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Review

Predictors of tacrolimus pharmacokinetic variability: current evidences and future perspectives

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Abstract

Introduction: In kidney transplantation, tacrolimus (TAC) is at the cornerstone of current immunosuppressive strategies. Though because of its narrow therapeutic index, it is critical to ensure that TAC levels are maintained within this sharp window through reactive adjustments. This would allow maximizing efficiency while limiting drug-associated toxicity. However, TAC high intra- and inter-patient pharmacokinetic (PK) variability makes it more laborious to accurately predict the appropriate dosage required for a given patient.

Areas covered: This review summarizes the state-of-the-art knowledge regarding drug interactions, demographic and pharmacogenetics factors as predictors of TAC PK. We provide a scoring index for each association to grade its relevance and we present practical recommendations, when possible for clinical practice.

Expert opinion: The management of TAC concentration in transplanted kidney patients is as critical as it is challenging. Recommendations based on rigorous scientific evidences are lacking as knowledge of potential predictors remains limited outside of DDIs. Awareness of these limitations should pave the way for studies looking at demographic and pharmacogenetic factors as well as gut microbiota composition in order to promote tailored treatment plans. Therapeutic approaches considering patients' clinical singularities may help allowing to maintain appropriate concentration of TAC.

Keywords: tacrolimus, kidney transplantation, pharmacokinetics, pharmacogenetics, demographic factors, drug interactions.

Article highlights

- TAC concentrations can be affected by several factors such as genetics, demographics, drug-drug interactions or microbiota composition.
- Taking this information into account might allow individualized treatments with increased efficiency and reduced toxicity.
- We provide a scoring index for known associations between these factors and TAC PK to grade the relevance of these associations.
- For drug-drug interactions, we present clear guidelines to clinicians allowing for a better control of TAC PK.
- For demographics, ageing and ethnicity appear to be of relevance for explaining part of the TAC PK disparities.
- For Pharmacogenetics, several lines of evidences pinpoint the potential benefit of *CYP3A5* pre-emptive genotyping strategy for TAC dosage individualization.
- More recently, some clues have been highlighted for a possible involvement of microbiota in TAC PK.
- In the Expert opinion section, we recap the current state of knowledge and provide perspectives for future research into TAC PK inter- and intra-individual variability.

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1. Introduction

Solid-organ transplantation is the treatment of choice for patients suffering from end-stage organ disease. In 2018, more than 140,000 organ transplantations were recorded worldwide, of which 65% were kidney grafts [1]. Post-surgical treatment includes the implementation of a lifelong immunosuppressive (IS) therapy to prevent organ rejection. In kidney transplantation, the most commonly used combination for maintenance IS therapy is composed of one calcineurin inhibitor (CNIs), most often tacrolimus (TAC), one anti-metabolite, mycophenolate mofetil (MMF), and glucocorticoids [2, 3]. Among these IS agents, TAC has become a central part of IS protocols in organ transplantation due to its ability to inhibit T-cell activation. By forming a complex with FK binding protein-12, TAC blocks the serine-threonine phosphatase activity of calcineurin, thus preventing T-cell and antibody-mediated rejection after organ transplantation [4].

TAC is characterized by a narrow therapeutic index with drug overexposure linked to nephrotoxicity, neurotoxicity, and diabetes mellitus [5], while underexposure might result in graft rejection [6]. Considerable intra- and inter-individual variability has been reported in TAC pharmacokinetics (PK), highlighting the need of precise therapeutic drug monitoring (TDM). Thus, drug levels are to be constantly maintained within the sharp therapeutic window through reactive adjustments, in order to limit drug-associated toxicity while maximizing efficacy [2, 7].

Oral bioavailability of TAC is highly variable among patients, ranging from as low as 4 to 89% [8]. With the prolonged-release tablet formulation (Advagraf®) given once daily, TAC has the capacity to be released and absorbed throughout the gastrointestinal tract until the distal gut [9, 10]. There is extensive and highly variable pre-systemic metabolism in the gut wall and the liver, mainly driven by cytochromes P450 (CYP) 3A isoenzymes [11], with CYP3A5 being a better catalyst than CYP3A4 [12]. TAC is also subjected to active transport, directed by efflux proteins, chiefly ABCB1 (P-glycoprotein, P-gp) [13] which modulates gastrointestinal absorption and cellular distribution [14]. After absorption, the remaining fraction is extensively bound to erythrocytes, and in the plasma, 90% of TAC is fixed to proteins [8]. CYP450-mediated metabolism gives rise to at least 15 metabolites, resulting from *O*-demethylation, hydroxylation and/or oxidative metabolic reactions [8]. Among these, 13-*O*-desmethyl-TAC is the major metabolite with an IS activity reduced to merely one-tenth compared to TAC itself. By contrast, 31-*O*-desmethyl-TAC is the only metabolite as active as TAC but is quantitatively negligible. Thenceforth the contribution of TAC metabolites to its IS efficacy is likely insignificant [8]. Eventually these metabolites are excreted in the bile [8]. Besides this phase I metabolic process, TAC glucuronidation by UGT1A4 was shown, but little is known about the physiological abundance of these glucuronides' derivatives [15-17]. Furthermore, it was evidenced *in vitro* that gut bacteria can also metabolize TAC with the production of a distinct TAC metabolite through a C-9 keto-reduction [18]. All in all, the intrinsic PK properties of TAC, including erratic absorption, variable first-pass effect, and microbial metabolism, are responsible for its large PK intra- and inter-patient variability.

Clinically significant variability within individual patients can be defined as an alternation between episodes of over- and under-exposure within a timeframe in which the dosage itself remains constant [19]. In renal transplantation, intra-patient variability in TAC drug exposure is now recognized as a predictor of poor clinical outcome [20, 21]. Indeed, persistent variability might be associated to alloimmune activation during low exposure [22], and toxicity and/or low immunity during overexposure. This inconsistent situation is commonly observed early after the engraftment and leads to suboptimal outcomes.

