Multicenter comparison of automated procalcitonin immunoassays

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A B S T R A C T

Objectives: A multicenter study to compare results of BRAHMS Kryptor PCT with those obtained using four BRAHMS-partnered procalcitonin (PCT) automated immunoassays (DiaSorin Liaison, BioMérieux Vidas, Roche Cobas E601 and Siemens Advia Centaur) and the Diazyme immunotubidimetric assay implemented on four clinical chemistry platforms (Abbott Architect c16000, Siemens Advia 2400, Roche Cobas C501 and Beckman Coulter AU5800).

Design and methods: One hundred serum samples from in-patients with PCT values between 0.10 and 58.7 ng/mL were divided into aliquots and tested with the nine different reagents and analyzers. BRAHMS PCT Kryptor results were used as reference.

Results: Compared to BRAHMS PCT Kryptor, significant differences in results were observed on Vidas, Advia Centaur, Architect, Cobas C501 and AU5800. However, the correlation coefficients (r) with BRAHMS PCT Kryptor were between 0.899 and 0.988. The mean bias was less than ±1.02 ng/mL, except for Vidas (2.70 ng/mL). The agreement at three clinically relevant cut-offs was optimal: between 83–98% at 0.50 ng/mL, 90–97% at 2.0 ng/mL, and 98% at 10 ng/mL. The comparison of Diazyme PCT across the four clinical chemistry analyzers yielded high correlation coefficients (r between 0.952 and 0.976), a mean bias less than 0.9 ng/mL, acceptable agreement at 0.5 ng/mL (>82%), and high concordance at the 2.0 ng/mL (>97%) and 10 ng/mL (>98%) cut-offs.

Conclusions: The methods and applications evaluated in this multicenter study are aligned with BRAHMS PCT Kryptor and can be used for predicting the risk of progression to systemic inflammation in patients with bacterial infections using the conventional PCT diagnostic thresholds.

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1. Introduction

Procalcitonin (PCT), a 116 amino acid peptide with molecular weight 14.5 kDa, belongs to the calcitonin superfamily of peptides. The protein is encoded by the CALC-1 (Calcitonin Gene-related Peptide 1) gene on chromosome 11 [1]. The original product of this gene, a 141 amino acid peptide known as pre-PCT, undergoes a proteolytic cleavage of the 25 amino acid signal peptide to produce the PCT molecule, which is then further processed to generate the mature, 32 amino acid-long calcitonin. Under physiological conditions, the transcription of CALC-1 gene is suppressed in non-neuroendocrine tissues, so that the C cells of the thyroid gland are responsible for the entire synthesis of PCT and calcitonin [1].

In the presence of microbial infections, the synthesis of PCT is dramatically amplified in all parenchymal tissues and differentiated cell types, so that its concentration in blood may increase by several orders of magnitude, up to one thousand-fold [1]. The PCT response to the infection closely mirrors the severity of the inflammatory reaction. More specifically, higher values are associated with severe disease,
whereas a declining concentration usually reflects disease resolution [2]. This paradigmatic behavior is now widely used for diagnosis and monitoring of bacterial infections. In particular, PCT values between \( \geq 0.5 \) and \( 2.0 \) ng/mL are suggestive of moderate risk for progression to systemic inflammation, between \( 2.0 \) and \( 10 \) ng/mL are suggestive for high risk for progression to systemic inflammation, whereas values \( \geq 10 \) ng/mL are associated with a high probability of developing severe sepsis and septic shock [1]. Recent evidence also suggests that the measurement of PCT may be helpful for the diagnosis of bacterial peritonitis [3], as well as for guiding antibiotic therapy in patients with localized (i.e., community acquired pneumonia) and systemic bacterial infections [4].

Although there is currently no agreed reference technique for PCT measurement, the BRAHMS PCT Kryptor method has been directly developed from the original BRAHMS luminometric immunoassay (LIA), and was the first automated method cleared by the FDA for the diagnosis of severe sepsis and septic shock [5]. Several diagnostic companies such as DiaSorin, BioMérieux, Siemens and Roche have since partnered with BRAHMS for developing automated PCT assays on their proprietary platforms, using different technologies (i.e., immunoluminometric, enzyme-linked immunofluorescent, chemiluminescent and electrochemiluminescent immunoassays). More recently, a latex-enhanced immunotubidimetric assay has also been developed by Diazyme, to be used on a vast array of clinical chemistry analyzers. Nevertheless, due to the lack of a PCT reference material, it remains uncertain whether the current clinical PCT cut-offs would apply to all the available procalcitonin immunoassays, or if monitoring using different methods would be clinically reliable when patients are transferred between healthcare facilities. Therefore, the aim of this multicenter study was to compare results of the BRAHMS Kryptor PCT with those obtained using four BRAHMS-partnered PCT automated immunoassays and the Diazyme immunotubidimetric assay implemented on four different clinical chemistry platforms.

