REX HEALTHCARE

Laboratory Bulletin...

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Parathyroid Hormone Assay Now Available at Rex

An intact (whole molecule) parathyroid hormone (PTH) assay is now available at REX Healthcare Laboratory. The stimulus for developing this assay was intraoperative PTH testing (*vide infra*), but routine testing will also be available for inpatient and Outreach use. PTH is an 84 amino acid peptide responsible for maintaining ionized calcium levels in the desired range. Decreased ionized calcium levels result in PTH secretion, which increases renal and gastrointestinal absorption of calcium. As calcium concentrations return to normal, PTH secretion is inhibited. The intact molecule has a short half-life (2 – 5 minutes). The intact molecule is subject to both glomerular filtration and peritubular uptake, as well as tubular reabsorption. Any remaining intact PTH is generally cleaved between amino acids 34 and 35, to produce a "N-terminal" (1-34) peptide and a "C-terminal" (35-84) peptide. The N-terminal fragment is also biologically active and has a short (< 5 min.) half-life. The C-terminal fragment is biologically inactive, and has a longer half-life (25-30 min.). Normally, the concentration of the C-terminal fragment is 10 times that of intact PTH, while the N-terminal fragment is present at a much lower concentration. In renal failure, the glomerular filtration of the C-terminal fragment is impaired, resulting in increased levels of C-terminal peptide.

Early PTH assays measured the C-terminal fragment, as it was present in the highest concentration. Assays for the N-terminal and "mid-molecule" (44-68) fragments were developed later for use in patients with chronic renal failure. Within the past 10 years, methods that measure the intact molecule have replaced the earlier assays. These "two-site" intact assays allow better separation between hyperparathyroid and nonparathyroidal hypercalcemic patients. These immunometric methods are usually 2 step procedures, where a polyclonal antibody to the C-terminal peptide fixed to a solid phase (e.g. bead) binds any intact (and C-terminal fragments of) PTH in the patient's plasma. After a wash step, enzyme-labeled or chemiluminescent-labeled polyclonal antibody to the N-terminal peptide is added, binding only to the intact PTH already affixed to the bead. The intact PTH concentration is proportional to the amount of substrate catalyzed by the enzyme label (or the amount of light produced by chemiluminescent label).

Indications for PTH testing

PTH is helpful in the evaluation of calcium disorders, particularly hypercalcemia. It distinguishes between "parathyroid" and "non-parathyroid" causes of hypercalcemia (Table 1). Primary hyperparathyroidism (1° HPT) is the most common cause of hypercalcemia in ambulatory patients. The hypercalcemia is often associated with decreased serum phosphate, decreased serum chloride, and increase alkaline phosphatase (Table 3). Most patients with 1° HPT (80-90%) have elevated PTH values, although some may have values in the upper limit of the reference range. However, normal PTH levels in a patient with persistent hypercalcemia are inappropriate, and suggest some type of PTH-dependent disorder. (PTH-independent hypercalcemia should result in PTH levels below the lower limit of the reference range.) "Pseudohyperparathyroidism" refers to the ectopic production of PTH by a non-parathyroid tumor. This is quite rare, particularly when compared to tumors secreting "parathyroid related peptide" (*vide infra*). Tertiary hyperparathyroidism refers to the development of autonomous PTH hypersecretion (or the development of a true parathyroid adenoma) in the setting of secondary hyperparathyroidism due to renal failure or following renal transplantation. Familial hypocalciuric hypercalcemia (FHH) is a congenital disorder, but mild cases may escape detection until adulthood. Patients may present with laboratory findings indistinguishable from primary hyperparathyroidism. [1] The clinical history is the most helpful element in distinguishing FHH from 1° HPT. A history of a previous documented normal calcium level in a patient with ambient hypercalcemia would suggest 1° HPT. Some have suggested that the ratio of urine calcium

clearance to urine creatinine clearance is helpful in discriminating between FHH and 1° HPT (1° HPT > FHH). [1] Chronic lithium therapy may produce hypercalcemia and even mild hyperparathyroidism.

