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Validation of the procalcitonin (PCT) assay: Experience in a pediatric hospital☆

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ABSTRACT

Objectives: Procalcitonin (PCT) is a potential early biomarker used to differentiate sepsis from systemic inflammation. Serial PCT measurement is useful in reducing the duration of antibiotic exposure without increasing treatment failure. Our aim was to establish and evaluate an automated quantitative PCT assay at Texas Children's Hospital.

Methods: We validated the analytical and clinical performance of the automated miniVIDAS B.R.A.H.M.S PCT® assay (BioMerieux®, France) at Texas Children's Hospital. Analytical performance parameters included precision, linearity, accuracy, correlation, and effect of different common interferents (free Hb, bilirubin, triglyceride and rheumatoid factor). Also, the interference of high calcitonin (CT) on PCT assay was tested. We performed clinical correlation of PCT to blood culture, WBC counts and CRP in sepsis patients.

Results: The PCT assay showed good precision with %CV of <5% for intra-assay and %CV of 6.5% for inter-assay precision. The assay was linear across the measurement range (0.05 μg/L–200 μg/L). Correlation studies showed a good correlation ($r > 0.9$). No significant effects on PCT levels were seen with common interferents however, calcitonin concentrations of 1000 ng/L or more showed cross-reactivity with PCT values. Fourteen (78%) out of the total eighteen patients with positive blood culture, showed median PCT concentrations greater than the cut-off values of 0.15 μg/L.

Conclusion: The miniVIDAS PCT assay can be used for diagnostic purposes in clinical laboratories. We envision that serial PCT monitoring along with clinical correlation will be beneficial in critically ill patients.

Introduction

Procalcitonin (PCT) is a well-established, potentially specific and early marker of bloodstream infection (BSI) and particularly sepsis in children. It has been widely recognized as a prognostic and therapeutic indicator of bacteremia [1]. Procalcitonin (PCT), a 116 amino acid polypeptide precursor of calcitonin (CT) is produced physiologically by the thyroid C-cells and to a lesser extent by the neuroendocrine cells in response to humoral stimuli [2]. PCT is used for management of unexplained fever, commonly seen in children presenting to emergency room [3]. In pathological conditions, PCT is released by most of the cells in the human body in response to proinflammatory stimulation and particularly bacterial endotoxins [4]. In sepsis, PCT starts to increase after three hours and remains elevated throughout the episode even though complications such as multiple organ failure ensue [5]. Additionally, it has been found that bacterial and fungal infections are more prone to show consistent elevations compared to viral diseases [6]. Host response biomarkers such as PCT and C-reactive protein (CRP) are now being used along with PCT in the diagnostic process [4]. In Europe, PCT guided antibiotic therapy in neutropenic children has been shown to be very successful. A review of recent pediatric literature reveals PCT is a good predictor of bacteremia in patients with central line associated blood stream infections (CLABSI) [3]. The PCT assays used in previous studies had limited functional sensitivity of 0.3 μg/L The original reference method B.R.A.H.M.S PCT Kryptor® assay [7] is laborious and technique dependent, however, a new rapid quantitative miniVIDAS® B.R.A.H.M.S PCT® assay (BioMérieux®, Marcy L'Etoile, France), is now available and is fully adapted for emergency conditions with a functional sensitivity of 0.09 μg/L, (99th percentile for normal) according to the manufacturers [8]. The aim of our study is to evaluate

Abbreviations: (AMR), analytical measurement range; (BSI), bloodstream infection; (CRP), C-reactive protein; (CT), calcitonin; (CLABSI), central line associated blood stream infection; (CLSI), Clinical and Laboratory Standards Institute; (CRR), clinical reportable range; (ELFA), enzyme linked fluorescent detection; (ESR), erythrocyte sedimentation rate; (IQR), inter quartile range; (PCT), procalcitonin; (RF), rheumatoid factor; (SPR®), Solid Phase Receptacle; (SIRS), systemic inflammatory response syndrome; (WBC), white blood cell; (TEa), total allowable error.

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the analytical and clinical performance of this automated quantitative PCT assay in our pediatric population at Texas Children's Hospital and to compare the results to other markers of sepsis in a small study.

Materials and methods

We conducted a validation study for evaluation of the miniVIDAS B.R.A.H.M.S PCT® assay at the Texas Children's Hospital laboratory and we received ethics approval to use anonymized samples for method validation and for analysis of left-over samples for correlation studies. Study design included the evaluation of various analytical performance parameters including precision, linearity, accuracy, correlation, clinical utility and effect of different interferences. The study was conducted using the pediatric samples received in the main laboratory for routine chemistry analysis. An automated one step sandwich immunoassay combined with an enzyme linked fluorescent detection (ELFA) was performed using human serum or plasma (lithium heparinate). In this assay, the Solid Phase Receptacle (SPR®) serves as the solid phase with pre-dispensed reagents in the sealed reagent strips. The sample is transferred into the wells containing anti-procalcitonin antibodies labeled with alkaline phosphatase (conjugate). The sample/conjugate mixture passes through the SPR® several times enabling the binding of antigen with the antibody and conjugate to form a sandwich. Two detection steps are performed successively. During each step, the conjugate enzyme catalyzes the hydrolysis of the substrate (4-Methyl-umbelliferyl phosphate) into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigen present in the sample. The assay only takes 20 min enabling a quick turnaround of samples that are sent for procalcitonin testing.

