a region-of-interest (ROI) on the PACS workstation. A spatial cursor key in the PACS that enabled exact matching of the corresponding sites on different images was used. The largest possible circular ROI was freely drawn on the axial slice at the largest cross-sectional area of the tumour, to ensure uniformity of ROI placement (median, 25.1 mm^2; range, 10.6–79.4 mm^2). When the ROI was drawn on the index tumour, areas of necrosis, haemorrhage and MR imaging artefacts were avoided. These measurements were performed two times for each lesion and values were averaged to minimise measurement error [8].

Clinicopathologic review

Patients' medical records were reviewed regarding age, sex, operative methods for GBC and types of adjuvant treatments. In general, fluoropyrimidine or gemcitabine-based adjuvant chemotherapy or chemoradiation treatment was used when the patients had positive margins or more than T2 stage or lymph node metastasis on the specimen according to the National Comprehensive Cancer Network (NCCN) guidelines. In addition, histologic subtype of GBC, tumour differentiation, margin status of the specimen (R0, no residual; R1, microscopic residual; R2, macroscopic residual disease) and TNM stage were evaluated. All histopathological findings from the resected specimen were analysed by one pathologist specialising in liver pathology (K.T.J., with 17 years of experience in hepatobiliary disease). Tumour differentiation was based on the World Health Organization 2010 classification and TNM stage was based on the 7th AJCC staging system.

Survival analysis

The clinical outcome investigated was DFS. It was defined as the interval between the date of curative resection and the date of tumour recurrence or the time of the last follow-up at the outpatient clinic before 31 January 2017. Tumour recurrence was defined as any type of recurrence after initial surgical resection identified on follow-up imaging studies. This survival analysis was conducted on patients who had an R0 resection margin because a residual tumour after surgery is one of the confounding factors that affect prognosis [24].

Statistical analysis

A comparison of findings from MR imaging of GBCs according to the histologic tumour differentiation was performed using the Kruskal–Wallis test for analysis of continuous variables and a Fisher's exact test for categorical variables. Spearman's correlation analysis was used to determine the association between the ADC value and tumour differentiation or AJCC stage. The mean ADC value from both observers was used in all analyses except for calculating interobserver variability of ADC measurements. For an agreement in the analysis of measured ADC values between two observers, interobserver variability was assessed by using an intraclass correlation coefficient (κ) as follows: 0.00–0.20, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good; and 0.81–1.00, excellent agreement. In addition, the Bland–Altman plot was used in describing the interobserver variability of measured ADC. DFS was estimated by the Kaplan–Meier method with the log-rank test. For evaluating the prognostic factor of DFS, multivariable Cox regression analysis was performed with the forward stepwise selection method, based on the Akaike information criterion. In multivariable analysis, the following variables were used: age, sex, AJCC tumour stage, tumour differentiation, presence of hepatic invasion on histopathological report, presence of hepatic resection, presence of adjuvant treatment and ADC category (binary). The cut-off point of ADC for DFS to discriminate between good vs. poor prognosis was analysed by the Youden index from a receiver operating characteristic analysis [25]. All statistical analyses were performed using R version 3.3.1 (The R Foundation for Statistical Computing, Vienna, Austria). *P* values less than 0.05 were considered to be statistically significant.

Results

Baseline characteristics

The clinicopathologic characteristics of the 79 patients are described in Table 2. Median age of study patients was 63 years (range, 37–86 years). Patients with GBCs

Table 2 Clinicopathological characteristics of study patients

Characteristics	Patients (n = 79)
Age (years)	63 (37–86)
Male	36 (45.6)
Morphologic type of GBC	
Wall-thickening type	41 (51.9)
Polypoid or mass-forming type	32 (40.5)
Gallbladder replacement type	6 (7.6)
Tumour location	
Fundus	25 (31.7)
Body	42 (53.2)
Neck	7 (8.9)
Diffuse	5 (6.3)
Tumour size (cm)	2.8 (0.6–5.7)
Presence of coexistent gallstone	26 (32.9)
Tumour differentiation	
Well-differentiated	18 (22.8)
Moderately differentiated	35 (44.3)
Poorly differentiated	26 (32.9)
AJCC stage	
I	11 (13.9)
II	28 (35.4)
IIIA	4 (5.1)
IIIB	28 (35.4)
IVA	1 (1.3)
IVB	7 (8.9)
Type of surgery	
Simple cholecystectomy	16 (20.3)
Extended cholecystectomy[a]	63 (79.7)
Resection margin	
R0	71 (89.9)
R1	4 (5.1)
R2	4 (5.1)
Treatment	
Adjuvant chemotherapy	11 (13.9)
Adjuvant chemoradiation therapy	16 (20.3)

Continuous variables are described as median with range in parentheses and categorical variables are described as number of patients with a percentage in parentheses

[a] Extended cholecystectomy includes open cholecystectomy and en bloc hepatic resection with regional lymph node dissection with or without bile duct excision for malignant involvement

consisted of 18 well-differentiated (22.8%), 35 moderately differentiated (44.3%) and 26 poorly differentiated tumours (32.9%). The most common AJCC stage of GBCs was stage III (n = 32, 40.5%), followed by stage II (n = 28, 35.4%), stage I (n = 11, 13.9%) and stage IV (n = 8, 10.2%). Two patients (2.5%) had pancreaticobiliary maljunction on MR imaging.

