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Therapeutic drug monitoring of immunosuppressive drugs in hepatology and gastroenterology

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ABSTRACT

Immunosuppressive drugs have been key to the success of liver transplantation and are essential components of the treatment of inflammatory bowel disease (IBD) and autoimmune hepatitis (AIH). For many but not all immunosuppressants, therapeutic drug monitoring (TDM) is recommended to guide therapy. In this article, the rationale and evidence for TDM of tacrolimus, mycophenolic acid, the mammalian target of rapamycin inhibitors, and azathioprine in liver transplantation, IBD, and AIH is reviewed. New developments, including algorithm-based/computer-assisted immunosuppressant dosing, measurement of immunosuppressants in alternative matrices for whole blood, and pharmacodynamic monitoring of these agents is discussed. It is expected that these novel techniques will be incorporate into the standard TDM in the next few years.

1. Introduction

Therapeutic drug monitoring (TDM) refers to the practice to dose drugs based on their concentrations in biofluids, usually whole blood or plasma. TDM is most often performed for so-called narrow-therapeutic index drugs. These are drugs for which the difference between toxic and effective concentrations is relatively small compared to the different concentrations seen in patients and that are therefore easily over- or under-dosed [1,2]. TDM is now considered standard practice during the treatment with most immunosuppressive drugs after solid organ transplantation [3]. Cyclosporine A (CsA), the first calcineurin inhibitor (CNI), was the first immunosuppressant in transplantation to be dosed following the principle of TDM and this has undoubtedly improved the efficacy and safety of CsA therapy [4]. Nowadays and in addition to CsA, TDM is routinely performed after transplantation for tacrolimus and the mammalian target of rapamycin inhibitors (mTORi) sirolimus and everolimus. However, there is ongoing debate about the benefits of TDM for mycophenolic acid (MPA) and azathioprine [5]. Moreover, TDM is not routinely performed for many immunosuppressants when prescribed for non-transplantation indications, such as inflammatory bowel disease (IBD) and autoimmune hepatitis (AIH), and there remains some debate about how to best perform TDM [6,7]. In this review, the principles of TDM and its use in liver transplantation and other gastro-enterology indications are described. The focus is on the most frequently used immunosuppressive drugs for liver transplantation, IBD, and AIH, namely tacrolimus, MPA, mTORi, and azathioprine.

2. Principle of therapeutic drug monitoring

TDM of a drug can be considered when the following requirements are met [8,9].

(a) *There exists a clear correlation between the concentration of a drug in a bodily fluid and the biological effect of that drug (either efficacy or toxicity)*;
(b) *There exists a small difference between the effective concentration and either the non-effective or toxic concentrations of that drug, i.e. the drug has a narrow therapeutic index.* The therapeutic index is

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generally defined as the ratio of the toxic dose (in 50% of subjects) to the effective dose (in 50% of subjects). As illustrated in Fig. 1, drug A has a wide therapeutic index and TDM is not required. In contrast, drug B has a narrow therapeutic index and should be closely monitored by TDM since the margin between efficacy and toxicity is small. Most immunosuppressants are considered narrow therapeutic index drugs;

(c) *There are no other outcomes reflecting a drug's action that are easier to assess than the drug's concentration.* For example, blood pressure or the glucose concentration when monitoring the effect of antihypertensive drugs and glucose-lowering drugs, respectively;

(d) *There exists a high inter-patient variability in drug exposure following fixed-dosing of that drug.* The phenomenon of a high inter-patient variability following fixed-dosing is illustrated in Fig. 2. Mycophenolate mofetil (MMF) was originally marketed as a fixed-dose drug [10]. However, when prescribed in a one-size-fits-all dose, the resulting MPA concentrations will vary considerably [11]. Drugs that can benefit from TDM should have both a high inter-patient variability and narrow therapeutic index. TDM would not be necessary if the therapeutic index of MPA was wider than its inter-patient variability;

(e) *There exists a small variability in drug exposure within a single patient over time when treated with a stable dose of that drug.* This so-called intra-patient variability (IPV) can be calculated in several ways and describes the fluctuation of a drug's concentration over time when the dose is unaltered (Fig. 3) [12]. TDM of a drug with a high IPV is generally not recommended as the drug concentration measured at a certain time point has little predictive value for the next time point. Patients in whom the drug concentration tends to vary little over time may be suitable candidates for TDM as the measured concentration may accurately predict the concentration on the next occasion;

(f) *The duration of drug treatment must be long enough to benefit from TDM;*

(g) *The analytic methods for the measurement of the drug of interest need to be reliable and standardized.*

In the following paragraphs we will describe the evidence for TDM of tacrolimus, MPA, (mTORi), and azathioprine following these basic principles. Areas of uncertainty and future research directions are described.

3. Tacrolimus

3.1. Pharmacokinetics

Tacrolimus is the mainstay of immunosuppressive therapy after liver transplantation and more recently has been studied in the treatment of active IBD and as second or third line treatment for AIH [13–15]. The drug has a poor bioavailability which averages around 30% [16]. The drug is a substrate of the efflux pump ABCB1 (also known as P-glycoprotein) and the metabolizing enzymes cytochrome P450 (CYP) 3A4 and 3A5. Both ABCB1 and CYP3A are expressed in the intestine and are responsible for tacrolimus' low bioavailability by actively excreting the drug from the enterocyte and substantial first pass-metabolism, respectively. The majority of the absorbed tacrolimus binds to its receptor FK-binding protein-12 (FKBP-12) which has a high concentration in erythrocytes. Of the absorbed tacrolimus, ~80% is located inside erythrocytes, whereas ~15% is plasma-protein bound. The free fraction of tacrolimus is small and is around 0.5% [17].

The systemic metabolism of tacrolimus is dependent on hepatic CYP3A4 and CYP3A5 expression and activity, which differs markedly between individuals. Based on their CYP3A metabolic activity, patients can be classified as poor, intermediate and fast metabolizers (discussed below). Tacrolimus is extensively metabolized by CYP3A and less than 1% of the drug is excreted unchanged in urine and feces. Approximately 95% of the tacrolimus metabolites is excreted via the biliary route, whereas only 2% is excreted by the kidneys [18].

