



^{68}Ga -FAPI-04 Versus ^{18}F -FDG PET/CT in the Detection of Hepatocellular Carcinoma

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Background: Fibroblast activation protein (FAP) is commonly expressed in activated stromal fibroblasts in various epithelial tumours. Recently, ^{68}Ga -FAPI-04 has been used for tumour imaging in positron emission tomography/computed tomography (PET/CT). This study aimed to compare the diagnostic performances of ^{68}Ga -FAPI-04 PET/CT and ^{18}F -FDG PET/CT in hepatocellular carcinoma (HCC), and to assess factors associated with ^{68}Ga -FAPI-04 uptake in HCC.

Materials and Methods: Twenty-nine patients with suspiciously HCC who received both ^{18}F -FDG and ^{68}Ga -FAPI-04 PET/CT were included in this retrospective study. The results were interpreted by two experienced nuclear medicine physicians independently. The maximum and mean standardized uptake values (SUV_{max} and SUV_{mean}) were measured in the lesions and liver background, respectively. The tumour-to-background ratio (TBR) was then calculated as lesion's SUV_{max} divided by background SUV_{mean} .

Results: A total of 35 intrahepatic lesions in 25 patients with HCC were finally involved in the statistical analysis. ^{68}Ga -FAPI-04 PET/CT showed a higher sensitivity than ^{18}F -FDG PET/CT in detecting intrahepatic HCC lesions (85.7% vs. 57.1%, $P = 0.002$), including in small (≤ 2 cm in diameter; 68.8% vs. 18.8%, $P = 0.008$) and well- or moderately-differentiated (83.3% vs. 33.3%, $P = 0.031$) tumors. SUV_{max} was comparable between ^{68}Ga -FAPI-04 and ^{18}F -FDG (6.96 ± 5.01 vs. 5.89 ± 3.38 , $P > 0.05$), but the TBR was significantly higher in the ^{68}Ga -FAPI-04 group compared with the ^{18}F -FDG group (11.90 ± 8.35 vs. 3.14 ± 1.59 , $P < 0.001$). SUV_{max} and the TBR in ^{68}Ga -FAPI-04 positive lesions were associated with tumour size (both $P < 0.05$), but not the remaining clinical and pathological features (all $P > 0.05$).

Conclusions: ^{68}Ga -FAPI-04 PET/CT is more sensitive than ^{18}F -FDG PET/CT in detecting HCC lesions, and ^{68}Ga -FAPI-04 uptake is correlated mainly with tumour size.

Keywords: ^{68}Ga -FAPI-04, ^{18}F -FDG, hepatocellular carcinoma (HCC), cancer-associated fibroblast (CAF), fibroblast activating protein (FAP)

INTRODUCTION

Hepatocellular carcinoma (HCC), the most frequent primary liver cancer, is the fourth most common cause of cancer-related death worldwide (1, 2). The majority of HCCs occur in patients with underlying liver disease, mostly as a result of hepatitis B or C virus (HBV or HCV) infection or alcohol abuse (3). Unlike many other malignant tumours, HCC can be diagnosed by imaging based on non-invasive criteria without confirmatory pathology (4). Therefore, imaging plays a critical role in the detection and diagnosis of HCC. Conventional imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound, are mainly utilized for anatomical evaluation, with limited value in the assessment of morphologically atypical lesions (5). In contrast, positron emission tomography/computed tomography (PET/CT) as one of the functional imaging approaches has the potential to provide relevant biological information in HCC and to improve response assessment (6). Furthermore, a subset of HCCs cannot be diagnosed non-invasively either because the patients do not have cirrhosis or the lesions do not follow established enhancement patterns on contrast-enhanced CT or MRI (1, 3). Therefore, molecular imaging with PET/CT offers potential additional advantages to non-invasively confirm a diagnosis of HCC. However, the most widely available clinical PET tracer, ¹⁸F-FDG, shows poor sensitivity for the detection of HCC ranging from 40% to 68%, mainly because of the relatively high glucose-6-phosphatase activity found in low-grade HCC (6). In addition, it is not useful for the detection of small HCC lesions (7, 8). Therefore, several new tracers have been developed and applied for HCC detection, including ¹¹C-acetate, ¹¹C-choline, ⁶⁸Ga-PSMA (9–12).

Cancer-associated fibroblasts (CAFs) are among the most crucial components of the tumour microenvironment that creates a favourable microenvironment for tumour growth, invasion and metastasis (13, 14). Fibroblast activation protein (FAP), a cell surface glycoprotein belonging to the serine protease family, is commonly expressed in activated stromal fibroblasts in various epithelial tumours (15, 16). Recent investigations indicated that ⁶⁸Ga-labelled FAP inhibitor (FAPI) shows an equal or even improved tumour imaging with lower background uptake in the liver and the brain in comparison to ¹⁸F-FDG in various cancers (17, 18). ⁶⁸Ga-FAPI-04 PET/CT was also revealed to have high sensitivity in detecting hepatic malignancies (19–21). Therefore, ⁶⁸Ga-FAPI-04 may be a potential tracer for visualizing HCC by targeting CAFs that are abundant in the tumour microenvironment. This study aimed to comparatively assess the diagnostic performances of ⁶⁸Ga-FAPI-04 PET/CT and ¹⁸F-FDG PET/CT in HCC and to assess factors associated with the uptake of ⁶⁸Ga-FAPI-04 in HCC.

MATERIALS AND METHODS

Patients

This is a post-hoc analysis of a prior prospective study conducted at the Huashan Hospital of Fudan University. Twenty-nine

patients with suspiciously incipient or recurrent HCC determined by clinical manifestations and conventional imaging techniques (CT, MRI and ultrasound) were included in this retrospective study. They underwent both ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT examinations with an interval of one day before surgical treatment. In patients who underwent surgery or biopsy, the definitive diagnosis was confirmed by pathology. In patients who underwent transarterial chemoembolization (TACE), HCC diagnosis was based on a specific imaging pattern of hyperenhancement in the arterial phase and washout in the venous or delayed phase, on contrast-enhanced CT or MRI in the setting of liver cirrhosis (1, 3). This study was approved by the institutional ethics committee, and written informed consent was obtained from all patients.

PET/CT Imaging

Whole-body static FDG PET/CT scans were obtained as a routine procedure on a dedicated PET/CT scanner (Biograph mCT Flow scanner, Siemens, Germany). Whole-body ⁶⁸Ga-FAPI-04 PET/CT scans were obtained on another PET/CT scanner (μ MI510, Union imaging, Shanghai, China) within 60 min after intravenous injection of \sim 185 MBq (\sim 5 mCi) of ⁶⁸Ga-FAPI-04. Low-dose CT scans were obtained for attenuation correction and image fusion. PET images were acquired in the 3D mode, and reconstructed by the ordered subset expectation maximization 3D (OSEM 3D) method.

Because two different PETs were applied in this study, SUVs were normalized after data collection for PET/CT system performance harmonization. NEMA IEC body phantom (Data Spectrum Corporation, Durham, NC, USA) with 6 simulated lesion spheres (diameters of 10 mm, 13 mm, 17 mm, 22 mm, 28 mm and 37 mm, respectively) and 2, 4, 8, and 16 times of background activity (2 kBq/mL of background activity concentration) based on routine scan protocols was applied for SUV normalization.