MR imaging findings

Qualitative analysis

Among the various MR imaging findings, pericholecystic infiltration (p = 0.025) and regional lymph node enlargement (p = 0.038) were significantly different according to the histologic grades of GBCs. However, other imaging findings including adjacent hepatic invasion and biliary dilatation did not show significant differences among these groups (Table 3).

Quantitative analysis

According to the tumour differentiation, the median ADC value was 1.32×10^{-3} mm^2/s (range, 0.91–2.10 $\times 10^{-3}$ mm^2/s) in the well-differentiated group, 1.17×10^{-3} mm^2/s (range, 0.61–2.29 $\times 10^{-3}$ mm^2/s) in the moderately differentiated group and 0.91×10^{-3} mm^2/s (range, 0.50–1.78 $\times 10^{-3}$ mm^2/s) in the poorly differentiated group (Table 3) (Figs. 2 and 3). These ADC values and the tumour differentiation of GBCs were negatively correlated in a statistically significant manner ($\rho = -0.47$, $p < 0.001$). In addition, there was an inverse correlation between the ADC values and each component of the TN staging or AJCC stage system (T staging, $\rho = -0.37$, $p = 0.001$; N staging, $\rho = -0.31$, $p = 0.005$; AJCC stage, $\rho = -0.39$, $p = 0.001$) (Fig. 4). Regarding the interobserver agreement for the measured ADC values, the intraclass correlation coefficient was 0.93 (95% confidence interval [CI] = 0.88, 0.95) showing an excellent interobserver agreement (Fig. 5).

Survival analysis

Among 79 patients with GBCs, eight with R1 or R2 resection and two with synchronous common bile duct cancer were excluded from the DFS analysis. Sixty-nine patients were followed up for a range of 2.0–92.4 months (median, 23.5 months). As of January 2017, there were 17 cases (24.6%) of tumour recurrence after curative resection. With the cut-off ADC determined to be 1.04×10^{-3} mm^2/s for discrimination of DFS, 44 patients (63.8%) and 25 patients (36.2%) were classified into the high and low ADC groups, respectively. Diagnostic performance of this cut-off value for DFS is described in supplementary material Fig. E2. According to this classification of tumour ADC value, DFS rates were significantly different between the two groups (p = 0.004) (Fig. 6a). The 1- and 5-year DFS rates were 84.8% (95% CI = 74.2%, 97.0%) and 84.8% (95% CI = 74.2%, 97.0%) in the high ADC group and 54.0% (95% CI = 35.9%, 81.2%) and 47.2% (95% CI = 29.1%, 76.7%) in the low ADC group, respectively. In addition, DFS rates were significantly different according to the

Table 3 AJCC stage and MR imaging findings of gallbladder cancers according to the tumour differentiation

Variables	Well-differentiated (n = 18)	Moderately differentiated (n = 35)	Poorly differentiated (n = 26)	p value
AJCC stage				0.003
I	7 (38.9)	4 (11.4)	0	
II	8 (44.4)	12 (34.3)	8 (30.8)	
III	3 (16.7)	14 (40.0)	15 (57.7)	
IV	0	5 (14.3)	3 (11.5)	
Qualitative analysis				
T1-weighted image				0.489
Hypointense	11 (61.1)	23 (65.7)	20 (76.9)	
Iso- to hyperintense	7 (38.9)	12 (34.3)	6 (23.1)	
T2-weighted image				0.176
Hyperintense	17 (94.4)	34 (97.1)	22 (84.6)	
Iso- to hypointense	1 (5.6)	1 (2.9)	4 (15.4)	
Pericholecystic infiltration	2 (11.1)	14 (40.0)	13 (50.0)	0.025
Adjacent hepatic invasion	3 (16.7)	11 (31.4)	10 (38.5)	0.328
Biliary dilatation	0	1 (2.9)	2 (7.7)	0.597
Regional LN enlargement	3 (16.7)	11 (31.4)	14 (53.8)	0.038
Quantitative analysis				
ADC value ($\times 10^{-3}$ mm^2/s)	1.32 (0.91–2.10)	1.17 (0.61–2.29)	0.91 (0.50–1.78)	< 0.001
ADC group[a]				< 0.0001
High ADC	17 (94.4)	24 (68.6)	9 (34.6)	
Low ADC	1 (5.6)	11 (31.4)	17 (65.4)	

