Clinical Biochemistry 49 (2016) 16–21

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Analytical and clinical validation of parathyroid hormone (PTH) measurement in fine-needle aspiration biopsy (FNAB) washings

Hemamalini Ketha, Michael A. Lasho, Alicia Algeciras-Schimnich *

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 12 August 2015
Accepted 11 September 2015
Available online 15 September 2015

Keywords:
Parathyroid hormone (PTH)
Fine needle aspirate biopsy (FNAB)
FNAB needle washings

ABSTRACT

Background: Parathyroid hormone (PTH) quantitation in fine needle aspirate biopsy (FNAB) saline washings complements current modalities for parathyroid tissue localization.

Objectives: To establish the performance characteristics of the Roche Elecsys intact PTH immunoassay in FNAB needle washings and its diagnostic performance for the identification of parathyroid tissue.

Design and methods: Accuracy, precision, reportable range, and analytical specificity and sensitivity for the intact PTH immunoassay in FNAB needle washings were established. For clinical validation, 93 specimens from 79 patients were evaluated. Diagnostic cut-offs were established via receiver operator characteristic (ROC) curve analysis. Performance of PTH in FNAB needle washings was compared to cytology.

Results: Measurement of the PTH in FNAB needle washings demonstrated a matrix interference that was overcome by supplementation of the samples with a protein based matrix prior to analysis. ROC area under the curve (AUC) was 0.96 for PTH in FNAB needle washings. A PTH concentration ≥100 pg/mL showed 100% specificity and 82% sensitivity for identifying parathyroid tissue. On histology-confirmed parathyroid specimens, 21/38 (55%) were correctly identified by cytology; whereas 31/38 (82%) were identified by PTH.

Conclusions: Measurement of PTH in FNAB washings complements cytology for identification of parathyroid tissue. Analytical validation to exclude interference in the PTH immunoassay and proper localization of the parathyroid tissue by ultrasound is necessary to ensure the robustness of the method.

1. Introduction

Parathyroid hormone (PTH) measurement in serum is useful for the diagnosis of primary hyperparathyroidism. Causes of primary hyperparathyroidism include parathyroid gland hyperplasia and parathyroid adenomas (single or multiple). Bilateral intraoperative neck exploration to identify and remove the hyper-functioning parathyroid tissue is the gold standard for the treatment of hyperparathyroidism [1]. However, a small subset of patients will present with persistent or recurrent hyperparathyroidism requiring re-exploration and reoperation.

Preoperative localization of the parathyroid lesion is critical for successful reoperation, and to identify candidates suited for minimally invasive parathyroidectomy. Ultrasonography (US) and sestamibi scintigraphy (technetium-99-sestamibi scanning, MIBI) are widely used imaging techniques for preoperative localization of a hyper-functioning parathyroid gland or adenomas but have been shown to have a high false positive rate [2].

In conjunction to imaging studies, PTH measurement in FNAB needle washings following US-guided biopsy has gained popularity to localize suspected parathyroid lesions and to differentiate parathyroid tissue from thyroid nodules [3–11]. In previous studies a range of PTH diagnostic cut-offs (40–132 pg/mL) has been proposed to differentiate parathyroid from non-parathyroid lesions when analyzing FNAB needle washings [3–5,12]. However, the PTH assay used and its performance characteristics in the wash matrix, the FNAB needle washing technique including the collection volume is often missing from published literature. This information is critical when determining the diagnostic performance of PTH in FNAB needle washings for discrimination of parathyroid and non-parathyroid tissues. At our institution (Mayo Clinic, Rochester, MN) measurement of PTH in FNAB needle washings has been in clinical use since June 2008. The objectives of our study were to: 1) establish the performance characteristics of the Roche Elecsys intact PTH assay in saline washings of FNAB needles; 2) establish a diagnostic cutoff for PTH in FNAB needle washings that would detect parathyroid-derived tissue with 100% specificity; and 3) compare the performance of PTH in FNAB needle washings to cytology.

2. Materials and methods

2.1. Study subjects

This was a retrospective study approved by the Mayo Clinic Institutional Review Board. Mayo Clinic (Rochester, MN) patients on whom

* Corresponding author at: Mayo Clinic, Hilton 740D, 200 1st Street SW, Rochester, MN 55905, USA.
E-mail address: algecirasschimnich.alicia@mayo.edu (A. Algeciras-Schimnich).

http://dx.doi.org/10.1016/j.clinbiochem.2015.09.006
0009-9120/

PTH measurement on FNAB needle washings was ordered for clinical management from June 2008 through September 2013 were included. Classification of parathyroid or non-parathyroid tissues was based on the review of the medical record information regarding cytology, histology, and imaging studies which included MIBI and high-resolution ultrasonography.

2.2. FNAB needle washings

Ultrasound-guided FNAB was performed with a 25-gauge needle. The needle was moved in a back-and-forth motion within the mass, obtaining material from the target lesion via capillary action, without the use attached suction. Averages of 6 passes were made. After the needle was removed from the biopsied site, an air filled 10 cm^3 syringe was attached. Contents of the biopsy needles were expelled onto glass slides for cytological analysis. To obtain the washings, 0.1 to 0.5 mL of normal saline (0.9% *w*/*v* of NaCl) was then drawn into the needle, filling the needle hub which is then expelled through the needle into a polystyrene tube with no additives. Washes from all needles were pooled (final volume 0.5–1.0 mL) and sent to the laboratory refrigerated. Blood-contaminated FNAB needle washes were spun and the clear supernatant removed for PTH testing; blood-free samples were tested without manipulation.