Several factors have been proposed as contributing to TAC intra- and/or inter-patient variability, including concomitant intake of food or drugs [7], genetic polymorphisms [23, 24], demographic variables (gender [25, 26], age [27], ethnicity [28]), gastrointestinal disturbances [29], low serum protein [30], hematocrit [31], time-post transplantation [32], non-adherence [33], circadian rhythm [34], drug-disease interactions [35], and possibly change in gut microbiota composition [36].

To advance the understanding of the factors able to influence TAC PK, in the present review, we summarize studies investigating drug interactions with TAC, as well as demographic and pharmacogenetic predictors of TAC PK. Our aim is to identify clinical and genetic covariates able to explain the variability, and to provide dosing recommendations safeguarding appropriate drug exposure in kidney transplant recipients.

To help the reader in the identification of relevant associations, for each section, we have defined an ordinal scoring index reflecting the strength of reported associations between the different covariates and TAC PK. The clusters were scaled in four groups according to their relative relevance: either none, weak, moderate or strong effect. This classification aims to pool different aspects evaluating the strength and magnitude of the observations previously reported in the literature, including the consistency and repeatability, the quality as well as the size of the studies, and if there is *in vitro*/mechanistic-based data supporting the association. To consider these factors in clinical practice, recommendations are proposed in some sections, where appropriate. As it reflects our judgment, this proposed scoring should be interpreted carefully and ideally adapted to each individual situation.

2. Drug-drug interactions

Drug-drug interactions occur (DDI) when the PK of a medication is altered by the concomitant administration of another medication [37], the likelihood of DDI thus increases with the number of medications a patient is taking. For transplant patients, in order to achieve adequate immunosuppression while limiting side effects, the current guidelines involve combining medications from different classes. The use of polypharmacy is thus unavoidable; therefore, it is important to study the impact it might have on TAC PK and vice-versa.

As we previously established, monitoring TAC concentration is essential. Amongst some of the factors interfering with its PK, it was reported that DDI, by their impact on absorption, distribution, metabolism, and excretion (ADME), might directly affect blood levels, with TAC also able to affect the PK of other drugs [38]. To date, more than 707 drugs interacting to some extent with TAC have been reported, in addition to several alcohol/food interactions and diseases interactions [39]. The majority of food-drug interactions are only moderate to minor [7], still food is a factor to consider when studying TAC PK. In table 1, we present the main interactions relevant to a transplanted patient's regimen.

TAC is extensively metabolized by the CYP3A isoenzymes, with CYP3A5 being the main protagonist [12]. Therefore, DDI associated with TAC are mostly mediated by these enzymes. An inhibition of CYP3A activity leads to increased TAC blood levels, caused by decreased clearance through competitive inhibition, potentially causing significant overdosing. Such elevation of blood concentration has been reported with calcium channels blockers (diltiazem [40], verapamil [41], nicardipine [42], amlodipine [43]), which are potent inhibitors of CYP3A enzymes [44, 45]. Similar effects have been reported for antibiotics erythromycin and clarithromycin [46, 47], and suggested for some food products such as grapefruit [48, 49] and pomegranate [50, 51] including several cases of severe interactions. On the contrary, drugs inducing CYP3A enzymes such as anticonvulsants phenytoin [52-54], carbamazepine [55], and phenobarbital [56] reduce TAC blood levels, thus decreasing its bioavailability and possibly causing under dosing, increasing the risk of graft failure.

Though, TAC is also a substrate for ABCB1 expressed by different epithelial and endothelial cells including enterocytes, hepatocytes, lymphocytes, site of therapeutic action, and kidney, where its toxicity is exerted. Further to effects mediated by CYP3A enzymes, as emphasized above, TAC bioavailability is also highly influenced by this efflux transport [57]. Hence, drugs acting as ABCB1 inducers and inhibitors can interact with TAC PK leading to changes in TAC absorption and bioavailability, with a direct impact on blood exposure [7, 39]. It was described that in combination with corticosteroids inhibiting TAC metabolism while also acting on ABCB1 induction, the

bioavailability of TAC is initially reduced and a higher dosage is required to achieve target trough levels [58-60]. However, this effect is only observed in the short term as the impact of induction gradually decreases after several days, due to compensatory mechanisms [61]. Likewise, the toxicity of colchicine, also an ABCB1 substrate, was reported to increase at therapeutic doses in combination with TAC [62, 63].

Of even higher importance are the several drugs able to act on both CYP3A and ABCB1 activities. Their use, combined to administration of TAC, can lead to dangerous changes in blood levels with subsequent adverse effects. This is particularly true for anti-viral medications such as anti-HIV protease inhibitors, where TAC levels can be increased up to 140-fold together with an important decrease in clearance [64-67]. Furthermore, in co-medication with the anti-arrhythmic amiodarone, an ABCB1 inhibitor, TAC levels have been reported to increase significantly [68, 69]. When taken together, drugs of azole antifungal family and TAC would maximize their effects resulting in increased blood concentrations [70-72]. Meanwhile, rifampicin, an antibiotic and anti-tuberculosis agent causing ABCB1 induction, is reducing TAC levels when given concomitantly [73, 74]. In these cases, and whenever possible, drug substitution is strongly recommended to maintain TAC at optimal levels.

Both these metabolic pathways are critically involved in the PK of a wide range of commonly used drugs. CYP3A are important in the metabolism of drugs, while intestinal ABCB1 appears to influence the peak concentration of orally administered drugs in the systemic circulation. Therefore, it is essential to evaluate and consider the PK and potential DDI profiles of drugs used in IS protocols to avoid significant detrimental clinical effects. A better consideration of principles underlying TAC DDI in clinic will heavily contribute to more efficient and more adapted therapies for transplant patients.

