Clin. Lab. 2020;66:XXX-XXX

REVIEW ARTICLE

The Total Testing Process of Intra-Operative Parathyroid Hormone. A Narrative Review

Elisabetta Stenner [1], Sara Sandic [1], Chiara Dobrinja [2], Maurizio Ruscio [1], Stella Bernardi [3]

[1] *Department of Health Services. Laboratory Division of ASUITs, Burlo, Gorizia e Monfalcone, Italy*
[2] *Division of General Surgery, Department of Medical, Surgical and Health Sciences, University of Trieste, Cattinara Teaching Hospital, Italy*
[3] *Department of Medical Sciences, University of Trieste, Cattinara Teaching Hospital, Italy*

SUMMARY

Background: **Primary hyperparathyroidism (pHPT) is a common endocrine disorder, due to an excessive secretion of parathyroid hormone (PTH) from one or more parathyroid gland(s), where the only cure remains surgery. The surgical approach has become less invasive over the years, thanks to the advances in the preoperative localization of the enlarged parathyroid gland, as well as to the possibility to measure intra-operative parathyroid hormone (IOPTH). After the targeted removal of a parathyroid gland, IOPTH can confirm biochemically the cure of pHPT, such that it helps the surgeon to judge if the parathyroidectomy has been successful and there is no need of additional dissection. As with all laboratory tests, the quality of IOPTH total testing process is essential to the best utilization of patients' results. However, this can be affected by errors occurring in different phases. This review aims to describe the total testing process of IOPTH.**
Methods: **We performed a search in Pubmed and a review of the literature on the current management of pHPT and the total testing process of IOPTH measurement.**
Results: **Compared to previous studies focusing on single aspects of the IOPTH testing process, here we have analyzed all the steps crucial for the quality of IOPTH from the "pre-pre" to the "post-post" analytical phase.**
Conclusions: **Clinicians and laboratory scientists should be aware of all the potential sources of errors in IOPTH measurement in order to improve their daily management of pHPT.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190411)

Correspondence:
Elisabetta Stenner
Piazza Ospitale 1
34100 Trieste
Italy
Phone: +39 403992032
Fax: +39 403994872
Email: elisabetta.stenner@asuits.sanita.fvg.it
elisabetta.stenner@uslnordovest.toscana.it

Review Article accepted July 16, 2019

KEY WORDS

intra-operative parathyroid hormone, hyperparathyroidism, minimally invasive parathyroidectomy, clinical laboratory, CoreLab

INTRODUCTION

Primary hyperparathyroidism (pHPT) is a common endocrine disorder, usually due to an excessive secretion of parathyroid hormone (PTH) from one or more parathyroid gland(s), whose only cure remains surgery [1]. The surgical approach has become less invasive over the years, thanks to the advances in the preoperative localization of the enlarged parathyroid gland, as well as to the possibility to measure intra-operative parathyroid hormone (IOPTH) [2]. After the targeted removal of a parathyroid gland, IOPTH can confirm biochemically

the cure of pHPT, such that it helps the surgeon to judge if the parathyroidectomy has been successful and there is no need of additional dissection [2].
It has been argued that at least 30% of clinical diagnoses/choices depend on laboratory tests [3]. Consistent with its ability to demonstrate the biochemical cure of pHPT, IOPTH is the paradigm of a laboratory test on which to base clinical decisions, as it indicates whether to stop or go on with the neck exploration [4-7].
As with all laboratory tests, the quality of IOPTH total testing process (TTP), from the pre-preanalytical phase right through to the post-post analytical phase (Figure 1), is essential to the best utilization of patients' results [8,9]. Errors can happen in each phase leading, at best, to a waste of time that is crucial during surgery, or to surgery failure, in the worst-case scenario. Recent studies have shown that errors are more frequent during extra analytical phases [8,9]. Nevertheless, the analytical phase also matters to the quality of IOPTH, as does the lack of standardization and harmonization of laboratory assays. In addition, the existence of numerous sources of interference and errors in this phase can lead to an adverse clinical outcome [9,10].
Compared to previous studies focusing on single aspects of the testing process, here we aim to describe the total testing process of IOPTH in order to analyze all the steps that are crucial for its quality and help both clinicians and laboratory scientists avoid potential sources of error.

