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Letter to the editor

Falsely high sirolimus concentrations due to everolimus cross-reactivity in the Siemens sirolimus immunoassay: Corrective actions implemented

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Dear Editor:

Interference in immunoassays is a widely recognized problem [1]. Herein, we describe a case of interference in a Siemens sirolimus immunoassay resulting in falsely increased whole-blood sirolimus concentrations, and we describe the corrective actions taken to prevent reoccurrence.

A 68-year-old male idiopathic pulmonary fibrosis patient received a lung transplant in 2016, and his maintenance immunosuppressive therapy initially comprised tacrolimus, mycophenolate, and prednisone. Due to persistent, treatment-resistant cytomegalovirus (CMV), the mycophenolate was switched to sirolimus, which has been found to have anti-CMV effects. Thereafter, the patient experienced side effects, including fatigue, headache, loss of concentration, and rapid thoughts, so the sirolimus was switched to everolimus. However, as the sirolimus dose was tapered and the everolimus instituted, the patient's sirolimus concentrations remained persistently increased. According to the Siemens package insert (Siemens Healthcare Diagnostics Ltd.), sirolimus has a mean half-life of about 62 h; after 5 half-lives (~13 days), approximately 96.9% of the drug should be cleared from the patient's system. However, this patient had significant sirolimus concentrations (10.1–17.8 ng/ml) two months after discontinuing it.

An analytical investigation began when a pharmacist contacted us regarding several high sirolimus concentrations. We repeated the sample in triplicate and performed serial dilutions of the original specimen using the manufacturer's zero whole-blood calibrator as diluent, producing an average sirolimus concentration of 10.0 ng/ml. Serial dilution (1:2, 1:4, 1:8) of the original specimen gave a linear response with a regression line calculated as $y = 1.11x - 1.08$ ($r = 1.0$, $S_{y/x} = 0.20$) with y = measured and x = expected values.

We investigated heterophile antibody interference on the sirolimus assay of the patient's specimen using heterophile blocking reagents (HBR) (Scantibodies™; Laboratory Inc.), but the sirolimus concentrations after HBR incubation remained 10.0 ng/ml. Heterophile antibodies are usually directed to reagents in the immunoassay rather than to sirolimus molecules, so their interaction with sirolimus reagents is nonlinear [2]. The linearity of the sirolimus results with a serial dilution of the patient's specimen and the HBR results suggested no heterophile immunoassay interference.

We ensured that the patient's sample was not mislabeled and identified no specimen integrity issues. We reviewed our quality-control charts, calibrations, proficiency-testing data, and instrument-maintenance logs, finding no analytical deficiencies. The clinical chemistry laboratory had been notified of no previous problems with sirolimus tests, indicating that the discrepancy was likely unique to this patient. A conversation with Siemens technical support shed no light on the cause. The patient's specimen was sent to the Mayo Medical Laboratory (MML) for quantitative sirolimus testing using liquid-chromatography-tandem mass spectrometry (LC-MS/MS), which produced a level of < 1 ng/ml, indicating no sirolimus present in the patient's specimen.

Another specimen was tested for sirolimus 18 weeks later, showing a sirolimus concentration of 8.3 ng/ml. An aliquot of the original specimen and this second specimen were sent to the MML for quantitative everolimus testing using LC-MS/MS, which showed everolimus concentrations of 5.9 and 5.0 ng/ml, respectively. Given that LC-MS/MS uses no antibodies to detect either sirolimus or everolimus and is the reference method for both, we interpreted these results as indicating that the sirolimus concentrations measured by the Siemens immunoassay were due to cross-reactivity to everolimus [3–6].

To confirm that everolimus cross-reacts with sirolimus in the Siemens assay, we pooled blood from patients who had taken sirolimus and spiked the samples with varying concentrations of everolimus. The percent cross-reactivity for everolimus in the absence of sirolimus (whole-blood pool A) was 98–140%. In the presence of three different whole-blood pools of sirolimus (B, 5.4 ng/ml; C, 14.8 ng/ml; and D, 23.6 ng/ml), the percentages were 162–164%, 148–174%, and 156–176%, respectively (See Table 1.). These findings indicated that structural similarity between the sirolimus and everolimus molecules causes cross-reaction with antibodies in the sirolimus assay across the whole-blood therapeutic ranges [3–6]. Indeed, everolimus metabolites may cross-react with antibodies in the sirolimus assay [3–6], possibly explaining why the sirolimus concentration produced using the Siemens immunoassay method was higher than the everolimus concentration when using the LC-MS/MS method.

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Table 1
Cross-reactivity (%) of everolimus in the Siemens sirolimus assay.

Everolimus added (ng/ml)	Whole-blood pool A (0.0 ng/ml)	Whole-blood pool B (5.4 ng/ml)	Whole-blood pool C (14.8 ng/ml)	Whole-blood pool D (23.6 ng/ml)
0.0	0.0 (0%)[a]	5.4 (0%)[b]	14.8 (0%)[b]	23.6 (0%)[b]
5.0	4.9 (98%)[a]	13.5 (162%)[b]	23.5 (174%)[b]	32.4 (176%)[b]
16.7	22.7 (136%)[a]	32.4 (162%)[b]	39.5 (148%)[b]	49.7 (156%)[b]
25.0	35.0 (140%)[a]	46.3 (164%)[b]	51.8 (148%)[b]	66.2 (170%)[b]

Sirolimus assay in whole-blood pools A (no sirolimus); B (sirolimus = 5.4 ng/ml); C (sirolimus = 14.8 ng/ml); and D (sirolimus = 23.6 ng/ml).

[a] Percent cross-reactivity in absence of sirolimus = [apparent sirolimus concentration measured by Siemens instrument / everolimus concentration in sample] × 100%.

[b] Percent cross-reactivity in the presence of sirolimus = ([apparent sirolimus concentration measured by Siemens instrument in the presence of a particular everolimus added − apparent sirolimus concentration measured by Siemens instrument in the absence of everolimus] / everolimus concentration in sample) × 100%.

Our laboratory implemented corrective actions to prevent falsely increased sirolimus concentrations due to everolimus cross-reactivity, including 1) adding a comment to the testing results stating that our current immunoassay method gives falsely increased sirolimus concentrations for patient administered everolimus; 2) educating hospital and laboratory staff about the cross-reactivity; 3) revising standard operating procedure for sirolimus to include cross-reactivity; 4) including a question on everolimus cross-reactivity with the sirolimus immunoassay on the laboratory staff's annual written competencies; and 5) informing Siemens of the everolimus cross-reactivity and asking them to include it in the sirolimus package insert.

To preclude unnecessary diagnostic investigations or unfortunate therapeutic decisions, laboratory professionals must work with clinicians to determine the cause of discrepancies in patients' sirolimus results. In-vitro diagnostic manufacturers should provide everolimus cross-reactivity data in sirolimus package inserts. We conclude that specimens from patients receiving both everolimus and sirolimus or everolimus alone should be tested using LC-MS/MS rather than the Siemens sirolimus immunoassay.

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