Inter-laboratory Variability of Current Immunoassay Methods for Tacrolimus among Japanese Hospitals

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Received March 11, 2016; accepted May 18, 2016; advance publication released online June 4, 2016

The aim of this study was to assess inter-hospital laboratory variability (coefficient of variation; CV) of immunoassay methods for tacrolimus and the comparability of control samples and results obtained by immunoassay measurements. One hundred seven hospital laboratories routinely performing therapeutic drug monitoring (TDM) of tacrolimus participated in the study. Thirteen spiked samples with known tacrolimus concentrations in the range of 0–26.0 ng/mL were prepared. Each spiked sample was analyzed according to the manufacturer’s instructions using an affinity column-mediated immunoassay (ACMIA) on a Dimension® analyzer, the enzyme multiplied immunoassay technique (EMIT) on a Viva-E® analyzer, a chemiluminescent enzyme immunoassay (CLIA) on the Architect® system, and the electro-chemiluminescence immunoassay (ECLIA) on a cobas® analyzer. The 20% coefficient of variation values for the CLIA, ACMIA, EMIT, and ECLIA assays in the hospital laboratories were 1.82, 5.36, 4.59, and 0.89 ng/mL, respectively. CLIA and ECLIA had positive biases at concentrations of tacrolimus above 12 ng/mL relative to the spiked concentration, whereas the assay bias for ACMIA tended to be more negative at concentrations of tacrolimus above 6 ng/mL. EMIT had positive biases over the wide concentration range of 0.0–26.0 ng/mL (mean of mean errors 1.224). CLIA and ECLIA provided adequate precision at the target tacrolimus concentration of 3.0 ng/mL, whereas ACMIA and EMIT were unable to respond to target concentrations between 3.0 and 5.0 ng/mL for renal transplant recipients. Appropriate assessment of tacrolimus concentration by an assay having higher sensitivity, precision, and accuracy is necessary to ensure long-term survival of transplant recipients receiving tacrolimus.

Key words tacrolimus; immunoassay; coefficient of variation; quality control

Tacrolimus is a potent immunosuppressive agent that is used to prevent organ rejection after transplantation. Because tacrolimus has a narrow therapeutic range, large intra- and inter-individual pharmacokinetic variability, and a poor correlation between dose and blood concentration, therapeutic drug monitoring (TDM) of tacrolimus is an important tool for reducing the risk of nephrotoxicity and allograft rejection. Many clinical assay methods have been developed to monitor the concentration of tacrolimus in whole blood after transplant surgery and during lifetime immunosuppressive therapy. In particular, the routine assay of tacrolimus for more than 20 years has been dominated by one particular immunoassay, the microparticle enzyme immunoassay (MEIA, Abbott, Tokyo, Japan), performed on the IMx® analytical system. However, the MEIA assay disappeared from the market in 2009 because of cessation of production. As alternatives to the MEIA assay, other immunoassay methods appeared at this time including an affinity column-mediated immunoassay (ACMIA) on the Dimension® analyzer (Siemens, Tokyo, Japan), the enzyme multiplied immunoassay technique (EMIT) on the Viva-E® analyzer (Siemens, Tokyo, Japan), and a chemiluminescent enzyme immunoassay (CLIA) on the Architect® system (Abbott Laboratories, Chiba, Japan). Furthermore, since 2013, the electro-chemiluminescence immunoassay (ECLIA, cobas®, Roche, Tokyo, Japan) has also been marketed in Japan.

The target whole blood trough concentrations \( (C_0) \) of tacrolimus was reported to range from 5 to 20 ng/mL based on concentrations assayed by the MEIA method for all transplant populations. In the maintenance phase after renal transplantation, a target tacrolimus \( C_0 \) of 5 ng/mL is recommended in combination with other immunosuppressive drugs such as mycophenolate mofetil and steroids. Recently, the addition of everolimus into the immunosuppressive therapy including tacrolimus has been used to reduce the risk of tacrolimus nephrotoxicity in renal transplant recipients, a regimen that lowered the target \( C_0 \) of tacrolimus to 3 ng/mL. Thus, the target concentration range of tacrolimus could be reduced by combining it with other immunosuppressive therapeutics. Consequently, immunoassay methods for tacrolimus with higher sensitivity or higher precision and accuracy at a lower \( C_0 \) of 3 ng/mL are needed. In addition, a system that allows patients to obtain a checkup at any institution during lifetime immunosuppressive therapy and delivers consistent clinical results is also required. Therefore, external quality control for immunosuppressive drugs such as tacrolimus is necessary.

