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Parathyroid Adenomas and Carcinomas (3M)

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National Academy of Clinical Biochemistry Guideline for the Use of Tumor Markers in Parathyroid Gland Adenomas and Carcinomas

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Abbreviations: FHH, familial hypocalciuric hypercalcemia; FIHPT, familial isolated hyperparathyroidism; HPT, hyperparathyroidism; HPT-JTS, hyperparathyroidism-jaw tumor syndrome; IRMA, immunoradiometric assay; LOE, level of evidence; LOH, loss of heterozygosity; MEN, multiple endocrine neoplasia; MEN1, multiple endocrine neoplasia type 1; NACB, National Academy of Clinical Biochemistry; NIH Panel, National Institutes of Health; PTG, parathyroid gland; PTH, parathyroid hormone; TMUGS, tumor marker grading system; SPECT, single photon emission computed tomography ; ULN, upper limit of normal.

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INTRODUCTION

The incidence of primary parathyroid gland (PTG) carcinoma is extremely low, with an estimated annual prevalence of about 0.005%, i.e. five cases of primary PTG carcinoma/100,000 cases of primary hyperparathyroidism (HPT) or less than 1% of all cases of HPT (1). Parathyroid adenomas, on the other hand, account for 85% of all cases of this disease, and they are more prevalent in older women. In addition to the solitary or sporadic PTG tumors, approximately 5% of all PTG tumors are associated with hereditary cancer syndromes such as multiple endocrine neoplasia (MEN) types 1 and 2A, familial isolated HPT (FIHPT), and HPT-jaw tumor syndrome (HPTJG) (2). The incidence of HPT among individuals with the HPTJG syndrome is reported to be 90% with 15% of these cases due to PTG carcinoma (3). In contrast to HPTJG, about 85% to 95% of MEN type 1 cases eventually develop adenomas and HPT (4).

Most PTG tumors are functional and cause over-activity of this gland and excessive secretion of parathyroid hormone (PTH), resulting in HPT. Widespread testing of calcium in blood using automated analyzers in the clinical laboratory has led to the detection of hypercalcemia early in the course of HPT and in the majority of cases before other signs of HPT are apparent. More advanced disease is accompanied by bone pain, kidney stones, or abdominal disturbances (the triad of "bones, stones, and groans"). A palpable neck mass and hoarseness may be the major clinical manifestations, particularly for patients with non-PTH secreting PTG carcinoma. The 2002 National Institutes of Health (NIH) consensus panel on the management of asymptomatic primary hyperparathyroidism (2002 NIH Panel) classified primary HPT into two categories: asymptomatic and symptomatic (5). Asymptomatic primary hyperparathyroidism accounts for 75 to 80% of all HPT cases (6). In both asymptomatic and symptomatic HPT, hypercalcemia in concert with an increased serum PTH concentration is the biochemical hallmark for the diagnosis of primary HPT.

Parathyroidectomy of the affected gland(s) is the definitive primary treatment. Surgical resection should be offered to patients who are symptomatic or who meet the criteria for surgery established by the 2002 NIH Panel (5). In cases where hyperplasia is the underlying abnormality affecting all parathyroid glands, a portion of the glands are left *in situ*, or surgically excised and immediately auto-transplanted into the forearm to maintain long-term calcium homeostasis. Preoperative localization of affected gland(s) using sestamibi scanning or SPECT intra-operative PTH measurement before and after gland excision promotes surgical success (7). In the case of parathyroid gland carcinoma, a complete *en bloc* surgical resection, including the adherence to surrounding tissue is performed. Metastatic PTG carcinoma is treated medically for the signs and symptoms of hypercalcemia. Data are insufficient to assess the

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effectiveness of radiation therapy and chemotherapy in the treatment of metastatic PTG carcinoma (8).

Although surgery is considered the ultimate therapy for parathyroid gland tumors, asymptomatic patients who meet the criteria recommended by the 2002 NIH Panel may be monitored for the onset of disease by measuring serum calcium every six months, and serum creatinine and bone density annually (5). Estrogen, oral bisphosphonates, calcimimetics and raloxifene are administered to reduce bone resorption and calcium levels (5). Recurrent disease is treated with aggressive resection.

Subsequent to parathyroidectomy, patients are followed-up at regular intervals with clinical history, physical examination, and serum markers, such as calcium, PTH, and creatinine. The serum calcium level is the most reliable indicator of disease recurrence (9). Patients with metastatic disease usually manifest recurrent progressive hypercalcemia together with an elevated PTH level (10).

