The performance of [68Ga]Ga-FAPI-04 PET/CT in head and neck squamous cell carcinoma: a prospective comparison with [18F]FDG PET/CT

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Abstract

Purpose

This study aimed to compare the performance of [^{68}Ga]Ga-FAPI-04 and [^{18}F]FDG PET/CT for initial staging and recurrence detection of head and neck squamous cell carcinoma (HNSCC).

Methods

Prospectively, 77 patients with histologically proven or highly suspected HNSCC, who presented for either initial staging (n = 67) or restaging (n = 10), were referred to paired [^{18}F]FDG and [^{68}Ga]Ga-FAPI-04 PET/CT within one week. The diagnostic performance was compared for the two imaging approaches, especially for N staging. SUVmax, SUVmean, and target-to-background ratio (TBR) were assessed for paired positive lesions. Furthermore, change in management by [^{68}Ga]Ga-FAPI-04 PET/CT and histopathologic FAP expression of some lesions were explored.

Results

[^{18}F]FDG and [^{68}Ga]Ga-FAPI-04 PET/CT exhibited comparable detection rates for primary tumors (100%) and recurrence (62.5%). In the twenty-nine patients receiving neck dissection, [^{68}Ga]Ga-FAPI-04 PET/CT showed higher specificity and accuracy in evaluating preoperative N staging than [^{18}F]FDG based on patients ($p = 0.031$ and $p = 0.070$), neck sides ($p = 0.002$ and $p = 0.006$) and neck levels ($p < 0.001$ and $p < 0.001$). As for distant metastasis, [^{68}Ga]Ga-FAPI-04 PET/CT detected more positive lesions than [^{18}F]FDG (25 *vs* 23), and with higher SUVmax (7.99 ± 9.04 *vs* 3.62 ± 2.68, $p = 0.002$) by the lesion-based analysis. The types of neck dissection in 9 cases (9/33) were altered by [^{68}Ga]Ga-FAPI-04. Overall, clinical management was significantly changed in 10 patients (10/61). Three patients underwent a follow-up [^{68}Ga]Ga-FAPI-04 PET/CT after neoadjuvant therapy: one showed complete remission, and the others showed progression. The [^{68}Ga]Ga-FAPI-04 uptake intensity was confirmed to be consistent with FAP expression.

Conclusion

[^{68}Ga]Ga-FAPI-04 outperforms [^{18}F]FDG PET/CT in evaluating preoperative N staging in patients with HNSCC. Furthermore, [^{68}Ga]Ga-FAPI-04 PET/CT also shows the potential in clinical management and monitoring response to treatment.

Clinical Trial Registration:

This prospective study was reviewed and approved by the Medical Ethics Committee of Zhongnan Hospital, Wuhan University, and was registered online at NIH ClinicalTrials.gov (NCT05034146 & NCT05030597).

Introduction

Head and neck squamous cell carcinoma (HNSCC) ranks as the sixth most common type of malignant tumor worldwide [1, 2]. These heterogeneous tumors occur in the epithelium of multiple anatomical sites within the head and neck region, characterized by aggressive local invasion and overall poor prognosis. Meanwhile, recurrence and metastasis are frequent events leading to HNSCC mortality [3, 4]. Therefore, optimal imaging of HNSCC is crucial for accurate initial staging, selection of the primary therapy, and follow-up to detect local recurrence or metastatic spread as early and as completely as possible.

Computed tomography (CT) and magnetic resonance imaging (MRI) are the conventional imaging modalities for the preoperative evaluation and staging of HNSCC. Their sensitivity is mainly limited based on morphologic diagnostic criteria, such as nodal size and contrast enhancement patterns [5–7]. Combining functional and anatomical imaging, [^{18}F]FDG PET/CT has been widely and successfully applied in diagnosis, staging, restaging, and recurrence detection for HNSCC. Moreover, [^{18}F]FDG PET/CT is recommended to determine the initial treatment strategy for head and neck cancers [8, 9]. However, as a non-specific tracer, [^{18}F]FDG remains certain disadvantages due to possible false-positive findings in the presence of cervical lymphadenitis, brown fat, or even healthy benign structures [10, 11].

Cancer-associated fibroblasts (CAFs) are the predominant cell type within the tumor stroma, bestowing a favorable microenvironment for tumor cell growth and proliferation [4]. Fibroblast activation protein (FAP) overexpression on CAFs has been confirmed in more than 90% of epithelial carcinomas, including head and neck cancers [12]. In contrast, it is largely absent in normal adult tissues, rendering it a rather specific marker of CAFs in the tumor microenvironment for tumor imaging [13–15]. Recently, tracers based on FAP inhibitors (FAPIs) have been introduced as promising tumor imaging agents and have attracted much attention. Several researchers have reported the excellent performance of radioactive FAPIs in head-and-neck cancers for primary staging and recurrence detection [16–24]. However, these studies contained a relatively small sample size. Furthermore, studies reported so far were performed with a limited number of various pathological types of head and neck cancers. Moreover, analysis of the performance of FAPI-based probes in judging the status of cervical lymph nodes may be inadequate as most of the studies reported in the literature did not use the final pathology results as a reference standard. Therefore, the clinical value of [^{68}Ga]Ga-FAPI PET/CT for HNSCC should be further investigated, especially in exploring the diagnostic efficacy of cervical neck lymph node metastases.

Herein, we aimed to further investigate the clinical utility of [^{68}Ga]Ga-FAPI PET/CT for primary staging and recurrence detection in patients with newly diagnosed or relapsed HNSCC compared with [^{18}F]FDG PET/CT, especially for N staging.

Materials And Methods

Patients

This prospective study was reviewed and approved by the Medical Ethics Committee of Zhongnan Hospital, Wuhan University, and was registered online at NIH ClinicalTrials.gov (NCT05034146 & NCT05030597). All participants provided written informed consent prior to participation. Between February 2021 and June 2022, a total of 114 patients were preliminarily enrolled in this study consecutively. According to the inclusion and exclusion criteria, 77 patients were included in the final analysis. Inclusion criteria were as follows: (i) adult patients (aged 18 years or older); (ii) pathologically confirmed or highly suspected HNSCC; (iii) clinically suspected recurrence of HNSCC; (iv) underwent both [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT within one week. The exclusion criteria were as follows: (i) start of treatment within 3 months before PET/CT scan; (ii) patients with unknown primary tumor.

Preparation Of Radiopharmaceuticals

[^{18}F]FDG was purchased from Wuhan HTA Pharmaceutical Co., Ltd., (Wuhan, China) with radiochemical purity greater than 95%. The good-manufacturing-practice (GMP) grade precursor, DOTA-FAPI-04, was obtained commercially from CSBio Ltd. (Shanghai, China). [^{68}Ga]Ga-FAPI-04 (abbreviated as [^{68}Ga]Ga-FAPI) was prepared using the iTM ^{68}Ge/^{68}Ga generator and iQS-TS automated synthesis module (Isotope Technologies Munich GmBH, Munich, Germany). The detail preparation of [^{68}Ga]Ga-FAPI are shown in the supplementary information. The radiochemical purity was > 96% of the final product measured using radio-HPLC.

Pet/ct Scan

The paired [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT scans were performed within one week for each participant. The detail imaging techniques are found in the supplementary information.

