Therapeutic drug monitoring of selected antiviral and antifungal agents using the volumetric absorptive-microsampling device (VAMS) in the pediatric population with sepsis: bioanalytical aspects of ANTISEPSIS study

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Background: Currently, it is estimated that infections are one of the five leading causes of death in the pediatric population. Incorrect dosing of antimicrobials increasing resistance of microorganisms, and toxic effects of drugs. Therefore, the therapeutic drug monitoring of antifungal and antiviral agents should be a crucial part of the therapy of pediatric patients with sepsis or septic shock in the ICU (intensive care units). ANTISEPSIS is a Polish, prospective, randomized, single-blind study in a population of antimicrobial-treated children with suspected or confirmed infection.

Methods: In the first part of the analytical stage of the ANTISEPSIS project, the LC-MS/MS (liquid chromatography-tandem mass spectrometry) methods for acyclovir (ACV), ganciclovir (GCV), fluconazole (FLU), voriconazole (VOR) and amphotericin B (APB) have been developed and validated. The methods were based on two different analytical runs: for antivirals and antifungals respectively. For methods, the Mitra™ (Trajan, Australia) microsampling device (10µL) was used, subsequently extracted with methanol-water solution, and analyzed with LC-MS/MS system in electrospray positive ionization mode. The stable isotope labelled internal standards of GCV, ACV, FLU, VOR were used during method optimization and validation.

Results: The methods were optimized using simple sample precipitation and/or liquid-liquid extraction (LLE), respectively. The methods were successfully validated and optimized in calibration, ranging from 5 to 20000 ng/mL (different ranges for each analyte). The validation parameters fulfilled EMA (European Medicines Agency), FDA (Food and Drug Administration) and IATDM&CT guidelines. No hematocrit, matrix and carry-over effects were observed. The first stage of stability examination under different stress conditions has been initiated.

Conclusions: In the next step, fully optimized and validated methods will be implemented in TDM of clinical samples from pediatric patients (N=5000) treated with antifungals and antivirals. The clinical validation of the drug levels in the serum and VAMS samples will be correlated for appropriate results interpretation.

Key Words: antivirals, antifungals, sepsis, ANTISEPSIS, VAMS