CLINICAL STUDY

Thyroid diseases cause mismatch between MIBI scan and neck ultrasound in the diagnosis of hyperfunctioning parathyroids: usefulness of FNA–PTH assay

Francesco Boi, Cira Lombardo, Maria Chiara Cocco, Mario Piga¹, Alessandra Serra¹, Maria Letizia Lai², Pietro Giorgio Calò³, Angelo Nicolosi³ and Stefano Mariotti

Endocrinology and ¹Nuclear Medicine, Department of Medical Sciences 'M. Aresu', University of Cagliari and A.O.U. Presidio di Monserrato, SS 554, I-09042 Monserrato-Cagliari, Italy, ²Department of Cytomorphology and ³Department of Surgery, University of Cagliari, Monserrato, Cagliari, Italy
(Correspondence should be addressed to F Boi; Email: francesco.boi@tin.it)

Abstract

Design: To evaluate the efficacy of the main tools in the diagnostic localization of hyperfunctioning parathyroids (HP) in primary hyperparathyroidism (pHPT) with concomitant thyroid diseases.

Methods: Forty-three patients with pHPT associated with nodular goiter (NG, $n=32$) and/or autoimmune thyroid diseases (AITDs, $n=11$) for a total of 63 neck lesions were considered. Sixteen patients displaying HP (16 lesions), unequivocally localized by sestaMIBI scintigraphy (MIBI) and neck ultrasound (US) (group I), were compared with 27 patients (47 neck lesions) displaying equivocal parathyroid localization (group II). In all cases, neck US, MIBI scan, cytology, and parathyroid hormone assay in fine-needle aspiration washout fluid (FNA–PTH) were performed. All patients finally underwent surgery.

Results: According to histological examination, high FNA–PTH values (>103 pg/ml) correctly identified all HP in both groups of patients (100% of sensitivity and specificity). Both MIBI and US correctly identified all HP only in group I patients; in contrast, four patterns of mismatch between these techniques were observed in group II patients, leading to low diagnostic performances of neck US (71.4% sensitivity and 78.9% specificity) and of MIBI scan (35.7% sensitivity and 42.1% specificity). The latter was due to both false-negative (mainly in AITD) and false-positive (mainly in NG) scan images.

Conclusions: Coexistent thyroid diseases are responsible for mismatch between MIBI and US images resulting in equivocal HP localization. In these cases, FNA–PTH resulted in the most accurate tool to identify HP. However, although safe, it should be advised only to patients with uncertain HP localization.

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Introduction

Primary hyperparathyroidism (pHPT) is an endocrine disease mainly caused by single or multiple hyperfunctioning parathyroid (HP) lesions (adenomas, hyperplasia, or rarely carcinomas) (1, 2). The identification of HP is an important step to plan surgery, which represents the definitive therapeutic approach of pHPT (3). Although bilateral neck exploration (BNE) performed by an experienced surgeon may allow the intraoperative identification of HP (4), in some cases, such as in the presence of concomitant thyroid nodular disease, this approach may fail. For this reason, preoperative localization studies have been regarded as necessary but their use is still controversial (5). Some authors suggest that these studies may reduce surgical time, technical failures, complication rates (6), improving

pHPT operation by minimally invasive parathyroidectomy (MIP) (7), and radioguided (8, 9) surgery. Several imaging techniques such as neck ultrasound (US) (10, 11), computerized tomography (CT) (12), and dual-phase ^{99m}technetium sestaMIBI (MIBI) scan associated with single-photon emission CT (SPECT)–CT are currently available (13), although neck US and MIBI scan are generally used as first tools in the diagnostic approach of pHPT (14).

MIBI is a lipophilic monovalent cation showing increased uptake in epithelial cells rich in mitochondria such as HP cells (15, 16). MIBI scan is able to localize HP in pHPT patients with high sensitivity (71–93%) and specificity (90%) and, for this reason, it has been used as the technique of choice to guide MIP (17). However, small dimension of HP, oxyphil cell content, and various degree of apoptosis or necrosis may reduce

MIBI uptake causing false-negative results (18, 19). On the other hand, MIBI retention is not parathyroid specific and is also observed in hypermetabolic thyroid nodules (TNs) such as oncocytic tumors (20) and autonomous thyroid adenomas (TA) (20, 21). Neck US is the least invasive and cheapest method (11, 22) to accurately localize HP, but its efficacy is related to the operator experience and may be reduced in the presence of concomitant nodular goiter (NG) (23, 24). No studies on the potential interference of autoimmune thyroid disease (AITD), such as Hashimoto's thyroiditis (HT) and Graves' disease (GD) in HP localization by MIBI scan and neck US, have been so far performed. Finally, other potential conditions leading to uncertain HP localization by these techniques are represented by ectopic parathyroid location, multiglandular HP hyperplasia, and exceptionally lymphadenopathies (20, 24, 25).