Imaging Interpretation

Visual analysis of both PET/CT images was performed independently by three qualified and experienced nuclear medicine physicians. The reviewers were blinded to clinical data, histological findings, and other imaging results. Any disagreements were resolved by the consensus between the reviewers. Primary tumors and metastatic lesions were considered positive when a focal accumulation was visually higher than surrounding healthy tissue and not related to physiological uptake. The location and number of the primary tumor, nodal, and distant metastasis were recorded. Furthermore, second primary tumors were also recorded if present on PET/CT images. Cervical lymph nodes were delineated according to the delineation of neck node levels for head and neck tumors (updated version in 2013) [25].

Tracer uptake in positive lesions was quantified with SUVmax, which was automatically measured from the volume of interest (VOI) drawing at a 40% isocontour. The tumor-to-background ratio (TBR) and tumor-to-blood pool ratio (TBPR) were measured for primary tumors. The TBR was determined by dividing lesion SUVmax by the SUVmean of contralateral normal tissue. The TBPR was calculated from the lesion SUVmax divided by the mean SUV of the blood pool.

For primary tumors and distant metastasis, all lesions were assessed. As for cervical lymph node metastases, if there were multiple positive lesions at each level, all (< 5) or five lymph nodes (≥ 5) with the most intense tracer uptake were evaluated. Confluent lymphoid nodules, which cannot be distinguished, were denoted as one lymph node. Since it is unavailable to perform precise one-to-one spatial correlations between PET/CT images and histopathology for lymph nodes, analysis was restricted to node levels and neck sides. If at least one positive lymph node was present on PET/CT images in a given neck side and level, this neck side and level were designated as positive, which also applies to the patient-based analysis.

Histopathology served as the gold standard for final diagnosis. For those cases without histopathology results, radiographic follow-up data of more than 3 months were requested. Lesions were considered malignant during follow-up based on (i) typical malignant features confirmed by multi-modality medical imaging, and (ii) a significant reduction in size and/or number of lesions after anti-cancer treatment (chemo, radio, targeted therapy, and/or immunotherapy) according to the RECIST 1.1 guideline [26]. Disease progression was defined as the increase in lesion size and/or the number of metastatic lesions.

Clinical Staging

The staging was determined based on the 8th edition of the American Joint Committee on Cancer (AJCC) [27]. Changes in TNM staging according to [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT were recorded for patients. Subsequent changes in oncologic management ([^{18}F]FDG-based *vs.* [^{68}Ga]Ga-FAPI-based) were further evaluated.

Fap Immunohistochemistry

Immunohistochemistry (IHC) staining was performed in 7 cases to validate the expression of FAP, including 5 primary tumor samples and 8 cervical lymph nodes samples. IHC staining (antibody ab207178, Abcam, 1:100) was performed according to standard protocols. Semiquantitative IHC analyses of FAP expression was determined by combining the proportion score (percentage of positive stained area) with the staining intensity score by an experienced pathologist (composite score: - No reactivity; + low; ++ moderate; +++ strong).

Statistical analysis

Statistical analyses were performed using SPSS software (22.0, IBM Inc.). Data were expressed as means (SD) or median and range, as appropriate. Paired-samples *t*-test or Wilcoxon signed-rank test were applied to assess the differences of various parameters between [^{68}Ga]Ga-FAPI and [^{18}F]FDG. The sensitivity, specificity and accuracy of the two PET/CT scans were compared using McNemar's chi-squared test to evaluate the diagnostic efficacy in primary tumors, nodal metastases, and distant metastasis. The correlation between the two parameters was determined using the Spearman test. Two-tailed *P* values were adopted, and $P < 0.05$ was considered significant.

Results

Patients' characteristics

The flow diagram of the study design is presented in Fig. 1. Finally, seventy-seven patients (Male 61, Female 16; median age, 58 [range, 20 ~ 89]) were analyzed, including 67 treatment-naïve patients for initial staging and 10 for detection of recurrence. Thirty-three patients (30 initial-treated patients and 3 patients with suspected recurrence) underwent surgery. Patient characteristics are summarized in Table 1.

Table 1
Subject demographics

Patients' characteristics (N = 77)	Value (%)
Age	
Median [Min, Max]	58 [20, 89]
Sex	
Male	61 (79%)
Female	16 (21%)
Status and indication	
Treatment-naïve (staging)	67 (87%)
Recurrence (restaging)	10 (13%)
Primary tumor location	
Nasopharynx	18 (23%)
Oral cavity	19 (25%)
Oropharynx	14 (18%)
Larynx	16 (21%)
Hypopharynx	7 (9%)
Nasal cavities and Paranasal sinuses	3 (4%)
Treatment	
Surgery with neck dissection	30 (39%)
Surgery without neck dissection	3 (4)
Non-surgical treatment	44 (57%)
Histopathology	
Available	73 (95%)
Unavailable	4 (5%)

Evaluation Of Primary Tumors, Local Recurrence Detection And T Staging

Histopathological results were available for all 67 treatment-naïve patients. A total of 73 primary lesions in 67 patients were observed (tumor size, 3.5 ± 1.6 cm; range, 0.4 ~ 8.2 cm) by [^{68}Ga]Ga-FAPI and

[^{18}F]FDG PET/CT, respectively. All primary lesions were well detected by [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT with a 100% positive detection rate. The difference of SUVmax and SUVmean between [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT was not statistically significant (17.73 ± 7.02 *vs* 16.34 ± 7.28 ($p = 0.06$), 10.29 ± 4.08 *vs* 9.46 ± 4.19 ($p = 0.05$), respectively). TBR between [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT also showed no significant difference (9.78 ± 6.16 *vs* 9.31 ± 5.61, $p = 0.525$). However, TBPR (13.89 ± 6.98) of [^{68}Ga]Ga-FAPI was higher than that of [^{18}F]FDG PET/CT (8.37 ± 4.29, $p = 0.000$). As for the location analysis, primary tumors occurred in nasopharynx and oral cavity showed significantly higher SUVmax of [^{68}Ga]Ga-FAPI than [^{18}F]FDG PET/CT ($p = 0.021$ and $p = 0.048$) (Table S1). Representative PET/CT images of primary tumors with different location are shown in Fig. 2.

Ten patients underwent PET/CT examination for detection of recurrence. Nine positive lesions in 8 cases were observed on both methods. The other two patients who were suspected of regional lymph node metastases without local recurrence were analyzed in the next section. Local recurrences were confirmed pathologically by surgery or biopsy in four patients and clinically by sequential radiologic follow-up in the other four patients. [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT showed similar sensitivity (62.5%) and accuracy (62.5%) in recurrence detection (Table S2).

In the assessment of T staging, upstaging was identified in one patient (1/67) for initial staging and three patients (3/10) for restaging (Table S4 and Fig. S1). Skull base and intracranial invasion was clearly delineated in four patients on [^{68}Ga]Ga-FAPI PET/CT, while these lesions was masked by high physiological [^{18}F]FDG uptake of normal brain tissues on [^{18}F]FDG PET/CT.