Overview of primary hyperparathyroidism and its management

Primary hyperparathyroidism is a common endocrine disorder due to an excessive secretion of PTH from one or more parathyroid glands [1]. This disease is generally sporadic and caused by a solitary adenoma (90%), but it can also be due to multiple adenomas or hyperplasia of the parathyroid glands. In about 10% of cases, pHPT is part of hereditary endocrine syndromes, such as multiple endocrine neoplasia type 1 (MEN1), MEN2A, or MEN4 [1]. Parathyroid carcinoma is extremely rare (< 1%) [1].
Usually, the principal regulators of PTH secretion are calcium and 1,25-dihydroxy-vitamin D [11]. In particular, when calcium levels are low, PTH is secreted, and this response allows maintaining the stability of circulating calcium (with a daily excursion of values lower than 6%). PTH increases circulating calcium by resorption of bone tissue, kidney reabsorption of calcium, and the production of 1,25-dihydroxy-vitamin D, which increases calcium absorption in the small intestine [12]. The process is regulated by a negative feedback mechanism, with rising blood calcium levels inhibiting the release of additional PTH.
Having said that, in all forms of pHPT, the most likely mechanisms underlying the excessive secretion of PTH seem to be either an increase of parathyroid cell mass or a reduction of the calcium-sensing receptor [13]. Both cause a loss of the negative feedback of circulating calcium upon PTH release, leading to a state where higher levels of calcium are required to suppress PTH [1]. In this setting, higher levels of PTH promote bone demineralization, osteoporosis, and fragility fractures. In the kidney, pHPT causes nephrolithiasis, nephrocalcinosis, and renal function abnormalities. In addition, overproduction of PTH leads to hypercalcemia, with subsequent direct gastrointestinal and neurological manifestations.
Over the past decades, the presentation of pHPT has markedly changed [14]. While initially pHPT was a symptomatic disease, characterized by the presence of kidney stones, fragility fractures, peptic ulcer, and neuropsychiatric symptoms, today, it is most often an asymptomatic disease, discovered by biochemical screenings. The diagnosis of pHPT should be established biochemically, by documenting a hypercalcemia with simultaneously elevated (or inappropriately normal i.e., > 20 pg/mL) intact PTH levels [15]. Of note, PTH is synthesized as a large polypeptide (pre-proPTH) containing 115 amino acids and it undergoes two proteolytic cleavages to yield the 84-amino-acid molecule, (1 - 84) PTH. When assessing for pHPT, PTH levels should be measured with assays that do not cross-react with PTH-related peptides, such as long C-terminal fragments [16].
The only opportunity for a definitive cure of pHPT is surgery [2]. Surgical resection of the abnormal gland(s) is associated with resolution of biochemical abnormalities, bone mass density improvement [17], as well as with a decreased risk of bone fracture [18] and kidney stones. Consistent with these findings, indications for surgery include symptomatic pHPT or at least one of the following: hypercalcemia, hypercalciuria, nephrolithiasis, nephrocalcinosis, osteoporosis, and/or fragility fractures [19,20].
The standardard operation performed in case of pHPT has always been bilateral neck exploration (BNE) for the visualization of all parathyroid glands and the removal of those that are enlarged [2,21]. However, the increasing sensitivity of preoperative localization methods, such as 99Tc-sestamibi scanning and ultrasound, in combination with the possibility to measure IOPTH have led to the use of minimally invasive procedures in parathyroid surgery [2,4,5,7,22-28]. These include open minimally invasive parathyroidectomy (MIP), minimally invasive video-assisted parathyroidectomy (MIVAP), minimally invasive radio-guided parathyroidectomy (MI-RP), purely endoscopic parathyroidectomy (EP) and video-assisted parathyroidectomy by the lateral approach (VAP-LA) with unilateral neck explorations [2, 4,7]. These minimally invasive procedures allow for reduced operative and recovery time, less postoperative pain, and lower complication rates, in terms of parathyroid gland and recurrent laryngeal nerve injuries [27, 28].
These minimally invasive procedures are recommended for most patients with at least one preoperative localization of a single parathyroid adenoma, measuring less

than 3 cm, with no evidence of multiglandular disease (MGD) [2]. They consist on the targeted removal of an image-identified abnormal gland without further dissection, based on IOPTH, which provides real-time assessment of parathyroid function. In particular, if the peripheral PTH value drops after excision of the gland(s), this confirms that the procedure has been successful and it can be terminated without further exploration. Interestingly, it has been demonstrated that if IOPTH assay is available, the cure rate following MIP is around 99%, basically equal or even better than that of conventional BNE with resection of abnormal glands [5,6,27-29].

Intra-operative parathyroid hormone: from the pre-preanalytical phase right through to the post-post analytical phase

Pre-Preanalytical phase

Eligibility criteria

IOPTH assay monitoring is recommended for patients undergoing MIP [4-7]. According to the literature, the minimally invasive surgical approach is indicated for patients with sporadic pHPT presenting with a single well-localized adenoma, not bigger than 3 cm, without simultaneous thyroid disease or malignancy and without previous irradiation of the neck [2]. However, nowadays, the eligibility criteria are less strict and it is possible to perform these procedures on a larger number of patients, such as patients with uncertain pre-operatory localization, bigger adenomas, and multiglandular disease, at least in part due to the availability of IOPTH [4-7,24-30]. Furthermore, previous radiation and simultaneous thyroid disease no longer represent absolute contraindications for MIP [30].

Exclusion criteria

Patients with previous conventional neck surgery, persistent or recurrent hyperparathyroidism, mediastinal adenomas or concomitant large goiter are not suitable for MIP approaches [2].

Sample type

PTH is usually measured in serum tubes with or without gel [31], in order to assay calcium or other analytes, too [31,32]. However, EDTA coated tubes are preferred for IOPTH due to their faster processing time in comparison to serum. The sample can be centrifugated immediately, in order to shorten the turnaround time (TAT) by reducing the clotting time [32].

Sampling site

The site of sampling is usually a peripheral vein (peripheral arterial accesses are occasionally used, too [33]). Central veins can be also considered, for the advantage of having only one invasive access for blood drawing. In this case, it has to be noted that PTH levels are higher when measured in central venous samples taken before and 10 minutes after gland resection, but they become comparable to those measured in peripheral venous samples 15 minutes after excision [34-36]. Therefore, both peripheral and central sample sites are acceptable for use but the recommendation is to choose only one site for all the samples [38]. Nevertheless, in cases where peripheral sampling is compromised, changing from a peripheral to a central site should not alter the predictive accuracy of MIP [37].

Sampling time

The time of sampling is critical for the correct interpretation of the post-excision results. Consistent with this, it is recommended to use a validated protocol of IOPTH measurement [2]. The Miami protocol was the first method to be described and one of the most used today. It consists of taking a pre-incision sample before skin incision, a pre-excision (T0) sample before clamping the blood supply to the parathyroid gland; and a 5- and a 10-minute sample after gland resection (T1). If PTH values drop more than 50% compared to the highest level (either pre-incision or pre-excision) to that at 10 minutes after excision, the surgical procedure can be stopped [38,39].

