The role of $^{99m}$Tc-HFAPi SPECT/CT in patients with malignancies of digestive system: first clinical experience

Xi Jia  
Xian Jiaotong University Medical College First Affiliated Hospital

Xinru Li  
Xian Jiaotong University Medical College First Affiliated Hospital

Bing Jia  
Peking University

Ye Yang  
Xian Jiaotong University Medical College First Affiliated Hospital

Yuanbo Wang  
Xian Jiaotong University Medical College First Affiliated Hospital

Yan Liu  
Xian Jiaotong University Medical College First Affiliated Hospital

Ting Ji  
Xian Jiaotong University Medical College First Affiliated Hospital

Xin Xie  
Xian Jiaotong University Medical College First Affiliated Hospital

Yu Yao  
Xian Jiaotong University Medical College First Affiliated Hospital

Guanglin Qiu  
Xian Jiaotong University Medical College First Affiliated Hospital

Huixing Deng  
Xian Jiaotong University Medical College First Affiliated Hospital

Zhaohui Zhu  
Peking Union Medical College Hospital

Si Chen  
Foshan Atomic Medical Equipment

Aimin Yang  
Xian Jiaotong University Medical College First Affiliated Hospital

Rui Gao  (jacky_mg@xjtufh.edu.cn)  
Xian Jiaotong University Medical College First Affiliated Hospital  https://orcid.org/0000-0003-4841-5929

Research Article

Keywords: $^{99m}$Tc-HFAPi, SPECT/CT, digestive system cancer, diagnostic efficiency

Posted Date: October 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2100885/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Recently, PET/CT imaging with radiolabelled FAP inhibitors (FAPIs) has been widely evaluated in diverse diseases. However, rare report has been published using SPECT/CT, a more available imaging method, with $^{99m}$Tc-labelled FAPI. In this study, we evaluated the potential effect of $^{99m}$Tc-HFAPi in clinical analysis for digestive system tumours.

Methods

This is a single-centre prospective diagnostic efficiency study (Ethic approved No.: XJTU1AF2021LSK-021 of First Affiliated Hospital of Xi’an Jiaotong University and ChiCTR2100048093 of Chinese Clinical Trial Register). 40 patients with suspected or confirmed digestive system tumours underwent $^{99m}$Tc-HFAPi SPECT/CT between January through June 2021. For dynamic biodistribution and dosimetry estimation, whole-body planar scintigraphy was performed at 10, 30, 90, 150, and 240 min post-injection. Optimal acquisition time was considered at 60–90 min post-injection and semi-quantified using SUV$_{max}$ and T/B ratio. The diagnostic performance of $^{99m}$Tc-HFAPi were calculated and compared with those of contrast-enhanced CT (ceCT) using McNemar test, and the changes of tumour stage and oncologic management were recorded.

Results

Physiological distribution of $^{99m}$Tc-HFAPi was observed in the liver, pancreas, gallbladder, and to a lesser extent in the kidneys, spleen and thyroid. The diagnostic sensitivity of $^{99m}$Tc-HFAPi for non-operative primary lesions was similar to that of ceCT (94.29% [33/35] vs 100% [35/35], respectively; $P = 0.5$); in local relapse detection, $^{99m}$Tc-HFAPi was successfully detected in 100% ($n = 3$) of patients. In the diagnosis of suspected metastatic lesions, $^{99m}$Tc-HFAPi exhibited higher sensitivity (89.66% [26/29] vs 68.97% [20/29], respectively, $P = 0.03$) and specificity (97.9% [47/48] vs 85.4% [41/48], respectively, $P = 0.03$) than ceCT, especially with 100% (24/24) specificity in the diagnosis of liver metastases, resulting in 20.0% (8/40) changes in TNM stage and 15.0% (6/40) changes in oncologic management.

Conclusion

$^{99m}$Tc-HFAPi demonstrates a greater diagnostic efficiency than ceCT in the detection of distant metastasis, especially in identifying liver metastases.

Introduction

An in-depth understanding of the tumour microenvironment has revealed a new player: cancer-associated fibroblasts (CAFs) [1]. The majority of epithelial tumours recruit fibroblasts and other non-malignant cells, stimulating them to become CAFs. This often leads to overexpression of membrane serine protease fibroblast activating protein alpha (FAP-alpha, also known as prolyl endopeptidase FAP), which is estimated to be overexpressed in approximately 90% of human cancers [2–4]. As FAP is mostly absent in healthy tissue, inhibitors of FAP (FAPIs) can be used in nuclear medicine for imaging [5]. Indeed, a large number of FAPI-based radiopharmaceuticals have been developed for PET/CT imaging, and a promising role for $^{68}$Ga-FAPI PET/CT in the diagnosis, staging, and radiotherapy planning of digestive tract cancers has been demonstrated [6–8].