Evaluation Of Neck Nodal Metastasis In Patients Undergoing Neck Dissection

Twenty-nine patients undergoing neck dissection were included in the final analysis of neck node metastasis, including a total of 40 neck sides, 130 neck levels and 906 lymph nodes. According to the pathological interpretations, metastatic lymph nodes were found in 21 of 29 patients (72.4%), involving 25 neck sides (62.5%), 40 neck levels (30.8%) and 78 lymph nodes (8.6%). Of 40 neck levels, 8 were located in level I, 18 in level II, 9 in level III, 4 in level IV and 1 in level V.

Patient-based analysis

The sensitivity, specificity and accuracy of the diagnosis of the positive neck nodes based on patient were 85.7%, 87.5% and 86.2% for [^{68}Ga]Ga-FAPI PET/CT and 85.7%, 12.5% and 65.5% for [^{18}F]FDG PET/CT, respectively. The specificity of [^{68}Ga]Ga-FAPI PET/CT was considerably higher than the corresponding parameters of [^{18}F]FDG PET/CT ($p = 0.031$) (Table 2). The concordance rate on N staging between PET/CT and histopathology for [^{68}Ga]Ga-FAPI (69.0% (20/29)) was significantly higher than that for [^{18}F]FDG PET/CT (31% (9/29), $p = 0.001$). Strikingly, the diagnostic accuracy of the N0 and N1 neck

status of [^{68}Ga]Ga-FAPI (80.0% (12/15)) was significantly higher than that for [^{18}F]FDG PET/CT (20.0% (3/15), $p = 0.004$) (Table 3 and Table S3).

Neck side-based analysis

The sensitivity, specificity and accuracy for the diagnosis of neck side metastasis were 84.0% *vs* 84.0%, 93.3% *vs* 26.7% and 87.5% *vs* 62.5% for [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT, respectively (Table 2). The specificity and accuracy of [^{68}Ga]Ga-FAPI PET/CT were significantly higher than those of [^{18}F]FDG PET/CT ($p = 0.002$ and $p = 0.006$, respectively). [^{68}Ga]Ga-FAPI PET/CT showed greater accuracy than [^{18}F]FDG PET/CT for evaluating the N0 neck side status (14/15 *vs* 4/15, $p = 0.002$).

Neck node level-based analysis

The sensitivity, specificity and accuracy were 82.1% *vs* 82.1%, 96.7% *vs* 74.7% and 92.3% *vs* 76.9% for [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT, respectively (Table 2). [^{68}Ga]Ga-FAPI had significantly higher specificity and accuracy compared with [^{18}F]FDG PET/CT (both $p < 0.001$). Representative [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT images of lymph nodes are shown in Fig. 3.

Both [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT (7.83 ± 7.55 *vs* 2.40 ± 1.25, $p < 0.01$ and 7.89 ± 8.00 *vs* 4.13 ± 1.40, $p < 0.01$) showed significantly higher SUVmax of true-positive neck lymph nodes compared to that of false-positive neck lymph nodes. Based on neck levels, the cut-off value of SUVmax for metastatic lymph nodes was determined by receiver operating characteristic (ROC) curves and the Youden's Index. The best cut-off value of SUVmax for diagnosing metastatic lymph nodes in [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT was 4.24 (AUC 0.703 [95% CI 0.612 ~ 0.795], sensitivity 66.7%, specificity 78.4%, $p < 0.0001$) and 2.02 (AUC 0.843 [95% CI 0.747 ~ 0.940], sensitivity 68.4%, specificity 83.8%, $p < 0.0001$), respectively (Fig. S2).

Table 2

Diagnostic performance of [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT in assessment of neck region lymph node

Basis and modality	Sensitivity (%)	Specificity (%)	Accuracy (%)
Based on patients			
FAPI *vs* FDG	85.7 (18/21) *vs* 85.7 (18/21)	87.5 (7/8) *vs* 12.5 (1/8)	86.2 (25/29) *vs* 65.5 (19/29)
p	**1.000**	**0.031**	**0.070**
Based on neck sides			
FAPI *vs* FDG	84.0 (21/25) *vs* 84.0 (21/25)	93.3 (14/15) *vs* 26.7 (4/15)	87.5 (35/40) *vs* 62.5 (25/40)
p	**1.000**	**0.002**	**0.006**
Based on neck levels			
FAPI *vs* FDG	82.1(32/39) *vs* 82.1(32/39)	96.7 (88/91) *vs* 74.7 (68/91)	92.3(120/130) *vs* 76.9 (100/130)
p	**1.000**	< **0.001**	< **0.001**

Table 3

N staging by [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT based on patients underwent neck dissection

N staging		Pathology (n)	FAPI agreement with pathology (n)	FDG agreement with pathology (n)
N0		8	**7**	**1**
N1		7	**5**	**2**
N2	N2a	1	0	0
	N2b	7	5	3
	N2c	3	2	2
N3	N3a	0	0	0
	N3b	3	1	1

Changes In N Staging On [ga]ga-fapi Pet/ct

In terms of N staging, downstaging was observed in 33 cases (33/77) and upstaging was found in 2 cases (2/77) on [^{68}Ga]Ga-FAPI PET/CT when compared to [^{18}F]FDG PET/CT. [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT seemed to display greater inconsistency (35/77) on N staging (Table S4).

Detection Of Distant Metastases, M Staging And Second Primary Tumor Detection

Distant metastasis was observed on PET/CT in two patients, distributed in mediastinal lymph node, lung, bone, muscle and hypodermis (Table S5 and Fig. S3). [^{68}Ga]Ga-FAPI PET/CT detected more positive lesions than [^{18}F]FDG (25 *vs* 23). Overall, the [^{68}Ga]Ga-FAPI SUVmax was higher than that of [^{18}F]FDG (7.99 ± 9.04 *vs* 3.62 ± 2.68, $p = 0.002$) by the lesion-based analysis, especially bone metastasis (17.64 ± 10.32 *vs* 5.26 ± 3.09, $p = 0.012$). Regarding M staging, distant metastases in two patients (2/67) were well detected by both [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT (Table S4).

A second primary tumor was detected in the submandibular gland of one patient. Interestingly, the primary lesion was not found at the early scan on [^{68}Ga]Ga-FAPI PET/CT, while it appeared on delayed imaging at 2 h (SUVmax 7.07, TBR 3.91). The lesion was identified as secretory carcinoma by subsequent surgical pathology. Physiological high [^{68}Ga]Ga-FAPI uptake of the normal submandibular gland at early imaging will mask the lesions, so a delayed scan is required when necessary.

Change In Management

As shown in Fig. 4A, among 33 patients who underwent surgery, the type of neck lymph node dissection was altered in 9 patients. Of those patients, 7 patients were with a change from bilateral cervical lymph node dissection to unilateral neck dissection, 1 patient underwent unilateral neck node dissection instead of no need of neck node dissection, and another patient changed to no need of neck node dissection (Fig. 4B).

For the total 77 patients, management of therapy was available in 61 cases (79.2%). [^{68}Ga]Ga-FAPI PET resulted in intended management changes in 10 of 61 patients (16.4%) (Fig. 4C). Six (60.0%) of the ten patients had a monotherapy instead of intended combination therapy (e.g., from cisplatin radiotherapy (CCRT) to radiotherapy alone, from surgery plus chemoradiotherapy to surgery alone). A treatment shift toward systemic therapy occurred in another 4 (40.0%) patients (Fig. 4D).

