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Reviewer A

This study evaluates the performance of parathyroid scintigraphy with tomographic acquisition (SPECT/CT) in a small patient population with parathyroid cysts.

This article does not add significant novelty and the small patient population is a significant limitation.

Furthermore, parathyroid scintigraphy with SPECT/CT will be soon substituted by PET/CT for detection and localization of hyperfunctioning parathyroid glands.

Minor point: the nomenclature of the radiopharmaceutical used is wrong and it should be corrected.

Reply: Thank you for your suggestion. For the error of the nomenclature of the radiopharmaceutical, we have modified in the revised manuscript (All ^{99m}Tc -MIBI in this article has been changed to $^{99\text{m}}\text{Tc}$ -MIBI).

Although mentioned in the limitations of this study, the small number of patients was indeed an important limiting factor in this study, mainly because PCs are a rare case. We have modified our text in the revised manuscript (see Page 13, line 1).

$^{99\text{m}}\text{Tc}$ -MIBI SPECT/CT scintigraphy is the current standard method for detecting hyperfunctioning parathyroid glands (1), and the conventional first-line presurgical imaging method (2), usually supplemented by ultrasonography, which is generally accepted.

PET/CT has recently emerged as a complementary second-line imaging technique with the advantage of a higher resolution and a shorter acquisition time than SPECT/CT (3), the higher resolution of PET/CT imaging allows for better detection of smaller lesions that cannot be visualised by SPECT/CT. Increasing evidence suggested that PET/CT had high sensitivity in localizing hyperfunctioning parathyroid glands. However, the disadvantages are that cyclotron-produced radionuclides for PET/CT parathyroid imaging are relatively expensive, and there are no standardized guidelines for image acquisition (4).

If the results of SPECT/CT parathyroid scintigraphy are negative, equivocal, inconsistent with ultrasound findings, or persistence or recurrence of HPT after surgery, an alternative investigation involving hybrid

1 Petranović Ovčariček P, Giovanella L, Carrió Gasset I, et al. The EANM practice guidelines for parathyroid imaging. *Eur J Nucl Med Mol Imaging* 2021; 48:2801-22.

2 Mathey C, Keyzer C, Blocklet D, et al. 18F-Fluorocholine PET/CT Is More Sensitive Than 11C-Methionine PET/CT for the Localization of Hyperfunctioning Parathyroid Tissue in Primary Hyperparathyroidism. *J Nucl Med* 2022;63:785-91.

3 Imperiale A, Taïeb D, Hindié E. 18F-Fluorocholine PET/CT as a second line nuclear imaging technique before surgery for primary hyperparathyroidism. *Eur J Nucl Med Mol Imaging* 2018;45:654-7.

4 Petranović Ovčariček P, Giovanella L, Carrió Gasset I, et al. The EANM practice guidelines for parathyroid imaging. *Eur J Nucl Med Mol Imaging* 2021; 48:2801-22.

PET/CT technique is recommended (5). As stated by the reviewers, parathyroid scintigraphy with SPECT/CT will be substituted by PET/CT for detection and localization of hyperfunctioning parathyroid glands. Recently published parathyroid imaging guidelines suggested, when possible, ¹⁸F-FCH PET/CT was a potential “alternative” first-line option in patients with pHPT (6).

We added some data about SPECT/CT and PET/CT imaging (see Page 4, line 21- Page 5 line 10).

Reviewer B

This manuscript by Yushan Wei and colleagues presents an initial single-center experience regarding the diagnostic role of ^{99m}Tc-MIBI SPECT/CT in functional parathyroid cysts (FPCs).

Overall, this manuscript aligns with the Journal's scope and lines of research.

However, there are some fundamental points that need to be addressed by the authors before accepting the manuscript:

1) According to current guidelines, preoperative imaging is not recommended for diagnostic purposes but only to locate the abnormal parathyroid glands in patient with hyperparathyroidism. Therefore, why did the seven patients with normal calcium levels undergo ^{99m}Tc-SPECT/CT?

Reply: Thank you very much for pointing out this issue.