Beside these conventional DDI, drug-mediated alterations of gut microbiota composition are other underexplored likely sources of DDI. Indeed, microbiota composition is very sensitive to any shift in environmental factors, including pharmaceutical regimen. This intestinal microbial community influence the PK processes of xenobiotics (including drugs) in the human body through direct or indirect mechanisms [75]. We can expect that drugs, and particularly antibiotics, also alter indirectly TAC PK through changes in microbiota-influenced PK processes.

In summary, co-administration with a drug known to interact with ABCB1 and/or CYP3A, can cause alterations in TAC bioavailability and metabolism [39], thus leading to dangerously high IS levels with subsequent toxicity, or to inadequately low levels and increased probabilities of organ rejection [76]. In consequence, additionally to anticipated dosage modifications, TDM is of primary importance in clinical practice for transplant patients, even more in cases where adjustments to patient's regimen are required.

3. Demographic predictors

Apart from medication and potential interactions, patient's demographic parameters are another major factor to consider in TAC dosage adjustment. Recently several studies have highlighted a relation between demographic characteristics such as race, gender, or age to TAC PK [77, 78]. Therefore, analyses aimed at determining if demographic predictors could improve the understanding of tolerability and safety profiles within key subpopulations. In table 2, we summarize the main demographic factors relevant to a transplanted patient's regimen.

Biological aging is a complex multifactorial process characterized by structural and functional changes affecting most molecular, cellular, and organ systems, resulting in an overall reduced homeostatic capacity and a decreased physiological reserve in response to stress [79]. Aging induces significant changes in body composition, hepatic and renal function [80]. This has a direct impact on the pharmacodynamic (PD) and PK, resulting notably in an increased bioavailability for some drugs, including for TAC [81]. In the elderly population, understanding alterations in PK is particularly

important since age-related impairments have been reported to have a critical impact on TAC metabolism and excretion, leading to a prolongation of half-life [82]. Despite a lower dose-to-bodyweight, increased TAC exposure (up to 50%) was observed in older transplant patients (≥ 65 years) compared to younger adults, possibly due to reduced clearance capacities [27]. Alongside the impact on PK, higher prevalence of TAC-mediated toxicity was reported after 60 years of age and associated with higher rate of TAC withdrawal [83]. Moreover, because of genetic and environmental modulators, patients at the same age will show different trajectories of age-related decline [84]. Taken together, these observations partly explain the increasing inter-individual variability observed in TAC PK as people get older, but also give hints to explain the intra-variability observed for patients over time [82, 85]. We recommend to increase vigilance and reinforce TDM for older patients as they are also more vulnerable.

Research has shown that sex differences may contribute to variations observed between women and men in PK. It was previously suggested that sex could affect drug ADME leading to differences in drug response [86]. These PK differences can mostly be explained by body composition and weight since the majority of drugs are currently administered at fixed doses for adult patients rather than on an mg/kg basis [87]. Still, using fixed doses might lead to higher exposure in women compared to men, resulting in potential adverse effects [88]. However, for TAC, initial doses are prescribed on an mg/kg basis. Consequently, a part of the impact of gender on TAC exposure is indirectly considered. This could also explain why, in most studies, the gender hasn't been identified as a covariate of TAC exposure [25, 26]. Yet, recent literature suggests that, even with weight adjustment, sex differences might persist based on additional indirect and concomitant factors such as hormonal distribution, age, polypharmacy, genetic specificities or polymorphisms, and disease state [89].

Despite the high prevalence of TAC as an IS in transplantation and the aforementioned issues of intra- and inter-variability, surprisingly, few studies have attempted to clarify the impact of ethnic disparities on TAC exposure. It has, however, been established that ethnic factors might affect the bioavailability, distribution, metabolism, and elimination of drugs [90]. Recent studies have provided evidence that African American transplant recipients require higher doses of TAC in order to achieve therapeutic drug concentrations and to decrease the risk of acute rejection. Although these doses would also significantly increase the risk of TAC-induced nephrotoxicity [91]. Indeed, African Americans were described as having a greater risk of renal failure than the general population [92]. On the other hand, it was demonstrated that Asian populations display greater TAC bioavailability compared to other ethnic groups, suggesting that these patients may require lower TAC doses [93]. The differential prevalence of *CYP3A5* polymorphisms in these populations is the main explanation for these differences. We consider that with regards to the current knowledge on the effect of specific SNPs on drug metabolism, it is pharmacogenetics and not ethnicity per se that need to be considered as the central variable.

4. Pharmacogenetic predictors

Proteins implicated in TAC ADME pathways are highly polymorphic, providing alternative explanations to the observation that some patients have relatively low drug exposure with possibly fast drug clearance, while others display a higher drug exposure and slower drug elimination rate. Pharmacogenetic studies have provided interesting clues to clarify the reasons behind TAC PK variability (Table 3). With the exception of a single nucleotide polymorphism (SNP) in *CYP3A5* that correlates with significant changes in TAC PK, most of the associations are still controversial. Confidently, for *CYP3A5*, some findings have been validated and repeated across multiple studies. Nevertheless, the systematic application of pharmacogenetic testing strategies in daily clinical practice is not as universally accepted as it can be for DDI, and still requires stronger guidelines before clinical implementation.