2. Materials and methods

2.1 Study design

This multicenter study was based on 100 serum samples from inpatients (52 from the intensive care unit, 12 from the emergency department, 15 from the infective diseases unit and 21 from other medical and surgical wards) referred to the laboratory of the University Hospital of Parma for routine PCT measurement during a single working day. The samples were selected to cover the most clinically significant PCT concentrations (0.10–58.7 ng/mL) measured using the local routine immunoassay (BRAHMS PCT Kryptor system, BRAHMS, Hennigsdorf, Germany) (Fig. 1). The blood samples were immediately centrifuged upon arrival in the laboratory, serum was separated and divided into seven aliquots of 0.6 mL each. The first aliquot was used for routine PCT assessment, whereas the remaining six aliquots were frozen at \(-70^\circ \text{C}\). After one week of storage, the aliquots were transported to the participating centers, under controlled conditions of temperature and humidity. Specifically, four reusable dry gel packs containing carbopol gel (2 \( \times \) 200 mL and 2 \( \times \) 500 mL) were frozen at \(-70^\circ \text{C}\) and then placed into certified transport boxes at the sample collection center [6]. The mean time of shipment to the participating centers was 92 min (range: 54–106 min). Upon arrival, the aliquots were stored frozen at \(-70^\circ \text{C}\) until all sites had received the shipment. The aliquots were then thawed at room temperature on the same day, and simultaneously analyzed in each center with all reagents and methods, including retesting on BRAHMS PCT Kryptor.

2.2 Methods description

The complete description of reagents and instrumentation is shown in Table 1. In brief, the BRAHMS PCT Kryptor system is an
<table>
<thead>
<tr>
<th>Site</th>
<th>Company</th>
<th>Method</th>
<th>Assay principle</th>
<th>Sample volume (µL)</th>
<th>Measuring range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parma, Italy</td>
<td>BRAHMS, Hennigsdorf, Germany</td>
<td>Kryptor BRAHMS PCT sensitive</td>
<td>Time-Resolved Amplified Cryptate Emission (TRACE) immunoassay</td>
<td>50</td>
<td>0.02–1000</td>
</tr>
<tr>
<td>Asti, Italy</td>
<td>Diasorin, Saluggia (VC), Italy</td>
<td>Liaison BRAHMS PCT</td>
<td>Two-site immunoluminometric assay</td>
<td>100</td>
<td>0.02–100</td>
</tr>
<tr>
<td>Lodi, Italy</td>
<td>bioMérieux, Marcy l’Etoile, France</td>
<td>Vidas BRAHMS PCT</td>
<td>Enzyme-linked fluorescent immunoassay (ELFA)</td>
<td>200</td>
<td>0.05–200</td>
</tr>
<tr>
<td>Milano, Italy</td>
<td>Roche Diagnostics, Mannheim, Germany</td>
<td>Roche BRAHMS PCT</td>
<td>Electrochemiluminescence immunoassay (ECLIA)</td>
<td>30</td>
<td>0.02–100</td>
</tr>
<tr>
<td>Alessandria, Italy</td>
<td>Siemens Diagnostics, Marburg, Germany</td>
<td>Siemens BRAHMS PCT</td>
<td>One-step chemiluminescence immunoassay</td>
<td>100</td>
<td>0.02–75</td>
</tr>
<tr>
<td>Bassano del Grappa, Italy</td>
<td>Abbott Diagnostics Abbott Park, Illinois, U.S.A</td>
<td>Diazyme procalcitonin</td>
<td>Latex-enhanced immunotubidimetric assay</td>
<td>20</td>
<td>0.16–56</td>
</tr>
<tr>
<td>Alessandria, Italy</td>
<td>Siemens Healthcare Diagnostics, Marburg, Germany</td>
<td>Diazyme procalcitonin</td>
<td>Latex-enhanced immunotubidimetric assay</td>
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<td>Roche Diagnostics, Mannheim, Germany</td>
<td>Diazyme procalcitonin</td>
<td>Latex-enhanced immunotubidimetric assay</td>
<td>20</td>
<td>0.16–56</td>
</tr>
<tr>
<td>Parma, Italy</td>
<td>Beckman Coulter Inc., Brea, CA, USA</td>
<td>Diazyme procalcitonin</td>
<td>Latex-enhanced immunotubidimetric assay</td>
<td>20</td>
<td>0.16–56</td>
</tr>
</tbody>
</table>

* Diazyme procalcitonin, Diazyme Laboratories, Poway, CA, USA.
immunofluorescent, fully automated and highly sensitive PCT assay. The measurement principle is based on Time-Resolved Amplified Cryptate Emission (TRACE) technology, entailing the measurement of a signal emitted from an immunocomplex with time delay. The functional sensitivity and total imprecision (coefficient of variation, CV) of the assay were previously found to be 0.04 ng/mL and 0.7–8.3%, respectively [7]. Serum procalcitonin was also measured using four BRAHMS-partnered PCT automated immunoassays, the analytical characteristics of which have been previously described elsewhere and are briefly reported in Table 1 [8–12].