The aim of this study was to evaluate the recently devel-
oped CLIA, ACMIA, EMIT, and ECLIA technologies for monitoring tacrolimus blood concentrations and to compare tacrolimus concentrations obtained from these methods using blind blood samples spiked with tacrolimus in the range from 0.0 to 26.0 ng/mL among 107 Japanese hospital laboratories.

MATERIALS AND METHODS

Ethylenediaminetetraacetic acid (EDTA) blood specimens were obtained from healthy volunteers. None of the healthy volunteers had a history of significant medical illness or hypersensitivity to any drug. All subjects were nonsmokers. The study protocol was approved by the Ethics Committee of Yokohama Minoru Clinic, and all subjects gave written informed consent before participating in the study. Tacrolimus powder was donated by Astellas Pharma Inc. (Tokyo, Japan). We made appropriate dilutions of tacrolimus stock solution with tacrolimus-free blood to produce 13 spiked samples with nominal tacrolimus concentrations in the range of 0–26.0 ng/mL. These samples were prepared for every examination, and 4 of the 13 spiked samples per each examination were analyzed using 4 different analytical techniques, CLIA and ECLIA technology in each institution with a rule to analyze immediately after arrival. The same spiked specimens were analyzed using 4 different analytical techniques, CLIA (n=58–67), ACMIA (n=18–19), EMIT (n=8–17), and ECLIA (n=5–13), in different Japanese hospital laboratories (Table 1). The flex reagent cartridge (TACR Flex; Siemens Healthcare Diagnostics, Tokyo, Japan) used for ACMIA on the Dimension® analyzer was used in older version kits released during the time period up to September 2015.

Statistics The precision of the method at each concentration was determined by comparing the coefficient of variation (CV), obtained by calculating the standard deviation (S.D.) as a percentage of the calculated mean concentration from each institution. The accuracy of each immunoassay was calculated according to the following formula: mean prediction error\(^1/n\)\(\sum_{i=1}^{n}\) (immunoassay–spiked concentration). The imprecision was calculated as the tacrolimus concentration at 20% CV, using a linear regression curve fit of CV% versus concentration of spiked sample of tacrolimus of 1, 1.5, 1.7, 2, 3, 5, 6, 8, 12, 14, 25, and 26 ng/mL in different laboratories.\(^{31}\) In the same way, single linear regression analysis was used to evaluate the correlation between theoretical values and actual measurements of tacrolimus concentrations. The goodness of fit of the linear regression curves was assessed on the basis of the determination coefficient (r\(^2\)). The range of values for each method is shown in the box-and-whisker plots. Two-way repeated or non-repeated measures ANOVA was used to evaluate differences in mean prediction error (immunoassay–spiked concentration) between tacrolimus concentrations and between immunoassay methods, and those interactions. A p-value less than 0.05 was considered statistically significant. Statistical analysis was performed with SPSS 20.0 for Windows (SPSS IBM Japan Inc., Tokyo, Japan).

RESULTS

Plots of the CVs of CLIA, ACMIA, EMIT, and ECLIA determined from different measurements in different hospital laboratories at each concentration are shown in Fig. 1. The CV values calculated from the mean tacrolimus concentration data for CLIA, ACMIA, EMIT, and ECLIA were 1.82, 5.36, 4.59, and 0.89 ng/mL, respectively.

Box-and-whisker plots of CLIA, ACMIA, EMIT, and ECLIA for lower target tacrolimus concentrations (1.0, 2.0, 3.0, 5.0 ng/mL) are shown in Fig. 2A. With lower target tacrolimus concentrations, the inter-laboratory variabilities for ACMIA and EMIT analytical methods were very large. On the other hand, the precision for CLIA and ECLIA in the low concentration area, especially 1.0–3.0 ng/mL, was high. The distribution of boxes in the box-and-whisker plots of CLIA and ECLIA is tight, showing that results obtained by CLIA and ECLIA were similar among the laboratories. Similarly, in the medium and high target concentrations (8.0, 12.0, 14.0, 25.0 ng/mL), box-and-whisker plots of CLIA, ACMIA, EMIT, and ECLIA are shown in Fig. 2B. In the medium and high concentration regions, the distribution of boxes in the box-and-whisker plots assayed using the ACMIA method tended to be low; however, the median tacrolimus concentrations obtained by ACMIA were nearest the spiked concentration from all methods.

The mean prediction errors (ME) for CLIA, ACMIA, EMIT, and ECLIA at each concentration and the mean (95% CI minimum–maximum) at all concentrations are shown in Figs. 3A and B, respectively. CLIA and ECLIA had positive biases at higher concentrations of tacrolimus above 12 ng/mL relative to the spiked concentration, whereas the assay for ACMIA tended to have negative biases at higher concentrations of tacrolimus above 6 ng/mL. EMIT had positive biases.

