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Liquid chromatography – tandem mass spectrometry outperforms fluorescence polarization immunoassay in monitoring everolimus therapy in renal transplantation


Abstract

There is a need to monitor everolimus blood concentrations in renal transplant recipients as a result of its high pharmacokinetic variability and narrow therapeutic window. However, analytical methods to determine blood concentrations often differ in performance. Therefore, we investigated whether two commonly used therapeutic drug monitoring methods for everolimus were in agreement and to what extent their differences could lead to differences in dosage advice. Six hundred twelve whole blood samples were obtained from 28 adult renal transplant recipients receiving everolimus and prednisolone therapy. These samples included 286 everolimus trough concentrations. The remaining samples were obtained up to 6 hours post everolimus intake and allowed calculation of 84 AUCs. All samples were analyzed with fluorescence polarization immunoassay (FPIA) on an Abbott TDxFLx analyzer and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Everolimus blood concentrations measured with FPIA and LC-MS/MS were not in agreement. Concentrations determined by FPIA were, on average, 23% higher than concentrations quantified by LC-MS/MS. Moreover, concentrations lower than 15 mg/L or AUC determined with FPIA could be twofold higher than with LC-MS/MS. This variability can lead to clinically relevant differences in dose adjustment of up to 1.25 mg everolimus despite using a correction factor of 23%. Finally, when trough concentrations were measured with FPIA, higher intra-patient variability was observed compared with the use of LC-MS/MS. LC-MS/MS outperforms FPIA for clinical drug monitoring and intervention of everolimus therapy in adult renal transplant recipients on dual therapy with prednisolone. Specifically, the use of FPIA can lead to clinically relevant differences in everolimus dosage advice and higher intra-patient variability.
Introduction

Everolimus (Certican; Novartis, Basel, Switzerland) is an orally administered immunosuppressive agent targeting the mammalian target of rapamycin receptor and is used in the prevention of acute and chronic rejection of solid organ transplants. Its high pharmacokinetic variability together with a narrow therapeutic window makes therapeutic drug monitoring (TDM) crucial for dose individualization [1]. Everolimus concentrations are determined in whole blood because over 75% of the drug is partitioned into red blood cells [2]. Currently, immunoassays such as fluorescence polarization immunoassay (FPIA) and chromatographic methods such as high-pressure liquid chromatography (HPLC) or the more sophisticated HPLC combined with (tandem) mass spectrometry (LC-MS/MS) are the most commonly used analytical techniques for TDM of everolimus [3–7]. These methods may differ in specificity and sensitivity leading to altered accuracy and precision. Inaccuracy in dosage advice caused by these differences could impact on patient outcomes such as toxicity or increased risk for transplant rejection. In this study, two of the most applied analytical techniques for everolimus, FPIA on an Abbott TDxFLx analyzer and LC-MS/MS, were compared. A large number of blood samples were obtained from stable adult renal transplant patients receiving everolimus therapy. This comparison was aimed at identifying whether differences between the two techniques could lead to different everolimus dosages in clinical practice.

Materials and methods

Patients and Samples

Whole blood samples from 28 adult renal transplant recipients (18 male and 10 female) were obtained. Mean age was 52 years (± 10) and ranged from 35 to 69 years. Stable renal transplant recipients treated with immunosuppressive therapy consisting of everolimus (Certican; Novartis) and prednisolone [8] were studied from 6 months up to 2 years after transplantation. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and patients gave written informed consent. Everolimus therapy was started at an oral dose of 3 mg twice daily and was supported by routine TDM based on trough concentrations and AUC_{0–12h}. Routine TDM samples were obtained throughout time after starting everolimus therapy and were analyzed by FPIA. The target AUC_{0–12h} for FPIA was set at 150 µg*h/L [8], which roughly corresponds with an everolimus trough
concentration of 7 to 11 µg/L. All samples were also quantified with LC-MS/MS. Finally, TDM resulted in a between-patient everolimus range from 1 mg to 4.5 mg twice daily. A total of 612 whole blood samples were obtained. This number corresponds to 286 trough concentration measurements and 326 samples drawn at 1, 2, 3, 4, 5, or 6 hours after dose intake. The measurements obtained up to 6 hours, representing 27 full (seven or six time points) and 57 sparsely sampled (four time points) AUCs, allowed the calculation of 84 AUC_{0-12h}.