$^{68}$Ga-FAPI PET/CT showed a higher sensitivity than $^{18}$F-FDG PET/CT in the detection of primary and metastatic lesions of various types of cancers [6, 9]. Recently, Koerber et al reported the first clinical use of $^{68}$Ga-FAPI PET/CT for tumours in the lower intestinal tract [7]. Their results revealed that both primary and metastatic malignancies in the lower gastrointestinal tract can be reliably detected using $^{68}$Ga-FAPI PET/CT, leading to relevant changes in TNM status and oncologic management.

Due to its lower cost, SPECT/CT with technetium-99m ($^{99m}$Tc) is a more widely available, and $^{99m}$Tc-labelled FAPIs are generally applicable tracers that are attractive options for imaging in clinical management when PET imaging is inaccessible or limited [10]. Lindner et al reported $^{99m}$Tc-labelled FAPIs and evaluated their biodistribution in tumour-bearing mice [10], and found $^{99m}$Tc-FAPI-34 showing strong and constant tumour accumulation. The preclinical application has indicated that it is a good candidate for scintigraphic
imaging owing to the high contrast obtained via rapid tumour uptake and clearance from the rest of the body. Nevertheless, reliable clinical data are lacking, which only applied in two patients with ovarian and pancreatic cancer. Here, we report our first clinical experience with $^{99m}$Tc-HYNIC-FAPI-04 ($^{99m}$Tc-HFAPi) SPECT/CT applied in a cohort of patients with digestive system tumours. After quantifying tracer uptake in primary tumours and metastases, we compared the diagnostic efficiency of the $^{99m}$Tc-HFAPi with the recommended conventional imaging ceCT.

**Materials And Methods**

**Radiopharmaceutical Preparation**

For $^{99m}$Tc radiolabelling, 1 mL of 925–1,295 MBq (25–35 mCi) of $^{99m}$TcO$_4^-$ saline solution was added to 25 µg of hydrazinonicotinamide-FAPI-04 (HYNIC-FAPI-04, abbreviated to HFAPi, Fig. S1), 3.0 mg of Trisodium triphenylphosphine-3,3′,3″-trisulphonate, and 2.0 mg of tricine, then incubated at 100°C for 15 min. The radiochemical purity (RCP) was analysed by radio-HPLC and ITLC-SG, and the specific operation method is detailed in the supplementary materials. For clinical use, the RCP was always greater than 95%. The reaction mixture was then filtered through a 0.20-mm Millex-LG filter (EMD Millipore) before agent injection.

**Patients**

This is a single-centre prospective diagnostic efficiency study of $^{99m}$Tc-HFAPi SPECT/CT in digestive system tumours, with ceCT serving as the reference method, approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Xi’an Jiaotong University (Ethic approved No.: XJTU1AF2021LSK-021) and Chinese Clinical Trial Register (Registration No.: ChiCTR2100048093). From January to June 2021, patients with suspected digestive system tumours who needed preoperative initial staging or posttreatment restaging were consecutively recruited at the First Affiliated Hospital of Xi’an Jiaotong University with written informed consent. Detailed Eligibility criteria is provided in the supplementary informations. After a standard work-up including but not limited to ceCT, additional $^{99m}$Tc-HFAPi SPECT/CT was performed (generally within 7 days).

**Scintigraphy and SPECT/CT**

Based on the previous reference dose [$^{10–12}$], $^{99m}$Tc-HFAPi was administered intravenously in amounts ranging from 790.4 to 930.2 MBq (21.36 to 25.14 mCi). Whole-body planar scintigraphy was performed at 10, 30, 90, 150 and 240 min for dynamic biodistribution in four representative patients; $^{99m}$Tc-HFAPi imaging was performed 60–90 min following tracer injection. Whole-body scans were performed via GE Discovery 670 pro scanner system (GE Healthcare) equipped with low-energy high-resolution (LEHR) collimators in 18 cm/min velocity. Low-dose CT was performed for attenuation correction and anatomic localization. The patients were asked to self-report any abnormalities at 30 min after the examination was completed.

**Biodistribution and Dosimetry Estimation**

Visual analysis was applied to determine the integral biodistribution of the tracer as well as the transient and intersubject stability. For each subject, regions of interest (ROIs) were delineated over the identified organs: the heart, liver, lungs, kidneys, pancreas, spleen, brain, thyroid and salivary glands. The geometric mean count was determined for every organ from the background-corrected anterior and posterior counts. The results are expressed as a percentage of the initial injected activity after decay correction (%ID/organ). For dosimetry estimation, absorbance dose of different organs and effective dose were calculated using OLINDA/EXM 1.0 software (Vanderbilt, University, Nashville, TN) as previous described [$^{12}$].