As exposed above, after oral administration, TAC is metabolized by intestinal and hepatic CYP3A isoenzymes, with CYP3A5 being a better catalyst than CYP3A4 [12]. The expression of CYP3A5 is

largely determined by genetic polymorphisms with only 15% of Caucasians expressing a functional enzyme at a detectable level [94, 95]. The *CYP3A5*3* allele (rs776746) is an intronic SNP that creates a cryptic splice site causing the addition of an extra exon (exon 3B) in the mRNA [94]. This splicing defect leads to the introduction of a premature stop codon, ending up with the translation of an inactive truncated protein. As a consequence, carriers of two loss-of-function (LOF) alleles do not express a functional CYP3A5, and are therefore classified as CYP3A5 non-expressers, whereas carriership of at least one functional allele (*CYP3A5*1*) is associated with a significant CYP3A5 effective expression (CYP3A5 expressers). *CYP3A5*3* is the main genetic predictor of TAC PK and it was shown that CYP3A expressers require a double dose when compared to carriers of two LOF alleles [24, 25, 96-106]. The consistency of this association was confirmed by meta-analyses, regardless of the time post-transplantation or the ethnicity of the patient [107-109]. The dominance of the explicative value of *CYP3A5* allelic status was illustrated by the fact that, in a population of 446 kidney transplant recipients, among a panel of more than 2000 SNPs in PK genes, no variant other than *CYP3A5*3* was significantly correlated with concentration-to-dose ratio and, by itself, the *CYP3A5*3* SNP explained up to 39% of the variability in dose requirement [110]. Additionally, multiple population PK models describing the PK effect of *CYP3A5*3* have been developed so far in distinct types of transplant populations. They revealed that introducing the *CYP3A5*3* genetic status of the patient in the model explained on average 30% of the total variability in the TAC clearance [26, 31, 105, 111-121]. Subsequent to these convincing observations, it was expected that pharmacogenetics-based TAC dosage would decrease the time needed to achieve target concentration range. This hypothesis was investigated in three independent randomized clinical trials (RCT) [122-124]. In all three of these trials, the patients were assigned to receive either a standard, body-weight-based or a *CYP3A5* genotype-based TAC starting dose, with CYP3A5 expressers receiving a higher TAC dose when compared to CYP3A5 non-expressers. In the first RCT [122], it was observed that, in the group receiving the *CYP3A5*-adapted dose, a higher proportion of patients had values within the targeted trough concentration (C_0) range at day 3 after TAC initiation. They also required fewer dose modifications, and the targeted C_0 was achieved sooner by 75% of these patients. However, the benefit of reaching therapeutic TAC concentrations earlier did not translate into better clinical outcomes. In the second RCT [123], it was shown by Min *et al.* that a pre-emptive *CYP3A5* genotyping strategy allows shortening the time to reach target concentrations in pediatric transplant recipients. By contrast, in the third trial [124], at day 3, no difference in the proportion of patients having a TAC exposure within the target range was observed between the standard-dose and genotype-based groups despite CYP3A5 expressers still required a higher Tac dose to achieve target concentrations compared with CYP3A5 nonexpressers. Given the implication of CYP3A5 in TAC PK, these negative results are quite surprising and might possibly be attributed to variances in study design. In this third RCT, like in others, the incidence of acute rejection was comparable between both groups. All together, these results indicate that despite the fact that *CYP3A5* genotype has the power to explain a substantial part of TAC PK variabilities, it is not sufficient by itself to improve the clinical outcome. Although it has been prospectively shown in a pilot study that a genotype-based approach was safe and allowed deferring TDM interventions [125], it was recently reported that genotype-guided initial doses and achieving target therapeutic TAC levels at day 3 do not decrease the cost associated with TDM after transplantation [126]. However, the authors have also shown that CYP3A5 expressers generate higher additional hospitalization cost for kidney transplants when compared to CYP3A5 non-expressers [126]. This last observation suggests that genotyping is a cost-conscious tool that can assist the clinicians and somewhat support the Clinical Pharmacogenetics Implementation Consortium (CPIC) recommendations that endorse increasing the TAC starting dose 1.5–2 times for CYP3A5 expressers [127].

In addition to *CYP3A5* genotype, other SNPs might explain the residual TAC PK variability, especially in CYP3A5 non-expressers where CYP3A4 seems to compensate the loss of CYP3A5 activity. The activity of CYP3A4 is extremely variable and depends on multiple demographic, physiologic but also genetic factors. It was suggested that up to 90% of CYP3A4 activity variability would have a genetic

basis [128]. Among the different SNPs that have been tested for association with TAC PK, the *CYP3A4*1B* SNP, which is defined by an A to G transition at position -392 in the promoter region of *CYP3A4*, was associated with an increased *CYP3A4* activity [129]. It was shown that the dose requirement (*i.e.* the through concentration-to-dose ratio) was increased among carriers of the G variant allele [101, 102, 106]. However, it is suspected that this association is mostly due to the strong linkage disequilibrium with the *CYP3A5*1* functional allele and not from a substantial change in *CYP3A4*-mediated metabolism [103]. By contrast, the *CYP3A4*22* allele which is defined by the presence of the rs35599367C>T SNP in intron 6 was associated with reduced *CYP3A4* hepatic expression and activity leading to a decreased TAC clearance for *CYP3A4*22* carriers [130-133]. Kidney transplant recipients who carry the *CYP3A4*22* variant allele require significantly lower TAC doses compared to non-carriers [134-138]. Additionally, *CYP3A5* non-expressers who carry a *CYP3A4*22* allele have a higher risk of TAC overshoot during the first 3 days after kidney transplantation [136, 139, 140]. This suggests that TAC dosage adjustment based on *CYP3A4*22*–*CYP3A5*3* combined allelic status might be more informative than an algorithm considering the *CYP3A5* genotype exclusively [141, 142]. These findings were further corroborated through statistical investigation and discrimination analysis, showing that *CYP3A* clustering according to both *CYP3A4*22* and *CYP3A5*3* better fits with TAC PK reality when compared to regrouping according to *CYP3A5*3* genotype solely and refining of the CPIC recommendations has been proposed accordingly [143]. This observation is also supported by the fact that population PK (popPK) models including *CYP3A4*22* are more accurate than those not including this SNP [105, 113, 120, 144]. However, this specific SNP seems to be relevant in whites not expressing *CYP3A5* only because *CYP3A4*22* alone does not significantly improve the performance of TAC popPK models. It is thus possible that in population where *CYP3A5*1* is more frequent, other SNPs might have a predictive value for explaining differences in TAC PK. In that way, it was proposed that *CYP3A5* activity is affected by SNPs in the P450 oxidoreductase (*POR*) gene, which codes for a protein essential for *CYP450* activity [145-147]. *POR*28* is the most common nonsynonymous SNP reported for *POR* and was associated with increased TAC dose requirement in *CYP3A5* expressers [148-151]. Likewise, the rs4253728 SNP in *PPAR-alpha* was linked with decreased *CYP3A4* activity [152] and further associated with the development of new-onset diabetes (NODAT) in kidney transplant recipients treated with TAC [153, 154]. In Asians, it seems that an additional predictive value can be credited to the *CYP3A4*18B* allele to explain TAC inter-individual variability [155-157] as it is the case for *CYP3A4*20* in the Spanish population [158].