The association between TNs with pHPT occurs frequently in iodine-deficient areas (Italy or Central Europe) where the incidence of NG varies from 20 to 60%, and this has been reported to reduce the diagnostic performance of MIBI scan to ~60–70%. In such a condition, the combined use of MIBI scan and neck US may also improve the diagnostic efficacy, but a 15–20% of inaccurate localization (20, 26, 27) still remains. In these last years, the assay of intact parathyroid hormone in washout fluid from fine-needle aspirate (FNA–PTH) has been proposed as a further diagnostic tool to improve the detection of HP in patients with NG (28, 29, 30). However, this procedure is still a matter of controversy, mainly for the concern of subsequent technical difficulties in HP surgery (31) and potential mistakes in histological interpretation (32).

With these concepts in mind, this study was aimed to reevaluate the potential interference of the most frequent thyroid diseases (NG and AITD) on MIBI scan and neck US diagnostic accuracy and redefine the diagnostic role and value of FNA–PTH in HP localization.

Materials and methods

Patients

We conducted a retrospective cohort study based on hospital records of 43 consecutive patients (ten males and 33 females, aged 37–80 years, and mean 59 years) with diagnosis of pHPT and concomitant NG ($n=32$) or AITD ($n=9$ HT and $n=2$ GD) who were referred to the out clinic service of our Endocrinology Unit during a 6-year period (2003–2008). The diagnosis of pHPT was based on elevated serum PTH along with elevated or high normal range serum calcium (see Tables 1 and 2). The diagnosis of AITD was made in the presence of positive serum antithyroid autoantibodies (ATA), typical thyroid US hypoechoic pattern, and/or primary hypo/hyperthyroidism. The diagnosis of NG was made by neck US in the presence of increased thyroid volume and/or in the presence of single or multiple TNs. All patients were also submitted to an accurate physical examination, MIBI scan, FNA–PTH measurement, and FNA cytology of their neck masses (a total of 63 neck lesions), performed as detailed later. The results of all diagnostic procedures were retrospectively reviewed. Written informed consent was obtained from each patient after full explanation of the purpose and nature

Table 1 Clinical, biochemical, and pathological data in patients of group I: MIBI scan and neck US images fully concordant with HP localization.

Patient	Age	Sex	Serum PTH (pg/ml)	Serum Ca (mg/dl)	NG (+/–)	AITD (+/–)	US (+/–; mm/site)	MIBI (+/–)	Cytology	FNA–PTH (pg/ml)	Histology
Group I											
1	57	F	107	11.2	+	–	+12/D	+	Comp	3693	PA
2	51	F	155	11.5	+	–	+14/N	+	Comp	8673	PA
3	58	F	96	10.8	+	–	+20/N	+	Comp	2999	PA
4	57	F	1187	12.3	+	–	+50/D	+	Foll	16 439	PA
5	55	M	103	10.9	+	–	+16/N	+	ND	>2500	PA
6	37	M	156	11.8	–	–	+10/D	+	Comp	>2500	PA
7	46	F	288	10.8	–	–	+15/N	+	Comp	517	PA
8	45	F	236	11.3	+	–	+10/N	+	Comp	>2500	PA
9	51	M	155	10.5	–	–	+10/I	+	ND	1215	PA
10	74	F	461	12.2	+	–	+20/N	+	Comp	>2500	PA
11	63	F	90	10.8	–	–	+12/N	+	Foll	>2500	PA
12	62	F	1367	14.1	–	–	+11/D	+	ND	>2500	PA
13	65	F	460	11.4	+	–	+21/N	+	Comp	>2500	PA
14	69	M	407	13.34	+	–	+19/N	+	ND	989	PA
15	80	F	335	10.9	+	+	+10/D	+	Comp	>5000	PH
16	52	M	110	11.3	+	–	+6/I	+	Foll	643	PA

Sex: F, female; M, male; neck US and MIBI scan (+, positive; –, negative for HP); HP site: D, distant; N, near; and I, inside the thyroid. NG and AITD (+, presence; –, absence); cytology: comp, compatible with HP; foll, follicular pattern; ND, not diagnostic. Histology of hyperfunctioning parathyroids: PA, parathyroid adenoma; PH, parathyroid hyperplasia.

Table 2 Clinical, biochemical, and pathological data in patients of group II: group II-A, patients with negative MIBI scan; group II-B, patients with MIBI uptake projected on a neck nodule not corresponding to HP; group II-C, patients with a double focus of MIBI uptake; and group II-D, patients with MIBI focus projected in a neck area where two adjacent lesions were present.