Continuous variables are described as median with range in parentheses and categorical variables are described as number of patients with percentage in parentheses

LN lymph node, *ADC* apparent diffusion coefficient

[a] Cut-off value of ADC was determined as 1.04×10^{-3} mm^2/s

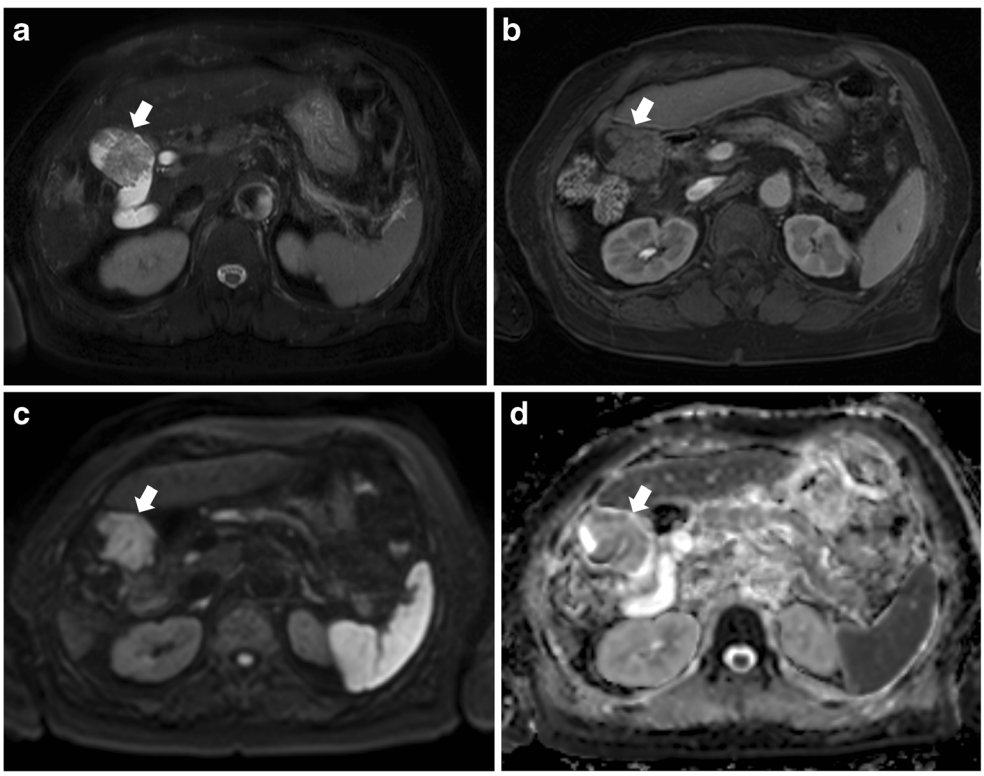

Fig. 2 Images of an 80-year-old woman with a well-differentiated adenocarcinoma of the gallbladder. **a** On T2-weighted axial MR image, a 5.7-cm intraluminal polypoid mass (white arrow) is located in the gallbladder body. **b** On the portal phase, the mass is confined within the gallbladder lumen without adjacent liver invasion. **c** On DW image with a b value of 800 /mm^2, the mass shows high signal intensity (white arrow) compared to the background liver. **d** On the ADC map, tumour ADC value was 1.57×10^{-3} mm^2/s. The patient underwent surgical resection and the mass was revealed to be well-differentiated adenocarcinoma (pT1N0M0, AJCC stage I)