3.2. Monitoring strategies

For TDM of tacrolimus the pre-dose (or trough concentration (C_0)) is most widely used in every day clinical practice. Ideally, the measurement of tacrolimus exposure should be performed by measuring the 12-h (the dosing interval) area under the concentration *versus* time curve (AUC). However, measuring a full AUC is impractical. For tacrolimus, the correlation coefficient between C_0 and AUC in general is acceptable with an r of 0.7 and higher [19,20]. However, this correlation ranges between 0.34 and 0.60 in some patients [21–23]. The problem is that such patients may be considered to have an adequate exposure to tacrolimus if only a C_0 is measured, whereas the true exposure is off target. *Vice versa* some patients may have a C_0 that is outside the target range, whereas their exposure (measured by AUC) is in fact adequate. Reports have been published of patients experiencing acute rejection from inadequate exposure despite having a C_0 within the target range [24].

In clinical practice, C_0 is generally used for TDM because of its feasibility and simplicity, although the correlation with total tacrolimus exposure is not perfect. To better estimate tacrolimus exposure, limited sampling strategies (LSS) have been developed. LSS utilize tacrolimus concentrations measured at 2–3 time points (rather than ≥8 time-points in the full 12-h AUC), to *estimate* an AUC. The correlation coefficient between the full AUC and its estimation by LSS (using multiple linear regression) is good with $r \geq 0.90$ [25]. Moreover, when LSS is combined with Bayesian estimation, which uses information from *a priori* estimated population pharmacokinetic parameters (such as drug clearance

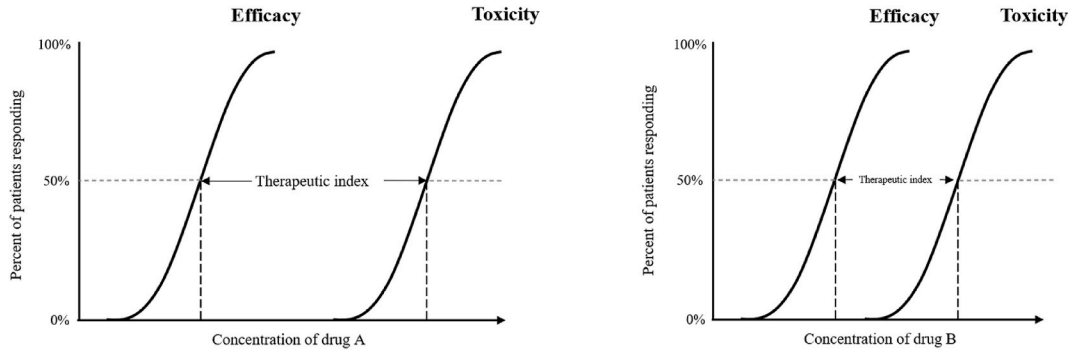

Fig. 1. The therapeutic index. The therapeutic index of a drug is defined as the ratio between the toxic and effective concentration in 50% of the patients. Drug A has a wide therapeutic index, while drug B has a narrow therapeutic index. Therapeutic drug monitoring in general is not necessary for drugs with a wide therapeutic index but is recommend for drugs with a small therapeutic index.

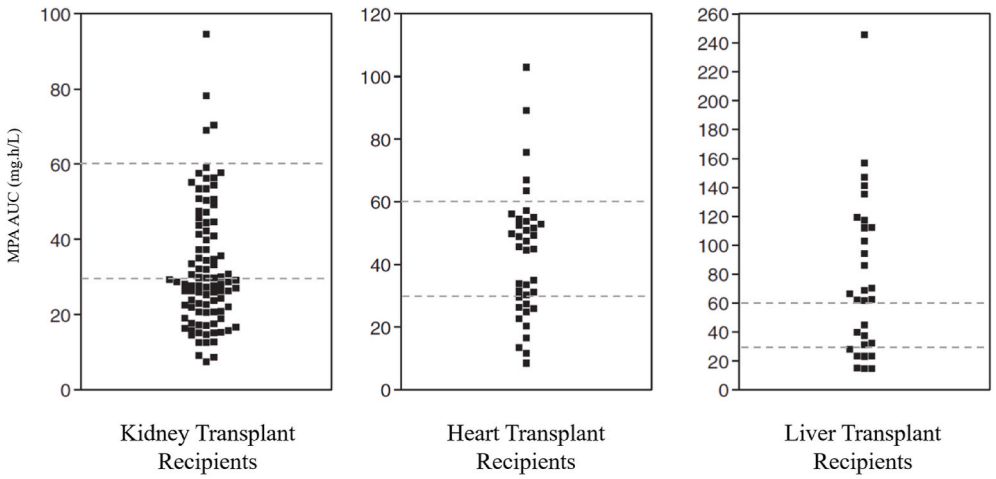

Fig. 2. MPA exposure following fixed-dose MMF. Depicted is the exposure to MPA (measured as area-under the concentration *versus* time curve; AUC) in solid organ transplant recipients in response to MMF 1 g *b. i.d.* The resulting MPA exposure varies greatly between individual patients (reproduced with permission from Shaw et al. ref 11). Dash lines represent the narrow therapeutic index of MPA AUC (30–60 ng h/L).