Nevertheless, a unanimous agreement on the number and timing of withdrawals has still not been reached. The baseline timepoint (T0) most frequently used is the pre-incision stage, corresponding to the collection of blood in the operating room before skin incision [40, 41]. Several studies have addressed the issue of the optimal time for collecting pre-incision samples. In particular, Garbutt et al. [42] observed that baseline PTH measured 10 minutes after positioning the patient and inducing general anesthesia was significantly lower and therefore better than that measured before the induction of anesthesia, when the Miami criteria for results interpretation were used [42]. When a MGD is suspected, however, Hong et al. [43] recommend to collect the T0 samples in the pre-induction phase, in order to avoid possible errors ascribed to more elevated post-induction values. Some surgeons are used to measuring PTH in pre-incision and pre-excision samples and then choosing the higher of the two as the baseline PTH value [44]. Nevertheless, it has to be noted that the manipulation of the area, especially with surgeons that do not have much experience, may induce a false PTH increase in pre-excision samples that can lead to an incorrect interpretation of PTH changes [44,45].

Due to the half-life of PTH, which is less than 5 minutes, the second sample (T1) can be withdrawn 5 minutes after gland resection. However, the T1 sample is more frequently collected after 10 minutes, as it seems to be the most accurate time at predicting a complete excision [46-48]. Then, if there is a 50% reduction of PTH between T0 and T1, surgery can be considered successful and terminated without further exploration [42,47]. If this is not the case, surgeons can proceed as follows: a) they can extend the exploration, repeating the protocol for blood sampling after each excision; b) they can obtain another sample after 20 minutes from excision (T2). Then if there is at least a 50% reduction of PTH between T0 and T2, the procedure can be stop-

Table 1. Intra-instrument turnaround time (time from starting the assay to obtaining the result) for parathyroid hormone (PTH) using routine and/or intra-operative calibration, when available.

Test	Manufacturer	Generation	Method	Intra-instrument turnaround time
ARCHITECT Intact PTH	Abbott	2°	CMIA	routine: 40 minutes intraoperative: 18 minutes
Access Intact PTH	Beckman Coulter	2°	CMIA	routine: 30 minutes intraoperative: 15 minutes
Liaison N - tact PTH	DiaSorin	2°	CLIA	routine: 25 minutes
Liaison 1 - 84 PTH	DiaSorin	3°	CLIA	routine: 30 minutes
Vitros iPTH	OCD	2°	ELISA	routine: 32 minutes
Elecsys 2010 PTH	Roche	2°	ECLIA	routine: 20 minutes
ADVIA Centaur iPTH	Siemens	2°	CLIA	routine: 18 minutes
VIDAS PTH (1 - 84)	BioMérieux	3	ELFA	routine: 24 minutes
Lumipulse Whole PTH	Fujirebio	3°	CLEIA	routine: 35 minutes
QuiCk-Intraoperative Bio-Intact PTH (1 - 84)	Nichols Institute Diagnostics	3°	CMIA	intraoperative: 8 minutes
Cobas 1 - 84 PTH	Roche	3°	ECLIA	routine: 18 minutes intraoperative: 9 minutes
STAT-IO-I-PTH	Future Diagnostics	3°	CLIA	intraoperative: 8 minutes

CMIA - chemiluminescent magnetic microparticle immunoassay, CLIA - chemiluminescence immunoassay, ELISA - enzyme-linked immunosorbent assay, ECLIA - electrochemiluminescence immunoassay, ELFA - enzyme-linked fluorescence assay, CLEIA - chemiluminescent enzyme immunoassay system, CMIA - chemiluminescent microparticle immunoassay.

Table 2. The most common intra-operative parathyroid hormone (IOPTH) monitoring criteria used for prediction of outcomes of parathyroid surgery.

Criterion	Definition
Miami [70,71]	IOPTH drop of ≥ 50% from the highest of either pre-incision or pre-excision PTH level at 10 minutes after excision of hyperfunctioning parathyroid gland(s)
Vienna [72]	IOPTH drop of ≥ 50% from the pre-incision baseline value within 10 minutes after excision
Rome [48]	IOPTH drop greater than 50% from the highest pre-excision level, and/or IOPTH concentration within the reference range at 20 minutes post-excision, and/or ≤ 7.5 ng/L lower than the value at 10 minutes post-excision
Halle [72]	IOPTH decay into the low normal range (≤ 35 ng/L) within 15 minutes after excision of hyperfunctioning parathyroid gland(s)

ed without further exploration. Otherwise, further dissection/exploration should continue until the Miami criterion is fulfilled or a complete BNE has been done [47, 49].

Pre-analytical phase

Preparation of the sample

If the measurements are performed directly in the operating room, using a point of care testing (POCT), the whole blood collected at each time point can be immediately used to perform the IOPTH test. If the measurements are performed in a central laboratory, each EDTA sample, at each time point, should immediately be delivered to the laboratory. There, the laboratory staff should centrifuge blood samples, as they arrive, for 5 minutes at 2,200 g, and they should perform the IOPTH assay using a dedicated instrument.

Sample acceptability

Hemolysis of IOPTH samples occurs commonly and can falsely decrease IOPTH levels by 20 to 50% [50, 51]. It is important to know that such a decrease can be sufficient to contribute to either a false negative or a false positive result, leading to failed parathyroidectomy or unnecessary additional exploration [50,51]. As a consequence, hemolyzed samples should be rejected, according to manufacturer's instruction.

