High Sensitivity Measurement of Parathyroid Hormone Related Protein by LC-MS/MS for Diagnosing Disorders of Calcium Regulation

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Parathyroid hormone related protein (PTHrP) has close homology with parathyroid hormone (PTH), and some of the physiological functions of PTHrP are similar to the functions of PTH. In health, circulating concentrations of PTHrP are very low and commercial assays are often unable to detect endogenous concentrations. PTHrP is known to activate pathways that promote formation of bone metastasis from tumor cells and cause hypercalcemia of malignancy. PTHrP can be present in elevated concentrations in blood of patients diagnosed with squamous cell lung cancer, squamous cell head and neck cancers, breast, lung, uterus, and skin cancers.

We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the measurement of PTHrP. Sample preparation was performed as follows: stable isotope labeled internal standard was added to the samples and PTHrP was enriched using anti-PTHrP antibody conjugated to magnetic beads. After incubation, the beads were washed, PTHrP was digested with trypsin and the samples were analyzed by LC-MS/MS. The ratio of quantitation from two mass transitions was used to assess the specificity of analysis in every sample.

The lower limit of quantification and upper limit of linearity were 0.6 and 580 pmol/L, respectively. Total imprecision of triplicate measurements in serum samples over seven days was < 9.4%. No interferences were observed in over 2000 samples analyzed by the method. Poor agreement was observed with a commercial RIA (Immunotech), which is likely explained by low sensitivity and nonlinearity of the RIA. As an alternative to the regression analysis, we assessed agreement of the quantitative measurements using the LC-MS/MS with qualitative results of the RIA using logistic regression analysis (results by RIA were considered positive at concentrations above the upper limit of the current reference interval of 4 pmol/L). Area under ROC curve for

the logistic regression analysis was 0.82. Analytical sensitivity of the method was sufficient for measuring PTHrP in the blood of healthy individuals. Reference intervals of PTHrP in healthy adults (n=238) established with this method were 0.6-3.3 pmol/L in women, and 0.6-2.2 pmol/L in men; measurable concentrations of PTHrP were observed in all analyzed samples. In order to assess performance of the assay we analyzed PTHrP in sets of plasma samples from patients with unexplained hypercalcemia (calcium >10.5 mg/dL, and PTH <15 pg/mL; n=88); 42% of these samples had concentration of PTHrP above the established reference interval, suggesting that PTHrP was the cause of hypercalcemia.

This method is highly specific for PTHrP and has acceptable performance characteristics for use in clinical diagnostic applications. Sensitivity of the method is sufficient for quantitative measurements of PTHrP in samples of healthy and pathologic individuals.