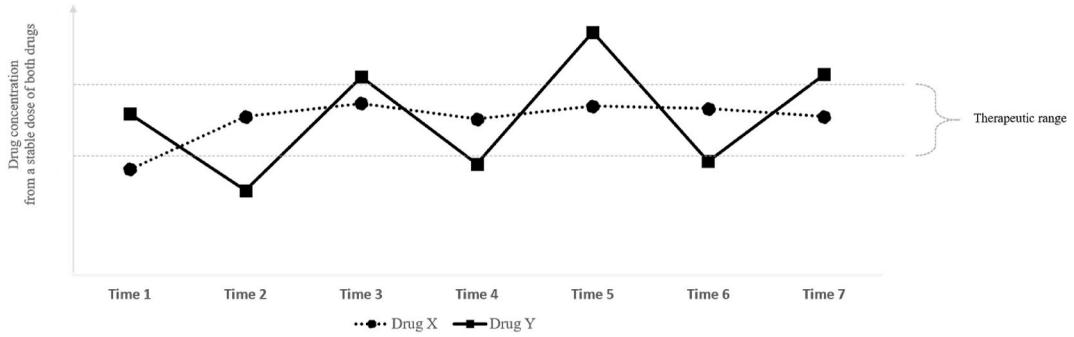

Fig. 3. Intra-patient variability and TDM. Depicted are the concentrations over time of drug X and drug Y. The concentration of drug X is a relatively stable over time with an unaltered dose, while the concentration of drug Y fluctuates greatly despite the patient receiving a stable dose. For drug X TDM may be indicated and because the concentration at a certain time corresponds to the concentration at the next, this is a sensible strategy. The concentrations of drug Y however, have little predictive power for the concentration measured on the next occasion and this drug may therefore not be a suitable candidate for TDM.

and volume of distribution), the individualized AUC for each patient can be generated after fitting the LSS to the population-based model. This enhances the predictive value of LSS for tacrolimus exposure in an individual patient to $r \geq 0.95$ [26]. Ideally, the AUC should be evaluated in every patient before hospital discharge but this might not be practical in all transplant centers. Clinicians can select patients who are suspected of having a poor correlation between their tacrolimus C_0 and AUC, *i.e.* patients who develop toxicity or rejection despite an C_0 within the target concentration range, and these may have an indication for an (abbreviated) AUC measurement.

A number of studies has been conducted to identify pharmacodynamic biomarkers that can be used in combination with classic pharmacokinetic TDM of tacrolimus. These biomarkers are molecules that form part of the pathway targeted by tacrolimus, and include the phosphatase activity of calcineurin, the nuclear translocation of the nuclear factor of activated T cells (NFAT; measured by flow cytometry), NFAT-regulated gene expression, NFAT cytoplasmic 1 (NFATc1) amplification, and interleukin (IL)-2 concentration (measured either by the IL-2 concentration or IL-2 messenger RNA expression). The results from these studies are limited by their relatively small study populations and the fact that these were not controlled clinical studies [27–31]. Most importantly, measuring the tacrolimus concentration is much easier to perform compared to the measurement of these pharmacodynamic biomarkers which requires sophisticated analytic procedures and which have no demonstrated clinical benefits (yet). As a result, none of the markers for pharmacodynamic TDM has currently been implemented in routine clinical practice, although this is a subject of active and ongoing research.

3.3. Concentration-effect relationship

The optimal tacrolimus concentration range that is associated with the lowest incidence of toxicity and rejection is poorly characterized. Several studies have reported conflicting results and have not been able to define the exact cutoffs for the upper and lower limits of the target concentration range [32].

Only target C_0 (not AUC) has been studied in liver transplantation for the concentration-effect relationship. Backman et al. observed that the C_0 of patients with acute rejection *versus* those with stable graft function was within the same range (15–30 ng/mL), but that higher C_0 were associated with more nephrotoxicity and neurotoxicity [33]. As a result, the initial recommended tacrolimus target C_0 for liver transplant recipients used to be 10–15 ng/mL in the first 4–6 weeks, followed by a reduction to 5–10 ng/mL, thereafter [34]. Nashan et al. subsequently demonstrated in a randomized, controlled trial (RCT) that the 1-year incidence of acute rejection, graft loss, and death among liver transplant recipients maintained at a tacrolimus C_0 of 5–8 ng/mL was not different from patients maintained at 10–15 ng/mL [35]. A meta-analysis of 20 RCTs comparing different tacrolimus-based immunosuppressive regimens demonstrated no difference in the acute rejection rate between liver transplant recipients maintained at a C_0 of 10–15 ng/mL compared with 6–10 ng/mL in the first month after

transplantation.[34] [34] The international consensus on managing modifiable risk in transplantation (COMMIT) recommends a tacrolimus C_0 of 6–10 ng/mL in the first month after liver transplantation, which is reduced to 4–8 ng/mL thereafter [36].

Tacrolimus-associated nephrotoxicity is one of the most feared complications after organ transplantation, and occurs in as much as 60% of patients depending on its definition [34]. For tacrolimus-associated nephrotoxicity, a clear and positive correlation has been demonstrated with the tacrolimus C_0 in liver transplant recipients. Lin et al. showed that patients with a tacrolimus C_0 of 5–10 ng/mL in the first week after liver transplantation had a significantly better renal function at 3 months post-transplantation compared with patients who had a C_0 of 10–15 ng/mL [37]. In the "ReSpECT study", a multicenter RCT, patients with reduced-dose tacrolimus (target $C_0 \leq 8$ ng/mL) had a lesser eGFR decline and dialysis requirement at 52 weeks, compared with patients who were assigned to a standard tacrolimus exposure ($C_0 > 10$ ng/mL) [38]. In a meta-analysis, liver transplant recipients with a $C_0 > 10$ ng/mL were found to have an approximately 2-fold higher risk of renal impairment compared with patients maintained at $C_0 < 10$ ng/mL [34].

Ongoing attempts are made to further lower the tacrolimus target C_0 in order to decrease its nephrotoxicity. Strategies to achieve this include the combination of several immunosuppressants with tacrolimus, *i.e.* combining tacrolimus with either MPA or everolimus, as compared with tacrolimus monotherapy (which often requires an exposure $C_0 > 10$ ng/mL) [39]. The recent consensus from the Italian Working Group in liver transplantation recommends that in the first 3 months and in case of a standard immunological risk recipient, the target tacrolimus C_0 should be 3–5 ng/mL when combined with MPA, and 5–10 ng/mL when used together with everolimus (both with the same level of recommendation), and to avoid the use of tacrolimus monotherapy. Three months after liver transplantation, the C_0 can be reduced to 2–3 ng/mL (when combined with MPA) or tapered slowly to 3–6 ng/mL (if used with everolimus) [40]. However, these recommendations require clinical studies to demonstrate their proposed renal benefit and safety in terms of rejection.

