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Cyclosporine A Concentrations in Blood Measured with the Immunoassays on Roche e601® and ADVIA Centaur XP® Analysers- What is the Extent of the Agreement?

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Abstract: Monitoring of cyclosporine A (Cs A) concentrations is inevitable for efficient and safe immunosuppression. Currently, immunoassays are the most often used method. The study compared the Cs A concentrations in EDTA-blood samples of 50 patients, measured on Roche e601® and ADVIA Centaur XP® analyzers. The Cs A concentrations on e601® were between 30.00 and 573.00 ng/mL. On Centaur XP® they were in the range 30.2-395.2 ng/mL. For all data the correlation coefficient (95% confidence interval (CI)) was 0.98 (0.97-0.99), while in the groups with concentrations below and above 100 ng/mL it was 0.90 (0.74-0.93) and 0.98 (0.94-0.99), respectively. The slope (95% CI) in the Passing-Bablok analysis on all results was 0.73 (0.67-0.83), and the intercept (95% CI) was 12.53 (6.66-17.78). In the group with results below 100 ng/mL, the slope was 0.92 (0.77-1.12) and the intercept 3.05 (from -8.45 to 12.09). For the Cs A concentrations above 100 ng/mL the slope was 0.71 (0.64-0.84) and the intercept 9.31 (from -8.86 to 24.27). The proportional and systematic errors were present in a wide range of Cs A concentrations measured on the evaluated analyzers. The concordance was satisfactory for concentrations below 100 ng/mL.

INTRODUCTION

Cyclosporine A (Cs A) is an immunosuppressant drug belonging to the family of calcineurin inhibitors (Tapia *et al*., 021). It has a narrow range between the pharmacologic and toxic doses. If overdosed, the common adverse effects include hypertension and nephrotoxicity (Pollard 2004; Damiano *et al.* 2015). Also, the extensive metabolism makes the pharmacokinetics of Cs A highly unpredictable (Survase *et al*.,2011; Soldin et al., 2010).

Therefore, monitoring Cs A concentrations in blood is inevitable in achieving efficient and safe immunosuppression in transplanted patients.

The commercial immunoassays represent usual methods for measuring the Cs A concentration in a majority of the routine clinical laboratories (Vogeser *et al*., 2014). Their main advantages are simple sample preparation and automatization (Zhang and Zhang, 2018). Nevertheless, caution is necessary when interpreting the results because the Cs A metabolites can interfere with the assays (Seger *et al*.,2016).

Roche® diagnostics and Siemens Healthcare® developed competitive immunoassays to measure the Cs A concentration in the whole blood (Soldin *et al*., 2010; Vogeser *et al*., 2014). The Roche assay employs precipitation with zinc sulfate and extraction with methanol for sample preparation and electrochemiluminescence for quantification of Cs A (Vogeser *et al*., 2014). The Siemens assay uses a single-step precipitation-free extraction for sample preparation and direct chemiluminescent technology on the ADVIA Centaur for quantification (Soldin *et al*., 2010). For both assays, the validation included a comparison with the liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Soldin *et al*., 2010; Vogeser *et al*., 2014), the "gold standard" for quantitation of the immunosuppressants (Seger *et al*., 2016). Nevertheless, no study has compared the results of these two immunoassays. This study aimed to evaluate the agreement between the Cs A concentrations in blood

measured using the immunoassays on Roche e601® and Siemens Healthcare ADVIA Centaur XP® analyzers.

EXPERIMENTAL

Samples

We used the surplus of samples from 50 patients on therapy with Cs A. Between October 2014 and January 2015, we collected venous blood samples into vacuum tubes with K_2EDTA (BD Vacutainer). After the manual pretreatment, the samples were analyzed within 6 hours from sampling, simultaneously on both analyzers, according to the manufacturer's instructions.