**SPECT/CT Imaging Review**

$^{99m}$Tc-HFAPi SPECT/CT scans were evaluated by 1 certified radiologist and 2 certified nuclear medicine physicians. They reach consensus when there is disagreement. Conventional imaging was interpreted by 2 certified radiologists with consensus but blind to the $^{99m}$Tc-HFAPi SPECT/CT results. Fused SPECT/CT images were viewed on the Xeleris Workstation (version AW 4.7, GE Healthcare). For quantitative and semi-quantitative analyses, ROIs were drawn on transaxial images over the tumour with focally increased uptake. The tumour-to-background (T/B) ratio was determined by dividing the maximum tumour uptake by the maximum contralateral psoas muscle uptake.

**Diagnosis and follow-up**

Histopathology of biopsy/resected surgical specimens served as the gold standard for the final diagnosis. In cases in which the diagnosis of malignancy was not applicable, follow-up data after the SPECT/CT scans were requested. Referring to a similar study [$^{9}$],...
the disease was defined as malignancy when (a) typical malignant features were confirmed by multi-modality imaging, (b) significant progression on follow-up imaging (significant increase in size), or (c) a significant decrease in size after anticancer treatment. All suspected lesions were followed up for no less than 6 months.

**Immunohistochemistry (IHC) of FAP expression**

FAP expression in 4 representative patients was analysed by immunohistochemistry, heat-mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0. The sections were incubated with 1:250 humanized anti-fibroblast activation antibody (Abcam, ab207178) at 4°C overnight. After incubation with the labelled streptavidin-biotin (LSAB) complex, the slides were stained and visualized using the iView DAB detection system (ZSGB-BIO, Beijing, China). Typical lesions in high-power fields were photographed for visual comparison.

**Statistical analyses**

All statistical analyses were conducted using SPSS 25.0 statistical analysis software (IBM, Armonk, NY, USA). For organ biodistribution, the percentage of initial injected activity after decay correction (%ID) and was used. To determine lesion uptake, the T/B ratio and $\text{SUV}_{\text{max}}$ were used with the median ± interquartile range (IQR) because of non-normal distribution. McNemar test and chi-square test were employed to compare the diagnostic values between $^{99m}$Tc-HFAPi SPECT/CT and ceCT. A receiver operating characteristic (ROC) curve was constructed to quantify the diagnostic performance of the T/B ratio and $\text{SUV}_{\text{max}}$ by assessing the respective areas under the curve (AUCs). Two-tailed $P$ values < 0.05 were considered significant.

**Results**

**Radiopharmaceutical Preparation**

The structure of $^{99m}$Tc-HFAPi was shown in (Fig. S1). The average radiochemical purity of $^{99m}$Tc-HFAPi prepared from lyophilized kits, determined by radio-HPLC (Fig. S2) and ITLC-SG (Fig. S3), was over 95% with < 1% of free $^{99m}$Tc$\text{O}_4^-$ and $^{99m}$Tc-coligand as well as < 0.5% of $^{99m}$Tc-colloid. $^{99m}$Tc-HFAPi could be readily prepared in high specific activity (> 7.5 Ci/µmol), and it was stable in the kit matrix as well as in the saline for > 6 h. More data on $^{99m}$Tc-HFAPi preparation and preclinical studies will be reported in detail in a separate research paper.

**Patient characteristics**

For 40 patients (25 male) enrolled, thirty-five patients had yet to undergo cancer-related surgery (30 treatment naïve and 5 with chemo/radio-therapy). Another 5 patients had already undergone surgery with/without chemotherapy and/or radiotherapy. The characteristics of the patients and primary lesions information are summarized in Table 1. Ultimately, 39 patients were confirmed to have malignant disease, whereas 1 was confirmed to have benign tumour (spindle cell tumour of intestine). The scheme of the study design is presented in Fig. 1.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>40</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>64.5</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>54.3–71.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
</tr>
<tr>
<td>Site and pathology of primary disease</td>
<td></td>
</tr>
<tr>
<td>Rectum Adenocarcinoma</td>
<td>15</td>
</tr>
<tr>
<td>Gastric Adenocarcinoma</td>
<td>13</td>
</tr>
<tr>
<td>Colonic Adenocarcinoma</td>
<td>8</td>
</tr>
<tr>
<td>Esophageal Squamous carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Intestinal Spindle cell tumor</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Anal malignant melanoma</td>
<td>1</td>
</tr>
<tr>
<td>Clinical status before imaging</td>
<td></td>
</tr>
<tr>
<td>Treatment-naive</td>
<td>30</td>
</tr>
<tr>
<td>Resection surgery</td>
<td>3</td>
</tr>
<tr>
<td>Neoadjuvant chemo/radio-therapy</td>
<td>5</td>
</tr>
<tr>
<td>Chemo/radio-therapy after surgery</td>
<td>2</td>
</tr>
<tr>
<td>Other imaging</td>
<td></td>
</tr>
<tr>
<td>Contrast enhanced CT</td>
<td>40</td>
</tr>
<tr>
<td>Gastrointestinal endoscope</td>
<td>38</td>
</tr>
<tr>
<td>B ultrasound</td>
<td>7</td>
</tr>
<tr>
<td>DWI</td>
<td>11</td>
</tr>
<tr>
<td>(^{18}\text{F}-\text{FDG})</td>
<td>1</td>
</tr>
<tr>
<td>Clinical questions for (^{99}\text{Tc})-HFAPI</td>
<td></td>
</tr>
<tr>
<td>Staging of cancer before surgery</td>
<td>35</td>
</tr>
<tr>
<td>Identification of disease recurrence and restaging</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 2
Diagnostic efficiency of $^{99m}$Tc-HFAPi in suspected lesions compared with ceCT