Diazyme PCT (Diazyme Laboratories, Poway, CA, USA) is a latex-enhanced immunoturbidimetric assay, which has been developed for use on a wide array of laboratory analyzers capable of reading absorbance at 600 nm. According to the test procedure, the PCT present in the serum sample binds to specific anti-PCT antibodies coated on latex particles and trigger their agglutination. The turbidity is then measured at 600 nm and is directly proportional to the concentration of PCT in the sample. The final PCT value is calculated by interpolation of optical signal against a 6-point calibration curve. The amount of sample required for testing is 20 µL and the first test result is available in 10 min. In this study, Diazyme PCT was measured on four different clinical chemistry platforms, using the same assay protocol (Table 1). According to a previous analytical evaluation, the lowest measurable value and the total imprecision were found to be 0.16 ng/mL and 2.9–7.7%, respectively [13].

According to the manufacturers’ literature, the inter- and intra-assay imprecision (CV) of the other immunoassays used in this study is 2.0–3.8% and 3.2–9.5%, respectively for Liaison BRAHMS PCT; 1.9–4.6% and 3.6–7.0%, respectively for Vidas BRAHMS PCT; and 1.1–7.1% and 1.6–8.7%, respectively for Roche BRAHMS PCT. The manufacturer’s quoted total imprecision for Siemens BRAHMS PCT is <11%.

2.3 Statistical analysis

The results obtained using the different methods and analyzers were compared with those obtained with BRAHMS PCT Kryptor. Method comparison was based on Wilcoxon–Mann–Whitney paired rank sum test, Deming fit, Spearman’s correlation coefficient, Bland–Altman difference plots, receiver operating characteristics (ROC) curves, and agreement at three clinically relevant cut-offs for bacterial infections (i.e., 0.50 ng/mL, 2.0 ng/mL and 10 ng/mL) with kappa statistic. The statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK) and the level of statistical significance was set at $p < 0.05$. The analytical evaluation was entirely based on pre-existing inpatient serum samples referred for routine PCT testing, so that informed patient consent was unnecessary. However, the study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

3. Results

No significant decay of PCT immunoreactivity was observed after one week of storage at −70 °C, as attested by the negligible bias observed between fresh and frozen serum analyzed with BRAHMS PCT Kryptor (mean −0.05 ng/mL; 95% CI, −0.12 to 0.010 ng/mL; $p = 0.097$). The distribution of PCT values obtained with different reagents and analyzers is shown in Fig. 1. Statistically significant differences compared to BRAHMS PCT Kryptor were observed for two BRAHMS-partnered PCT automated immunoassays (Vidas BRAHMS PCT and Advia Centaur BRAHMS PCT) and for three Diazyme PCT applications on Architect, Cobas C501 and AU5800 (Table 2). Nevertheless, the correlation between BRAHMS PCT Kryptor and the different methods and applications was always satisfactory, with correlation coefficients of 0.899 and 0.988 (all $p < 0.001$). The mean bias was also limited and always lower than ±1.02 ng/mL, with the exception of Vidas BRAHMS PCT (2.70 ng/mL) (Table 2 and Fig. 2). Interestingly, this bias was mostly attributable to overestimation at PCT values higher than 10 ng/mL with Vidas BRAHMS PCT (mean bias 11.6 ng/mL, 95% CI 8.1–15.1 ng/mL for PCT values > 10 ng/mL compared to mean bias of 0.6 ng/mL and 95% CI 0.04–1.19 ng/mL for values < 10 ng/mL) (Fig. 2). Other interesting information emerged from the comparison. Specifically, the bias between results obtained with BRAHMS PCT Kryptor and Advia Centaur BRAHMS PCT appeared to follow a specific linear pattern, characterized by overestimation of lower PCT values and underestimation of higher PCT concentration with the Advia immunoassay. Some outliers were also evident in almost every plot, but were more frequently observed for higher PCT values measured with Diazyme PCT applications (Fig. 2).