Apart from the oxidative metabolism, TAC is a known substrate for ABCB1, formerly known as the P-gp [159]. ABCB1 is an efflux pump belonging to the ATP-binding cassette (ABC) super-family and is responsible for the active transport of substrates across cell membranes from the intra- to the extra-cellular environment. ABCB1 expression is quite ubiquitous and plays a key role in absorption, distribution and excretion of drugs [160]. By its expression in enterocytes, ABCB1 limits TAC bioavailability. It is also expressed in lymphocytes where it might limit the access of TAC to its therapeutic target. Finally, it is expressed in excretory organs like the kidney, where TAC (or its metabolites) toxicity occurs, and the liver, where metabolites are excreted. SNPs in *ABCB1* have been widely considered for association with TAC PK [99, 106]. The most common SNPs in *ABCB1* are 1236C>T, 2677G>T/A and 3435C>T and are in strong linkage disequilibrium and, like *CYP3A* SNPs, their allelic frequencies vary among ethnic groups. Much attention has been focused on the synonymous coding 3435C>T SNP which variant allele 3435T was associated with reduced mRNA expression and stability but also differential substrate affinity [161, 162]. Meta-analyses have attempted to unravel the different controversial observations on the influence of *ABCB1* SNPs on TAC PK in renal transplantation [107, 163]. The results suggest a limited impact of the 3435C>T as well as 1236C>T and 2677G>T/A SNPs on TAC blood concentrations. It was hypothesized that changes in ABCB1 activity could be more relevant to explain differences in TAC drug tissue distribution than differences in TAC blood concentration. Indeed, it seems that *ABCB1* SNPs have a greater influence on TAC local cellular concentration and compartmentalization than on systemic

drug exposure, as it was shown that both 1199G>A and 3435C>T SNPs are significantly associated with increased TAC cellular concentrations in hepatocytes and in lymphocytes [164-168]. These observations were further confirmed *in vitro* either in recombinant cellular models or in cultured cells obtained from renal tissue [13, 169]. Interestingly, the *ABCB1* SNPs have been more consistently linked with TAC PD outcomes, suggesting that TAC local exposure is more closely related to the drug activity than the blood concentrations [170-172].

Other genetic factors might potentially explain differences in metabolism. For instance, the pregnane X receptor (PXR), encoded by *NR1I2*, is a key nuclear receptor controlling the expression of multiple CYP and transporter proteins [173]. In a mixed cohort of kidney transplant patients treated with either TAC or cyclosporin, the rs2276707C>T *NR1I2* SNP in donors has been associated with an increased expression of PXR in the kidney [174]. This gain of function in the engrafted kidney carrying the TT variant genotype was shown to be related to an increased risk of delayed graft function [174]. It was hypothesized by the authors that renal induction of CYP3A5 and *ABCB1* expression by activation of PXR may be more pronounced in recipients of kidneys from TT-carrying donors and lead to an increased risk for the development of delayed graft function. However, to our knowledge, these results have not been repeated so far and still await confirmation [175]. *In vitro*, it was shown that the rs3814055C>T SNP in *NR1I2* is associated with a down regulation of PXR expression and activity either when assessed in liver samples or through luciferase assay in recombinant models [176, 177]. In this later study, the authors also investigated the effect of this SNP on the TAC PK in 42 healthy volunteers. They showed that the greater the number of T alleles at rs3814055 in the *NR1I2* gene, the greater the mean exposure to TAC was and that the effect was independent from *CYP3A5**3. Quantitatively, they observed that the area under the concentration-time curve to the last quantifiable time point (AUClast) was 3.42 times greater in *CYP3A5* non-expressers with the variant TT genotype for rs3814055 when compared to wild-type individuals. Supporting this observation, a clinical trial in 32 kidney transplant patients showed that TAC clearance decreased gradually with the number of variant alleles for the rs3814055C>T SNP in *NR1I2* [178].

Other genetic associations have been reported, for instance in *ABCC2* or *CYP2C8*, but the sense of these findings is not clear. These associations have not been repeated despite the high number of studies published in the field, indicating possibly their poor clinical relevance [99, 106, 179].

5. Recent perspectives in PK variability research

Although the different factors developed here above have the potential to advance the understanding of PK variabilities, it is clear that the current body of knowledge is not sufficient to explain inter- and intra-individual variabilities. This appears as particularly important for intra-individual PK variability, as it has been related to the risk of treatment failure [20, 21].

Of old, researchers have perceived that microbiota, and especially gut microbiota, must take part in the becoming of drugs, and thus in individual susceptibilities [180, 181]. A decade ago, pharmacomicrobiomics has been defined as a new branch of pharmacology that focuses on variations in responses to drug disposition, action, and toxicity in which the variable is the combined genetic makeup of the human-associated microbes (microbiome) and their metabolic potential [182]. The role played by gut microbiota in drug efficiency and safety has attracted more and more attention over the last years. This field has also benefited from pioneering examples of gut microbiota interacting with drugs such as digoxin and irinotecan [183, 184].

