Study protocol

1. Title: Head-to-head comparison of ^{68}Ga-FAPI-46 and ^{18}F-FDG PET/CT for evaluation of head and neck squamous cell carcinoma: a single-center exploratory study
2. Objective: A head-to-head comparison of the performance of ^{68}Ga-FAPI-46 PET/CT and standard ^{18}F-FDG PET/CT imaging for detecting primary cancer and metastatic lesions in patients with head and neck squamous cell carcinoma.
3. Outcome

   3.1 Primary outcome: Concordance of FAPI and FDG PET/CT

   3.2 Secondary outcome: Diagnostic accuracy of FAPI and FDG PET/CT

   3.3 Tertiary outcome: Comparison of semiquantitative parameters
4. Study design: Observational study
5. Study population: Patients with pathologically confirmed head and neck squamous cell carcinoma who were referred for PET scan with indication for initial staging or suspected recurrence.
6. Inclusion criteria

   6.1 Patients with pathologically confirmed head and neck squamous cell carcinoma

   6.2 Patients who were > 18 years old.

   6.3 Patients who were scheduled for PET/CT with indication for initial staging or suspected recurrence.
7. Exclusion criteria

   7.1 Patients with fasting blood sugar > 150 mg/dL

   7.2 Patients who were pregnant or breast feeding.

   7.3 Patients who were unwilling to participate.

8. Study procedure

8.1 Patient was scheduled ^{18}F-FDG PET/CT and ^{68}Ga-FAPI-46 PET/CT within 2 weeks apart

8.2 ^{18}F-FDG PET/CT day

8.2.1 Patient fasted for 6 h prior

8.2.2 Plasma glucose level was determined to ensure it is ≤ 150 mg/dL.

8.2.3 Patient had 2.59 MBq/kg of ^{18}F-FDG intravenous injection, uptake time 60 min

8.2.4 PET/CT acquired from the vertex to the proximal thigh (with arms in the down-position) using a 64-slice Siemens/Biograph vision PET/CT scanner (Siemens Healthcare GmbH, Erlangen, Germany) in the three-dimensional mode. The continuous bed motion method with a speed of 1.6–1.8 mm/s. The matrix size 440 x 440.

8.2.5 Reconstruction methods: True X and Time of Flight (Ultra HD PET).

8.2.6 CT parameters: tube voltage of 120 kV, current of 25 mAs with Siemens CARE Dose technology, and a slice thickness of 3.0 mm.

8.3 ^{68}Ga-FAPI-46 PET/CT day

8.3.1 No specific patient preparation

8.3.2 Patient had 2.0 MBq/kg of ^{68}Ga-FAPI-46 intravenous injection, uptake time 60 min

8.3.3 PET/CT acquisition, reconstruction and CT parameter as ^{18}F-FDG PET/CT

8.4 PET/CT analysis

8.4.1 ^{18}F-FDG and ^{68}Ga-FAPI-46 PET/CT scans were separately interpreted by three board-certified nuclear medicine physicians who were unaware of the clinical data or histopathological results at the time of review.

8.4.2 An area of focal tracer uptake higher than that of the surrounding background was considered positive by visual analysis. The lesion was categorized as a primary tumor, involved lymph node or distant metastasis.

8.4.3 Three-dimensional voxels of interest were drawn around the lesions seen on visual analysis by using threshold of 40% of SUVmax. Semiquantitative analysis including SUVmax, SUVmean, tumor-to-background ratio, metabolic tumor volume, total lesion glycolysis, FAP expression tumor volume, total lesion FAP expression were calculated. Tumor-to-background ratio was determined by dividing SUVmax with SUVmean of the contralateral normal tissue.

8.5 Data analysis

8.5.1 Histopathology served as the gold standard for diagnostic accuracy calculation. For non-biopsied lesions, anatomical abnormality observed on CT or MRI was used as reference.

8.5.2 Anatomical abnormal criteria for non-biopsied lesion as the follow;

8.5.2.1 Nodal metastasis: a cluster of at least 3 size-independent nodes were present at one site or if fewer than 3 lymph nodes were present and at least 1 of them measured ≥ 1 cm along the short axis or spherical form or showed central necrosis.

8.5.2.2 Lung metastasis: solid pulmonary nodules, nodules with a reticulonodular pattern, cavitating nodules, or a lymphangitis carcinomatosis pattern.

8.5.2.3 Bone metastasis: lytic or sclerotic lesions with cortical breakthrough, periosteal reaction, expansile appearance, or pathological fracture observed by CT scan or an abnormal marrow signal intensity observed on MRI.

8.5.2.4 Distant metastasis at another site: a nodule or mass forming lesion

8.5.3 Lesions showing a focal increased tracer uptake beyond the background and corresponding anatomical abnormality criteria were defined as true positives. Patients with negative PET/CT findings were followed up clinically for at least 3 months to confirm a true negative result.

8.5.4 For initial staging, the clinical TNM stage of head and neck squamous cell carcinoma was based on the eighth edition of the American Joint Committee on Cancer staging system.

8.6 Statistical analysis

8.6.1 Concordant rates between ^{18}F-FDG PET/CT and ^{68}Ga-FAPI-46 PET/CT for initial staging and recurrence detection. For initial staging, concordance is the agreement of PET/CT results in all T and N and M staging. For recurrence detection, concordance is the agreement of PET/CT studies in detecting recurrent lesions, either positive or negative results.

8.6.2 Diagnostic accuracy of ^{18}F-FDG PET/CT and ^{68}Ga-FAPI-46 PET/CT defined by sensitivity, specificity, positive predictive value, negative predictive value, and accuracy.

8.6.3 Differences in semiquantitative parameters between ^{18}F-FDG and ^{68}Ga-FAPI-46 PET/CT were analyzed using paired t-tests.

8.6.4 A P value of < 0.05 was considered statistically significant.