Tacrolimus is now considered as the second (or third) line option for the treatment of AIH [41]. However, no study compared the association of different tacrolimus C_0 ranges and clinical outcomes of AIH. The current recommendation from the European Reference Network is to aim for a C_0 of 6–8 ng/mL until full biochemical remission is achieved, which is then tapered to 3–5 ng/mL thereafter [42].

3.4. Inter-patient variability

Demographic factors, drug-drug interactions, and genetics are causes of inter-patient variability. Several single-nucleotide polymorphisms (SNPs) have been identified in the *CYP3A4* and *CYP3A5* genes. The most studied SNP is *CYP3A5*1/*3*. The *CYP3A5*3* variant allele causes alternative splicing, leading to the absence of functional CYP3A5 protein and decreased CYP3A5 activity compared with the *CYP3A5*1* allele. Patients with the *CYP3A5*1/*1* and *CYP3A5*1/*3* genotype are considered CYP3A5 expressors or "rapid metabolizers" and need 1.5–2.0-times higher doses of tacrolimus to achieve the same target concentration compared with CYP3A5 non-expressors (individuals with the *CYP3A5*3/*3* genotype) [43]. In liver transplantation, the effect of *CYP3A5* genotype on enzyme activity is more complicated. Since both the recipient's intestinal and donor's hepatic CYP3A5 contribute to the metabolism of tacrolimus, the genotype of both the donor and the recipient needs to be considered [44]. Two meta-analyses have shown that the recipient's *CYP3A5* genotype influences the tacrolimus dose requirement in the first month post-transplantation, whereas the donor's genotype becomes the major determinant after 1 month [45,46]. This finding is likely explained by the gradually recovering function of the liver allograft.

*CYP3A4*22* is a variant allele which has been associated with decreased CYP3A4 metabolic activity [47]. Based on the combination of *CYP3A4* and *CYP3A5* genotype, rapid (*CYP3A4*1/*1* plus the *CYP3A5*1/*1* or the *CYP3A5*1/*3* genotype), and slow metabolizers (*CYP3A4*1/*22* or *CYP3A4*22/*22* plus *CYP3A5*3/*3*) can be identified. While the rest of the combinations are considered CYP3A intermediate metabolizers [48]. The combination of *CYP3A4* and *CYP3A5* genotype has been studied in kidney transplantation patients only and such information in liver transplantation is lacking.

Several RCTs in kidney transplantation have shown that *CYP3A5* genotype-guided tacrolimus (start) dosing can lead to a more rapid achievement of the target concentration compared with the standard, bodyweight-based starting dose [49–51]. However, this has not been a universal finding [52]. Possibly, more advanced dosing regimens using computerized/algorithm-based dosing can further optimize tacrolimus therapy. A *maximum a posteriori* Bayesian estimation (MAP-BE) technique is currently an accepted method to estimate the AUC that involves the use of large patient databases with concentration-time profiles [53]. Woillard et al. demonstrated that machine-learning algorithms can further improve the accuracy of AUC estimation from MAP-BE [54]. Moreover, the same group of authors has shown that machine-learning algorithms that used population parameters of previously published population pharmacokinetics (instead of the large patient databases used in MAP-BE) can yield a comparable estimation of tacrolimus AUC with less than 5% bias compared with the MAP-BE [55]. However even if such an algorithm-guided tacrolimus dosing strategy is shown to lead a better exposure to the drug, a clinical benefit in terms of less rejection and toxicity, remains to be demonstrated [50,51,56]. For liver transplantation, no studies on *CYP3A5* genotype-guided tacrolimus dosing have been conducted.

Other factors that contribute to inter-patient variability include clinical factors and drug-drug interactions [57]. Tacrolimus is distributed widely in erythrocytes and anemia increases the concentration of unbound tacrolimus, without changing the total whole blood concentration. Patients with hepatic dysfunction have a decreased CYP3A activity and this may result in higher whole blood tacrolimus exposure. Finally, drug-drug interactions interfere with CYP3A enzyme activity and affect tacrolimus exposure (for an extensive review see van Gelder et al.) [58].

3.5. Intra-patient variability

Intra-patient variability in tacrolimus exposure (IPV) denotes the variability in tacrolimus concentrations over time without changes in the dose. IPV can be calculated in several ways but the most frequently used is the coefficient of variability (CV) [12]. Patients having a high IPV have large fluctuations in their exposure to tacrolimus and will likely spend less time within the therapeutic range and more time in the supra-therapeutic or sub-therapeutic concentration ranges.

Medication non-adherence is considered the most common cause of a high tacrolimus IPV, which is usually defined as a CV higher than 25–30%. Leino et al. showed that in a cohort of kidney and liver transplant recipients with 99.9% medication adherence, the tacrolimus CV was 16.8% in the former and 14.4% in the latter group [59]. This information supports the notion that the intrinsic IPV of tacrolimus is low in the absence of medication non-adherence. However, missed-doses and fluctuation of the dosing interval (*i.e.* taking the drug too early or later than scheduled) are not uncommon in the real-word, in addition to other factors that affect tacrolimus IPV, such as the hemoglobin concentration, hypoalbuminemia, gut dysmotility, and drug-drug interactions [57].

Several studies have investigated the association between tacrolimus IPV and kidney transplantation outcomes (reviewed by Shuker et al.) [12]. A high tacrolimus IPV was associated with an increased risk of acute rejection, more *de novo* DSA formation, worse allograft function, more rapid evolution of chronic histologic lesions suggestive of tacrolimus nephrotoxicity, and an increased risk of allograft loss [60,61]. These findings are in line with the hypothesis that patients with a high

tacrolimus IPV will more often be subject to both under- and over-immunosuppression and the related complications of rejection and chronic tacrolimus-induced nephrotoxicity.