<table>
<thead>
<tr>
<th>Basis of analysis and modality</th>
<th>Sensitivity TPR (%)</th>
<th>Specificity TNR (%)</th>
<th>Negative predict value NPV (%)</th>
<th>Positive predict value PPV (%)</th>
<th>Accuracy ACC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFAPi-meta</td>
<td>89.7 (26/29)</td>
<td>97.9 (47/48)</td>
<td>94.0 (47/50)</td>
<td>96.3 (26/27)</td>
<td>94.8 (73/77)</td>
</tr>
<tr>
<td>95% CI</td>
<td>71.5–97.3</td>
<td>87.5–99.9</td>
<td>82.5–98.4</td>
<td>79.1–99.8</td>
<td>87.0–98.4</td>
</tr>
<tr>
<td>ceCT-meta</td>
<td>69.0 (20/29)</td>
<td>85.4 (41/48)</td>
<td>82.0 (41/50)</td>
<td>74.1 (20/27)</td>
<td>79.2 (61/77)</td>
</tr>
<tr>
<td>95% CI</td>
<td>49.0–84.0</td>
<td>71.6–93.5</td>
<td>68.1–90.9</td>
<td>53.4–88.1</td>
<td>68.8–86.9</td>
</tr>
</tbody>
</table>

TPR: true positive rate  
TNR: true negative rate  
CI: confidence intervals

No drug-related side effects occurred during or after $^{99m}$Tc-HFAPi injection, and SPECT/CT imaging was tolerated well by all patients. Vital parameters remained stable, and no patient reported any new symptoms during the observation period.

**Dynamic biodistribution in organs and dosimetry estimation**

The dynamic physiological biodistribution of $^{99m}$Tc-HFAPi in vital organs at 10, 30, 90, 150, and 240 min was measured in 4 patients and summarized using %ID/organ (patients’ information Table S1). Physiological distribution of $^{99m}$Tc-HFAPi was observed in the liver, pancreas, gallbladder, and to a lesser extent in the kidneys, lungs, spleen, salivary glands, and thyroid glands, with rapid clearance of the radiotracer from these organs (Fig. 2a). Representations of a coronal section from whole-body SPECT are shown in Fig. 2b.

A summary of dosimetric parameters for various organs is given in Table S2, and the mean effective dose equivalent of the whole body was $1.26\times10^{-3}$ mSv/MBq, which is consistent with those for other molecules labelled with $^{99m}$Tc [12, 13].

**$^{99m}$Tc-HFAPi for diagnosing primary lesions**

35 patients with unresected primary digestive system lesions, which were all pathologically confirmed as malignant lesions, were detected by $^{99m}$Tc-HFAPi SPECT/CT with a nearly identical sensitivity of 94.29% (33/35) as ceCT (35/35, 100%, $P=0.5$). The two false-negative of $^{99m}$Tc-HFAPi were found highly differentiated rectal adenocarcinoma by pathology. Representative 3 true-positive and 1 false-negative lesions were stained with the anti-FAP antibody by IHC. As illustrated in Fig. 3, patient (P4) with false-negative lesions on $^{99m}$Tc-HFAPi SPECT/CT showed the lowest expression of FAP compared with true-positive patients (P1-3), indicating that the uptake of $^{99m}$Tc-HFAPi was associated with the expression of FAP.

Local recurrence was found in 60% (3/5) patients by pathology or follow up imaging, all of which were positively detected by ceCT and $^{99m}$Tc-HFAPi SPECT/CT (Table S3).

