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COMPARISON OF ARCHITECT I 2000 FOR DETERMINATION OF CYCLOSPORINE WITH AXSYM

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Original paper
ABSTRACT
Background: Cyclosporine has been shown effective drug in suppressing acute rejection in recipients of allograft organ transplants. ***Methods:*** The cyclosporine concentration of 96 blood samples was determined using CMIA (chemiluminesecent microparticle immnoassay) Architect i 2000 and FPIA (fluorescence polarization immunoassay) AxSYM Abbott diagnostic. All patients have transplantation of kidneys and were hospitalized at Department of Nephrology at the Clinical center of University of Sarajevo. The reference serum range of cyclosporine for kidney organ transplantation for maintenance lies between 50 and 150 ng/mL. The quality control, precision and accurancy of Architect i 2000 were assessed. ***Results:*** The quality control was done using quality control serums for low (= 91 ng/mL), medium (= 328 ng/mL) and high (= 829 ng/mL). We have used commercial BIORAD controls and got reproducibility CV 5.83 % to 13 % for Architect i 2000. It was established that the main difference between Architect i 2000 and AxSYM and it was statistically significant for $P < 0.05$ according to Student t-test. Correlation coefficient was r = 0.903. ***Conclusion:*** The CMIA Architect assay has significant reduced cyclosporine metabolite interference relative to other immunoassay and is a convenient and sensitive automated method to measure cyclosporine in whole blood.
Key words: architect I 2000, determination, Cyclosporine, Axsym.

1. INTRODUCTION

Regular monitoring of cyclosporin A (CsA) in whole blood for dosage adjustment is considered mandatory. Immunosuppressive drugs in transplant patients represent life-long therapy and are the key to prevention of acute and chronic graft rejection. The specific pharmacokinetic profile of each immunosuppressive drug, low therapeutic index and potential interactions with numerous medications indicate the fact that monitoring of immunosuppressive therapy is the essential part of therapy protocol in transplant patients. Despite the possible role of CsA metabolites in immunosuppression and toxicity (1, 2), the consensus among clinicians and laboratory personal is that specific methods for measuring the parent drug only should be used (3, 4, 5). Following the widespread introduction of the micro emulsion formulation of CsA (Neoral®; Novartis Pharma), there has been a renewed interest in approaches to therapeutic drug monitoring TDM that are based on the original observations of Lindholm and Kahan (6). These authors demonstrated that total exposure to CsA, as reflected by the area under the concentration-time curve (AUC), was a better predictor of outcomes than predose (trough) CsA concentrations. Furthermore, several studies have shown that the AUC can be estimated with good reliability by means of a limited sampling strategy (7, 8). Recently, clinical studies utilizing CsA measurements made at single or multiple time points in the early period (0–6 h) after CsA ingestion have shown the potential of such measurements for improving clinical outcomes compared with the traditional, predose, approach. These studies have made recommendations for target CsA concentration ranges at either specific postdose time points (2 or 3 h) or for limited AUC measurements in the period 0–6 h post dose. The recommendations were based on particular immunoassay methods and were for either kidney or liver transplant patients (9, 10, 11, 12). The monoclonal antibody-based fluorescence polarization immunoassay (mFPIA) CsA assay has been adapted for the AxSYM (Abbott). The ARCHITECT CsA assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of cyclosporine in blood. Using patient samples collected in our laboratory we analyzed Cyclosporine concentration by CMIA and FPIA technology methods and compared the results.

2. MATERIAL AND METHODS

2.1. Patients

The patient samples of blood were collected in Na-EDTA Vacutainer test tubes (Becton Dickinson, Rutherford, NJ 07,070 U.S.) in volume of 3.5 mL. We used test tubes with Na-EDTA. The investigation was done

respecting ethical standards in the Helsinki Declaration. The investigation included patients (n=96) in period from February till September in 2012. The study included patients who were hospitalized at Department of Urology and Department for Kidney disease at the Clinical center of University of Sarajevo.

2.2. Methods

All immunoassays require the use of labeled material in order to measure the amount of antigen or antidody. A label is a molecule that will react as a part of the assay, so a change in signal can be measured in the blood after added reagent solution. CMIA is noncompetitive sandwich assay technology to measure analytes. The amount of signal is directly proportional to the amount of analyte present in the sample.

2.3. Chemiluminescent microparticle immunoassay – CMIA

Architect CsA asssay is two-step immunoassay to determine the presence cyclosporine in human serum using CMIA technology. In the first step, sample, assay diluent and anti- cyclosporine -I-antibody-coated paramagnetic particles are combined. CsA present in the sample binds to the anti- cyclosporine -I coated microparticles. After incubation and wash, anti- cyclosporine -I-acridinium-labeled conjugat is added in the second step.

