

CASE REPORT

Falsely Elevated Tacrolimus Levels Caused by Immunoassay Interference Secondary to β -Galactosidase Antibodies in an Infected Liver Transplant Recipient

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Careful interpretation of tacrolimus levels is essential to ensure optimal immunosuppressive therapy while avoiding toxicity. Interference with tacrolimus assays may be an underreported event that has the potential to result in negative patient outcomes through unnecessary modifications of therapy. We describe a 55-year-old liver transplant recipient who had falsely elevated tacrolimus levels that led to the eventual disruption of his immunosuppressive therapy and subsequent rejection of his allograft. Although his increased tacrolimus levels did not correlate with clinical signs and symptoms of tacrolimus toxicity, interruption of therapy in this patient was supported by an acute infection and a slight elevation in serum creatinine concentration. Tacrolimus levels were analyzed by using an antibody conjugated magnetic immunoassay method, and levels as high as 79.7 ng/ml were observed, despite discontinuation of tacrolimus. We conducted an evaluation for assay interference by using an alternative assay method (microparticle enzyme immunoassay), by testing plasma samples that were not hemolyzed, and by analyzing levels of an unrelated drug that uses the same technology as the initial tacrolimus assay. β -Galactosidase antibodies were ultimately confirmed as the cause of the immunoassay interference. In patients receiving tacrolimus, spuriously high tacrolimus levels should be carefully evaluated, and drastic adjustments to therapy should be made only within the context of clinical toxicity.

Key Words: transplant, immunology, infectious disease, pharmacokinetics, tacrolimus.

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Due to the narrow therapeutic index of tacrolimus, drug levels must be accurately interpreted in order to make dosage adjustments to maintain the therapeutic effect while avoiding drug toxicities. Elevations in tacrolimus trough levels correlate with increased rates of dose-

dependent adverse events, especially nephrotoxicity, neurotoxicity, metabolic effects, and overimmuno-suppression.¹ When confronted with a high tacrolimus level, clinicians should cautiously interpret the result while considering the patient's clinical status, dosage administered, time of blood sampling, type of assay used, potential changes in drug metabolism from altered organ function or a drug-drug interaction, as well as any possible causes of a false elevation. Several sources of interference with immunoassays, such as those used in tacrolimus monitoring, have been

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documented in the literature. These include serum proteins, heterophilic and antianimal antibodies, low hematocrit, and drug metabolites.^{2,3}

We describe a case of falsely elevated tacrolimus levels caused by immunoassay interference secondary to β -galactosidase antibodies in an infected liver transplant recipient, which led to the withdrawal of tacrolimus and subsequent allograft rejection.

Case Report

A 55-year-old southeast Indian man with end-stage liver disease secondary to cryptogenic cirrhosis was scheduled for orthotopic liver transplantation. His immediate preoperative course, however, was marked by pneumonia and urosepsis. A 14-day course of ertapenem was started, which was to be continued until postoperative day 2. The organism isolated in his urine culture was *Escherichia coli* with resistance due to acquired extended-spectrum β -lactamase. The patient was cleared for surgery and underwent transplantation with an allograft from a 58-year-old donor. Biopsies performed at the time of transplantation revealed mild macrosteatosis and minimal microsteatosis.

The patient's initial immunosuppressant regimen consisted of methylprednisolone 500 mg intraoperatively, followed by maintenance therapy with tacrolimus 2 mg every 12 hours, mycophenolate mofetil 500 mg twice/day, and a steroid taper to prednisone 20 mg/day by postoperative day 4. The patient's tacrolimus target range was 7–12 ng/ml, which is the initial target range used at our institution. On postoperative day 2, a tacrolimus trough level of 25 ng/ml was determined by using an antibody conjugated magnetic immunoassay (ACMIA) (Siemens Dimension TACR; Siemens Healthcare Diagnostics, Inc., Newark, DE). The patient's next tacrolimus dose was withheld, and the dosage was decreased to 1 mg every 12 hours. On postoperative day 4, tacrolimus was withheld due to continuously elevated tacrolimus levels, as well as an elevation in serum creatinine concentration (from 1.2 mg/dl at baseline to 1.8 mg/dl). On postoperative day 7, the patient developed a repeat urinary tract infection with the same organism. Ertapenem was restarted, and mycophenolate mofetil was withheld; prednisone 5 mg/day was continued as his sole immunosuppressant.