Monitoring Response To Neoadjuvant Therapy

Three patients received neoadjuvant therapy (NAT), including three cycles of immunotherapy and two cycles of chemotherapy. [^{68}Ga]Ga-FAPI PET/CT scan was performed at baseline and post-NAT before surgery. In one patient with tongue squamous cell carcinoma, post-NAT PET/CT showed a significant decrease in SUVmax of the primary tumor and metastatic LNs compared to baseline PET/CT (5.97 *vs* 26.23, 2.99 *vs* 16.90, respectively) (Fig. 5). The patient achieved pathological complete remission (pCR) for both primary tumor and metastatic LNs after NAT. Progressive disease occurred in the other two patients, with more metastatic lymph nodes, enlarged size and increased FAPI uptake in lesions.

Immunohistochemistry Analysis

A total of 5 primary tumor and 8 cervical lymph nodes samples from 7 patients undergoing surgery were available. In detail, 46.1% (6/13) of samples presented strong positive FAP immunostaining (score +++), 7.7% (1/13) moderate (score ++), 23.1% (3/13) low (score +) and 23.1% (3/13) negative (Fig. 6). What's more, the [^{68}Ga]Ga-FAPI uptake (SUVmax) was markedly correlated with FAP expression (r = 0.822, p = 0.001). Interestingly, the cervical lymphatic micro-invasion showed false negative on [^{68}Ga]Ga-FAPI PET/CT in a patient with tongue root cancer, and the corresponding IHC showed no or low expression of FAP (Fig. S4).

Discussion

This prospective study investigated the performance of [^{68}Ga]Ga-FAPI imaging in patients with HNSCC for initial evaluation and recurrence detection compared to [^{18}F]FDG. Our findings showed that [^{68}Ga]Ga-FAPI PET/CT demonstrated higher specificity and accuracy in evaluating preoperative N staging than did [^{18}F]FDG. Meanwhile, [^{68}Ga]Ga-FAPI PET/CT also showed the potential in clinical management and monitoring response to treatment. In a word, our study suggests that [^{68}Ga]Ga-FAPI imaging holds excellent promise as a new imaging modality in evaluating HNSCC.

CAFs are the major components in the tumor microenvironment and play a crucial role in facilitating tumor progression by promoting tumor growth, angiogenesis, and metastasis [4]. FAP, a vital surface marker of CAFs, is overexpressed in various human epithelial tumors, including head and neck cancers [22]. Quinoline-based radiotracers such as [^{68}Ga]Ga-FAPI is a promising imaging agent for tumor diagnosis, showing favorable tumor-to-background ratios in imaging [28]. In our study, [^{68}Ga]Ga-FAPI uptake and tumor-to-normal tissue of primary lesions showed slightly higher than that of [^{18}F]FDG, consistent with previous studies [16–19, 23, 24].

Accurate tumor T staging at diagnosis is of utmost importance in clinical decision-making, therapeutic management, and prognosis prediction for patients with HNSCC [27]. [^{18}F]FDG PET/CT is extensively used in diagnosing primary tumors and detecting local relapse for HNSCC [29]. However, skull-base and intracranial invasion are often hidden by the physiological [^{18}F]FDG uptake in the normal brain tissue, leading to an underestimate of T staging. [^{68}Ga]Ga-FAPI can help overcome these disadvantages due to nearly no uptake in brain tissue. Compared with [^{18}F]-FDG images, this study indicated that the T stage of four patients was upgraded on [^{68}Ga]Ga-FAPI images due to additional visualization of skull-base and intracranial invasion. Similar results have been reported in other studies [20, 24]. Besides the strength mentioned above, the two imaging modalities showed comparable diagnostic accuracy in primary lesions in 67 treatment-naive patients. Therefore, [^{68}Ga]Ga-FAPI PET/CT is comparable with or superior to [^{18}F]FDG on T staging and tumor detecting.

Accurately detecting lymph node metastases in HNSCC patients is essential for accurate staging and effective treatment planning [30]. It has also long been an issue seeking diagnostic imaging modality to obtain more accurate N staging for HNSCC. Our study analyzed the diagnostic efficiency of [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT in cervical lymph node metastasis. For 29 patients receiving neck dissection, [^{18}F]FDG PET/CT demonstrated to have a sensitivity of 82.1% and a specificity of 74.7%, as well as an accuracy of 76.9% based on neck levels, all of which were compatible with the results of previous studies [23, 31–34]. [^{68}Ga]Ga-FAPI PET/CT demonstrated identical sensitivity (82.1% *vs* 82.1%) in node metastases detection. However, [^{68}Ga]Ga-FAPI PET/CT showed significantly higher specificity (96.7% *vs* 74.7%) and accuracy (92.3% *vs* 76.9%) as compared to [^{18}F]FDG PET/CT, especially in the N0 and N1 status of the neck. Therefore, [^{68}Ga]Ga-FAPI PET/CT may be expected to improve the accuracy for preoperative N staging in HNSCC to avoid unnecessary neck damage and possible surgical complications caused by excessive neck level or side node dissection, which may improve the quality of life for HNSCC patients [35]. In general, a poor agreement for N staging between [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT mainly occurred in the inflammatory lymph nodes located in level I and II, whose uptake of [^{18}F]FDG was higher than normal tissue, but negative on [^{68}Ga]Ga-FAPI. In addition, physiological uptake of brown fat was observed in [^{18}F]FDG of one patient (Fig. 3C), which was easily mistaken for metastatic lymph nodes and yielded false positive results. Two patients showed false-negative for small lymph node metastases (length diameter < 5 mm) on both imaging modalities, which may be due to the limited spatial resolution of PET. One patient showed false-positive lymph nodes on [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT, and the pathological results demonstrated tuberculosis. Thus, we should pay attention to differentiating tuberculous lymphadenitis from metastatic lymph nodes, and combing morphologic imaging and a detailed clinical history may help decrease false positivity and negativity.

Regarding distant metastasis, [^{68}Ga]Ga-FAPI PET/CT detected more lesions than [^{18}F]FDG in the two patients with severe disease progression. What's more, the overall uptake of [^{68}Ga]Ga-FAPI of lesions was significantly higher than that of [^{18}F]FDG, especially in bone lesions, which was similar to the results of other studies [18, 24]. In the analysis of local recurrence, [^{68}Ga]Ga-FAPI PET/CT showed the same diagnostic accuracy (5/8) with [^{18}F]FDG. In two NPC patients with post-radical radiotherapy, false positive uptake in the inflammatory sites was observed on both [^{68}Ga]Ga-FAPI and [^{18}F]FDG PET/CT. False-positive [^{68}Ga]Ga-FAPI uptake post-therapy could have occurred because local radiotherapy and surgery may cause local damage to tissues, resulting in localized inflammation, which causes tissue fibrosis [24, 36, 37]. Therefore, images of post-treatment patients should be carefully considered.