Image Evaluation

¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT images were interpreted independently by two experienced nuclear medicine physicians blinded to other imaging and pathology results. The maximum standardized uptake value (SUV_{max}) was measured by delineating a spherical region of interest (ROI) for each lesion. The mean standardized uptake value (SUV_{mean}) of the liver background was measured by drawing a spherical ROI with 2 cm diameter in the non-tumour hepatic parenchyma of the right lobe in each patient. The tumour-to-background ratio (TBR) was calculated by dividing the lesion's SUV_{max} with the background SUV_{mean} . A lesion was considered to be positive on the basis of the visual judgment of elevated uptake in the tumour tissue by 2 experienced nuclear medicine physicians independently, supported by the calculation of the TBRs of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04, respectively. Any difference of opinion between these two physicians was settled by mutual agreement.

Statistical Analysis

All statistical analyses were conducted with the STATA statistical analysis software (StataCorp LLC, version 15.1). Categorical

variables were presented as frequency and percentage, and continuous variables as mean \pm standard deviation (SD). The McNemar's test and Fisher exact test were performed to compare categorical variables. Non-parametric tests were carried out for the comparison of continuous variables with non-normal distribution. The Spearman rank correlation coefficient was determined to assess the correlation between continuous variables with non-normal distribution. Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics

Twenty-nine patients were included in the current study, including 23 treated by hepatic surgery, 5 administered TACE, and one that underwent biopsy only. Except for 3 patients who were diagnosed with benign hepatic nodules, the remaining 26 patients were diagnosed with HCC. One recurrent HCC case who underwent surgical resection had extensive peritoneal dissemination but no intrahepatic lesions. Therefore, 25 HCC patients with 35 intrahepatic lesions were finally involved in the statistical analysis. The study flowchart is presented in **Figure 1**. According to microvascular invasion (MVI) number and distribution, 2, 8 and 10 patients were categorized into the M0 (no MVI), M1 (≤ 5 MVI in adjacent liver tissue ≤ 1 cm away from the HCC), and M2 (> 5 MVI or MVI in adjacent liver tissue > 1 cm away from the HCC) groups, respectively. According to the American Joint Committee on Cancer (AJCC) cancer staging system (8th Edition), 5, 10, 4 and 1 patients were categorized into stage I, II, III and IV, respectively. The general characteristics of the 25 HCC patients are summarized in **Table 1**.

TABLE 1 | Characteristics of the included HCC patients.

General characteristics	n = 25	%
Age (years)	59.40 \pm 6.90	
Gender (male)	24	96
HBsAg (+)	20	80
Anti-HCV (+)	0	0
Cirrhosis	19	76
AFP (> 20 ng/mL)	12	48
Tumour number		
Solitary tumour	15	60
Multiple tumours	10	40
MVI		
M0	2	8
M1	8	32
M2	10	40
AJCC TNM stage		
I	5	20
II	10	40
III	4	16
IV	1	4

Five HCC patients whose diagnosis was based on non-invasive criteria underwent TACE instead of hepatic surgery and, therefore, had no pathological data. HBsAg, hepatitis B surface antigen; Anti-HCV, anti-hepatitis C virus antibody; AFP, α -fetoprotein; MVI, microvascular invasion; AJCC TNM, American Joint Committee on Cancer tumour-node-metastasis.

Comparison of ⁶⁸Ga-FAPI-04 With ¹⁸F-FDG in Patient-Based Analysis

The results of the patient-based analysis of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT are summarized in **Table 2**. Of the 35 intrahepatic HCCs, 20 were tested positive by both ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT, 10 were tested positive by ⁶⁸Ga-FAPI-04 PET/CT alone, and 5 were not tested positive by either method. ⁶⁸Ga-FAPI-04 PET/CT showed a better sensitivity in detecting

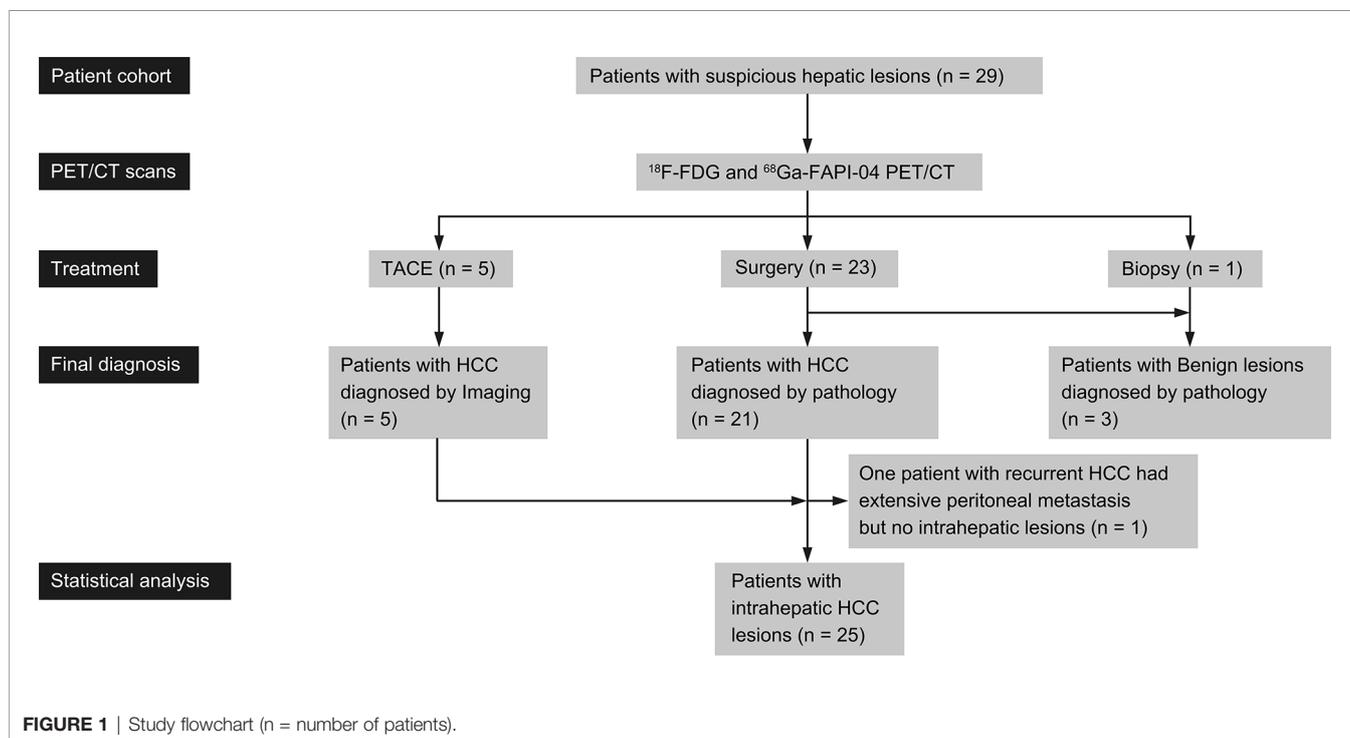


TABLE 2 | Sensitivities of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT in patient-based analysis.

