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Characterization of circulating antibodies with affinity to an epitope used in antibody-conjugated magnetic immunoassays from a case of falsely elevated cyclosporine A

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ABSTRACT

Background: We report a case demonstrating falsely elevated cyclosporine A (CsA) levels by using the antibody-conjugated magnetic immunoassay (ACMIA). To determine the cause of the false positive result, we examined the presence of an interferent in the patient's sample.
Methods: A 26-year-old male patient displayed a high CsA concentration eight weeks after discontinuation of CsA treatment when measured by ACMIA. The possibility of assay interference was examined using serial dilution tests, heterophilic blocking tubes, erythrocyte washing, polyethylene glycol (PEG) protein precipitation, and a Protein A sepharose bead immunoabsorption assay.
Results: The serial dilution test showed nonlinearity, suggesting the presence of an interferent. CsA concentrations in the patient's plasma pre- and post-heterophilic blocking tube treatment were similar. Erythrocyte washing and PEG protein precipitation tests indicated the existence of a protein interferent in the plasma. Moreover, the Protein A bead immunoabsorption assay identified the interferent as antibodies against a unique epitope on the monoclonal antibody-β-galactosidase (mAb-β-gal) enzyme conjugate used in CsA ACMIA.
Conclusions: We identified a circulating antibody against the unique epitope of the CsA ACMIA mAb-β-gal conjugate that induces falsely high CsA concentrations. These findings suggest that clinically aberrant CsA levels require immediate re-measurement by reference methods such as mass spectrometry.

1. Introduction

Cyclosporine A (CsA) is one of the most important immunosuppressive drugs commonly used following organ transplantation. CsA exhibits a narrow therapeutic range and various toxic effects, which are mostly concentration dependent. This drug also demonstrates poor correlation between dosage and blood concentration [1], therefore, therapeutic monitoring of CsA is essential in order to avoid toxic effects and to maintain the therapeutic blood level required for optimum immunosuppression.

The gold standard for measuring CsA concentration is liquid chromatography-tandem mass spectrometry (LC-MS/MS). However, this method requires expensive equipment, considerable time, and experienced staff; therefore, alternative immunoassays are widely employed in many laboratories. Of these, antibody-conjugated magnetic immunoassay (ACMIA, Dimension, Siemens Healthcare) is popular in many clinical laboratories since it does not require a pre-treatment step. Initial studies of the ACMIA method reported good agreement with other immunoassays [2]. However, several limitations have been recently reported for this method. A comparison between ACMIA and LC-MS/MS for CsA demonstrated proportional bias and additional constant bias [3]. In addition, ACMIA is not completely specific for the parent drug and exhibits cross-reactivity towards CsA metabolites [4]. Furthermore, since this method does not include a manual pre-treatment phase, there is no option to eliminate some potential interfering factors in the sample [5].

2. Patient and methods

2.1. Patient history

The patient, a 26-year-old male, underwent kidney transplantation on March 2003 because of focal segmental glomerulosclerosis. The patient continued treatment with immunosuppressants including CellCept® (mycophenolate mofetil, Roche) and Sandimmun Neoral® (CsA, Novartis) for several years. Although the patient was diagnosed with chronic graft rejection in July 2004 by renal biopsy, his subsequent condition was favorable and immunosuppressant treatment was maintained coupled with CsA blood level monitoring using the automated ACMIA method.

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Following years of continual follow-ups and CsA level monitoring, the patient's general state of health was favorable. However, on February 7th, 2015, the patient was admitted to the hospital because of pneumonia. The infection was severe and the patient underwent treatment in the intensive care unit. Therefore, CellCept® treatment was discontinued because of its immunosuppressive effect. However, the patient's condition did not improve and his blood CsA levels were persistently high. (Fig. 1) Consequently, Sandimmun Neoral® treatment was also stopped. Subsequently, the patient began to recover from his pneumonia infection and was discharged on February 27th, 2015.

Although CsA treatment had been discontinued, the blood CsA levels of the patient were persistently high (507–592 ng/ml) until discharge. Because of these unexpected and unusual CsA levels, the patient received two low-dose (25 mg per day) CsA treatments following discharge, on March 7th, 2015 and March 25th, 2015. However, the patient's blood CsA concentration was still high (233.9 ng/ml) on April 10th, 2015, as measured by the ACMIA method. Because of the discrepancy between dosage and blood CsA levels, this result was suspected as being a false positive. Thus, we decided to re-evaluate the patient's sample by using a reference method. In contrast to the results of the ACMIA, the CsA level as measured by LC-MS/MS was found to be below the lower limit of detection (<26.1 ng/ml). From that date, the patient's CsA levels were monitored using both ACMIA and LC-MS/MS and the patient's CsA levels were reported using LC-MS/MS.

Because the patient's CsA blood levels measured by ACMIA were consistently high compared to the prescribed dosage, we hypothesized that this false positive result was probably due to interference. To evaluate this hypothesis, we conducted several experiments using the patient's blood sample, which was collected with informed consent during a regular follow-up visit at the hospital on August 7th, 2015.