Immunoassays

In Elecsys Cs A assay (Roche Diagnostics GmbH, Mannheim, Germany), the pre-treated sample was incubated with a Cs A-specific biotinylated antibody and a Cs A derivative labeled with the ruthenium. Both the sample analyte and the ruthenium-labeled hapten interacted with the binding site on the labeled antibody. In the next step, the streptavidin–coupled paramagnetic particles on the solid phase bounded the entire complex. The intensity of the electrochemiluminescence signal was reciprocal to the Cs A concentration. The measuring range was 30-2000 ng/mL, and the within run coefficient of variation (CV) is 2.0-4.1 %. Concentrations of 1-hydroxy cyclosporine (AM1) and 4-N-desmethyl cyclosporine (AM4N) less than 2000 ng/mL showed a cross-reactivity of 2%. The analogous concentrations of 1, 9-dihydroxy cyclosporine (AM1,9) and 1- hydroxy-1-tetrahydrofuryl cyclosporine (AM1c) did not produce detectable cross-reactivity (Vogeser *et al*., 2014).

In Advia Centaur XP Cs A assay (Siemens Healthcare Diagnostics) Cs A from the pre-treated sample competed with the acridinium ester-labeled Cs A for binding to a biotin-labeled monoclonal anti-Cs A antibodies binding sites. In the next step, biotin-labeled anti-Cs A antibodies bound to the magnetic particles coated with streptavidin. The intensity of the generated chemiluminescent signal is inversely proportional to the concentration of Cs A. The measuring range was between 30 and 1500 ng/mL and the within run CV from 3.8 to 4.6%. The cross-reactivity with AM1, AM1c, AM4N, and AM1,9 in concentrations below 1000 ng/mL was less than 5 % (Soldin *et al*., 2010).

3. Statistical analysis

Statistical evaluation included Pearson correlation, Passing-Bablok, and Bland-Altman analyses, all performed in MedCalc® Statistical Software Version 12.5.0.0.

RESULTS AND DISCUSSION

Table 1 presents the obtained results. For all data, the correlation coefficient was 0.98 (0.97-0.99). In the groups with concentrations below and above 100 ng/mL was 0.90 (0.74-0.93) and 0.98 (0.94-0.99), respectively.

Our results indicate a close correlation between the wide range of Cs A concentrations measured on Roche e601® and ADVIA Centaur XP® analyzers. The previous articles reported the correlation between the concentrations obtained with the evaluated methods and LC-MS/MS (Soldin et al., 2010; Vogeser et al., 2014). The correlation was also present with the other immunoassays like chemiluminescent microparticle immunoassay (Soldin *et al*., 2010; Vogeser *et al*., 2014) and antibody-conjugated magnetic immunoassay (Vogeser *et al*.,2014).

The Bland-Altman analysis on all 50 samples identified only one point beyond the limits of agreement (Figure 1A). In the group with Cs A values below 100 ng/mL, two out of 32 points did not fit into the limits of the agreement (Figure 1B). Finally, for the samples with the concentrations of Cs A above 100 ng/mL, the Bland-Altman analysis showed one out of 18 points exceeding the limits of agreement (Figure 1C).