An ascending trend of hepatic transaminase and total bilirubin levels starting on post-

operative days 9–11 prompted the patient's first postoperative percutaneous biopsy, which revealed acute cellular rejection. On postoperative day 11, he was treated with pulse-dose methylprednisolone followed by a prednisone taper, and mycophenolate mofetil was restarted. Tacrolimus was restarted at 1 mg every 12 hours on postoperative day 13, when tacrolimus levels were within the target range. A hepatobiliary iminodiacetic acid scan and endoscopic retrograde cholangiopancreatography were performed on postoperative days 12 and 16, respectively, which showed no leak; however, a biliary stent was placed secondary to anastomatic narrowing. A repeat biopsy on postoperative day 17 revealed improving acute cellular rejection. With the dosage adjustments, the patient's tacrolimus levels remained therapeutic until postoperative day 18, when the trough level rose to 22.3 ng/ml. Tacrolimus was withheld for 2 days and was resumed at 0.5 mg every 12 hours when it once again fell below our upper-limit threshold of the target range.

On postoperative day 23, the patient's urine cultures were still positive, and a 14-day course of tigecycline was begun. On postoperative day 24, tacrolimus was again withheld after the morning dose, as the level was elevated at 12.9 ng/ml. Prompting further investigation, his plasma samples were sent to an outside laboratory for analysis of tacrolimus levels. At that laboratory, microparticle enzyme immunoassay (MEIA) (IMx Tacrolimus II; Abbott Laboratories, Abbott Park, IL) was used. On postoperative day 30, results from the MEIA showed undetectable levels, whereas the result from the same plasma sample using the ACMIA was 18.9 ng/ml. Tacrolimus continued to be withheld, and despite not receiving tacrolimus for 14 days, the patient's tacrolimus level results from the ACMIA continued to trend upward to a peak of 79.7 ng/ml on postoperative day 37. As we suspected that potential interference may be occurring with the ACMIA, we decided to use the MEIA results (outside laboratory) to monitor our patient's tacrolimus dosing. On postoperative day 38, tacrolimus was restarted at a dose of 0.5 mg every 12 hours, and the drug was titrated up to therapeutic levels. Figure 1 illustrates the relationship of the tacrolimus trough levels and the tacrolimus doses administered throughout the patient's postoperative course.

On discussion with the manufacturer of the Siemens Dimension TACR assay, the interference was determined to be due to the presence of

antibodies to β -galactosidase, which we suspect were secondary to the patient's *E. coli* infection. At the discretion of the outpatient hepatologist, the patient was changed to a cyclosporine-based immunosuppressant regimen 4 months after transplantation. Of note, 12 months after transplantation, his tacrolimus levels were still detectable (despite not having received the drug); he did, however, continue to develop several recurrent urinary tract infections during this time frame.

Discussion

Interferences with immunoassays have been described in great detail elsewhere. However, we performed a MEDLINE search and found only three published case reports of antibody interference with tacrolimus assays.⁴⁻⁶ All three cases involved heterophilic antibody interference. Our case is distinctive in that it illustrates interference with antibodies to β -galactosidase and highlights the importance of the careful evaluation of therapeutic drug levels and the ramifications of incorrectly interpreting an elevated tacrolimus level—in our patient, acute cellular rejection of his allograft. Our decision to modify the patient's immunosuppressant regimen was based on apparent elevations in tacrolimus level and was supported by an increase in his serum creatinine concentration and an overlapping acute infection. Causes of tacrolimus elevations by drug-drug interactions and metabolic changes were carefully analyzed but were not considered to be clinically significant.