Table 1
Differential Diagnosis of Hypercalcemia (abridged)*

PTH-Dependent	PTH-Independent
Primary Hyperparathyroidism	Neoplasms
Adenoma (85-90%)	PTHrP-dependent (humoral hypercalcemia)
Hyperplasia (10-15%)	Osteolytic metastases
Carcinoma (0-5%)	Hematologic malignancies (TNF, OAF)**
Pseudohyperparathyroidism (very rare)	Medications (e.g. thiazides, Vit. A, Vit. D, theophylline)
Tertiary Hyperparathyroidism	Endocrine (adrenal insufficiency, hyperthyroidism)
Familial hypocalciuric hypercalcemia	Granulomatous disease (e.g. sarcoidosis, TB)
Lithium	Renal failure (acute, chronic w/ aplastic bone disease)
	Immobilization

* Adapted from Wilson *et al* and Jialal *et al* [1,3]
** TNF = tumor necrosis factor, OAF = osteoclast activating factor (not available as diagnostic tests)

As noted above, PTH-independent hypercalcemia should produce PTH levels below the lower limit of the reference range. This can be particularly helpful in distinguishing 1° HPT from humoral hypercalcemia of malignancy. This latter condition is observed in certain tumors (including some visceral squamous carcinomas and insulinomas) which secrete a "parathyroid related peptide" (PTHrP) structurally and chemically similar to (but immunologically distinguishable from) PTH. While direct assays for PTHrP have been developed, they are not recommended for general use due to lack of sensitivity. [2] PTHrP presence can often be inferred based on a decreased or suppressed PTH value and the clinical setting. The remaining causes of PTH-independent hypercalcemia can generally be deduced from the clinical setting supplemented with appropriate lab tests.

Hypocalcemic disorders may also be divided on the basis of PTH deficiency or excess, although PTH measurement is often not necessary for appropriate classification (Table 2). PTH deficiency may be congenital or acquired. Hypomagnesemia may suppress PTH secretion, but may also produce PTH resistance, ultimately leading to an increase in PTH. Most of the remaining causes of hypocalcemia are due to vitamin D deficiency, vitamin D resistance or PTH resistance; and produce an elevated PTH. Chronic renal failure is the most common cause of secondary hyperparathyroidism, but gastrointestinal malabsorptive syndromes may also produce this picture.

Table 2
Differential Diagnosis of Hypocalcemia (abridged)*

PTH Deficiency	PTH Excess
Congenital absence	Chronic renal failure (secondary hyperparathyroidism)
Neonatal (transient)	Malabsorption (secondary hyperparathyroidism)
Surgical removal	PTH resistance (pseudohypoparathyroidism)`
Autoimmune	Vitamin D deficiency
Metastatic malignancy	Phosphate therapy
Hemochromatosis	Drug (cis-platinum, calcitonin, bisphosphonates)
Hypomagnesemia	

* Adapted from Jialal *et al* [3]

Table 3

Laboratory Findings in Calcium Disorders*

Condition	Ca^{+2}	PO_4	PTH	Alk. Phos.
Primary hyperparathyroidism	↑	↓/N	↑/N	↑/N
Pseudohyperparathyroidism	↑	↓/N	↑	↑/N
Tertiary hyperparathyroidism	↑	N/↑	↑	↑/N
Familial hypocalciuric hypercalcemia	↑	↓/N	N/↑	N
Lithium therapy	↑	N/↓	N/↑	N/↑
PTHrP-dependent humoral hypercalcemia of malignancy	↑	↓	↓	N/↑
Non-PTHrP-dependent hypercalcemia	↑	N/↑	↓	N/↑
Hypoparathyroidism (congential, acquired)	↓	↑	↓	N
Hypomagnesemia	↓	↑	↓/N/↑	N
Secondary hyperparathyroidism (renal failure)	↓/N	↑	↑	N/↑
Secondary hyperparathyroidism (GI malabsorption)	N/↓	↓	↑	↑
PTH resistance (pseudohypoparathyroidism)	↓	↑	↑	↑
Vitamin D deficiency	↓	↓	↑	↑

