Letter to the Editor:
Failure of Systematic Error Detection with Internal Quality Control Program

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Introduction

Everolimus (Certican®, Novartis Pharma AG, Basel, Switzerland), a macrolide antibiotic derived by chemical modification of a natural product, sirolimus (Rapamune®, Wyeth-Ayerst, Princeton, NY, USA), is a potent immunosuppressive agent that prevents the rejection of solid organ grafts [1, 2]. Therapeutic drug monitoring (TDM) plays a key role in maintaining blood everolimus concentrations within the narrow therapeutic range and helps to optimize the dosage regimen, maintain immunosuppressive efficacy, and minimize toxicity [3].

For the detection of analytical errors in TDM, quantification of control materials is generally effective, as routinely done in clinical chemistry laboratories. However, we recently experienced an episode in which the internal quality control procedure for everolimus, which uses control materials provided by the same manufacturer as the reagent, failed to detect a systematic error in the analysis of patient samples.

Materials and Methods

Since 29 January 2007, everolimus has been measured in our laboratory for renal transplant recipients using an immunoassay system (Seradyn Innofluor® Certican® Assay System, Seradyn, Inc., Indianapolis, IN, USA), a homogeneous fluorescence polarization immunoassay (FPIA), on a TDxFLx analyzer (Abbott Diagnostics, Chicago, IL, USA). Specimen preparation prior to analysis followed the manufacturer’s instructions. An aliquot (300 μl) of each control and patient sample was transferred to a centrifuge tube containing 350 μl of methanol and 50 μl of the Innofluor Certican precipitation reagent. After vortexing and centrifugation, the supernatants were loaded into the TDxFLx analyzer. Preventive maintenance for the analyzer, including photocheck, was performed according to the manufacturer’s instructions and the optical assembly was checked properly.

Calibration was done with Seradyn Innofluor® Certican® Calibrator sets [Catalog no. 0373407, lot 601234]. Seradyn Innofluor® Certican® Control sets [Catalog no. 0373399, lots 601101 (from 29 January 2007 to 5 March 2007) and 603325 (from 13 March 2007 to 31 August 2007); Seradyn Inc.] were used to monitor the assay performance, the quality of analysis, and the stability of the calibration curve. We established and used our own quality control ranges after verifying 10 times that results of the control materials were in the ranges supplied by the manufacturer (level 1, [2.20; 5.11] ng/ml; level 2, [6.95; 11.66] ng/ml; level 3, [13.25; 21.24] ng/ml (lot 601101); and level 1, [3.13; 4.70] ng/ml; level 2, [8.49; 12.74] ng/ml; level 3, [17.38; 26.08] ng/ml (lot 603325)) (Fig. 1A to 1C).

As part of an inter-laboratory quality control program, the data from our control materials were compared with a peer laboratory using reagents with the same lot number. In addition, since the implementation of the assay, every patient sample was sent to a reference laboratory that measured the everolimus concentration by liquid chromatography-mass spectrometry (LC-MS), so that we could compare the results from our laboratory and the reference laboratory.

Results and Discussion

The everolimus concentrations in each level of control material were not significantly different between our laboratory and the peer laboratory (p = 0.994 in level 1; 0.304 in level 2; 0.176 in level 3; Table 1). However, in terms of the patient samples...
from 29 January 2007 to 23 March 2007, the everolimus concentrations from our laboratory were higher than the reference laboratory (Fig. 1D; \( p < 0.001 \)). The percent difference of the patient samples was 154 ± 64% (mean ± SD) and the difference of the concentrations was 8.06 ± 3.64 ng/ml (mean ± SD). The differences in everolimus concentrations in the patient samples were calculated using a paired t-test (\( p < 0.05 \) was considered statistically significant). From the time that we noticed the difference in inter-laboratory comparison results of patient samples, the analytical results from the reference laboratory were reported to the clinicians.

We immediately launched an investigation to determine the source of the analytical error. We checked the expiration dates and storage conditions of the reagents, the calibrator, and the control materials and we exchanged each of them with new ones of same lot. Photocheck had been passed and the sample processing procedure used by the laboratory technician was reviewed. However, the source of the error was not identified. We verified that the patients were not being treated with any of the potentially interfering drugs listed on the package insert of the assay kit.

We withheld the reports of everolimus concentrations for patient samples and continued the inter-laboratory comparisons with the peer and reference laboratories. Special maintenance with overhaul of the TDxFLx analyzer was performed by the manufacturer and a problem with the optical assembly was detected. After replacement of the optical assembly, the positive bias in the comparison of the patient samples with the reference laboratory was markedly decreased (Fig. 1D). The percent difference changed from 154 ± 64% to 19.4 ± 21.0%, and the mean concentration changed from 8.06 ± 3.64 ng/ml to 1.01 ± 0.96 ng/ml.