Table 1. Number of Japanese Hospital Laboratories Participating in Surveys

<table>
<thead>
<tr>
<th>Trials</th>
<th>Tacrolimus spiked concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Assays</td>
<td></td>
</tr>
<tr>
<td>CLIA</td>
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</tr>
<tr>
<td>ACMIA</td>
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</tr>
<tr>
<td>EMIT</td>
<td>8</td>
</tr>
<tr>
<td>ECLIA</td>
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</tr>
<tr>
<td>Total</td>
<td>99</td>
</tr>
</tbody>
</table>

in the wide concentration range of 0.0–26.0 ng/mL.

On the other hand, these 4 immunoassays detected a tacrolimus concentration for the control blood sample without tacrolimus (Fig. 4). EMIT calculated an average concentration of 1.5±1.2 ng/mL and a maximum concentration of 3.2 ng/mL.

The correlation between tacrolimus concentrations obtained from each immunoassay method and spiked tacrolimus concentrations is shown in Table 2. The actual tacrolimus concentration measured by CLIA relative to expected concentration gave a slope of 1.106 (95% CI: 1.092–1.119) and an intercept of −0.144 (95% CI: −0.195−0.093). Relationships between actual and expected tacrolimus concentrations were obtained as follows: a slope of 0.874 (95% CI: 0.820–0.928) and an intercept of 0.430 (95% CI: 0.222–0.639) for ACMIA, a slope of 1.045 (95% CI: 0.958–1.133) and an intercept of 0.696 (95% CI: 0.345–1.047) for EMIT, and a slope of 1.109 (95% CI: 1.074–1.144) and an intercept of 0.024 (95% CI: −0.115–0.163) for ECLIA.

DISCUSSION

We conducted an external quality control for tacrolimus to prevent acute rejection and to ensure long-term survival of Japanese transplant recipients administered tacrolimus. Among 107 Japanese hospital laboratories routinely performing TDM of tacrolimus after organ transplantation, there was no hospital laboratory that assessed tacrolimus concentration using liquid chromatography/tandem mass spectrometry (LC-MS/MS); either the CLIA, ACMIA, EMIT, or ECLIA immunoassay method was used. In the present study of different laboratories, the 20% CV values for CLIA and ECLIA were 1.82 and 0.89 ng/mL, respectively, providing adequate precision at the target tacrolimus concentration of 3 ng/mL. A limit of quantitation (LOQ) value of 1 ng/mL is reported to be essential for measuring tacrolimus concentrations between 3–5 ng/mL. However, the 20% CV values for ACMIA and EMIT were 5.36 and 4.59 ng/mL, respectively, and these immunoassays appear unable to analyze target concentrations between 3.0 and 5.0 ng/mL for renal transplant recipients. When recipients have a checkup in a different institution that uses the same immunoassay, for tacrolimus concentrations greater than the 20% CV values of 1.82, 5.36, 4.59, and 0.89 ng/mL for CLIA, ACMIA, EMIT, and ECLIA, respectively, tacrolimus trough concentrations of those patients would be similar to those obtained in their previous institution. Thus, we should consider as a set of the immunoassay method and tacrolimus concentrations.

LOQ values for CLIA and ECLIA are reported to be 0.5–0.8 ng/mL and 0.2–0.3 ng/mL, respectively. These LOQ values were calculated by Architect® analyzer or cobas® analyzer.

![Fig. 1. Plot of the CVs for CLIA (Architect®), ACMIA (Dimension®), EMIT, and ECLIA (Cobas®) Determined from Different Measurements in Different Hospital Laboratories at Each Concentration](image-url)
within one laboratory, i.e., an intra-laboratory CV of 20% (6–10); however, in the present study, the 20% CV values were calculated by multiple Architect® analyzers (n = 58–67) or cobas® analyzers (n = 5–12) in multiple institutions, i.e., an inter-laboratory CV of 20%. Therefore, the 20% CV values obtained from the present study might be lower than the reported LOQ values. However, the sensitivity and precision of CLIA and ECLIA would be almost equal.
On the other hand, the LOQ value for ACMIA is reported to be 2.5–3.12 ng/mL. The 20% CV values for ACMIA on 19 different Dimension® analyzers in the present study were slightly lower than the reported LOQ values. Although the linearity for ACMIA is reported to be up to at least 31.4 ng/mL, ACMIA tended to have a negative bias at higher tacrolimus concentrations. The present results completely agree with the previous report using spiked samples by Levine et al. In addition, Hashi et al. also reported that the ME for ACMIA at tacrolimus concentrations greater than 10 ng/mL has a more negative bias. The negative bias observed in ACMIA with the Dimension® analyzer might depend on the automated sample preparation from whole blood containing high concentrations of tacrolimus, which takes several minutes for one sample. Furthermore, in the present study, the results by the ACMIA assay showed a large concentration distribution compared to CLIA and ECLIA. Hashi et al. suggested that the larger distribution of tacrolimus concentrations by ACMIA is caused by the automated sample preparation with the Dimension® analyzer, because during the wait time between samples, cellular components of the blood samples might precipitate by gravity. At the spiked tacrolimus concentration of 26 ng/mL, the mean (minimum–maximum) concentration of 19 hospital laboratories assayed by ACMIA was 22.0 (17.8–25.8) ng/mL, and overall this assay gave lower values, whereas the mean concentrations determined by CLIA and ECLIA were 27.8 and 27.6 ng/mL, respectively. Although we cannot fully explain this phenomenon, it was not caused by a large deviation from one institution. There may have been a problem with the flex reagent cartridge at the time of the assays. Consequently, our results indicate that quantification of tacrolimus by ACMIA might ideally be in the range from 5.0 to 25 ng/mL, and multiple assays might be required. However, in the present study, the flex reagent cartridge used was in the older version kits and was not the improved type of cartridge released in December 2015. Therefore, our results might be limited to the present study, and the next quality examination by a future study group covering a new version of ACMIA flex cartridge for the Dimension® analyzer could be favorable in comparison with CLIA, ECLIA and EMIT.

