Analytical methods of Measurement for Immunosuppressive Drug Concentrations

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Scope of the lecture:
The lecture presents state-of-the-art and the guidelines for proper analytical performance of measurement procedures for TDM of immunosuppressive drugs on the basis of recommendations of the IATDMCT Immunosuppressive Drug Scientific Committee (*Ther. Drug Monit. 2016; 38, 170-189.*)

Learning objectives:
1. The identification of factors responsible for reliability of the analytical method.
2. The list of crucial parameters to be included in method validation.
3. Pros and cons of choosing chromatography and immunoassay.

Extended abstract:
Therapeutic drug monitoring (TDM) plays an invaluable role for individualization of immunosuppressive therapy. The treatment is based on simultaneous administration of drugs with complementary mode of action. The choice of a specific scheme of treatment depends on transplant organ, patient's characteristics and conditions related to transplant centre. Primary immunosuppressive drugs (ISDs): cyclosporine (CSA), tacrolimus (TAC), everolimus (EVE) and sirolimus (SIR) require whole blood monitoring, whereas mycophenolic acid (MPA) needs to be determined in plasma. Availability of reliable analytical method is the basis for successful TDM. The value of analytical tool depends on quality of three factors: properly validated and maintained analytical procedure, properly used and qualified analytical equipment and finally, properly educated and trained TDM laboratory personnel.

The starting point for implementing TDM services for ISDs is to identify clinical expectations and to match it with laboratory potential and ability. The creation of analytical goals allows to select the best methodology for individual purposes and to choose between chromatography and immunoassays. The following step is method development, then method validation and/or verification. „A full method validation should be performed for any analytical method whether new or based upon literature." Reasonable definition of appropriate acceptance criteria ensures that validation will justify clinical application of the method. When the assay is implemented to TDM practice, the phase of permanent quality control commences. As the ISDs are used in life-long treatment of most transplant patients thus the method consistency over time for long-term clinical usage must be guaranteed.

There is no official guideline dedicated for validation of analytical method used for TDM purposes. The preparation of a proper validation plan is in the hands of individuals responsible for method implementation. Validation parameters are usually well-known and described in particular guidelines, however, we can identify those especially important for TDM: the range with lower limit of quantification (LLOQ), specificity, precision, accuracy and stability tests.

The range should cover concentration of ISD expected in patient sample. The concentration level reflects ISD dose administered, and multi-drug therapeutic scheme, although it is also to consider TDM strategy applied (i.e. predose concentration, Cmax/C2

monitoring, AUC monitoring). Considering the range, LLOQ parameter is particularly important to ensure a reliable quantification of low ISD concentrations and to detect inappropriate low dosing or patient noncompliance. Therefore, "analyte quantification should be at least one-third to half of the lower limit of the target concentration window" (therapeutic range). For example, an LLOQ close to 1 ng/mL should be achieved for TAC, SIR, EVE determinations to allow meaningful TDM at 2-3 ng/mL drug concentrations; similarly: LLOQ at 20 ng/mL for CSA and at 0.2 μg/mL for MPA will be accepted. Certainly, imprecision and inaccuracy ≤20% must be demonstrated for LLOQ concentration. On the other hand, dilution protocol should be validated in case of incidental measures of samples with ISD concentration at a toxic level exceeding upper limit of the range.

Analytical range is described by satisfactory accuracy and precision. In general, for ISDs a CV ≤10% or even ≤6% should be achieved for between-day imprecision. The limits are the result of total analytical error estimate calculation presented in detail in the IATDMCT Recommendations (*Ther. Drug Monit. 2016; 38, 170-189.*). This approach is highly more restrictive than CV ≤15% presented in the EMEA and FDA guidelines, which are however not intended for TDM routine measurements. The accuracy is usually defined as the closeness of agreement between a single result and the true concentration of the drug. The reader needs to distinguish between analytical accuracy in itself validating the method of measurement and a bias which is a result of a difference between two methods. Intralaboratory accuracy and precision is in the hands of individual laboratory while interlaboratory bias strongly depends on reference materials and procedures.

For ISD there are numerous different (mainly ligand-binding) assays available on the market. Some of them produce different results due to cross-reactivity or improper calibration, what may lead to wrong decisions in dose adjustment and to questioning the trust in analytical methods in general. EVE QMS immunoassay may serve as an example - its calibration strategy was based on value-assigned calibrators and QC samples, probably to compensate for cross-reactivity with EVE metabolites. It is therefore advisable for laboratory management to evaluate the performance of incoming immunoassay using chromatography as a reference method, working on real patient samples before releasing the assay for its clinical application. Generally, method comparison is presented by means of Passing-Bablok or Deming regression as well as using Bland-Altman plots for bias, all supported by proper statistical analyses. Unfortunately, LC-MS/MS should not be taken unquestioningly as a synonym for reference because individual LC-MS/MS methods may also produce sizable bias for ISD determinations. It is naturally related to the differences in individual analytical procedure but also to a lack of certified worldwide available reference materials (except TAC). Despite this, it is generally well recognized that a properly validated chromatographic method is superior to immunoassay because of its specificity. Other parameters like stability, carryover and technique-related interfering factors should also be evaluated with care.

Currently, methods for measuring ISDs are divided into chromatography and automated immunoassays. The choice of analytical technique is very dependent on resources available and on the number of samples to be analyzed by the laboratory. The reference chromatographic techniques are preferred at large TDM centers employing highly educated and trained analysts, often located at academic hospitals. Small, local or transplant oriented medical laboratories rather prefer ready-to use, automated immunoassays easily offered by analytical companies. So far, medical diagnostic laboratories in many countries did not benefit enough from new chromatographic tools, especially LC-MS/MS. In spite of shortening turnaround time, lowering reagents consumption and decreasing cost of LC-MS/MS equipment, a common opinion that such technique is too sophisticated for routine analyses still exists. In summary, there is currently a place for both: chromatography and immunoassays, although the information on analytical method used should be obligatorily

included in each ISD TDM analytical report.

Since a method is working for TDM of ISDs, a continuous supervision needs to be established in order to guarantee the method performance and reliability of the measurements. This procedure includes the use of certified reference materials for calibration, control samples of reliable quality, participation in recognized proficiency testing schemes simultaneously with well-maintained analytical equipment. Education and training should be a routine procedure addressed not only for laboratory staff but also for clinical personnel. The understanding cooperation is absolutely essential for successful TDM. Even perfect analytical work becomes useless following pre-analytical errors caused by incorrect sampling time or improper sample obtaining and handling.