Given its mode of administration, its low intestinal absorption, and its biliary excretion after metabolism [8], TAC is in close contact with the gut microbiota, which substantiates the likelihood of direct interactions with microorganisms *in vivo* [75]. Several clues support the hypothesis of an interplay between TAC and the gut microbiota. For instance, post-transplant diarrhea and enterocolitis might be associated with gut dysbiosis and have been repeatedly associated with altered drug levels, caused by a paradoxical increased bioavailability, possibly resulting in additional

toxicity and the need of dose reduction [185-187]. Furthermore, co-administration of antibiotics was linked to variations in TAC levels [188-190]. In addition, TAC dose escalation during chronic therapy in kidney transplant patients was correlated with *Faecalibacterium prausnitzii* (*F. prausnitzii*) abundance in the first week after kidney transplantation and was also positively correlated with future TAC dosing at 1 month after engraftment [36]. However, given the small number of participants enrolled (n = 5 patients in “dose escalation group”, n = 14 patients in “stable group”) and the relatively minor correlation observed ($Rho = 0.57$, $p = 0.01$), the results reported in this clinical study must be interpreted with caution. Following up on this study, *F. prausnitzii*, but also several other *Clostridiales*, were proposed as being able to metabolize TAC *in vitro* into a much less potent immunosuppressive metabolite, the C-9 keto-reduction product. This newly discovered metabolite is specific to bacterial metabolism since it was shown that hepatic microsomes were not able to produce it [18]. However, the physiological relevance of this bacterial metabolite is still unclear.

On the other hand, microbial metabolites can also indirectly affect the PK processes of drugs through interactions with host pathways [75]. In the case of TAC, we believe that microbial alterations could cause a change in CYP3A-mediated metabolism and/or ABCB1 efflux, resulting in variable TAC exposure. Indeed, it was demonstrated that, in germ-free mice, the hepatic mRNA expression and activity of *cyp3a11* (mice orthologous of CYP3A4) is decreased [191]. The same trend is observed after antibiotics-mediated microbial depletion [192]. However, current knowledge is insufficient to predict how a change in intestinal microbiota can result in alterations of TAC exposure, through modification of these host metabolic pathways.

Thanks to their β -glucuronidase, some intestinal bacteria possess the metabolizing capacity to deconjugate glucuronide metabolites [193]. Then, the restored parent compound can be reabsorbed in the intestine through enterohepatic circulation [75]. Such a process is responsible for the intestinal toxicity of the chemotherapeutic agent irinotecan [184]. For MMF, enterohepatic circulation contributes for almost 40% of drug exposure in humans [194]. Considering TAC, in a clinical study on liver transplantation, Tron et al. observed a second peak in TAC profile in 15% of patients, that might indicate the existence of an enterohepatic circulation of the drug [17]. While optimizing TAC quantification by HPLC-MS/MS in human bile sample, they observed a co-eluted peak using the same MS/MS *m/z* transition than TAC [195]. This co-eluted peak disappeared when samples were pre-treated with β -glucuronidases before extraction, resulting in increased TAC total peak area. This result suggests that TAC glucuronide metabolites were back converted into TAC by β -glucuronidases treatment. Similar observations were previously described by Firdaous et al. in 1997 [15]. Therefore, biliary excretion of TAC glucuronide derivatives could participate to such enterohepatic circulation. Alteration of microbiota composition could affect this re-absorption process, and consequently be a source of microbial-mediated TAC PK variability.

It is now accepted that the composition of the intestinal microbiota impacts on uremic toxins generation [196]. However, in patients with chronic kidney disease, the problem is bidirectional as the uremic status in turn affects the composition of gut microbiota and, consequently, the inflammation status. An inflammatory environment is known to lead to the phenoconversion of drug-metabolizing enzymes, meaning that the presence of some pro-inflammatory cytokines (e.g. IL-6, TNF α , IL-1 ...) reduces their expression and/or activity [197]. More particularly, IL-6 represses the expression of the nuclear receptors PXR and CAR (constitutive androstane receptor) and their target genes, including CYP3A4/5 [198]. This phenoconversion process could explain unexpected overdoses of tacrolimus reported in two clinical cases where patients suffered from acute inflammatory episodes [199]. Indeed, a very recent study highlighted that the median daily weight-based TAC dose requirement was associated with inflammation and oxidative stress markers [35]. In this context, the contribution of the gut microbiota to an inflammatory environment, possibly still present after the transplantation, could contribute to the PK variability of TAC.

Hopefully, improving the knowledge about gut microbiota, including as a regulating factor of TAC PK will fuel the state-of-the-art in regard to TAC therapy optimization and provide new insights for precision pharmacotherapy. In contrast to genetics, microbial composition and metabolic function evolve all life long, and therefore, could also explain TAC intra-patient variability. So, we trust this is a promising track that should be explored further for TAC management therapy.

6. Conclusion

Immunosuppression is a critical part of current transplantation protocols, with TAC being increasingly more prevalent which is not expected to decrease in the decade to come. However, because TAC is also characterized by a narrow therapeutic index, it is essential that optimal dosage is achieved immediately after transplantation and at all time to ensure immunosuppression efficiency. Furthermore, despite its popularity, high incidence of intra- and inter-individual variability is still being reported, and still leads to devastating outcomes in some cases. In this review, we provide a state-of-the-art update on the potential influence of demographics, genetic polymorphisms and drug interactions on TAC PK and variability. In addition, we discuss in this paper how the gut microbiota might constitute a promising avenue to address this issue in the future. Further prospective studies are essential to determine optimal dosing strategies for TAC based on patients' specific characteristics, including demographics and pharmacogenetics. We believe that a better knowledge of all of these interfering parameters collectively will advance the understanding of inter-individual response differences. Thus, enabling better predictions of dosage requirements leading to an improvement in TAC management in transplant patients.