CURRENTLY AVAILABLE MARKERS FOR PARATHYROID TUMORS: NACB RECOMMENDATIONS

Table 1 summarizes the current National Academy of Clinical Biochemistry (NACB) guidelines for the use of parathyroid tumor markers, calcium (total and ionized), PTH, and molecular genetic markers (i.e., mutated MEN1 and HRPT2 genes) in the detection and management of parathyroid tumors. Each of these tests is accompanied by information on its phase of development and the level of supporting evidence (LOE), evaluated according to the tumor marker grading system of Hayes et al. (11) [*see Section 1*]. A more detailed discussion of these tests is presented below.

PARATHYROID HORMONE AND CALCIUM

Before discussing the clinical utility of PTH and calcium measurements in the assessment of PTG adenoma and carcinoma, it is useful to review the principles and advantages and disadvantages of assays for the quantitative measurement of PTH and the use of total versus ionized calcium measurements in the assessment of in vivo calcium levels.

The nascent, undegraded PTH molecule consists of 84 amino acids. Several PTH fragments of various lengths arise from the degradation of this PTH molecule within either the parathyroid glands or peripheral tissues. These fragments include N-terminal, mid-molecule (MM), and C-terminal portions of the nascent, undegraded PTH molecule (12). C-terminal fragments are cleared from the blood, principally by glomerular filtration (13), and their levels are increased in individuals with renal failure (14,15). Confusion over the nomenclature for the

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PTH molecule quantified by various immunoassays stemmed from the use of the term "intact PTH" to refer to the PTH molecule thought to be measured exclusively by 1st-generation IRMAs. Subsequently, these assays were shown to measure both nascent, undegraded PTH and the PTH fragment consisting of amino acids 7 through 84. Therefore, the newer term, "whole molecule PTH" is used in lieu of "intact PTH" to refer to immunometric assays (IMAs) that measure only nascent, undegraded PTH. Thus, to avoid confusion over the terminology of PTH fragments and the analytical specificity of various PTH assays, it is commonplace for these assays to specify the amino acid sequence of the PTH molecule quantified by the antibodies used in these assays. We recommend that PTH_{1-84} be used to refer to nascent, undegraded PTH containing amino acids 1 through 84, also known as "whole molecule PTH." Similarly, PTH_{35-84} refers to a C-terminal PTH molecule consisting of amino acids 35 through 84.

Early PTH radioimmunoassays, which used antibodies directed against epitopes of the mid-molecule or C-terminal regions of PTH_{1-84}, had low diagnostic sensitivity and were also of limited value in the differential diagnosis of mild primary HPT or secondary HPT in patients with end-stage renal disease. The 1st-generation of two-site IRMAs for the quantitative serum measurement of PTH used two antibodies, a capture antibody attached to a solid phase support medium (e.g., a plastic bead), usually directed against an epitope in the C-terminal region of PTH_{1-84}, and a signal antibody, usually directed against an epitope in the N-terminal region of PTH_{1-84} and containing a radioactive label or signal [e.g., iodine-125 (^{125}I)]. Lack of absolute specificity for PTH_{1-84} occurred because both the capture and signal antibodies recognized epitopes that did not include the first six amino acids in the N-terminal region of the PTH_{1-84} molecule (16-18). These assays therefore overestimated the true concentration of PTH_{1-84} in sera from patients with renal failure and decreased clearance of PTH fragments (15,19). The 2nd-generation PTH IMA uses a signal antibody directed against an epitope containing the first four amino acids of PTH_{1-84} and therefore, measures only PTH_{1-84} (19,20). This assay offers improved diagnostic sensitivity over RIAs and 1st generation PTH immunoradiometric assays (IRMAs) in identifying patients with primary HPT. The diagnostic sensitivity of 2nd-generation PTH IRMAs versus RIAs and 1st-generation PTH IRMAs is typically, 96%, 63%, and 73%, respectively (21). The 2nd-generation PTH IRMAs are also more reliable when determining post-operatively whether all hypersecreting PTG tissue has been successfully removed during parathyroidectomy for primary and secondary HPT, because the PTH_{1-84} molecule declines more rapidly than other PTH fragments (22).