**$^{99m}$Tc-HFAPi in the diagnosis of suspected metastatic lesions**

After $^{99m}$Tc-HFAPi examination, 77 suspected lesions were detected. Among them, 29 lesions in 9 patents were confirmed metastatic lesions by pathology (n = 4), multi-modality imaging (n = 11), or follow-up (n = 14). In total, $^{99m}$Tc-HFAPi-positive metastases were observed in 8 patients with 26 lesions, including liver (n = 15), bone (n = 8), abdomen (n = 1), pelvis (n = 1) and mediastinum tissue (n = 1). Compared to ceCT, $^{99m}$Tc-HFAPi exhibited higher sensitivity in the diagnosis of suspected metastatic lesions (89.66% [26/29] vs 68.97% [20/29], respectively, $P=0.03$). For 48 benign lesions, $^{99m}$Tc-HFAPi showed higher specificity than ceCT (97.9% [47/48] vs. 85.4% [41/48], respectively; $P=0.03$). The only one false positive case turned out to be tuberculosis has been reported previously [9, 14]. A comparison of diagnostic efficiency on benign or metastatic lesions between ceCT and $^{99m}$Tc-HFAPi SPECT/CT is shown in Table 3. It is worth noting that for liver metastasis determination, 100% (24/24) specificity was achieved using $^{99m}$Tc-HFAPi SPECT/CT, with 83.3% (20/24) by...
ceCT (Table 4). Although the non-statistical difference ($P = 0.13$) might be related to the relatively small sample size, subsequent studies with larger sample size are still worthwhile.

Table 3
Diagnostic efficiency of $^{99m}$Tc-HFAPi in liver metastasis compared with ceCT

<table>
<thead>
<tr>
<th>Basis of analysis and modality</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Negative predict value</th>
<th>Positive predict value</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFAPi-liver</td>
<td>88.2 (15/17)</td>
<td>100.0 (24/24)</td>
<td>92.3 (24/26)</td>
<td>100.0 (15/15)</td>
<td>95.1 (39/41)</td>
</tr>
<tr>
<td>95% CI</td>
<td>62.2–97.9</td>
<td>82.8–100.0</td>
<td>73.4–98.7</td>
<td>74.7–100.0</td>
<td>83.0–99.5</td>
</tr>
<tr>
<td>ceCT-liver</td>
<td>100.0 (17/17)</td>
<td>83.3 (20/24)</td>
<td>100.0 (20/20)</td>
<td>81.0 (17/21)</td>
<td>90.2 (37/41)</td>
</tr>
<tr>
<td>95% CI</td>
<td>77.1–100.0</td>
<td>61.8–94.5</td>
<td>79.9–100.0</td>
<td>57.4–98.7</td>
<td>76.9–96.7</td>
</tr>
</tbody>
</table>

TPR: true positive rate
TNR: true negative rate
CI: confidence intervals

Table 4
Changes in metastatic staging and oncologic management according to $^{99m}$Tc-HFAPi

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>State</th>
<th>Primary tumor</th>
<th>Metastasis stage and site from ceCT</th>
<th>Metastasis stage and site from HFAPi</th>
<th>Ways to confirm</th>
<th>Changing in stage</th>
<th>Clinical decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>69</td>
<td>Treatment-naive</td>
<td>Adenocarcinoma of rectum</td>
<td>Mx</td>
<td>M1 with occipital bone</td>
<td>CT with bony change</td>
<td>Staging up</td>
<td>Palliative operation</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>58</td>
<td>Treatment-naive</td>
<td>Adenocarcinoma of the cecum</td>
<td>M1 with liver</td>
<td>M0 without liver</td>
<td>DWI: hepatic cyst</td>
<td>Staging down</td>
<td>Tend to operation</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>81</td>
<td>Treatment-naive</td>
<td>Adenocarcinoma of rectum</td>
<td>M1 with liver</td>
<td>M0 without liver</td>
<td>DWI: Hepatic spongy hemangioma</td>
<td>Staging down</td>
<td>Tend to operation</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>55</td>
<td>Treatment-naive</td>
<td>Gastric adenocarcinoma</td>
<td>M1 with liver</td>
<td>M0 without liver</td>
<td>DWI: no obvious abnormality</td>
<td>Staging down</td>
<td>Tend to operation</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>22</td>
<td>Treatment-naive</td>
<td>Gastric adenocarcinoma</td>
<td>M0</td>
<td>M1 with skull, posterior bulbar tissue, peritoneum</td>
<td>Pathology of peritoneum, CT with bony change of the skull</td>
<td>Staging up</td>
<td>Palliative surgery and intraperitoneal chemotherapy</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>58</td>
<td>Treatment-naive</td>
<td>Gastric adenocarcinoma</td>
<td>Mx</td>
<td>M0 without liver</td>
<td>Follow up</td>
<td>Staging down</td>
<td>Tend to operation</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>51</td>
<td>Neoadjuvant chemotherapy</td>
<td>Adenocarcinoma of rectum</td>
<td>Mx</td>
<td>M0 without spleen</td>
<td>DWI: Splenic hemangioma</td>
<td>Staging down</td>
<td>Tend to operation</td>
</tr>
</tbody>
</table>

