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COMPARISON OF TWO COMMERCIAL CYCLOSPORIN ASSAYS

POREĐENJE DVA KOMERCIJALNA TESTA ZA ODREĐIVANJE CIKLOSPORINA A

Marina Vučeljić[1], Nataša Lalić[2], Zoran Mijušković[1], Janko Pejović[1]

[1]Institute of Medical Biochemistry, Military Medical Academy, Belgrade, Serbia
[2]Institute of Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

Summary: A new Dade Behring Cyclosporin A (CsA) assay has been made commercially accessible recently. The aim of this study was to examine the analytical performance of this assay and the correlation with the Abbott Diagnostics assay. The accuracy and precision within-run were good, but precision between-run slightly exceeded the manufacturer's recommendation on one quality-control material. Dade Behring CsA whole blood results from the kidney recipients (n = 104) were lower ($p < 0.001$) than the Abbott results. The correlation between these two assays was significant ($y = -15.61 + 0.751x$; $r = 0.974$; $p < 0.001$). Dade Behring CsA assay demonstrates adequate performance characteristics for routine clinical use and the main advantage is the full automatization without the need for samples pretreatment.

Keywords: cyclosporin A, transplantation, Dade Behring assay, Abbott Diagnostics assay, correlation

Kratak sadržaj: Odnedavno je komercijalno dostupan novi Dade Behring test za određivanje CsA u krvi. Naš cilj bio je da ispitamo analitičke karakteristike ovog testa i korelaciju sa Abbott Diagnostics testom. Tačnost i preciznost u seriji Dade Behring testa bili su zadovoljavajući, dok je preciznost iz dana u dan blago prevazilazila preporuku proizvođača u jednoj kontroli. Određivanjem CsA u krvi pacijenata kojima je izvršena transplantacija bubrega (n=104) Dade Behring testom, dobijene su niže vrednosti ($p<0,001$) nego Abbott testom. Korelacija ove dve metode bila je statistički značajna ($y = -15,61 + 0,751x$; $r = 0,974$; $p<0,001$). Dade Behring test za određivanje CsA pokazuje odgovarajuće karakteristike za rutinsku kliničku upotrebu, a glavna prednost je potpuna automatizacija testa bez potrebe za prethodnom pripremom uzorka.

Ključne reči: ciklosporin A, transplantacija, Dade Behring test, Abbott Diagnostics test, korelacija

Introduction

Cyclosporin A (CsA) is an immunosuppressant used in graft rejecting prevention following solid organ and bone marrow transplantation. Today, however, this drug is also used for the treatment of some autoimmune diseases. Cyclosporin A is a cyclic polypeptide of fungal origin which specifically inhibits T-lymphocyte activation (1, 2). It may be administered intravenously or orally, and its absorption, distribution and clearance are variable. Following oral application, up to 50% of the drug resorbs depending on the individual, while the peak blood concentration occurs 2–4 hours later (1). At room temperature and at therapeutic concentrations, under *in vitro* conditions, CsA is partitioned between erythrocytes (40–50%), leukocytes (10–20%) and plasma where it is primarily bound to lipoproteins (3). Partitioning of the drug between these compartments is dependent on temperature, hematocrit and metabolite concentration (4, 5). CsA is metabolized in the liver via cytochrome P-450 mediated processes, producing thus more than thirty metabolites, most of which are inactive (1, 2). Liver dysfunction can alter absorption or elimination of the drug, and therefore the management of CsA therapy in liver recipients is very complex. Moreover, CsA interacts with some drugs that may change its bioavailability and metabolism. Lately, an increasing interest has been shown in the impact of genetic polymorphisms for metabolic enzymes and transporter proteins on dose requirements or on drug efficacy (6). CsA metabolites elimination is mainly performed through the bile, with only about 6% through urine.

Address for correspondence:

Marina Vučeljić
Institute of Medical Biochemistry, Military Medical Academy
Crnotravska 17, 11000 Belgrade
e-mail: vucmina@eunet.yu

Serious adverse effects related to CsA treatment are nephrotoxicity, hepatotoxicity, neurotoxicity, hypertension (7, 8). Considering the potential toxicity and variability in pharmacokinetics, therapeutic drug monitoring for this drug is essential.