Patient characteristics	No. of patients	No. of lesions	¹⁸ F-FDG		⁶⁸ Ga-FAPI-04		P between 2 tracers
			Positive lesions (%)	P	Positive lesions (%)	P	
All	25	35	20 (57.1)		30 (85.7)		0.002*
Clinical features							
Cirrhosis	19	29	16 (55.2)	0.680	24 (82.8)	0.561	0.008*
Non-cirrhosis	6	6	4 (66.7)		6 (100)		0.500
AFP (ng/mL)							
≤ 20	13	17	11 (64.7)	0.500	17 (100)	0.045*	0.031*
> 20	12	18	9 (50.0)		13 (72.2)		0.125
Tumour number							
Solitary tumour	15	15	10 (66.7)	0.492	14 (93.3)	0.365	0.125
Multiple tumours	10	20	10 (50.0)		16 (80.0)		0.031*
MVI							
M0 + M1	10	14	4 (28.6)	0.057	10 (71.4)	0.326	0.031*
M2	10	14	10 (71.4)		13 (92.9)		0.250
AJCC TNM staging							
I	5	5	0 (0)	0.016*	4 (80.0)	0.423	0.125
II	10	16	8 (50.0)		12 (75.0)		0.125
III + IV	5	7	6 (85.7)		7 (100)		1

Five HCC patients whose diagnosis was based on non-invasive criteria underwent TACE instead of hepatic surgery and, therefore, had no pathological data. *, statistically significant; AFP, α -fetoprotein; MVI, microvascular invasion; AJCC TNM, American Joint Committee on Cancer tumour-node-metastasis.

intrahepatic lesions compared with ¹⁸F-FDG PET/CT (85.7% vs. 57.1%, $P = 0.002$). In subgroup analysis, ⁶⁸Ga-FAPI-04 PET/CT was more sensitive than ¹⁸F-FDG PET/CT in the detection of intrahepatic lesions in patients with cirrhosis, low α -fetoprotein (AFP), multiple tumours, and non-serious MVI (M0 and M1) (all $P < 0.05$). Moreover, ⁶⁸Ga-FAPI-04 PET/CT detected 4 of the 5 lesions in patients with stage I disease, whereas ¹⁸F-FDG PET/CT did not reveal any abnormal finding in these patients (**Figure 2**). The sensitivity of ¹⁸F-FDG PET/CT was associated with AJCC TNM stage ($P = 0.016$), while that of ⁶⁸Ga-FAPI-04 PET/CT was

correlated with serum AFP levels ($P = 0.045$). These findings suggested that ⁶⁸Ga-FAPI-04 PET/CT was more sensitive than ¹⁸F-FDG PET/CT in the detection of intrahepatic lesions, particularly in patients with cirrhosis, low AFP, multiple HCCs, and non-serious MVI.

Comparison of ⁶⁸Ga-FAPI-04 With ¹⁸F-FDG in Lesion-Based Analysis

The results of lesion-based analysis of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT are summarized in **Table 3**. ⁶⁸Ga-FAPI-04 PET/CT

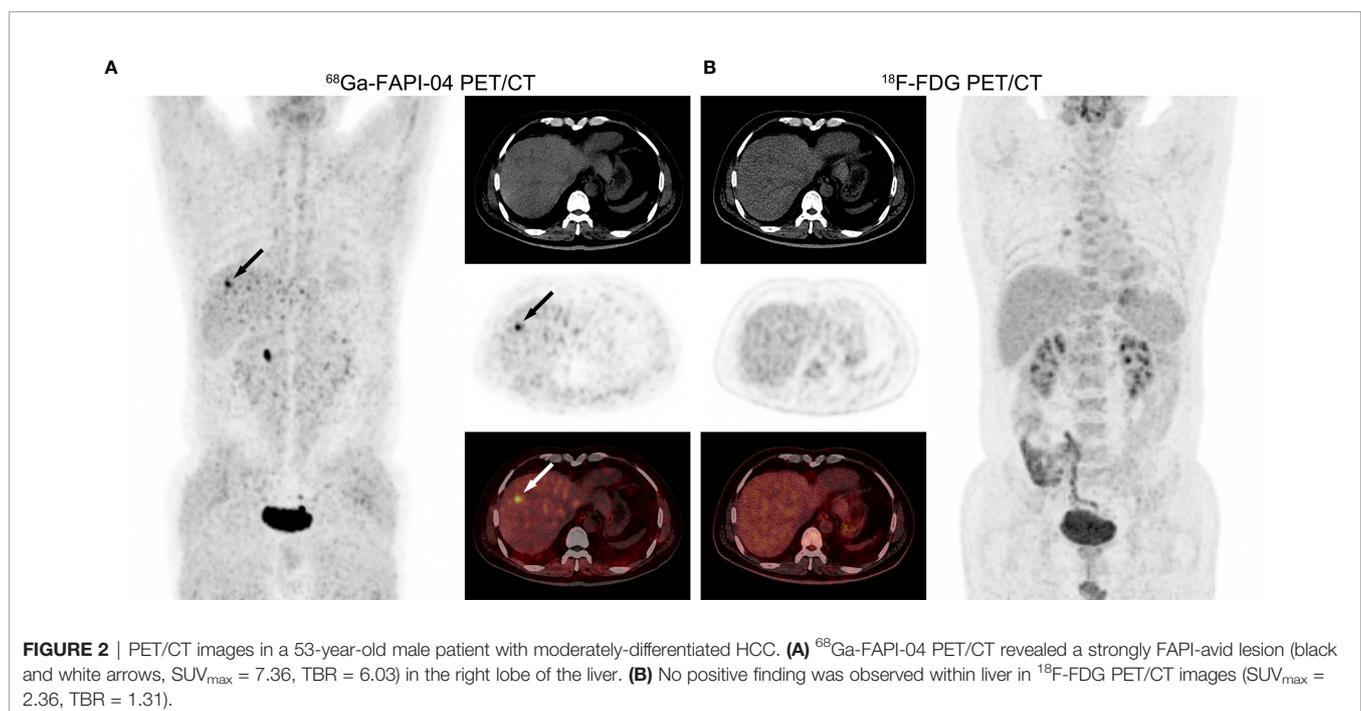


TABLE 3 | Sensitivities of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT in lesion-based analysis.

Lesion characteristics	No. of lesions	¹⁸ F-FDG		⁶⁸ Ga-FAPI-04		P between 2 tracers
		Positive lesions (%)	P	Positive lesions (%)	P	
Diameter (cm)						
≤ 2	16	3 (18.8)	<0.001*	11 (68.8)	0.038*	0.008*
> 2, ≤ 5	11	9 (81.8)		11 (100)		0.500
> 5	8	8 (100)		8 (100)		1
Histologic grade						
I + II	12	4 (33.3)	0.252	10 (83.3)	1	0.031*
III + IV	15	9 (60.0)		12 (80.0)		0.250

Eight lesions had no pathological data.

*Statistically significant.

was more sensitive than ¹⁸F-FDG PET/CT in detecting small HCCs (≤ 2 cm in diameter) ($P = 0.008$) and well- or moderately-differentiated HCCs ($P = 0.031$), but there were no significant sensitivity differences between the 2 tracers in the detection of HCCs > 2 cm in diameter (both $P > 0.05$) and poorly-differentiated or undifferentiated HCCs ($P > 0.05$). The sensitivities of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 PET/CT were significantly related to the size of intrahepatic lesions (both $P < 0.05$). These findings indicated that ⁶⁸Ga-FAPI-04 PET/CT was more sensitive than ¹⁸F-FDG PET/CT in the detection of small and well- or moderately-differentiated HCCs.

