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ORIGINAL ARTICLE

Additional value of integrated ^{18}F-choline PET/4D contrast-enhanced CT in the localization of hyperfunctioning parathyroid glands and correlation with molecular profile

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Abstract

Purpose The localization of hyperfunctioning parathyroid gland(s) (HPTG) in patients with primary hyperparathyroidism (PHPT) with negative or inconclusive first-line imaging is a significant challenge. This study aimed to evaluate the role of integrated ^{18}F-choline PET/4D contrast-enhanced computed tomography (4DCeCT) in these patients, compare its detection rate and sensitivity with those of ^{18}F-choline PET/CT and (4DCeCT), and analyse the association between choline metabolism and morphological, biochemical and molecular parameters of HPTG.

Methods We prospectively enrolled 44 PHPT patients with negative or inconclusive first-line imaging. ^{18}F-Choline PET/CT and 4DCeCT were performed at the same time, and integrated ^{18}F-choline PET/4DCeCT images were obtained after coregistration. Experienced physicians examined the images. The SUVratio and degree of contrast enhancement were recorded for each positive finding. Histopathology, laboratory and multidisciplinary follow-up were used as the standard of reference. Both the detection rates and sensitivities of the three imaging modalities were calculated retrospectively. Immunohistochemistry was performed to evaluate the molecular profile of HPTGs.

Results ^{18}F-Choline PET/4DCeCT was positive in 32 of 44 patients with PHPT (detection rate 72.7%), and 31 of 31 surgically treated patients (sensitivity 100%). These results were significantly ($p < 0.05$) better than those of ^{18}F-choline PET/CT (56.8% and 80%, respectively) and those of 4DCeCT (54.5 and 74%, respectively). A significant correlation between SUV and calcium level was found. In a multivariate analysis, only calcium level was significantly associated with ^{18}F-choline PET/4DCeCT findings. SUVratio and Ki67 expression were significantly correlated.

Conclusion Integrated ^{18}F-choline PET/4DCeCT should be considered as an effective tool to detect PHPT in patients with negative or inconclusive first-line imaging. Choline metabolism is correlated with both calcium level and Ki67 expression in HPTG.

Keywords Hyperparathyroidism · ^{18}F-Choline · Elderly · 4DCeCT · Molecular profile

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Introduction

Primary hyperparathyroidism (PHPT) is defined as high serum parathyroid hormone (PTH) levels due to the presence of enlarged hyperfunctioning parathyroid gland(s) (HPTG) [1]. Generally, high serum calcium values are recorded but calcaemia may be normal. Rarely, PTH levels are inappropriately normal with concomitant hypercalcaemia. Notably, PHPT patients can be considered symptomatic when specific signs and symptoms of the disease (e.g. nephrolithiasis and osteoporosis) are present, or asymptomatic when complications are absent. PHPT includes a heterogeneous group of patients. Symptomatic PHPT patients require treatment and traditionally undergo parathyroidectomy which is the only definitive therapy [2], but those who are unfit for surgery are managed with medical therapy. However, surgery is also a relevant option in asymptomatic patients who may develop hypercalcaemia and target organ involvement later in life [2, 3]. Thus, the localization of the HPTG plays a significant role in the management of PHPT patients [2, 3]. First, it allows a tailored surgical approach (i.e. minimally invasive parathyroidectomy) with a consequent significant reduction in complications and the avoidance of a difficult bilateral neck exploration [4]. In addition, old patients with important comorbidities can be referred for medical therapy more confidently when ectopic mediastinal or thoracic HPTG is detected.

Neck ultrasonography (US) and ^{99m}Tc-sestamibi imaging (combined with either SPECT or SPECT/CT) can localize HPTG in the majority of patients and are still the first-line imaging procedures in patients with PHPT [5]. However, the reliability of both US and ^{99m}Tc -sestamibi imaging is limited in specific conditions. US cannot detect HPTG in ectopic sites such as retrotracheal, retrooesophageal and intrathoracic sites [5–7], and coexisting multinodular goitre reduces the accuracy of ^{99m}Tc-sestamibi imaging. In addition, small HPTG may be missed by both tools [5, 8]. In the presence of negative or inconclusive first-line imaging, second-line imaging should be considered, and two methods, four-dimensional computed tomography (4DCeCT) and ^{18}F-choline PET/CT, have been developed and proposed [9–16]. 4DCeCT combines standard multiplanar CT scanning (non-contrast, arterial and venous phases) with the fourth dimension of changes in contrast attenuation over time, providing both functional and anatomical information about the abnormal gland. Indeed, some studies have investigated the role of 4DCeCT and have shown high sensitivity, reaching 86%, in detecting "culprit" adenomas associated with persistent or recurrent PHPT [10–13].