Compared with [^{18}F]FDG, [^{68}Ga]Ga-FAPI PET/CT resulted in upstaging in 6 patients and downstaging in 33 patients. Concerning T staging, our study showed that [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT have good agreement (73/77), and the upstaging mainly occurred in tumors with skull base and intracranial invasion, which was in accordance with the results of other studies [20, 24]. Notably, we observed poor agreement (42/77) between [^{18}F]FDG and [^{68}Ga]Ga-FAPI PET/CT in N staging. [^{68}Ga]Ga-FAPI PET-guided N staging seemed to be more accurate compared to [^{18}F]FDG since [^{18}F]FDG tended toward

overestimation [38, 39], which still needs further more investigations. What's more, [^{68}Ga]Ga-FAPI has revised the type of neck dissection in 9 (9/33) patients and led to intended management changes in 10 of 61 patients. The role of [^{68}Ga]Ga-FAPI in cancer management is of concern and warrants further research.

In this study, an additional follow-up [^{68}Ga]Ga-FAPI PET/CT was conducted in three oral cancer patients with neoadjuvant therapy. One patient was in complete remission, and the other two showed progression. Therefore, our results suggested that [^{68}Ga]Ga-FAPI had great potential in monitoring treatment response.

There are some limitations in our study. First, although the total sample size is relatively large compared with the relevant published studies [16, 18, 22], the number of each specific cancer is modest. Second, some patients (48/77) in this cohort did not undergo surgery or biopsy for metastatic lymph node and distant metastasis to confirm the pathology, but the follow-up and other imaging data (such as MRI or CT) were available as a reference. Further studies with more pathological results are required. Finally, our study focused on head and neck squamous cell carcinoma. The next step is to increase the cumulative number of cases for specific cancers.

Conclusion

The results of this prospective clinical study reveal that [^{68}Ga]Ga-FAPI PET/CT has a comparable diagnostic performance with [^{18}F]FDG for detecting primary tumors and local recurrence in patients with HNSCC. We further found that [^{68}Ga]Ga-FAPI PET/CT showed higher specificity and accuracy for preoperative N staging compared to [^{18}F]FDG. The potential of [^{68}Ga]Ga-FAPI imaging in clinical management and evaluation of therapeutic response should be explored in future studies.

Declarations

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Ethics declarations

Ethics approval

All procedures involving human participants were carried out in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any experiments with animals.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Conflict of interest

The authors declare no competing interests.

Authors' contributions

Y.H., J.J. and Y.J. contributed to the conception and design. All authors contributed to patient enrollment. Z.X. contributed to the preparation of [^{68}Ga]Ga-FAPI and quality control. D.X. contributed to image acquisition. C.L., Y.T. and Y.H. contributed to the analysis and interpretation of image. Y.J. and Y.H. contributed to the statistical analysis. Y.J. drafted the initial version of the manuscript. Y.H., J.J., B.W. and

Y.J. revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Availability of data and material

Data generated or analyzed during the study are available from the corresponding author by request.

Code availability

Not applicable.

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Supplementary information

Supplementary information are presented as Supplementary Materials (.doc), attached.