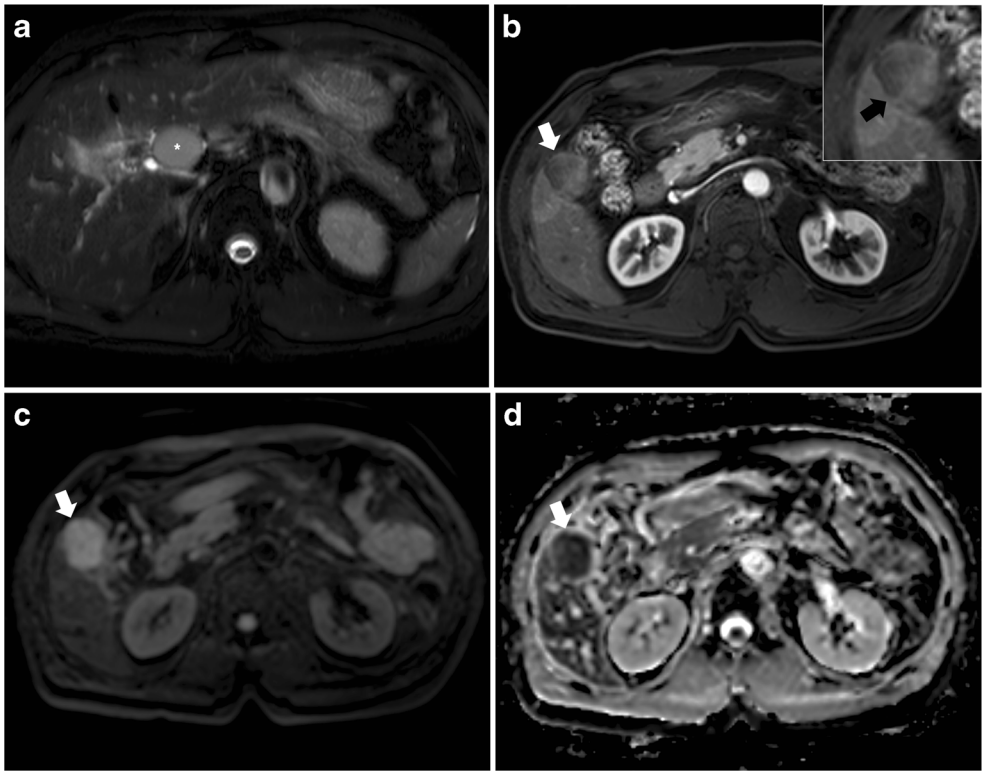

Fig. 3 Images of a 56-year-old man with a poorly differentiated adenocarcinoma of the gallbladder. **a** On the T2-weighted axial MR image, a well-defined nodular lesion with high signal intensity (asterisk) is seen as a metastatic lymph node around the hepatoduodenal ligament. **b** On the portal phase, gallbladder fundus is filled with a 4-cm mass (white arrow). There is pericholecystic infiltration around the gallbladder bed with direct hepatic invasion showing disruption of normal hepatic capsule (black arrow). **c** On DW image with a *b* value of 800 /mm2, the mass shows high signal intensity (white arrow) compared to the background liver. **d** On the ADC map, tumour ADC value was 0.84×10^{-3} mm^2/s. The patient underwent surgical resection and the mass was revealed to be poorly differentiated adenocarcinoma (pT3N1M0, AJCC stage IIIB)

AJCC stage ($p = 0.003$) (Fig. 6b). However, survival rates between patient groups determined by the degree of tumour differentiation did not show any significant difference ($p = 0.113$) (Fig. 6c).

Prognostic factor analysis for DFS

In the multivariate analysis, old age (hazard ratio [HR] = 1.08; 95% CI 1.02, 1.14, $p = 0.008$), adjuvant chemotherapy (HR = 14.15; 95% CI 3.41, 58.69, $p < 0.001$), adjuvant chemoradiation therapy (HR = 5.80, 95% CI 1.72, 19.56, $p = 0.005$) and low ADC value (HR = 4.29; 95% CI 1.44, 12.77, $p = 0.009$) were significant prognostic factors for poor DFS (Table 4).

Discussion

Our results demonstrate the potential role of the ADC value in the identification of histologic tumour differentiation as well as AJCC stage in patients with resectable GBC. Furthermore, multivariate analysis showed that the specific cut-off tumour ADC value was an independent prognostic factor for long-term DFS. Therefore, tumour recurrence after curative surgical resection for GBC could be predicted by using DWI preoperatively.

We have demonstrated that the histologic tumour differentiation had a significantly inverse correlation with ADC values in patients with GBCs. Consistent with our results, a recent study [15] reported that the ADC values of well-differentiated adenocarcinomas in the gallbladder were significantly higher than those of higher-grade (moderately and poorly differentiated) adenocarcinomas. In general, well-differentiated adenocarcinomas are known to have more of a glandular structure and show less cellular density, which causes an increase in water diffusion, compared with moderately and poorly differentiated adenocarcinomas [26]. This discrepancy of tumour

