Clinical Efficacy of the Abbott Tacrolimus II Assay for the IMx®

REBECCA D. CANNON1
STEVEN H. Y. WONG1
SUNDARAM HARIHARAN2
MARK B. ADAMS3
CHRISTOPHER P. JOHNSON3
ALAN M. ROZA3
M. LYNN PEARSON1
CINDA L. WERNER4

1Department of Pathology,
2Department of Nephrology,
3Department of Transplant Surgery,
Medical College of Wisconsin,
4Children’s Hospital of Wisconsin,
Milwaukee, WI USA

ABSTRACT

Monitoring tacrolimus is essential to maintain therapeutic concentrations. Performance of the new Abbott Tacrolimus assay (FK II) was evaluated and compared to the original tacrolimus assay (FK I). 189 trough whole blood samples from transplant cases were included in the study. Samples (n = 117) with FK I concentrations > 5 ng/mL were reanalyzed with the FK II assay. Patient samples (n = 43) that had FK I concentration < 5 ng/mL with apparent mean and range of 3.1 ng/mL and 0.7 to 4.5 ng/mL, respectively, were also reanalyzed with FK II to yield a mean of 5.9 ng/mL with a range of 2.9 to 10.8 ng/mL. Checking for patient compliance, samples (n = 10) with a FK I concentration of 0 ng/mL were re-analyzed. With one exception of a mislabeled cyclosporine sample, all samples (n = 9) showed FK506 levels greater than 2 ng/mL with the FK II assay. The FK II assay was shown to be a clinically efficacious assay, with improved sensitivity and acceptable precision versus the previous FK I assay.

Introduction

FK506 (Tacrolimus, Prograf) is a large, cyclic macrolide antibiotic with potent immunosuppressive activity. As the use of FK506 by transplant centers increases, more information is obtained regarding the correlation between dose, response, and adverse effects. Therefore it is necessary to monitor patient FK506 levels to maximize the immunosuppressive activity while minimizing adverse effects.1-3 In 1995, the International Consensus Conference on Immunosuppressive Drugs developed a set of guidelines for the clinical monitoring of FK506.4 Recommendations include: 1) FK506 shows moderate variability in absorption and clearance; thus, the therapeutic drug monitoring of FK506 is recommended; 2) a 12 hour
trough whole blood sample should be used, and the recommended therapeutic range is 5 to 20 ng/mL; 3) EDTA should be used as the anticoagulant in the collection tube, and the samples are stable for one week at room temperature and stable for six months at −4 degrees centigrade; 4) monitoring of FK506 should begin on the second or third day after the initial dose, then biweekly for two weeks; 5) and a laboratory quality control program is essential.

Previously, the two primary methods available to the clinical laboratory for the monitoring of FK506 are the microparticle enzyme immunoassay (MEIA) for the IMx® by Abbott Diagnostics and the enzyme linked immunosorbent assay (ELISA) by IncStar Corporation.5–7 Both assays use the same monoclonal antibody; however, the linearity and turnaround time differed significantly. The IncStar ELISA method (ProTrac®) has a linearity of 0.5 to 40 ng/mL with a turnaround time of 4 hours for 24 samples. The Abbott MEIA method (the original “Tacrolimus”, or FK I assay) had a linearity of 5 to 60 ng/mL with a turnaround time of 35 minutes for 24 patient samples. Recently, Abbott Diagnostics had developed a second generation MEIA assay (the “Tacrolimus II”, or FK II assay) for the therapeutic monitoring of FK506 by the IMx.8 The new MEIA method has a linearity of 1.5 to 30 ng/mL with the same turnaround time of 35 minutes for 24 samples. The correlation between FK I and FK II seems to be site-dependent.9 Our clinical toxicology and therapeutic drug monitoring lab initially chose the Abbott Tacrolimus assay (FK I) due to its quick turnaround time and ease of operation—a whole blood precipitating step followed by a fully automated assay. However, the assay was not as sensitive as the IncStar method. The new generation Tacrolimus II assay (FK II) has an increased sensitivity with the linearity of 1.5 to 30 ng/mL, comparable to the ELISA assay. This study was performed to test the clinical efficacy of the new Abbott FK II assay versus the original FK I assay used in the clinical laboratory.