The performance of the assays was investigated by the quality control (QC) samples, which were included in each series of everolimus patient samples. Accuracy bias for FPIA and LC-MS/MS was determined by calculating the concentration of the control sample as a percentage of the nominal concentration (determined by the manufacturer) using the formula: 

\[
\frac{(C_c - C_n)}{C_n} \times 100\%
\]

with \(C_c\) as the concentration of the control sample and \(C_n\) as the nominal concentration. Assay performance, in terms of limits of quantification, was in agreement with the guidelines regarding bioanalytical method validation of Shah et al. [9].

**Innofluor Certican Assay System**

Quantification of everolimus blood concentrations was performed with FPIA (Seradyn Inc, Indianapolis, IN) on a TDxFLx instrument from Abbott Diagnostics (Abbott Park, IL). FPIA is a homogeneous fluorescence polarization assay using a polyclonal rabbit antibody directed against everolimus [10]. Routine measurements were performed according to manufacturer’s guidelines [10]. Everolimus calibrators (0, 2.5, 5.0, 10.0, 20.2, and 39.1 µg/L) and controls levels 1, 2, and 3 were obtained from Seradyn (Seradyn Inc). Calibrators and controls were prepared by gravimetric addition of everolimus to a human blood hemolysate matrix. Each calibrator was value-assigned by the manufacturer’s reference laboratory using a validated HPLC-MS method [11,12].

Blood samples as well as calibrator and control samples (600 µL) were pretreated with methanol (700 mL) (Merck, Darmstadt, Germany) and precipitation reagent (100 µL), vortexed for at least 10 seconds, and centrifuged for 5 minutes at 4000 rpm. Subsequently, 700 µL of each supernatant was divided into two identical samples of 350 µL and transferred into two reaction cells.

According to the manufacturer’s specifications, the lower limit of quantification was 2.0 µg/L, whereas the upper limit of quantification was 40 µg/L [10]. Samples with values above 40 µg/L were diluted four times with calibrator A (everolimus-free). Lot-dependent calibrators were used. FPIA within-run accuracy and precision were determined by
analyzing two controls in duplicate. Between-run precision and accuracy were evaluated by analyzing the QC results of each determination for the duration of the study. Controls used for FPIA were: control level 1: 4.0 µg/L displayed an accuracy bias of 13.0% and an imprecision with a coefficient of variation (CV) of 19.9%; control level 2: 11.5 µg/L had an accuracy bias of 1.6% and an imprecision with a CV of 15.4%; and finally control level 3: 23.0 µg/L showed an accuracy bias of 2.2% and an imprecision with a CV of 13.5% (n = 78).

**Liquid Chromatography–Tandem Mass Spectrometry Assay**

Quantification of everolimus with LC-MS/MS was performed with a validated assay capable of analyzing everolimus, sirolimus, and tacrolimus simultaneously. The system consisted of an Ultimate 3000 autosampler, a thermostatted column compartment TCC 100, and a p680 HPLC dual low-pressure gradient pump (analytical). All were purchased from Dionex Benelux BV (Amsterdam, The Netherlands). The MS/MS used was a Quattro micro API Tandem Quadrupole system from Waters Corporation, Milford, MA. Two hundred microliters of blood samples, controls, or calibrators were diluted with 200 µL 0.1M ZnSO₄ and 500 µL internal standard solution. Internal standard solution consisted of 100 µL 16 µg/L desmethyl sirolimus in methanol and 25 mL acetonitrile (LiChrosolv; Merck KGaA, Darmstadt Germany). A 6 + 1 multilevel calibrator set (0, 2.1, 6.0, 12.3, 18.2, 25.3, 46.5 µg/L) was used, which was obtained from Chromsystems (Munich, Germany). Blood control levels 1, 2, and 3 were obtained from RECIPE (Munich, Germany). After diluting, vortex mixing for 2 minutes followed by 5 minutes of centrifugation at 13,000 rpm was conducted. After centrifugation, the supernatant was transferred into a cylindrical crimp neck autosampler vial.