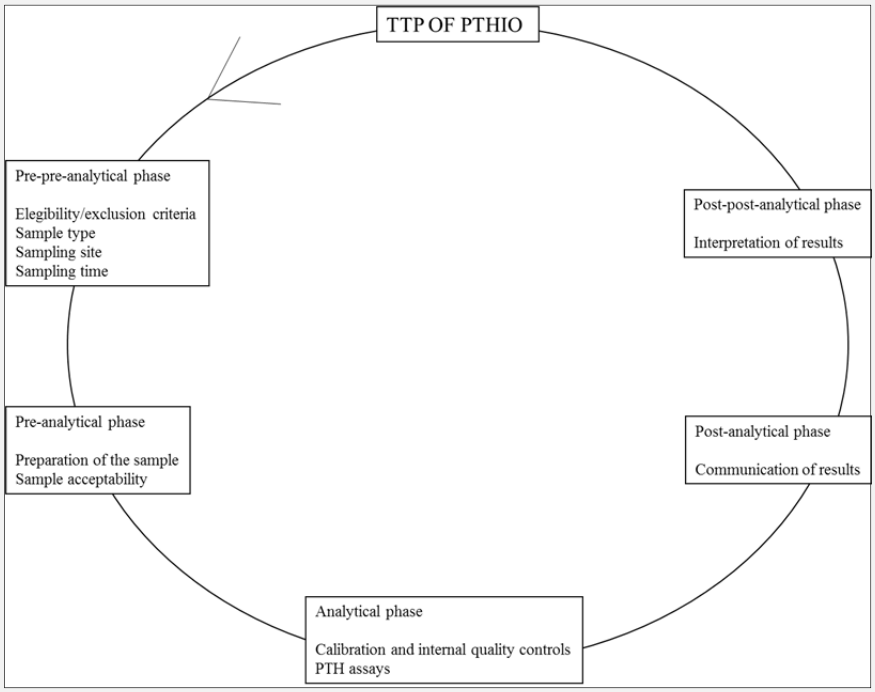

Figure 1. The total testing process (TTP) of intra-operative parathyroid hormone (IOPTH).

Analytical phase

Calibration and internal quality controls

IOPTH usually needs a proper calibration (intraoperative mode) of the assay, according to manufacturer's instructions, to allow a reduction of assay time with respect to routine mode. Consequently, when a surgeon schedules a MIP with IOPTH, the laboratory staff should be advised at least one day before in order to have time to perform the specific calibration, if available, [7] and verify internal quality controls (IQC) [52]. Moreover, the surgery staff should also communicate to the laboratory staff the patient's identification, the telephone number of the surgery room, and the time when the MIP will be performed.

The latter is essential to plan IQC in time to have the instrument ready for the first sample arrival. Laboratory scientists should be aware of possible failures of the desirable quality specifications of IQC, as they might need additional time necessary to re-run IQC or re-calibrate the assay, if necessary. External quality assurance/proficiency testing should also be periodically performed, when available [52,53].

PTH assays

PTH is currently measured with "second" and "third" generation assays, which use similar detection systems and are referred to as "sandwich" assays. The "first generation" assays are not used anymore, mostly due to their lack of specificity, since they also detected inactive C-terminal PTH fragments. The "second generation" assays are also known as "intact" PTH assays, as they were initially thought to recognize only intact (1 - 84) PTH. These "intact" PTH assays were developed to detect PTH with two different antibodies: a capture antibody directed towards the carboxyl terminal and a detection antibody directed towards the amino terminal (first 34 amino acids) [54,55]. Unfortunately, it was later demonstrated that these assays showed cross-reactivity with non-(1 - 84) PTH fragments (e.g., (7 - 84) PTH [56], (10 - 84) PTH, (15 - 84) PTH [57-58]), which represent approximately 20% of circulating PTH in healthy individuals and up to 50% in terminal renal failure patients [59]. The (1 - 84) PTH is the biologically active fragment on bones and kidneys and its activity is mediated by type 1 PTH/PTHrP receptor. By contrast, other PTH fragments, above all (7 - 84) PTH, interact with C-PTH receptors and may have different or even inhibitory actions with respect to (1 - 84) PTH [57,60-62]. As a consequence, when second generation assays are used in patients with renal failure, who tend to accumulate these fragments, biologically active PTH may be overestimated. Moreover, although the results of different intact PTH assays normally correlate well, a remarkable discrepancy in PTH concentrations has been observed

in CDK patients [55].
For these reasons, "third generation" assays were developed. They are known as "whole" PTH assays as they recognize only the biologically active (1 - 84) PTH. This recognition is guaranteed by the use of an anti-N-terminal antibody that is directed towards the very first amino acids (1 to 4) of the peptide while the anti-C-terminal antibody is similar to the one of the "intact" PTH assays [63]. However, not even this third-generation assay is perfect, as it cross-reacts with a N-terminal form of PTH, phosphorylated on Ser-17, which is overproduced in parathyroid carcinoma but rarely in cases of severe pHPT [61,62]. This form is called amino-PTH and is not recognized by the second-generation assays. It contributes to the inter-method variability of PTH assays, since the recognition may vary between them [55]. Finally, it is well acknowledged by now that the PTH peptide contains 2 methionines, in position 8 and 18 [55,63]. These methionines can be oxidized and, as such, PTH becomes biologically inactive. To date, the accessible immunoassays are not capable of making a distinction between the oxidized and non-oxidized PTH and therefore cross-react with this inactive form. Hence, the development of a "fourth-generation" assay, which would recognize only the non-oxidized (1 - 84) PTH, has been taken into consideration [63].
Usually, PTH concentrations are lower when measured with the third-generation assay and therefore the third/second generation PTH ratio is < 1 [64]. It was noted, however, that when amino-PTH is overproduced, PTH concentrations measured with the third-generation assays are higher than those measured with the second one and there is an inversion of the third/second generation PTH ratio (> 1) [63,64]. Some authors suggest that the inverted ratio could be used as a parathyroid cancer marker [63,65]. Cavalier et al. reported a sensitivity of 81.8% and specificity of 97.3% in detecting parathyroid cancer among pHPT patients [66].
Several studies showed that when it comes to the diagnosis of pHPT, the diagnostic sensitivity of both the generations is similar [60,67,68]. However, it should be kept in mind that the assays are different and therefore not interchangeable.
In the intra-operative monitoring during the surgery, the importance is given to the trend evaluation of PTH (delta value) and that being so, it does not matter if the assay is second or third generation, although one must choose one method or the other and be consistent. However, in the intra-operative monitoring during the surgery, the processing time (time from starting the assay to obtaining the result) is crucial (Table 1); as a consequence, faster methods should be the preferable choice.

