Individual dose adjustment of cyclosporine (CsA) and tacrolimus (TAC), which are critical-dose drugs widely used in transplantation, is important not only to prevent acute rejection but also to 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 (a) strong relationships with therapeutic and/or adverse effects; (b) low intraindividual variability; (c) ideally, validated target values; and (d) easy and fast measurement.

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

Early on in the use of CsA, monitoring of the predose (trough, C₀) concentration became the standard of care (2). However, no trial has been conducted in transplantation patients to determine whether C₀ monitoring is superior to no therapeutic monitoring with respect to efficacy or toxicity (2), and conducting such a trial now would not be ethically acceptable given the obvious improvements brought to transplantation patients by therapeutic drug monitoring. However, it has long been recognized that the results of C₀ monitoring are suboptimal, and retrospective analysis of transplantation populations monitored on C₀ has shown that the full interdose area under the concentration-time curve (AUC₀–12h), abbreviated AUCs (AUC₀–6h or AUC₀–4h), and Cmax (maximum concentration) have stronger links with the residual inefficacy or toxicity episodes than C₀. Because of the impracticability of routine AUC₀–12h monitoring, limited sampling strategies have been proposed as a more practical approach. In 2002, the CONCERT (Consensus on Neoral C₂: Expert Review in Transplantation) “International Consensus Statement” even recommended monitoring all de novo and maintenance renal and liver transplant recipients with the CsA blood concentration measured 2 h postdose (C₂), based on the findings that C₂ is the best single time-point predictor of AUC₀–4h, which represents the period of greatest interindividual variability of CsA pharmacokinetics and the best surrogate for Cmax, and which coincides with the period of maximal calcineurin and interleukin-2 (IL-2) inhibition (2, 3). A recent report critically reviewed the randomized trials comparing the clinical benefits of C₂ and C₀ monitoring (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 C₂-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 C₂ monitoring resulted in a reduction in mean CsA dose, without obvious clinical benefit. Therefore, clinical evidence of the superiority of limited sampling strategies or C₂ over C₀ monitoring is still weak (2).

C₀ monitoring has been the standard of care since TAC was approved for use, although since then its therapeutic ranges have changed. A recent consensus conference emphasized the lack of concentration-controlled trials investigating the relationships between TAC concentrations and clinical outcome, from which TAC 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 compared with regimens based on low-dose CsA, low-dose sirolimus, or standard-dose CsA without induction (6). No randomized trial has been performed to investigate the
efficacy of alternative strategies to TAC \( C_0 \) monitoring, such as other time points or AUC\(_{0-12} \) Bayesian estimation. Therefore, although \( C_0 \) may not be a good surrogate to TAC interdose AUC in all situations, available evidence indicates it is still impossible to conclude whether one particular therapeutic drug-monitoring 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 posttransplantation period, CsA intracellular concentrations or AUC\(_{0-12h} \) 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 interindividual variability (7). However, at the present time, the extemporaneous isolation of PBMC from whole blood is laborious. Liquid chromatography–tandem mass spectrometry is the only technique that allows 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 by use of a marker of cell number, all of which are rather imprecise.

Studies in liver transplant recipients receiving TAC or CsA showed that intrahepatic CNI concentrations were lower in patients with acute rejection than in those without and that there was an excellent correlation of TAC intragraft 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 intracellular proteins called immunophilins, and the CNI-immunophilin complex then inhibits calcineurin phosphatase activity. Calcineurin is involved in T-cell activation through dephosphorylation of NFAT (nuclear factor of activated T cells), which enables its translocation to the nucleus. In the nucleus calcineurin binds to the interleukin 2 (IL2)\(^5\) and interferon, gamma (INFG) gene promoter regions, resulting in increased secretion of the corresponding cytokines by the lymphocytes. Calcineurin also directly activates the lymphocytes by upregulating the expression of T-cell surface receptors, including the IL-2 receptor \( \alpha \) chain CD25 and the transferin receptor CD71.

Different approaches to pharmacodynamic monitoring of CNI therapy have been explored. The absence or weak inhibition of IL-2 production in whole blood (8) or in CD8\(^+\) lymphocytes (9) were described to be linked with a high incidence of acute rejection in renal and liver transplant recipients, respectively. Also, patients with a high pretransplantation IL-2 secretion were at higher risk of acute rejection after liver transplantation (9). However, to our knowledge no attempt has been made to 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 \( C_g \)) in kidney transplantation patients and with TAC \( C_g \), but not \( C_0 \), in liver and renal transplantation patients (10). Not all studies have found a correlation between CaN and CNI blood concentrations, however, a situation that might be attributable to the imprecision of CaN measurement, as well as imprecision of standardization by the number of cells or the protein content. However, the intrindividual 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 h, which is not practical for routine use.

Predose 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), a finding that 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. 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-vs-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 pretransplantation CaN; the degree of activation of T lymphocytes; the intralymphocyte 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.

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5 Human genes: IL2, interleukin 2; INFG, interferon, gamma; CSF2, colony stimulating factor 2 (granulocyte-macrophage).
Another approach is to assess, by flow cytometry on T lymphocytes stimulated in whole-blood cultures, the expression and downregulation of cell surface markers (e.g., CD25, CD71) as an estimate of cell activation, the percentages of cells expressing intracellular cytokines (IL-2, tumor necrosis factor-α, interferon γ) as an indicator of T-cell function, and T-cell proliferation (1). In liver transplant recipients on CsA or TAC, pretransplantation IL-2 expression by CD8+ T cells (but not calcineurin activity) was higher in patients who subsequently developed acute rejection (9). The same was true for intracellular IL-2 at the time of the rejection episode, and a positive correlation was found between the percentage of IL-2–expressing T cells and calcineurin activity. Tumor necrosis factor-α and IL-2 expression correlate with transplantation patient 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 yet been investigated.

Interestingly, T-lymphocyte activation and proliferation are not specifically linked with CNI effects, because they are also affected by inosin monophosphate dehydrogenase and mammalian target of rapamycin 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 h of collection, which is far from being routinely applicable.

Finally, the detection and quantification by real-time PCR of mRNAs encoding inflammatory cytokines after ex vivo cell activation might be used to monitor T-cell response. A weak expression of the NFAT-regulated IL2, INFG, and colony stimulating factor 2 (granulocyte–macrophage) (CSF2) 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 h postdose 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 workup, and so far none have been validated prospectively by comparison to traditional therapeutic drug monitoring, 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 (Fig. 1) are probably not straightforward, and these relationships must be investigated in large patient populations before PD monitoring can be considered for individual dose adjustment.

In conclusion, pharmacokinetic monitoring of CsA and TAC has been improved over the years by a trial-and-error process. Although formal clinical evidence is weak, TAC monitored on C0 and/or CsA monitored on C2 have become the basis of immunosuppression in most countries, associated with very low rejection rates during the first year posttransplantation. These procedures, however, could possibly be (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 pretransplantation PD and/or pharmacogenetic tests, as well as on posttransplantation pharmacokinetic and PD monitoring to compensate for posttransplantation changes, environmental factors, and intraindividual 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 such monitoring can be envisaged as a treatment personalization tool in organ transplantation, careful validation is needed, potentially with prospective trials.
transplantation, much more investigation is needed, starting with the best marker of immunity to use, the influence of the physiological changes and immune system stimulation occurring posttransplantation, the effects on these biomarkers of each immunosuppressive drug and their combinations, 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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