A growing body of evidence indicates that ^{18}F-choline PET/CT is more sensitive than US and ^{99m}Tc-sestamibi imaging [17–20], with sensitivities as high as 85–96% [21–25], even in patients with prior negative/inconclusive first-line imaging [21, 22]. To date, no solid information on the relative value of these two procedures exists [23]. Thus, whether we should use ^{18}F-choline PET/CT rather than 4DCeCT in clinical practice is still unknown. In addition, although hybrid molecular and radiological imaging seems to be promising in this setting [24], no data are yet available on the performance of ^{18}F-choline PET/CT and 4DCeCT in a single integrated diagnostic modality.

Following the reported clinical and technical background, the present study was undertaken to evaluate the use of integrated ^{18}F-choline PET/4DCeCT for detecting HPTG in PHPT patients with negative or inconclusive first-line imaging results as defined by strictly standardized criteria, and to compare its performance in terms of detection rate and sensitivity with that of ^{18}F-choline PET/CT and 4DCeCT. In addition, the association between several morphological, biochemical and molecular parameters and choline metabolism was evaluated. In particular, we examined the molecular profile in each HPTG and assessed correlations between these findings and ^{18}F-choline uptake.

Materials and methods

Patient selection

The study period was between May 2016 and December 2017 and two nuclear medicine departments (Galliera Hospital, Genoa, Italy, and the Oncology Institute of Southern Switzerland, Bellinzona, Switzerland) were involved. Consecutive patients with biochemically proven PHPT according to literature guidelines [3] were initially prospectively enrolled. The study design required that all PHPT patients referred to our centres were examined, assessed for therapy or no therapy, and asked to undergo both neck US and ^{99m}Tc-sestamibi SPECT/CT. According to our study aim, as the main inclusion criterion, only those PHPT patients with negative/inconclusive first-line imaging were included in the study series and these patients were offered second-line imaging using ^{18}F-choline PET/CT and 4DCeCT. Patients who refused the study were excluded. Figure 1 illustrates the study design. Our institutional review board approved the study and patients of the final study series gave their informed consent.

First-line imaging procedures

All patients underwent US and ^{99m}Tc-sestamibi SPECT/CT on the same day. Images were acquired according to standard procedures. First, all patients were examined using neck US. The US parameters evaluated included lesion diameter, structure (i.e. solid or mixed), echogenicity (hypoechoic, hyperechoic, isoechoic), and the presence of microcalcifications. Using power Doppler US analysis, nodule blood flow was classified as positive if there was evidence of intralesional blood flow or negative if blood flow was peripheral or absent. ^{99m}Tc-sestamibi

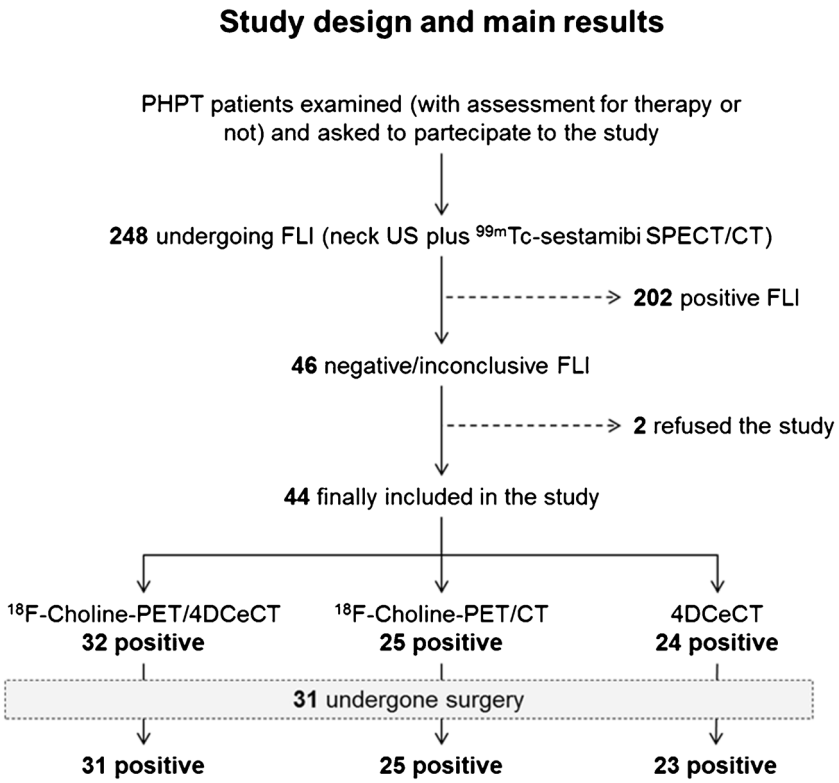

Fig. 1 Study design and main results (*FLI* first-line imaging)