Post-analytical phase
IOPTH is a critical test [69], consequently any result should be rapidly communicated to the surgery staff, likely using a call-back communication. To the best of our knowledge, there are no indications regarding how to report IOPTH results but according to the best laboratory practice, a report should contain both IOPTH results and comments regarding sampling time.

Post-post-analytical phase
Interpretation of results
The IOPTH protocol to use for predicting the success of surgery is still questioned [6,47]. The most common IOPTH protocols are Miami, Halle, Rome, and Vienna [6,47] (Table 2), and their overall accuracy, sensitivity, specificity, positive predictive value, and negative predictive values, depending on the patient population, have already been described in detail [6,39,47,69-71]. Nevertheless, given the lack of standardization of baseline PTH samples, sampling times, sampling frequency, and the cutoff percentage of PTH decrease [6], it is still controversial as to which is the best IOPTH protocol to use.
Notwithstanding these limits, it has been argued that the Miami and Vienna protocols/criteria are the most effective in predicting surgery success intraoperatively [39, 47]. On the contrary, in case of multiglandular diseases, the Rome and Halle criteria seem to be more precise [47]. However, using these on patients with concordant pre-operative imaging would lead to a remarkably higher number of needless additional explorations and to only a minimal increase of success rate [39].
False positive and false negative results should always be considered [39,69,72]. A false positive result is observed when, after the removal of suspicious glands, PTH values drop to a level indicating the correctness of the gland excision but the patient remains hypercalcemic. It is more likely to occur in patients with double adenomas [72], renal impairment [73,74], simultaneous thyroid surgery, and parathyroid cancer [7]. On the contrary, a false negative result is observed when PTH values remain elevated after parathyroid removal but the exploration does not show any additional enlarged/abnormal parathyroid gland and the patient becomes normocalcemic. It is more likely to occur when more specific criteria, like Rome and Halle, are used [72]. As said before, false negative results can also be due to the use of pre-excision samples for the baseline PTH value (instead of pre-incision), as excessive manipulation of the adenoma could result in a pre-excision spike [75]. This spike could mask the success of the operation despite the eradication of the pathology, due to post-excision IOPTH level apparently not falling adequately. For this reason, it has been suggested [76] to monitor IOPTH also at 20 minutes after gland resection, preventing unnecessary BNE.
Finally, one of the major causes of false positive and negative results is unrecognized hemolysis, which significantly reduce PTH [50,51]. Specifically, if this is the case of pre-incision and pre-excision samples, it can mask a sufficient drop in IOPTH after the resection of the abnormal gland, leading to unnecessary exploration or conversion to BNE and, consequently, unnecessary morbidity. Conversely, if it is the post-excision sample that is hemolyzed, the IOPTH drop observed after the

resection of the abnormal gland could erroneously meet the criteria for cure, leading to possible failure of parathyroidectomy and the need for reoperation [50,51].

CONCLUSION

The possibility to measure IOPTH represents a clear advantage for patients and physicians, which has changed the surgical management of hyperparathyroidism over the years. Nevertheless, it is important to know the potential sources of errors that can occur in the whole testing process, in order to use and interpret IOPTH correctly.

Acknowledgment:
The authors would like to thank Dr. Piero Cappelletti for collaborating on the editing of the Table 1.

Funding:
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for profit sectors.

Disclaimers:
None.

Declaration of Interest:
There is no conflict of interest.

References:

1. Bilezikian JP, Cusano NE, Khan AA, Liu JM, Marcocci C, Bandeira F. Primary hyperparathyroidism. Nat Rev Dis Primers 2016;2:16033 (PMID: 27194212).

2. Wilhelm SM, Wang TS, Ruan DT et al. The American Association of Endocrine Surgeons Guidelines for Definitive Management of Primary Hyperparathyroidism. JAMA Surg 2016;151: 959-68 (PMID: 27532368).

3. Wilke MH, Schenker M, Hoffmann G. Detection and documentation of DRG-relevant comorbidities using laboratory tests. Aust Health Rev 2002;25:152-160 (PMID: 12136557).

4. AACE/AAES Task Force on Primary Hyperparathyroidism. The American Association of Clinical Endocrinologists and the American Association of Endocrine Surgeons position statement on the diagnosis and management of primary hyperparathyroidism. Endocr Pract 2005;11(1):49-54 (PMID: 16033736).

5. Bobanga ID, McHenry CR. Is intraoperative parathyroid hormone monitoring necessary for primary hyperparathyroidism with concordant preoperative imaging? Am J Surg 2017;213:484-8 (PMID: 28017299).

