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CASE REPORT

# Falsely elevated whole-blood tacrolimus concentrations in a kidney-transplant patient: potential hazards

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## Summary

Tacrolimus-based immunosuppression is the most frequently prescribed immunosuppression for kidney-transplant (KT) patients. Because tacrolimus has a narrow therapeutic window, drug monitoring is mandatory. Of the many methods used to assess whole-blood trough levels, antibody-conjugated magnetic immunoassay (ACMIA) is popular because, compared with microparticle enzyme-linked immunoassays (MEIA), there is no need to pretreat samples, thus reducing time taken by the laboratory technician. Herein, we report on a KT tacrolimus-treated patient who experienced falsely elevated whole-blood tacrolimus concentrations after using the ACMIA method. ACMIA gave trough levels of 24 ng/ml, whereas the actual trough level, when measured by enzyme-multiplied immunoassay technique (EMIT) and high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS), was nil. After a workup we only found one factor that might have caused the elevated concentration: positive anti-double stranded DNA autoantibodies. We conclude that, when ACMIA produces surprisingly high tacrolimus concentrations in organ-transplant patients, these should be reassessed immediately using either LC-MS/MS or another immunoassay in order to eliminate falsely elevated results.

## Introduction

Kidney-allograft transplantation, a well-established therapy for end-stage kidney disease, maintains good long-term results only if patients are given an immunosuppressive therapy. To date, the most popular regimen is the one that is tacrolimus-based. A recent prospective, controlled trial in *de novo* kidney-transplant (KT) patients showed that the best immunosuppressive regimen was that based on low-dose tacrolimus/mycophenolate mofetil (MMF)/steroids when compared with those based on standard- or low-dose cyclosporine A (CsA) or low-dose sirolimus (SRL), all combined with MMF/steroids [1]. However, because tacrolimus has a narrow therapeutic window, transplant physicians have to closely monitor whole-blood trough levels in order to avoid underdosage, which can result in potential rejection, or overdosage, which can cause side-effects such as renal insufficiency, hypertension, or infections [2].

The gold standard to measure tacrolimus blood levels is high-performance liquid chromatography generally coupled with mass spectrometry (LC-MS or LC-MS/MS), as this effectively measures the low therapeutic levels of this drug. However, because this technique is labour-intensive and expensive, the following alternative techniques are now mainly used: microparticle enzyme-linked

 

immunoassay (MEIA), enzyme-multiplied immunoassay technique (EMIT), antibody-conjugated magnetic immunoassay (ACMIA), and chemiluminescent microparticle immunoassay (CMIA) [3].

Herein, we report on a KT tacrolimus-treated patient who experienced falsely elevated whole-blood tacrolimus concentrations that were caused by the ACMIA method.

## Case report

A 47-year-old female underwent kidney transplantation in April 2004 for end-stage renal disease resulting from glomerulopathy. Baseline immunosuppression relied on CsA, MMF, and low-dose steroids after basiliximab induction. In April 2007, she was converted from CsA- to SRL-based immunosuppression because of repeated breast dysplastic lesions. In February 2009, she underwent mastectomy because of *in situ* intracanalaire carcinoma. At this point, MMF was stopped and SRL whole-blood trough levels were from 5–8 ng/ml. Her renal function was excellent: serum creatinine was 97 μmol/l. Hemoglobin (Hb) level was 12 g/dl.

Because of this surgery and the potential risks of delayed wound healing, SRL was stopped and replaced by tacrolimus (Prograf®; ASTELLAS Pharma Europe, London, UK) at 3 mg twice a day, aiming at trough levels of 5–10 ng/ml Trough levels were assessed in our hospital laboratory using the ACMIA technique run on a Dimension RXL® system (Siemens Healthcare Diagnostics, Deerfield, IL, USA). The first trough level of 6.9 ng/ml was measured 6 days after initiation of tacrolimus. The next measurement, at 1 month, was 60 ng/ml. Despite this high level, her serum creatinine was unchanged (Fig. 1) and there were no clinical symptoms to suggest tacrolimus overdosage. Hemoglobin level was unchanged (Fig. 1). The next day, a repeat test for tacrolimus trough levels gave 50.3 ng/ml. At this point tacrolimus therapy was suspended and the patient remained on steroid monotherapy (prednisolone 10 mg/day). Two days later, tacrolimus trough levels had decreased to 26 ng/ml. Repeat trough-level measurements during the following 4 weeks remained almost unchanged, i.e., 18.9–27 ng/ml (Fig. 1). Throughout this period, both serum creatinine and Hb levels remained remarkably stable.

On 24 April, arising out of our suspicion of falsely positive tacrolimus whole-blood trough levels, a blood sample was referred to another laboratory that used EMIT (run on a Cobas-Mira system) and LC-MS/MS [4] techniques: a nil concentration was reported, as compared to 24 ng/ml given by the ACMIA method the same day.

A subsequent workup from this false positive result showed serum creatinine 97 μmol/l; Hb of 13.7 g/l; total complement level as well as C3 and C4 subfractions were within the normal range; antinuclear autoantibodies were negative, but anti-double stranded DNA autoantibodies were positive at 14 (N: 0–10); rheumatoid factors, antineutrophil cytoplasmic autoantibodies, and cryoglobulins were negative. Serology for hepatitis B and C viruses and HIV were also negative. A kidney biopsy was normal, i.e., Banff score of t0, i0, g0, v0, ptc0, aah2, cg0, ci0, ct1, cv0, mm0. Immunostaining for C4d was negative. At this point, we decided to resume sirolimus at 3 mg/day to achieve trough levels of approximately 5 ng/ml. Two months later, her serum creatinine was found to be unchanged.