scans were obtained 20 and 120 min after injection of 740 MBq of ^{99m}Tc-MIBI. All images were obtained in the anterior projection of the neck using a gamma camera equipped with a high-resolution parallel-hole low-energy high-resolution collimator. The images were obtained in a 256 × 256 matrix using a digital zoom of 1.8. The acquisition time was set to 900 s with a 20% window centred at 140 keV in all scans. All patients underwent a dual-isotope ^{99m}Tc-sestamibi/^{99m}Tc pertechnetate subtraction protocol in addition to the dual-phase protocol described above, after a subsequent injection of 111 MBq of ^{99m}Tc pertechnetate. A supplementary SPECT/CT acquisition was performed in all patients 2 h after the ^{99m}Tc-MIBI injection. The SPECT/CT acquisition comprised 64 projections of 35 s each. A low-dose CT scan (120 kV, effective 50 mAs) was performed for anatomical correlation only.

Second-line imaging procedures

All patients underwent ^{18}F-choline PET/CT and 4DCeCT, which were acquired at the same time using a PET/CT-scanner within 30 days of first-line imaging. The scans were acquired with the patients in the supine position with their arms along the body. Patients were required to fast for at least 6 h before the ^{18}F-choline PET/CT scan. ^{18}F-Choline was administered intravenously (administered activity 100 MBq). and data were acquired in 3D mode 10 min after injection using the PET/CT system discussed above. The PET scan was acquired from the upper neck to the abdomen with 3 min per bed position and sequential fields of view each covering 12 cm (matrix of 256 × 256). A low-dose CT scan was acquired for both attenuation correction and topographic localization. The CT parameters used were 140 kV, 80 mA, 0.5 s per rotation and pitch 6:1, with a slice thickness of 3.25 mm.

Dynamic 4DCeCT scanning was performed on the 16 detector-row CT scanner of the PET/CT tomograph from the angle of the mandible to the carina. Three phases were acquired: noncontrast, arterial, and venous phases. Iodinated contrast medium (80–100 ml of iohexol, 350 mg iodine/ml at 3.3–4 ml/s, followed by a 30-ml saline chaser) was injected into an antecubital vein (when possible of the right arm). Arterial phase images were acquired 10/15 s after enhancement of the thoracic arch (100 HU threshold), and venous phase images were acquired 50 s after the end of the arterial phase. The CT parameters of the arterial and venous phases were: 120 kV, 80–300 mA (using tube current modulation with a noise index of 11), 0.5 s rotation time, pitch 1,375:1, and a slice thickness of 5 mm. Arterial and venous phases were reconstructed at a slice thickness of 1.25 mm for reformatting in the axial, coronal and sagittal planes.

Integrated ^{18}F-choline PET/4DCeCT images were automatically obtained by coregistration and fusion of the 4DCeCT and PET images using a commercially available image registration software tool (Xeleris, GE Healthcare).

Image interpretation

Neck US and ^{99m}Tc-sestamibi SPECT/CT were performed during the same session and the images were compared and interpreted simultaneously by two double board-certified endocrinologist/nuclear medicine physicians (M.C., L.G.) with more than 25 years of experience in the field. In the event of discordant findings, the procedure provided a face-to-face comparison to reach a consensus. Interpretation criteria are summarized in Table 1. Notably, first-line imaging was classified as negative if the results of both procedures were negative, and inconclusive if the result of one of the two procedures was negative and the second inconclusive (defined as a doubtful and not readily interpretable finding). In particular, ^{99m}Tc-sestamibi SPECT/CT was classified as inconclusive if faint uptake not corresponding to any cervical or thoracic lesion discriminable from thyroid tissue was present.

^{18}F-Choline PET/CT studies were interpreted visually and semiquantitatively by an expert nuclear medicine physician (G.B.) in terms of SUVmax and SUVratio (ratio of lesional SUVmax to neck background SUVmax) using dedicated software to review fused PET/CT images. On ^{18}F-choline PET/CT, any focal nonphysiological uptake corresponding to any cervical or thoracic lesion discriminable from thyroid tissue and positioned in typical parathyroid sites or in ectopic areas that were not typical lymph node sites was considered as positive.

4DCeCT studies were interpreted by an expert radiologist (F.P.) unaware of the ^{18}F-choline PET/CT data. Any cervical or thoracic lesion discriminable from thyroid tissue characterized by any enhancement after injection of contrast medium (i.e. faint or intense enhancement) and positioned in typical parathyroid sites or in ectopic areas that were not typical lymph-node sites was considered as positive. Each lesion considered as positive on 4DCeCT was further classified according to the intensity and time course of contrast enhancement: *score 0* lesions with contrast intensity lower than that of normal adjacent thyroid tissue with slow wash-out; *score 1* lesions with contrast intensity equal to or slightly higher than that of thyroid tissue with slow wash-out; *score 2* lesions with contrast intensity significantly higher than that of thyroid tissue with slow wash-out; *score 3* lesions with contrast intensity significantly higher than that of thyroid tissue with rapid wash-out.