6. Patel KN, Caso R. Intraoperative Parathyroid Hormone Monitoring: Optimal Utilization. Surg Oncol Clin N Am 2016;25:91-101 (PMID: 26610776).

7. Shawky MS, Abdel-Aziz TE, Kurzawinski TR. Modern Parathyroid Surgery and Intra-Operative Hormone Monitoring; Present Status, Future Concepts. J Steroids Horm Sci 2018, 9:1. DOI: 10.4172/2157-7536.1000190.

8. Plebani M, Laposata M, Lundberg GD. The brain-to-brain loop concept for laboratory testing 40 years after its introduction. Am J Clin Pathol 2011;136:829-33 (PMID: 22095366).

9. Plebani M. Towards a new paradigm in laboratory medicine: the five rights. Clin Chem Lab Med 2016;54:1881-91 (PMID: 27732 557).

10. Bayat H. Selecting multi-rule quality control procedures based on patient risk. Clin Chem Lab Med 2017;55:1702-8 (PMID: 282366 26).

11. Goltzman D, Mannstadt M, Marcocci C. Physiology of the Calcium-Parathyroid Hormone-Vitamin D Axis. Front Horm Res 2018;50:1-13 (PMID: 29597231).

12. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol 2015;10:1257-72 (PMID: 25287933).

13. Brown EM. Role of the calcium-sensing receptor in extracellular calcium homeostasis. Best Pract Res Clin Endocrinol Metab 2013;27:333-43 (PMID: 23856263).

14. Bilezikian JP, Silverberg SJ. Asymptomatic Primary Hyperparathyroidism. N Engl J Med 2004;350:1746-51 (PMID: 15103001).

15. Walker MD, Silverberg SJ. Primary hyperparathyroidism. Nat Rev Endocrinol 2018;14:115-25 (PMID: 28885621).

16. D'Amour P, Brossard JH. Carboxyl-terminal parathyroid hormone fragments: role in parathyroid hormone physiopathology. Curr Opin Nephrol Hypertens 2005;14:330-336 (PMID: 159310 00).

17. Rubin MR, Bilezikian JP, McMahon DJ, et al. The Natural History of Primary Hyperparathyroidism with or without Parathyroid Surgery after 15 Years. J Clin Endocrinol Metab 2008;93:3462-70 (PMID: 18544625).

18. Vestergaard P, Mosekilde L. Parathyroid surgery is associated with a decreased risk of hip and upper arm fractures in primary hyperparathyroidism: a controlled cohort study. J Intern Med 2004;255:108-14 (PMID: 14687246).

19. Bilezikian JP, Brandi ML, Eastell R, et al. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the Fourth International Workshop. J Clin Endocrinol Metab 2014;99:3561-9 (PMID: 25162665).

20. Udelsman R, Åkerström G, Biagini C, et al. The surgical management of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. J Clin Endocrinol Metab 2014;99:3595-606 (PMID: 25162669).

21. Madkhali T, Alhefdhi A, Chen H, Elfenbein D. Primary hyperparathyroidism 2016;32(1):58-66 (PMID: 26985167).

22. Barczynski M, Golkowski F, Konturek A, et al. Technetium-99m-sestamibi subtraction scintigraphy vs. ultrasonography combined with a rapid parathyroid hormone assay in parathyroid aspirates in preoperative localization of parathyroid adenomas and in directing surgical approach. Clin Endocrinol (Oxf) 2006;65:106-13 (PMID: 16817828).

23. Lombardi CP, Raffaelli M, Traini E, De Crea C, Corsello SM, Bellantone R. Video-assisted minimally invasive parathyroidectomy: benefits and long-term results. World J Surg 2009;33:2266-81 (PMID: 19219494).

24. Dobrinja C, Stenner E, Trevisan G, Micheli W, Biasioli B, Liguori G. [Minimally invasive video-assisted parathyroidectomy and intraoperative parathyroid hormone monitoring]. G Chir 2010;31:319-21 (PMID: 20646382).

25. Norman J, Chheda H. Minimally invasive parathyroidectomy facilitated by intraoperative nuclear mapping. Surgery 1997;122: 998-1004 (PMID: 9426412).

26. Goldstein RE, Billheimer D, Martin WH, Richards K. Sestamibi scanning and minimally invasive radioguided parathyroidectomy without intraoperative parathyroid hormone measurement. Ann Surg 2003;237:722-30 (PMID: 12724639).

27. Laird AM, Libutti SK. Minimally Invasive Parathyroidectomy Versus Bilateral Neck Exploration for Primary Hyperparathyroidism. Surg Oncol Clin N Am 2016;25(1):103-18 (PMID: 26610777).

28. Walsh NJ, Sullivan BT, Duke WS, Terris DJ. Routine bilateral neck exploration and four-gland dissection remains unnecessary in modern parathyroid surgery. Laryngoscope Investig Otolaryngol 2018;4:188-92 (PMID: 30828638).

29. Norman, J, Lopez J, Politz D. Abandoning unilateral parathyroidectomy: why we reversed our position after 15,000 parathyroid operations. J Am Coll Surg 2012;214:260-9 (PMID: 22265807).

30. Dobrinja C, Santandrea G, Giacca M, Stenner E, Ruscio M, de Manzini N. Effectiveness of Intraoperative Parathyroid Monitoring (ioPTH) in predicting a multiglandular or malignant parathyroid disease. Int J Surg 2017;41:S26-S33 (PMID: 28506410).

31. World Health Organization. Diagnostic Imaging and Laboratory Technology. (2002). Use of anticoagulants in diagnostic laboratory investigations. Geneva: World Health Organization. (http://www.who.int/iris/handle/10665/65957)

32. Cavalier E, Plebani M, Delanaye P, Souberbielle JC. Considerations in parathyroid hormone testing. Clin Chem Lab Med 2015; 53:1913-9 (PMID: 26035114).