* Adapted from Jialal *et al* [3] ↑ = increased N = normal ↓ = decreased

Intraoperative PTH Testing

Intraoperative assay of parathyroid hormone (PTH) is a useful tool in the surgical treatment of hyperparathyroidism. Because of the short half-life of PTH (2 – 5 minutes), a significant decrease in blood PTH can be observed following successful surgical removal of hyperfunctioning parathyroid tissue. In practice, this is generally accomplished by analyzing pre-excision and post-excision blood specimens collected in the operating room for PTH assay. A 50% decrease in PTH* 15-20 minutes after removing the hyperfunctioning parathyroid(s) predicts a good medical outcome with regard to calcium metabolism.[4] Early intraoperative PTH assays were performed on dedicated instruments brought to the Operating Room, but because of cost concerns most institutions offering intraoperative PTH assays have migrated to central laboratory testing.[5,6,7] The instrument used at Rex is **not** dedicated to PTH testing, and is used for other tests. (This helps reduce the overall cost of the intraoperative PTH at Rex.) To be placed in a mode to measure PTH requires instrument software changes at the time of measurement. If properly alerted, the Laboratory can provide PTH results within 20 –30 minutes of specimen receipt. If **not** notified in advance, results may not be available for 1 hour or longer. Cooperation and coordination between the Dept. of Surgery, Operating Room and Pathology Laboratory will assure optimum patient care.

* (It should be noted that all current intact PTH assays are subject to interference from large PTH degradation fragments [e.g. PTH(7-84)]. These large fragments result from short amino-terminal truncation and are biologically inactive, but will be measured as "intact PTH". These fragments may be markedly increased in renal failure. This interference has not been found to be clinically significant, with the possible exception of intraoperative PTH testing. Patients with chronic renal failure undergoing parathyroidectomy may not experience a 50% decrease in PTH levels following removal of all hyperfunctioning parathyroid tissue.) [7]

PTH testing at Rex

The Immulite Turbo® Intact PTH has been validated by comparison studies with the intact PTH assay used at Mayo Medical Laboratories. A total of 20 specimens were assayed with a correlation coefficient of 0.99. There will be a slight revision in the reference range to reflect the method change. The specimen should be collected in an EDTA (purple top) tube. It is important to completely fill the tube or the excess EDTA will interfere with the assay, falsely depressing the results.[8] The intact PTH is stable in EDTA tubes at room temperature for 72 hours after collection. Routine PTH testing (order "PTH" in hospital information system) is performed twice weekly. Concurrent measurement of calcium, phosphate, and creatinine requires separate orders for these tests and collection in a serum separator or red top tube. Intraoperative PTH testing (order "PTHOR" in hospital information system) is "by appointment only" through OR Scheduling (784-3197). Mayo Medical Laboratories

offers a "PTH profile" comprising PTH, calcium, phosphate, and creatinine. This may be ordered as "PTH profile (reference)" on an Outreach requisition (or by entering "ICMA" in the hospital information system).

John D. Benson, MD
Elaine Patterson, MT(ASCP)
Gwen Frisch, MT(ASCP)

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8. Immulite Turbo® Intact PTH product specification sheet.

Normal ALT/AST r/o acute hepatitis

While the results may appear intuitive to some, a recent study confirmed the value of liver enzymes (specifically AST and ALT) in excluding the diagnosis of acute hepatitis. [1] The retrospective study looked at 274 patients who had "comprehensive viral hepatitis serologic testing" performed. The serologic tests included anti-HBs, HBsAg, anti-HBc (total), anti-HBc (IgM), anti-HAV (total), anti-HAV (IgM), and anti-HCV. Serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) were measured within 48 hours of the time blood was collected for the viral serology. Elevated ALT and/or AST were observed in 171 of the 274 patients (62%). Of these, 44 (26%) had negative viral serology, 127 (74%) had positive viral serology of any type, and 11 (9%) had positive serology for acute viral hepatitis (6 HBsAg alone, 5 HAV (IgM) alone). More importantly, **normal ALT/AST** levels were present in 103 patients (38%). **Not one** of these patients had serologic findings indicative of **acute** hepatitis, although 71% had serologic findings indicative of chronic or past infection. The authors conclude that acute viral hepatitis can be confidently excluded in patients with normal ALT/AST, and that serologic testing for acute hepatitis A and B can be eliminated, with resultant cost savings.

John D. Benson, MD

Reference

1. Sharp SE *et al*. Utilizing Liver Enzymes for Screening Purposes prior to Hepatitis Testing. *Laboratory Medicine* 2002: 33:429-30.

For further information, call the Laboratory (784-3040). Telephone extensions are: Pathologists' Direct Line (3201), Sharon Logue (Lab Director 2400), Robin Ivosic (Microbiology Lab Manager 3053), Elaine Patterson (Core Lab Manager 3054), Jackie Okoth (Core Lab PM Manager 4248), Diane Young (Anatomic Pathology Manager 3888), Nga Moore (Customer Service Manager 3396)