DEVELOPMENT AND VALIDATION OF NEW LC-MS/MS METHOD FOR DETERMINATION OF UNBOUND TACROLIMUS IN PLASMA IN CYP 3A5 EXPRESSORS AND NON-EXPRESSORS

Organ transplantation is the best and often the only method of renal and hepatic failure treatment. After transplantation the treatment with multidrug immunosuppression is necessary. This prevents rejection of the transplant but also reduces the function of patient's immune system. Currently, the primary drug in this group is tacrolimus with effectiveness and safety confirmed in numerous reviews. Since the introduction of tacrolimus, the percentage of acute organ rejection episodes has been reduced from 50-60% to 10-20% in transplant recipients. However, after thirty years of clinical experience, tacrolimus therapy remains associated with a high risk of complications.

Tacrolimus is characterized by a narrow therapeutic window, as well as high inter- and intra-individual variability in pharmacokinetics. This means that even small differences in drug concentrations in the blood can significantly change its effect in patients, which caused by many factors, including genetics (expression of the CYP3A5 enzyme metabolizing tacrolimus). Therefore, the whole blood tacrolimus concentration needs to be monitored with the target range between 4 and 12 ng/mL. Both under- and overexposure may lead to severe adverse effects, such as acute rejection of allogenic organ, nephrotoxicity, neurotoxicity, hypertension, diabetes mellitus, infections, malignancies and cardio-vascular incidences. Occurrence of these complications significantly increase morbidity and mortality rate in the population of organ recipients.

Currently, the concentration of tacrolimus is monitored in whole blood by taking a sample before administration of the drug (concentration C0). Based on this measurement, doctors modify the dose of tacrolimus to prevent adverse effects of the drug. However, numerous reports indicate poor concentration C0-effect on complication possibility. As there are over 15.000 patients in Poland on continuous tacrolimus therapy, the identification of new, more sensitive monitoring methods is of utmost importance.

The aim of this project is to evaluate clinical usefulness of different therapeutic drug monitoring protocols in patients after kidney and liver transplantation, which include measurements of C0 in the whole blood and free tacrolimus C0 in plasma ultrafiltrate. It is believed that only protein-unbound drug is the pharmacologically active compound and can better represent therapeutic drug effectiveness. Therefore, we suspect that free tacrolimus level will be better correlated with acute rejection episodes and adverse tacrolimus-related effects than C0 measurement. We expect to find a mathematical relationship between C0, albumin concentration and hematocrit level to calculate free fraction of tacrolimus. Nexera Liquid Chromatography System with LCMS-8050 triple quadrupole MS will be used for sample analysis.

Additional aim of the study is to address CYP3A5 expression in each patient. Presence of this enzyme results in faster drug metabolism and lower tacrolimus C0 in whole blood. In this project, better correlation of free tacrolimus and CYP3A5 expression than C0 is assumed. This would give a stronger rationale for genotyping in drug dose adjustment. Genotyping will be performed with the use of QuantStudioTM 12 K Flex Real-Time PCR.

The project will significantly contribute to understanding tacrolimus pharmacokinetics and body response to drug exposure. The proposed project is the first attempt to integrate both different therapeutic drug monitoring measurement methods (whole blood C0 and plasma ultrafiltrate free TAC) and patient genetics in a prospective study with the assessment of the tacrolimus-related adverse effects. We anticipate providing an argument for implementation of even more personalized immunosuppressive therapy, which means individual dosage adjustment even before the transplantation. That may result in improvement of overall patient and graft survival.