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Summary of doctoral dissertation prepared based on scientific articles series

Therapeutic drug monitoring of tacrolimus and mycophenolic acid using volumetric absorptive microsampling coupled with LC-MS/MS technique in pediatric renal transplant recipients

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Transplantation is considered the best option for renal replacement therapy, particularly for end-stage renal failure. In addition to difficulties arising from organ availability and surgical or immunological aspects, tools are still being sought to optimize the best immunosuppressive therapy necessary for the rest of the patient's life. Particular difficulties are observed in pediatric patients, where non-adherence to therapeutic recommendations, mainly concerning medication intake, is the cause of more than 70% of rejection episodes in the first year after transplantation.

The cornerstone of immunosuppressive treatment is tacrolimus (TAC), a macrolide antibiotic with a narrow range of therapeutic concentrations. Another problem is the high variability in individual response to the drug (intraindividual variability) and the difficulty in establishing appropriate population algorithms for matching drug doses (interindividual variability). This means that the drug dose must be adjusted individually for the patient based on therapeutic drug monitoring (TDM). TAC binds almost entirely to erythrocytes; therefore, in routine clinical practice, a threshold whole-blood concentration of the drug is quantified, that is, the TAC concentration before the daily dose of the drug (C_0), which should be within the therapeutic range of 5-20 ng/ml.

Another drug equally often used in a therapeutic immunosuppressive regimen is the inosine monophosphate dehydrogenase (IMPDH) inhibitor- mycophenolate mofetil (MMF), which is a prodrug of mycophenolic acid (MPA). Monitoring of the concentration of this drug is not performed in some transplant centers but is recommended because of the need to avoid adverse effects (bone marrow or gastrointestinal toxicity). Although the pharmacokinetics of MPA are assumed to be linear, the high intra- and inter-individual variability argue in favor of

determining the concentration of this drug. MPA binds extensively to plasma proteins, making this blood fraction the recommended biological matrix to determine C_0 concentrations, which are assumed to be 1-3,5 μg/ml.

As TDM is based on frequent blood sampling, an alternative method of obtaining biological material is being sought, especially for children for whom the classical collection is challenging and painful. The recently introduced volumetric-absorptive microsampling (VAMS) technique allows a defined, relatively small volume of blood to be collected (10 – 30 μl) after puncturing the finger with a lancet, similar to standard blood glucose measurement. After drying and using appropriate extraction reagents, the collected samples were subjected to an appropriate analytical process, usually based on high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Additionally, the VAMS technique is considered to be independent of individual hematocrit levels, in contrast to the more popular dried blood spot (DBS) technique.

Given the specificity of administering immunosuppressive therapy to pediatric renal transplant recipients, the overarching aim of this dissertation was to develop LC-MS/MS analytical methods for determining TAC and MPA in capillary blood samples collected by VAMS in the pediatric renal transplant population.

This main aim is supported by the specific objectives: optimization of the collection strategy for VAMS in children, selection of conditions related to sample preparation for LC-MS/MS determination, optimization of chromatographic and MS detector conditions, validation of LC-MS/MS analytical methods for the determination of TAC in whole blood and VAMS, and MPA in whole blood, plasma, and VAMS, following the European Medicines Agency (EMA) international guidelines. In addition, this dissertation aimed to study the stability of VAMS samplers under different storage conditions to evaluate potential adverse hematocrit effects, cross-validation, and clinical validation with routinely used laboratory diagnostics methods.

The planned LC-MS/MS analytical methods were successfully validated within the calibration ranges for TAC (0-60 ng/ml) and MPA (0-15 μg/ml). The validation parameters met the acceptance criteria of the EMA and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDM&CT) guidelines. For the validation of the VAMS-based methods, no effect of hematocrit level on analyte recovery from the biological matrix was observed. The developed methods determined TAC and MPA concentrations in samples from pediatric renal transplant patients (n=50). The described validation protocols were cross-validated, proving the equivalence of the whole blood and VAMS-based methods

for TAC and MPA. In addition, owing to differences in plasma and VAMS sample concentrations, a concentration-correction model based on statistical regression was validated, allowing the resulting MPA concentration determined in the VAMS samples to be converted to the predicted plasma concentration. This is extremely important because the current therapeutic range characterizes the plasma MPA concentrations.

This model was further validated on a group of independent VAMS samples, resulting in the first correlation formula described in the literature that meets the stringent acceptance criteria formulated by IATDM&CT.

The dissertation presented here is based on a monothematic series of three publications, including two experimental papers, which comprehensively describe the LC-MS/MS methods developed for determining TAC and MPA, their analytical, cross-validation, and clinical validation. The developed analytical protocols have been successfully applied to the therapeutic monitoring of immunosuppressive drugs in pediatric renal transplant patients treated at the Children Memorial Health Institute (CMHI) in Warsaw.

The VAMS technique offers new possibilities for TDM, and not only for immunosuppressive drugs. Both the manufacturer's recommendations and the results presented in this dissertation testify to the long-term stability of the collected samples under various conditions, allowing for self-sampling by the patients or their caregivers at home without qualified medical personnel. Once properly secured, the collected sample can be transferred to a diagnostic laboratory, and the attending physician can communicate the obtained results and therapeutic recommendations via communication and information systems.

The VAMS technique can be used as a motivational strategy for young patients regarding therapeutic adherence, enabling more frequent monitoring of the C_0 parameter of immunosuppressive drugs, whose fluctuations are a direct consequence of the non-adherence phenomenon. In addition, the use of VAMS in daily practice seems attractive because of recent limitations in access to health care during the SARS-CoV-2 pandemic, as well as the logistical difficulties faced by patients and their families (the only pediatric transplant center in Poland is the CMHI).

To the best of our knowledge, the application of the VAMS technique to monitor immunosuppressive drugs in the pediatric population described in this paper is one of the first described in the literature. Hopefully, this will begin the widespread optimization of pharmacotherapy and TDM based on microsampling techniques.