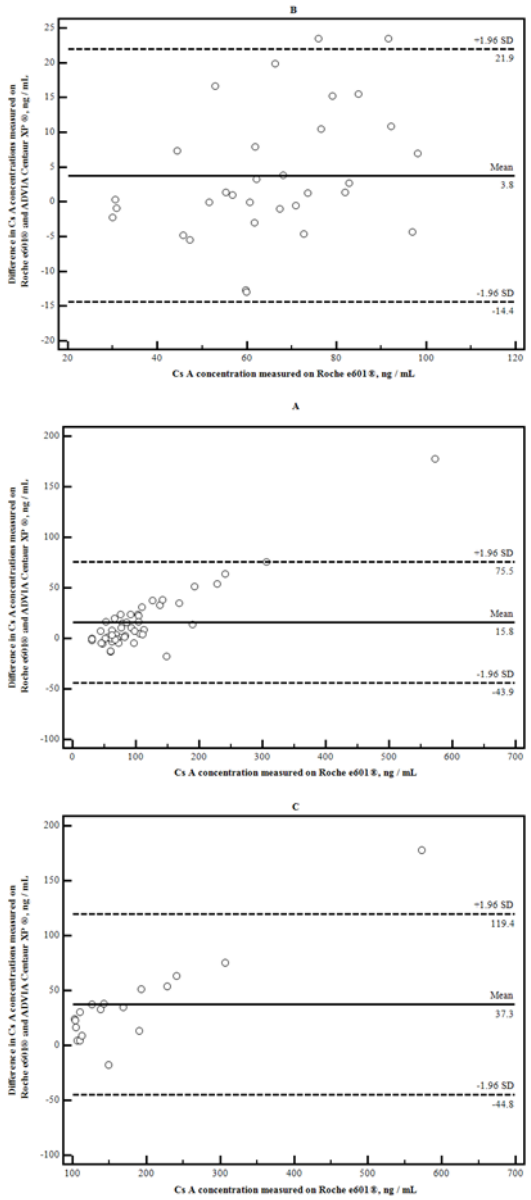

Figure 1. Bland-Altman analysis for (A) all samples, (B) samples with Cyclosporine A (Cs A) concentrations lower than 100 ng/mL, and (C) samples with Cs A concentrations higher than 100 ng/mL.

The Bland–Altman plots suggested a satisfactory agreement between Roche e601® and ADVIA Centaur XP® platforms regardless of the Cs A concentration in the

sample. In the initial evaluation study of the ADVIA Centaur XP®, the Bland–Altman analysis revealed the close agreement with the LC-MS/MS, the substantial specificity for Cs A, and the absence of the significant interference of the Cs A metabolites (Soldin *et al*., 2010; Vogeser et al., 2014). Similar findings occurred during the multicentric evaluation of Roche e601 (Vogeser et al., 2014), although a mild bias, acceptable according to the consensus recommendations (Wallemacq (2004), raised the possibility of the eventual cross-reactivity with the Cs A metabolites.In the Passing-Bablok regression (Figure 2A), the slope (95 % confidence interval) was 0.73 (0.67-0.83) and the intercept 12.53 (6.66-17.78). Also, the slope 0.92 (0.77-1.12) and the intercept 3.05 (from -8.45 to 12.09) were the results of the Passing-Bablok analysis (Figure 2B). The slope and the intercept calculated in the Passing-Bablok analysis (Figure 2C) were 0.71 (0.64-0.84) and 9.31 (from -8.86 to 24.27).

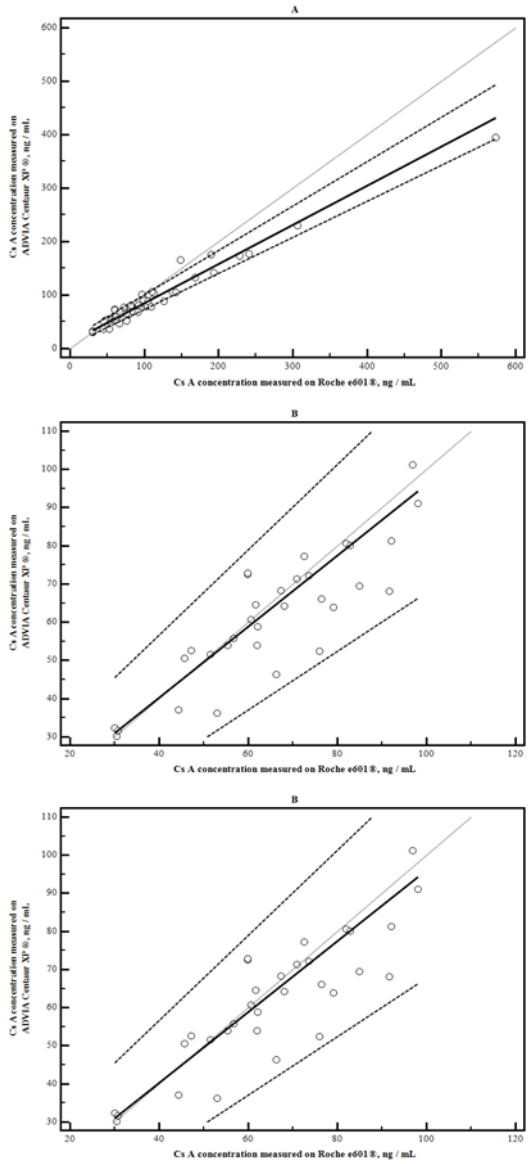

Figure 2. Passing-Bablok regression analysis for (A) all samples, (B) samples with Cyclosporine A (Cs A) concentrations lower than 100 ng/mL, and (C) samples with Cs A concentrations higher than 100 ng/mL.

