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Title

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Permalink

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Journal

Therapeutic drug monitoring, 38(3)

ISSN

0163-4356

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Publication Date

2016-06-01

DOI

10.1097/ftd.0000000000000252

Peer reviewed

Evaluation of the Waters MassTrak LC-MS/MS Assay for Tacrolimus and a Comparison to the Abbott Architect Immunoassay.

Running title (Short Title): Tacrolimus analysis using LC-MS/MS

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Conflicts of Interest and Source of Funding: Clinical chemistry fellowships were funded by Waters Corporation (Alec Saitman) and Roche Diagnostics (Imir G. Metushi). Donald S. Mason is employed by and owns stock in Waters Corporation. Robert L. Fitzgerald has nothing to declare.

This work was supported, in part, by funding from Waters Corporation.

ABSTRACT

Background: Tacrolimus (Prograf®, Advagraf®, FK-506) is the most commonly prescribed calcineurin inhibitor following kidney and liver transplantation. The use of tacrolimus (in conjunction with other drugs) has successfully contributed to the maintenance of solid organ allografts; however, it also exhibits toxic side effects. Therapeutic drug monitoring of tacrolimus is used as an aid to achieve drug concentrations within a narrow therapeutic window.

Methods: The Waters® MassTrak™ Immunosuppressants assay (LC-MS/MS) for the quantification of tacrolimus in whole blood was evaluated for precision, linearity, lower limit of quantification, matrix effects, and accuracy. A method comparison with the Abbott Architect® Tacrolimus immunoassay was also performed.

Results: The mean concentration (ng/mL) and coefficient of variation (CV) for low, mid, and high patient pools were; $0.6 \pm 19.9\%$, $16.0 \pm 5.4\%$, and $31.2 \pm 5.8\%$, respectively. The MassTrak assay was linear from 0.5 to 30.0 ng/mL. While the MassTrak and Architect assays correlated well ($R^2 = 0.97$) for patient samples, the MassTrak assay displayed an average negative bias of 18.5 % vs. Architect (range of 0.0 to 36.7 %). Analysis of a certified tacrolimus reference material in human whole blood (ERM-DA110a, LGC Standards) on both platforms failed to completely explain the observed difference for patient samples.

Conclusions: Two widely used assays for therapeutic drug monitoring of tacrolimus are not in agreement with one another. Care should be exercised when interpreting results generated on these two assay platforms.

Key words: tacrolimus, immunosuppressants, LC-MS/MS, immunoassay, therapeutic drug monitoring

Introduction

Immunosuppressive drugs have become a cornerstone of solid organ transplantation [1, 2]. Tacrolimus, a 23 membered macrocyclic lactone natural product, is the most widely prescribed immunosuppressant following kidney transplantation [3]. Tacrolimus is a calcineurin inhibitor which acts by inhibiting T-cell signal transductions and ultimately IL-2 transcription [4-6]. Tacrolimus has a narrow therapeutic index which suggests that its blood concentration needs to be tightly controlled in order to provide efficacy and to reduce the risk of adverse drug reactions. Quantitative analysis of tacrolimus in whole blood is essential for dose adjustments and is partially responsible for the long term success of solid organ transplants [7].

Several analytical methods are available for analysis of tacrolimus in whole blood including immunoassay [8] and liquid chromatography—tandem mass spectrometry (LC-MS/MS) [9-11]. Immunoassays utilize monoclonal antibodies raised against tacrolimus. However, tacrolimus is metabolized by the CYP3A subfamily of enzymes giving rise to metabolites which are structurally similar to the parent drug and may cross react with the assay antibody [12]. While most immunoassays are FDA cleared for the quantification of tacrolimus, most MS based assays in clinical laboratories are laboratory developed tests (LDTs). Waters Corporation has recently released the first FDA cleared mass spectrometry based assay for tacrolimus quantification, the MassTrak™ Immunosuppressants Kit [13]. This kit has a stated analytical measurement range of 0.5 ng/mL to 30.0 ng/mL of tacrolimus. The accuracy of this assay is increased by monitoring tacrolimus along with an internal standard, ascomycin, added to the specimen before analysis

(Figure 1). In this study, we verified performance claims of the Waters MassTrak assay and performed a patient comparison against the Abbott Architect® Tacrolimus immunoassay.

3. Results and Discussion

3.1. Matrix Effects and Lower Limit of Quantification

The difference in recovery based on area counts of tacrolimus and the internal standard produced a negative bias of 8.0 % and 7.5 % respectively when compared to the matrix free (water) medium. Importantly, for the whole blood specimens, ascomycin area counts changed in the same relative amount as tacrolimus area counts demonstrating that the internal standard was performing appropriately. Both of the bias for tacrolimus and ascomycin were less than 10 % which are within acceptable criteria limits. The average concentration and % CV were determined for the 25 samples spiked with tacrolimus and were found to be 0.34 ng/mL $\pm$ 30.2 % respectively. The % CV for the LLOQ was higher than claimed by the manufacturer (precision of < 20 % at 0.34 ng/mL LLOQ). The range of tacrolimus concentrations obtained in the LLOQ experiment was 0.16-0.52 ng/mL.