Patient	Age	Sex	Serum PTH (pg/ml)	Serum Ca (mg/dl)	NG (+/–)	AITD (+/–)	US (+/–; mm/site)	MIBI (+/–)	Cytology	FNA–PTH (pg/ml)	Histology
Group II-A											
1	60	M	140	12.07	–	+	(HT) +20/D	–	Comp	> 2500	PC
2	57	M	110	13.35	+	–	+25/N	–	Foll	> 2500	PA
3	68	F	121	10.3	+	–	+5/N	–	Comp	> 2500	PA
4	68	F	392	15.06	+	–	+35/D	–	ND	> 2500	PA (cystic)
5	58	F	225	11.05	–	+	(HT) +11/N	–	Comp	830	PA
6	37	F	107	9.07	+	+	(HT) +15/N	–	ND	366	PA
7	57	M	99	11	–	+	(GD) +14/N	–	Foll	> 2500	PA
8A	64	F	222	11.2	+	–	–14/D	–	Comp	> 2500	PA
8B	64	F	222	11.2	+	–	–10	–	Foll	4.4	PTC
9A	63	F	113	10.8	+	+	(HT) –8/I	–	ND	> 2500	PA
9B	63	F	113	10.8	+	+	(HT) –25	–	Foll	6	TA
10A	53	F	119	12	+	+	(HT) –9/I	–	Comp	> 2500	PH
10B	53	F	119	12	+	+	(HT) –15	–	Foll	16	HTN
Group II-B											
1A	62	F	157	11.5	+	–	+15/N	–	Comp	741	PA
1B	62	F	157	11.5	+	–	–10	+	Foll	<3	HTN
2A	60	M	128	10.5	+	–	+14/D	–	Comp	> 2500	PA
2B	60	M	128	10.5	+	–	–12	+	ND	34.3	Lymphoma
3A	60	F	200	12	+	+	(HT) +27/I	–	Foll	> 2500	PH
3B	60	F	200	12	+	+	(HT) –38	+	Foll	12.4	TA (toxic)
4A	79	F	294	10.02	+	+	(HT) –22	+	Foll	29.6	FC (oncocytic)
4B	79	F	294	10.02	+	+	(HT) –22	+	Foll	22.2	HTN
4C	79	F	294	10.02	+	+	(HT) +10/D	–	ND	> 2500	PA
Group II-C											
1A	74	F	362	10.02	+	–	+18/N	+	Foll	366	PA
1B	74	F	362	10.02	+	–	–15/N	+	Comp	> 2500	PA
2A	62	F	344	12.3	+	–	+17/N	+	Foll	2571	PA
2B	62	F	344	12.3	+	–	–20	+	Foll	6	PTC
3A	63	F	107	10.6	+	–	+23/D	+	Comp	> 2500	PA
3B	63	F	107	10.6	+	–	–21	+	Foll	5.9	PTC
4A	74	F	152	11.36	+	–	+14/D	+	Comp	> 2500	PA
4B	74	F	152	11.36	+	–	–22	+	NA	NA	Lung cancer
Group II-D											
1A	59	F	111	11.8	+	–	–16	+	Foll	<3	HTN
1B	59	F	111	11.8	+	–	–20/N	+	Foll	> 2500	PA
2A	60	F	221	11	+	–	+10/D	+	ND	> 2500	PA
2B	60	F	221	11	+	–	–15	+	ND	15.5	TA
3A	56	F	1365	13	+	+	(HT) –8	+	Foll	<3	HTN
3B	56	F	1365	13	+	+	(HT) +25/D	+	Comp	> 2500	PA
4A	56	F	244	10.52	+	–	–15	+	ND	19.01	HTN
4B	56	F	244	10.52	+	–	–15/N	+	Comp	2332	PA
5A	64	F	178	10.99	+	–	+20/D	+	Comp	> 2500	PA
5B	64	F	178	10.99	+	–	–9	+	Foll	<3	PTC
6A	51	F	180	11.6	+	–	–7/I	+	Foll	> 2500	PA
6B	51	F	180	11.6	+	–	–13	+	ND	3.07	HTN
7A	65	F	147	10.6	+	–	–13/N	+	ND	> 2500	PA
7B	65	F	147	10.6	+	–	–9	+	Foll	33.6	TA (oncocytic)
8A	65	F	95	12.4	+	–	–24	+	ND	<3	PTC (oncocytic)
8B	65	F	95	12.4	+	–	+11/D	+	Comp	> 2500	PA
9A	42	M	381	12.6	+	+	(HT) –8	+	Foll	4.4	PTC
9B	42	M	381	12.6	+	+	(HT) +20/D	+	Comp	> 2500	PA

Sex: F, female; M, male; neck US and MIBI scan (+, positive, –, negative for HP); HP site: D, distant; N, near; and I, inside the thyroid. NG and AITD (+, presence; –, absence); cytology: comp, compatible with HP; foll, follicular pattern; ND, not diagnostic; NA, not available. Histology of hyperfunctioning parathyroids: PA, parathyroid adenoma; PH, parathyroid hyperplasia; PC, parathyroid carcinoma. Histology of thyroid nodules: HTN, hyperplastic thyroid nodule; TA, thyroid adenoma; PTC, papillary thyroid carcinoma.

of all procedures used. The Institutional Review Board of University Hospital of Cagliari reviewed and approved the protocol of this study.

