

Letter to the Editor

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Analytical performance specifications for measurement uncertainty in therapeutic monitoring of immunosuppressive drugs

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To the Editor,

The measurement uncertainty (MU) estimates and the definition of its maximum allowable limits for the clinical application of measurements represents one of the mainstays to produce standardized laboratory results suitable for clinical use, together with the definition of reference measurement systems and the establishment of a proper post-market surveillance of *in vitro* diagnostic (IVD) medical device quality [1]. Criteria and models for defining analytical performance specifications (APS) for MU have been firmly established in the last years, with special reference to endogenous biochemical measurands [2]. Therapeutic drug monitoring (TDM) is a clinical practice for measuring drugs in biological matrices to optimize individual dose regimens. This approach is usually adopted in patients treated with narrow therapeutic index (NTI) drugs, such as digoxin, antiepileptics, anti-infective agents, and immunosuppressive drugs. Nevertheless, the role of TDM in drugs with a wider therapeutic index is being increasingly recognized, especially in complex clinical setting, such as intensive care units [3]. However, the estimation of APS for drugs undergoing TDM has received little attention. For instance, in a recent study defining APS for 23 common laboratory tests only digoxin was included [4]. Seger et al., on behalf of the International Association of Therapeutic Drug

Monitoring and Clinical Toxicology Immunosuppressive Drug Scientific Committee, did an interesting discussion about the theoretical options that can be used to define APS for immunosuppressive drug TDM [5]. However, these authors did not elaborate concepts regarding MU and did not provide practical recommendations for individual drugs, just indicating that for immunosuppressive drug TDM, an analytical CV of approximately 6 % should be aimed for. On the other hand, the IFCC Working Group on Immunosuppressive Drugs does not mention in its terms of reference and projects the establishment of APS for MU of these drugs.

In this study, we therefore aimed to extend these findings by precisely defining the APS for MU for NTI immunosuppressive drugs. We considered cyclosporine, everolimus, sirolimus, and tacrolimus (available on the market as immediate- and prolonged-release formulations). For the derivation of corresponding APS for MU, the previously proposed hybrid model (using concepts belonging to the Milan models 1 and 2) was employed [4], in agreement with concepts first given by Callum Fraser in 1987 for desirable standards of performance for TDM [6]. Based on this model, the desirable APS for MU of NTI drugs undergoing TDM can be estimated as $\frac{1}{4} [(2^{T/t} - 1) / (2^{T/t} + 1)] \times 100$, where T is the time interval between doses and t is the average elimination half-life. The acceptability quality level can be then modulated to minimum goal, established as 50 % greater than the desirable APS, as previously described [4].

Table 1 shows the estimated APS for standard MU of the four evaluated immunosuppressive drugs when employed in adults. Only pharmacokinetic data and average elimination half-life of drugs related to adult transplant recipients were considered because pediatric patients may differ significantly in terms of frequency of drug administration (e.g., sirolimus in pediatrics is given both once and twice daily) and average terminal half-life, adding further sources of variability to the reported information.

We focused on cyclosporine, sirolimus, everolimus, and tacrolimus because these drugs are universally considered NTI drugs and, accordingly, their optimal dosage is established for each patient by TDM of whole blood concentrations

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Table 1: Analytical performance specifications (APS) for standard measurement uncertainty on clinical samples (u_{result}) (desirable and minimum quality level) for the four studied immunosuppressive drugs undergoing therapeutic monitoring in adults (data on frequency of drug administration and average terminal half-life were derived from [8]).

Drug	Frequency of administration, h	Terminal half-life, h	APS for u_{result} , %	
			Desirable	Minimum
Cyclosporine	12	8.4	11.5	17.2
Everolimus	12	25.6	4.03	6.04
Sirolimus	24	60.0	3.44	5.17
Tacrolimus, immediate release	12	12.1	8.27	12.4
Tacrolimus, prolonged release	24	36.0	5.68	8.51
Tacrolimus, extended-release tablets (Envarsus XR®)	24	30.0	6.76	10.1

in most transplant centers [7, 8]. Mycophenolic acid derivatives were not included in the present investigation because they are not considered as NTI and the role of TDM for these drugs is still debated. The same applies for azathioprine and corticosteroids, with blood concentrations poorly correlating with clinical outcome, both in terms of drug efficacy and tolerability [9].

Few studies have dealt with the issue of MU of immunosuppressive agents and, to the best of our knowledge, none has defined the APS for MU using appropriate models. Rigo-Bonnin et al. reported for whole blood tacrolimus concentrations an APS for standard MU of 7.15 % based on “components of biological variation data of the measurand” [10]. However, if we consider that the biological variation theory defines the *physiological fluctuation* of an analyte around its homeostatic set point, it is difficult to apply this classical model for deriving APS for measurements of drugs employed to suppress a physiologic function. By the way, those authors estimated a standard MU for tacrolimus measurements of approximately 6.0–6.5 % by using a procedure based on an ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method, a figure that seems to fulfil the desirable APS reported in Table 1 when the immediate-release formulation is administered. The same authors also reported a standard MU of 3.6–3.7 % for whole blood cyclosporine concentrations measured by UHPLC-MS/MS that appeared widely fulfil our proposed desirable APS [11]. More recently, Grote-Koska et al., using the model based on the state-of-the-art, have proposed a unique APS for standard MU of 10–12.5 % for all

four immunosuppressive drugs with respect to the data presented in their study and the technical status quo [12]. The marked difference in pharmacokinetics and elimination half-life of the drugs reported in Table 1 appear however to not support the indiscriminate use of a single APS value for all immunosuppressants, as drugs with small dosing intervals or long half-lives required better MU. Although all these studies used LC-MS/MS, other commercial methods are available on the market, with immunoassays largely used in most of medical laboratories [7]. It will be therefore important to verify if the proposed APS for MU can be achieved also by these methods. Considering the APS for MU reported in Table 1 for everolimus and sirolimus, the current performance of available methodology could also not easily permit to fulfill these goals. Alternative models to establish APS for MU in TDM based upon iatrogenic variability have been developed in the past. It is however of interest that the goals derived using the different strategies do have a number of similarities [13]. What in our opinion should be avoided is to use the state-of-the-art of the current technology performance as a ‘rescue’ model for establishing APS when data correctly obtained with other more appropriate models for a certain drug appear too stringent. We believe that the presented data should be considered as a starting point for medical laboratories and IVD manufacturers involved in the TDM of immunosuppressive drugs to verify the quality of their procedures. If needed, all attempts should be made to improve critical situations and sufficiently reduce MU to achieve the proposed APS. The involved stakeholders should be prepared to improve their performance whenever the clinical application of the test is made questionable by the failure to achieve APS and, at last, decisions can be made as to whether the employed method should be replaced with another one performing better.

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