The main reasons are as follows: Firstly, the long case collection period of 11 years in this study and the clinicians' limited knowledge of parathyroid cysts (PCs) and the relevant guidelines at the beginning of this study may have been the main reason for performing SPECT/CT parathyroid scintigraphy in the seven patients with elevated PTH and normal blood calcium. On the other hand, PCs are very rare and difficult to diagnose clinically. Seven patients with elevated PTH and normal calcium were suspected of having HPT, and MIBI imaging was performed to differentiate them from early HPT. With the summary of this study, I believe that SPECT/CT imaging should not be performed if a similar case presents in our hospital.

2) Four patients were considered affected by FPCs. However, the authors do not specify what criteria they used to distinguish NFPCs from FPCs in the Methods section. Is it the presence of a diagnosis of primary hyperparathyroidism?

Reply: Thank you very much for pointing out this issue.

PCs were defined as having elevated PTH in the cystic fluid or parathyroid cells on puncture fluid cytology. FPCs were defined as PCs patients with a clinical diagnosis of PHPT, or with biochemical changes of PHPT

5 Mathey C, Keyzer C, Blocklet D, et al. ¹⁸F-Fluorocholine PET/CT Is More Sensitive Than ¹¹C-Methionine PET/CT for the Localization of Hyperfunctioning Parathyroid Tissue in Primary Hyperparathyroidism. *J Nucl Med* 2022;63:785-91.

6 Petranović Ovčariček P, Giovanella L, Carrió Gasset I, Hindié E, Huellner MW, Luster M, et al. The EANM Practice Guidelines for Parathyroid Imaging. *Eur J Nucl Med Mol Imaging* 2021; 48:2801–22.

before surgery and excluding vitamin D deficiency, malabsorption, renal disease or drug-induced factors. Thus, cases diagnosed as FPCs also had a diagnosis of primary hyperparathyroidism. In contrast, NPCs were defined as PCs patients with normal serum PTH levels before resection. We have modified our text as advised in the revised manuscript (see Page 6, line 2-6).

a) The authors, however, do not specify whether these four patients had a diagnosis of primary hyperparathyroidism. Three of them had elevated PTH and calcium, but the last one had only mildly elevated PTH with normal calcium levels. This could be secondary hyperparathyroidism to vitamin D deficiency, malabsorption, kidney disease, or medications, hence explaining the negative SPECT/CT results. Were these factors excluded before defining the patient as having a FPC?

Reply: Thank you for your insightful suggestion. In order to answer this question, several medical experts from different departments discussed this case, and results of the discussion as follows:

In this study, one patient with NFPCs who was initially misdiagnosed as normocalcemic PHPT, had a significantly elevated in cyst fluid PTH (1152 pg/mL), in three tests persistently elevated PTH levels, consistently normal calcium levels, and vitamin D deficiency, malabsorption, renal disease-related and some drug-related secondary hyperparathyroidism were excluded.

The definition of normocalcemic PHPT is that persistently elevated serum PTH levels in the presence of consistently normal albumin-corrected and ionized calcium levels after excluding other causes of PTH elevation (7), and the most accepted concept is that it is an early form of the classic PHPT (8). Normocalcemic PHPT is an exclusionary diagnosis and should be made with great caution. Natalie et al. followed 108 patients provisionally identified as normocalcaemic PHPT for 8 years; only 1.6% (1/64) were confirmed and 20% (13/64) went on to show evidence of normocalcaemic PHPT (9).

For this case, it was suggested that medications that affect PTH levels, such as bisphosphonates, anticonvulsants, diuretics, lithium, denosumab, phosphorus, etc, should be excluded. However, the latest follow-up results showed that serum 25-hydroxyvitamin D, PTH, calcium and phosphorus were normal, and parathyroid ultrasound and puncture results showed PC. Therefore, the international guidelines suggest that patients with normocalcaemic PHPT should have consistently normal values of calcium.; it has also been suggested that the diagnosis of normocalcaemic PHPT may be made in patients with multiple episodes of

7 Schini M, Jacques RM, Oakes E, Peel NFA, Walsh JS, Eastell R. Normocalcemic Hyperparathyroidism: Study of its Prevalence and Natural History. *J Clin Endocrinol Metab.* 2020;105:e1171–86.