Clinical values of $^{99m}$Tc-HFAPi

In suspected metastatic lesions which were not diagnosed coincidently by ceCT and $^{99m}$Tc-HFAPi SPECT/CT, follow-up data were requested as described in methods (Diagnosis and follow-up). As a result, $^{99m}$Tc-HFAPi SPECT/CT lead to M classification restaging in 8/40 (20.0%) patients (1 patient staged up and 7 staged down). Among the restaging, 6 of 8 patients changed the oncologic regimen, including 1 with new findings for bone metastasis who changed to systemic therapy and 5 for whom curative surgery was performed.
staging of cancer and treatment modication. These results further demonstrate the diagnostic advantage of FAPI imaging in identifying more lesions in skeletal metastases than ceCT is commonly used for diagnosing liver metastasis, its accuracy does not always meet clinical requirements. The liver is the main site of metastasis and a major cause of death in digestive system malignancies. The detection of liver metastasis is typically based on multi-modality imaging, including ceCT, MRI, and $^{18}$F-FDG PET/CT, but all have limitations. Although ceCT is commonly used for diagnosing liver metastasis, its accuracy does not always meet clinical requirements. ceMRI is suggested to have advantage over ceCT in detecting small liver metastases (<10 mm); however, criticism has been directed towards it because the cost does not match the clinical benefit. \cite{19, 21, 22}. $^{18}$F-FDG PET/CT is not routinely indicated for initial staging of digestive tract tumours because of its low sensitivity for liver metastasis, particularly in patients who have received preoperative chemotherapy. The low background of the normal liver leads to the potential application of FAPI tracers in patients with suspected liver metastases. Our findings support the implementation of $^{99m}$Tc-HFAPi SPECT/CT in the identification of liver metastasis. Basically, $^{99m}$Tc-HFAPi demonstrated satisfactory sensitivity (88.2\%) for detecting liver metastasis. Moreover, due to the low expression of FAP in benign liver lesions, an extremely high specificity (100\%) of $^{99m}$Tc-HFAPi was achieved. Four cases of suspected liver metastasis in ceCT were negatively detected by $^{99m}$Tc-HFAPi with minimal uptake, and the lesions were proven to be benign by biopsy or multi-modality imaging, thus excluding from metastasis and restaging from M1 to M0 and allowing the chance for instead of systemic therapy, consistently with those of $^{99m}$Tc-HFAPi imaging results. Changes in the clinical oncologic regimen are given in Table 5. The results indicate that $^{99m}$Tc-HFAPi SPECT/CT can provide a strong basis for clinical decision-making.

**SUV$_{\text{max}}$ and T/B ratio in the diagnosis of benign and malignant disease**

Our team previously designed an algorithm, which has been patented (Patent number: US11189374B2) \cite{15}, to calculate the SUV$_{\text{max}}$ based on SPECT/CT and linearized it against the standard T/B ratio. The result indicating an obvious linear correlation with $R^2$ of 0.735 (Fig. 4, $P < 0.001$) between SUV$_{\text{max}}$ and T/B ratio. For primary malignant lesions, the median T/B ratio and SUV$_{\text{max}}$ were 6.35 (IQR: 3.64 to 8.10) and 9.52 (IQR: 5.73 to 14.52), respectively; for all metastases, they were 3.65 (IQR: 2.47 to 4.79) and 6.05 (IQR: 4.43 to 9.09), respectively. The SUV$_{\text{max}}$ and T/B ratio for different malignant lesions by $^{99m}$Tc-HFAPi are shown in Fig. 5a, b and Table S3, S4. High SUV$_{\text{max}}$ and T/B ratios were found in gastric cancer and liver metastasis. For non-malignant lesions, few showed intense uptake of $^{99m}$Tc-HFAPi, with a median T/B ratio and SUV$_{\text{max}}$ of 1.32 (IQR: 1.01–1.74) and 2.11 (IQR: 1.68–2.75), respectively, both were significantly lower ($P < 0.001$) than malignancies (Fig. 5c).

The ROC curve built with 67 malignant lesions and 48 benign lesions yielded an AUC of 0.938 (95\% CI, 0.895–0.981, $P < 0.001$) for the T/B ratio and 0.913 (95\% CI, 0.859–0.966, $P < 0.001$) for SUV$_{\text{max}}$ (Fig. 6, left). Youden's index analysis revealed several optimal cut-off values for discriminating malignant from non-malignant lesions (Fig. 6, right).