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Total versus Ionized Calcium

About 45% of the total calcium level in whole blood circulates as free (or ionized) calcium, which is physiologically active. The remaining calcium is either bound to protein (40%), mostly albumin, or complexed with anions such as bicarbonate, citrate, phosphate, or lactate. The 2002 NIH Panel did not recommend using ionized calcium to monitor patients with hyperparathyroidism who underwent parathyroidectomy, presumably because ionized calcium measurement requires the use of arterial whole blood specimens, special instrumentation not routinely available in most clinical laboratories, and special handling of specimens, including collection of anti-coagulated whole blood in tubes or syringes that maintain anaerobic conditions, are kept refrigerated, not frozen, on wet ice, and are delivered promptly to the laboratory for testing (23). Direct measurement of ionized calcium concentration is typically performed using a blood gas analyzer. Since ionized calcium is the physiologically active form of the circulating calcium and is not affected by alterations in serum albumin concentration, ionized calcium measurements are recommended over corrected total calcium values whenever possible. In lieu of direct ionized calcium measurement, many laboratories determine a "corrected" total serum calcium concentration that corrects the total serum calcium concentration based on the serum albumin concentration because albumin is the major serum protein to which calcium is bound and the albumin concentration affects the total calcium level. A variety of formulas have been proposed for correcting measured values for serum total calcium levels using formulas based on the total protein or albumin concentration of the patient's specimen. Examples of these formulas are provided below (24,25):

$$[\text{Total calcium, mg/dL}]_{\text{corrected}} = \frac{[\text{Total calcium, mg/dL}]_{\text{observed}}}{0.6 + ([\text{Total protein, g/dL}]/18.5)}$$

$$[\text{Total calcium, mg/dL}]_{\text{corrected}} = [\text{Total calcium, mg/dL}]_{\text{observed}} + \{0.8 * (4.0 - [\text{Albumin, g/dL}])\}$$

Corrected total calcium values, however, do not always allow clinicians to diagnose hypercalcemia in patients with HPT, chronic renal failure, or liver disease (26). Moreover, it may be more appropriate to measure ionized calcium concentration in neonates, in patients receiving large volumes of blood or plasma containing calcium-binding anticoagulants, and in patients undergoing hemodialysis (26).

The following sections discuss the clinical utility and supporting evidence for the use of PTH and calcium measurements in the diagnosis of HPT.

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Clinical utility of PTH and calcium in the diagnosis of HPT

In the presence of persistent hypercalcemia, an elevated PTH concentration virtually establishes the diagnosis of HPT (5) and excludes a diagnosis of hypercalcemia of malignancy associated with tissues other than the parathyroid gland(s). However, HPT may not necessarily be due to parathyroid adenoma, hyperplasia, or carcinoma. Other conditions, such as a defective calcium sensor, familial hypocalciuric hypercalcemia (FHH), and chronic lithium therapy may have the same clinical presentation as HPT. Moreover, mild hypercalcemia should be confirmed by repeated measurement of serum calcium concentration (preferably ionized calcium) especially in cases of borderline hypercalcemia (27). The degree of PTH elevation has been correlated with various disease states (28). Mean levels of PTH and serum total calcium concentrations are higher in patients with PTG carcinoma than in those with PTG adenoma. About two-thirds of all cases of PTG carcinoma are associated with a mean total serum calcium value between 3.75 mmol/L and 4.0 mmol/L (15 mg/dL and 16 mg/dL), while only 10% of individuals with parathyroid gland adenomas have total calcium values of 2.75 mmol/L to 3.0 mmol/L (11 to 12 mg/dL) (29,30). Moreover, the PTH level in patients with parathyroid gland carcinoma is consistently higher than that found in patients with benign parathyroid gland disease. Approximately 70% of patients with parathyroid gland carcinoma have a PTH level more than 5-fold higher than the upper limit of normal (ULN) for PTH (29,31).

Clinical utility of parathyroid hormone and calcium in treatment

The 2002 NIH Panel recommended that asymptomatic patients with a serum total calcium level more than 0.25 mmol/L (1.0 mg/dL) above the ULN should be considered candidates for parathyroidectomy (5). The members of the NACB Panel agree with this recommendation.