There are many analytical methods for the measurement of whole blood CsA concentrations. High-performance liquid chromatography (HPLC) assay is considered the gold standard (9, 10) since it provides the most specific measurement of the parent drug, although it is a labor-intensive and time-consuming process. In clinical practice, however, the most frequently used are immunoassays with monoclonal and polyclonal antibodies (11, 12). These assays require sample pretreatment, including lysing of erythrocytes, before assay performance. Our laboratory most commonly uses the Abbott Diagnostics assay. Recently, however, no pretreatment requiring and monoclonal antibody-based immunoassay of the Dade Behring has appeared. The aim of this study was to compare these two assays.

Materials and Methods

The procedure of the Dade Behring assay accuracy evaluation included duplicate calibrators determining as samples (Csa Calibrator levels 1, 2, 3, 4, 5, Dade Behring), while the accuracy of the method was calculated by linear and regression analyses. Three quality control materials were used for quality control: QC 1, 2 and 3 (Dade Behring). Precision within-run was calculated by measuring quality control materials in duplicate 20 times in a single analytical run. Precision between-run was calculated by measuring quality control materials in duplicate within 20 days. For quality control of the Abbott Diagnostics assay and between-run precision we used two quality control materials: low and medium control (Abbott Diagnostics).

A total of 104 samples from 70 kidney recipients were examined. Blood was sampled into vacuum collection tubes with potassium-EDTA as anticoagulant. The samples were stored at –20 °C for a week. After thawing, CsA was determined on both the Dade Behring assay using a Dimension Xpand analyzer, and the Abbott Diagnostics assay using a TDx analyzer at the same time.

The procedure for the Dade Behring Dimension Xpand assay is as follows: 200 μL of whole blood is added to a sample cup by the operator. The Dimension uses the ultrasonic probe to mix the sample, lyses the blood cells by the use of a saponin-based buffer and sonication, and then adds an anti-CsA antibody/β-galactosidase conjugate that binds CsA in the sample. After CsA-coated magnetic beads are added to the reaction mixture, unbound conjugate is removed magnetically. The supernatant containing the CsA-antibody-enzyme complex is then transferred to a measuring cuvette, where it is mixed with chlorophenol red galactoside, which is hydrolyzed by the β-galactosidase to chlorophenol red. The change in absorbance at 577 nm directly correlates to the amount of CsA in the sample. If CsA concentrations exceeded 500 μg/L, manual dilution was recommended, using the Dimension CsA Level 1 Calibrator (0.0 μg/L).

Abbott Diagnostics TDx CsA assay is an immunoassay with monoclonal antibody requiring the sample pretreatment step. Sample pretreatment is performed in several steps. First, an operator transfers 150 μL of whole blood to the centifuge tubes, then adds 50 μL of a solubilization reagent which lyses the erythrocytes, taking care not to have any air bubbles present in order to prevent foaming which may affect the results. After that, 300 μL of precipitation reagent should be added. Vortex tubes for at least 10 seconds to reach homogeneity. Centrifuge tubes in a microfuge at 9500×g for at least 5 minutes. Decant supernatant into corresponding sample cup, which is subsequently put into the analyzer for further automated handling assay based on the fluorescence polarization immunoassay (FPIA) technology.

The values were expressed as mean ± standard deviation. Statistical tests were performed by the statistical package Statistic for Windows (Stat for Windows, R. 4.5, USA). The correlation was analyzed by the Pearson linear regression test. Values of $p<0.05$ were taken as statistically significant.

Results

The Dade Behring assay accuracy results were presented in *Table I*. The statistically significant correlation between labeled and measured CsA values was obtained ($r=0.99968$; $p<0.001$), presented by the following equation: $y = -2.109 + 0.985x$, where y represented the measured CsA levels, and x labeled CsA levels. An intercept (–2.109) presented the systemic error of the method, which was not statistically significant ($p>0.05$), and a slope (0.985) was a percentage deviation of –1.5% (100% –98.5% = 1.5%) and was nonsignificant ($p>0.05$).