In liver transplantation, only a limited number of studies into the association between tacrolimus IPV and clinical outcomes have been conducted. A high tacrolimus IPV after liver transplantation was associated with a higher incidence of infection, acute rejection, *de novo* DSA formation, acute kidney injury, and graft loss [62–64]. These studies were conducted early or immediately after transplantation. To assess the association between tacrolimus IPV and outcomes in a more stable period when there is less interference from other perioperative factors such as bleeding, intestinal dysfunction, drug-drug interaction, and hemodynamic instability van der Veer et al. calculated the tacrolimus IPV between month 6 and month 18 after liver transplantation [65]. The authors found no association between a high IPV and immune-mediated graft injury or graft failure. However, a high tacrolimus IPV was associated with more renal function loss per year in patients with an estimated glomerular filtration rate (eGFR) less than 40 mL/min. This finding suggests that in patients with a low baseline eGFR, a high tacrolimus IPV leads to more annual eGFR loss, possibly from nephrotoxicity resulting from over-exposure.

So far, the most effective strategy to reduce tacrolimus IPV is to improve medication adherence. This may be accomplished by remote drug monitoring and drug-dosing assist software [61,66]. The tacrolimus IPV can also be reduced by switching from twice-daily tacrolimus to the once-daily, extended-release formulation [67]. However, this has not been a universal finding [68]. The lower tacrolimus IPV observed in patients taking the once-daily tacrolimus formulation likely results from the improvement in medication adherence rather than the pharmaceutical property of the drug itself [66].

3.6. Analytic methods

The most frequently used analytical methods to measure tacrolimus in whole blood are liquid chromatography-tandem mass spectrometry (LC-MS/MS) which is the gold standard, or an immunoassay. LC-MS/MS is now slowly replacing immunoassays [69]. The advantage of LC-MS/MS is its high sensitivity and specificity. However, well-trained laboratory personnel is needed and the apparatus is very expensive. An important disadvantage of immunoassays is their cross-reactivity with some of tacrolimus' metabolites which may lead to an overestimation of the true whole blood tacrolimus concentration [48,70].

Although whole blood is the routine matrix for TDM of tacrolimus, more evidence is accumulating that this may not be the best way to measure the drug. Since tacrolimus' site of action is within the immune cells, particularly the lymphocytes, intracellular (intra-lymphocytic) tacrolimus may better correlate with the drug's effect and transplant outcomes [71–73]. The landmark study by Capron et al. included 90 liver transplant recipients who received tacrolimus monotherapy. The authors found no association between the whole blood tacrolimus concentration and liver allograft histological rejection grade. However, the concentration of tacrolimus within peripheral blood mononuclear cells (PBMCs) did correlate negatively with the histological staging of acute liver transplant rejection. There was a poor correlation between the intra-PBMC and the whole blood tacrolimus concentration [71]. However, subsequent studies in liver, kidney, and heart transplantation could not confirm the association between the intracellular tacrolimus concentration and the risk and severity of rejection [74–76]. This may relate to differences in study design and analytical differences in the assays that were used. Currently, the measurement of intracellular tacrolimus is in the initial stage of its development. No cutoff for the intracellular tacrolimus concentration associated with a higher risk for acute rejection has been established. Further studies should also explore the tacrolimus concentration in specific PBMC subsets such as the T-lymphocyte.

Sallustio et al. explored the relationship between the intra-allograft and the whole blood concentration of tacrolimus in kidney transplant recipients [77]. They demonstrated that recipients who developed acute tacrolimus-nephrotoxicity had a significantly higher intra-allograft-to-whole blood ratio of tacrolimus. However, their finding was limited by the small sample size, low incidence of nephrotoxicity, and the different timing of blood sampling and allograft biopsies. Nonetheless, this study demonstrates that the intra-renal tacrolimus concentration is in fact related to its renal toxicity.

Microsample-based tacrolimus concentration monitoring by dried blood spot (DBS) is gaining more attention. This method is patient-friendly, minimally invasive (only a small volume of blood (10–20 μL) is needed), and can be performed by the patient at home. DBS will allow more frequent sampling within a dosing interval and more easily enables the determination of a full AUC [48]. In addition, this method can be used by patients in whom there are contra-indications for the standard venous blood sampling, such as children, those who are difficult to sample, and those living in remote areas. During the coronavirus pandemic, when frequent hospital visits pose a risk to the patient, DBS may serve as the alternative method for TDM. We believe that in the near future, DBS will be more generally used for TDM.

4. Mycophenolic acid

4.1. Pharmacokinetics

MPA is a potent, non-competitive, selective and reversible inhibitor of inosine-5′-monophosphate dehydrogenase (IMPDH). IMPDH is an essential enzyme in the *de novo* purine synthesis pathway, which is essential for lymphocyte proliferation and differentiation. Unlike other cells, lymphocytes cannot utilize the salvage pathway of purine synthesis [78]. Two forms of MPA are used in organ transplantation and immune-mediated disease: the prodrugs mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS). EC-MPS was developed to reduce the gastrointestinal side effects of MMF. However, clinical research has demonstrated that these two formulations differ little in their gastrointestinal side effect profile [79,80]. Although both are prodrugs of MPA, in terms of pharmacokinetics, it is important *not* to consider MMF and EC-MPS as bioequivalent. EC-MPS has a slower absorption, a longer time to maximum concentration (t_{max}), and higher C_0 [5,81–83]. In clinical use, 250 mg of MMF is equivalent to 180 mg of EC-MPS in terms of MPA dose since the mofetil group in MMF is heavier than the sodium atom in EC-MPS.