The choice of the surgical approach of pHPT was based on the results of all the diagnostic procedures employed to localize HP and on the basis of associated significant thyroid diseases, which may need surgical treatment. As detailed in the Results section, when patients obtained a satisfactory localization of their HP, a MIP was performed; in those with more than one suspicious HP or concomitant significant thyroid diseases, a BNE was preferred. Furthermore, in all cases, confirmation of radical pHPT treatment was reached either by the demonstration of intraoperative significant reduction of serum PTH and/or by persistent normalization of serum calcium levels.

Hormonal assays

Serum-free thyroxine (FT₄), free tri-iodothyronine (FT₃), and TSH were assayed by automatic ultrasensitive chemiluminescent assay (Ortho Clinical Diagnostic SpA, Milan, Italy). Anti-thyroperoxidase (TPOAb), anti-thyroglobulin (TgAb) autoantibodies, and PTH (in serum and in FNAB fluid) were measured by chemiluminescent assay: Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA; distributor Medical Systems Corporation, Genoa, Italy) and anti-TSH receptor autoantibodies (TRAb) by radio-receptor assay (TRAKhuman, BRAHMS, Hennigsdorf, Germany; distributor DASIT SpA, Milan, Italy). By these procedures, normal range values were as follows: FT₄ 7.7–21.9 pg/ml, FT₃ 2.77–5.27 pg/ml, TSH 0.2–3.0 µU/ml, TPOAb < 35 IU/ml, TgAb < 40 IU/ml, TRAb < 1.0 U/ml, and serum PTH 1–87 pg/ml. For FNA–PTH, see the paragraph related to the cutoff values. Patients with increased ATA titers were considered ATA positive.

US with color flow doppler sonography

US and color flow doppler sonography (CFDS) of the neck were performed by one of the authors (F Boi) using a Sonoline G60S (Siemens, Medical Solutions, Issaquah, WA, USA) color Doppler system with a 8–13 MHz linear electronic transducer. The examiner was unaware of any prior imaging results using other techniques. The examination (conventional gray-scale US, followed by CFDS) included the central neck from the subclavian vein to the submandibular glands. The images were obtained with hyperextended position and lateral rotation of the neck to better visualize the common parathyroid ectopic sites (paraesophageal, paravertebral regions, within the thyroid gland, etc.). Enlarged parathyroid glands typically appear as an oval or triangular mass with sharp borders, homogenous hypoechoic texture with hypervascularization at CFDS. Usually, HP were found lying posterior and

adjacent to the upper one-third of the thyroid lobes or adjacent to the lower pole. All neck masses were localized and their diameters were measured by US. The position of HP with respect to the thyroid gland was defined as follows: near to or inside the thyroid when HP were strictly adjacent or embedded in the thyroid, and distant when HP resulted clearly separated from thyroid lobes. US and CFDS standard criteria (20) were also employed to evaluate and select concomitant TNs to be submitted to FNA.

Parathyroid ^{99m}Tc-MIBI scintigraphy

The patients received 400 MBq ^{99m}Tc-MIBI (Cardiolite, Bristol-Meyers-Squibb, Brussels, Belgium) by i.v. injection. Anterior planar images of the neck and chest were obtained immediately after the injection (thyroid phase) and at 30 and 60 min later (parathyroid phase), each for 10 min, using a dual-head gamma camera (Varicam, General Electric Medical System, Milwaukee, WI, USA) equipped with high-resolution low-energy parallel-hole collimators. SPECT images of the same area were obtained after study of parathyroid phase with 120 frames at 25 s/frame and 3° angles over a 360° arc. The images were acquired into a 128×128 matrix and reconstructed using Hanning filter. Thyroid scan was performed the following day with the same gamma camera. Anterior planar images were acquired 20 min after an i.v. injection of 4 mCi (152 MBq) of ^{99m}Tc-pertechnetate. Visual analysis of the thyroid and MIBI scan were interpreted by experienced nuclear medicine physicians (M Piga and A Serra) who were unaware of US results. Patients were studied with standard MIBI scan integrated with SPECT/CT study to better evaluate the correspondence with neck US images. The MIBI scan was considered positive in locating abnormal parathyroid when one or more areas of increased and persistent MIBI uptake were detected in early and/or late acquisition and mismatched on ^{99m}TcO₄ uptake revealed by previous thyroid scan.

