BRIEF REPORT

Comparison of whole-blood tacrolimus concentrations measured by different immunoassay systems

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Introduction: Different measured values for tacrolimus were obtained with different automated immunoassays. We aimed to examine the differences in the blood tacrolimus concentrations measured by the major immunoassay systems commercially available in Japan.

Methods: Whole-blood samples from 118 patients were assayed by 3 commercial assays: chemiluminescent enzyme immunoassay (CLIA), affinity column-mediated immunoassay (ACMIA), and enzyme-multiplied immunoassay technique (EMIT). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for reference.

Key findings: The correlation coefficient of immunoassay vs LC-MS/MS was excellent for ACMIA (.83) and CLIA (.81) and good for EMIT (.71). The mean error was negative for ACMIA and positive for CLIA and EMIT. The mean absolute error and root-mean-square error were almost the same for ACMIA and CLIA and lower than those for EMIT.

Conclusions: The ACMIA and CLIA yield considerably better results than the EMIT for monitoring blood tacrolimus concentrations.

KEYWORDS

immunoassays, measurement error, tacrolimus, therapeutic drug monitoring

1 | INTRODUCTION

Tacrolimus or FK506 is an immunosuppressant discovered in 1984 by Fujisawa Pharmaceutical Co. Ltd. This drug has been proven to be effective for the prevention of organ rejection after transplantation and for the treatment of autoimmune diseases.^{1,2} Monitoring the blood tacrolimus concentration is required to adjust the optimal dose in each patient, because of the narrow therapeutic window and large individual variability in tacrolimus pharmacokinetics. Low blood concentrations may result in rejection, while high concentrations may result in

infection or nephrotoxicity. In the clinical setting, immunoassays are used to measure the concentrations of tacrolimus in whole blood.

Automatic immunoassay systems that can measure tacrolimus levels in a short time are used for routine monitoring of blood concentrations of tacrolimus. However, it has been reported that the measured results for the same sample vary when measured by different assay systems.^{3,4} Therefore, if a patient moves to another hospital, the blood tacrolimus concentrations cannot be compared if both hospitals used different systems. There is an increasing need to evaluate different immunoassay systems for consistency.

Abbreviations: ACMIA, affinity column-mediated immunoassay; CLIA, chemiluminescent enzyme immunoassay; EMIT, enzyme-multiplied immunoassay technique; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MAE, mean absolute error; ME, mean percent error; r^2 , correlation coefficient; RMSE, root-mean-square error; TDM, therapeutic drug monitoring

The reagents used in automated immunoassay systems have improved over the years. Flex cartridge TAC reagent has changed antibody, pretreatment reagent, and magnetic particle reagent compared to flex cartridge TACR reagent. As a result, TAC improves specificity, extraction efficiency, stability after dissolution than TACR. Hence, there is a possibility that the blood concentrations measured by the current assay systems may differ from those reported previously.⁵

This study aimed to examine the differences in blood tacrolimus concentrations measured by various immunoassay systems that are currently available commercially in Japan. We compared the blood tacrolimus concentrations measured by an affinity column-mediated immunoassay (ACMIA), a chemiluminescent enzyme immunoassay (CLIA), and an enzyme-multiplied immunoassay technique (EMIT) and validated the results using values obtained from the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as standard.

2 | PATIENTS AND METHODS

2.1 | Patients

Medical records of Japanese inpatients at Oita University Hospital between May 2013 and December 2013 were reviewed to identify those who received tacrolimus treatment. Patients who received tacrolimus to prevent rejection after organ transplantation or treatment of autoimmune diseases were included in this study. The following clinical data recorded during tacrolimus therapy were collected: gender, age, and body weight. This study was approved by the Ethics Committee of Oita University. As blood samples were collected as part of the routine patient care for therapeutic drug monitoring (TDM) and laboratory tests, written informed consents were not necessary.

2.2 | Automatic immunoassay systems

Tacrolimus concentration in the whole blood was determined by ACMIA using a Dimension™ Xpand Plus analyzer (Siemens Healthcare Diagnostics, Berlin, Germany), EMIT using a Viva-E™ analyzer (Siemens Healthcare Diagnostics), CLIA using an ARCHITECT® i1000SR analyzer (Abbott Diagnostics, North Chicago, IL, USA), and LC-MS/MS using an ACQUITY UPLC H-Class/XevoTQD system (Waters Corporation, Milford, MA, USA). ACMIA, EMIT, and LC-MS/MS were performed at Oita University Hospital, and CLIA was

performed at Oita Red Cross Hospital according to the manufacturer's instructions. For each measurement range, EMIT and CLIA are 2.0-30 ng/mL and ACMIA is 1.0-30 ng/mL (from the package insert).