**Discussion**

Recent PET/CT studies with FAP inhibitors have been well developed, revealing strong PET signals across dozens of major cancers, especially digestive system cancers \cite{6, 7, 16, 17}. However, limited data about $^{99m}$Tc-labelled FAPIs in clinical have been reported, with only application in two patients (ovarian and pancreatic cancer) \cite{10}. Here we evaluated the biodistribution of a newly developed $^{99m}$Tc-HFAPi and demonstrated its value for initial staging and restaging in digestive system cancers.

Based on time-dependent biodistribution of $^{99m}$Tc-HFAPi in nontarget organs, optimal T/B ratios with limited noise were achieved by image acquisition at 60–90 min after injection. Almost all primary malignancies showed marked uptake of $^{99m}$Tc-HFAPi, especially in gastric cancers, with median T/B ratios of 7.01. In addition, $^{99m}$Tc-HFAPi might be superior to anatomic assessment for detecting local relapse, which is particularly useful in the presence of elevated tumour markers but no clinical or morphological evidence, as previously indicated \cite{9}.

The liver is the main site of metastasis and a major cause of death in digestive system malignancies. The detection of liver metastasis is typically based on multi-modality imaging, including ceCT, MRI, and $^{18}$F-FDG PET/CT, but all have limitations. Although ceCT is commonly used for diagnosing liver metastasis, its accuracy does not always meet clinical requirements. ceMRI is suggested to have advantage over ceCT in detecting small liver metastases (<10 mm); however, criticism has been directed towards it because the cost does not match the clinical benefit. ceMRI is not routinely indicated for initial staging of digestive tract tumours because of its low sensitivity for liver metastasis, particularly in patients who have received preoperative chemotherapy. The low background of the normal liver leads to the potential application of FAPI tracers in patients with suspected liver metastases. Our findings support the implementation of $^{99m}$Tc-HFAPi SPECT/CT in the identification of liver metastasis. Basically, $^{99m}$Tc-HFAPi demonstrated satisfactory sensitivity (88.2\%) for detecting liver metastasis. Moreover, due to the low expression of FAP in benign liver lesions, an extremely high specificity (100\%) of $^{99m}$Tc-HFAPi was achieved. Four cases of suspected liver metastasis in ceCT were negatively detected by $^{99m}$Tc-HFAPi with minimal uptake, and the lesions were proven to be benign by biopsy or multi-modality imaging, thus excluding from metastasis and restaging from M1 to M0 and allowing the chance for radical surgery. Overall, $^{99m}$Tc-HFAPi provided an accurate diagnosis of suspected liver metastases, which may avoid unnecessary misdiagnosis, correct tumour staging and promote clinical oncological decisions.

According to a previous study, FAPI imaging identifies more lesions in skeletal metastases than $^{18}$F-FDG PET/CT \cite{9}. A similar result was observed in our study: 2 patients with multiple skeletal uptake of $^{99m}$Tc-HFAPi were considered to have bone metastasis, which was not detected by ceCT. These results further demonstrate the diagnostic advantage of $^{99m}$Tc-HFAPi for distant metastasis, thus improving the staging of cancer and treatment modification.
As previously reported, inflammation-induced fibrosis revealed positive uptake of FAPIs [30], and our study showed that tuberculosis can take up $^{99m}$Tc-HFAPi to some extent. Moreover, uterine fibroids demonstrated elevated $^{99m}$Tc-HFAPi uptake, which might be attributed to the activated fibroblasts, with similar results in previous studies [31, 32]. However, unlike a previous report in which hepatobiliary elimination of FAPI-19 resulted in a lack of tumour accumulation [8], in our study, significant and stable $^{99m}$Tc-HFAPi uptake was observed by tumours, and low liver uptake was observed in the 4-h period, indicating no redistribution to the liver during the enterohepatic cycle.

For 2 false-negative cases of primary malignancies, one showed obviously low expression of FAP by IHC staining, while the other had moderate expression, suggesting that the difference between FAP expression and imaging results may indicate changes in protein functional status; in fact, other membrane proteins have different functional states in different conformations [33] thus are inaccessible to inhibitors. Regardless, the reason remains to be explored.

There are several limitations to this study. First, a small patient cohort limited the statistical significance for some kinds of cancers, such as oesophageal, pancreatic, and gallbladder cancer. Second, although being the ideal reference standard, histopathological examination was not available in all lesions because of ethical and technical reasons. Third, due to relatively poor resolution of SPECT/CT, small lesions such as lung nodules and small lymph nodes were not well detected.

Despite these limitations, to the best of our knowledge, this article might be the first application of a new $^{99m}$Tc-labelled FAPI for digestive system tumours from a clinical perspective, and we confirmed its diagnostic efficacy in tumour staging and restaging, providing an important basis for clinical application and subsequent studies. Further prospective studies with larger populations in head-to-head comparisons of $^{99m}$Tc-HFAPi SPECT/CT and $^{68}$Ga-FAPI PET/CT are warranted to best comment on the superiority of the tracers to clarify the role of SPECT/CT.