FNA cytology and FNA–PTH

Written informed consent was obtained before US-guided FNA, which was performed using 22–25 gauge needles attached to a 10 ml syringe. One or no more than two passes were performed on each neck lesion. FNA cytology and histology were performed by an experienced pathologist (M L Lai), who was unaware of neck US and MIBI images. Cytological diagnosis of TNs and HP was expressed according to standard criteria (20, 33). For the purpose of this study, FNA cytology was considered 'positive' when suggestive of HP and 'negative' in the absence of HP features or inadequate sample. After smear preparation, the needle was washed out with 500 µl PTH-free serum dilution buffer and the solution processed for PTH measurement. Histological diagnosis on surgical specimens of HP and

TNs was made using standard criteria and appropriate pathological techniques (20, 33, 34).

Cutoff values for FNA–PTH

To correctly interpret the results of hormonal assays in FNA, we had to establish a cutoff above which FNA–PTH concentrations in washout fluid could be considered expression of local hormonal production, rather than the result of peripheral blood contamination. For this purpose, we retrospectively compared all hormonal FNA results from HP and not-HP of this series. On the basis of this analysis, all FNA–PTHs more than three times (103 pg/ml) the maximal concentration found in not-HP lesions (34.3 pg/ml) were considered positive and diagnostic for HP.

Statistical analysis

The sensitivity and specificity of the different tools to correctly detect HP were evaluated using Galen & Gambino formula (35). The potential association between AITD and NG with the presence/absence of MIBI uptake was evaluated using nonparametric test (χ^2 and exact Fisher's test, with 0.05 as the significance level) by Graphpad Prism® (Graphpad Software Co., San Diego, CA, USA).

Results

Performance of the diagnostic tools in HP localization

Morphological (US) and functional (MIBI scan) images allowed the precise localization of HP in 16 patients (indicated as group I), which were confirmed by histology. In the remaining 27 patients (included in group II), US and MIBI images were discordant and identified 47 neck lesions displaying some features of HP, but, due to the presence of several degrees of mismatch, not allowing unequivocal identification of pathological parathyroids.

The complete results obtained in group I patients are detailed in Table 1. All patients of this group showed a small goiter or a single small TN (<1 cm) both detected by neck US.

In this group, US and MIBI images were fully concordant, allowing, in each patient, the precise identification of a single well-distinct lesion, strongly suggestive of HP. Moreover, all these MIBI-positive lesions showed high FNA–PTH concentrations (517–>2500 pg/ml), resulting in all cases pathological HP (15 parathyroid adenomas (PAs) and one parathyroid hyperplasia (PH)) at histology. FNA cytology was positive only in 9/16 (56.2%) of cases.

In group II patients, palpation and neck US displayed a larger goiter and/or multiple TNs. In this group, we

were able to identify at least four different patterns of mismatch (described as subgroups A, B, C and D) of these procedures. All the results obtained in this group are detailed in Table 2.

In all patients of group II-A ($n=10$), MIBI uptake was always absent (Fig. 1a) and HP was suspected by neck US (Fig. 1b) in seven of ten. These seven lesions displayed very high FNA–PTH concentrations (366–>2500 pg/ml) and were confirmed HP at histology (six PA and one parathyroid carcinoma (PC)). Each of the remaining three patients displayed at US two neck lesions with a echographic pattern insufficient for a clear identification of parathyroid tissue. High FNA–PTH values (>2500 pg/ml) were found only in one lesion of each patient, which was confirmed HP (two PA and one PH) at histology. The other three lesions with low FNA–PTH concentrations (4.4–16 pg/ml) resulted in thyroid origin (one TA and one papillary thyroid carcinoma (PTC), and one hyperplastic TN (HTN)) at histology.

Each patient of group II-B ($n=4$) exhibited one or more MIBI-positive areas projected on neck nodule

Figure 1 Selected MIBI scan and neck US images of group II patients: (a) negative MIBI scan and (b) HP detected by neck US (circled) in a patient of group II-A. (c) MIBI uptake projected on a toxic TA and (d) MIBI-negative HP detected by neck US (circled) in a patient of group II-B. (e) Double focus of MIBI uptake (projected on a PTC and on a HP) and (f) HP at neck US (circled) in a patient of group II-C. (g) Single MIBI focus projected on two adjacent neck lesions (a HTN and a HP) and (h) HP at neck US (circled) in a patient of group II-D.