A 50 µL aliquot of supernatant was injected into an online solid phase extraction column (Cartridge Hysphere 5C18 HD, 7-µm particle size 10×2 mm; Spark, Emmen, The Netherlands) for enrichment. For sample cleanup, two mobile phases were used: mobile phase A: 0.1% v/v formic acid + 2 mM ammonium acetate in water and mobile phase B: 0.1% v/v formic acid + 2 mM ammonium acetate in methanol. The elution gradient used on the solid phase extraction column was 50% A and 50% B for 2 minutes followed by 0.8 minute 100% B and 1.5 minutes 50% A and 50% B for elution of everolimus and internal standard for isocratic liquid chromatography on the precolumn (Hypersil 4×2 mm; Phenomenex, Utrecht, The Netherlands) and analytical column (Hypersil Phenyl 50×3 mm, 3-µm particle size; Thermo Scientific, Geel, Belgium). The column oven was set at 55°C. The elution gradient for chromatographic separation to the MS was 10% A
and 90% B at a flow rate of 600 µL/min. Mass spectrometric detection was in positive ion mode using selected reactant monitoring (everolimus m/z 975.7→908.3, internal standard, desmethyl sirolimus, m/z 901.7→834.3).

The lower limit of quantification for everolimus was 0.2 µg/L determined with the following criteria: accuracy limits of 80% to 120% and imprecision CV less than 20%. LC-MS/MS within-run accuracy and precision were determined by analyzing the three controls in duplicate. Interassay precision and accuracy were evaluated by analyzing the QCs of each determination, which provided data for this study. The accuracy biases of the calibrators (2.1, 6.0, 12.3, 18.2, 25.3, 46.5 µg/L) were 3.3, –1.8, –1.1, –0.9, –0.4, and 0.5%, respectively with CVs for imprecision of 6.1%, 4.0%, 3.1%, 2.8%, 2.4%, and 1.6%, respectively (n = 105), QC samples used for LC-MS/MS were: control level 1 with a theoretical value of 3.3 µg/L had an accuracy bias of –7.3% and a CV for imprecision of 7.0%; control level 2: 10.5 µg/L had an accuracy bias of –2.7% and a CV for imprecision of 5.2%. Finally, control level 3: 17.2 µg/L had an accuracy bias of –2.2% and a CV for imprecision of 5.5% (n = 115).

**Statistical Analysis**

Agreement between LC-MS/MS and FPIA measurements of everolimus whole blood concentrations was determined using Bland and Altman analysis [13–15]. Passing-Bablok regression analysis was used to confirm the Bland-Altman results and to check for a linear relationship between the two methods. Analysis was performed with Microsoft Office Excel (Microsoft Inc, Redmond, WA) add-in Analyse it statistics software (Analyse-it Software, Ltd, Leeds, UK). Areas under the curve (AUCs) were calculated using the linear trapezoidal rule with everolimus trough concentrations used as 12-hour values. Figures were made with S-Plus (Insightful Corporation, Seattle, WA).

As suggested by Altman et al [16], we introduced a clinical acceptance limit to be able to decide whether two methods were in agreement. We chose the clinical acceptance limit to be a 20% range around the average difference between the methods. This clinical acceptance limit was based on the lowest everolimus oral dose available of 0.25 mg and a dose change that would be clinically relevant. This can be explained by the fact that the same exposure in terms of either trough concentration or AUC could be reached for instance with 1 mg for one individual versus 3 mg in another. A 20% difference in dose would mean a 0.2 mg (in clinical practice 0.25 mg) and 0.6 mg (in clinical practice 0.5 mg) dose difference, respectively.
Results

The everolimus concentrations ranged from 2.3 to 59.2 µg/L and 2.1 to 50.0 µg/L measured by FPIA and LC-MS/MS, respectively. Everolimus trough blood concentrations in 286 samples ranged from 2.3 to 25.0 µg/L and 2.1 to 18.0 µg/L measured by LC-MS/MS and FPIA, respectively. The everolimus whole blood pharmacokinetic curves constructed from the full AUCs collected 2 weeks after conversion from patients receiving 3 mg everolimus are presented in Figure 1. The mean (n = 20) AUC$_{0–12h}$ determined with FPIA was 166 µg*h/L (± 57) and the mean (n = 20) AUC$_{0–12h}$ determined with LC-MS/MS was 140 µg*h/L (± 41) (P < 0.001; paired Student t test).

![Figure 1: Mean everolimus whole blood concentration profile (0–6 hours after administration) of 3 mg everolimus start dose (n = 20) determined in renal transplant recipients using fluorescence polarization immunoassay (FPIA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Bars represent the standard deviation. AUC, area under the curve.](image-url)

First of all, a Passing-Bablok analysis was performed to give insight into the relationship between the two methods (Fig. 2A–B). In particular, linearity between the two methods and deviation from the line of identity were investigated. Figure 2A shows the relationship between trough concentrations measured with FPIA and LC-MS/MS, whereas a similar figure for all measurements is presented in Figure 2B. The slope of the regression equation for the trough concentrations was higher than the slope of the regression equation describing all concentrations. Furthermore, the regression equation for trough concentrations demonstrated a constant bias (95% confidence interval [CI]: 1.3–1.5) for the slope. In contrast, the
regression equation for all concentrations only showed a proportional bias for the slope (95% CI: –0.2 to –0.6). Finally, when the Passing-Bablok analysis for all concentrations was tested for linearity with the cusum test, a significant deviation from linearity was observed (P< 0.01), which was not the case for the trough concentrations (P > 0.10).