Intra-operative parathyroid hormone measurement and recommended procedures

Due to its short half-life ($t_{1/2}$ <5 min), intra-operative measurement of PTH, performed directly in the surgical suite, in close proximity to the surgical suite [i.e., "point-of-surgery testing" (POST) (32)], or in a central laboratory after prompt delivery of specimens collected in the surgical suite, is useful and should be performed in patients undergoing parathyroidectomy to assess whether all hyperfunctioning parathyroid tissue has been successfully removed (32-34). A decrease in the PTH concentration of >50% from the baseline level at 5-10 minutes after excision of all suspected hyperfunctioning parathyroid tissue suggests the absence of any residual hyperfunctioning tissue (32,33). The advent of intra-operative PTH testing has reduced the post-operative failure rate of initial parathyroidectomy surgery from 6.0% to 1.5% (35,36) and decreased the need for performing frozen sections (37). Moreover, when repeat surgery is

required due to a failed initial surgery, the success rate following re-operation improves from 76% to 94% (38,39). Intra-operative PTH measurement also accurately predicts the outcome of parathyroidectomy (i.e. normocalcemia versus persistent hypercalcemia) in patients with solitary adenomas and improves the accuracy of pre-operative localization for PTG tumors by 9% to 15% (40). Intra-operative PTH testing is relatively accurate for 85% of patients with a solitary parathyroid gland tumor but is not as accurate in detecting the presence of two or more parathyroid adenomas (41,42). Multiple adenomas in up to 50% of patients having these adenomas may not be detected following intra-operative PTH testing (41,42). Point-of-surgery testing is preferred over central laboratory testing for PTH during parathyroid surgery, unless patients' blood samples obtained intraoperatively can be rapidly and reliably transported to a central laboratory, tested promptly, and the results transmitted within 20 minutes to the surgeon in the operating room (43).

Carter and Howanitz (44) reviewed 165 published reports on intra-operative testing for PTH and proposed the following guidelines for such testing:

1. The baseline PTH concentration should be determined before any manipulation of the parathyroid gland has occurred and, preferably, immediately prior to the induction of anesthesia;

2. PTH should be tested at 10 minutes following the removal of any suspected hyperfunctioning PTG;

3. If the change in PTH concentration, compared to the baseline level, exceeds a 50% decline in PTH concentration, it can be assumed that all hyperfunctioning parathyroid tissue has been successfully removed;

4. If this criterion is not met, exploration for other hyperfunctioning parathyroid glands should be continued until this criterion is met; and,

5. To reduce variation in PTH test results, the PTH level in all intra-operative specimens obtained from the same patient should be determined using the same method/instrument and operator.

Clinical utility of parathyroid hormone and total calcium levels following parathyroid gland surgery.

Serum calcium and PTH levels decrease rapidly following successful parathyroid surgery. The serum total calcium concentration typically falls to a nadir within 24-36 hours post-surgery whereas the serum PTH level typically returns to the normal range within 30 hours post-surgery. Following parathyroid surgery, serum total calcium levels are determined immediately and then monitored serially, especially in patients with large parathyroid adenomas because such

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patients may develop symptomatic hypocalcemia due to "hungry bone" syndrome (45). In such patients, PTH should also be assessed. Hungry bone syndrome occurs in some patients with skeletal demineralization who have undergone parathyroid surgery for HPT and have subsequently been treated with vitamin D to promote bone remineralization in areas of bone loss, which requires calcium from the plasma. Once the process of bone mineralization has been initiated, it may occur so rapidly that too much calcium is taken out of the plasma to meet the needs of those areas of demineralization that are "hungry" to replace their lost bone tissue (and calcium) and the patient may become hypocalcemic and in need of calcium supplementation (45).

Persistent hypercalcemia and elevated PTH concentration following parathyroid surgery indicate the presence of a missed adenoma, failed management of parathyroid malignancy, or misdiagnosis of primary HPT (46). In addition, recurrence of HPT is not particularly rare and may occur more than 20 years after the initial treatment of patients with single or multiple parathyroid gland disease (47).

Serum calcium levels are the most reliable indicator of tumor recurrence, particularly parathyroid carcinoma, in patients who have undergone parathyroid surgery (9); however, the PTH level is often elevated first (48). Patients with parathyroid carcinoma and distant metastasis manifest progressive elevation of their PTH level. Most patients with parathyroid carcinoma who experience recurrent disease present within three years of their initial surgery (48).

Clinical utility of parathyroid hormone and total calcium measurements in monitoring asymptomatic patients who do not undergo parathyroid gland surgery

While the majority of asymptomatic HPT patients are relatively stable, about 25% of these patients will progress eventually to overt HPT. Therefore, serum total calcium and PTH levels should be monitored in asymptomatic patients who are suspected of having HPT and do not undergo parathyroid surgery (5,49,50). Other parameters, including serum creatinine concentration and bone density, should be monitored as well.