Figure 2 FNA-PTH concentrations found in histologically proven HP (filled circle) and in thyroid or other miscellaneous neck lesions (open circle). The dotted line indicates the cutoff for positive FNA-PTH (103 pg/ml).

(Fig. 1c) without US features of HP, while an other neck lesion suggestive of HP at US (Fig. 1d) did not show any MIBI uptake. All four MIBI-negative neck lesions identified by US had high FNA-PTH values (741–>2500 pg/ml) and all resulted HP at histology (three PA and one PH). All five MIBI-positive masses (a single lesion from three patients and a double mass from one case) showing low levels of FNA-PTH (<3–34.3 pg/ml) resulted in thyroid lesion (two HTNs, one hyperfunctioning TA, and one oncocyctic variant of follicular carcinoma (FC)) with exception of patient #2 who had a local recurrence of neck lymphoma at histology.

Each patient of group II-C ($n=4$) had two distinct and separate MIBI-positive areas; in three patients, MIBI focuses were projected in the lower third of the neck, and in the remaining case, one focus was projected in the jugular area and the other in the hilum of the left lung. Of these seven neck MIBI-positive lesions, 4/7 results were suggestive of HP at US examination and were confirmed HP at histology. High FNA-PTH (366–>2500 pg/ml) was found in a total of 5/7 neck lesions, all HP at histology (patient #1 had double PA and three patients had a single PA). In two patients (#2 and #3), the other neck MIBI-positive focus showed low FNA-PTH (5.9–6 pg/ml) and in both cases resulted PTC at histology: one of these cases (patient #2), a rare association between MIBI-positive PA and PTC (Fig. 1e and f), has been previously reported (26). In patient #4, the other MIBI-positive area, located in the lung, resulted in a recurrence of lung neoplasia.

In each patient of group II-D ($n=9$), a single MIBI focus was projected in a neck area where two similar, hypoechoic adjacent masses (Fig. 1g and h) were found at US. Of these 18 neck lesions, five results were suggestive of HP at US examination and were confirmed HP at histology. High FNA-PTH (2332–>2500 pg/ml) was found in a total of 9/18 lesions, all resulting HP at histology (nine PA). All the other nine lesions with low FNA-PTH (<3–33.6 pg/ml) resulted thyroid neoplasias ((four HTNs, two TAs (one classic and one oncocyctic

variant), and three PTC (two classic and one oncocyctic variant)) at histology.

The accuracy of all diagnostic procedures (MIBI scan, neck US, FNA-PTH, and FNA cytology) was calculated according to the Galen & Gambino formula (35). For this purpose, MIBI uptake was considered true positive when the mass was confirmed HP at histology, false positive when the lesion resulted not-HP (i.e. TN), true negative when the mass was confirmed not-HP, and false negative when HP resulted MIBI negative. For neck US, it was considered true positive when the mass suggestive of HP was confirmed at histology, false positive when the suspected lesion resulted in not-parathyroid origin, true negative when US correctly identified the other neck masses, and false negative when US failed to identify HP. For FNA-PTH, true positive was considered a value >103 pg/ml found in histologically proven HP and true negative was considered all values <103 pg/ml found in lesions of not-parathyroid origin. FNA cytology was considered true positive when the neck mass corresponded to HP at histology and false negative when cytology results were inadequate or not indicative of HP in histologically proven parathyroid lesions. In group I patients (Table 1), almost all techniques (MIBI scan, neck US, and FNA-PTH) allowed the correct identification of all HP, reaching 100% sensitivity, while FNA cytology resulted in only 56.2% sensitivity.

As shown in Fig. 2 and Table 3, in the whole series of group II patients, all neck masses with positive FNA-PTH values (366–>2500 pg/ml) resulted HP at histology, while low FNA-PTH concentrations (<3–33.6 pg/ml) were only found in not-HP lesions. MIBI scan was true positive only in 10/28 (35.7%) of HP lesions, while resulted false positive in 11/19 (57.9%) neck masses of not parathyroid origin. Neck US was able to immediately identify HP in 20/28 (71.4%) of cases and excluding a parathyroid origin in 15/19 (78.9%) of not parathyroid lesions. FNA cytology correctly identified 15/28 (53.6%) of HP, resulting not indicative for HP (follicular pattern) in 7/28 (33.3%)

Table 3 Comparison between MIBI scan, neck US, FNA-PTH, and FNA cytology vs histology of group II patients.