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Figures

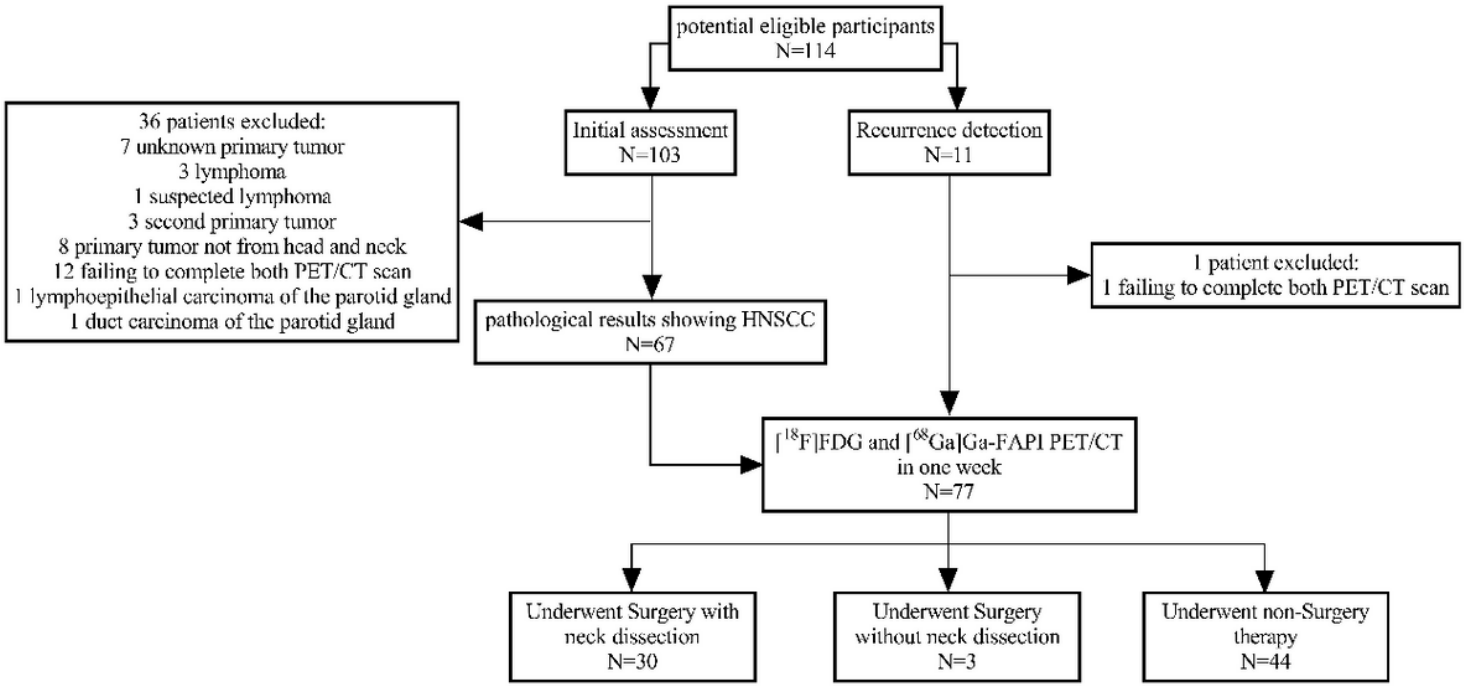

Figure 1

Flow diagram of the study design

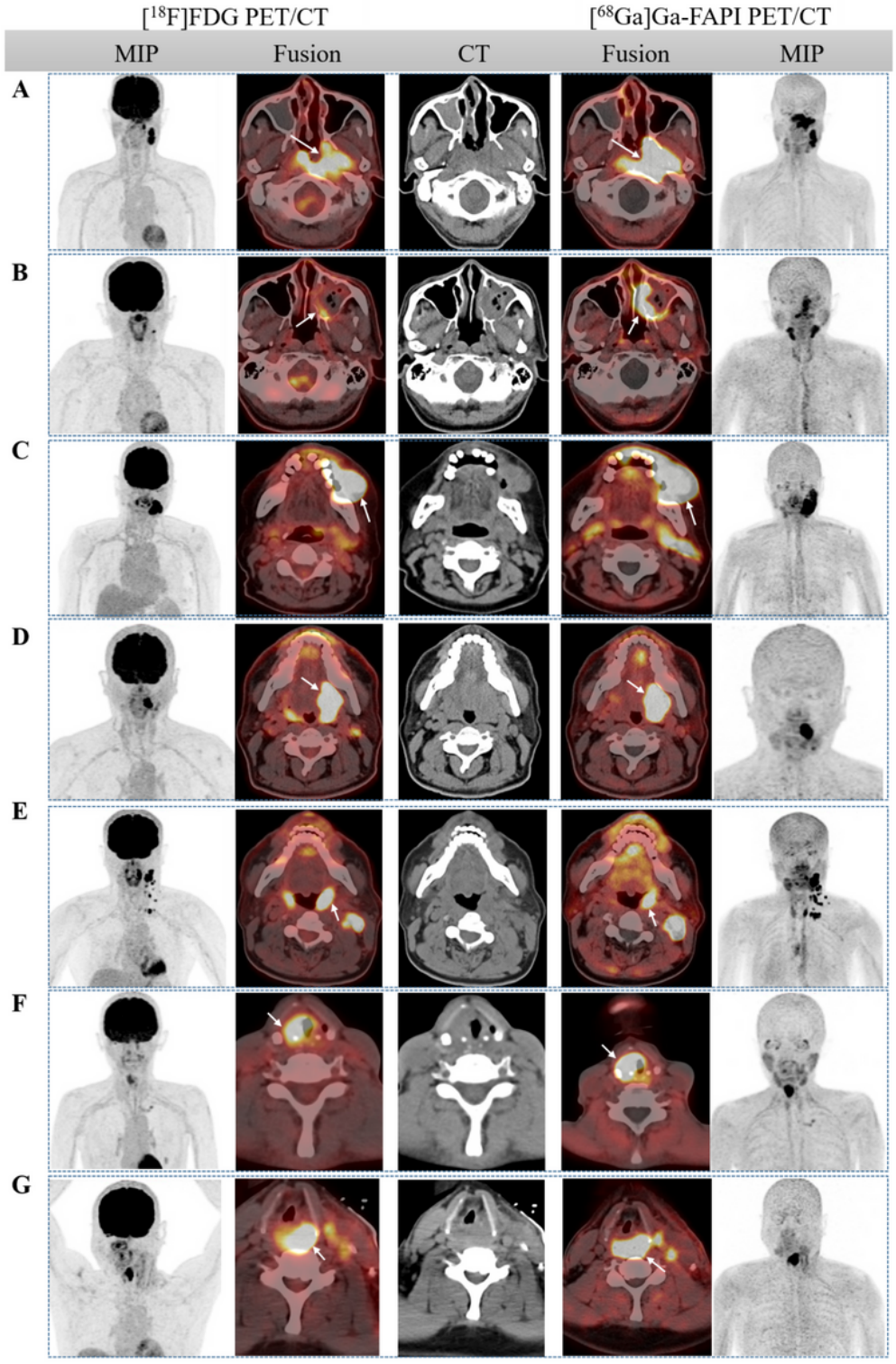

Figure 2

Representative images of primary tumor lesions located in nasopharynx (A), paranasal sinuses (B), oral cavity (C), oropharynx (D and E), larynx (F) and hypopharynx (G). [^{68}Ga]Ga-FAPI is comparable to [^{18}F]FDG in evaluation of primary lesions (similar SUVmax and detection rate). Arrows indicate the location of lesions.

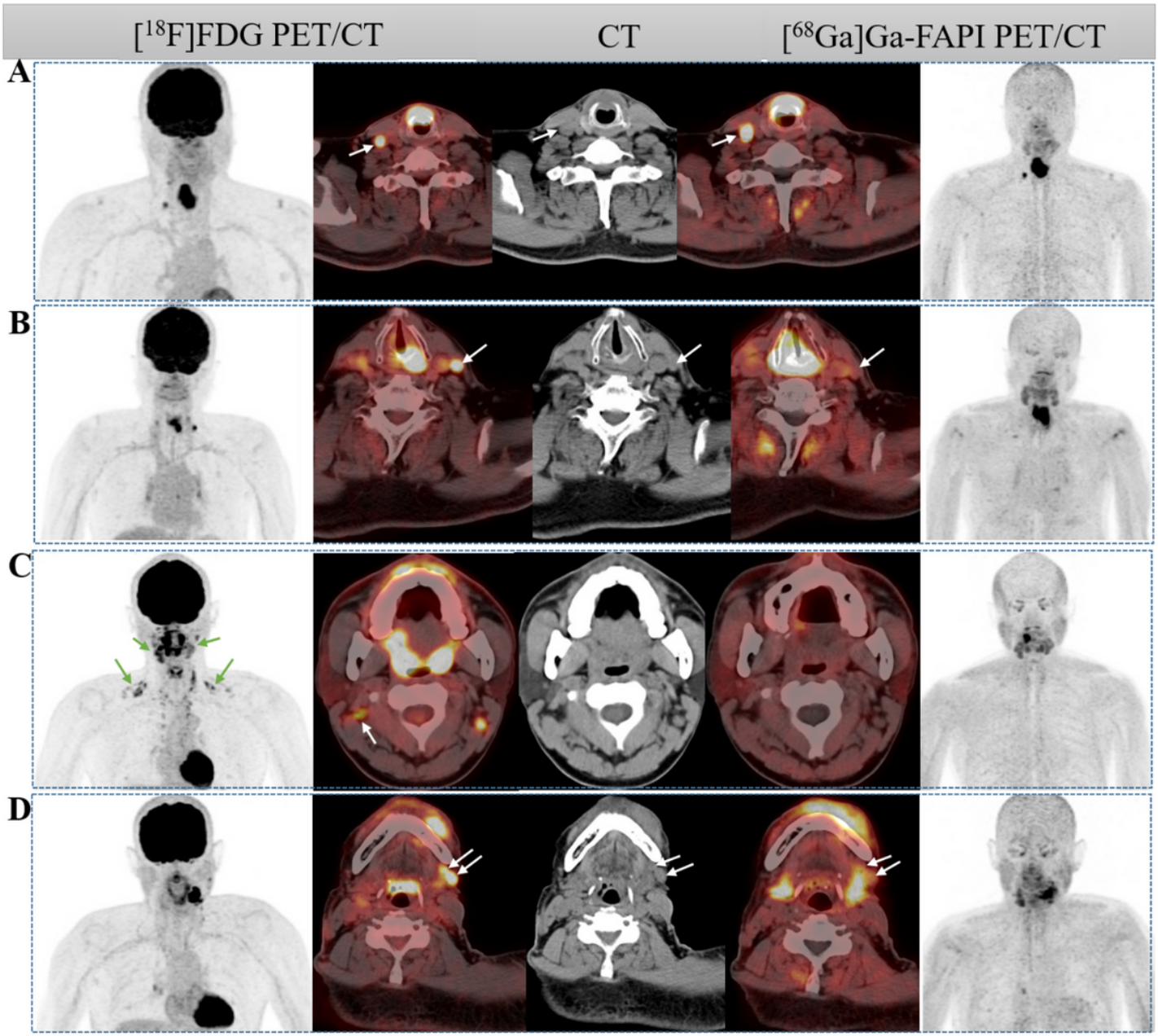

Figure 3

Representative images of cervical lymph nodes. (A) A 57-year-old male patient with laryngeal cancer. Both [^{18}F]FDG and [^{68}Ga]Ga-FAPI showed a visually positive lymph node at the right neck level III (white arrow, SUVmax 7.34 *vs* 12.05, size 1.80 × 1.41 cm). Pathological results confirmed a positive lymph node at the right neck level III (1/13). (B) A 79-year-old male patient with laryngeal carcinoma. [^{18}F]FDG showed visually positive lymph nodes (white arrow, SUVmax 9.96, size 0.80 × 0.55 cm), while [^{68}Ga]Ga-FAPI was visually negative. Pathological results confirmed that there was no lymph node metastasis. (C) A 30-year-old male patient with SCC of the right tongue root. Physiological [^{18}F]FDG uptake (green arrow) was observed in brown fat throughout the head and neck region, which was not seen on [^{68}Ga]Ga-FAPI. (D) A 65-year-old male patient with SCC of the lower left gingiva. Both [^{18}F]FDG and [^{68}Ga]Ga-FAPI showed

visually positive lymph nodes in the left level Ib (white arrows, SUVmax 8.26 *vs* 5.20, size 1.54 × 0.90 cm). Pathological results confirmed tuberculous lymphadenitis.