The three case reports we identified also utilized the ACMIA method to analyze tacrolimus levels. In the first case report, a 57-year-old man with end-stage liver disease secondary to hepatitis C virus had tacrolimus levels ranging from 49 ng/ml immediately after liver transplantation to 12.5 ng/ml 2 weeks after the discontinuation of tacrolimus.⁴ Levels were analyzed using ACMIA with the Siemens Dimension TACR analyzer. No clinical evidence of toxicity was noted, and rejection was not reported. Heterophilic antibody interference was confirmed by treating plasma samples with heterophilic blocking tubes as protein G resin. In the second case report, a 43-year-old man had tacrolimus level elevations 10 months after kidney transplantation.⁵ His clinicians then compared the ACMIA on an XPand analyzer (Siemens Healthcare Diagnostics, Inc.) with the enzyme multiplied immunoassay technique (EMIT) on a Cobas analyzer (Siemens Healthcare Diagnostics, Inc.). The patient had human immunodeficiency virus infection and was taking potentially interacting antiretrovirals; however, his tacrolimus levels had been previously stable during his antiretroviral therapy. Measurement of tacrolimus levels by using ACMIA resulted in a 3–7-fold elevation when compared with EMIT; the levels were also higher when compared with liquid chromatography with tandem mass spectrometry. An unidentified antibody, which was not a β -galactosidase antibody, was presumed to be responsible for the interference. In the third case report, 6 years after liver trans-

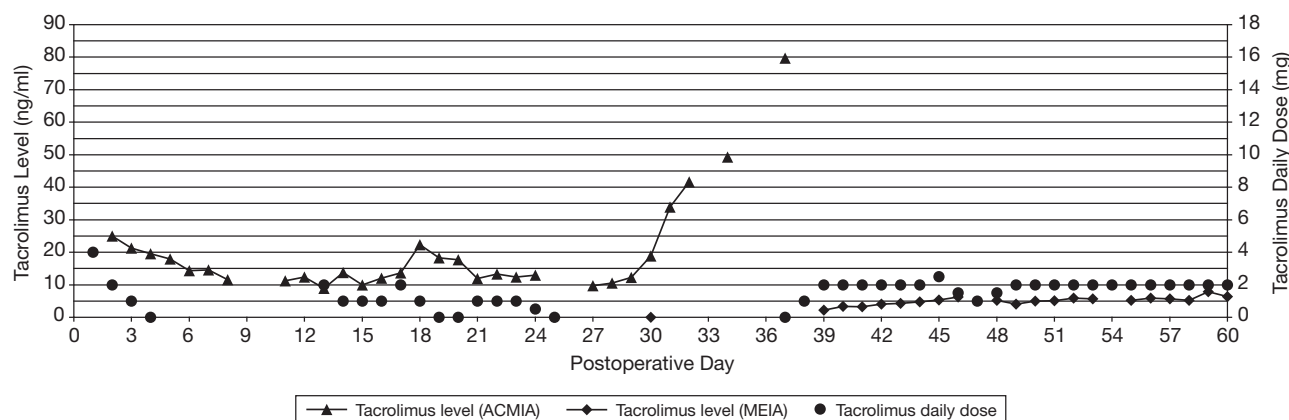


Figure 1. Timeline of the patient's tacrolimus levels (target range 7–12 ng/ml) in relation to his tacrolimus daily dose during his postoperative course. On postoperative day 30, the same plasma sample was also analyzed using a different immunoassay (microparticle enzyme immunoassay [MEIA]), which showed undetectable tacrolimus levels versus a level of 18.9 ng/ml with the antibody conjugated magnetic immunoassay (ACMIA). On postoperative day 39, the analysis method changed entirely to the analyzer using the MEIA, and standard doses of tacrolimus provided therapeutic levels.

plantation secondary to hepatitis C infection, a 61-year-old man had an increased tacrolimus level of 29 ng/ml using ACMIA on a Dimension XPand Plus analyzer (Siemens Healthcare Diagnostics, Inc.) compared with a level of 6.1 ng/ml using MEIA on an IMx analyzer.⁶ Interference by an unknown heterophilic antibody was suspected. Considering that the patient had a history of animal handling, it was presumed to be antianimal in nature; however, it was determined that it was not murine in origin.