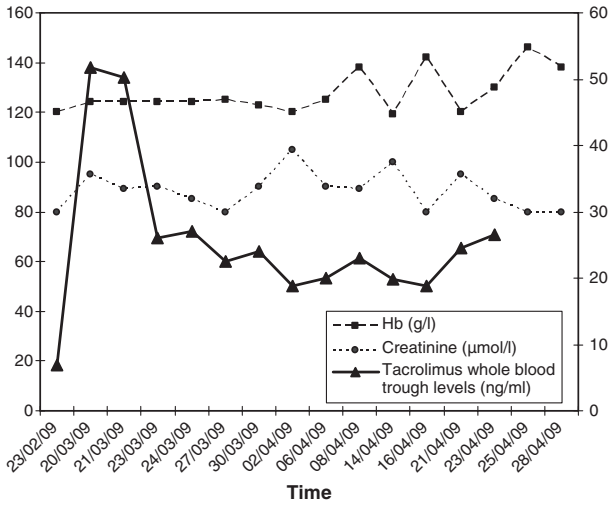


**Figure 1** Outcome of whole-blood tacrolimus concentrations (assessed by an affinity column-mediated immunoassay), hemoglobin and serum creatinine levels.

## Discussion

We report a case of falsely elevated whole-blood tacrolimus trough levels in a KT patient that was caused by interference within the ACMIA method. Recently, Barau *et al.* have reported the same findings for a 43-year-old HIV(+) HCV(+) male KT patient at 10 months post-transplantation [5]. That patient was also receiving antiretroviral therapy. However, in that case, when the falsely elevated results for tacrolimus were found using the ACMIA method, they were re-analysed using the EMIT method: ACMIA gave tacrolimus concentrations that were three- to sevenfold higher than those measured by the EMIT assay. Additionally, three tacrolimus concentrations were further verified by LC-MS to be similar to those determined by EMIT [5]. Thus, the authors hypothesized that the patient had an unidentified antibody that had led to interference with ACMIA.

The most accurate technique to assess whole-blood tacrolimus trough levels is LC-MS/MS. However, as this

method is time-consuming, immunoassay-based methods, e.g., EMIT and MEIA, or their recent replacements ACMIA and CMIA, are now mostly used. MEIA is of similar diagnostic value in both kidney- and liver-allograft recipients [6]. However, for anemic transplant patients, the EMIT method might be preferred to MEIA in determining whole-blood tacrolimus concentrations [7]. MEIA and ACMIA methods have also been compared to assess tacrolimus whole-blood trough levels [8,9]. As compared with other immunoassays, the ACMIA method does not need any sample pretreatment procedures, thereby resulting in significant decrease in time spent by the technician. ACMIA within- and between–run variation coefficients were acceptable (<10.8%) [9]. Tacrolimus levels determined by both MEIA and ACMIA were not influenced by hematocrit levels [8].

Elevated tacrolimus whole-blood trough concentrations in transplant patients have major implications. As a result of the narrow therapeutic window for tacrolimus, high blood concentrations could result in adverse effects such as renal impairment or central nervous system toxicity, e.g., seizures. When a high tacrolimus whole-blood concentration is observed for no apparent reason, analysis should be repeated on the same sample using different method(s), and the following day using at least two different methods.

Our patient had been recently converted from sirolimus- to tacrolimus-based immunosuppression. Initially, we thought that she overdosed when we were faced with high whole-blood tacrolimus concentrations as assessed by ACMIA. However, because they remained elevated despite discontinuation of tacrolimus, we wondered whether the analytical procedure was at fault. This was actually the case because when tacrolimus whole-blood concentration was measured by EMIT and LC-MS/MS techniques it was found to be nil, compared to the ACMIA result of 24 ng/ml value. As at that time, there was no circulating tacrolimus, a kidney-allograft biopsy ruled out subclinical acute rejection. An extensive workup to discover any potential factors that explained the falsely elevated tacrolimus concentration showed both HIV and HCV serologies to be negative. The autoimmune workup found only anti-double stranded DNA autoantibodies, though these were only slightly elevated (14; $n < 10$).

Recently, Heramida & Tutor reported that a liver-transplant patient who received tacrolimus therapy showed falsely increased blood tacrolimus concentrations after using the ACMIA assay; in this case, this was attributable to endogenous antibodies caused by rheumatoid factors [10]. Although they also showed that estimated whole-blood tacrolimus concentrations could be obtained from washed erythrocyte concentrations this seems difficult to implement in every center.

Borrows *et al.* compared factors linked to whole-blood trough-level bias when using MEIA as compared with HPLC [11]. They found that the time since transplantation, oral antimicrobials, and the recipient's age were independently and positively correlated with the bias, whereas estimated creatinine clearance, hematocrit, serum albumin, infective diarrhea, oral prednisolone, MMF-related diarrhea, and tacrolimus dose were independently and negatively associated with the immunoassay bias [11]. However, this study reported no bias as large as those reported by Heramida & Tutor [10] or in the present case report (where a nil concentration was taken as an elevated one).

We conclude that when high tacrolimus concentrations are observed in transplant patients for no apparent reason when the ACMIA method is used, these should be reassessed immediately using another technique to rule out falsely elevated or even false positive results.

## Authorship

LR, OC, AGJ, NK: took care of the patient. PM, ML, FSM: in charge of the lab data analysis.

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