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Counterpoint: Is Pharmacokinetic or Pharmacodynamic Monitoring of Calcineurin Inhibition Therapy Necessary?

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Individual dose adjustment of cyclosporine (CsA) or tacrolimus (TAC), which are critical-dose drugs widely used in transplantation, is important not only to prevent acute rejection but also to try and prolong graft (and patient) survival. New therapeutic regimens such as calcineurin inhibitor (CNI) sparing strategies have recently strengthened the need for fine individual dose adjustment of these drugs.

A good treatment-personalization strategy should employ a marker or a set of markers with 1) strong relationships with therapeutic and/or adverse effects; 2) low intra-individual variability; 3) established, and ideally validated, target values; and 4) easy and fast measurement.

So far, dose individualization has mainly been based on pharmacokinetics (PK), which monitors the handling of the drug by the body, but pharmacodynamics (PD), which focuses on the biologic effect of the drug on its target (*1*), might also be useful as a replacement or as an adjunct.

Early on in the use of CsA, pre-dose (trough, C_0) concentration monitoring became the standard of care (*2*). However, no trial has been conducted in transplantation to determine whether C_0 monitoring is superior to no therapeutic monitoring with respect to efficacy or toxicity (*2*) and it would not be ethically acceptable now given the obvious improvements brought to transplant patients by TDM. However, it has long been recognized that C_0 monitoring is suboptimal, and retrospective analysis of transplant populations monitored on C_0 has shown that the full inter-dose area under the concentration-time curve ($AUC_{0\text{-}12h}$), abbreviated AUCs ($AUC_{0\text{-}6h}$ or $AUC_{0\text{-}4h}$), or Cmax has a stronger link with the residual inefficacy or toxicity episodes than C_0. Because of the impracticability of routine $AUC_{0\text{-}12h}$ monitoring, limited sampling strategies have been proposed as a more practical approach. In 2002, the CONCERT International Consensus Statement even recommended monitoring all *de novo* and maintenance renal and liver transplant recipients with the CsA blood

concentration measured 2 hours post-dose (C_2), based on the findings that C_2 is the best single time-point predictor of $AUC_{0\text{-}4h}$, which represents the period of greatest inter-individual variability of CsA pharmacokinetics, the best surrogate for Cmax, and which coincides with the period of maximal calcineurin and interleukin-2 (IL2) inhibition (*2*,*3*). A recent paper critically reviewed the randomized trials comparing C_2 and C_0 monitoring in terms of clinical benefits (*4*). In de novo renal, hepatic, and cardiac transplant recipients the most consistent finding was a higher mean CsA dose in the early postoperative period in C_2 monitored patients, with no significant effect on the rate of acute rejection for a majority of these studies. In stable transplant recipients, the majority of studies showed that C_2 monitoring resulted in a reduction in mean CsA dose, without obvious clinical benefit. Therefore, clinical evidence of the superiority of limited sampling strategies or C_2 over C_0 monitoring is still weak (*2*).

C_0 monitoring has been standard of care since tacrolimus approval, although its therapeutic ranges have since changed. A recent consensus conference emphasized the lack of concentration-controlled trials investigating the relationships between tacrolimus concentrations and clinical outcome, and from which tacrolimus target blood concentrations could be derived (*5*). However, a large randomized trial demonstrated that a regimen based on TAC with low therapeutic ranges may be advantageous for renal function, allograft survival, and acute rejection rates, as compared with regimens based on low-dose cyclosporine, low-dose sirolimus, or standard-dose cyclosporine without induction (*6*). No randomized trial investigated the efficacy of alternative strategies to TAC C_0 monitoring, such as other time points or $AUC_{0\text{-}12}$ Bayesian estimation. Therefore, although C_0 may not be a good surrogate to TAC inter-dose AUC in all situations, based on the available evidence it is still impossible to conclude whether one particular TDM strategy is more effective than the others (*5*).

CNI concentrations in lymphocytes or in the transplanted organ might better predict efficacy than whole blood concentrations. Preliminary data suggest that in the early post-

transplantation period, CsA intracellular concentrations or $AUC_{0\text{-}12h}$ in peripheral blood mononuclear cells (PBMC) would be lower in patients experiencing acute rejection or would decrease during the week preceding the acute rejection episode, and that TAC concentrations in PBMC would exhibit a very high inter-individual variability (*7*). However, at the present time, the extemporaneous isolation of PBMC from whole blood is laborious, LC-MS/MS is the only technique allowing the measurement of drug concentrations in such small sample volumes (even more so in patients with leucopenia), and these concentrations must be standardized per million cells or using a marker of cell number, all of which are rather imprecise.

Studies in liver transplant recipients receiving TAC or CsA showed that intra-hepatic CNI concentrations were lower in patients with, than in those without, acute rejection, and that there was an excellent correlation of TAC intra-graft concentration with the severity of rejection, contrary to TAC whole blood concentrations (*7*). However, the determination of tissue CNI concentrations is unlikely to make its way into clinical practice (*7*).