2.3 | LC-MS/MS

Tacrolimus whole-blood concentration was determined by LC-MS/MS according to the method reported by Said et al.⁶ Briefly, 1.25 mL of 2.18 ng/mL ascomycin in 0.1% formic acid methanol (internal standard) was added to 50 µL of whole blood in a glass tube. The mixture was then briefly vortexed for 5 minutes, followed by centrifugation at 6000 g for 2 minutes. The extract was subjected to Micro Extraction Packed Sorbent (MEPS)[™] sample preparation. Sample loading was performed using 6 replicate 100-µL aliquots of the diluted blood sample. Next, the MEPS sorbent was washed once with 100 µL of water/methanol (95:5, v/v). The analytes were eluted and injected by withdrawing 50 µL methanol/isopropanol/acetonitrile/water (50:30:10:10 v/v/v/v), and 20 µL was injected into the LC sample injector. An Acquity UPLC® BEH C18 column (50 × 2.1 × 1.7 µm) was used for analysis. An Acquity UPLC® BEH C18 VanGuard™ Pre-Column (5 × 2.1 × 1.7 µm) was used as a guard column. Data on LC-MS/MS intraday and interday validation analysis including accuracy and accuracy meet the criteria of FDA guidelines (Table 1).

2.4 | Statistical analysis

The blood tacrolimus concentration obtained by reference method LC-MS/MS was compared using a scatter diagram correlated with ACMIA method, EMIT method, and CLIA method. The tacrolimus blood concentrations obtained by the 3 indirect immunoassays were compared between each other. Tacrolimus concentration measured by an immunoassay ($Tacrolimus_{immunoassay}$) was compared with the value obtained by LC-MS/MS ($Tacrolimus_{LC-MS/MS}$) by calculating the mean percent error (ME), mean absolute error (MAE), and root-mean-square error (RMSE) for precision and bias, using the following equations.

$$ME = 1/n \sum_{i=1}^n (Tacrolimus_{immunoassay} - Tacrolimus_{LC-MS/MS})$$

$$MAE = 1/n \sum_{i=1}^n |Tacrolimus_{immunoassay} - Tacrolimus_{LC-MS/MS}|$$

QC FK concentration (ng/mL)	Intraday variation		Interday variation	
	Measured concentration (ng/mL)	CV (%)	Measured concentration (ng/mL)	CV (%)
3.5	3.53 ± 0.28	8.0	3.55 ± 0.42	11.9
6.5	6.65 ± 0.74	11.1	6.34 ± 0.60	9.5
13	13.18 ± 0.66	5.0	12.45 ± 0.53	4.3

N = 6 Median ± SD.

TABLE 1 Data of intraday and interday validation assay of LC-MS/MS

TABLE 2 Patient characteristics and clinical laboratory data

Patient	118
Gender (male/female)	136 (65/71)
Age (y)	52 (13-86)
Height (cm)	160 (128.6-185)
Body weight (kg)	57 (27.8-91.61)
TAC dose (mg/day)	3 (1-16)
TAC administration dates (day)	30 (1-30)
WBC ($\times 10^3/\mu\text{L}$)	6.57 (0.52-27.85)
RBC ($\times 10^6/\mu\text{L}$)	4.04 (2.54-5.12)
HGB (g/dL)	12.1 (7.8-15.5)
HCT (%)	36.4 (21.5-46.3)
CRP (mg/dL)	0.085 (0.01-6.02)
Alb (g/dL)	4 (2.26-4.96)
TP (g/dL)	6.635 (4.59-10.65)
T.Bil (mg/dL)	0.56 (0.24-1.35)
AST (IU/L)	17.35 (6.1-322)
ALT (IU/L)	15.1 (3.6-294.1)
ALP (IU/L)	200 (25.4-765)
γ -GTP (IU/L)	26.3 (9.1-617.2)
BUN (mg/dL)	19.8 (5.4-57.8)
Cr (mg/dL)	0.865 (0.3-12.21)
CCR (mL/min)	56.38 (8.48-199.01)

Data are expressed as median and interquartile range unless otherwise stated.

$$\text{RMSE} = [1/n \sum_{i=1}^n (\text{Tacrolimus}_{\text{immunoassay}} - \text{Tacrolimus}_{\text{LC-MS/MS}})^2]^{1/2}$$

The ME, MAE, and RMSE were calculated to compare the reproducibility of the immunoassays. Analyses were performed using the Predictive Analysis Software (PASW) Statistics version 18 (SPSS Inc., IL, USA), and MS Excel (Microsoft Corp, Redmond, WA, USA).

