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Therapeutic Drug Monitoring (TDM) in Renal Transplant (RT): Reasons to Choose the LC-MS/MS Method

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Pick an Issue, A Problem, A Question

The role of TDM in the setting of renal transplantation

Over the past three decades, renal transplantation has become established as the treatment of choice for many patients with end-stage renal failure, the only alternative being dialysis. The establishment of transplantation has been made possible by the introduction of immunosuppressants. People who undergo renal transplantation are required to receive life-long (or at least, long-term) treatment with immunosuppressive drugs. When selecting these treatments, the risk of immunologically mediated graft failure for any donor–recipient pair needs to be balanced against the drug's side effects for the recipient. The ultimate aim of treatment is to prolong patient and graft survival.

Survival of renal organ transplant recipients has improved greatly over the years, particularly since the introduction of new powerful drugs. However, despite greatly improved early results, the rate of late allograft loss remains relatively constant [1]. Pathologic changes in kidneys during late allograft dysfunction are the result of a constellation of immunologic and non-immunologic factors involved in the development and progression of histological injury [2].

The immunosuppression is the cornerstone of the therapeutic treatment of patient who underwent a renal transplant. Cyclosporine, tacrolimus, sirolimus, everolimus and other drugs are successfully applied in kidney transplantation, but their narrow therapeutic index requires caution regarding the dosages of these immunosuppressants. Critical dose drugs are defined by a narrow therapeutic window in which elevated concentrations can cause significant toxicity or under dosing can result in serious consequences from ineffective treatment: a too low immunosuppression carries a high risk of rejection, although with minimal adverse effects; on the other hand, high blood levels are responsible for nephrotoxicity, a particular complication of some immunosuppressive regimens, notably the calcineurin inhibitors, which may increase the risk of chronic graft dysfunction; elevated risk of infections and cancer, especially lympho proliferative disorders.

In this setting, therapeutic drug monitoring (TDM) plays an important role in the optimal use of immuno suppressants in patients carrying a renal transplant [3]. There are many important clinical problems involving the use of TDM: first of all, these drugs show a great degree of interindividual and intraindividual pharmacokinetic and pharmacodynamic variability; moreover, the therapeutic ranges of the different immunosuppressants are also dependent on the period after transplantation [4,5]. The variable pharmacokinetics of immunosuppressants within and between patients as a result of variations in absorption, distribution and/or elimination makes it impossible to reliably predict the best dose for each patient [6]. The resulting variability in trough blood concentration can reach up to 50% [6]. Thus, frequently patient blood levels are complex and unpredictable [5].

Moreover, there is no consensus about the choice of the best pharmacokinetic marker: currently, used markers are area under the curve monitoring, C2 (2 hours after administration), and through blood levels. Which one of the TDM approaches produces the optimal clinical outcome is still under debate.

In the last years the number of organ transplantations is increased; in addition, immuno suppressive drugs are also used for other diseases, such as steroid-resistant nephritic syndrome, psoriasis, and other autoimmune disorders.

Pose One or More Possible Answers

How to perform TDM ?

As with other monitored drugs, the clinical laboratory has two main choices in technologies: immunoassay or chromatography based methods.

Currently, several analytical methods have been developed for the determination of immunosuppressive drugs, among which the following immunoassays (IAs): fluorescence polarization immunoassay (FPIA), microparticle enzyme immunoassay (MEIA), enzyme multiplied immunoassay, radioimmunoassay, enzyme-linked immunosorbent assay and high-performance liquid chromatography (HPLC)-UV and HPLC and tandem mass spectrometry (HPLC-MS/MS) methods [7]. Recently, also liquid chromatography and tandem mass spectrometry (LC-MS/MS) method with the use of a single LC-MS/MS system, has been proposed [8].

Nowadays, TDM is mainly based on immunoassays, which are suited to a routine laboratory with excellent automation and high throughput. However, there are some well known major drawbacks of this technology, such as the frequent lack of specificity for the parent drug, as, for example in the major and variable overestimation by immunoassay of the immunosuppressant drugs, tacrolimus and cyclosporine [9,10]; non-specific binding of the antibody resulting in overestimation with immunoassays [11-13], and the confounding effect of hematocrit level [14] have also been well documented with immuno suppressant. Moreover, the analysis costs of IA techniques are

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relatively high (eg, for tacrolimus and everolimus). The lower limit of quantification (LLOQ) of the IA techniques is often not low enough, especially for tacrolimus in case of liver transplantation in young children [8]. It has been demonstrated an important cross-reactivity between drugs, such as with everolimus and sirolimus [15], thus for patients who switch from sirolimus to everolimus medication, the selectivity of the IA technique is insufficient. IA techniques have been shown to be inaccurate in the case of Cyclosporin and its metabolites because of a significant cross-reactivity with this technique [16].