In the same manner, ^{18}F-choline PET/4DCeCT images were interpreted by one expert nuclear medicine physician (A.P.) and one radiologist (L.B.). In this case, any focal nonphysiological uptake corresponding to any cervical or thoracic lesion characterized or not by enhancement after injection of contrast medium discriminable from thyroid tissue and positioned in typical parathyroid sites or in ectopic areas that were not typical lymph-node sites was considered as positive.

Histology and molecular profile

The surgically removed parathyroid glands were fixed in formalin and submitted for histological evaluation. A pathologist examined the samples and recorded the major diameter. The samples were then reduced and embedded in paraffin wax. Paraffin sections of thickness 5 μm were stained with haematoxylin/eosin for microscopic evaluation to establish the diagnosis of parathyroid adenoma (PTA) according to the WHO Classification of Tumours of Endocrine Organs [26] and to rule out malignancy. Where appropriate, paired sections were treated for immunohistochemical analysis with anti-PTH antibody (clone MRQ31, Cell Marque) to confirm the parathyroid origin of the lesions. After histological diagnosis, adjunctive immunohistochemistry was performed in patients surgically treated at Galliera Hospital, Genoa, Italy. This study included antibodies to parafibromin (clone 2H1, Santa Cruz), PGP 9.5 (polyclonal, Cell Marque), galectin 3 (clone 9C4, Cell Marque) Ki-67 Mib 1 (clone 30-9, Ventana), p53 (clone DO-7, Ventana), BCL1 (clone SP4-R, Ventana) and BCL2 (clone124, Ventana). Immunohistochemistry was performed using an automated instrument (BenchMark

Table 1 Interpretation of first-line imaging provided by the combination of neck US and parathyroid scan

Neck US	Parathyroid scan	Imaging interpretation
Positive	Positive	Positive
	Inconclusive	Positive
	Negative	Positive
Inconclusive	Positive	Positive
	Inconclusive	Positive (if concordant)
		Negative (if discordant or suspected for thyroid lesion)
	Negative	Inconclusive
Negative	Positive	Positive
	Inconclusive	Inconclusive
	Negative	Negative

Only PHPT patients with inconclusive/negative first-line imaging (grey cells) were included in the study

ULTRA, Ventana). Staining was considered positive when more than 10% of the lesional cells showed the presence of reaction product. Ki-67 and p53 reactions are reported as the percentage of adenomatous cells with positive nuclear staining.

Standard of reference

Histological examination after surgery was used as the gold standard in this study to calculate the sensitivity of the imaging procedures. Diagnostic sensitivity was calculated as: number of patients with positive findings on the imaging procedure/number with histologically proven PHPT. Detection rates were calculated as: number of patients with positive findings on the imaging procedure/total number of patients with biochemically proven PHPT. The combination of histological findings and biochemical normalization after surgery was considered as the gold standard. In addition, high persistent PTH and calcium levels over time (6 to 12 months after PET/CT) was considered as proof of the presence of PHPT in patients not treated with surgery.

Statistical analysis

The main descriptive statistics were absolute and relative frequencies, mean, standard deviation, median, minimum, maximum and interquartile range. Fisher's exact test was used to compare independent categorical factors and McNemar's exact test was used to compare detection rates among the detection methods in the same subjects. Spearman's correlation coefficient (rho) was used to evaluate the correlations between semiquantitative PET parameters (SUVmax, SUVratio) and contrast enhancement parameters, and all other demographic and clinical, pathological and laboratory parameters. Logistic regression analysis was used to evaluate the associations between positivity on ^{18}F-choline PET/4DCeCT and levels of PTH and calcium, adjusting for age and sex. All analyses were conducted using STATA (version 14.2, StataCorp.; College Station, TX, USA). Two-tailed probabilities are reported, and p values less than 0.05 were considered statistically significant.

Results

According to the study design a series of 44 PHPT patients were finally enrolled (32 patients at Galliera Hospital, Genoa, Italy, and 12 at the Oncology Institute of Southern Switzerland, Bellinzona, Switzerland). Of these patients, 25 were positive on ^{18}F-choline PET/CT, 24 were positive on 4DCeCT and 32 were positive on integrated ^{18}F-choline PET/4DCeCT. Seven patients showed discordant findings between ^{18}F-choline PET/CT and 4DCeCT (four positive on ^{18}F-choline PET/CT and negative on 4DCeCT; three with the opposite pattern). The main clinical, laboratory and imaging characteristics of the patients are presented in Table 2.

Minimally invasive parathyroidectomy was performed in 31 of 32 patients with positive results on second-line imaging (^{18}F-choline PET/CT, 4DCeCT, and integrated ^{18}F-choline PET/4DCeCT). A single PTA was found in all patients, and all patients showed normalization of serum PTH and calcium levels. In contrast, 13 nonsurgically treated patients (12 with negative second-line imaging and 1 with positive second-line imaging) showed persistent PHPT over the follow-up period of 6 to 12 months. A detailed description of the multidisciplinary standard of reference considered for each patient is provided in Fig. 2.