Pre-analytical and specimen storage issues

The PTH molecule is extremely labile and is stable in serum for only 2 hours at room temperature and 8 hours at refrigerator temperatures (2-4 ^{0}C). Therefore, specimens should be promptly tested or stored frozen at -80 ^{0}C. Since the serum and/or ionized calcium level has been established previously in patients undergoing parathyroid surgery and intra-operative PTH testing, and limiting the amount of time that the patient is kept under anesthesia is of paramount importance, blood samples for intra-operative PTH testing can be collected in EDTA

anticoagulant to reduce the time required for clotting and to inhibit various serum proteases that may degrade the PTH molecule (33).

For routine measurement of serum calcium concentration, serum is the preferred specimen. Serum calcium is stable at refrigerator temperatures for several months. EDTA and oxalate anticoagulants must not be used because these anticoagulants bind calcium extremely tightly and interfere with its measurement.

MUTATION ANALYSIS OF GENES (*HRPT2, MEN1, CYCLIN D1/PRAD1*) ASSOCIATED WITH HYPERPARATHYROIDISM AND PARATHYROID GLAND TUMORS

HRPT2 Gene

The *HRPT2* gene, located on chromosome 1q25, is a recently discovered gene encoding the putative tumor suppressor protein, parafibromin. Several studies have shown that inactivation of the *HPRT2* gene by either mutations or loss of heterozygosity (LOH) is the cause of specific HPT-associated familial tumor syndromes such as HPT-jaw tumor syndrome (HPT-JTS) (52-54) and a subset of familial isolated hyperparathyroidism (FIHPT) (53). Germ line mutations in the *HRPT2* gene were found in HPT-JT and FIHPT patients (52,53). An additional somatic mutation or loss of heterozygosity (LOH) was identified in patients with HPT-JT or FIHPT who subsequently developed PTG carcinoma (52-54).

These findings are consistent with Knudson's "two hit" hypothesis for the tumor suppressor gene [i.e., inactivation of both copies of a tumor suppressor gene by one hereditary germ line mutation on one allele (the first "hit") and another acquired somatic mutation on the second allele (the second "hit")]. In addition to the familial tumor syndromes, somatic mutations in the *HRPT2* gene have also been found in patients with sporadic PTG carcinoma (53,54). Moreover, *HRPT2* germ-line mutations were identified in three patients with sporadic PTG carcinoma (54). Except for 4% of cases of sporadic cystic PTG adenoma, no LOH or mutations of any kind in the *HRPT2* gene have been found in patients with benign PTG lesions, including those with a sporadic adenoma, a lithium-associated tumor, or secondary or tertiary PTG hyperplasia (52). The strong association between the inactivation of the *HRPT2* gene and PTG malignancy indicates that this gene is a marker for aggressive or malignant PTG tumors (52,53). However, correlations have not been determined between mutation genotype and the presence of PTG disease alone or together with other lesions. Similarly, no correlations have been established between the severity of the mutations or the number of "hits" in the *HRPT2* gene and the clinical presentation of familial tumors as benign, cystic, or malignant (53).

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Approximately 80% of the mutations in the *HRPT2* gene occur within exons 1, 2, and 7; however, mutations affecting other regions of the HRPT2 gene have also been found (53).

For genetic analysis of parathyroid tissue, tissue should be frozen in liquid nitrogen immediately after surgical removal and stored at -70°C or below. In addition, a peripheral blood sample for germ line mutation analysis should be collected in tubes containing EDTA anticoagulant and stored at -70°C. Currently, there are no commercially available kits for *HRPT2* gene mutation analysis. Recommendations for performing *HRPT2* gene mutation analysis on tissue samples from patients with PTG tumors include:

1. Given the strong association between mutations in the *HPRT2* gene and PTG carcinoma, analysis of the *HRPT2* gene for intragenic mutations is recommended as a marker of parathyroid gland malignant potential in patients with both sporadic and familial tumors, including HPT-JT and probably, a subset of individuals with FIHPT.

2. Genetic analysis of the *HRPT2* gene is advised for patients suspected of having a parathyroid malignancy because of severe metabolic disease, the presence of hard tumor masses or fibrous bands, or for patients whose tumors have a high mitotic index by histopathology.