The results of the Dade Behring assay precision within-run and between-run analyses are showed in *Table II*. The coefficients of variation (CV%) values for the within-run precision were 3–5.2%, with those for the between-run ranging 7.5–15.1%. The results variation was greater at lower concentrations.

The results of the Abbott Diagnostics assay precision between-run analyses are showed in *Table III*. The coefficients of variation ranged 5.3–6.4%, and they were greater than the manufacturer's recommendations.

The levels of CsA in whole blood of transplanted patients, measured by the Dade Behring assay, were lower than the Abbott Diagnostics assay *(Figure 1)*. The mean CsA measured by the Dade Behring assay

Table I Accuracy of CsA assay on Dade Behring analyzer.

Calibrators CsA (g/L)	0.0	80.0	180.0	330.0	500.0
Measured values* CsA (g/L)	1.6	78.3	166.6	324.7	492.5

* Mean of two measurements of calibrators as sample.

Table II Precision of CsA Dade Behring assay.

Sample	Sample number (n)	Expected value CsA (μg/L)	Mean value CsA (μg/L)	Sd (μg/L)	CV (%)	CV (%) Manufacturer's recommendation
Within-run						
Control 1	20	44.0	46.3	2.41	5.2	–
Control 2	20	114.0	118.2	5.32	4.5	4.9
Control 3	20	303.0	314.5	9.43	3.0	5.4
Between-run						
Control 1	40	44.0	49.3	7.42	15.1	–
Control 2	40	114.0	120.8	15.82	13.1	11.3
Control 3	40	303.0	328.4	24.75	7.5	7.8

Table III Between-run precision of CsA Dade Behring assay.

Sample	Sample number (n)	Expected value CsA (μg/L)	Mean value CsA (μg/L)	Sd (μg/L)	CV (%)	CV (%) Manufacturer's recommendation
Low control	40	150.0	154.7	9.90	6.4	2.2
Medium control	40	400.0	422.1	22.37	5.3	2.0

was 57.3 ± 41.88 mg/L, while the Abbott Diagnostics was 97.02 ± 54.34 mg/L. The mean difference was statistically significant ($p<0.001$). Also, a significant correlation between these two assays was observed: $y = -15.61 + 0.751x$; $r=0.974$; $p<0.001$; y – represented Dade Behring assay and x – Abbott Diagnostics assay *(Figure 2)*.

Discussion

The Dade Behring CsA assay showed good accuracy. The obtained CV% values for the within-run precision were 3–5.2%, which was in accordance with the manufacturer's recommendation. Similar results were published (11, 13) for the Abbott Diagnostics method (CV 2–6%). However, CV% values for the between-run precision (7.5–15.1%) slightly exceeded the manufacturer's recommendations and what the Consensus document recommends for CsA assays. Namely, the Consensus document recommends the following performance characteristics for CsA assays: CV ≤10% at concentration of 50 μg/L, and CV ≤5% at concentration of 300 μg/L (10). We obtained CV=15.1% at concentration of 44 μg/L, and CV=7.5% at concentration of 303 mg/L. Our precision between-run results of the Dade Behring assay were in accordance with other authors' results (15). At the same time, the Abbott Diagnostics assay showed better precision between-run, but it should be noted that the variations of the results were greater than the manufacturer's recommendations *(Table III)*. However, it should be pointed out that many CsA immunoassays do not have satisfactory between-run precision (11–13).

The linearity range for Dade Behring assay is from 30 to 500 μg/L, which is acceptable for routine clinical use, because therapeutic ranges are generally 75–400 mg/L (13). Immunosuppression requires trough whole blood concentration of at least 100 μg/L. It has been found that whole blood concentration exceeding 600 μg/L is associated with toxic effects. Therapeutic trough blood concentration of CsA for renal transplants is 100 to 300 μg/L, whereas 200 to 350 μg/L is used as the target concentration for cardiac, hepatic and pancreatic transplants. However, there is no universally established therapeutic range for CsA in the blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of CsA, co-administration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of this drug (2).