The pharmacokinetics of MPA is more complex compared with tacrolimus, as MPA undergoes enterohepatic circulation. After ingestion, MMF is rapidly de-esterified to MPA in the stomach, where it is partly absorbed (the remainder is absorbed in the proximal small intestine). In contrast, EC-MPS is hydrolyzed to MPA in the small intestine where it is easily dissolved in the more neutral pH. MPA is distributed mainly in the plasma compartment and up to 97% of MPA is bound to albumin. MPA is metabolized to the pharmacologically inactive 7-*O*-glucuronide of MPA (MPAG) mainly by hepatic UGT1A9, and to a lower extent in the gastrointestinal tract and kidney. MPAG is then excreted via the multidrug resistance-associated protein 2 (MRP2, also known as ABCC2) into the biliary tract [84]. In the gut, MPAG undergoes colonic bacterial de-glucuronidation and is then reabsorbed into the circulation as MPA. This enterohepatic (re)circulation is responsible for 30–40% of the total MPA exposure. The use of drugs that interfere with enterohepatic circulation, such as cholestyramine, can lower the MPA exposure by as much as 40% (Table 1) [85]. Eventually, MPAG is eliminated from the body by glomerular filtration and renal tubular secretion and more than 95% of orally administered MPA is excreted in the urine [86,87]. Both MPA and MPAG have a high albumin-binding capacity (97% and 82%, respectively). Free (unbound) MPA is considered as pharmacologically active. As a result, any condition that interferes with the protein binding of MPA (or MPAG), may result in a change of the pharmacokinetics and pharmacodynamics of free MPA (Table 1) [78,82,

Table 1
Factors affecting MPA exposure.

Variables	Mechanism	Total MPA AUC	Free MPA concentration
Hypoalbuminemia (hyperbilirubinemia)	Decreased protein binding of MPA (bilirubin displaces MPA from albumin)	↓	↔ (Free MPA fraction is increased from the reduction of protein binding, but the free MPA concentration remains the same if renal clearance is intact)
Renal impairment	Decreased renal clearance of MPAG, MPAG displaces MPA from albumin	↑ (increased MPA from MPAG enterohepatic circulation, uremic toxin inhibits UGT enzyme)	↑
		↓ (in presence of cyclosporine that inhibits enterohepatic circulation)	↑
Hepatic impairment	Decreased protein binding of MPA, decreased enterohepatic circulation	↓	↔
Duration after transplantation	Improved albumin, less inflammation	↑	↔
Diarrhea	Impaired absorption	↓	↔
Cyclosporine	Inhibit enterohepatic circulation (MRP2 inhibition)	↓	↓
Corticosteroid	Increased UGT activity	↓	?
Resin, metal ions	Reduced MPA and MPAG absorption in gastrointestinal tract	↓	?
PPI	Reduced MMF absorption in stomach (but not MPS)	↓	?
Cholestyramine	Inhibit enterohepatic circulation	↓	?
Antibiotics	Reduced colonic bacterial glucuronidation (and enterohepatic circulation)	↓	?
Rifampicin	Increased UGT activity (also possibly inhibit enterohepatic circulation via decreased MRP2 function)	↓	↓

88–91]. Patients who have severe renal impairment or hypoalbuminemia are at risk for developing MPA toxicity as they may develop higher concentrations of free MPA despite the same total MPA AUC. Renal insufficiency reduces the clearance of MPAG which subsequently binds to albumin and displaces MPA resulting in a higher free fraction and thus increasing the risk of toxicity. Measuring the free MPA concentration in these patients might increase the accuracy of MPA exposure interpretation [82].

4.2. Monitoring strategies

MPA was initially designed as a one-dose-fits-all drug. However, post-launch studies clearly showed a high inter-patient variability with at least a 10-fold difference in exposure among patients using the same dose, supporting the use of TDM [91]. The pre-dose concentration is a suboptimal surrogate for total MPA exposure since it has a poor-to-moderate correlation with the AUC with a r of only 0.4–0.7, which can be explained by the large effect of enterohepatic circulation [92]. Recent studies have shown that the exposure to MPA may be better predicted by equations based on LSS, with the use of 2–4 sampling points. These LSS equations have a good correlation with AUC with an improved r of 0.90–0.94, and are therefore a more appropriate tool for TDM than C_0 [90,93]. Consequently, LSS is the preferred method for the estimation of MPA AUC in clinical practice, particularly the LSS based on Bayesian estimators [82,89]. However, these estimating methods should be validated in each population before they can be routinely used in that particular population, and should consider co-medication, type of organ transplant, time after transplantation, and genetic background (ethnicity) [83].

With regard to pharmacodynamic monitoring of MPA therapy, IMPDH enzyme activity is the most investigated target biomarker. The activity of IMPDH correlates inversely with the MPA plasma concentration [94]. Many studies in kidney and liver transplant recipients demonstrated that a high pre-transplantation IMPDH activity/gene expression in PBMC is associated with an increased risk of allograft rejection, whereas a low pre-transplant IMPDH activity/gene expression is associated with an increased risk of MPA-related adverse events requiring dose reduction [95–97]. However, TDM based on IMPDH activity in PBMC during the post-transplantation period is more complex. It has been shown that IMPDH activity slowly increases within the first year after transplantation as a biological phenomenon, indicating the need of dynamic cutoff values adjusted for the time after transplantation [96]. Clinical trials that evaluate the role of TDM based on IMPDH activity to guide MPA dosing are lacking and more studies (with adjusted cutoff values at different time points) are needed before IMPDH activity assessment can be utilized in clinical practice. The turn-around time of IMPDH activity measurement also require shortening.

4.3. Concentration-effect relationship

The relationship between MPA exposure and clinical outcomes has been clearly demonstrated in kidney transplantation. Hale et al. found that an MPA AUC within the range of 30–60 mg/L.h was associated with a low risk of acute rejection (10–15%) without excessive drug withdrawal because of adverse effects [98]. This finding was consistent and reproducible, and as a result, the recommended target AUC of MPA is 30–60 mg/L.h for kidney transplantation [91,99]. Data in liver transplantation are limited, and the target MPA AUC is derived from the studies of kidney transplantation (30–60 mg/L.h). There is no study that compared the effect of different MPA AUCs to clinical outcomes in liver transplantation, although there is some evidence from studies using C_0. Tredger et al. observed that in liver transplant recipients (with either cyclosporine or tacrolimus as co-medication) an MPA C_0 of less than 1 mg/L was associated with a 2.5-fold higher risk of acute rejection, whereas an MPA C_0 concentration of 3–4 mg/L was associated with a 3-fold higher risk of leukopenia, infection, and gastrointestinal disturbance [100]. This MPA C_0 range (between 1 and 3.5 mg/L) can be used in liver transplant recipients if the estimation of the MPA AUC (such as from LSS) is unavailable [83]. Data on the TDM of MPA as a second line treatment in AIH are even more scarce and recommended doses have been adapted from organ transplantation [41].