Diagnostic tools	Results	HP ($n=28$)	Not-HP ($n=19$)
MIBI scan	Positive	10	11
	Negative	18	8
Neck US	Positive	21	4
	Negative	7	15
FNA-PTH	Positive	28	0
	Negative	0	19
FNA cytology	Positive	15	0
	Negative	13	19

HP, hyperfunctioning parathyroid at histology; not-HP, lesions different from HP at histology. MIBI scan pos, positive MIBI uptake; MIBI scan neg, absent MIBI uptake. Neck US pos, identification; neck US neg, not identification of HP by ultrasound. FNA-PTH pos, FNA-PTH greater than the cutoff; FNA-PTH neg, FNA-PTH lower than cutoff. FNA cytology pos, compatible with HP; FNA cytology neg, inadequate or not compatible with HP.

Figure 3 Sensitivity (black bar) and specificity (gray bar) of MIBI scan, neck US, FNA-PTH, and FNA cytology in the identification and localization of HP in group II patients.

and inadequate in 6/28 (21.4%). In summary, as displayed in Fig. 3, in group II patients, FNA-PTH resulted in the best procedure able to identify all HP reaching 100% sensitivity and specificity; neck US reached 71.4% sensitivity and 78.9% specificity; MIBI scan 35.7% sensitivity and 42.1% specificity; and FNA cytology 53.6% sensitivity and 100% specificity.

Thanks to the above procedures, in patients with a fully satisfactory localization of HP (all 16 patients of group I) and in several cases with significant thyroid or other diseases (the first eight cases of group II-A, the patient with associated recurrence of neck lymphoma (group II-B), the case with mediastinum recurrence of lung neoplasia (group II-C) and four patients (#3, #5, #7, and #9) with small thyroid tumors of group II-D) who displayed an unequivocal identification of HP by FNA-PTH assay (a total of 14 of 27, 52% of patients of group II), an adequate MIP (unilateral neck exploration) was performed. The remaining patients (13 of 27, 48% of patients of group II) who exhibited more than one suspicious HP or a large NG/thyroid tumors, which required an extensive surgical treatment, were submitted to a BNE.

Finally, in the postoperative 6-month follow-up, almost all (39/40, 97.5%) patients with histological diagnosis of PA reached radical pHPT treatment, while only one case displayed recurrence of pHPT. On the other hand, one of the three patients with PH and the patient with PC, showed persistence of pHPT.

Thyroid diseases and HP localization: effect of thyroid autoimmunity on MIBI scan

When the results obtained with MIBI scan in group II patients were analyzed according to the nature of associated thyroid disorders, a significant increase of false-negative MIBI scans were found in the ten patients with AITD (8/10, 80%) when compared with patients with NG (6/17, 35.3%; $P < 0.05$). In contrast, no false-positive MIBI scans were found in patients with

associated AITD. Almost all (10/12, 83.3%) cases of false-positive MIBI uptake were caused by TNs. In particular, between the five thyroid carcinomas, two were an oncocyctic variant of FC and PTC and the remaining were three cases of classic variant of PTC; between the five benign lesions, there were three TA (one oncocyctic variant, one hyperfunctioning, and one classic variant) and the remaining were two HTNs. The other two false-positive MIBI scans were represented by a neck lymphoma and a recurrence of lung neoplasia.

On the whole, as displayed in Tables 1 and 2, FNA cytology allowed the correct identification of seven concomitant malignant thyroid tumors ($n=6$ PTC, $n=1$ FC) and 11 benign thyroid lesions ($n=3$ TA and $n=8$ HTNs) associated with HP, which needed to be removed in the same surgical time.

Discussion

Neck US and MIBI scan represent the first diagnostic tools to correctly plan surgery of HP (10) in the absence of concomitant cervical masses (14). False-negative neck US results may be rarely found in cases with small and ectopic HP, intrathyroidal HP, and underscent HP situated along the common carotid artery or recurrent laryngeal nerve, while false-negative MIBI scans have been observed in the presence of small size, low oxyphil cell content, and morphological changes (cystic degeneration, apoptosis, or necrosis) of HP (18, 19). On the other hand, difficulty in neck US and MIBI scan interpretation has been described in the presence of TNs (20, 26, 36) with high rate of false-positive images (15–20%) for both techniques (24, 37); blood vessels, esophagus, longus colli muscle, and enlarged lymph nodes (37) may represent other rare sources of false-positive results. In contrast with nodular thyroid diseases, no studies on the potential interference of concomitant AITD in the diagnostic approach of pHPT by MIBI scan and neck US have been so far performed. Despite the usefulness of FNA-PTH in cases with inconclusive neck US and MIBI scan results, some authors reported (31) that FNA technique may be responsible for HP damage leading to fibrosis, with potential incomplete removal of HP, increased risk of laryngeal nerve injury, and the induction of false worrisome histology, which can be mistaken for carcinoma (32). These authors concluded that the routine use of this technique is unnecessary and should be avoided. This topic is still a matter of controversy, as Abraham *et al.* (38) rebutted that the above complications were caused by the use of large-bore needles and excessive number of FNA passes performed by operators of unknown skill levels.