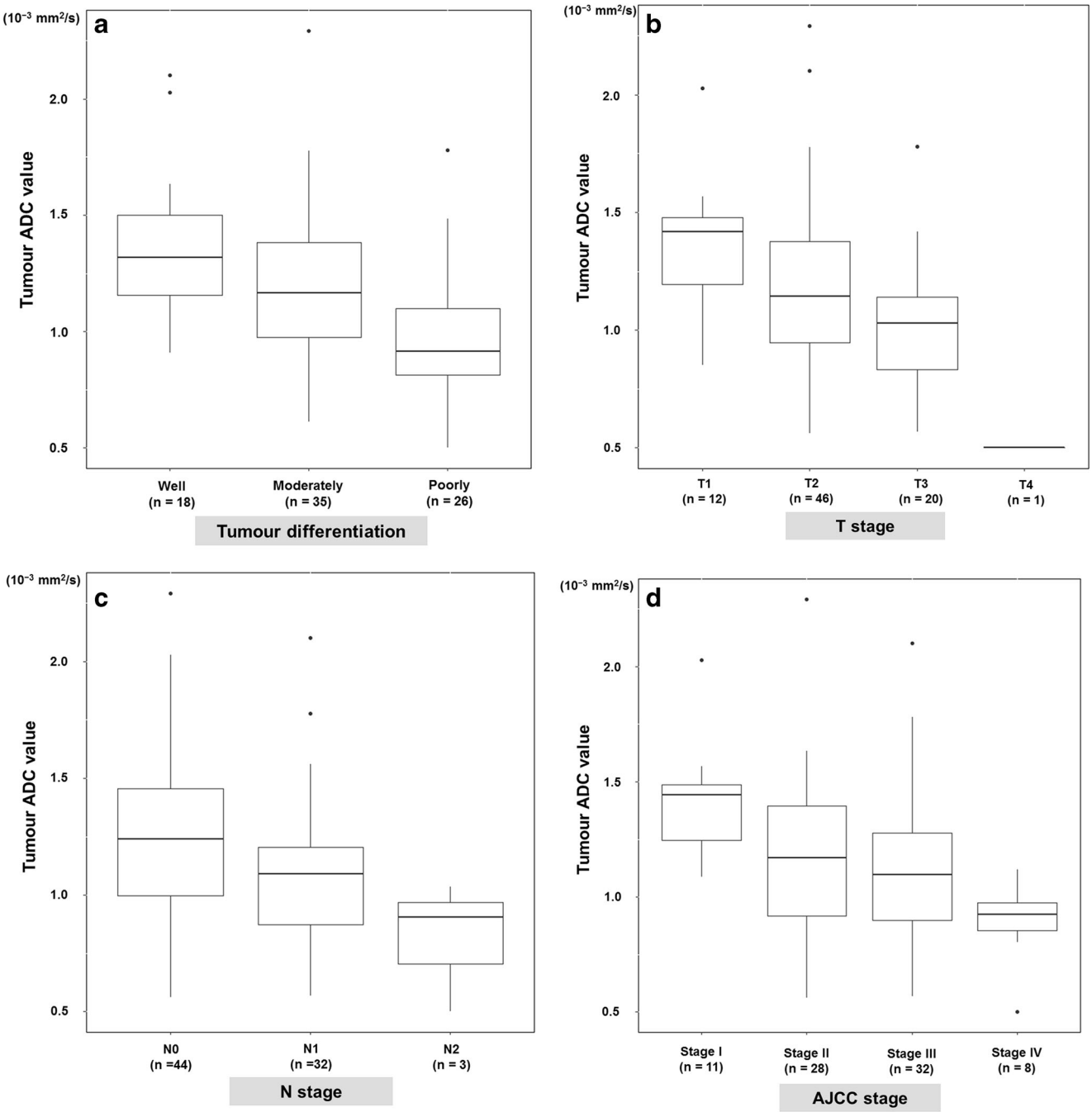

Fig. 4 Box-and-whisker plot of the ADC values of gallbladder cancer and tumour differentiation, each TN staging and AJCC staging. **a** Tumour ADC values according to the tumour differentiation. Line within the box represents the median of each group. Top and bottom boxes are 25th and 75th percentiles, respectively. Circles outside the box are outliers. **b** Tumour ADC values according to the tumour stage. **c** Tumour ADC values according to the nodal stage. **d** Tumour ADC values according to the AJCC stage

cellularity, according to the tumour differentiation, would lead to a significant correlation between the ADC value of GBC and tumour differentiation.

In our analysis, the ADC value of GBCs decreased as the tumour or nodal stage and AJCC stage increased. As observed in previous studies [3, 27], the depth of tumour invasion and regional nodal status of GBCs are well-known prognostic indicators. However, they do not alter tumour cellular architecture directly, which can affect the diffusion properties of water within GBCs. Thus, it is interesting that there was a significant difference in ADC values according to tumour or nodal stage of GBCs. This may be partly explained by the fact that the histologic tumour differentiation and tumour