To test whether the two methods were in agreement, the Bland-Altman plot of the difference between the two methods against the mean of the two methods was constructed as shown in Figure 3A. This Bland-Altman plot shows that the absolute difference ranged from –6.0 to 16.7 µg/L with a mean difference of 2.5 µg/L. Differences between FPIA and LC-MS/MS increased with higher mean concentrations of both methods. As has been described previously [13,14,16], the influence of this trend should be taken into account by either a log transformation of the data or using a concentration ratio (FPIA:LC-MS/MS). For interpretation purposes, we chose the second option [14]. The ratio was plotted against the mean concentration. The proportional trend disappeared and the range of the difference decreased with higher mean concentrations (Fig. 3B). The geometric mean and median of the concentration ratio were 1.23, but the ratio ranged from 0.65 to 2.14. Moreover, the range of the concentration ratio FPIA:LC-MS/MS below a mean concentration of 15 µg/L is much larger than above a mean concentration of 15 µg/L.

![Figure 2: Passing-Bablok comparison plot of the everolimus trough concentrations of renal transplant recipients (n = 286) (A) and all measurements (n = 612) (B) obtained by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and fluorescence polarization immunoassay (FPIA) with the line of identity. Dashed lines represent the 95% confidence intervals. The solid line within the dashed lines represents the regression equation.](image)
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(0.65–2.14 below compared with 0.78–1.69 above). At the start of this comparison, we set the clinical acceptance limits at 6 ± 20% of the mean ratio. As can be seen from Figure 3B, this acceptance limit falls well within the 95% CI (mean ± 1.96 standard deviation). More specifically, 19% (119 of 612) of the data points exceeded the clinical acceptance limits. The majority of these data points (80%) were below a mean concentration of 15 µg/L. Trough concentrations represented 73% of the data points that were lower than 15 µg/L and were outside the upper acceptance limit (36 of 49). This indicates that concentrations in the lower range such as trough concentrations vary more between the two methods. Indeed, this is likely to be the case because the Passing-Bablok analysis also showed a difference for the two methods regarding linearity and the regression equation when comparing trough concentrations and all concentrations.

Figure 3: Bland-Altman plot of difference (A) and concentration ratio (B) against mean everolimus concentration measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and fluorescence polarization immunoassay (FPIA) (n = 612) showing data range, mean (2A: 2.5 µg/L, 2B: 1.23) with 95% limits of agreement and clinical acceptance limits (1.48 and 0.98).

Agreement between FPIA and LC-MS/MS was also investigated using AUC0–12h values as presented in the AUC ratio plot (FPIA:LC-MS/MS) in Figure 4. The ratio plot showed a mean ratio and median of 1.24 meaning that, on average, AUC0–12h measured with FPIA resulted in a 24% higher AUC0–12h than with LC-MS/MS. The ratio ranged from 0.92 to 1.94 showing a large variability. All except two data points were within the 95% CIs of the mean ratio, but the acceptance limit was crossed by 10% of the data points, again confirming that the two methods are not in agreement.
Figure 4: Bland-Altman plot of ratio against mean area under the curve (AUC) obtained by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and fluorescence polarization immunoassay (FPIA) \((n = 84)\) showing data range, mean (1.24) with 95% limits of agreement, and clinical acceptance limits (1.48 and 0.99).

To investigate whether this variability would have clinical implications, dose adjustments were calculated using the 84 AUCs\(_{0–12h}\) from 28 patients. The target AUC\(_{0–12h}\) for FPIA was 150 µg*h/L and therefore target AUC\(_{0–12h}\) for LC-MS/MS was set at 120 µg*h/L because we found that FPIA results were 24% higher than LC-MS/MS results. Figure 5 shows the
difference in individual dosage advice based on everolimus AUC_{0–12h} as determined by FPIA or LC-MS/MS. The differences ranged from −1.18 mg to 0.85 mg with an average difference of 0.04. Differences of 0.5 mg or higher would cause clinical concern. All extreme outliers (greater than 0.75 mg) were from different patients. The graph shows a large variability in everolimus doses leading to actual differences in dose adjustments up to 1.25 mg when using the average ratio (FPIA:LC-MS/MS) as a correction factor.