CsA and TAC bind to intra-cellular proteins called immunophilins, and the CNI-immunophilin complex then inhibits calcineurin phosphatase activity. Calcineurin is involved in T-cell activation through dephosphorylation of the nuclear factor of activated T-cells (NFAT), enabling its translocation to the nucleus where it binds to the IL2 and interferon-γ (*INF-γ*) gene promoter regions, resulting in an increased secretion of the corresponding cytokines by the lymphocytes. Calcineurin also directly activates the lymphocytes by up-regulating the expression of T-cell surface receptors, including the IL-2 receptor α chain CD25 and the transferin receptor CD71.

Different approaches to pharmacodynamic monitoring of CNI therapy have been explored. The absence or weak inhibition of IL2 production in whole blood (*8*) or in CD8+ lymphocytes

(*9*) were described to be linked with a high acute rejection incidence in renal and liver transplant patients, respectively. Also, patients with a high pre-transplantation IL2 secretion were at higher risk of acute rejection after liver transplantation (*9*). However, to our knowledge no attempt has been made so far to try and individualize the CNI dose based on this biomarker.

The inhibition of calcineurin phosphatase activity (CaN) can be measured reproducibly in PBMC isolated by Ficoll density gradient centrifugation. Most studies have shown that CaN correlates with blood CsA concentrations (in particular C_2) in kidney transplant patients and with tacrolimus C_2, but not C_0, in liver and renal transplantation (*10*). However, not all studies have found a correlation between CaN and CNI blood concentrations, which might be due to the imprecision of CaN measurement, as well as imprecision of standardization by the number of cells or the protein content. However, the intra-individual variability in CaN in patients on CNI would be much larger than the assay variability, suggesting that PD monitoring should be performed on each occasion in each patient. Unfortunately, calcineurin activity measurement in PBMC currently takes almost 24 hours, which is not practical for routine use.

Pre-dose CaN has been found to be an acceptable surrogate of the area under the CaN-time curve in renal and liver transplantation. Moreover, in both conditions as well as *ex-vivo*, CsA displayed deeper CaN inhibition than TAC (*11*), which is not consistent with the greater *in-vitro* and clinical immunosuppressive effect of TAC, and suggests that this drug may have another mechanism of action. Anyway, owing to its inhibitory effect on hepatitis C virus replication not shared by TAC, CsA is also suspected to have a secondary mechanism of action. The relationships between CaN and clinical outcome are not clear. Conflicting results were found between CaN and graft-versus-host disease in stem cell graft recipients. In a rather small group of patients on TAC, nephrotoxicity was linked with lower CaN and acute rejection with higher CaN; however, CaN did not seem to be more predictive than C_0 (*12*).

More generally, in patients on a CNI, the residual CaN in lymphocytes probably results from their pre-transplant CaN; the degree of activation of T lymphocytes; the intra-lymphocyte concentrations of CNI; and the intrinsic sensitivity of calcineurin to the CNI (*13*). Also, the relationships of enzyme inhibition with biologic function and, eventually, graft outcome remain to be clearly established.

Another approach is to assess, by flow cytometry on T-lymphocytes stimulated in whole-blood cultures, the expression and down-regulation of cell surface markers (e.g., CD25, CD71) as an estimate of cell activation, the percentages of cells expressing intracellular cytokines (IL2, TNFα, INFγ) as an indicator of T-cell function, and T-cell proliferation (*1*). In liver transplant recipients on CsA or TAC, pre-transplantation IL2 expression by CD8+ T cells (but not calcineurin activity) was higher in patients who subsequently developed acute rejection (*9*). The same was true for intra-cellular IL2 at the time of the rejection episode, and a positive correlation was found between the percentage of IL2 expressing T cells and calcineurin activity. TNFα and IL2 expression correlates with transplant patients' age, and in healthy volunteers lymphocyte proliferation is higher in women (*14*), suggesting that a variety of factors can influence these biomarkers, many of which have probably not been investigated yet.

Interestingly, T-lymphocyte activation and proliferation are not specifically linked with CNI effects as they are also affected by IMPDH or mTOR inhibition. Consequently, these tests might be used as markers of cellular immunosuppression rather than as markers of CNI effects, provided that their link with acute rejection and/or graft function is further demonstrated. However, such flow cytometry tests are still expensive, laborious, lengthy (up to 3-day incubation), and require that blood samples be analyzed within 24 hours of collection, which is far from being routinely applicable.

Finally, the detection and quantification by real-time PCR of mRNAs encoding inflammatory cytokines following *ex-vivo* cell activation might be used to monitor T-cell response. A weak expression of the NFAT-regulated *IL2*, *INFγ*, and *GM-CSF* genes was closely linked with the frequency of infections and cancer in kidney transplant recipients on CsA (*15*). In patients with CsA dose tapering, expression of the same genes measured at 2 hours post-dose significantly increased after CsA dose reduction (*16*). However, the superiority of gene expression measurement over classic therapeutic drug monitoring has not been demonstrated. Such methods still need to be tested for their clinical pertinence (*1*).