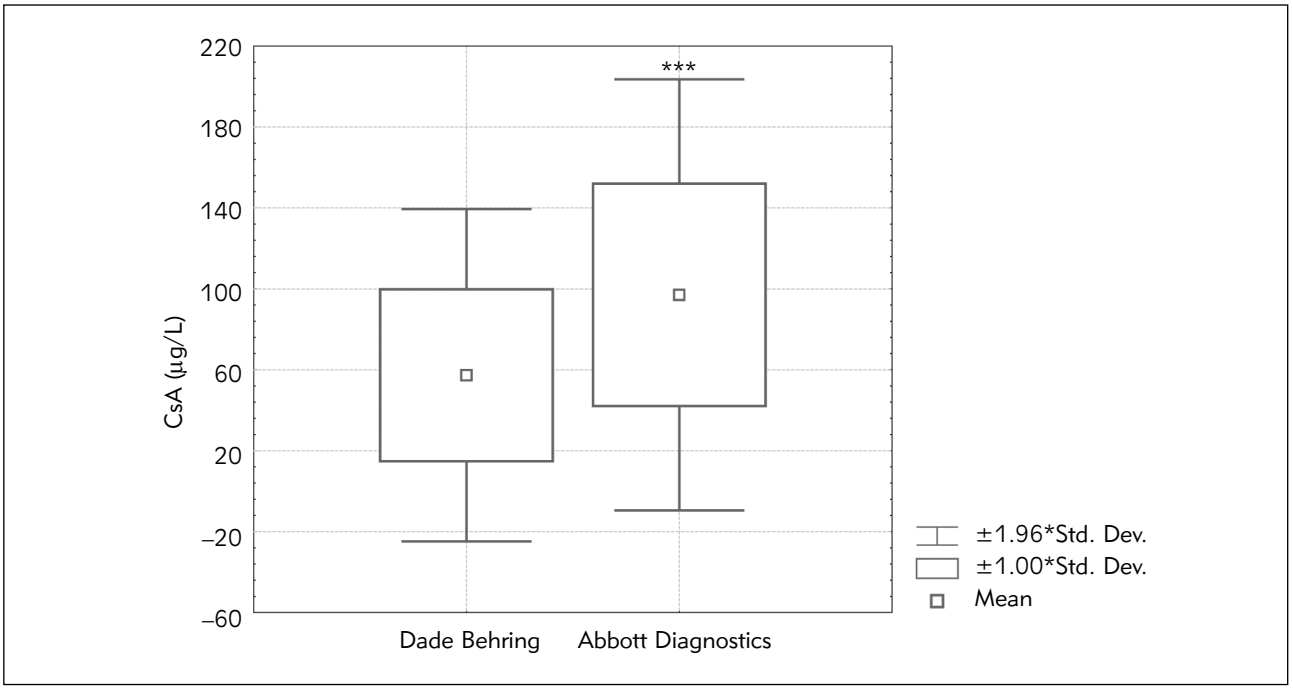

Figure 1 Presentation of the results obtained by CsA whole blood assay in kidney recipients using the Dade Behring and Abbott Diagnostics (n=104).