8 Schini M, Jacques RM, Oakes E, et al. Normocalcemic Hyperparathyroidism: Study of its Prevalence and Natural History. *J Clin Endocrinol Metab* 2020;105:e1171–86.

9 Cusano NE, Maalouf NM, Wang PY, Zhang C, Cremers SC, Haney EM, Bauer DC, Orwoll ES, Bilezikian JP. Normocalcemic hyperparathyroidism and hypoparathyroidism in two community-based nonreferral populations. *J Clin Endocrinol Metab.* 2013;98:2734-41.

elevated PTH concentrations and consistently normal calcium for at least three months (10).

We have modified our text as advised in the revised manuscript (see Page 8, line 9-12; Page 11, line 16- Page 12, line 9).

3) Assuming that a diagnosis of primary hyperparathyroidism was made, three patients had a clear indication for surgery (i.e., two patients had hypercalcemia >2.76 mmol/l and one patient was <50 years old). However, why did other three patients undergo surgery (Size? Recurrence of cysts after repeated aspiration? Suspicion of non-functioning parathyroid carcinoma?). Please provide clarification.

Reply: Thank you for your suggestion. Three of the seven NFP cases underwent surgical treatment, two of which were due to recurrence of the cysts after repeated aspiration, with lesion sizes of $28 \times 16 \times 30$ mm and $30 \times 25 \times 10$ mm, respectively; one was due to obvious compression symptoms, with a lesion size of $51 \times 39 \times 60$ mm. . We have modified our text as advised (see Page 7, line 11-15).

4) Once the previous points have been addressed, please review the diagnostic accuracy of SPECT/CT in your case series (i.e., verify the correct diagnosis of FPC in the non-operated patient and decide whether to include her as a “false-negative”)

Reply: Thank you.

This patient had multiple elevations of PTH, normal calcium levels, and vitamin D deficiency, malabsorption, kidney disease -induced secondary hyperparathyroidism were excluded. There are many drugs that cause secondary HPT, but not all of them can be excluded, and because of this, this patient was previously misdiagnosed with normocalcaemic PHPT.

Based on your question, several medical experts from different departments discussed this case again, and the patient's diagnosis was corrected. See question 2 for details.

In addition, we performed parathyroid puncture in four cases (including this case) with NFPC who did not undergo surgery, and the US-guided fine needle puncture results of four patients (including this one) were parathyroid cysts.

Again, we thank the reviewers for their rigors. We have modified our text as advised (see Page 8, line 9-12; Page 11, line 16- Page 12, line 9; Page 19, line 1 and Page 19, Table 2).

5) In the Conclusions, please specify that the current role of SPECT/CT according to current guidelines and clinical practice is for localization rather than diagnosis.

Reply: Thank you for your suggestion. We have modified our text as advised (see Page 13, line 12-14).

In addition, I would like to provide some additional comments and corrections:

1. Some authors have reported rare cases of cystic parathyroid carcinoma (e.g., Wright JG, Brangle RW, 1985

10 Cusano NE, Cetani F. Normocalcemic primary hyperparathyroidism. Arch Endocrinol Metab 2022;66:666-77.

Carcinoma in a parathyroid cyst; Cocorullo et al., 10.11138/gchir/2017.38.5.243 ; El-Housseini, Y. et al., Unusual presentations of functional parathyroid cysts: a case series and review of the literature. <https://doi.org/10.1186/s13256-017-1502-1>). Consider including parathyroid carcinoma in the differential diagnosis of PCs in the Introduction and/or Discussion sections.

Reply: Thank you for your suggestion. We have modified our text as advised. Changes in the text (see Page 10 line 16- Page 11 line 2).

2. Page 4, Lines 3-5: Please review citation #4 as the citation chain seems to lead to contradictory information. The reference cites a paper that cites another paper not saying "1-2% of FPCs present with hyperparathyroidism." I suggest verifying the accuracy of this statement, which may be misleading. As a suggestion, you could cite the large study by McCoy and colleagues (your citation #13) and adjust the sentence to something like "Occasionally, FPCs are a rare cause of primary hyperparathyroidism, with an estimated prevalence of 2.3% in a large surgical case series by McCoy et al. (cit.)". Alternatively, you could refer to Pontikides and colleagues (your citation #16) for more incidence rates of PCs.