RCTs that compared the outcomes of a TDM-based approach to MPA dosing with a fixed-dose MPA regimen have been conducted in kidney transplantation only. The APOMYGRE study used a target concentration intervention (TCI) strategy which provided a dose optimization feedback loop by using a Bayesian estimator to achieve the target MPA AUC of 40 mg/L.h. The authors successfully demonstrated the benefit of MPA TDM over the fixed-dose regimen as the former strategy lowered the

incidence of acute rejection [101]. No such RCT has been performed in liver transplantation.

4.4. Inter-patient variability

Factors that affect MPA inter-patient variability are summarized in Table 1. The hepatic enzyme UGT1A9 is responsible to more than 50% of MPA metabolism. Several SNPs in *UGT1A9* have been identified and have been associated with increased MPA glucuronidation and a lower total MPA exposure. These SNPs include *UGT1A9 275T > A, 2152C > T, 440C > T*, and *331T > C* [102]. Patients carrying these SNPs need a higher dose of MPA to achieve the same (target) AUC exposure compared with patients who do not carry these variant alleles [89]. However, no clinical benefit of a pharmacogenetics-guided MPA dosing approach has been demonstrated, at least when TDM is performed [90]. When TDM is not practiced for MPA, *UGT1A9* genotyping may have a role and can be used to predict the initial dose of MPA [103].

Other genes that are associated with MPA pharmacokinetics are *UGT1A8* and *UGT2B7*, *SLCO1B1* and *SLCO1B3* (organic anion transporter polypeptides in the hepatocytes), and *ABCC2* (the MRP2 transporter for biliary excretion of MPAG). Genetic variants in *IMDPH1* and *IMPDH2* influence the activity of IMPDH and thus MPA pharmacodynamics. However, studies investigating the effects of variation in these genes have provided inconsistent data and lack clear evidence of a clinical benefit [102,104]. Taken together, at present, little evidence is available to support a pharmacogenetics-guided approach to MPA treatment.

Finally, ethnicity also impacts MPA exposure. Differences in MPA pharmacokinetics are most significant between Caucasians and Asians. A systematic review by Li et al. showed that Asian transplant recipients need a 20–46% lower MPA dose compared with Caucasians or African Americans to reach the same target AUC [105]. In addition, differences between individuals in renal function, plasma protein concentration, and concomitant medication all contribute to inter-patient variability (Table 1) [83].

4.5. Intra-patient variability

The IPV of MPA is generally low with a CV of less than 20%. However, the CV can increase to 25% in patients with renal dysfunction [106]. This low intra-patient variability, combined with its considerable inter-patient variability, supports the use of TDM for MPA.

4.6. Analytic methods

MPA is largely distributed in the extracellular compartment and plasma or serum are the appropriate matrices for the measurement of MPA. Some studies have explored the role of pharmacologically active MPA, including the free MPA and intracellular (intra-PBMCs) MPA, but these studies were limited by their low number of included patients, and the evidence that measuring MPA in an alternative matrix improves clinical outcomes is lacking [83].

Similar to tacrolimus, DBS monitoring of MPA is gaining more interest and is considered as a promising tool for MPA TDM. This method will facilitate the monitoring of MPA therapy by measuring abbreviated AUC. However, the correlation to the MPA concentration measured in venous blood is still required to establish the appropriate therapeutic range for concentrations measured in finger pricks [83].

5. Mammalian target of rapamycin inhibitors

5.1. Pharmacokinetics

Two mTORi have been registered for the prevention of solid organ transplant rejection, sirolimus and everolimus. mTORi bind to the intracellular receptor FKBP12. The mTORi-FKBP12 complex inhibits mTOR kinase and interrupts the intracellular mTOR signaling pathway [107]. The mTOR signaling pathway consists of the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). While both complexes are involved in cell metabolism, mTORC1 mainly regulates cell growth and proliferation, whereas mTORC2 mainly affects cytoskeletal organization and cell migration [108]. In preclinical and animal studies, everolimus was shown to inhibit mTORC2 more potently than sirolimus, while they both have the same potency for mTORC1 inhibition [107].

Everolimus has a higher oral bioavailability than sirolimus (16% *versus* 10%). After ingestion, both drugs are distributed into erythrocytes (75–80% for everolimus and 94% for sirolimus) [109,110]. Both drugs are metabolized by intestinal and hepatic CYP3A4 and are excreted mainly via the biliary route [109,110]. The major difference is in their half-lives which is shorter for everolimus (half-life 28 h; and requires twice-daily administration), compared with once-daily administration in sirolimus (half-life 62 h). This difference also leads to a quicker achievement of the steady state for everolimus [111].

5.2. Monitoring strategies

C_0 allows a reliable estimation of mTORi AUC [112]. The correlation between the C_0 and AUC of everolimus and sirolimus is good with *r* of 0.9 for both drugs [113,114]. The use of a LSS for TDM of mTORi is thus unnecessary in most cases.

mTOR activity is a potential drug-specific pharmacodynamic biomarker. Previous studies examined the phosphorylation of mTORi downstream targets including 4E-BP1, S6 kinase beta-1, p70S6K1 activity, and ribosomal S6 protein [103,115]. The development of these molecules as biomarkers is still in the experimental hypothesis-testing stage. In addition, their measurements have a long turn-around time and are complex and non-standardized. At present, pharmacodynamic monitoring of mTORi therapy is not recommended.