Chromatographic based methods represent an alternative to immunoassays [17]. In general, these techniques have higher selectivity than antibody-based methods and allow a simultaneous analysis because of their common features. In fact, they are soluble in organic solvents like alcohols, acetonitrile and they are practically insoluble in water. They all, except for mycofenolic acid (MPA) , can be measured in whole blood due to high distribution in erythrocytes, between 40–60% for cyclosporine A (CsA), about 95% for sirolimus (SIR), 95–98% for tacrolimus (TAC) and 75% for everolimus [18].

Gas chromatographic methods have been used for therapeutic drug monitoring, but the limitation of this technique is that the drug must be volatile to be measured. Derivatization of the analyte may be required to increase volatility and these additional steps add complexity to the method. Liquid chromatography with ultra-violet detection (LC-UV) is another chromatographic technique that has been used for therapeutic drug monitoring. However, LC-UV often requires extensive sample preparation due to the limited specificity of the detection mode and the poor ultra-violet absorbance of some compounds. Moreover, LC-UV can be used for the measurement of cyclosporin, sirolimus, everolimus and mycophenolic acid [19], but not tacrolimus, because of the lack of chromophores and low circulating concentrations. While it is relatively easy to measure mycophenolic acid by LC-UV (due to the high circulating concentrations), the other drugs require extensive sample cleanup and typically long chromatographic analysis time. Thus this approach is not ideal for routine TDM.

Nowadays, the technique of choice may be liquid chromatography with tandem mass spectrometry (LC-MS/MS) because of its selectivity, sensitivity, and flexibility [7].

Mass spectrometry is already well established as a quantitative tool for small molecules, and is based on producing, differentiating and detecting ions in the gas phase. Conversion of dissolved analyte eluting from a separation system into gas-phase ions occurs in the ion source and is generally associated with evaporation, pressure reduction and an ionization process.

Several LC-MS/MS methods have been described so far, although most of them require online extraction procedures [20-23].

Weight the Evidence Supporting Possible Answers

LC-MS/MS as the best choice for immunosuppressants TDM

In the past few years, high-pressure liquid chromatography with mass spectrometry (HPLC-MS) has been popularly utilized in drug quantitation and pharmacokinetics studies and now is considered to be the gold standard analytical method in TDM [24]. The main attraction of HPLC-MS is high selectivity and sensitivity because this technique allows the quantification of the main drug independently of its metabolites. Very frequently, the immunosuppressive agents are used in combined regimens; in these cases HPLC-ESI- mass spectrometry is the best option for simultaneous analysis of several compounds in one short run. All HPLC-MS methods need less laborious sample preparation when compared with HPLC with UV detection. All these advantages of HPLC-MS methods can shorten total cycle time and save reagent usage.

Given the analytical advantages this technique has over other methods, it is not surprising that LC-MS/MS is now used in a wide variety of TDM settings [17].

LC–MS/MS offers the simultaneous measurement and detection of CsA, tacrolimus, sirolimus, and everolimus. Various LC–MS/(MS) methods have been published for the pre- and post-dosage TDM of these immunosuppressants in daily clinical routine, referring both single analyte and simultaneous measurement of these drugs [8,25-27]. Simultaneous whole blood measurement of calcineurin and mTor inhibitors can be performed since these drugs have similar physicochemical properties, are neutral and ionize in a similar manner and are highly bound to red blood cells.

Methods for the measurement of mycophenolic acid (MPA) and its glucuronide metabolite (MPAG) are generally based on HPLC–UV or immunological methods [28]. Very recently, LC–MS/(MS) methods for the determination of MPA and MPAG were also described [29]. The measurement of MPA in plasma by LC-MS/MS is easier, as its circulating concentrations are in the milligram per litre concentration range. Nowadays, it is possible to perform with an unique LC–MS/MS platform the measurement of all immunosuppressants (CsA, tacrolimus, sirolimus, everolimus, and MPA) in one analytical mode [30]. MPA is determined from EDTA–plasma using the same sample pretreatment protocol (sample volume, precipitation and centrifugation) and analytical setup (on-line SPE and HPLC–MS/MS), which are used for the other immunosuppressants. The method reported by Ceglarek et al. [30] is rapid and reproducible, needing only 50 µl EDTA–plasma. A rapid HPLC step (5 min) is used for the separation of MPA and MPAG to prevent interferences of insource fragmentation.

Assess Counter-Evidence and Conclude With An Answer

With improvements in instrumentation, particularly faster scan rates, simultaneous high-throughput analysis of cyclosporin, tacrolimus, sirolimus and everolimus is becoming standard practice. Liquid chromatography with mass spectrometry detection is a major breakthrough in therapeutic drug monitoring of immunosuppressive agents and is considered as the method of choice in TDM of immunosuppressants. Despite the initial high cost for the instrumentation, HPLC-MS is more cost effective than microparticle enzyme immunoassay, see the case of tacrolimus [31].

The main important features of LC-MS/MS methodology for immunosuppressive drugs are the shortened analysis time, an increased throughput, higher selectivity and low cost of analysis [24].

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