Reply: Thank you very much for pointing out this issue. We have modified our text as advised in the revised manuscript (see Page 4, line 3-4).

3. Page 4-5: consider adding more information on SPECT imaging in FPCs. E.g., the above cited work by McCoy et al. reports an accuracy rate of 68% for FPCs lateralization compared to 97.4% for parathyroid lesions overall.

Reply: Thank you very much for pointing out this issue. We have modified our text as advised in the revised manuscript (see Page 4, line 21- Page 5, line 10).

4. Page 6, Lines 17-19: Please rephrase to clarify that the patients did not have thyroid cysts and tumors in addition to FPCs, rather that these were initial wrong interpretations based on CT images.

Reply: Thank you. We have modified our text as advised in the revised manuscript (see Page 7, line 7-9, 17-18).

5. Page 7, Lines 3-4: "In the remaining seven cases, the pathological findings were PCs". According to the Tables, this appears to be incorrect. Please rephrase to clarify: two of these patients underwent surgery, with a pathological diagnosis of PCs, and the remaining five cases did not undergo surgery, hence lacking pathological examination.

Reply: Thank you. In seven cases of NFPCs, no ^{99m}Tc-MIBI uptake lesions were found in the periphery of the cysts (Figure 3), and three of these patients underwent surgery, with a pathological diagnosis of PCs, and the remaining four cases did not undergo surgery, underwent parathyroid puncture, and the puncture findings were parathyroid cysts.

We have modified our text as advised in the revised manuscript (see Page 7, line23- Page 8, line 1-3).

6. Page 7, Line 8: "One of the six surgically resected patients [...]": what about the other four patients, especially the one clinically diagnosed as FPCs due to a mild increase of PTH? Were they treated with aspiration? Was the FPC diagnosis confirmed at follow-up? Did the patient develop hypercalcemia? Did she eventually undergo surgery?

Reply: Thank you. A patient with cyst fluid PTH of 1152 pg/ml, serum PTH of 74.3 pg/ml and normal calcium was initially misdiagnosed with FPCs, but the latest follow-up results that serum 25-hydroxyvitamin D, PTH, calcium and phosphorus were normal, and parathyroid ultrasound and puncture results showed PC. Therefore, the diagnosis was changed to NFPCs.

We have modified our text as advised in the revised manuscript (see Page 8, line 9-12).

7. Page 7, Lines 9-10: "and in one case, ^{99m}Tc-MIBI SPECT/CT parathyroid imaging was normal in one case at six months postoperatively": Could you provide information about the laboratory follow-up? If unavailable, please indicate so.

Reply: Thank you.

One of the six surgically resected patients was lost to follow-up; serum PTH, serum calcium and serum phosphorus were normal in five cases at three years, and in one case, ^{99m}Tc-MIBI SPECT/CT parathyroid imaging was normal at 6 and 14 months postoperatively, respectively, and with serum PTH of 26.2, 32.2 and 26.2 pg/mL (15 ~ 65pg/mL), serum calcium of 2.25, 2.21 and 2.13 mmol/L (2.1 ~ 2.51mmol/L), serum phosphorus of 1.05, 1.06 and 0.92 mmol/L (0.9 ~ 1.5mmol/L) at 32, 37 and 39 months postoperatively, respectively.

We have modified our text as advised in the revised manuscript (see Page 8, line 6-12).

8. Page 7, Line 15: "with an incidence of only 0.08% to 3.4% (14)". Please verify citation #14, as I couldn't find these incidence rates in this paper. These rates are, in fact, reported in another paper by Silva et al: 10.1136/bcr-2019-232017 , but I can't find their original source. I suggest referring to McCoy et al., or Pontikides et al., as recommended in comment 1.

Reply: I am very sorry for the error in reference 14.

"Parathyroid cyst is an exceedingly rare etiology, with an incidence of only 0.08% to 3.4%" is from McCoy's and Silva's article (11), which contains a statement about the incidence of parathyroid cysts but no references. In a retrospective study of 6621 patients undergoing neck ultrasound for various reasons, only five PCs were diagnosed and the overall prevalence of PCs was 0.075% in an unselected population (12).McCoy et al.