The Passing–Bablok analysis revealed the concentration-dependent disparity between the evaluated methods. In the wide range of concentrations, the combination of negative proportional and positive systematic error appeared after the comparison. The errors were absent when the analysis included only samples with a Cs A concentration less than 100 ng/mL. On the contrary, the negative proportional error persisted for the blood samples with a Cs A concentration higher than 100 ng/mL. The validation studies reported that in comparison with the LC-MS/MS, ADVIA Centaur XP® had shown the negative proportional error (Soldin *et al*., 2010), while for Roche e601®, there had been both positive proportional and systematic error (Vogeser *et al*., 2014). The negative proportional error characterized both ADVIA Centaur XP® and Roche e601® in comparison with the other immunometric platforms (Soldin *et al*., 2010; Vogeser *et al*., 2014), while the negative systematic error was present for Roche e601®
The observed disparity presumably has at least two causes. One could be the cross-reactivity with the Cs A metabolites. They can occur in concentrations between 5 and 150% relative to the Cs A concentration (Seger *et al*., 2016). The findings of the validation studies showed the neglectable interference from metabolites in ADVIA Centaur XP® assay (Soldin *et al*., 2010). For the Roche e601®, they ranged between 2 and 6% (Vogeser *et al*., 2014), and although they characterized the concentrations much higher than those measured in this study, the fact that we confirmed a concentration-dependent proportional error supports their potential significance.

Also, the disparity could result from the difference in the sample preparation between the evaluated methods. Approximately 50% of Cs A in the blood is in the erythrocytes, and the rest is tightly bound to proteins (Seger *et al*., 2016). Therefore, the manual pre-treatment of the sample makes the Cs A accessible for the antibodies in both assays (Oellerich *et al*., 1995). For ADVIA Centaur XP® the pre-treatment had included single-step Cs A extraction (Soldin *et al*., 2010), instead of the precipitation and extraction, as it had been for Roche e601® (Vogeser *et al*., 2014). Notwithstanding that the single-step Cs A extraction had been technically more convenient, the advantage of precipitation could be the removal of the potentially interfering heterophilic antibodies (Bartoli *et al*., 2010).
A relatively small number of participants may represent a limitation of the study. However, the results contribute to the evaluation of the equivalence between the Cs A concentrations measured with different immunometric platforms. Therefore, they serve as a reliable starting point for future larger studies.

CONCLUSION

In a wide range, the proportional and systematic errors were present after the comparison of the Cs A concentrations measured on Roche e601® and ADVIA Centaur XP ® analyzers. The concordance is satisfactory for Cs A concentrations less than 100 ng/mL.

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Summary/Sažetak

Praćenje koncentracija ciklosporina A (Cs A) u krvi je neizostavan element postizanja efikasnog i bezbjednog imunosupresivnog efekta. Trenutno se u tu svrhu najčešće primjenjuju imunohemijske metode. U ovom istraživanju, poređene su koncentracije Cs A u uzorcima EDTA krvi 50 pacijenata, izmjerenih na analizatorima Roche e601 i ADVIA Centaur XP. Interval koncentracija izmjerenih na Cobas e601 analizatoru kretao se od 30.00 do 573.00 ng/mL. Na Centaur XP analizatoru taj interval bio je od 30.2 do 395.2 ng/mL. Uzevši u obzir sve podatke, koeficijent korelacije (interval pouzdanosti 95% (CI)) iznosio je 0.98 (0,97-0,99), dok je u grupama sa koncentracijama Cs A iznad i ispod 100 ng/mL iznosio 0.90 (0.74-0.93) i 0.98 (0.94-0.99). Passing-Bablok analizom ukupnih podataka (95% CI), dobijena je vrijednost nagiba od 0.73 (0.67-0.83), dok je vrijednost odsječka iznosila 12.53 (6.66-17.78). Za grupu podataka sa koncentracijama Cs A manjim od 100 ng/mL, nagib i odsječak iznosili su 0.92 (0.77-1.12) i 3.05 (8.45 - 12.09). Za koncentracije Cs A iznad 100 ng/mL vrijednost nagiba bila je 0.71 (0.64-0.84), a odsječka 9.31 (8.86-24.27). Proporcionalne, kao i sistemske greške bile su zastupljene u širokom području koncentracija Cs A izmjerenih na ispitivanim analizatorima. Pri koncentracijama nižim od 100 ng/mL, slaganje metoda je zadovoljavajuće.