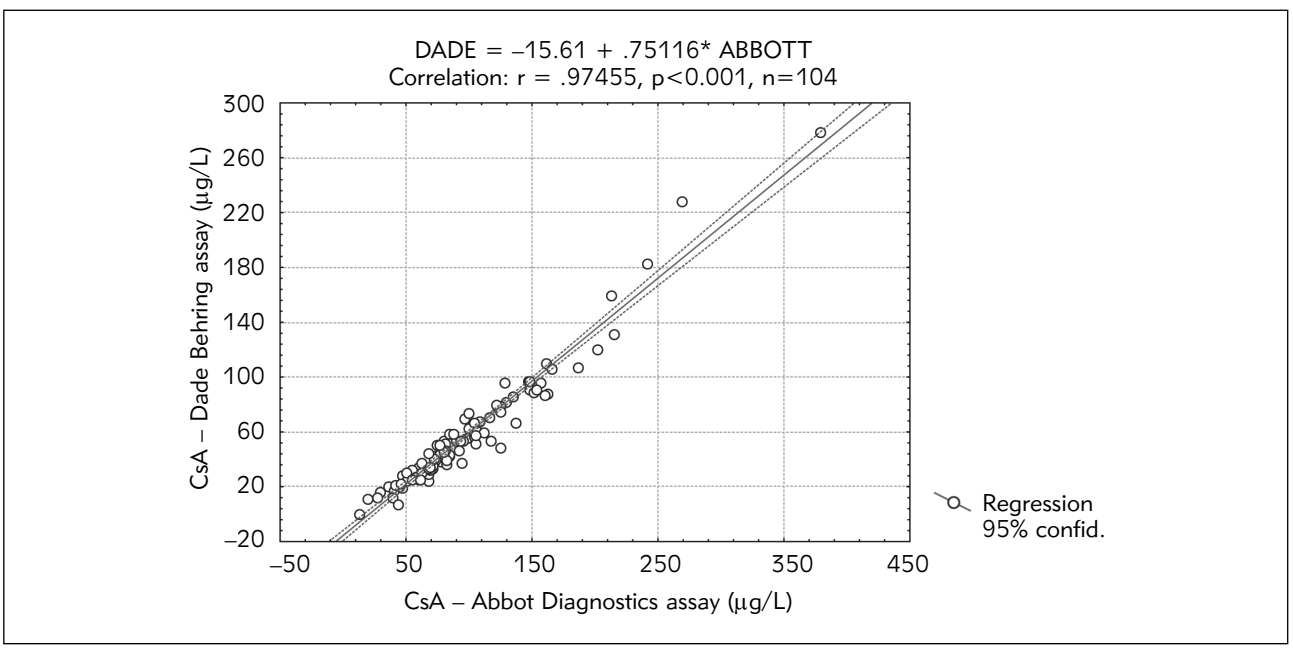

Figure 2 Correlation between the results of CsA whole blood assays using the Dade Behring and Abbott Diagnostics.

Evaluation of a no-pretreatment CsA assay results using the Dade Behring method is comparable to that obtained by the Abbott method. In our study, we obtained slightly lower results using the Dade Behring assay than the Abbott one. These assays use different monoclonal antibodies, which is the likely reason for the differences in the results. Terrell at al. (13) compared these two methods with HPLC and showed that the Dade Behring assay has a constant positive error when compared to HPLC, but lower than that showed by the Abbott assay. These results are presumably attributed to variable cross-reactivities with CsA metabolites, since the Dade Behring assay showed less cross-reactivity with all tested metabolites except AM4N (11, 13).

Our opinion, thus, is that the lower cross-reactivity assays are better to use, because the role of metabolites in immunosuppression remains to be a subject of discussion. Anyhow, the use of different evaluation of CsA assays provides the results which differ 3.1–51.8% from the results obtained by the HPLC method (6, 14).

Turnaround time can be important when assaying immunosuppressants, and results should be generated within a dosing interval to let changes in the next dose be made if necessary. An assay for CsA on a random-access analyzer, such as the Dade Behring Dimension Xpand, substantially decreases turnover time, since it is fully automated and eliminates the requirement for a manual pretreatment. In addition, being fully automated, it decreases error possibility. As we know, the less manual steps, the less error possibility. According to the above said, it can be concluded that the benefits of the Dade Behring assay as compared to the Abbott assay are less cross-reactivity with CsA metabolites, that is, higher specificity and less turnover time, and a full automatization implying more comfort and a decreased preanalytic error. The advantage of the Abbott assay showed to be the higher between-run precision.

In conclusion, the new no-pretreatment CsA assay on the Dade Behring analyzer demonstrates adequate performance characteristics for routine clinical use. The results obtained with the Dade Behring assay correlate with those obtained with the Abbott assay.

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