To investigate the variability of the methods with time during clinical monitoring of everolimus, dose-corrected trough concentrations from six different patients as determined by the two methods were plotted chronologically in Figure 6.

Figure 6: Dose-corrected trough concentrations at monitoring occasions measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and fluorescence polarization immunoassay (FPIA) showing variability in difference between FPIA and LC-MS/MS in six patients.

This means that all concentrations were scaled to an everolimus dose of 3 mg twice daily to correct for dose-related concentration differences. Next to the two lines of concentrations measured with both methods, a third line is introduced, which indicates the differences between the FPIA line and LC-MS/MS line. The horizontal axis reflects the occasion number. The FPIA line ascends and descends, whereas the LC-MS/MS line remained more
stable, resulting in pronounced fluctuations of the difference line. If both methods showed no difference at all, the difference line would be a flat line. Overall, FPIA-determined everolimus trough concentrations were higher than those determined by LC-MS/MS, but the difference between the two methods was not constant. This is illustrated by multiple peaks with the FPIA methods in this figure, whereas a straight line is observed for LC-MS/MS. For instance, this is the case for Patient B at occasion 12 or Patient C at occasion 7.

Discussion

In this study, the two widely used analytical techniques for everolimus blood concentration measurement, FPIA and LC-MS/MS, were compared using a large series of blood samples from stable adult renal transplant recipients. Overall, this study demonstrated that these two methods were not in agreement, because the preset acceptance limit was exceeded. Furthermore, this study showed that everolimus concentrations determined by FPIA are, on average, 23% higher than LC-MS/MS. However, the variability found between FPIA and LC-MS/MS could be twofold for concentrations lower than 15 µg/L or AUC$_{0–12h}$. This suggests a relatively large effect on variability of FPIA versus LC-MS/MS when monitoring trough concentrations. Moreover, the large variability of the everolimus concentrations determined with FPIA can lead to differences in everolimus doses of 1.25 mg compared with LC-MS/MS when applying dose adjustments based on a preset target AUC despite using a correction factor of 23%. Finally, the within-patient variability for trough concentrations appeared to be higher using the FPIA method.

Previous method comparisons for everolimus [3,4,17,18] were based mainly on trough concentration measurements and not AUCs. Differences between the methods using trough concentrations, other concentration time points, or the AUC were not investigated, and implications of the differences between the methods on dose adjustments remained unclear. Moreover, in previous studies, samples were obtained from patients on a cyclosporine A, prednisolone, and everolimus based immunosuppressive regimen. Because an interaction between cyclosporine A and everolimus has been described [19], this could affect the results.

When comparing the regression equations obtained with this study and earlier studies, the regression equation: FPIA = 1.21 x LC-MS/MS + 0.2 was comparable to those of Salm et al: FPIA = 1.19 x HPLC-MS + 0.5118 and Koster et al: 1.34 x LC-MS/MS + 0.855 found
in patients on a everolimus with a calcineurin inhibitor regimen. Dially et al. found, with a smaller number of samples of renal and heart transplant patients; FPIA = 0.851 × LC-MS/MS + 1.773 [4], a larger difference in slope but a higher intercept, possibly caused by use of different internal standards [4,20]. Khoschsorur et al. [17] found in their trough concentration comparison of FPIA versus HPLC an equation of FPIA = 1.11 × HPLC + 0.378 [17]. The difference with Khoschsorur et al. [17] in the trough concentration equation could be caused by higher process efficiency of LC-MS/MS, population differences, and cyclosporine A use.

In the present study, different QC samples were used for determining within-day and between-day precision for the two methods. It would be best to use the same controls for both methods but this was not possible as a result of the prefabricated packages with FPIA. However, the controls used were similar to those used by other research groups [3,4,17,18]. This study used a large number of samples obtained from 28 individuals. This means that multiple samples from individual patients were used for the comparison. To exclude any bias from repeated measurements we repeated all the analyses on data sets containing only one measurement per individual. All conclusions remained the same when applying that procedure. In fact, one should not consider these as repeated measurements because different days with different clinical situations (alternating hematocrit, co-medication) and different everolimus doses within an individual were compared.