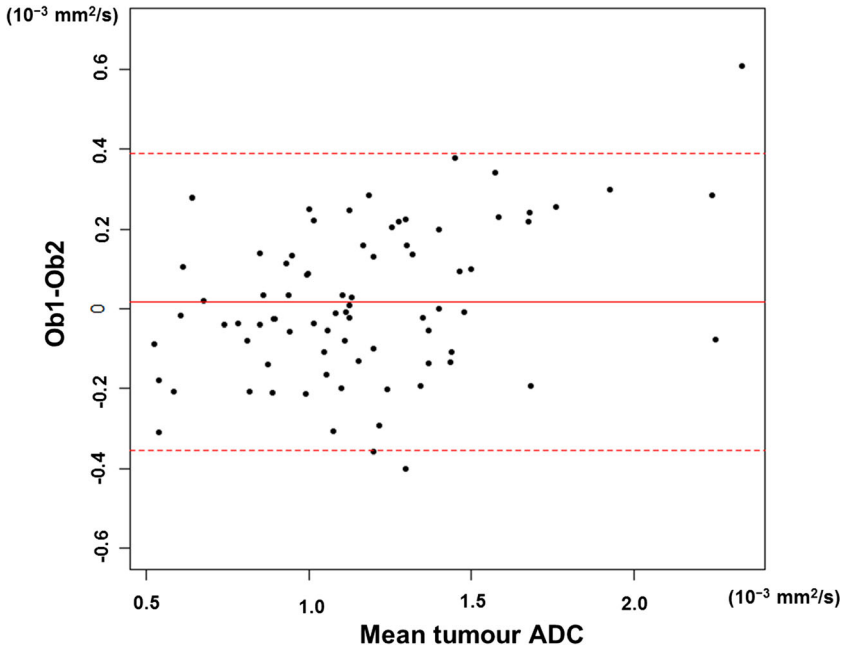

Fig. 5 Altman–Bland plot demonstrating the interobserver agreement for the ADC values of gallbladder cancers. The red solid line represents the mean; the red dashed lines represent ± 2 standard deviations. The intraclass correlation coefficient for the measured ADC values was 0.93 (95% CI = 0.88, 0.95)

aggressiveness tend to be positively correlated. Poor differentiation of GBCs showing low ADC values could be manifested as a high tumour or nodal stage due to tumour aggressiveness. In line with this assumption, pericholecystic infiltration and regional lymph node enlargement were more frequently observed in patients with poorly differentiated GBCs, compared to those with well-differentiated tumours in our qualitative MR imaging analysis.

In our results, the low ADC value of GBCs was one of the independent predictive factors for poor long-term DFS, given that other variables were adjusted in a multivariate analysis. Use of adjuvant chemotherapy or chemoradiation treatment was one of the other significant factors for this outcome. Although AJCC tumour stage was not entered into our multivariate model during stepwise selection, the degree of tumour invasion or presence of lymph node metastasis as a major component of the AJCC staging system could have an important role as a confounding factor. This might lead to a poor prognosis in our cohort, who underwent chemotherapy or chemoradiation treatment for a locally advanced disease. In addition, therapeutic efficacy of adjuvant treatment may be ineffective in the setting of R0 resection for patients with GBC, as observed in our cohort. Similarly, a previous randomised controlled trial [28] and a nationwide study using the National Cancer Data Base [29] demonstrated that patients with GBC

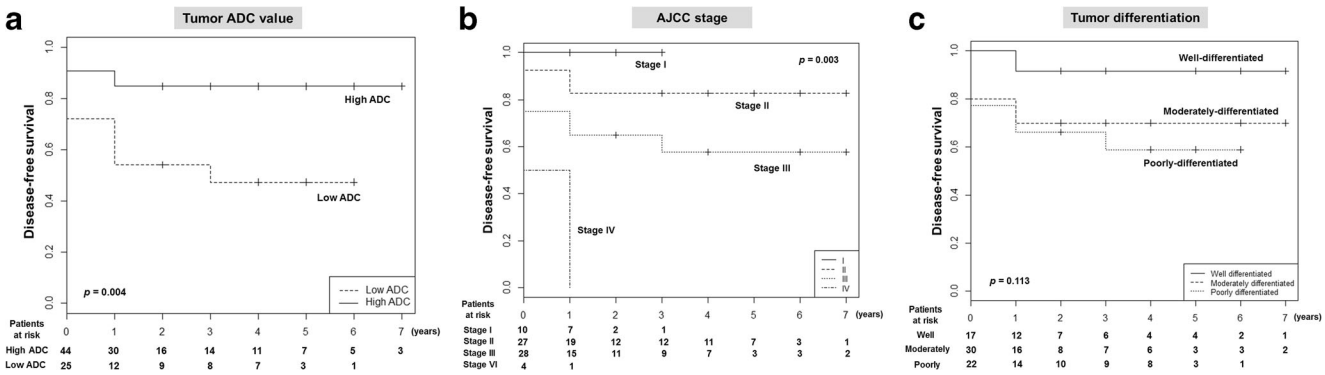

Fig. 6 Disease-free survival after curative resection in 69 patients with gallbladder cancer. **a** Disease-free survival rates according to the measured tumour ADC value. The cut-off value of ADC was determined as 1.04 × 10⁻3 mm2/s. **b** Disease-free survival rates according to the AJCC stage. **c** Disease-free survival rates according to the histologic tumour differentiation

Table 4 Prognostic factor analysis for disease-free survival in patients with R0 resection