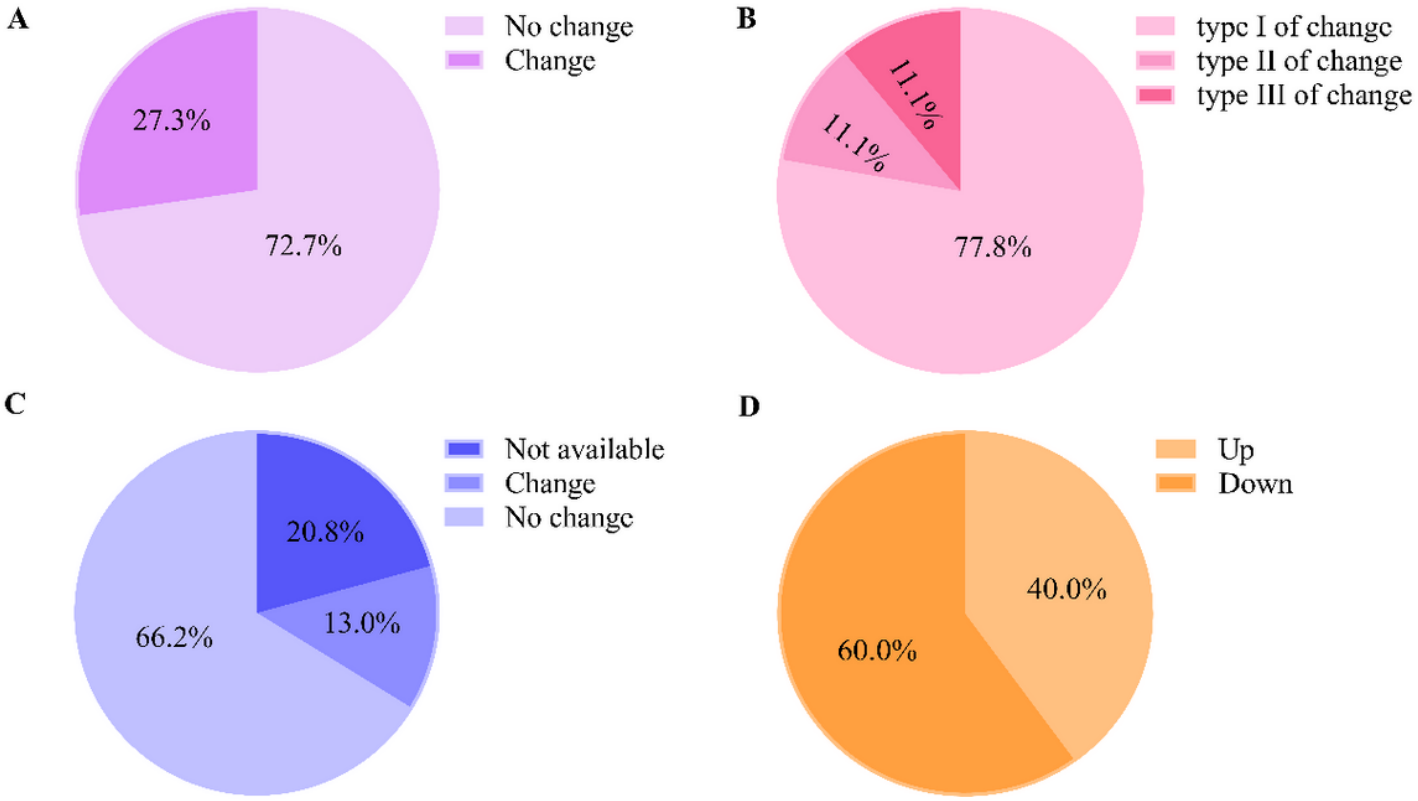

Figure 4

Change in management. Pie chart of implemented management change of surgical type of neck dissection after [^{68}Ga]Ga-FAPI PET for 33 patients underwent surgery (A and B). Types of changes were categorized as type I (Change from bilateral neck node dissection to unilateral neck node dissection), type II (Change from no need of neck node dissection to unilateral neck node dissection) and type III (Change from unilateral neck node dissection to no need of neck node dissection). [^{68}Ga]Ga-FAPI PET resulted in intended management changes in 10 of the total 61 patients (C and D). Up: from intended local therapy to systemic therapy; Down: from intended combination therapy to monotherapy.