Uptake Intensities of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 in HCC

Among the 25 HCC patients with 35 intrahepatic lesions, uptake of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 in positive lesions was assessed,

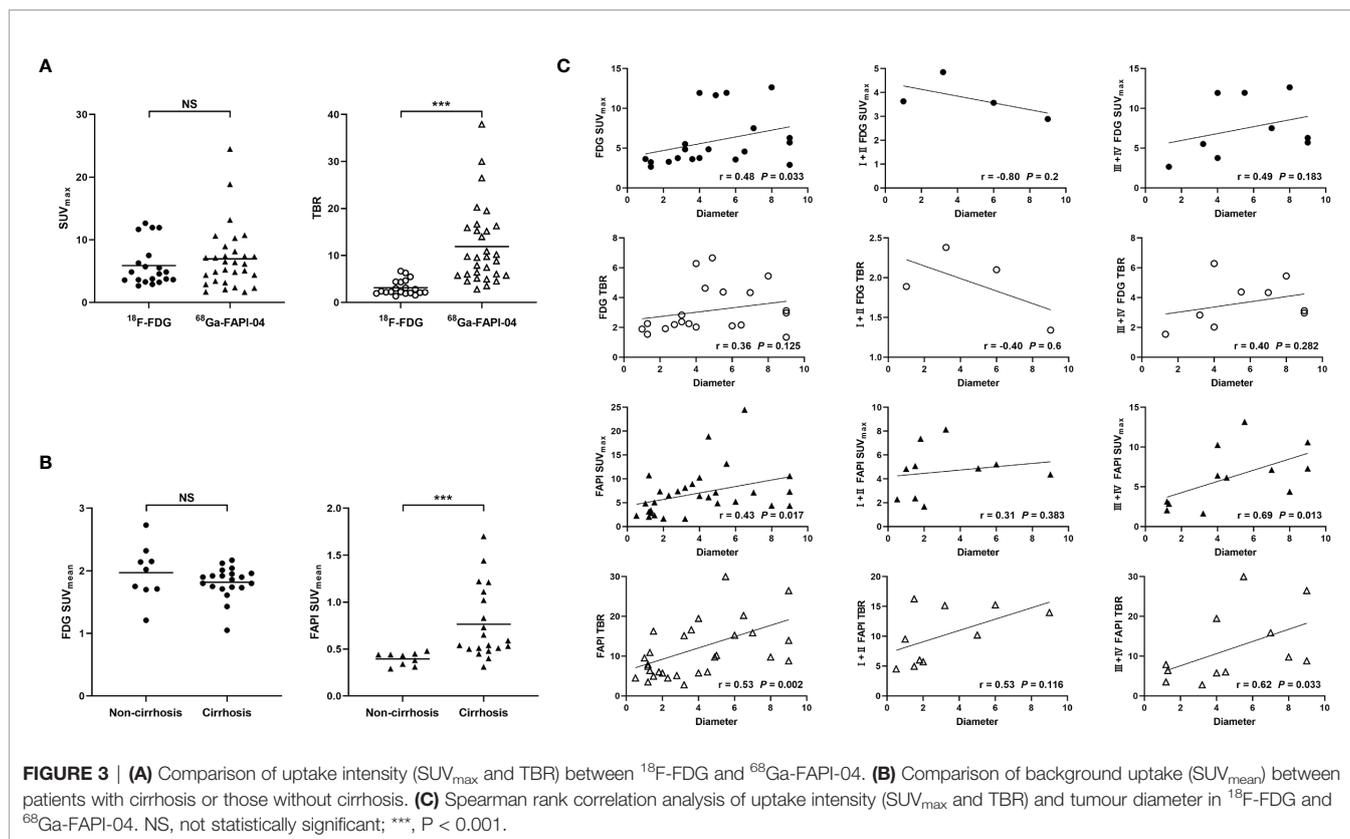
respectively (**Table 4**). Although the lesion uptake (SUV_{max}) of ⁶⁸Ga-FAPI-04 was similar to that of ¹⁸F-FDG ($P > 0.05$), its TBR was significantly higher than that of ¹⁸F-FDG ($P < 0.001$) (**Figure 3A**). Particularly, the background uptake (SUV_{mean}) of ⁶⁸Ga-FAPI-04 was much lower than that of ¹⁸F-FDG in each patient. The SUV_{mean} of ⁶⁸Ga-FAPI-04 in patients with cirrhosis was significantly higher than that of patients without cirrhosis (0.76 ± 0.39 vs. 0.40 ± 0.07 , $P < 0.001$); however, no significant difference was obtained in SUV_{mean} of ¹⁸F-FDG between these two groups (1.82 ± 0.39 vs. 1.97 ± 0.44 , $P > 0.05$) (**Figure 3B**).

The SUV_{max} of ¹⁸F-FDG in positive lesions was associated with the degree of MVI ($P = 0.048$) and tumour differentiation ($P = 0.045$), while the TBR was only associated with tumour differentiation ($P = 0.045$). In contrast, the SUV_{max} and TBR of ⁶⁸Ga-FAPI-04 in positive lesions were associated only with tumour size (both $P < 0.05$), but not with other clinical and

TABLE 4 | Uptake intensities of ¹⁸F-FDG and ⁶⁸Ga-FAPI-04 in positive lesions.

Characteristic	¹⁸ F-FDG					⁶⁸ Ga-FAPI-04				
	No.	SUV_{max}	P	TBR	P	No.	SUV_{max}	P	TBR	P
All	20	5.89 ± 3.38		3.14 ± 1.59		30	6.96 ± 5.01		11.90 ± 8.35	
Clinical features										
Cirrhosis	16	5.43 ± 2.79	0.706	3.09 ± 1.57	0.925	24	7.29 ± 5.27	0.351	11.33 ± 8.41	0.300
Non-cirrhosis	4	7.77 ± 5.25		3.32 ± 1.92		6	5.61 ± 3.91		14.15 ± 8.47	
AFP (ng/mL)										
≤ 20	11	4.92 ± 2.55	0.239	2.60 ± 1.32	0.119	17	7.02 ± 5.27	0.818	10.72 ± 6.33	0.517
> 20	9	7.09 ± 4.01		3.79 ± 1.73		13	6.88 ± 4.86		13.44 ± 10.52	
Tumour number										
Solitary tumour	10	7.15 ± 4.26	0.326	3.73 ± 1.98	0.326	14	8.53 ± 6.43	0.271	14.69 ± 9.73	0.074
Multiple tumours	10	4.64 ± 1.58		2.54 ± 0.81		16	5.58 ± 2.89		9.45 ± 6.26	
MVI										
M0 + M1	4	3.64 ± 0.86	0.048*	2.02 ± 0.47	0.120	10	4.86 ± 2.04	0.852	10.45 ± 4.52	0.804
M2	10	7.16 ± 3.74		3.48 ± 1.58		13	5.85 ± 3.69		11.60 ± 8.78	
AJCC TNM staging										
I	0	–	0.093	–	0.121	4	4.07 ± 2.59	0.404	6.73 ± 2.36	0.194
II	8	4.92 ± 2.96		2.64 ± 1.53		12	4.99 ± 2.54		10.27 ± 5.70	
III + IV	6	7.79 ± 3.85		3.63 ± 1.37		7	6.94 ± 3.87		15.03 ± 9.56	
Diameter (cm)										
≤ 2	3	3.17 ± 0.49	0.079	1.90 ± 0.36	0.215	11	4.17 ± 2.75	0.023*	7.56 ± 3.62	0.019*
> 2, ≤ 5	9	5.91 ± 3.42		3.46 ± 1.89		11	7.84 ± 4.28		12.12 ± 10.15	
> 5	8	6.89 ± 3.65		3.23 ± 1.39		8	9.58 ± 6.77		17.55 ± 7.56	
Histologic grade										
I + II	4	3.74 ± 0.82	0.045*	1.93 ± 0.44	0.045*	10	4.62 ± 2.10	0.391	10.17 ± 4.70	0.895
III + IV	9	7.55 ± 3.74		3.66 ± 1.57		12	6.26 ± 3.67		11.90 ± 9.05	

