Successful Implementation of Immunosuppressant Drugs (ISDs) Monitoring Using Liquid Chromatography Mass Spectrometry (LC-MS/MS)

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Background: Due to the narrow therapeutic index of immunosuppressant drugs (ISDs), therapeutic drug monitoring is required to prevent transplant rejection due to inadequate dosage, as well as to minimize toxic side effects due to excessive dosage [1]. Tacrolimus, CyclosporineA and Sirolimus have previously been monitored by chemiluminescent microparticle immunoassay (CMIA) at Children's Hospital Los Angeles. Liquid chromatography-mass spectrometry (LC-MS/MS) has now become the preferred analytical method for therapeutic monitoring of ISDs due to its low cost, improved sensitivity and selectivity over immunoassay-based methods. It can also quantitate all four ISDs simultaneously with a single analytical run including Everolimus. We hereby report our experience of implementing ISDs using LC-MS/MS, which now replaces the previous immunoassay technique.

Methods: A Thermo-PRELUDE-TSQ-QUANTIVA LC-MS/MS was purchased and a quantitative MRM (Multiple Reaction Monitoring) analytical method was validated utilizing the positive ion mode to quantitate Tacrolimus (m/z 821.5/768.5), Cyclosporine (m/z 1202.8/425.3), Sirolimus (m/z 931.6/864.5) and Everolimus (m/z 975.7/908.5) using Tacrolimus-13C, D2 (m/z 824.4/771.0), Cyclosporine-D12 (m/z 1214.9/437.4), and Sirolimus-D3 (m/z 934.5/867.6) as

internal standards. The chromatography is carried out by a gradient program through an analytical column (Accucore C_8, 2.6 um, 30 X 3 mm) protected with a Turbo Flow column (Cyclone P. 0.5 X 50 mm) at 70°C for 4 minutes. 50 µL of EDTA whole blood was lysed with 75 µL of a 0.1 M $ZnSO_4$ solution in a 1.5 mL centrifuge tube. Samples were vortexed at the highest speed on a multi tube vortexer (FisherScientific, Waltham, USA) for 2 minutes. Protein was precipitated with 125 µL of methanol with internal standards. Samples were vortexed again at the highest speed on a multi tube vortexer before centrifugation at 8,000 RCF for 10 minutes to remove precipitated protein. 20µL of clean supernatant was injected for LC-MS/MS analysis.

Results: The Recoveries for all four ISDs were between 88% and 113%. The intra-assay CVs for all four ISDs ranged from 1.8% to 7.6%. The inter-assay CVs for all four ISDs ranged from 4.1% to 13.7%. Accuracy was evaluated by analyzing CAP proficiency testing samples; comparing with other facilities using same LC-MS/MS methodology; also comparing with CHLA immunoassay on real patients' samples. For Sirolimus, a constant bias was observed between CHLA LC-MS/MS and immunoassay. However, the agreement between CHLA and another facility using same LC/MS-MS methodology was excellent with a correlation coefficient R=0.9870. Analytical sensitivity (LOQ) were 0.51 ng/ml for Tacrolimus, 14.53 ng/ml for Cyclosporine A, 0.60 ng/ml for Sirolimus and 0.75 ng/ml for Everolimus.

Conclusion:A very simple, fast, sensitive, LC-MS/MS method was successfully implemented at CHLA for simultaneous quantitation of ISDs replacing immunoassays in three months, which resulted in better optimization of therapeutic drug levels, decreased sample volumes, and significant cost savings.

References:

Christians U, Klawitter J, Clavijo CF. Bioequivalence testing of immunosuppressants: concepts and misconceptions. *Kidney Int Suppl*. **2010** (115):S1-7.