In the overall series, the detection rate of integrated ^{18}F-choline PET/4DCeCT was 72.7%, significantly higher than those of ^{18}F-choline PET/CT (56.8%) and 4DCeCT (54.5%; Table 3). In patients with histopathological confirmation, the sensitivities of integrated ^{18}F-choline PET/4DCeCT, ^{18}F-choline PET/CT and 4DCeCT were 100%, 80% and 74%, respectively (Table 3). Among the 32 patients with positive second-line imaging, one patient with intrathoracic adenoma was positive on integrated ^{18}F-choline PET/4DCeCT and 4DCeCT and negative on ^{18}F-choline PET/CT; he was not treated surgically due to comorbidities. ^{18}F-Choline PET/CT was also false-negative in six patients with a very small PTA close to the thyroid gland. The PTAs in all these six patients were characterized by faint uptake similar to that of the thyroid, making discrimination between thyroid and parathyroid tissue particularly difficult. In this setting, integrated ^{18}F-choline PET/4DCeCT allowed the PTA to be distinguished from thyroid gland as a result of parathyroid tissue enhancement

Table 2 Characteristics of the final series of 44 enrolled patients

Characteristic	Value
Age (years), median (min–max)	65.5 (45–85)
Sex, *n* (%)	
Male	9 (20)
Female	35 (80)
PTH level (pg/ml), median (min–max)[a]	120.7 (71.8–545)
Calcium level (mg/dl), median (min–max)[b]	10.7 (9–13.4)
Creatinine (mg/dl), median (min–max)[c]	0.8 (0.5–0.9)
Neck US result, *n* (%)	
Inconclusive	7 (18)
Negative	37 (82)
Parathyroid scan result, *n* (%)	
Inconclusive	8 (16)
Negative	36 (84)

[a] Normal range 15–65 pg/ml

[b] Normal range 8.2–10.2 mg/dl

[b] Normal range 0.5–0.9 mg/dl

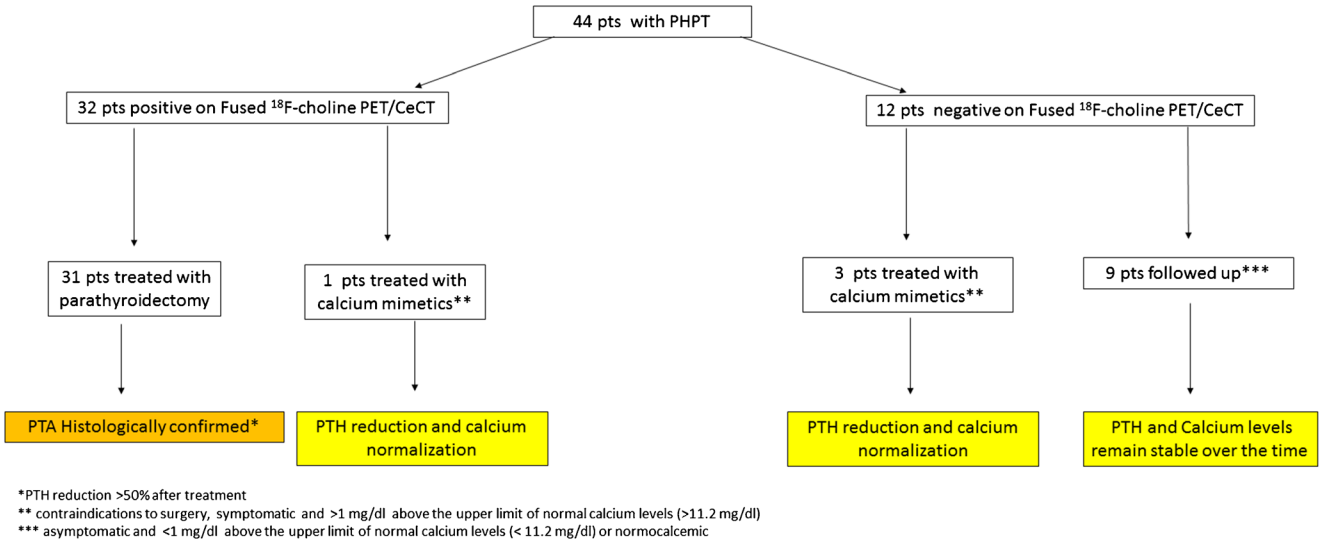

Fig. 2 Concordance between ^{18}F-choline PET/4DCeCT results and the multidisciplinary standard of reference (combination of histological findings and biochemical normalization after surgery) for each patient. In particular, histopathological confirmation (orange box) was available in 31 out of the 44 patients with evidence of PHPT

(Fig. 3). On the other hand, patients negative on 4DCeCT included eight patients with a small PTA characterized by very low or absent contrast enhancement (Fig. 4).