33. Urquhart AC, Wiley C. Arterial and venous parathyroid hormone levels in minimally invasive surgery. Arch Otolaryngol Head Neck Surg 2005;131:137-9 (PMID: 15723945).

34. Woodrum DT, Saunders BD, England BG, Burney RE, Doherty GM, Gauger PG. The influence of sample site on intraoperative PTH monitoring during parathyroidectomy. Surgery 2004;136: 1169-75 (PMID: 15657572).

35. Beyer TD, Chen E, Ata A, DeCresce R, Prinz RA, Solorzano CC. A prospective evaluation of the effect of sample collection site on intraoperative parathormone monitoring during parathyroidectomy. Surgery 2008;144:504-9 (PMID: 18847632).

36. Edwards CM, Folek J, Dayawansa S, et al. Central venous parathyroid hormone monitoring using a novel, specific anatomic method accurately predicts cure during minimally invasive parathyroidectomy. Am J Surg 2016;212:1154-61 (PMID: 27793324).

37. Abdel-Misih SR, Broome JT, Li X, et al. Changing intraoperative parathyroid hormone collection sites from peripheral to central does not affect predictive accuracy during minimally invasive parathyroidectomy. Ann Surg Oncol 2011;18:771-6 (PMID: 20949324).

38. Irvin GL, Carneiro DM. Rapid parathyroid hormone assay guided exploration. Oper Tech Gen Surg 1999;1:18-27. (https://www.sciencedirect.com/science/article/pii/S1524153X99800049)

39. Barczyński M, Konturek A, Hubalewska-Dydejczyk A, Cichon S, Nowak W. Evaluation of Halle, Miami, Rome, and Vienna intraoperative iPTH assay criteria in guiding minimally invasive parathyroidectomy. Langenbecks Arch Surg 2009;394:843-9 (PMID: 19529957).

40. Callender GG, Udelsman R. Surgery for primary hyperparathyroidism. Cancer 2014;120:3602-16 (PMID: 25042934).

41. Perrier ND, Dickson PV, Figueroa AS. Parathyroid exploration for primary hyperparathyroidism. 2018. (https://www.uptodate.com/contents/parathyroid-exploration-for-primary-hyperparathyroidism)

42. Garbutt L, Sigvaldason H, Sharaf Eldin MH, Dembinski T, Nason RW, Pathak KA. What is the most appropriate intraoperative baseline parathormone? A prospective cohort study. Int J Surg 2016;25:49-53 (PMID: 26639086).

43. Hong JC, Morris LF, Park EJ, Ituarte PH, Lee CH, Yeh MW. Transient increases in intraoperative parathyroid levels related to anesthetic technique. Surgery 2011;150:1069-75 (PMID: 22136823).

44. Harrison BJ, Triponez F. Intraoperative adjuncts in surgery for primary hyperparathyroidism. Langenbecks Arch Surg 2009;394: 799-809 (PMID: 19590891).

45. Gioviale MC, Damiano G, Altomare R, et al. Intraoperative measurement of parathyroid hormone: A Copernican revolution in the surgical treatment of hyperparathyroidism. Int J Surg 2016;28: S99-102 (PMID: 26708859).

46. Naik AH, Wani MA, Wani KA, Laway BA, Malik AA, Shah ZA. Intraoperative Parathyroid Hormone Monitoring in Guiding Adequate Parathyroidectomy. Indian J Endocrinol Metab 2018;22: 410-6 (PMID: 30090736).

47. Barczyński M, Gołkowski F, Nawrot I. The current status of intraoperative iPTH assay in surgery for primary hyperparathyroidism. Gland Surg 2015;4:36-43 (PMID: 25713778).

48. Shawky MS. Intraoperative parathyroid hormone monitoring in primary hyperparathyroidism; resolving controversies and debates. J Parathyr Dis 2017;5:3-10. (http://www.jparathyroid.com/Abstract/JPD_20160924144547)

49. Calò PG, Pisano G, Loi G, et al. Intraoperative parathyroid hormone assay during focused parathyroidectomy: the importance of 20 minutes measurement. BMC Surg 2013;13:36 (PMID: 24044556).

50. Moalem J, Ruan DT, Farkas RL, et al. Prospective evaluation of the rate and impact of hemolysis on intraoperative parathyroid hormone (IOPTH) assay results. Ann Surg Oncol 2010;17:2963-9 (PMID: 20552403).

51. Moalem J, Ruan DT, Farkas RL, et al. Hemolysis falsely decreases intraoperative parathyroid hormone levels. Am J Surg 2009;197:222-6 (PMID: 19095211).

52. CLSI. Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions. 4th ed. CLSI guideline C24. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. (https://clsi.org/standards/products/clinical-chemistry-and-toxicology/documents/c24/)

53. Topic E, Nikolac N, Panteghini M, et al. How to assess the quality of your analytical method? Clin Chem Lab Med 2015;53: 1707-18 (PMID: 26408611).

54. Couchman L, Taylor DR, Krastins B, Lopez MF, Moniz CF. LC-MS candidate reference methods for the harmonisation of parathyroid hormone (PTH) measurement: a review of recent developments and future considerations. Clin Chem Lab Med 2014;52: 1251-63 (PMID: 24762644).

55. Kakuta T, Ishida M, Fukagawa M. Critical Governance Issue of Parathyroid Hormone Assays and its Selection in the Management of Chronic Kidney Disease Mineral and Bone Disorders. Ther Apher Dial 2018;22:220-8 (PMID: 29781225).