Following another incubation and wash, pre-trigger and trigger solutions are then added to the reaction mixture. The pre-trigger solution (hydrogen peroxide) creates an acidic environment to prevent early release of energy (light emission), helps to keep microparticles from clumping and splits acridinium dye off the conjugate bound to the microparticle complex (this action prepares the acridinium dye for the next step). The trigger solution (sodium hydroxide) dispenses to the reaction mixture. The acridinium undergoes an oxidative reaction when is exposed to peroxide and an alkaline solution. This reaction causes the occurence of chemiluminescent reaction. N-methylacridone forms and releases energy (light emission) as it returns to its ground state. The resulting chemiluminescent reaction is measured as relative light units (RLU). A direct relationship exists between the amount of SCC in the sample and RLU detected by Architect System optics.

2.4. Manual pretreatment procedure

The ARCHITECT CsA assay requires a manual pretreatment step for all whole blood patients speciments, calibrators and controls. Each sampler sould be mixed by slow inversion of the container 5-10 times. We add a 200 µL of sample, 100 µL of ARCHITECT CsA whole solubilisation reagent and 400 µL ARCHITECT CsA whole blood precipitation in centrifuge tube. The added blood and all regagents we vortex vigorously for 5-10 seconds and centrifuge for 4 minutes. The supernatant we take to transplant pretreatment tube (13, 14).

2.5. Fluorescence polarization immunoassay- FPIA

FPIA is a type of homogeneous competitive fluorescence immunoassay. With competitive binding, antigen from the speciment and antigen-fluorescein (AgF) labeled reagent compete for binding sites on the antibody. As a homogeneous immunoassay, the reaction is carried out in a single reaction solution, and the bound Ab-AgF complex does not require a wash step to separate it from „free“ labeled AgF. Typically antigen is labeled with fluorescemt label and competes with unlabeled antigen from the speciment. The relatively slow rotation of large molecule as well as the ability of slow-moving particles to polarize light are utilized to obtain a measure of the number of large antibody-antigen-fluorescein particles in solution. In this competitive format, the concentration of the analyte present is indirectly proportional to the amount of the signal measured. Fluorescein absorbs light energy at 490 nm and releases this energy at a higher wave length 520 nm as fluorescent light.

2.6. Manual pretreatment procedure

The AxSYM CsA assay requires a manual pretreatment step for all whole blood patients speciments, calibrators and controls. Each sampler sould be mixed by slow inversion of the container 5-10 times. We add a 150 µL of sample, 50 µL of AxSYM CsA whole solubilisation reagent and 300 µL AxSYM CsA whole blood precipitation in centrifuge tube. The added blood and all regagents we vortex vigorously for 10 seconds and centrifuge for 5 minutes. The supernatant we take to sample cup (13, 15).

2.7. Quality control

The low, medium and high CsA controls of commercially available BIORAD controls for ARCHITECT ABBOTT and AxSYM ABBOTT CsA controls were used. The precision (intra-day variation) was tested by measuring (n = 20) of three different controls of CsA. The reproducibility (inter-day variation) for same controls was tested all controls once a day over 10 consecutive days. The accuracy of measuring was tested in 96 whole blood EDTA samples of patient who were determined CsA. Measures were obtained by ARCHITECT CMIA and AxSYM FPIA technology.

2.8. Statistics

The results were statistically analyzed using NCSS and statistical software SPSS version 12.0 software. Determined by the average value (), standard deviation (SD), Pearson correlation coefficient (r), equations of linear regression and Student t test with statistical significance level of $P < 0.05$.

3. RESULTS

3.1. Quality control testing

three controls low, medium and high Abbott technology (n = 20) were measured for quality control testing. Measurements were done during 10 days period. The average value (), standard deviation (SD) and coefficient of variation (CV) are shown in Table 1. The coefficients of variation (CVs) for the three controls using ARCHITECT CsA BIORAD controls assay were 5.1-7.3 %. Reproducibility was determined by running controls in the morning over

Concentration spiked (ng/mL)	Concentration found intra-day (mean SD, n= 20) (ng/mL)	Precision intra-day (%)	Concentration found inter-day (mean SD, n= 20) (ng/mL)	Reproducibility (%)
Architect Cyclosporine assay CMIA technology				
91	97±5.3	7.3	118.7±15.4	13.0
328	335±15.4	6.0	401±30.8	7.69
829	835±37.2	5.1	920±53.7	5.83
AxSYM Cyclosporine assay MEIA technology				
70	69.8±5.31	7.2	69.8±8.9	12.0
300	305.4±12.52	4.3	299.7±16.8	8.94
600	596±26.37	6.1	611±28.6	10.24

Table 1. Quality control testing

Suggested maintenance therapeutic ranges (50-100 ng/mL)	X_{sr}	S.D.	S.E.
ARCHITECT CMIA	76.63	11.00	2.75
AxSYM FPIA	91.91	18.38	4.59
Suggested maintenance therapeutic ranges (100-150 ng/mL)	X_{sr}	S.D.	S.E.
ARCHITECT CMIA	131.72	19.55	4.48
AxSYM FPIA	143.61	14.95	3.43
Suggested initiation therapeutic ranges (150-300 ng/mL)	X_{sr}	S.D.	S.E.
ARCHITECT CMIA	234.65	75.14	26.56
AxSYM FPIA	231.32	66.89	23.65

Table 2. The mean concentration of cyclosporine determined in different methods

10 consecutive day. CV for the reproducibility of CsA assay varied from 5.83 to 13 %. The CVs for the three controls using AxSYM CsA assay technology were 4.3–7.2 %. Reproducibility was determined by running controls in the morning over 10 consecutive day. CV for the reproducibility of Cyclosporine assay varied from 8.94 to 12.0 %.