Although the LOQ value for EMIT is reported to be 2.8–3.2 ng/mL, the 20% CV values in the present study were much lower than the reported values. In the present study, tacrolimus concentrations assayed by EMIT had a positive bias in the wide range of 0.0–26.0 ng/mL. In samples of transplant patients receiving tacrolimus, the EMIT assay has
been reported to have an average positive bias of 17–30% compared to LC-MS/MS\(^{(14)}\) or LC-MS\(^{(16,17)}\) methods, and an average positive bias of 17.5% compared to CLIA.\(^{(15)}\) Since the quantification of tacrolimus by EMIT overestimates the blood concentration of tacrolimus, monitoring the concentration of tacrolimus by EMIT might lead to a lower dosage of tacrolimus compared to other methods. Of note, in the present study, EMIT calculated a maximum concentration of 3.2 ng/mL for the control blood sample without tacrolimus. This finding might lead to clinical errors, especially in patients with much lower compliance. Assessment of tacrolimus concentration by apparatus with higher accuracy is required to ensure long-term survival of transplant recipients administered tacrolimus.

Our results must be interpreted within the context of the study limitations. In the present study, spiked samples with known tacrolimus concentrations were prepared using tacrolimus-free blood obtained from healthy volunteers. The major metabolites of tacrolimus are 13-O-demethyl tacrolimus (M-I), 31-O-demethyl tacrolimus (M-II), and 15-O-demethyl tacrolimus (M-III)\(^{(10)}\); however, in the present study, these metabolites were not included in the spiked samples. These tacrolimus metabolites exhibit cross-reactivity to varying degrees with the monoclonal antibodies used in the CLIA, ACMIA, EMIT, and ECLIA immunoassays.\(^{(10,13,19,20)}\) Therefore, our results might not agree with the results obtained using the blood of transplant patients. Furthermore, we cannot explain the cause of the inter-hospital laboratory variability for spiked tacrolimus concentration from the principle of each immunoassay. The difference in tacrolimus concentration among hospital laboratories on each immunoassay may be caused by a bias between the analyzers, the difference of version of hospital laboratories on each immunoassay may be caused by a bias between the analyzers, the difference of version of calibrators, calibration inaccuracy or the errors in the pretreatment step.\(^{(9)}\) In addition, large outliers from spiked concentrations that were calculated in some hospital laboratories may have been caused by small deviations from a calibration curve and micropipette maintenance issues. Periodic external quality control might be necessary to ensure long-term survival of transplant recipients.

**CONCLUSION**

CLIA and ECLIA provide adequate precision at the target tacrolimus concentration of 3.0 ng/mL, whereas ACMIA and EMIT appear unable to analyze target concentrations between 3.0 and 5.0 ng/mL. Appropriate assessment of tacrolimus concentration by an assay with higher sensitivity, precision, and accuracy is required to ensure long-term survival of transplant recipients administered tacrolimus.

**Acknowledgments** The authors would like to thank the Japanese hospital laboratories that carried out organ transplant plants for their participation in this study and Dr. Takenori Niioka (Akita University Hospital) for carrying out the statistical analyses.

**Conflict of Interest** Sogo Rinsho Medefi Co., Ltd., which prepared and sent the tacrolimus-spiked blood samples to each hospital laboratory, received research funding from Astellas Pharma Inc. (Tokyo, Japan).

**REFERENCES**