To summarize the data, immunosuppression biomarkers have sometimes exhibited association with CNI exposure and/or clinical outcome, but the best parameters and methods to measure immune cell function in a fast and cost-effective way have still to be defined (*1*). All these tests suffer from long and cumbersome work-up and none have been validated prospectively by comparison to traditional TDM so far, nor used in clinical practice. The relationships between enzyme inhibition and biologic function, T-cell function and cellular rejection, acute graft rejection and graft function or survival (Figure 1) are probably not straightforward and need to be investigated in large patient populations before PD monitoring can be considered for individual dose adjustment.

In conclusion, PK monitoring of cyclosporine and tacrolimus has been improved over the years by a try-and-error process. Although formal clinical evidence is weak, TAC monitored on C_0 and/or CsA monitored on C_2 have become the basis of immunosuppression in most countries, associated with very low rejection rates during the first year post-transplant. However, it could possibly (and in the present era of CNI minimization, may need to) be improved further by means of more sophisticated approaches, such as AUC Bayesian estimation, preferably after validation by randomized prospective trials. Treatment

personalization in transplantation may ultimately rely on the choice of the best drugs and the best starting doses using pre-transplant pharmacodynamic and/or pharmacogenetic tests, as well as on post-transplant pharmacokinetic and pharmacodynamic monitoring to compensate for post-transplantation changes, environmental factors and intra-individual variability (*13*). However, clinical evidence in favor of CNI PD monitoring is currently at the lowest level and clinical experience is close to nil. Before it can be envisaged as a treatment personalization tool in organ transplantation, many questions still have to be answered, starting with the best marker of immunity to use, the influence of the physiological changes and immune system stimulation occurring post-transplantation, the effects of each immunosuppressive drug and their combinations on these biomarkers, and leading up to the relationships between such markers and clinical outcome. Continued efforts should also be made for a better understanding of the impact of pharmacogenetics on immunosuppressant benefit/risk ratio.

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Figure legend
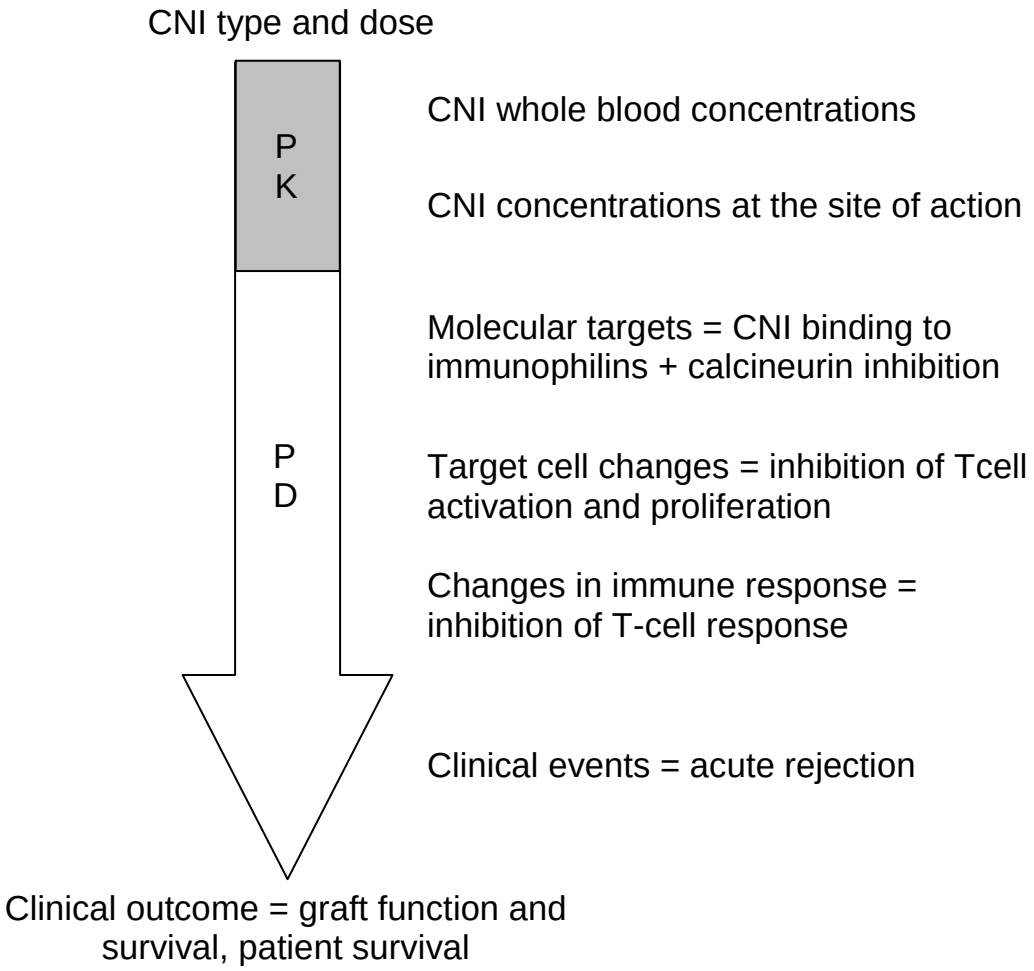
Figure 1: flow-chart of the pharmacokinetic fate and pharmacodynamic effects of calcineurin inhibitors.