No., number of positive lesions. *Statistically significant; AFP, α -fetoprotein; MVI, microvascular invasion; AJCC TNM, American Joint Committee on Cancer tumour-node-metastasis.



pathological features (all $P > 0.05$). In Spearman correlation analysis (**Figure 3C**), the SUV_{max} and TBR of ^{68}Ga -FAPI-04 in positive lesions were correlated with tumour size ($rSUV_{max} = 0.43$, $rTBR = 0.53$, both $P < 0.05$). Further subgroup analysis revealed these correlations in poorly-differentiated or undifferentiated HCCs ($rSUV_{max} = 0.69$, $rTBR = 0.62$, both $P < 0.05$), rather than well- or moderately-differentiated ones (both $P > 0.05$). For ^{18}F -FDG, SUV_{max} , but not TBR, was correlated with tumour size ($rSUV_{max} = 0.48$, $P = 0.033$). However, neither SUV_{max} nor TBR exhibited a correlation with tumour size in subgroup analysis (all $P > 0.05$).

Characteristics of Extrahepatic Metastases and Other Benign Lesions

Lymph node metastasis in one patient with poorly-differentiated HCC showed strong uptake of ^{68}Ga -FAPI-04, but undetectable uptake of ^{18}F -FDG (**Figure 4**). ^{68}Ga -FAPI-04 PET/CT detected a small metastatic lesion that was not revealed by ^{18}F -FDG PET/CT in another HCC patient with extensive peritoneal dissemination (**Figure 5**).

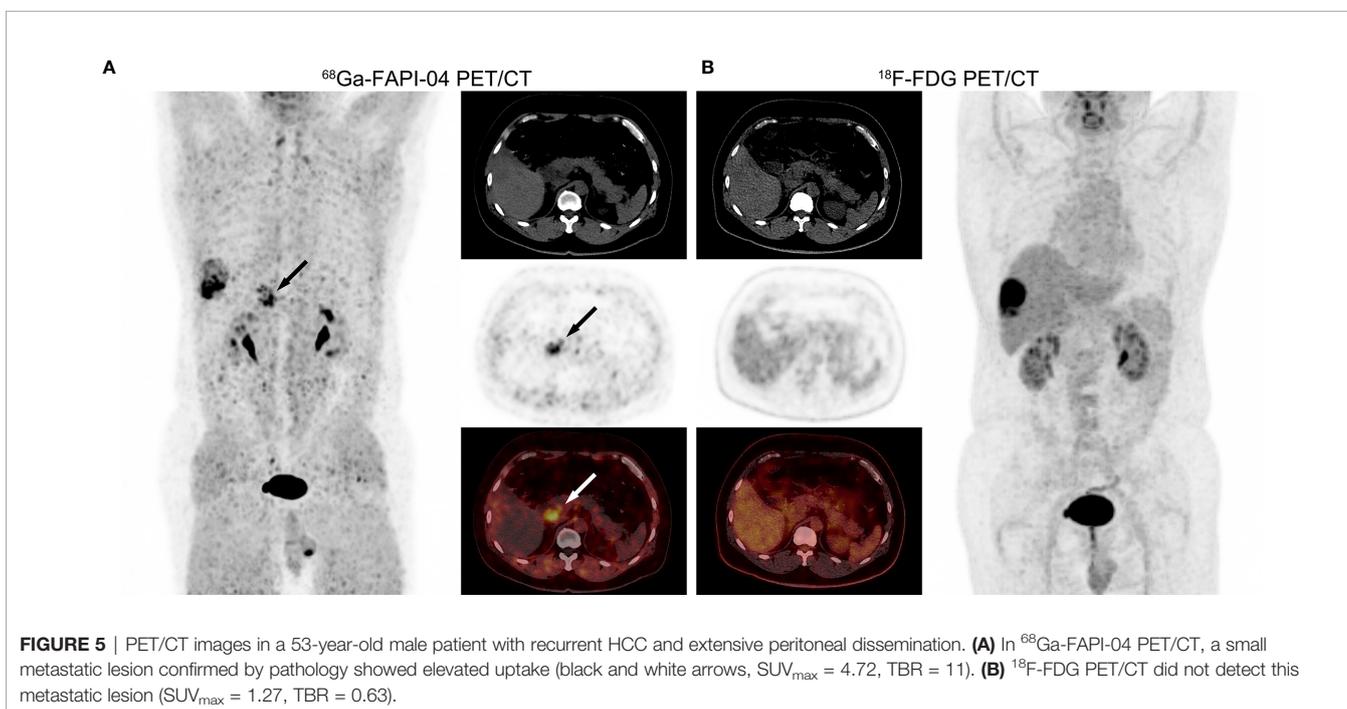
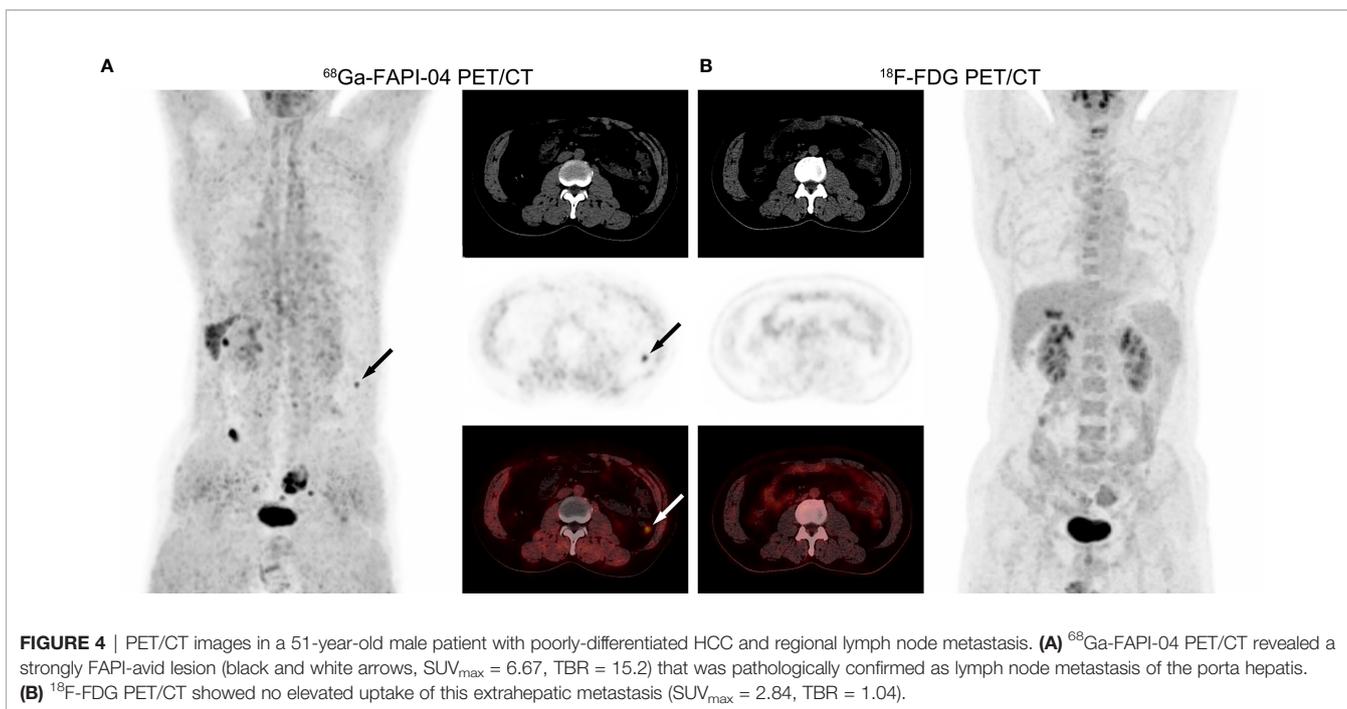
Of the benign lesions, angiomyolipoma (AML) presented strong uptake of ^{68}Ga -FAPI-04 ($SUV_{max} = 8.34$ and $TBR = 21.92$) and mildly increased uptake of ^{18}F -FDG ($SUV_{max} = 2.70$ and $TBR = 1.54$), whereas focal nodular hyperplasia (FNH) presented elevated uptake of ^{68}Ga -FAPI-04 ($SUV_{max} = 2.20$ and $TBR = 5.77$) and negative uptake of ^{18}F -FDG ($SUV_{max} = 1.51$ and $TBR = 0.86$). In two cases of inflammatory nodules in the liver, one showed positive uptake of ^{68}Ga -FAPI-04 ($SUV_{max} = 1.56$ and