2.3. Analytical validation of the intact PTH immunoassay

PTH in serum and FNAB needle washings was measured using the Roche Elecsys intact PTH immunoassay on a Roche Cobas 6000 (Roche Diagnostics, Indianapolis, IN). This assay is FDA-approved for analysis of PTH in serum. Analysis of PTH in saline washings is considered an off-label use on an FDA-approved test; therefore, the following assay performance characteristics were determined: accuracy, inter- and intra-assay imprecision, analytical measurement range, dilutions, limit of detection (LOD), limit of quantitation (LOQ), analytical specificity (hemoglobin and bilirubin interference) and sample stability.

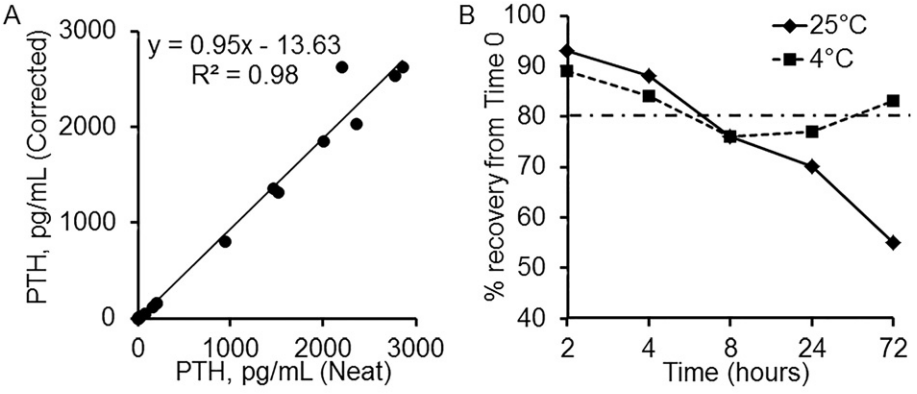
Prior to measurement, 50 μl of Elecsys MultiAssay diluent (Roche Diagnostics) was added to 450 μl of the FNAB needle washings sample. The Elecsys MultiAssay diluent consists of buffered equine serum matrix solution and provides the protein required for optimal performance of the assay (see results section and Fig. 1). Samples were tested immediately after addition of the Elecsys MultiAssay diluent. Results obtained from the instrument were corrected for the dilution factor by dividing by 0.9. The corrected result was used for further calculations and data analysis.

Accuracy was determined by spike recovery of FNAB needle washing samples. Eight samples with PTH concentrations between 17 and 287 pg/mL were spiked with PTH calibrator material (4396 pg/mL). For each sample, 50 μl PTH calibrator material was added to 450 μl of FNAB needle washings. Percent recovery was calculated for each sample as follows: measured concentration / expected concentration × 100.

Intra-assay imprecision was determined using three FNAB needle washings sample pools (Low: 17 pg/mL, medium: 76 pg/mL; high: 287 pg/mL). Each sample was analyzed 20 times in a single analytical run and the coefficient of variation (CV) was calculated. Inter-assay imprecision was determined by analyzing three FNAB needle washing sample pools 20 times (2 measurements at each level over 10 days). For the inter-assay impression study, individual aliquots for each pool were stored at −20 °C and a new aliquot was used for every run.

The LOD was assessed by running a saline blank and a FNAB washing sample with a low PTH concentration twenty times in one day. The following LOB and LOD calculations based on CLSI EP17-A [13] were used: Limit of Blank (LOB) = Mean $_{blank}$ + 1.645(SD_{blank}) and LOD = LOB + 1.645($SD_{low\ concentration\ sample}$).

Analytical measurement range was determined by sample admixtures of a low and high PTH concentration FNAB needle washing samples. Admixture ratios to cover a range of 6–5000 pg/mL were performed. This range was selected based on the serum PTH reportable range established by the manufacturer. Two admixture experiments (nine points for each admixture study) were performed and used to calculate the slope, intercept and correlation coefficient using Passing–Bablok regression fit. The reportable range was extended by validating dilutions from 2- to 1000-fold using Elecsys MultiAssay diluent. Five samples with a PTH concentration in the range of 2000 to 6000 pg/mL were serially diluted using Elecsys MultiAssay diluent to cover a range of 2- to 1000-fold dilution. For each sample at least 5 dilution series were performed. Accuracy of the dilutions was based on recovery of PTH in the dilution. Percent recovery was calculated for each



C

	Multi-assay (MA) diluent	Saline	Saline + MA diluent	Saline+MA diluent/0.9
N	10	20	20	20
Mean	3.3	17.2	7.5	8.3
SD	0.10	0.76	0.32	0.35
% CV	3.2	4.4	4.3	4.2

Fig. 1. Analytical validation of PTH measurement in FNAB needle washings. (A) Correlation between PTH measurements in untreated FNAB washings (X axis; neat) and in the same samples treated with Multi-Assay (MA) diluent prior to testing (Y axis; corrected); (B) PTH recovery in FNAB washing after 2–72 h at 4 °C and 25 °C; (C) effect of MA diluent in the measurable PTH concentration. Addition of MA diluent (50 μl MA + 450 μl saline) causes a decrease in the background observed in protein-devoid in saline.