2.2. Methods

2.2.1. Serial dilution test

The serial dilution test is a useful and informative assay for detecting inaccuracy and potential interference from endogenous antibodies in specific immunoassays [6]. To determine the existence of an interferent, we first performed a serial dilution test using a control sample with a similar CsA therapy regimen and the zero calibrator from the ACMIA assay kit as the diluent. The presence of a method-specific interferent in the patient's sample would be indicated by non-linear serial dilution test results compared with the control sample.

2.2.2. Erythrocyte washing and polyethylene glycol protein precipitation

CsA levels were measured in both washed erythrocytes and plasma from the patient [7]. The washed erythrocytes were re-suspended in saline equal in volume to the separated plasma in order to obtain the same hematocrit level as in the original whole blood sample. In addition, we measured supernatant CsA levels following polyethylene glycol (PEG) plasma protein precipitation [8]. PEG is relatively specific for immunoglobulin and immunoglobulin complexes. The patient's blood CsA concentration was also measured using an alternative chemiluminescence immunoassay (CMIA, Architect, Abbott Labs).

2.2.3. Evaluation of antibody characteristics

Next, we measured the levels of tacrolimus and sirolimus in the patient's whole blood sample using ACMIA in order to rule out the presence of endogenous β-galactosidase or autoantibodies against the β-galactosidase component of the mAb-β-gal conjugate used in ACMIA [2]. To further exclude the presence of heterophilic antibodies, the patient and two controls samples were incubated for 1 h in heterophilic blocking tubes (HBT, Scantibodies Labs) and the CsA levels were then re-measured by the ACMIA method [9].

2.2.4. Modified immunoabsorption experiment

Finally, a series of modified immunoabsorption experiments [10] using Protein A-sepharose beads were performed to confirm whether the interfering antibody was specific to the mAb-β-gal conjugate used in the CsA ACMIA (Fig. 2). IgG from 200 μl of patient or control (also treated with CsA) plasma was bound to 50 μl of 50% Protein A beads solution (Sigma-Aldrich) during an overnight incubation at room temperature (around 20 to 26 °C). Subsequently, the beads were washed twice with 500 μl of phosphate-buffered saline (PBS) and then incubated with 50 μl (300 μg) of rabbit IgG (Sigma-Aldrich) for 1 h to block the unbound Ig binding sites on the Protein A beads. The beads were washed twice and then incubated with 100 μl of CsA, tacrolimus, or sirolimus mAb-β-gal conjugate reagent, obtained from Siemens RxL Flex cartridges, for 1 h. Following incubation, the beads were washed and then 30 μl of PBS and 10 μl of chlorophenol red β-D-galactopyranoside (CPRG) obtained from Siemens RxL Flex cartridges were added. The absorbance, representing the catalytic reaction, was measured after 10 min at

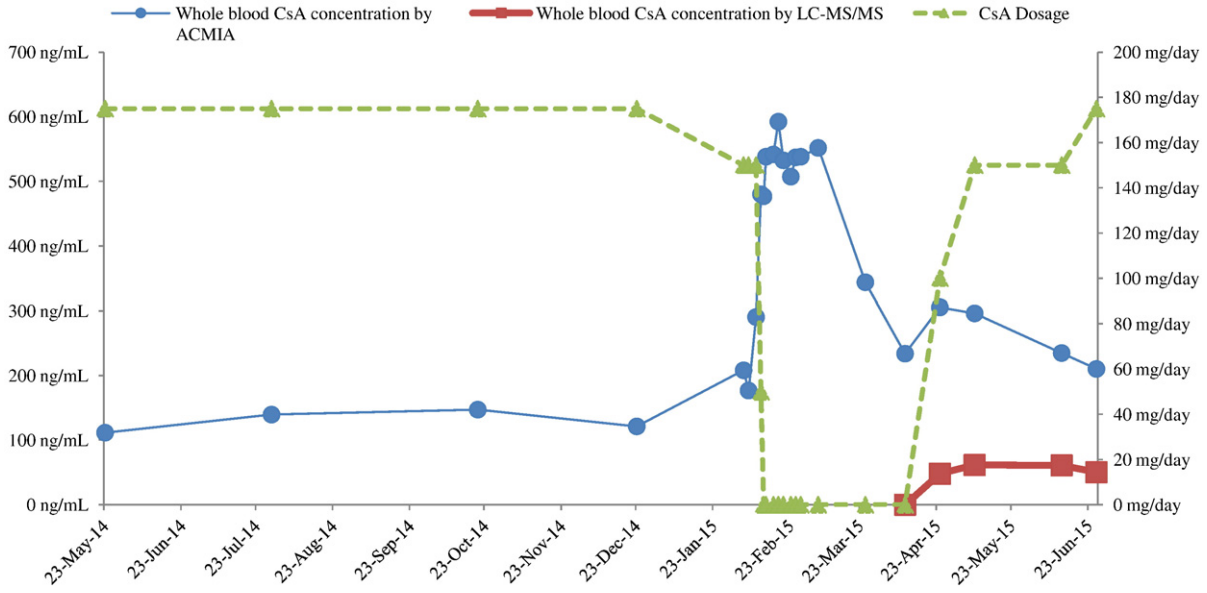

Fig. 1. Whole-blood CsA levels and administrated CsA dosage.