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p* value	HR	95% CI	*p* value
Age	1.03	0.98, 1.08	0.315	1.08	1.02, 1.14	0.008
Male (female)	1.26	0.49, 3.28	0.629	2.27	0.79, 6.49	0.127
AJCC stage (IV)			0.037			
Stage II	0.10	0.02, 0.48	0.004			
Stage III	0.30	0.08, 1.11	0.070			
Tumour differentiation (well-differentiated)			0.066			
Moderately differentiated	5.40	0.68, 43.18	0.112			
Poorly differentiated	6.84	0.86, 54.74	0.070			
Hepatic invasion on pathologic report (absence)	4.81	1.81, 12.77	0.002			
Hepatic resection (no)	3.92	0.52, 29.53	0.185			
Adjuvant treatment (no)			0.001			< 0.001
Adjuvant chemotherapy	10.27	2.84, 37.13	< 0.001	14.15	3.41, 58.69	0.001
Adjuvant chemoradiation therapy	4.81	1.61, 14.31	0.005	5.80	1.72, 19.56	0.005
Low ADC (high ADC)[a]	3.92	1.44, 10.63	0.007	4.29	1.44, 12.77	0.009

Multivariable Cox regression analysis was performed with the forward stepwise selection method based on the Akaike information criterion. The reference category for each variable is parentheses in the first column. There was no recurrence in patients with AJCC stage I

[a] Cut-off value of ADC was determined as 1.04×10^{-3} mm^2/s

who undergo R0, but not R1 resections, did not show survival benefit from adjuvant treatment.

Our study had several limitations. First, our retrospective study design may have resulted in selection bias. Second, we included only surgically resected GBCs, which may have led to the exclusion of GBCs at a more advanced stage with distant metastasis. Therefore, our results may not reflect the entire spectrum of GBCs. However, currently, the only curative treatment option of GBC is surgical resection. In this respect, our findings can provide useful information to physicians for predicting the long-term prognosis of patients with resectable GBC preoperatively. Third, although our interobserver agreement for measurement of ADC was excellent, some cases of wall thickening-type GBCs could make it difficult to measure the ADC value within the tumour. Fourth, an external validation study using another set of patients was not performed. Fifth, we did not perform the histogram analysis of ADC maps including the minimum and maximum ADC values. However, we think that the measurement of mean ADC value is a simple and widely used representative method in clinical practice. Sixth, our cases were enrolled from a single centre. Therefore, follow-up studies with a prospective multicentre design are warranted to confirm the usefulness of tumour ADC value as an imaging biomarker of patients with GBC. Although the role of adjuvant treatment is debatable in patients with R0 resection for GBC, using the ADC value for selected high-risk patients may lead to its enrolment into clinical trials of novel regimens beyond the NCCN-endorsed adjuvant treatments that are currently considered for patients with T3 or lymph node metastasis.

In conclusion, the measured ADC value on DW MR images was significantly correlated with histologic tumour differentiation as well as AJCC stage in patients with GBCs. In addition, patients with GBCs that had low ADC values were associated with poor DFS. Since measurement of tumour ADC value on DWI is simple and reliable, ADC may serve as an effective prognostic imaging biomarker in patients with GBC.

Funding The authors state that this work has not received any funding.

Compliance with ethical standards

Guarantor The scientific guarantor of this publication is Won Jae Lee.

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 (Soo Hyun Ahn) has significant statistical expertise.

Informed consent Written informed consent was waived by the institutional review board.

Ethical approval Institutional review board approval was obtained.