3.2. Accuracy testing

We compared CsA concentration measured in 96 whole blood by ARCHITECT CMIA and AxSYM FPIA technology. The results of the comparison between ARCHITECT CMIA and AxSYM FPIA technology analysis are shown in Figure 1. Sizable correlation was noted between Architect and AxSYM technology in the investigation of 96 blood samples (r = 0.902). Regression equation revealed a slope of 0.8744 and a y axis intercept of 25.842. The difference between the methods was statistically significant for P<0.05 according Student t-test.

The concentration range of measured blood samples of CsA using AxSYM FPIA technology was 38.7-375 ng/mL and for ARCHITECT CMIA technology was 32.7-385 ng/mL. The average concentration of CsA using ARCHITECT was 120.49+/-68.62 ng/mL and AxSYM was 131.20+/-64.13 ng/mL. Therefore, serum concentrations of CsA measured using ARCHITECT CMIA were significantly lower than those measured using AxSYM FPIA technology. The average concentration of CsA using ARCHITECT was lower for – 11.89 ng/mL to–15.28 ng/mL in compare with AxSYM FPIA technology in the suggested maintenance therapeutic ranges. In the suggested initiation therapeutic ranges the average concentration of CsA was same in both technologies. The results of mean CsA concentration are shown in Table 2.

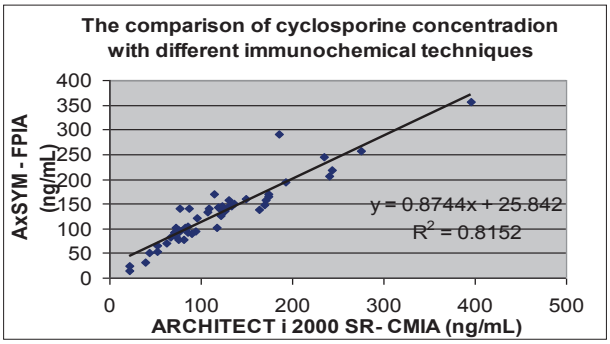

Figure 1. The correlation in cyclosporine concentration between ARCHITECT and AxSYM

4. DISCUSSION

The specificity of monoclonal immunoassays for CsA has been addressed in the past mostly by direct comparison with HPLC or by measurement of purified metabolites. Few data correlating the presence of metabolites in clinical samples and the irregular biases observed with monoclonal immunoassays are available. This is because HPLC procedures for measuring metabolite concentrations are labor-intensive and time-consuming. Ratios between nonspecific immunoassays and specific methods have been widely used to estimate overall metabolite concentrations in patient samples and to identify differences between certain populations. Large inter-individual differences have also been observed (16, 17, 18, 19, 20). Our results have shown precision CV for ARCHITECT Cyclosporine BIORAD controls assay were 5.1-7.3 %. The CVs for the three controls using AxSYM Cyclosporine assay technology were 4.3–7.2 %. The total precision %CV of the ARCHITECT and AxSYM Cyclosporine assay was determined to be less than to 12%. The other investigation groups have got a results for total precision less or equal to 15 % (21,22). In Architect CMIA and AxSYM FPIA technology using Levey – Jennings report for measurement of CsA was under range of two S.D. The accuracy testing shows that we found very good correlation between CMIA and FPIA with correlation coefficient r = 0.902, the other groups have correlation coefficient r ≥ 0.89. The average concentration of CsA using ARCHITECT was lower for 11.2 ng/mL then using AxSYM FPIA. However the cross-reactivity of the seven metabolites using the Abbott monoclonal assay matched closely with their pharmacological potency as measured in the MLC assay (21). FPIA whole blood CsA levels exhibit higher results than CMIA. The Wallemacq P.

and all have show that the measured average concentration of CsA using FPIA was higher for 4 to 53 ng/mL, then CMIA. The 95 % confidence interval of the ng/ml difference between methods bias is -17.17 ng/mL to – 34.34 ng/mL. The ARCHITECT CsA assay has significantly reduced CsA metabolite interference relative to other immunoassays and is a convenient and sensitive semi automated method to measure CsA in whole blood (22). The ARCHITECT is a fast, and sensitive analyser with a possibility for measurement of lower concentration CsA in whole blood .

5. CONCLUSION

The ARCHITECT assay cyclosporine metabolites are less active, the CMIA results are a better estimate for clinical use then FPIA. Method has low functional sensitivity and lower elimination of interferences: hematocrit, high values of cholesterol, triglycerides, bilirubine, total protein and uric acid, what means that this method is more specific in comparison to other routine methods. It is a sensitive automated method to measure cyclosporine in whole blood.

Conflict of interest: none declared.

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