$TBR = 3.08$) and negative uptake of ^{18}F -FDG ($SUV_{max} = 2.14$ and $TBR = 1.19$), while the other was neither ^{68}Ga -FAPI-04 avid ($SUV_{max} = 0.53$ and $TBR = 1.20$) nor ^{18}F -FDG avid ($SUV_{max} = 2.92$ and $TBR = 1.36$).

DISCUSSION

Nowadays, ^{18}F -FDG as the most widely available clinical PET tracer has been increasingly utilized for detecting extrahepatic metastases (22), TNM staging (23), selecting patients for liver transplantation (23), and predicting tumour progression or recurrence after treatments (24, 25). However, ^{18}F -FDG has limited value in the early diagnosis of HCC because of its low sensitivity (6, 26). Therefore, there remains an urgent need for highly sensitive tracers in the early diagnosis of HCC by PET/CT. Recently, ^{68}Ga -labelled FAPI was shown to be a novel tracer in PET/CT imaging of various cancers due to its high tumour-to-background contrast (17, 27). Furthermore, ^{68}Ga -FAPI-04 PET/CT has high sensitivity in detecting hepatic malignancies, including HCC and ICC (19, 21).

In line with previous studies reporting a range from 40% to 68%, the sensitivity of ^{18}F -FDG PET/CT in the detection of HCC was 57.1% in the present study (6). ^{68}Ga -FAPI-04 PET/CT had a better sensitivity (85.7%) than ^{18}F -FDG PET/CT in the detection of intrahepatic lesions in HCC patients. Of note, ^{68}Ga -FAPI-04 PET/CT was capable of detecting more than half of small HCC lesions (11 of 16, ≤ 2 cm in diameter) in the present cohort,



whereas ^{18}F -FDG PET/CT detected only three of the 16 lesions, which is consistent with previous studies that consider ^{18}F -FDG an inappropriate tracer for visualizing small HCCs (7, 8). Furthermore, ^{68}Ga -FAPI-04 PET/CT exhibited a relatively higher sensitivity in the detection of well- or moderately-differentiated HCCs (10 of 12, histologic grade I or II) compared with ^{18}F -FDG PET/CT (4 of 12). The poor sensitivity of ^{18}F -FDG

PET/CT in detecting low-grade HCC is probably related to enhanced glucose-6-phosphatase activity causing the dephosphorylation of ^{18}F -FDG-6- PO_4 , which is therefore not trapped in HCC cells, resulting in false-negative results (6, 28, 29).

In contrast, a CAF-targeting tracer can circumvent highly heterogeneous avidity exhibited by some tracers that target the tumour per se, because CAFs are among the most abundant

stromal components in the tumour microenvironment of many solid tumours, and are found even at the early stages of tumorigenesis (30, 31). The above correlation analysis of ⁶⁸Ga-FAPI-04 revealed that neither positive incidence nor uptake intensity was associated with tumour differentiation. Moreover, ⁶⁸Ga-FAPI-04 showed a high lesion-to-background contrast in the liver, which may partially increase sensitivity that is affected by the partial volume effect of PET/CT. As such, ⁶⁸Ga-FAPI-04 PET/CT can make up for the deficiencies of ¹⁸F-FDG PET/CT in the detection of low-grade HCC. Additionally, a higher detection rate was observed with ⁶⁸Ga-FAPI-04 PET/CT in high-grade HCC in comparison with ¹⁸F-FDG PET/CT, although this difference was not statistically significant. Therefore, ⁶⁸Ga-FAPI-04 PET/CT appears to be a promising new approach for the detection of intrahepatic HCC lesions with higher sensitivity compared with ¹⁸F-FDG PET/CT.

⁶⁸Ga-FAPI-04 PET/CT could not detect 5 intrahepatic HCC lesions with the diameter within 2 cm in this cohort. These negative results may be due to the similar uptake intensity of ⁶⁸Ga-FAPI-04 between small HCC lesions and the liver background of cirrhosis. Hypoxia may be a reasonable explanation for the positive correlation between the uptake intensity of ⁶⁸Ga-FAPI-04 and tumour size. It has been reported that the degree of hypoxia correlates positively with tumour size (32) and that hypoxia is a potent factor inducing the expression of FAP in CAFs (33). Therefore, the degree of hypoxia is mild in small HCCs, leading to the low uptake of ⁶⁸Ga-FAPI-04 in these lesions. In the cirrhotic liver, FAP is strongly expressed by activated hepatic stellate cells (34, 35). In line with a previous study (19), this work also found that patients with cirrhosis presented elevated uptake of ⁶⁸Ga-FAPI-04 in the hepatic parenchyma compared with those without cirrhosis. Therefore, small lesions have relatively lower uptake of ⁶⁸Ga-FAPI-04 in comparison with large ones, which makes them susceptible to being masked by the background of cirrhosis.

Although ¹⁸F-FDG PET/CT has a potential value in detecting extrahepatic metastases in HCC patients (7, 8, 36, 37), heterogeneous uptake of ¹⁸F-FDG in metastatic nodules remains a major reason limiting its wide application for tumour staging. CAFs play a critical role in constructing a microenvironment that favours tumour progression at the primary site, and are, moreover, responsible for creating a pre-metastatic niche in distal organs and triggering the subsequent metastatic events (30, 31, 38). Therefore, CAF-targeting tracers seem to be optimal candidates for PET/CT in the evaluation of extrahepatic metastases. In the present study, lymph node metastasis at the porta hepatis in one patient with poorly-differentiated HCC presented a clear visualization in ⁶⁸Ga-FAPI-04 PET/CT, but an obscure image in ¹⁸F-FDG PET/CT (**Figure 4**). Separately, one small metastatic lesion in another recurrent HCC case with extensive peritoneal dissemination was only clearly visualised by ⁶⁸Ga-FAPI-04 PET/CT (**Figure 5**). It appears that ⁶⁸Ga-FAPI-04 PET/CT may outperform ¹⁸F-FDG PET/CT in detecting extrahepatic lesions in patients with advanced HCC. Nevertheless, the comparison of ⁶⁸Ga-FAPI-04 with ¹⁸F-FDG in terms of applicability to the detection of

extrahepatic metastasis of HCC needs to be clarified in future studies.