Fig. 1. Chart plot showing everolimus concentrations of control materials at level 1 (n = 58; panel A), level 2 (n = 64; panel B), and level 3 (n = 60; panel C); percent difference ([our laboratory’s result – the reference laboratory’s result]/the reference laboratory’s result) plot against period under study from patient samples (n = 58) (panel D). The open circles show data obtained before replacement of the optical assembly; the solid circles show data obtained after replacement of the optical assembly. The mean values and the means ± 2 SD are represented by solid lines.
Regarding the control materials, although the everolimus concentrations in each level of the control material were significantly decreased after replacement of the optical assembly (p = 0.015 in level 1; 0.009 in level 2; and 0.010 in level 3; Table 1), most of the data were within the previously assayed range (Fig. 1A to 1C). The marked difference between the reduction of everolimus concentrations in the patient samples and the control materials after the systematic error correction suggests that a small adjustment in the net polarization value of the optical system resulted in greater change of the everolimus concentrations in the patient samples than in the control materials.

Our patients did not receive any possible interfering compounds. Although a previous study reported that monoclonal antibody used in the Abbott IMx® sirolimus microparticle enzyme immunoassay (MEIA) has a cross-reactivity with everolimus because of structural similarity between them [4], sirolimus was not co-administered to our patients. Our experience shows that the analysis of control materials did not detect a systematic error due to a matrix effect.

Sometimes the control material contains additives or preservatives that can introduce sources of error due to matrix problems with certain method reagents [5]. In this report, the control material was insensitive in detecting a systematic error of the analytical system that was corrected later by replacement of the optical assembly. This case highlights the importance of matrix effects on control materials. Therefore, manufacturers should inform the users as quickly and completely as possible about the matrix employed in their control materials [6].

Regarding the laboratory’s responsibility, either inter-laboratory comparisons of patient samples or proficiency testing with a pool of samples from patients, such as international proficiency testing scheme provided by Analytical Services International (ASI) [7], should be undertaken, especially during the early period after setup of a test. During the implementation of the everolimus assay, we conducted only an inter-laboratory comparison of control materials. Although an inter-laboratory comparison of patient samples with the reference laboratory in another country was started, we did not notice the difference of results from patient samples until 1-2 weeks later.

Internal quality control and proficiency testing of immunosuppressive agent assays are essential for clinical laboratories to meet reasonable clinical expectations for interpretation of assay results as an adjunct to dosage individualization and patient care [8]. Clinical laboratories are usually enrolled in inter-laboratory quality control programs (IQCP) from specialized manufacturers to evaluate the imprecision and accuracy of different methods, comparing their own monthly and cumulative statistics with those of a peer group of users employing the same lot number of control materials [6]. It is important to be aware that even IQCP and internal quality control programs with control materials of which the matrix is different from patient samples can fail to detect systematic errors in the analysis of patient samples.

In conclusion, even in assays that seem well controlled by analysis of control materials, systematic errors can exist in the analysis of patient samples. For accurate measurement of everolimus concentrations in patient samples with the Seradyn InnoFluor® Certican® FPIA assay on an Abbott TDxFLx® analyzer, inter-laboratory comparisons and proficiency testing using patient samples should be performed at regular intervals.

Table 1. Everolimus concentrations (mean ± SD [ng/ml]) in control materials measured in our laboratory and a peer laboratory.

<table>
<thead>
<tr>
<th>Error correction</th>
<th>Period</th>
<th>Level 1</th>
<th></th>
<th>Level 2</th>
<th></th>
<th>Level 3</th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>Our lab</td>
<td>Peer lab</td>
<td>p value</td>
<td>Our lab</td>
<td>Peer lab</td>
<td>p value</td>
</tr>
<tr>
<td>Before</td>
<td>29 Jan '07</td>
<td>3.82±0.58</td>
<td></td>
<td>9.11±0.90</td>
<td></td>
<td>17.84±2.05</td>
<td></td>
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<tr>
<td></td>
<td>23 Mar '07</td>
<td>3.82±0.86</td>
<td>0.994</td>
<td>9.34±0.87</td>
<td>0.304</td>
<td>17.18±1.74</td>
<td>0.176</td>
</tr>
<tr>
<td>After</td>
<td>24 Mar '07</td>
<td>3.38±0.57</td>
<td></td>
<td>8.47±0.58</td>
<td></td>
<td>16.29±1.06</td>
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<td></td>
<td>31 Aug '07</td>
<td></td>
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<td></td>
<td>0.015</td>
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<td>p value</td>
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References