3. Patients with parathyroid carcinoma should be tested for germ line mutations in the *HRPT2* gene.

4. Detailed genetic screening and risk assessment should be conducted for family members of patients with mutations in their *HRPT2* gene to identify individuals carrying germ line *HRPT2* gene mutations. In addition, long-term biochemical monitoring of these individuals, including periodic total calcium testing to diagnose parathyroid carcinoma at an early stage, is also warranted.

MEN1 Gene

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant familial cancer syndrome characterized by the presence of multiple tumors involving the parathyroid glands, enteropancreatic endocrine tissues, the anterior pituitary and other tissues. Using tissue specimens from MEN1 patients, the *MEN1* gene was mapped to chromosome 11q13 (55). As tumor development among MEN1 patients follows Knudson's "two hit" hypothesis, the *MEN1* gene, which encodes the 610-amino acid protein, menin, is considered a putative tumor suppressor gene (55). Primary HPT is the most frequent, and usually the earliest, clinical manifestation of MEN1. Affected individuals typically develop HPT at the age of 20 to 25 years and by age 50, almost all affected individuals have parathyroid lesions (56).

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Somatic or germ-line mutations in the *MEN1* gene have been detected in about 12-20% of patients with sporadic parathyroid adenomas (57-60) and in more than 90% of families affected with MEN1 (61-65). More than 100 germ line mutations in the *MEN1* gene have been reported since this gene was first discovered (66). The nature of these mutations is diverse and includes missense, nonsense, and frameshift mutations as well as mRNA splicing defects. These mutations/defects are scattered throughout the nine coding exons and the intervening intronic sequences of the *MEN1* gene (66). Approximately two-thirds of the mutations in the *MEN1* gene are predicted to cause a nonfunctioning truncated menin protein, which is consistent with the proposed role of the *MEN1* gene as a tumor suppressor gene. The presence of an *MEN1* gene mutation, regardless of type of mutation, has not been correlated with disease phenotype (67,68). In patients with familial lesions, the *MENI* gene mutation profile is usually unique to an individual family and shared by the affected family members (usually 50% of the family members). While a mutated *MEN1* gene is found in the majority of MEN1 patients, up to 10-30% of cases have no detectable *MEN1* gene mutations (69,70), presumably because mutations may be located outside the region of the MEN1 gene tested for the presence of mutations, such as intervening intron sequences. In addition to patients with MEN1, *MEN1* gene mutations have also been found in patients affected by non-familial PTG hyperplasia (57,59). In this regard, the sporadic PTG multiglandular hyperplasia of approximately 40% of patients with this disease appears to be of monoclonal origin (71); however, to what extent *MEN1* gene mutations contribute to the severity and prognosis of PTG hyperplasia is currently unknown. Guidelines or recommendations for the diagnosis and treatment of MEN1 have been reported previously (4,72,73).

Recommendations for performing *MEN1* gene mutation analysis on tissue samples from patients with PTG tumors include the following:
1. *MEN1* genotype should be established in patients <35 years of age with early onset of HPT.
2. Genetic screening for *MEN1* gene mutations during early adolescence is advised for individuals with a family history of MEN1 and FIHPT.
3. Annual biochemical testing, including PTH and/or calcium, is recommended for life in the follow-up of individuals with germ line mutations in the *MEN1* gene.
4. Relatives without family-specific *MEN1* mutations do not require annual follow-up with biochemical tests.

Cyclin D1/PRAD1 Gene

The *Cyclin D1/PRAD1* oncogene is located on human chromosome 11q13. This gene encodes the cell cycle regulator protein, cyclin D1, which is involved in driving cells from the G1

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phase to the S phase of the cell cycle (74,75). The *Cyclin D1/PRAD1* proto-oncogene was discovered at the breakpoint of an inversion on chromosome 11 in a subset of patients with parathyroid adenoma. The inversion leads to the placement of the encoding sequences of the *Cyclin D1* gene on chromosome 11q13 directly under the control of the transcriptional regulatory sequences of the PTH gene on chromosome 11p15 with consequent over-expression of the structurally normal cyclin D1 protein (74,75). While at least 5% of patients with PTG adenoma possess the rearranged *Cyclin D1/PRAD1-PTH* gene (74,78), over-expression of cyclin D1 due to such rearrangement or to other mechanisms is frequently associated with other types of parathyroid adenomas (39%), hyperplasia (61%), and carcinomas (91%) (77,78).