7. Expert opinion

Several criteria are necessary before implementing this type of recommendations into practice. First, the strength of the association must be validated, widely established and ideally, mechanistically explained. Second, recommendations must be clear and clinicians who makes use of them should be deeply briefed about all aspects of the issue to ensure adequate compliance from patients. Given the data we summarize in the present paper, and although some recent progresses have been achieved, one can easily appreciate that lots of hurdles are to be cleared before we can convert theoretical knowledges about TAC PK into clear enforceable messages that can be implemented in practice, in order to address variabilities.

In the present review some suggestions and solutions are offered depending on the current knowledge to simplify the situation and to help the reader detangle valuable associations from insignificant observations. For some of the stronger findings regarding drug interactions, we have sketched their possible integration into practice by giving advices for dosage adjustment anticipations. Nowadays, the expansion of screening software provides an important tool to assist clinicians in the detection and management of DDIs, including for TAC. Yet, for pharmacogenetic and demographic factors, and even for strongly substantiated associations, translation into clinical recommendations is still marginal in clinical practice (*e.g. CYP3A5*3*).

Therapeutic drug monitoring currently remains the tool of choice to master TAC PK variabilities but more factors might be considered as additional means to help refining these recommendations. These will have to include genetic polymorphisms and study of demographic predictors. Particular enthusiasm is also set on gut microbiota with the emergence of pharmacomicrobiomics, a field that is yet to be fully explored in the case of TAC. Once validated, all recommendations will have to demonstrate their benefits through prospective trials. From there, a robust and ideally cost-effective framework will have to be implemented to ensure proper clinical application. The road to clinics is still long, but we expect that the advances summarized here will raise awareness in the clinical community to better understand and manage the issue of TAC PK variabilities.

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Conflicts of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Graphical abstract

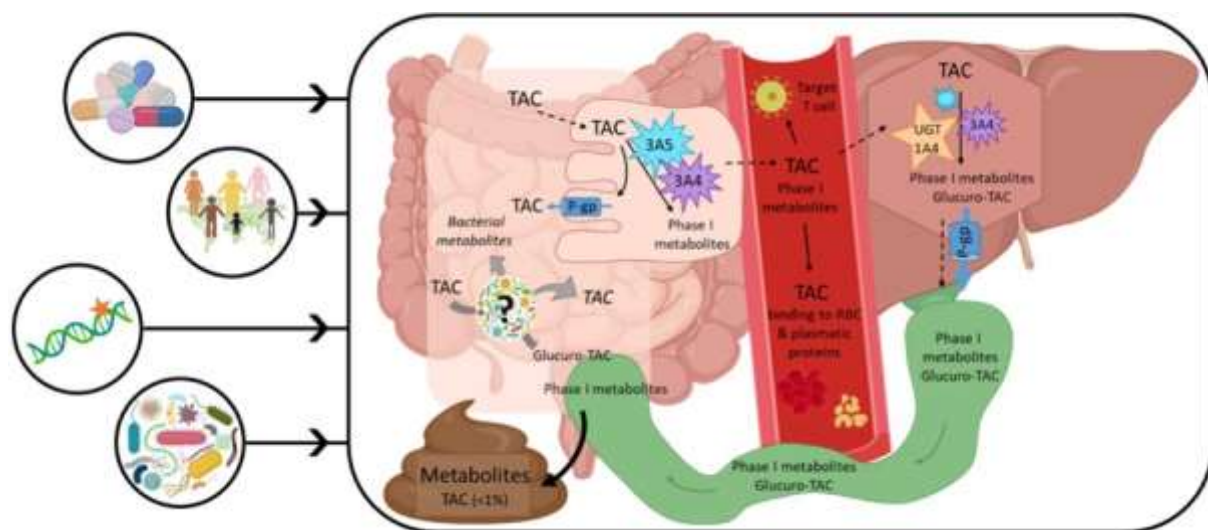


Table 1. Drug-drug interactions affecting tacrolimus pharmacokinetics and associated recommendations. TAC = tacrolimus, ↑ = increased, ↓ = decreased.