Methodology
- Retrospective
- Prognostic study
- Performed at one institution

References

1. Kanthan R, Senger JL, Ahmed S, Kanthan SC (2015) Gallbladder cancer in the 21st century. J Oncol 2015:967472
2. Cubertafond P, Gainant A, Cucchiaro G (1994) Surgical treatment of 724 carcinomas of the gallbladder. Results of the French Surgical Association Survey. Ann Surg 219:275–280
3. Lim H, Seo DW, Park DH et al (2013) Prognostic factors in patients with gallbladder cancer after surgical resection: analysis of 279 operated patients. J Clin Gastroenterol 47:443–448
4. Henson DE, Albores-Saavedra J, Corle D (1992) Carcinoma of the gallbladder. Histologic types, stage of disease, grade, and survival rates. Cancer 70:1493–1497
5. Bartlett DL (2000) Gallbladder cancer. Semin Surg Oncol 19:145–155
6. Kayahara M, Nagakawa T, Nakagawara H, Kitagawa H, Ohta T (2008) Prognostic factors for gallbladder cancer in Japan. Ann Surg 248:807–814
7. Alizadeh AA, Aranda V, Bardelli A et al (2015) Toward understanding and exploiting tumor heterogeneity. Nat Med 21:846–853
8. Kim M, Kang TW, Kim YK et al (2016) Pancreatic neuroendocrine tumour: Correlation of apparent diffusion coefficient or WHO classification with recurrence-free survival. Eur J Radiol 85:680–687
9. Jang KM, Kim SH, Lee SJ, Choi D (2014) The value of gadoxetic acid-enhanced and diffusion-weighted MRI for prediction of grading of pancreatic neuroendocrine tumors. Acta Radiol 55:140–148
10. Kang TW, Kim SH, Jang KM et al (2015) Gastrointestinal stromal tumours: correlation of modified NIH risk stratification with diffusion-weighted MR imaging as an imaging biomarker. Eur J Radiol 84:33–40
11. Akashi M, Nakahusa Y, Yakabe T et al (2014) Assessment of aggressiveness of rectal cancer using 3-T MRI: correlation between the apparent diffusion coefficient as a potential imaging biomarker and histologic prognostic factors. Acta Radiol 55:524–531
12. Shankar S, Kalra N, Bhatia A et al (2016) Role of diffusion weighted imaging (DWI) for hepatocellular carcinoma (HCC) detection and its grading on 3T MRI: a prospective study. J Clin Exp Hepatol 6:303–310
13. Irie H, Kamochi N, Nojiri J, Egashira Y, Sasaguri K, Kudo S (2011) High b-value diffusion-weighted MRI in differentiation between benign and malignant polypoid gallbladder lesions. Acta Radiol 52:236–240
14. Lee NK, Kim S, Kim TU, Kim DU, Seo HI, Jeon TY (2014) Diffusion-weighted MRI for differentiation of benign from malignant lesions in the gallbladder. Clin Radiol 69:e78–e85
15. Lee NK, Kim S, Moon JI et al (2016) Diffusion-weighted magnetic resonance imaging of gallbladder adenocarcinoma: analysis with emphasis on histologic grade. Clin Imaging 40:345–351
16. Kang TW, Kim SH, Park HJ et al (2013) Differentiating xanthogranulomatous cholecystitis from wall-thickening type of gallbladder cancer: added value of diffusion-weighted MRI. Clin Radiol 68:992–1001
17. Kim SJ, Lee JM, Kim H, Yoon JH, Han JK, Choi BI (2013) Role of diffusion-weighted magnetic resonance imaging in the diagnosis of gallbladder cancer. J Magn Reson Imaging 38:127–137
18. Kang TW, Rhim H, Lee J et al (2016) Magnetic resonance imaging with gadoxetic acid for local tumour progression after radiofrequency ablation in patients with hepatocellular carcinoma. Eur Radiol 26:3437–3446
19. Hwang JA, Kang TW, Kim YK et al (2017) Association between non-hypervascular hypointense nodules on gadoxetic acid-enhanced MRI and liver stiffness or hepatocellular carcinoma. Eur J Radiol 95:362–369
20. Haradome H, Grazioli L, Tsunoo M et al (2010) Can MR fluoroscopic triggering technique and slow rate injection provide appropriate arterial phase images with reducing artifacts on gadoxetic acid-DTPA (Gd-EOB-DTPA)-enhanced hepatic MR imaging? J Magn Reson Imaging 32:334–340
21. Chun KA, Ha HK, Yu ES et al (1997) Xanthogranulomatous cholecystitis: CT features with emphasis on differentiation from gallbladder carcinoma. Radiology 203:93–97
22. Kim SJ, Lee JM, Lee ES, Han JK, Choi BI (2015) Preoperative staging of gallbladder carcinoma using biliary MR imaging. J Magn Reson Imaging 41:314–321
23. Levy AD, Murakata LA, Rohrmann CA Jr (2001) Gallbladder carcinoma: radiologic-pathologic correlation. Radiographics 21:295-314; questionnaire, 549-255
24. Shirai Y, Sakata J, Wakai T, Ohashi T, Hatakeyama K (2012) "Extended" radical cholecystectomy for gallbladder cancer: long-term outcomes, indications and limitations. World J Gastroenterol 18:4736–4743
25. Fluss R, Faraggi D, Reiser B (2005) Estimation of the Youden index and its associated cutoff point. Biom J 47:458–472
26. Sun Y, Tong T, Cai S, Bi R, Xin C, Gu Y (2014) Apparent diffusion coefficient (ADC) value: a potential imaging biomarker that reflects the biological features of rectal cancer. PLoS One 9:e109371
27. Murakami Y, Uemura K, Sudo T et al (2011) Prognostic factors of patients with advanced gallbladder carcinoma following aggressive surgical resection. J Gastrointest Surg 15:1007–1016
28. Takada T, Amano H, Yasuda H et al (2002) Is postoperative adjuvant chemotherapy useful for gallbladder carcinoma? A phase III multicenter prospective randomised controlled trial in patients with resected pancreaticobiliary carcinoma. Cancer 95:1685–1695
29. Mantripragada KC, Hamid F, Shafqat H, Olszewski AJ (2016) Adjuvant therapy for resected gallbladder cancer: analysis of the National Cancer Data Base. J Natl Cancer Inst 109 https://doi.org/10.1093/jnci/djw202