Bland-Altman analysis of all everolimus concentrations showed large variability and a lack of agreement between the two methods. The majority of the data points that exceeded the acceptance limits were below a mean concentration of 15 µg/L. The ratio between AUC_{0-12h} determined with FPIA and AUC_{0-12h} determined with LC-MS/MS had a large variability corresponding to large differences in dosage advice. The dosing differences between 0.25 mg and −0.25 mg were considered not relevant because the lowest tablet dose available is 0.25 mg. Nevertheless, a large number of data points exceeded the ± 0.25-mg line, resulting in clinically relevant dosing differences. However, a difference of 0.25 mg on a total dose of 3 mg has less impact than a difference of 0.25 mg on a total dose of 1 mg. This clinical impact is taken into account by our clinical acceptance limit. A 20% difference on a dose of 1 mg or 3 mg leads to a maximum adjustment of the everolimus exposure of 20%. The large number of data points that exceeded the clinical acceptance limit indicated that the two methods cannot be interchanged. All data points exceeding or near the 20% clinical acceptance limits in Figure 4 correspond with large differences in dosing advice in Figure 5. Therefore, it is not desirable or acceptable to use the average AUC ratio of 1.24 or the
average concentration ratio of 1.23 as correction factors, because this could lead to clinically relevant differences in everolimus dose adjustments. The differences in dosage advice that were found could raise the question whether the target AUC of LC-MS/MS was calculated correctly. We investigated this as follows; compared with a target AUC of 115 µg*h/L or 125 µg*h/L, the chosen target AUC of 120 µg*h/L resulted in a mean difference closest to zero, which means that the average dosage advice of FPIA and LC-MS/MS is similar. With any other approach, the dose differences are higher.

The variability of the concentration ratio was higher for concentrations less than 15 µg/L. This of course could be the result of nonspecific binding of the antibodies [10]. The majority of the concentrations lower than 15 µg/L were trough concentrations. The concentrations are normally relatively low at the trough concentration (Fig. 3) and metabolites are present in relatively high concentrations before the next dose. Therefore, crossreactivity of these metabolites would probably have a greater impact on trough concentrations. For cyclosporine A, which has a similar metabolism [21], Schütz et al. [22] demonstrated that the relative cyclosporine A metabolite concentration was higher at trough concentration than at 2 hours after dose intake. Johnston et al.[23] showed higher crossreactivity at trough concentration for cyclosporine A as compared with 2 hours after dose intake. Crossreactivity of the FPIA assay was investigated by Tobin Strom et al. [24,25]. They identified metabolite patterns of everolimus in trough blood samples of renal transplant patients and found metabolite concentrations of the three main metabolites: 46-hydroxy 44.1% (0–784%), 24 hydroxy 7.7% (0–85.6%), and 25-hydroxy 14.4% (0–155.4%) (25). For FPIA, they found crossreactivity of 1% or less for 46-hydroxy and 24-hydroxy everolimus and 6% or less for 25-hydroxy everolimus [24]. This suggests that at least part of the overestimation of FPIA may be caused by crossreactivity.

The variability in differences in dosage advice showed that the risk of suboptimal dosage advice is present and clinically relevant. With FPIA, the question raises if an elevated or reduced trough concentration of a patient sample result is correct or the result of the variability of the assay. In the first 6 months after transplantation (patients using cyclosporine A and everolimus), an incorrect dose adjustment of 25% (0.75 on 3 mg total) resulting in too low an exposure to everolimus increases the risk of rejection up to 10% [26]. The risk of toxicity such as trombocytopenia after a similar incorrect dose adjustment resulting in an everolimus exposure 25% too high could increase up to 7% depending on the everolimus blood concentrations reached [26]. In general, LC-MS/MS is a more specific, more stable, and more accurate method for everolimus TDM. LC-MS/
MS is able to simultaneously measure several immunosuppressive drugs in a single run and can provide high specificity and sensitivity. The limitations are high initial capital investment and highly trained analysts for operation and maintenance. Because of this mainly financial limitation, not every clinical laboratory has a LC-MS/MS at its disposal [6]. Although FPIA is easy to operate, the analysis costs are relatively high.

Conclusion

The analytical methods FPIA and LC-MS/MS are not in agreement. Everolimus blood concentration measurement using FPIA results in higher everolimus concentrations compared with LC-MS/MS. Furthermore, LC-MS/MS outperforms FPIA for clinical monitoring and intervention of everolimus therapy in adult renal transplant recipients on duo therapy with prednisolone. Specifically, the use of FPIA can lead to clinically relevant differences in everolimus dosage advice and higher intra-patient variability.
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