Materials and Methods

A clinical laboratory study comparing the performance of the FK II assay to the FK I assay was conducted on 189 whole blood random samples of solid organ transplant recipients. The FK I values for this study were determined by the clinical laboratory personnel, then the samples were stored at −20°C to be re-analyzed with the FK II assay. The results of this study were then divided into three groups: The first group (n = 117) was selected to demonstrate the overall linear correlation between the FK II assay versus the FK I assay; the second group consisted of a set of patient samples (n = 43) with FK concentrations below the assay range of the FK I assay, and the samples were reanalyzed with the new, more sensitive FK II assay. The final group consisted of patient samples (n = 10) with FK I assay values less than 0.2 ng/mL, possibly indicative of noncompliance.

Results

Figure 1 shows the correlation between the FK II and the FK I assays for n = 117. Samples with FK I values less than 5 ng/mL (the assay cutoff) were not included in the analysis. The correlation was found to be FK II = 0.95*FK I + 1.62 with a correlation coefficient of r = 0.93. The overall sample distribution, which includes patient samples below 5 ng/mL with the FK I assay, is shown in table I. The therapeutic range in table I is proposed by the Consensus Document.4 Sixty percent of the samples yielded FK506 concentrations below the therapeutic range with the FK I assay were within therapeutic range with the FK II assay. There was no change in the distribution of samples within and above therapeutic range.

To further investigate the trend found in samples below 5 ng/mL, the FK I assay, 43 additional samples less than 5 ng/mL were re-evaluated with the FK II assay (group 2). Results are also shown in table I. Sixty-seven percent (n = 29) of the less than 5 ng/mL
samples were determined to be within the therapeutic range with the FK II assay. The mean concentration and range were 5.9 ng/mL, and 2.9 to 10.8 ng/mL respectively. The lowest value reported from the 43 samples with the FK II assay was 2.9 ng/mL, which was above the FK II assay range and therefore reportable, unlike the FK I assay.

Ten suspected noncompliant patients (less than 0.2 ng/mL with the FK I assay) were reanalyzed with the FK II assay. Nine of the 10 samples were above the minimum of the assay range of FK II. One sample showed a concentration of 0.0 ng/mL with the FK II assay. Upon further investigation, this was a mislabeled cyclosporine whole blood sample.

Further, the clinical efficacy of the new FK II assay was demonstrated by the case studies of the following patients from the Medical College of Wisconsin/Froedtert Memorial Lutheran Hospital Department of Transplant Surgery:

**Case 1-example of low FK506 blood concentration.** Patient was a 35 yr. old African-American male with stable renal function after transplantation. On routine follow-up, the patient was maintained with FK506 concentrations (measured with the FK II assay) between 4–6 ng/mL. The first generation assay, FK I, would not be able to measure this patient’s FK506 whole blood concentrations (FK I assay limit of 5 ng/mL).

**Case 2-example of low FK506 concentrations due to toxicity.** Patient was a 46 yr. old Caucasian male renal transplant recipient with chronic rejection. This patient was kept on low dose of FK506 to limit the nephrotoxic effects of FK506. Thus, this
patient’s FK II concentrations were generally below 5 ng/mL. In this case, monitoring the FK506 blood levels with the FK I assay was not possible.

Case 3-example of non-compliance. Patient was a 42 yr. old African-American female renal transplant recipient. On routine follow-up, the patient’s FK506 blood concentrations lowered to less than 1.5 ng/mL (the detection limit of the FK II assay). Upon investigation, it was discovered the patient was non-compliant.

In conclusion, the new generation Tacrolimus assay by Abbott Diagnostics, FK II or Tacrolimus II assay, is fast and easy to use with improved sensitivity and precision compared to the original Tacrolimus assay. The FK II assay is superior to the FK I assay in managing transplant recipients with low but clinical efficacious FK506 concentrations.

References