With the above issue in mind, to reevaluate the real impact of the main thyroid diseases and to redefine the diagnostic role of FNA-PTH in HP diagnostic evaluation, all data from pHPT patients with coexistent NG

or AITD were retrospectively compared. We found that, in the presence of concomitant large NG and/or HT (group II patients), MIBI scan and neck US images were discordant, resulting in an uncertain HP localization with four well-defined different patterns of mismatch between these techniques. In keeping with a recent study (39), coexistent NG was responsible for low diagnostic accuracy (sensitivity of 35.7% and specificity of 42.1%) of MIBI scan and unsatisfactory diagnostic performance (71.4% sensitivity and 78.9% specificity) of neck US. On the other hand, in the presence of concomitant small NG and absence of HT (group I patients), both neck US and MIBI scan displayed an excellent diagnostic performance in HP detection. Independently from the entity of associated thyroid diseases and the results of the above imaging techniques, FNA-PTH resulted in the best diagnostic tool (100% sensitivity and specificity) in the identification of all HP. As shown in Fig. 2, this excellent result was obtained with a rather arbitrary cutoff of 103 pg/ml corresponding to three times the highest FNA-PTH concentration (34.3 pg/ml) found in our series of not-HP lesions. Indeed, FNA-PTH technique helped to identify the site of the neck harboring HP when preoperative localization studies (US and MIBI scan images) resulted negative or discordant (e.g. in presence of large NG and/or AITD) and also allowed to select an adequate minimally invasive parathyroid surgical approach (e.g. unilateral neck exploration instead of BNE) in these cases. Finally, FNA cytology displayed low sensitivity both in group I and in group II patients (56.2 and 53.6% respectively) independently from the associated thyroid disorders. This phenomenon is in keeping with the known difficulties in distinguishing parathyroid from thyroid cells (33, 34). The different diagnostic accuracy between these FNA techniques is explained by the minimal amount of material needed to assay PTH-FNA, which was not sufficient for cytology.

The analysis of false-negative MIBI results of group II patients showed only in 2/14 cases the presence of known interfering factors of MIBI uptake, such as small size and cystic degeneration of two PA (patients #3 and #4 of group II-A), while in the remaining 8/12 cases (66.7%) a concomitant AITD (seven HT and one GD) was detected. It may be hypothesized that the inflammatory process related to AITD might be responsible for MIBI-negative scan by interferences on the mechanism of uptake, retention, and/or washout time of MIBI by parathyroid cells. To our knowledge, this is the first description of this association and further investigations are needed to better characterize the real prevalence of AITD in pHPT patients and clarify the potential interfering mechanism on MIBI function.

This study also confirms that MIBI false-positive scans (all detected in group II) were mainly caused by increased MIBI uptake of concomitant TNs. As previously reported (20), MIBI retention was observed in hypermetabolic oncocyctic tumors (one oncocyctic

variant of TA and two oncocyctic variants of PTC and FC) and TA (one hyperfunctioning adenoma and one classic variant), while the remaining five MIBI-positive scans were represented by three classic variants of PTC and two HTNs. Moreover, two other sources of MIBI false-positive uptake were represented by a recurrence of neck lymphoma and a mediastinum recurrence of lung neoplasia.

In our study, FNA was well tolerated, no complications and fibrosis were observed in any of the aspirated glands; no difficulties in operating setting and in distinguishing benign from malignant HP were reported when comparing this series with patients not submitted to this procedure (not reported in this paper). This good performance was obtained by adopting some technical precautions, as the use of small thin-bore needles (22–25 gauge) and a limited number (not more than one or two) of not-traumatic passes on all neck masses.

Accordingly, with Abraham *et al.* (38), FNA resulted in a safe technique when the above-described procedures were applied. However, it should be noted that in the presence of suspicious multiple parathyroid gland disease (or in patients with concomitant significant thyroid diseases), as well as in the presence of a true-positive result of PTH-FNA, other diagnostic imaging techniques (e.g. CT or magnetic resonance) may be proposed before a BNE in order to correctly find all HP and avoid recurrence or persistence of pHPT. We conclude that US-guided FNA-PTH should be used appropriately only in selected cases (i.e. when US and MIBI scan give conflicting results such as MIBI-negative cases, atypical location, multiple positive MIBI areas, as may happen in AITD/NG patients) and not applied routinely in pHPT. This technique helps the surgeon to perform a quicker operation with a higher degree of success and as an added benefit enables the identification of incidental thyroid tumors that coexist with pHPT (40) that can be effectively addressed during the same surgery.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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