A positive correlation was found between choline metabolism (SUVratio) and PTA volume (Spearman's rho = 0.55, p = 0.001). No correlation was found between PTA volume, PTH or calcium level (data not shown). No positive correlation was found between SUVratio and the intensity of contrast enhancement (Fig. 5) or PTH level (Spearman's rho = 0.19, p = 0.3). On the other hand, a low but significant direct correlation was found between SUV parameters and calcium level (Spearman's rho = 0.38, p = 0.03). In the logistic regression analysis, only calcium level was significantly associated with ^{18}F-choline PET/CeCT-positive findings (Table 4).

In all 17 patients with PTA whose molecular profile was analysed, galectin 3 was negative. On the other hand, none showed loss of parafibromin (the rate was 100% in all patients). The chromogranin index was ≥70% in all patients and ≥90% in 13. The Ki67 index was ≤4% in all except one patient and the p53 positivity rate was 1% in all except five patients in whom the rate was 10%. PGP 9.5 was expressed in five patients and in all positive patients the rate was ≤40%. BCL1 and BCL2 were positive in all patients and the rates were ≥80% in eight and three patients, respectively.

In the 17 patients with PTA whose molecular profile was analysed, analysis of the correlation between choline metabolism of PTA and the molecular profile showed a significant correlation between SUVratio and Ki67 expression (Spearman's rho = 0.67, p = 0.005). On the other hand, an inverse correlation was found between SUV parameters and p53 expression (Spearman's rho = −0.52, p = 0.04). No other correlations were found between SUVratio and the other molecular parameters studied.

Discussion

We evaluated the performance of ^{18}F-choline PET/CT, 4DCeCT, and integrated ^{18}F-choline PET/4DCeCT in the localization of HPTG in PHPT patients with prior negative or inconclusive first-line imaging. The main findings were that ^{18}F-choline PET/CT and 4DCeCT had quite similar detection rates (57% and 55%, respectively) and sensitivities (81% and 74%, respectively), while integrated ^{18}F-choline PET/4DCeCT showed higher reliability (73% detection rate and 100% sensitivity). Our data extend the information from a previously reported case series [23], and suggest for the first time that integrated ^{18}F-choline PET/4DCeCT should be introduced as second-line imaging in these patients.

Several studies on the use of ^{18}F-choline PET/CT in this context have been published. Among these, only a limited number included patients with prior negative/inconclusive

Table 3 Detection rate and sensitivities of each diagnostic modality

	^{18}F-Choline PET/4DCeCT	^{18}F-Choline PET/CT	p value[a]	4DCeCT	p value[a]
Detection rate	32/44 (72.7%)	25/44 (56.8%)	0.039	24/44 (54.5%)	0.02
Sensitivity	31/31 (100%)	25/31 (81%)	0.03	23/31 (74%)	0.0087