CLASS	DRUGS	PK IMPACT	STRENGTH	RECOMMENDATIONS
Antibacterials	Clarithromycin, erythromycin ^[45, 46]	↑ concentration (up to 6-fold)	Strong	Consider substitution for a drug less likely to interact with CYP3A4/5 (e.g. azithromycin, spiramycin).
	Rifampicin ^[71, 72]	↓ concentration (2 to 4-fold)	Strong	Consider substitution for a drug less likely to interact with CYP3A4 (e.g. isoniazide) or increase TAC dosage with blood concentration monitoring.
Calcium Channel Blockers	Diltiazem ^[39] , verapamil ^[40] , nifedipine ^[41] , amlodipine ^[42]	↑ concentration (up to 4-fold)	Strong	Monitor TAC blood concentration (especially if CYP3A5 non-expressers).
Antiretrovirals	Protease inhibitors ^[62-65] (e.g. lopinavir/darunavir + booster (ritonavir, cobicistat))	Extended ↑ concentration (> 10-fold)	Strong	Strong dosage reduction (0.5 – 1mg once a week) with Protease Inhibitors. Monitor TAC blood concentration.
	Efavirenz ^[63]	↓ concentration	Moderate	Monitor TAC blood concentration.
Antifungals	Fluconazole, voriconazole, posaconazole, itraconazole ^[68-70]	↑ concentration (up to 3-fold)	Strong	Consider dose adjustments (reduce by 2/3, except itraconazole monitoring only). Monitor TAC blood concentration, and Long QT prolongation.
Anti-convulsants	Phenobarbital ^[55] , carbamazepine ^[54] , phenytoin ^[51-53]	↓ concentration (up to 2-fold)	Strong	Consider progressive substitution for non-CYP3A4-inducer drug (e.g. gabapentine, pregabalin, but not primidone) or increase TAC dosage, both with blood concentration monitoring. Avoid extended release TAC with phenytoin.
Anti-inflammatory	Colchicine ^[60, 61]	↑ colchicine concentration	Strong	Monitor closely for colchicine toxicity. Consider colchicine dose reduction.
	Corticosteroids ^[58, 59]	↓ concentration	Strong	Monitor TAC blood concentration, when changing doses.
Anti-arrhythmics	Amiodarone ^[66, 67]	↑ concentration (≥ 4-fold)	Strong	Consider substitution for drugs not acting on CYP3A4 (e.g. flecainide, propafenone). Monitor TAC blood concentration, and potential QT interval prolongation.
	Quinidine ^[195]	/		
Hormones	Danazol ^[196] , testosterone ^[197]	↑ concentration	Moderate	Monitor TAC serum concentration, and toxicity.
Gastro-intestinals	Lansoprazole ^[198] , omeprazole, esomeprazole ^[199]	↑ concentration (Up 2 to 3-fold)	Moderate	Monitor TAC blood concentration. Consider substitution (e.g. pantoprazole, rabeprazole).
	Antacids ^[200] (Al(OH) ₃ - MgO - NaHCO ₃)	↓ absorption	Moderate	Consider 2 hours interval between TAC and antacids administration.

Anticoagulants	Rivaroxaban, apixaban ^[201, 202]	↑ concentration (Up to 1.3-fold)	Moderate	Consider substitution (e.g. dabigatran). Monitor anticoagulation. Avoid in patients with renal insufficiencies.
Antimalarials	Mefloquine ^[203]	↑ concentration	Moderate	Monitor TAC blood concentration and potential QT interval prolongation.

Table 2. Demographic factors affecting tacrolimus pharmacokinetics and associated recommendations. TAC = tacrolimus, ↑ = increased, ↓ = decreased, Cl/F = apparent clearance.

FACTOR	PK IMPACT	STRENGTH	RECOMMENDATIONS
Age (> 65 years) ^[27, 81]	↑ concentration (up to 50%) normalised to dose ↓ Cl/F	Strong	Consider dose reduction in elderly, based on regular TAC monitoring. Consider follow-up of TAC-induced neurotoxicity and nephrotoxicity.
Gender ^[25, 26]	/	Weak	Consider dosage adjustment on an mg/kg basis sufficient to limit gender variability.
Ethnicity ^[88-91]	Lower TAC concentration in Afro-American population Higher TAC bioavailability in Asian population	Strong	Ethnic factors highly correlate to genetic polymorphisms, (see pharmacogenetic section).

Table 3. Pharmacogenetic factors affecting tacrolimus pharmacokinetics and associated recommendations. TAC = tacrolimus, ↑ = increased, ↓ = decreased. Cau= Caucasians, As = Asians, Afr = Africans, His = Hispanics.

GENE	SNP	rs#	PK IMPACT	ADDITIONAL REMARKS	ALLELIC FREQUENCIES ^[204]				STRENGTH	REFERENCES
					Cau	As	Afr	His		
ABCB1	1199G>A	rs2229109	↑ TAC cellular concentrations	No effect on blood concentrations	0.03	0.00	0.00	0.03	Moderate	[162, 163, 167, 169]
	3435C>T (1236C>T, 2677G>T/A)	rs1045642 (rs1128503, rs1045642)	↑ TAC cellular concentrations	No clear effect on blood concentrations	0.48	0.60	0.85	0.54	Moderate	[105, 161-169]

CYP3A4	<i>CYP3A4*1B</i>	rs2740574	↑ TAC clearance ↓ TAC exposure	In strong linkage disequilibrium with <i>CYP3A5*1</i>	0.03	0.00	0.77	0.03	None	[99, 100, 104]
	<i>CYP3A4*18B</i>	rs28371759	↑ TAC clearance ↓ TAC exposure	Only relevant in Asians	0.00	0.01	0.00	0.00	Weak	[153-155]
	<i>CYP3A4*20</i>	rs67666821	↓ TAC clearance ↑ TAC exposure	Only relevant in Latinos	0.00	0.00	0.00	0.01	Weak	[156]
	<i>CYP3A4*22</i>	rs35599367	↓ TAC clearance ↑ TAC exposure	Only relevant in Caucasians	0.05	0.00	0.00	0.04	Strong	[103, 111, 118, 128-142]
CYP3A5	<i>CYP3A5*3</i>	rs776746	↓ TAC clearance ↑ TAC exposure	Pre-emptive genotyping tested in 3 independent RCT, CPIC guidelines available	0.06	0.29	0.82	0.07	Strong	[24-26, 31, 94-122]
NR1I2	c.938-17C>T	rs2776707	↑ risk of DGF for TT (kidney genotype)	Not repeated	0.16	0.17	0.02	0.22	None	[172, 173]
	c.-1135C>T	rs3814055	↓ TAC clearance ↑ TAC exposure	↓ PXR activity and expression	0.37	0.22	0.31	0.40	Moderate	[174-176]
POR	<i>POR*28</i>	rs1057868	↑ TAC clearance ↓ TAC exposure	Effect observed in CYP3A5 expressers only	0.30	0.37	0.17	0.28	Weak	[146-149]
PPARα	c.1055-17C>G	rs2276707	None	↓ CYP3A4 activity, ↑ risk of Tac-associated NODAT	0.17	0.47	0.42	0.18	Weak	[151, 152]