Despite the high sensitivity of ⁶⁸Ga-FAPI-04 PET/CT in the detection of malignancies, some benign lesions confirmed by pathological examinations in the current cohort presented positive results as well. Especially, AML, FNH, and one of two inflammatory nodules presented elevated uptake of ⁶⁸Ga-FAPI-04 in contrast to the hepatic background, perhaps because of the enhanced fibrosis around or within lesions. Moreover, increasing uptake of ⁶⁸Ga-FAPI-04 was observed in the postoperative area of the liver in one patient with recurrent HCC, which is consistent with a previous study that considered ⁶⁸Ga-FAPI-04 an inappropriate tracer for the discrimination between abnormal malignant progression and normal postoperative reaction (39). Nevertheless, negligible ⁶⁸Ga-FAPI-04 uptake has been observed in some other benign hepatic lesions such as adenoma (20, 39), dysplastic nodule (20), granuloma (21), and haemangioma (21). Collectively, great caution should be exercised when regarding intrahepatic lesions with elevated uptake of ⁶⁸Ga-FAPI-04 as malignancy.

There were several limitations in the present study. First, it is unethical to biopsy all lesions as part of a research study, although that is perhaps not practical or needed. As a result, the lack of pathological data in 5 HCC patients whose confirmative diagnosis was based on non-invasive criteria may yield latent bias. Another limitation is that the present cohort only included a small number of patients with suspicious HCC who were willing to receive both ⁶⁸Ga-FAPI-04 and ¹⁸F-FDG PET/CT examinations. Therefore, selection bias was inevitable. Finally, different scanners were used in this study for ⁶⁸Ga-FAPI-04 and ¹⁸F-FDG imaging because of the different production places of PET tracers. For this reason, SUV normalization was applied after data collection for PET/CT system performance harmonization.

CONCLUSIONS

⁶⁸Ga-FAPI-04 PET/CT is more sensitive than ¹⁸F-FDG PET/CT in detecting intrahepatic HCCs. This outperformance is more prominent in the detection of small and well- or moderately-differentiated HCCs. The uptake of ⁶⁸Ga-FAPI-04 was correlated mainly with tumour size in this study; therefore, ⁶⁸Ga-FAPI-04 PET/CT can be considered a very promising imaging modality in HCC diagnosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by ethics committee at Huashan Hospital of Fudan

University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LQ, FX, LL, WZ, and HW designed the study, interpreted the data and led the writing and review of the manuscript. HW, HJ, JC, WZ, and LL enrolled patients and collected clinical data. YG, JZ, QH, YK, and SR performed examinations. All authors contributed to the article and approved the submitted version.

REFERENCES

- Forner A, Reig M, Bruix J. Hepatocellular Carcinoma. *Lancet* (2018) 391 (10127):1301–14. doi: 10.1016/s0140-6736(18)30010-2
- Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A Global View of Hepatocellular Carcinoma: Trends, Risk, Prevention and Management. *Nat Rev Gastroenterol Hepatol* (2019) 16(10):589–604. doi: 10.1038/s41575-019-0186-y
- Villanueva A. Hepatocellular Carcinoma. *N Engl J Med* (2019) 380(15):1450–62. doi: 10.1056/NEJMra1713263
- Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* (2018) 68(2):723–50. doi: 10.1002/hep.29913
- Oliva MR, Saini S. Liver Cancer Imaging: Role of CT, MRI, US and PET. *Cancer Imaging* (2004) 4 Spec No A(Spec No A):S42–6. doi: 10.1102/1470-7330.2004.0011
- Goh V, Sarker D, Osmany S, Cook GJ. Functional Imaging Techniques in Hepatocellular Carcinoma. *Eur J Nucl Med Mol Imaging* (2012) 39(6):1070–9. doi: 10.1007/s00259-012-2096-x
- Trojan J, Schroeder O, Raedle J, Baum RP, Herrmann G, Jacobi V, et al. Fluorine-18 FDG Positron Emission Tomography for Imaging of Hepatocellular Carcinoma. *Am J Gastroenterol* (1999) 94(11):3314–9. doi: 10.1111/j.1572-0241.1999.01544.x
- Park JW, Kim JH, Kim SK, Kang KW, Park KW, Choi JI, et al. A Prospective Evaluation of ¹⁸F-FDG and ¹¹C-Acetate PET/CT for Detection of Primary and Metastatic Hepatocellular Carcinoma. *J Nucl Med* (2008) 49(12):1912–21. doi: 10.2967/jnumed.108.055087
- Ho CL, Chen S, Yeung DW, Cheng TK. Dual-Tracer PET/CT Imaging in Evaluation of Metastatic Hepatocellular Carcinoma. *J Nucl Med* (2007) 48 (6):902–9. doi: 10.2967/jnumed.106.036673
- Castilla-Lièvre MA, Franco D, Gervais P, Kuhnast B, Agostini H, Marthey L, et al. Diagnostic Value of Combining ¹¹C-Choline and ¹⁸F-FDG PET/CT in Hepatocellular Carcinoma. *Eur J Nucl Med Mol Imaging* (2016) 43(5):852–9. doi: 10.1007/s00259-015-3241-0
- Kesler M, Levine C, Hershkovitz D, Mishani E, Menachem Y, Lerman H, et al. (68)Ga-PSMA is a Novel PET-CT Tracer for Imaging of Hepatocellular Carcinoma: A Prospective Pilot Study. *J Nucl Med* (2019) 60(2):185–91. doi: 10.2967/jnumed.118.214833
- Lopci E, Torzilli G, Poretta D, de Neto LJ, Donadon M, Rimassa L, et al. Diagnostic Accuracy of ¹¹C-Choline PET/CT in Comparison With CT and/or MRI in Patients With Hepatocellular Carcinoma. *Eur J Nucl Med Mol Imaging* (2015) 42(9):1399–407. doi: 10.1007/s00259-015-3079-5
- Kubo N, Araki K, Kuwano H, Shirabe K. Cancer-Associated Fibroblasts in Hepatocellular Carcinoma. *World J Gastroenterol* (2016) 22(30):6841–50. doi: 10.3748/wjg.v22.i30.6841
- Baglieri J, Brenner DA, Kisseleva T. The Role of Fibrosis and Liver-Associated Fibroblasts in the Pathogenesis of Hepatocellular Carcinoma. *Int J Mol Sci* (2019) 20(7):1723. doi: 10.3390/ijms20071723