[a] McNemar test, vs. ^{18}F-choline PET/4DCeCT

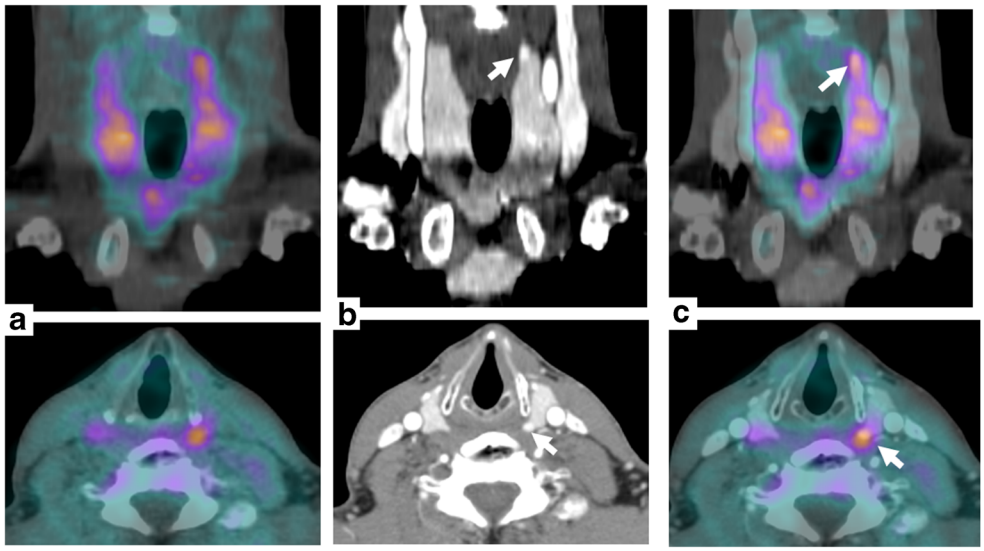

Fig. 3 A 63-year-old male patient with a large multinodular goitre and biochemical evidence of PHPT. **a** Coronal and axial ^{18}F-choline PET/CT images show inhomogeneous distribution of the tracer in the thyroid gland. No images showed suspicion of HPTG. **b** Coronal and axial 4DCeCT images (arterial phase), after injection of contrast medium, show a small lesion suspicious for PTA in the superior left position (*white arrows*). **c** Coronal and axial fused ^{18}F-choline PET/4DCeCT images show a perfect match between faint but focal choline uptake and the enhanced lesion in the superior left position (*white arrows*). Two months later the patient underwent surgery and a PTA in the superior left position was confirmed

first-line imaging findings, and of these, two [21, 22] showed very high sensitivity and detection rate (90% and 85%, and 76% and 81%, respectively). Our findings diverge from the latter and a brief discussion is warranted. First, one third of patients included in one of the previous studies [22] did not undergo SPECT during parathyroid scan or a dual tracer protocol, which indicates a high number of negative findings on first-line imaging and the introduction of a selection bias [8, 27]. This is confirmed by the very high rate of inconclusive or discordant first-line imaging results recorded (i.e. 85% and 44%, respectively). Second, in the other study [21], inconclusive findings (i.e. faint choline uptake without any CT corresponding finding) were considered as positive. If these latter findings were considered negative, the ^{18}F-choline PET/CT sensitivity would be quite similar to that found in our study (i.e. 76%). Third, in the study by Quak et al. [21], local or external radiologists performing neck US were unaware of the parathyroid scan findings and written US reports indicating no suspicious parathyroid enlargement were considered as negative on US. This approach could have significantly increased the number of false-negative US findings [28]. In this study, we performed first-line imaging in the same diagnostic session and the results were interpreted by the same panel of physicians who were aware of the neck US or ^{99m}Tc-sestamibi SPECT/CT findings. This protocol could have considerably reduced the false-negative rate of first-line imaging. In confirmation of this, only 15 patients (34%) had inconclusive first-line imaging results, including those with really doubtful and nonconcordant findings (Table 1).

To the best of our knowledge, this is the first study analysing the relationship between choline uptake and the rate of contrast enhancement after injection of contrast medium.

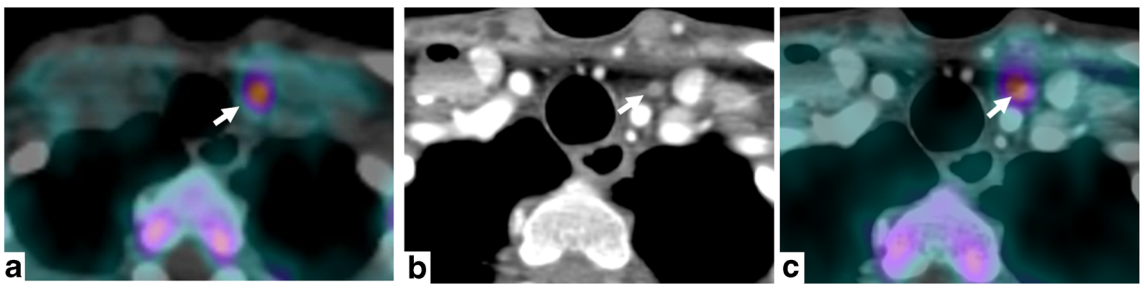

Fig. 4 A 57-year-old female patient with biochemical evidence of PHPT. **a** Axial ^{18}F-choline PET/CT images show focal uptake corresponding to a small lesion between the left common carotid artery and the ipsilateral subclavian artery (*white arrow*). **b** On the axial 4DCeCT image (arterial phase) the lesion does not show contrast enhancement (*white arrow*). **c** Axial fused ^{18}F-choline PET/4DCeCT image shows a perfect match between the focal choline uptake and the non-enhancing lesion (*white arrow*). Three months later the patient underwent surgery and an upper mediastinal PTA was confirmed