Overall, the agreement and the area under the curve (AUC) at the three clinically relevant cut-offs for bacterial infections were always satisfactory, with classification concordance between 83 and 98% at 0.50 ng/mL, between 90 and 97% at 2.0 ng/mL, and 98% at 10 ng/mL (Table 3). The direct comparison of data obtained with the Diazyme PCT assay on the four clinical chemistry analyzers yielded high correlation coefficients (between 0.952 and 0.976; all $p < 0.001$), a mean bias always less than ±0.9 ng/mL, an acceptable classification agreement at the 0.5 ng/mL threshold (82–94%), and a high classification concordance at both the 2.0 ng/mL (97–99%) and 10 ng/mL (98–100%) cut-offs (Table 4).

Table 2

Results obtained with procalcitonin immunoassays compared to BRAHMS PCT Kryptor.

<table>
<thead>
<tr>
<th>Method</th>
<th>Values (ng/mL)</th>
<th>P (Wilcoxon–Mann–Whitney)</th>
<th>Spearman’s correlation ($r^b$)</th>
<th>Deming fit$^b$</th>
<th>Mean bias and 95% CI (ng/mL)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kryptor</td>
<td>1.40</td>
<td>0.35–5.37</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Liaison</td>
<td>1.63</td>
<td>0.32–5.78</td>
<td>0.052</td>
<td>0.986 ($p &lt; 0.001$)</td>
<td>y = 1.02x + 0.22; 0.38 (−0.08 to 0.83)</td>
</tr>
<tr>
<td>Vidas</td>
<td>1.95</td>
<td>0.33–6.40</td>
<td>−0.001</td>
<td>0.979 ($p &lt; 0.001$)</td>
<td>y = 1.40x − 0.09; 2.70 (1.55 to 3.86)</td>
</tr>
<tr>
<td>Cobas E601</td>
<td>1.30</td>
<td>0.30–4.90</td>
<td>0.085</td>
<td>0.988 ($p &lt; 0.001$)</td>
<td>y = 1.12x − 0.47; 0.40 (−0.17 to 0.97)</td>
</tr>
<tr>
<td>Advia Centaur</td>
<td>1.35</td>
<td>0.32–5.11</td>
<td>0.001</td>
<td>0.984 ($p &lt; 0.001$)</td>
<td>y = 8.80x + 0.40; −1.02 (−1.67 to 0.36)</td>
</tr>
<tr>
<td>Diazyme (Architect)</td>
<td>1.27</td>
<td>0.28–4.53</td>
<td>0.038</td>
<td>0.899 ($p &lt; 0.001$)</td>
<td>y = 0.95x − 0.16; −0.51 (−1.07 to 0.06)</td>
</tr>
<tr>
<td>Diazyme (Advia 2400)</td>
<td>0.88</td>
<td>1.15–4.46</td>
<td>0.341</td>
<td>0.927 ($p &lt; 0.001$)</td>
<td>y = 1.01x − 0.23; 0.13 (−0.77 to 0.51)</td>
</tr>
<tr>
<td>Diazyme (Cobas C501)</td>
<td>0.80</td>
<td>0.48–4.38</td>
<td>0.004</td>
<td>0.895 ($p &lt; 0.001$)</td>
<td>y = 0.85x + 0.07; 1.01 (−1.76 to 0.26)</td>
</tr>
<tr>
<td>Diazyme (AU5800)</td>
<td>0.82</td>
<td>0.42–4.82</td>
<td>0.002</td>
<td>0.895 ($p &lt; 0.001$)</td>
<td>y = 0.87x − 0.04; 0.87 (−1.47 to 0.27)</td>
</tr>
</tbody>
</table>

* Median and interquartile range.

b Versus BRAHMS PCT Kryptor.
4. Discussion

The clinical value of measuring PCT for the diagnosis, follow-up and antibiotic guidance of severe bacterial infections has been confirmed in a large number of clinical trials [14–16], leading to the commercialization of many immunoassays to be used on conventional

Fig. 2. Bland and Altman plots of results obtained with different procalcitonin immunoassays (see Table 1 for details) compared to BRAHMS PCT Kryptor. The continuous line represents the mean bias, and the dotted lines are drawn at the 95% confidence interval (95% CI).
Conclusions

The results of our investigation demonstrate that the methods and applications evaluated in this multicenter study are substantially aligned with BRAHMS PCT Kryptor, and can hence be used for predicting the risk of progression to systemic inflammation, severe sepsis and septic shock in patients with bacterial infections using the conventional PCT diagnostic thresholds. Nevertheless, the varying bias between by the different methods indicates that longitudinal patient monitoring should preferably always be carried out using the same immunoassay.

Conflict of interest

None of the authors have any conflict of interest to report.
Acknowledgments

None.

References