Recommendations for performing *CYCLIN D1/PRAD1-PTH* gene mutation analysis on tissue samples from patients with PTG tumors include the following:

1. Routine use of *Cyclin D1/PRAD1-PTH* gene mutation analysis is not recommended because the clinical value of genetic testing for recombinant *Cyclin D1/PRAD1-PTH* gene sequences has not yet been established.

2. Routine use of over-expression of cyclin D1 protein as a marker of parathyroid tumors is also not recommended because such over-expression is not specific for parathyroid tumors and may occur in patients with parathyroid gland hyperplasia.

CONCLUSIONS

The NACB Panel recommends PTH and calcium, preferably ionized calcium, as the routine tumor markers for the diagnosis and follow-up of PTG tumors or for monitoring individuals susceptible to PTG tumors. Tissue-based genetic markers, such as *HRPT2* and *MEN1*, are probably more relevant to the identification or risk assessment of individuals with a family history of germ line mutations. While these genetic markers are strongly associated with PTG tumors, whether they are restricted solely to PTG tumors should be investigated further.

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Table 1. Currently available serum and tissue markers for parathyroid adenoma and carcinomas

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE	References
Serum-Based Markers				
Parathyroid hormone (PTH)	Combined with elevated serum calcium for diagnosis of primary hyperparathyroidism	In clinical use		5
	Intra-operative measurement of PTH assesses successful resection of parathyroid tumor	In clinical use	I	33
	Localization of diseased gland(s) by venous sampling of PTH	In clinical use	III, V	79,80
	Postoperative follow-up for successful resection and tumor recurrence	In clinical use		
	Follow-up for asymptomatic tumor patients	In clinical use		
Calcium	Combined with elevated serum PTH for diagnosis of primary hyperparathyroidism			5
	Suggestion of parathyroid carcinoma when a total serum calcium level is more than 15 mg/dL	In clinical use		5
	Indicator for parathyroidectomy when a serum total level is more than 1.0 mg/dL (0.25 mmol/L) above the upper limit of the reference range	In clinical use		5
	Postoperative follow-up for successful resection and tumor recurrence	In clinical use		
	Follow-up for asymptomatic tumor patients	In clinical use		5
Genetic/Tissue-Based Markers				
HRPT2	Indicator of carcinoma	In clinical use	V	52-54
	Risk assessment for family members of parathyroid carcinoma patients carrying germ line mutations	Evaluation/in clinical use	V	52-54
MEN1	Risk assessment for family members of parathyroid carcinoma patients carrying germ line mutations	Evaluation/in clinical use	III, V	70,81

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Table 2. NACB Recommendations for clinical diagnosis and management of patients with parathyroid gland tumors.

1. Serum total calcium and PTH are the primary laboratory tests for identifying patients with PTG tumors. Ionized calcium should be determined if the hypercalcemia based on total calcium values is borderline, if the clinical suspicion of HPT is high, or if factors that confound the interpretation of total calcium values are present.

2. Second generation immunometric assays, which detect only PTH_{1-84}, are the preferred assays for PTH testing.

3. Intra-operative PTH testing to confirm adequate excision of abnormal parathyroid tissue is recommended in patients with uniglandular disease and its efficacy in patients with multiglandular disease or PTG carcinoma has not been proven.

4. Serum total calcium and PTH measurements should be performed every 6 months (total calcium) and every 12 months (PTH) for asymptomatic patients suspected of having HPT who are not treated surgically. Other markers that can be monitored include annual measurement of serum creatinine and bone density.

5. Asymptomatic patients with a serum total calcium level > 1.0 mg/dL (0.25 mmol/L) above the ULN should be offered parathyroid surgery. To date, there are limited studies indicating an *io*nized calcium cutoff value for recommending PTG surgery. However, a serum total calcium increase of 1.0 mg/dL (0.25 mmol/L) corresponds to about 2 SD above the ULN. The corresponding 2 SD limit above the ULN for serum ionized calcium values is 0.32 mg/dL (0.08 mmol/L) (51).

6. Serial serum calcium and PTH determinations are recommended in the follow-up of any patient who has undergone parathyroid surgery. In patients who have undergone successful PTG surgery, serum calcium and PTH levels should be monitored at least every six months. If clinically indicated post-operatively, serum total calcium and PTH levels should be monitored every three months.

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