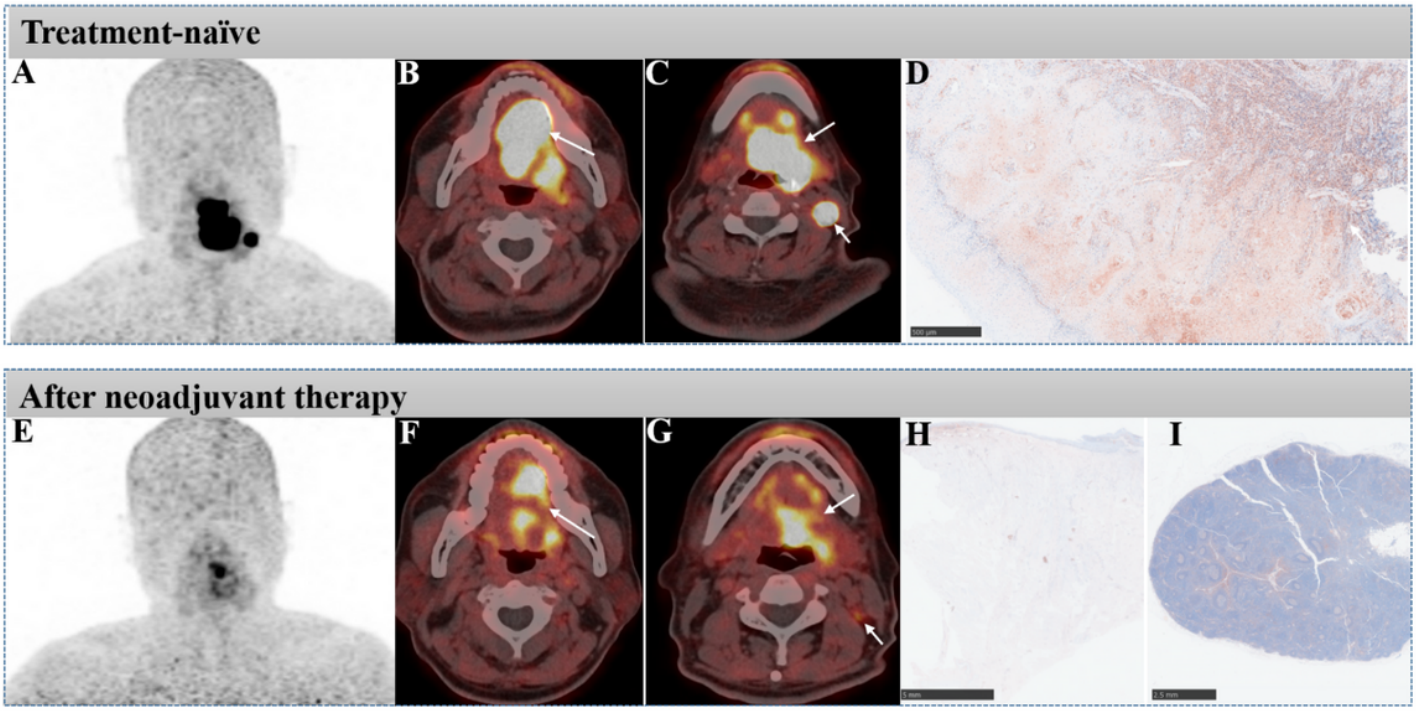

Figure 5

A 57-year-old man with squamous cell carcinoma of the tongue body and root (A~H). He underwent neoadjuvant therapy and had a follow-up [^{68}Ga]Ga-FAPI PET/CT, which showed a sharply decrease of [^{68}Ga]Ga-FAPI uptake in primary tumor and cervical metastatic lymph node (SUVmax 26.23 *vs* 5.97 for primary lesion and SUVmax 16.90 *vs* 2.99 for metastatic lymph node, respectively). His subsequent surgical pathology of primary tumor and neck nodes both showed negative. IHC analysis of FAP in primary tumor tissue at the initial treatment showed positive staining (D), while both primary tumor and lymph node sample presented FAP-negative staining after neoadjuvant therapy (H and I), which were consistent with [^{68}Ga]Ga-FAPI PET/CT images. Arrows indicate the location of lesions.

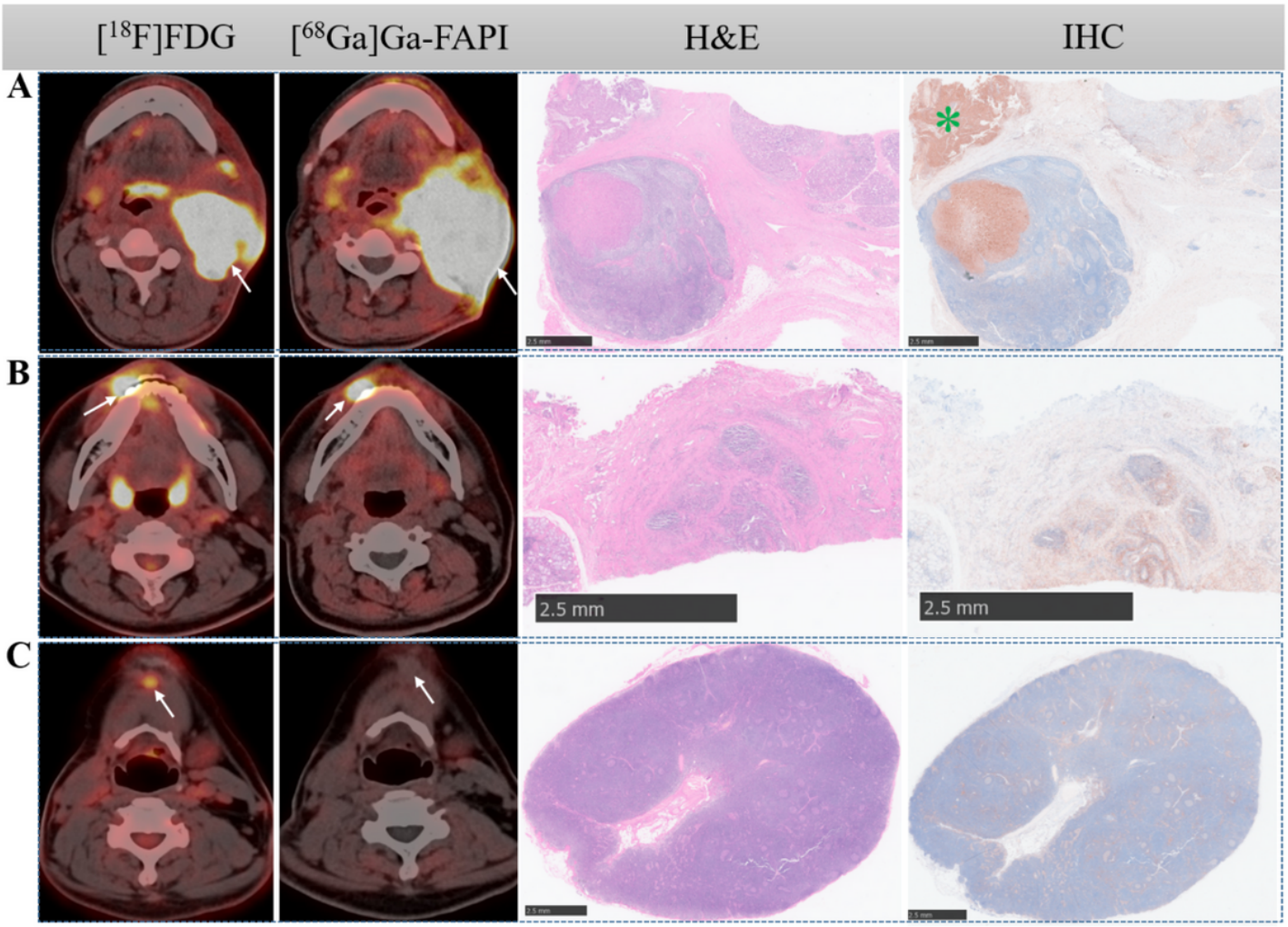

Figure 6

Representative IHC staining of FAP in primary tumors and cervical lymph nodes. (A) A 53-year-old male patient with buccal mucosa carcinoma was found to have developed cervical lymph node metastases at left level I~III. A high uptake by metastatic lymph nodes was observed at both [^{18}F]FDG and [^{68}Ga]Ga-FAPI (SUVmax 47.50 and 29.08, respectively). Correspondingly, IHC revealed strong staining (score +++) for FAP in the metastatic lymph nodes. (B and C) A 34-year-old male patient with tongue SCC was suspected local recurrence and lymph node metastasis after radical surgery. Final surgical pathologic findings showed no cancer in local lesion and cervical lymph nodes. Both [^{18}F]FDG and [^{68}Ga]Ga-FAPI presented positive uptake of local lesion (SUVmax 10.55 and 6.91, respectively), which was consistent with the IHC result (moderate, score ++). Futhermore, the suspected lymph node metastasis was positive at [^{18}F]FDG (SUVmax 3.89) but negative at [^{68}Ga]Ga-FAPI, while IHC showed no or low staining (score -~+). Arrows indicate the location of lesions. (* primary tumor sample, strong staining)

Supplementary Files

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

- Supplementarymaterials.docx