3 | RESULTS

A total of 118 patients (71 men and 47 women) were included in the study. Their mean age was 52 (range, 13-86) years, and the mean body weight was 57.0 (range, 27.8-91.2) kg (Table 2).

All the 3 commercial immunoassays produced results that were very consistent with those obtained by LC-MS/MS, with good

correlation for ACMIA (ACMIA = $.75 \times \text{LC-MS/MS} + 1.34$, $r^2 = .83$; Figure 1A), EMIT (EMIT = $.60 \times \text{LC-MS/MS} + 3.52$, $r^2 = .71$; Figure 1B), and CLIA (CLIA = $.74 \times \text{LC-MS/MS} + 2.08$, $r^2 = .81$; Figure 1C).

FIGURE 1 Scatter plots and regression line correlating blood tacrolimus concentrations (ng/mL) obtained by the reference method, LC-MS/MS, with those obtained by ACMIA (A), EMIT (B), and CLIA (C). ACMIA, affinity column-mediated immunoassay; CLIA, chemiluminescent enzyme immunoassay; EMIT, enzyme-multiplied immunoassay technique

Regression curve was as follows: $EMIT = .79 \times ACMA + 2.53$; $r^2 = .83$ (Figure 2A), $CLIA = .82 \times ACMA + 1.01$; $r^2 = .92$ (Figure 2B), $EMIT = .82 \times CLIA + 1.76$; $r^2 = .89$ (Figure 2C).

FIGURE 2 Scatter plots with fits correlating tacrolimus results (ng/mL) obtained by mutually comparing the data among the 3 indirect immunoassays: ACMA vs EMIT (A), ACMA vs CLIA (B), and CLIA vs EMIT (C). ACMA, affinity column-mediated immunoassay; CLIA, chemiluminescent enzyme immunoassay; EMIT, enzyme-multiplied immunoassay technique

TABLE 3 Analysis for precision and bias comparing whole-blood tacrolimus concentrations measured by the reference LC-MS/MS method vs those by each immunoassay

	ME	MAE	RMSE
ACMA vs LC-MS/MS	-0.572	1.877	2.841
CLIA vs LC-MS/MS	0.100	1.766	2.863
EMIT vs LC-MS/MS	0.465	2.424	3.662

ACMA, affinity column-mediated immunoassay; CLIA, chemiluminescent enzyme immunoassay; EMIT, enzyme-multiplied immunoassay technique; LC-MS/MS, liquid chromatography-tandem mass spectrometry method; ME, mean percent error; MAE, mean absolute error; RMSE, root-mean-square error.

Analysis for precision and bias comparing whole-blood tacrolimus concentrations measured by the reference LC-MS/MS method vs those by each immunoassay. The ME, MAE, and RMSE values were ACMA (-0.572, 1.877, and 2.841), CLIA (0.100, 1.766, and 2.863), and EMIT (0.465, 2.424, and 3.662; Table 3). In this study, the ME was negative for the ACMA and positive for CLIA and EMIT. MAE and RMSE were almost the same for ACMA and CLIA and lower than those for EMIT.

4 | DISCUSSION

The blood tacrolimus concentrations measured by the ACMA, CLIA, and EMIT showed good correlation with those obtained by the LC-MS/MS method. ACMA and CLIA showed a good correlation when compared directly, but ACMA and CLIA showed a low correlation with EMIT. However, the regression coefficients of all 3 immunoassays tended to be low. In particular, in this study, the blood tacrolimus concentrations determined by the ACMA and CLIA were almost equivalent to those obtained by the LC-MS/MS method. Although previous studies have shown that the ACMA yielded lower tacrolimus concentration values than the CLIA⁷⁻⁹ the present study showed that the 2 methods produced similar measured values. A possible reason for the disagreement with the previous report is that the reagent used by ACMA has improved in the past few years. In this study, as the ME of the ACMA was negative, the measured values obtained by ACMA may be lower than the true values. On the other hand, as the ME of the CLIA and EMIT methods were both positive, the measured values obtained by the CLIA and EMIT may be higher than the true values. In this study, the ACMA and the CLIA showed almost equivalent accuracy, indicating that the differences between the measured and true values of blood tacrolimus concentration are

due to the different principles of measurement by automated assay systems. As large discrepancies of whole-blood tacrolimus concentrations may arise between medical facilities, it is necessary to specify the method of measurement and obtain cooperation among medical facilities when implementing TDM enforcement of tacrolimus.

In conclusion, the present study indicated that the results of the 3 commercial assays were consistent with those of LC-MS/MS, and validation showed acceptable performance for the 3 assays. The ACMIA and CLIA provide considerably better results than the EMIT for monitoring blood tacrolimus levels.

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