3.2. Linearity

Regression analysis was performed and showed an $R^2 = 0.997$. We also applied linear, quadratic and cubic regression analyses to the data set and found that the quadratic and cubic functions were not significantly different than 0 indicating a high degree of linearity over the analytical measurement range of the MassTrak assay (Figure 2).

3.3. Accuracy and Precision

The mean $\pm$ % CVs for the low, mid, and high patient pools were; 0.6 ± 19.9 %, 16.0 ± 5.4 %, and 31.2 ± 5.8 % respectively. In addition to the patient pools, we conducted accuracy and precision studies by looking at the QC material provided by the manufacturer. The mean $\pm$ % CVs for the three levels of QC material were 2.1 ± 6.7 %, 8.8 ± 3.6 % and 24.7 ± 3.3 % for the low (1.7 – 2.5 ng/mL), mid (7.8 – 9.4 ng/mL) and high (21.7 – 26.5 ng/mL) levels of QC respectively. These measured values agreed well with the assigned target values and all had CVs of less than 10 %.

3.3.1. Patient comparison

As shown in figure 3, both assays correlated well using linear, quadratic and cubic regression analysis across the analytical measurement range with an $R^2 = 0.97$. A Bland-Altman plot with Abbott Architect as reference method was also constructed to evaluate potential constant and/or systematic biases between the two methods (Figure 4). As shown in figure 4, the MassTrak tacrolimus values were consistently lower than the immunoassay values with an average difference of 18.5 %. The difference between the two assays ranged from 0 to 36.7 %. The Z-value of the Wilcoxon Signed Rank test was found to be -5.44 indicating the difference in values is significant at $p \leq 0.01$, demonstrating the results of the two methods were statistically different. This large and variable bias likely has clinical implications. One possible explanation for the difference between the two methods is that metabolites present in patient whole blood samples may cross react with the Abbott Architect tacrolimus assay but not with the Waters MassTrak tacrolimus assay [15].

The certified concentration of tacrolimus in the whole blood European Reference Materials (ERM) was 7.82 ng/mL. The average of the quadruplicate analysis of the reference material

analyzed on the MassTrak assay was 7.60 ng/mL representing a negative bias of 2.8 % (vs. EMR material). The average of the duplicate analysis of the reference material analyzed on the Abbott Architect assay was 8.40 ng/mL representing a positive bias of 7.4 % (vs. EMR material). Although this difference in bias between the two assay platforms is 9.5 % (likely due to calibration differences between the methods) it does not account for all of the large negative bias that was observed during patient comparison studies.

4. Limitations

Some limitations exist in the data presented. Other medications the patient may have been concurrently taking or the patient's health status at the time when blood was drawn for tacrolimus levels was unknown. These unknown, but potentially important parameters may have contributed to the observed biases encountered especially when using the immunoassay.

5. Conclusions

This study presents an evaluation of the MassTrak assay for tacrolimus quantitation. The LLOQ stated in the MassTrak package insert was found to have an average, standard deviation, and % CV of 0.34 ng/mL, 0.10 ng/mL, and 30.2 %, respectively. The LLOQ is defined as the lowest concentration that meets acceptability accuracy (typically +/- 20 % of target) and precision (coefficient of variation < 20 %). This did not meet the manufacturer's claimed LLOQ. The mean $\pm$ % CVs for the low, mid, and high patient pools were; 0.6 ± 19.9 %, 16.0 ± 5.4 %, and 31.2 ± 5.8 % respectively. Our data demonstrate that the lower limit of quantification was 0.6 ng/mL, exceeding the recommendations of the consensus guideline for monitoring tacrolimus of less than 1 ng/mL [16]. The mean and % CVs for the low, mid and high levels of QC were 2.1 ± 6.7 %, 8.8 ± 3.6 %, and 24.7 ± 3.3 % respectively, agreeing well with the target concentrations

stated in the package insert. A statistically significant negative bias (average bias of 18.5 %) of the MassTrak tacrolimus assay as compared with the Abbott platform was observed. Analysis of the ERM reference material with the MassTrak assay agreed within 3 % of the material's assigned value, but analysis of this same material on the Architect assay demonstrated a bias of 7.4 %. This offers a partial explanation for the differences between the two different methodologies, but fails to explain the entire difference. This discordance between immunoassay and LC-MS/MS tacrolimus values has been demonstrated previously [17]. The higher measured concentrations in the immunoassay method are likely due to cross reactivity with tacrolimus metabolites by the antibody used in this assay [15, 16, 18]. This is also supported, in part, by the fact that a larger negative bias was observed between the LC-MS/MS method vs. Abbott immunoassay when patient samples were analyzed as opposed to the EMR reference material which only contained the parent drug (no metabolites). The metabolites of tacrolimus have been shown to have cross-reactivity with tacrolimus immunoassays of between 0 % and 100 % depending on the metabolite and assay platform [16]. Further studies supplementing whole blood with tacrolimus metabolites are needed to confirm these observations.

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Figure 1. Chemical structures for tacrolimus and ascomycin.

Tacrolimus

Ascomycin

Figure 2. Linearity studies performed by MassTrak™ LC-MS/MS method.

Figure 3. Correlation analysis using linear regression between Abbott and MassTrak™ Waters method.

Figure 4. Bland-Altman plot showing comparison of the Abbott Architect™ assay vs. Water's MassTrak™ assay.