11 Silva R, Cavadas D, Vicente C, Coutinho J. Parathyroid cyst: differential diagnosis. *BMJ Case Rep.* 2020;13:e232017.

12 Cappelli C, Rotondi M, Pirola I, De Martino E, Leporati P, et al. Prevalence of parathyroid cysts by neck ultrasound scan in unselected patients. *J Endocrinol Invest* 2009; 32:357-359.

reported an incidence of PCs lesions of 3% (48/1769) in a large sample of patients treated for PHPT (13). We have modified our text as advised in the revised manuscript (see Page 8, line 16-19).

9. Page 7, Line 18: For clarity, please rephrase, e.g.: "NFPCs account for 85% and are more common in females; FPCs, on the other hand, account for about 15% and are more common in males."

Reply: We are very sorry that we did not clarify our thought in the manuscript.

A network meta-analysis of 218 articles, reporting 359 well-documented cases of PCs showed that NFPCs were more frequent, representing 61.6% (220/357) of the total number of patients, and had a female predilection (male = 26.5%, female = 73.5%, $P < .0001$), whereas FPCs had a slight predominance in the female population (male = 48.5%, female = 51.5%, $P < 0.001$) (14).

We have modified our text as advised in the revised manuscript (see Page 8, line 22- Page 9, line 3).

10. Page 7, Lines 21-22: "renal damage in 32% to 84% of patients, and bone damage in 41% to 9% of patients". Please provide a more precise definition of "renal damage" (chronic kidney disease, urolithiasis, or both?) and "bone damage" (fractures, osteoporosis, osteopenia?). Please verify the prevalence of bone manifestations (41-9%?). A source for these statistics would be appreciated.

Reply: We are very sorry for making this mistake. the above statement is incorrect. These statistics are for HPT, not for FPCs. Thank you for the correction, and we have removed in the revised manuscript (see Page 9, line 4-5).

11. Page 8, Lines 1-2: Please add a supporting citation for your statement (e.g., Ippolito et al, reference #28).

Reply: Thank you. We have modified our text as advised in the revised manuscript (see Page 9, line 9).

12. Page 9, Lines 16-17: Please revise your citation. I would recommend reviewing the English for minor errors and enhancing clarity: e.g., page 7, line 20; page 9, lines 9-10; page 10, line 9.

Reply: We are very sorry that we did not clarify our thought in the manuscript.

We rewrite each of the corresponding sentences as follows: "such as hypercalcaemia, renal or bone damage" (see Page 9, line 4-5); "Since NFPCs may be caused by fusion of microcysts or fusion of embryonal remnants, ^{99m}Tc -MIBI imaging does not show nuclear uptake in the diagnosis of NFPC" (see Page 10, line 9-10); "The most likely reason for this is that ^{99m}Tc -MIBI uptake is mainly related to the fewer parathyroid cells remaining after cystic degeneration of the parathyroid adenoma." (see Page 12, line 13), respectively.

Lastly, consider adding a point about the need of exploring the role of choline/methionine PET/CT in

13 McCoy KL, Yim JH, Zuckerbraun BS, et al. Cystic parathyroid lesions: functional and nonfunctional parathyroid cysts. *Arch Surg* 2009;144:52–6.

14 Papavramidis TS, Chorti A, Pliakos I, et al. Parathyroid cysts: A review of 359 patients reported in the international literature. *Medicine (Baltimore)* 2018;97:e11399.

diagnosing PCs within the future perspectives of study.

Reply: Thank you. We have modified our text as advised in the revised manuscript (see Page 13, line 3-10).

In summary, more clarity is needed on how these patients were diagnosed, and managed (i.e., rigorous diagnosis of primary hyperparathyroidism and exclusion of secondary causes; indication to SPECT/CT; indication to surgery). A more accurate analysis is required before drawing conclusions on the role of SPECT/CT. In addition, some minor revisions are needed to correct some reference mistakes, and to enhance clarity and robustness of the manuscript.