**Conclusion**

In this work, we have developed a new $^{99m}$Tc labelled molecular probe and transformed it for the first time for digestive system tumours study. The findings indicate selective uptake of $^{99m}$Tc-HFAPi SPECT/CT and demonstrate a high target-to-background ratio for various types of digestive system cancers as well as related metastasis, especially liver metastasis, which contributes to the current literature on FAP inhibitor molecular imaging. This warrants further large-scale multicentre studies with a homogenous patient population and different cancer types should be examined with a head-to-head comparison of $^{99m}$Tc-HFAPi SPECT/CT molecules to best comment on the superiority of the tracers.

**Declarations**

**Author Contributions:** Rui Gao conceived of the study and participated in its design and is responsible for the development of clinical research. Xi Jia is primarily involved in registration of clinical trials, image processing and reviewing of reports. Xinru Li is primarily involved in results analysis, drafting the manuscript and substantively revising. Bing Jia is responsible for the designing and guidance of preclinical drug related experiments. Ye Yang is responsible for medical history collection and data processing. Yuanbo Wang and Yan Liu prepared the $^{99m}$Tc-HFAPi and collected SPECT/CT images. Ting Ji and Xin Xie wrote the primary medical report. Yu Yao, Guanglin Qiu, Huixing Deng and Aimin Yang are responsible for providing clinical cases and MDT discussion. Zhaohui Zhu is responsible for manuscript review and proofreading. Si Chen provides algorithm of SUV$_{max}$ calculating. All authors read and approved the final manuscript.

**Ethics approval and consent to participate:** This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Xi’an Jiaotong University (Ethic approved No.: XJTU1AF2021LSK-021) and Chinese Clinical Trial Register (Registration No.: ChiCTR2100048093), and all the patients gave written, informed consent before the study.

**Consent for publication:** All authors of the current manuscript meet the specified criteria for authorship and agreed to publish this manuscript.

**Conflict of interest:** The authors declare no competing interests.

**Funding:** The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.
Availability of data and material: The datasets used or analyzed during the current study are available from the corresponding authors on reasonable request.

References


Figures
Figure 1

The scheme of the study design.
Figure 2

Biodistribution of $^{99m}$Tc-HFAPi in different vital organs over time. **a**, % Injection Dose (ID)/organ of $^{99m}$Tc-HFAPi in heart, liver, lungs, kidneys, pancreas, spleen, brain, thyroid and salivary glands in different times. Data represent median ± interquartile range. **b**, Example of background ROIs in patient #002 on planar scintigraphy images: whole body $^{99m}$Tc-HFAPi scintigraphy was performed at 10, 30, 90, 150 and 240min post-injection.
Figure 3

SPECT/CT (left) and Immunohistochemistry staining (right) of representative primary lesions and tumor-adjacent tissue. The graphs above the dotted line were representations of true positive cases (P1-P3), while below the dotted line is a representation of false negative case (P4) by $^{99m}$Tc-HFAPI. Scale bar, 200 μm; T: primary tumor; NT: Tumor-adjacent tissue.
Figure 4

Linear relationship between $SUV_{max}$ and T/B ratio. $R$: linear correlation coefficient.

Fig. 5

(a) Relative uptake level in primary malignancies.
(b) Relative uptake level in metastatic lesions.
(c) Relative uptake level in malignancies and benign conditions.
Figure 5

Uptake of $^{99m}$Tc-HFAPi in different lesions. T/B ratio (blue bar) and SUV$_{\text{max}}$ (red bar) are shown in primary malignancies (a) and metastatic lesions (b). c, The comparison of $^{99m}$Tc-HFAPi uptake between benign and malignant lesions. Data represent median ± interquartile range. ***, $P<0.001$.

![Figure 5](image1)

Fig. 6

The performance of T/B ratio (red line) and SUV$_{\text{max}}$ (blue line) in the diagnosis of digestive system cancer by receiver operating characteristic (ROC) curves analysis (left). The charts (right) show several optimal cut-off values for discriminating malignant from non-malignant lesions. T/B ratio, tumour/background ratio; AUC, area under ROC curves; YI, Youden’s index.

![Figure 6](image2)

**Figure 6**

The performance of T/B ratio (red line) and SUV$_{\text{max}}$ (blue line) in the diagnosis of digestive system cancer by receiver operating characteristic (ROC) curves analysis (left). The charts (right) show several optimal cut-off values for discriminating malignant from non-malignant lesions. T/B ratio, tumour/background ratio; AUC, area under ROC curves; YI, Youden’s index.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformations.pdf