As of 2008, approximately 75% of transplant centers use immunoassays for therapeutic drug monitoring, and not the more specific, albeit costly and labor intensive, high-performance liquid chromatography with mass spectrometry (HPLC-MS).⁷ Immunoassays are susceptible to antibody interference from heterophilic antibodies, antianimal antibodies, or an antibody specific to a component of the assay—in our patient, β -galactosidase (Table 1). In addition to the antibody and physiologic interferences described, false elevations could be due to a poor specimen collected for analysis. More specifically, it has been observed that intravenous tacrolimus may adsorb to venous catheters and leach off when blood is collected through the same catheter, leading to levels significantly higher than from samples collected from other sites.¹⁸

In our patient, the initial tacrolimus levels were measured using the Siemens automated Dimension TACR method, ACMIA, an immunoassay in which free and tacrolimus-bound antibody-enzyme conjugates are separated using magnetic particles.^{15, 19} The reagent contains an antibody- β -galactosidase conjugate that binds to the tacrolimus present in a lysed whole blood sample. Tacrolimus-coated magnetic particles are added to bind the free antibody- β -galactosidase conjugate, which is then separated magnetically to remove the unbound conjugate. The tacrolimus that is bound to the antibody-enzyme complex is measured and reported. Although heterophilic antibodies are the most common cause of interference in immunoassays, a particular limitation of the Siemens TACR assay is that false results may occur if a patient's sample contains an abundance of antibodies to β -galactosidase as a result of bacterial infection. Although the TACR Flex reagent cartridge contains compounds to block antibody interference, patients may occasionally have titers that exceed the capability of the blocking reagent or that have antigenicity not recognized by the blocking reagent, resulting in a falsely elevated result.

False-positive results from immunoassay antibody interference should be considered when a spuriously high tacrolimus level coincides with absence of clinical toxicity. If an observed tacrolimus level seems to be inconsistent with clinical evaluation, the most readily available method for evaluating a suspected false-positive result would be to test a sample using an alternative assay type, as interfering substances may be specific to an assay type. In addition, methods for evaluating and confirming antibody interference of immunoassays, including serial dilutions of the sample, the use of heterophile-blocking agents, and screening for the presence of antimurine antibodies, have been documented.²⁰

Representatives of the assay manufacturer (Siemens Healthcare Diagnostics, Inc.) described their own experience in which the majority of the samples produced a tacrolimus level of greater than 15 ng/ml and oftentimes greater than 20 ng/ml (Donna Gavin, Senior Technical Administrative Support, written communication, April 2009). They recommended a troubleshooting approach to screen for suspected interference by antibodies to β -galactosidase. Since our patient had an *E. coli* infection at the time the elevated levels were obtained, the representatives suggested that the interference may have been due to the presence of β -galactosidase antibodies and suggested that we perform a digoxin assay on the same analyzer since it uses the same technology as the TACR assay. The analyzer showed the presence of measurable digoxin levels. Considering that our patient was not taking digoxin, this confirmed that the interference was indeed due to β -galactosidase antibodies.

The distribution of tacrolimus is mainly (95–98%) in erythrocytes; however, antibodies are freely circulating molecules in plasma.²¹ Measuring tacrolimus levels in nonhemolyzed plasma samples with ethylenediaminetetraacetic acid (EDTA) added should result in values less than 30% of the result from whole blood in the absence of interfering antibodies. In our patient, the results were also elevated in plasma. The assay manufacturer cautions that the plasma sample be nonhemolyzed since hemolysis may result in the release of intracellular tacrolimus. Furthermore, test results obtained from plasma samples are not reportable and should be used only for troubleshooting purposes. If the tacrolimus result from the EDTA plasma is greater than 30% of the result from whole blood, it is highly suggestive of nonspecific interference,

and the patient's blood sample should be analyzed by an alternative method. In this case, we tested the patient's sample with an MEIA analyzer, which does not use β -galactosidase in the reagent.

Our case report adds to the growing body of literature demonstrating antibody interference with tacrolimus immunoassays. When evaluating an elevated tacrolimus level without clinical signs and symptoms of toxicity, causes of false elevations should be considered when making the decision to modify a patient's immunosuppressant regimen. Based on our experience with this patient, we would recommend a thorough investigation, including the interventions discussed here, if applicable, before making an adjustment to a patient's immunosuppressant regimen.

Conclusion

Careful scrutiny of therapeutic drug levels is essential to ensure the safety and efficacy of tacrolimus in transplant recipients. When confronted with an elevated tacrolimus level, causes of a true elevation should be considered, and the decision to modify or withhold tacrolimus therapy should be made only if there is clinical evidence of toxicity. It is important for clinicians to be aware of possible false elevations due to assay interference, since the inappropriate modification of a regimen based on the incorrect interpretation of a level may be detrimental for the patient.

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