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- Garin-Chesa P, Old LJ, Rettig WJ. Cell Surface Glycoprotein of Reactive Stromal Fibroblasts as a Potential Antibody Target in Human Epithelial Cancers. *Proc Natl Acad Sci USA* (1990) 87(18):7235–9. doi: 10.1073/pnas.87.18.7235
- Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasí MP, Healey JH, et al. Molecular Cloning of Fibroblast Activation Protein Alpha, a Member of the Serine Protease Family Selectively Expressed in Stromal Fibroblasts of Epithelial Cancers. *Proc Natl Acad Sci USA* (1994) 91(12):5657–61. doi: 10.1073/pnas.91.12.5657
- Giesel FL, Kratochwil C, Lindner T, Marschalek MM, Loktev A, Lehnert W, et al. (68)Ga-FAPI PET/CT: Biodistribution and Preliminary Dosimetry Estimate of 2 DOTA-Containing FAP-Targeting Agents in Patients With Various Cancers. *J Nucl Med* (2019) 60(3):386–92. doi: 10.2967/jnumed.118.215913
- Chen H, Pang Y, Wu J, Zhao L, Hao B, Wu J, et al. Comparison of [(68)Ga]Ga-DOTA-FAPI-04 and [(18)F]FDG PET/CT for the Diagnosis of Primary and Metastatic Lesions in Patients With Various Types of Cancer. *Eur J Nucl Med Mol Imaging* (2020) 47(8):1820–32. doi: 10.1007/s00259-020-04769-z
- Shi X, Xing H, Yang X, Li F, Yao S, Zhang H, et al. Fibroblast Imaging of Hepatic Carcinoma With (68)Ga-FAPI-04 PET/CT: A Pilot Study in Patients With Suspected Hepatic Nodules. *Eur J Nucl Med Mol Imaging* (2020) 48 (1):196–203. doi: 10.1007/s00259-020-04882-z
- Guo W, Pang Y, Yao L, Zhao L, Fan C, Ke J, et al. Imaging Fibroblast Activation Protein in Liver Cancer: A Single-Center Post HOC Retrospective Analysis to Compare [(68)Ga]Ga-FAPI-04 PET/CT Versus MRI and [(18)F]FDG PET/CT. *Eur J Nucl Med Mol Imaging* (2021) 48(5):1604–17. doi: 10.1007/s00259-020-05095-0
- Shi X, Xing H, Yang X, Li F, Yao S, Congwei J, et al. Comparison of PET Imaging of Activated Fibroblasts and (18)F-FDG for Diagnosis of Primary Hepatic Tumours: A Prospective Pilot Study. *Eur J Nucl Med Mol Imaging* (2020) 48(5):593–603. doi: 10.1007/s00259-020-05070-9
- Lin CY, Chen JH, Liang JA, Lin CC, Jeng LB, Kao CH. ¹⁸F-FDG PET or PET/CT for Detecting Extrahepatic Metastases or Recurrent Hepatocellular Carcinoma: A Systematic Review and Meta-Analysis. *Eur J Radiol* (2012) 81 (9):2417–22. doi: 10.1016/j.ejrad.2011.08.004
- Cheung TT, Ho CL, Lo CM, Chen S, Chan SC, Chok KS, et al. ¹¹C-Acetate and ¹⁸F-FDG PET/CT for Clinical Staging and Selection of Patients With Hepatocellular Carcinoma for Liver Transplantation on the Basis of Milan Criteria: Surgeon's Perspective. *J Nucl Med* (2013) 54(2):192–200. doi: 10.2967/jnumed.112.107516
- Hong G, Suh KS, Suh SW, Yoo T, Kim H, Park MS, et al. Alpha-Fetoprotein and (18)F-FDG Positron Emission Tomography Predict Tumor Recurrence Better Than Milan Criteria in Living Donor Liver Transplantation. *J Hepatol* (2016) 64(4):852–9. doi: 10.1016/j.jhep.2015.11.033
- Song MJ, Bae SH, Lee SW, Song DS, Kim HY, Yoo Ie R, et al. ¹⁸F-Fluorodeoxyglucose PET/CT Predicts Tumour Progression After Transarterial Chemoembolization in Hepatocellular Carcinoma. *Eur J Nucl Med Mol Imaging* (2013) 40(6):865–73. doi: 10.1007/s00259-013-2366-2
- Ayuso C, Rimola J, Vilana R, Burrel M, Darnell A, Garcia-Criado Á, et al. Diagnosis and Staging of Hepatocellular Carcinoma (HCC): Current Guidelines. *Eur J Radiol* (2018) 101:72–81. doi: 10.1016/j.ejrad.2018.01.025

27. Kratochwil C, Flechsig P, Lindner T, Abderrahim L, Altmann A, Mier W, et al. (68)Ga-FAPI PET/CT: Tracer Uptake in 28 Different Kinds of Cancer. *J Nucl Med* (2019) 60(6):801–5. doi: 10.2967/jnumed.119.227967
28. Torizuka T, Tamaki N, Inokuma T, Magata Y, Sasayama S, Yonekura Y, et al. In Vivo Assessment of Glucose Metabolism in Hepatocellular Carcinoma With FDG-PET. *J Nucl Med* (1995) 36(10):1811–7.
29. Gallagher BM, Fowler JS, Gutterson NI, MacGregor RR, Wan CN, Wolf AP. Metabolic Trapping as a Principle of Oradiopharmaceutical Design: Some Factors Responsible for the Biodistribution of [18F] 2-Deoxy-2-Fluoro-D-Glucose. *J Nucl Med* (1978) 19(10):1154–61.
30. Kalluri R. The Biology and Function of Fibroblasts in Cancer. *Nat Rev Cancer* (2016) 16(9):582–98. doi: 10.1038/nrc.2016.73
31. Chen X, Song E. Turning Foes to Friends: Targeting Cancer-Associated Fibroblasts. *Nat Rev Drug Discovery* (2019) 18(2):99–115. doi: 10.1038/s41573-018-0004-1
32. Carmona-Bozo JC, Manavaki R, Woitek R, Torheim T, Baxter GC, Caracò C, et al. Hypoxia and Perfusion in Breast Cancer: Simultaneous Assessment Using PET/MR Imaging. *Eur Radiol* (2021) 31(1):333–44. doi: 10.1007/s00330-020-07067-2
33. Ammirante M, Shalpour S, Kang Y, Jamieson CA, Karin M. Tissue Injury and Hypoxia Promote Malignant Progression of Prostate Cancer by Inducing CXCL13 Expression in Tumor Myofibroblasts. *Proc Natl Acad Sci USA* (2014) 111(41):14776–81. doi: 10.1073/pnas.1416498111
34. Levy MT, McCaughan GW, Abbott CA, Park JE, Cunningham AM, Müller E, et al. Fibroblast Activation Protein: A Cell Surface Dipeptidyl Peptidase and Gelatinase Expressed by Stellate Cells at the Tissue Remodelling Interface in Human Cirrhosis. *Hepatology* (1999) 29(6):1768–78. doi: 10.1002/hep.510290631
35. Wang XM, Yu DM, McCaughan GW, Gorrell MD. Fibroblast Activation Protein Increases Apoptosis, Cell Adhesion, and Migration by the LX-2 Human Stellate Cell Line. *Hepatology* (2005) 42(4):935–45. doi: 10.1002/hep.20853
36. Khan MA, Combs CS, Brunt EM, Lowe VJ, Wolverson MK, Solomon H, et al. Positron Emission Tomography Scanning in the Evaluation of Hepatocellular Carcinoma. *J Hepatol* (2000) 32(5):792–7. doi: 10.1016/s0168-8278(00)80248-2
37. Sugiyama M, Sakahara H, Torizuka T, Kanno T, Nakamura F, Futatsubashi M, et al. 18F-FDG PET in the Detection of Extrahepatic Metastases From Hepatocellular Carcinoma. *J Gastroenterol* (2004) 39(10):961–8. doi: 10.1007/s00535-004-1427-5
38. Liu Y, Cao X. Characteristics and Significance of the Pre-Metastatic Niche. *Cancer Cell* (2016) 30(5):668–81. doi: 10.1016/j.ccell.2016.09.011
39. Chen H, Zhao L, Ruan D, Pang Y, Hao B, Dai Y, et al. Usefulness of [(68)Ga] Ga-DOTA-FAPI-04 PET/CT in Patients Presenting With Inconclusive [(18)F]FDG PET/CT Findings. *Eur J Nucl Med Mol Imaging* (2021) 48(1):73–86. doi: 10.1007/s00259-020-04940-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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