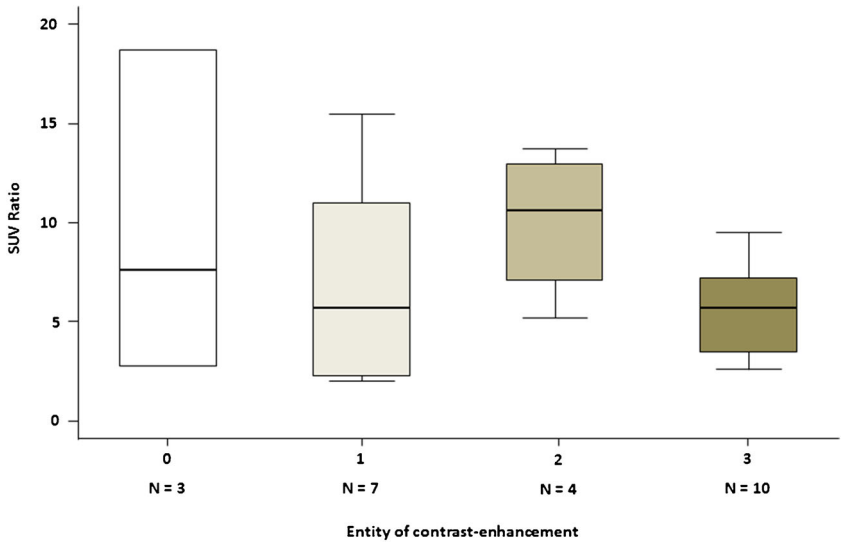

Fig. 5 Relationship between SUVratio and contrast enhancement intensity score

No correlation between parathyroid vascularization and choline metabolism was found. Among the 24 patients positive on 4DCeCT, in six of ten patients with a 4DCeCT intensity score of 3, the SUVratio was less than 6. On the other hand in three of five patients with a 4DCeCT intensity score of 0, the SUVratio was more than 7. These results indirectly support the use of the integrated procedure.

Little information is available on the relationship between choline uptake and PTA size, PTH levels and calcium values [29]. We found a low correlation with PTA dimensions, in agreement with what is known for MIBI uptake [30, 31]. SUVratio was correlated with calcium level. In contrast with the results of previous studies [18, 20], no correlation was found between SUVratio and PTH. Interestingly, calcium was the only parameter associated with positive findings on integrated ^{18}F-choline PET/4DCeCT in the multivariate analysis. This finding is of clinical interest since high calcium is a major factor in selecting patients for surgery [3]. In this setting, some studies have shown higher mean calcium and PTH serum levels in patients with positive choline PET/CT findings than in patients with negative findings, without a significant difference among the two groups [25, 32]. Overall, discordant findings have been reported on the possible correlation between radiolabelled choline uptake and PTH and calcium levels in PHPT patients [18, 20, 29, 33].

We also investigated the correlation between choline uptake and molecular profile in patients with HPTG. In particular, we analysed the relationship between SUVratio and expression of several gene products generally used to discriminate normal parathyroid tissue, adenoma/hyperplasia, and carcinoma. Because none of our patients had carcinoma, we evaluated the metabolism of choline in patients with PTA in relation to the expression of several genes and their related proteins able to regulate the cell cycle (particularly cell proliferation) and apoptosis. A direct correlation between SUVratio and Ki-67 expression and an inverse correlation between choline uptake parameters and p53 expression were found. Also, as found in a previous study on the use of ^{99m}Tc-sestamibi for imaging HPTG [34], the expression of Ki67 was relatively low in all histologically proven PTA. We speculate that intense uptake on ^{18}F-choline PET/CT identifies lesions with higher proliferative index that are likely to become clinically relevant over time.

The strengths and limitations of the present study deserve discussion. We assessed the reliability of the imaging techniques in a highly selected series of patients with PHPT. We evaluated their performance in second-line imaging in two different settings. We calculated the sensitivities as the true-positive rates on second-line imaging in surgical patients and calculated the detection rates as the number of positive second-line imaging results in a pretreatment setting regardless of postoperative confirmation. In the first case patients who were not surgically treated were excluded, introducing a selection bias. To account for this bias, we used clinical follow-up as a surrogate reference in patients refusing bilateral neck exploration (see Fig. 2). Conversely, analysing the

Table 4 Logistic regression model (dependent variable: positivity on fused ^{18}F-choline PET/4DCeCT)

	Odds ratio[a]	95% confidence interval	*p* value
Gender			
Male	1.0		
Female	0.14	0.01–1.68	0.1
Age (years)	0.98	0.90–1.07	0.7
Calcium level (mg/d)l	3.36	1.07–10.5	0.04
PTH level (pg/ml)	0.99	0.98–1.01	0.7

[a] For continuous predictors, odds ratio is an estimate of the risk of positivity for 1 unit increase in the factor considered

detection rate of each single imaging modality, potential selection bias (when only surgical patients were considered) and performance bias (when the physician and/or patient interfere with the decision on cure) were avoided.

Conclusion

The results of this study support the use of second-line imaging to localize PTA in patients with PHPT and previous negative conventional evaluation. The novel result of this study is that integrated ^{18}F-choline PET/4DCeCT was positive in three quarters of patients and had 100% sensitivity on histological follow-up. Since its performance was superior to that of ^{18}F-choline PET/CT and 4DCeCT, ^{18}F-choline PET/4DCeCT should be considered as the most effective second-line imaging modality in these patients. Also, choline metabolism was found to be correlated with both calcium levels and Ki-67 expression in PTAs.

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Compliance with ethical standards

Conflicts of interest None.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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

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