Volumetric-absorptive microsampling as novel approach to tacrolimus concentration measurement in pediatric population during immunosuppressive therapy

Arkadiusz Kocur[1*], Dorota Marszałek[1], Tomasz Pawiński[1], Jacek Rubik[2], Agnieszka Czajkowska[3]

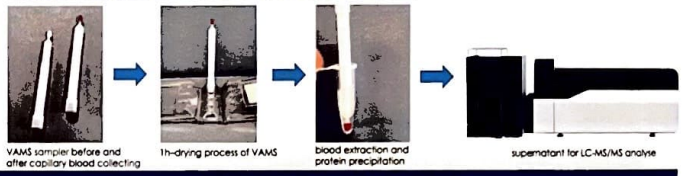
[1)] Department of Drug Chemistry, Medical University of Warsaw, Poland
[2)] Department of Nephrology, Kidney Transplantation and Arterial Hypertension, The Children's Memorial Health Institute, Warsaw, Poland
[3)] Department of Biochemistry, Radioimmunology and Experimental Medicine, Pharmacokinetics Laboratory, The Children's Memorial Health Institute, Warsaw, Poland

INTRODUCTION

- **Non-compliance with physician guidelines and therapeutic regimen is the most difficult problem during immunosuppressive therapy** in pediatric population after transplantation (estimated causes up to **70% graft rejections after kidney transplantation** (KTX)).
- **Tacrolimus (TAC) is widely used in post-transplant therapeutic schemes**, also in the pediatric population. Monitoring of blood levels is required due to the narrow therapeutic index of TAC. In routine clinical practice, a few milliliters of the blood are collected to check the concentration level of the TAC, which is not comfortable and less ethical for young patients. For that reason, the Mitra-VAMS® technology (Neoteryx®, Torrance, CA, USA) seems to be a better alternative to the classic sampling. **In our research, the method of TAC concentration measurement in samples collected by VAMS® was developed and the results were compared with reference method.**
- **Mitra devices are developed for analyses even in the least equipped laboratory- extraction with simply solvent, there is no need for centrifuge, transfer, or freezing.** Stability of Mitra after blood collecting is estimated to up to 1 month (we confirmed that in our previously study presented on Accord Conference in Warsaw, May 2022). After extraction, the Mitra® devices may be easily common with classic LC-MS/MS analyses.

METHODS

- LC/MS/MS analyses were performed by Nexera®-LC system coupled with **Shimadzu-MS-8050** (Shimadzu®, Tokyo, Japan)
- Stationary phase: **ThermoFisher column HyPurity C_{18}** (50x2,1mm; 50µm).
- Mobile phase: **A (methanol : acetonitrile, 50:50 (v/v)) with ammonium fluoride and acetic acid; B (water) with ammonium fluoride and acetic acid.**
- Flow rate – **0,75 mL/min** (gradient)
- Internal standard: **ascomycin** (ASC)
- Multiple Reaction Monitoring (MRM) pairs (m/z):
  - **for TAC: 821,49 > 768,40** and **821,49 > 576,20;**
  - **for ASC: 809,20 > 765,55** and **809,20 > 564,35.**
- Extraction from the VAMS by **pure delonized water** (150µL), with shaking.
- Blood extraction from the VAMS by water, and protein precipitation by **mixture of $ZnSO_4$: acetonitrile** (50:50, (v/v)).
- Steps of analytical process is presented below:



DEMOGRAPHIC DATA

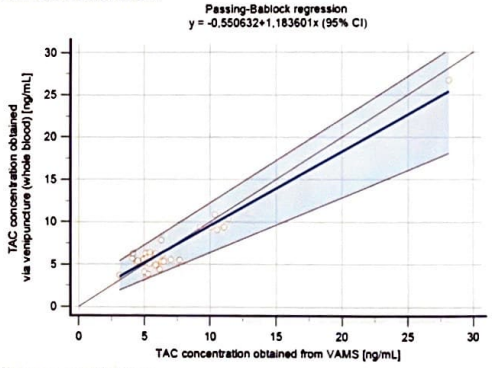
The 30 patients after KTX in *The Children's Memorial Health Institute* in Warsaw, Poland were included to the study – **demographic data below:**

PATIENTS CHARACTERISTICS	MEAN ± STANDARD DEVIATION
age [years]	12,40 ± 4,40
gender [male/female]	20/10
weight [kg]	46,3 ± 20,89
height [cm]	141,7 ± 24,09
daily dose of TAC [mg]	5,00 ± 3,17
dose per kg of body weight [mg/kg]	0,10 ± 0,08
time post KTX [months]	35,9 ± 35,13
creatinine [mg/dL]	1,00 ± 0,52
hematocrit (HT) [%]	37,2 ± 4,67
eGFR [mL/min/m²]	68,9 ± 19,62
treated by Advagraf® / Prograf®	7/21

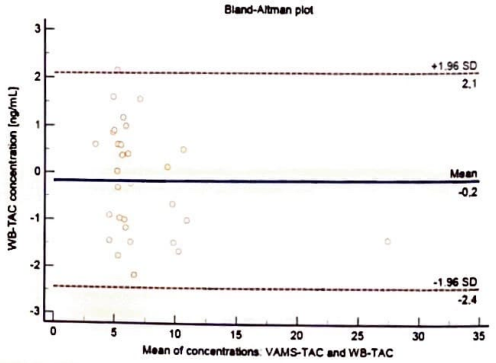
Approval of the bioethics committee number: **17/KBE/2022**

STATISTICAL ANALYSIS

- **Passing –Bablok correlation:**



- **Bland – Altman correlation:**



- **Pearson's correlation: $r^2 = 0,912$**

CONCLUSIONS

A satisfactory correlation between results confirmed, that method based on VAMS® is equal according to classic venipuncture method. The method is based on VAMS®-collected samples and may be used in clinical practice according to equivalence with the reference method and quite friendliness for pediatric patients. They may perform sampling without leaving home, which in case of pandemic reality and limited pediatric transplant centers in Poland seems to be the best alternative in opposition to classic venipuncture.

REFERENCES

1. Vethe NT. et al. "Tacrolimus can be reliably measured with volumetric absorptive capillary microsampling throughout the dose interval in renal transplant recipients." *Therapeutic Drug Monitoring* 41.5 (2019): 607-614.
2. Kindem IA. et al. "Tacrolimus Measured in Capillary Volumetric Microsamples in Pediatric Patients—A Cross-Validation Study." *Therapeutic Drug Monitoring* 43.3 (2021): 371.
3. Kocur A. et al. "Validation a new LC-MS/MS method of tacrolimus concentration measurment in the blood samples collected by volumetric-absorptive microsampling." *Accord, Interdisciplinary Conference on Drug Sciences*, Warsaw 2022– poster presentation.

The research was supported by MUW grant no FW22/1/F/MB/N/22 (F/MB/01/22)

* contact: Arkadiusz Kocur, MSc MPharm, ... Medical University of Warsaw, akocur@wum.edu.pl