Precision studies

The guidelines by Clinical and Laboratory Standards Institute (CLSI) EP5-A2 document were adopted to conduct intra-assay and inter-assay precision studies [9]. For intra-assay precision, two levels of AUDIT micro controls (MicroFD PCT controls) were analyzed in ten replicates in the same run. All the different levels of AUDIT micro controls were assayed in ten different runs over ten days to determine inter-assay precision. In addition to the manufacturer's controls, we performed precision studies on two separate serum pools (n = 2), representing low PCT concentration (0.16–1.20 μg/L), high PCT concentration (13.70–15.0 μg/L) and PCT was measured ten times on the same day.

Linearity and dilution studies

Linearity studies were conducted in accordance with the EP06-A CLSI guidelines. Analyses were done using MicroCV procalcitonin linearity set which consisted of five different pools (0.25–173.0 μg/L) and sample pool with high PCT value to fulfill the CLSI requirement to represent the entire analytical measurement range (0.05–200 μg/L). The serum sample with high PCT value (278.3 μg/L) was used and diluted concentrations were obtained for evaluation to complete the requirement. For samples with concentration of >200 μg/L, dilution studies were conducted using PCT negative sera (<0.05 μg/L) in a ratio of 1:10 (Fig. 1).

Accuracy

Audit PCT method validation set consisting of twenty five levels of bovine based serum albumin were used. Out of these 25 samples, 22 samples represented the entire measureable range and the last three samples were zero levels (0 μg/L) i.e. PCT negative samples.

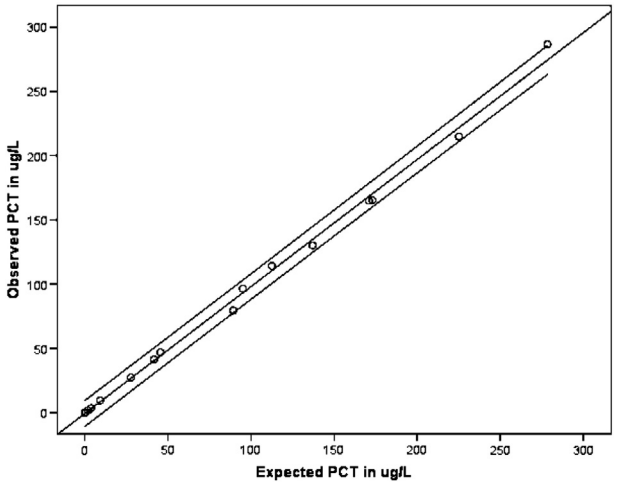

Fig. 1. Linearity studies for procalcitonin (PCT) assay for n = 19 samples run in duplicates. Observed and expected PCT values show a linear correlation (r = 0.98). The linear regression analysis equation is represented as y = 0.9809x − 0.3491. The upper and lower bounds represent the 95% confidence limits.

Interference studies

We conducted interference studies by evaluating the effect of free hemoglobin, triglycerides, bilirubin and rheumatoid factor. The typical concentrations for hemoglobin, triglycerides and bilirubin provided in the kit (INT-01 Routine Interferents ASSURANCE™ Interference Test Kit by Sun Diagnostics®, LLC) were used to obtain diluted operational concentrations to conduct the studies. The effect of these substances was thereby established on procalcitonin assay measurement. We analyzed the impact of mild (free hemoglobin: 1.5 g/L), moderate (free hemoglobin: 3.0 g/L), significant (free hemoglobin: 6.0 g/L) and gross (free hemoglobin: 12.0 g/L) hemolysis on three different sample pools measured for procalcitonin. Similarly, mild (triglycerides = 1.71 mmol/L), moderate (triglycerides = 4.56 mmol/L) and severe (triglycerides = 11.4 mmol/L) lipemic samples were assayed for procalcitonin. Three concentrations of bilirubin (100 μM, 250 μM and 500 μM) were added to sample pools to study their effect on PCT measurements. Each spiked serum sample result was further compared with the baseline sample pool PCT value. We evaluated the effect of rheumatoid factor (RF) at two concentrations (60 IU/mL and 300 IU/mL) on two sample pools using an Assurance interference kit (INT-03 Routine Interferents ASSURANCE™ Interference Test Kit by Sun Diagnostics®, LLC). All testing on samples were performed in triplicate and compared to baseline values.

We also obtained three serum samples from known medullary thyroid carcinoma patients with very high calcitonin levels to further evaluate the maximum measurable range of PCT. In addition, we studied the interference of high concentrations of calcitonin in measuring structurally similar PCT by miniVIDAS B.R.A.H.M.S® method. The entire data was presented as mean and standard deviation (SD). Total allowable error (TEa) was determined using the equation TEa = bias + 1.96SD. Percent coefficient of variation (%CV) was determined from the data generated. Pearson's correlation coefficient was calculated for the two sets of data obtained from TCH laboratory and the reference laboratory. A correlation coefficient of $r > 0.9$ and $p < 0.05$ was considered statistically significant.

Correlation studies

Correlation studies were performed taking into consideration EP09 CLSI guidelines (n ≥ 40). The new miniVIDAS B.R.A.H.M.S PCT® (LLoQ of 0.05 μg/L) method was compared against the B·R·A·H·M·S

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