56. Sturgeon CM, Sprague S, Almond A, et al. Perspective and priorities for improvement of parathyroid hormone (PTH) measurement - A view from the IFCC Working Group for PTH. Clin Chim Acta 2017;467:42-7 (PMID: 27746210).

57. D'Amour P. Circulating PTH molecular forms: what we know and what we don't. Kidney Int Suppl 2006;102:S29-33 (PMID: 16 810307).

58. Cappelletti P, Tozzoli R. Laboratory & Hypercalcemia. Hypercalcemia Pathophysiology and Treatment - Franco Lumachi. Bentham Science Publishers 2012;45-61. (http://www.eurekaselect.com/51729/chapter/laboratory-%2526-hypercalcemia)

59. D'Amour P, Brossard JH, Räkel A, Rousseau L, Albert C, Cantor T. Evidence that the amino-terminal composition of non-(1 - 84) parathyroid hormone fragments starts before position 19. Clin Chem 2005;51:169-76 (PMID: 15514090).

60. Bonanséa TC, Ohe MN, Brandão C, et al. Experience with a third-generation parathyroid hormone assay (BIO-PTH) in the diagnosis of primary hyperparathyroidism in a Brazilian population. Arch Endocrinol Metab 2016;60:420-5 (PMID: 27812604).

61. Silva BC, Bilezikian JP. Anabolic and Catabolic Pathways of Parathyroid Hormone on the Skeleton. The Parathyroids: Basic and Clinical Concepts. Academic Press 2015;233-244. (https://www.sciencedirect.com/science/article/pii/B9780123971661000151)

62. Fuleihan GE, Jüppner H. Parathyroid hormone assays and their clinical use. 2018. (https://www.uptodate.com/contents/parathyroid-hormone-assays-and-their-clinical-use)

63. Cavalier E, Delanaye P, Nyssen L, Souberbielle JC. Problems with the PTH assays. Ann Endocrinol (Paris) 2015;76:128-33 (PMID: 25916762).

64. Einbinder Y, Benchetrit S, Golan E, Zitman-Gal T. Comparison of Intact PTH and Bio-Intact PTH Assays Among Non-Dialysis Dependent Chronic Kidney Disease Patients. Ann Lab Med 2017; 37:381-7 (PMID: 28643486).

65. Caron P, Maiza JC, Renaud C, Cormier C, Barres BH, Souberbielle JC. High third generation/second generation PTH ratio in a patient with parathyroid carcinoma: clinical utility of third generation/second generation PTH ratio in patients with primary hyperparathyroidism. Clin Endocrinol (Oxf) 2009;70: 533-8 (PMID: 18782355).

66. Cavalier E, Betea D, Schleck ML, et al. The third/second generation PTH assay ratio as a marker for parathyroid carcinoma: evaluation using an automated platform. J Clin Endocrinol Metab 2014;99:E453-7 (PMID: 24423313).

67. Boudou P, Ibrahim F, Cormier C, Chabas A, Sarfati E, Souberbielle JC. Third- or second-generation parathyroid hormone assays: a remaining debate in the diagnosis of primary hyperparathyroidism. J Clin Endocrinol Metab 2005;90:6370-2 (PMID: 16189258).

68. Eastell R, Brandi ML, Costa AG, D'Amour P, Shoback DM, Thakker RV. Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. J Clin Endocrinol Metab 2014;99:3570-9 (PMID: 25162666).

69. Vaghaiwalla TM, Khan ZF, Lew JI. Review of intraoperative parathormone monitoring with the miami criterion: A 25-year experience. World J Surg Proced 2016;6:1-7. (https://www.wjgnet.com/2219-2832/full/v6/i1/1.htm)

70. Riss P, Kaczirek K, Heinz G, Bieglmayer C, Niederle B. A "defined baseline" in PTH monitoring increases surgical success in patients with multiple gland disease. Surgery 2007;142:398-404 (PMID: 17723893).

71. Carneiro DM, Solorzano CC, Nader MC, Ramirez M, Irvin GL 3rd. Comparison of intraoperative iPTH assay (QPTH) criteria in guiding parathyroidectomy: which criterion is the most accurate? Surgery 2003;134:973-9 (PMID: 14668730).

72. Sitges-Serra A, Díaz-Aguirregoitia FJ, de la Quintana A. Weight difference between double parathyroid adenomas is the cause of false-positive IOPTH test after resection of the first lesion. World J Surg 2010;34:1337-42 (PMID: 20107797).

73. Liu SN, Yusufali AH, Mao ML, Khan ZF, Farrá JC, Lew JI. Stricter ioPTH criterion for successful parathyroidectomy in stage III CKD patients with primary hyperparathyroidism. Surgery 2018 Dec;164(6):1306-10 (PMID: 30037429).

74. Egan RJ, Iliff H, Stechman MJ, Scott-Coombes DM. Intraoperative Parathyroid Hormone Assay Remains Predictive of Cure in Renal Impairment in Patients with Single Parathyroid Adenomas. World J Surg 2018 Sep;42(9):2835-9 (PMID: 29497805).

75. Riss P, Krall C, Scheuba C, Bieglmayer C, Niederle B. Risk factors for "PTH spikes" during surgery for primary hyperparathyroidism. Langenbecks Arch Surg 2013;398:881-6 (PMID: 23828733).

76. Khan ZF, Picado O, Marcadis AR, Farrá JC, Lew JI. Additional 20-Minute Intraoperative Parathormone Measurement Can Minimize Unnecessary Bilateral Neck Exploration. J Surg Res 2019 Mar;235:264-9 (PMID: 30691805).