5.3. Concentration-effect relationship

The exposure to mTORi has a clear correlation with its clinical effects and TDM is recommended [116]. Currently, in solid organ transplantation, mTORi are mostly used in CNI-minimization regimens (together with tacrolimus or cyclosporine) to reduce CNI (nephro-) toxicity. Levy et al. showed that an everolimus $C_0 \leq 3$ ng/mL was significantly associated with a higher rate of liver allograft rejection compared with a C_0 of >3 ng/mL when the drug was combined with reduced-dose cyclosporine and corticosteroids [117]. Regarding the upper limit of the everolimus therapeutic window, a $C_0 > 8$ ng/mL has been shown to associate with a higher incidence of dyslipidemia, thrombocytopenia, and proteinuria [111,118]. These findings have been confirmed in an RCT of liver transplantation by the H2304 Study Group. The authors found that the combination of everolimus (target C_0 3–8 ng/mL) and reduced-dose tacrolimus (target C_0 3–5 ng/mL) was not inferior to tacrolimus monotherapy in the control arm (which targeted a tacrolimus C_0 of 8–12 ng/mL) in terms of the composite primary endpoint (which consisted of acute rejection, graft loss, or death). Everolimus with reduced-dose tacrolimus combination therapy also had a significantly lower rate of acute rejection and resulted in a better renal function compared with the tacrolimus monotherapy control arm. However, the drug discontinuation rate was higher in the combination group [119]. Base on this evidence, the target everolimus C_0 when used with reduced-dose tacrolimus is 3–8 ng/mL [40]. The same therapeutic window applies to sirolimus [120,121].

5.4. Inter-patient variability

CYP3A4 is the main metabolizing enzyme of mTORi, hence the same drug-CYP interactions as for CNIs can be expected. Interestingly, the mTORi dose needs to be significantly increased to achieve the target concentration when used together with cyclosporine but not with

tacrolimus [116,118]. This may possibly relate to a relatively larger role of CYP3A4 in the metabolism of both mTORi and cyclosporine, compared with tacrolimus which is mainly metabolized by CYP3A5.

Although it is clear that CYP3A4 is the main metabolizing enzyme of mTORi, the role of *CYP3A4* genetic variation in the pharmacokinetics of mTORi is still controversial [104,116]. Genotyping for *CYP3A5*, which has a smaller role in mTORi metabolism, and *ABCB1*, which influences the intestinal absorption and intracellular concentration of mTORi, has not shown any benefits in terms of mTORi therapy nor with transplant outcomes [104]. Based on the current evidence, no recommendation can be made regarding pharmacogenetics-based TDM of mTORi [116].

5.5. Intra-patient variability

Only a few studies of intra-patient variability of sirolimus and everolimus have been conducted and those are limited to kidney transplantation. Wu et al. found that kidney transplant recipients with chronic allograft nephropathy (CAN) and who had a sirolimus CV >22.9% had a greater risk of progressive deterioration of allograft function [122]. In a study by Valero et al. that explored the CV of mTORi in 279 kidney transplant recipients, the %CV of sirolimus was significantly lower than the %CV of everolimus (23.8% *versus* 27.1%). A %CV >28.5% (for both mTORi) was associated with a lower death-censored graft survival [123]. No study investigating the IPV of mTORi in liver transplantation has been conducted.

5.6. Analytical methods

LC-MS/MS is the preferred method for mTORi quantification. However, commercial immunoassays are still used in centers [116]. The disadvantage of immunoassays is their cross-reactivity between sirolimus and everolimus, and this can lead to confusion when a patient needs to switch between these two drugs. Whole blood is the matrix to measure the mTORi concentration. To date, there is no study that explores the association between the intracellular concentration of mTORi and transplant outcomes. However, since both sirolimus and everolimus are largely distributed in the erythrocytes which are immunologically inactive, the measurement of the free concentration or the intra-PBMC or maybe even the intra-allograft mTORi concentration might be better correlated with transplant outcomes. A study regarding the benefits of monitoring mTORi by DBS is lacking.

6. Azathioprine

Azathioprine is now rarely used as a first-line immunosuppressant for solid organ transplantation due to the superior outcomes with MPA in preventing acute rejection [124]. However, it is the immunosuppressant of choice for pregnant transplant recipients [125], and still has an important role in the treatment of IBD and AIH. Azathioprine has a unique mode of action and pharmacokinetics which is closely linked with its pharmacogenetics.

6.1. Pharmacokinetics

The oral bioavailability of azathioprine ranges from 50 to 72% [126]. Azathioprine is a prodrug which after absorption is rapidly converted to 6-mercaptopurine (6-MP) by glutathione-S-transferase. 6-MP is further metabolized by 3 competing enzymes. The first is xanthine oxidase (XO) which oxidizes 6-MP to inactive 6-thiouric acid which has no toxicity. The second is hypoxanthine guanine phospho-ribosyltransferase (HGPRT) pathway which metabolizes 6-MP to 6-thioguanine nucleotides (6-TGN). 6-TGN are the active metabolites of azathioprine that have the immunosuppressive effect but are also responsible for its myelosuppressive toxicity. 6-TGN incorporates into DNA and RNA and blocks the *de novo* purine synthesis pathway, causing cell death. The third pathway is thiopurine-S-methyl-transferase (TPMT). TPMT converts 6-MP to the hepatotoxic 6-methylmercaptopurine (6-MMP). The activities of XO, HGPRT, and TPMT determine the efficacy and toxicity of azathioprine and its metabolites [127]. Concomitant allopurinol use strongly inhibits the XO pathway and causes a shunting of 6-MP metabolism into the other two pathways which may lead to life-threatening toxicity More than 95% of azathioprine metabolites are excreted by the kidney [128].

6.2. Monitoring strategies

The time to steady state of azathioprine is longer than for other immunosuppressants (2–4 weeks) [129]. Standard laboratory monitoring of azathioprine treatment includes complete blood count (CBC) and liver function tests to detect leucopenia and hepatitis. It is recommended to monitor every 2 weeks in the first 2 months after starting azathioprine followed by every 3 months thereafter [130]. The British Society of Gastroenterology consensus recently recommended that during azathioprine treatment of IBD, thiopurine metabolites should also be monitored to prevent toxicity and inadequate dosing [131]. However, no recommendation can be made regarding liver transplantation or AIH since all the evidence comes from the studies of IBD. The monitoring of thiopurine metabolites can be done by measuring 6-TGN and 6-MMP concentrations in erythrocytes. The azathioprine dose can then be adjusted, aiming to maintain a normal range of both 6-TGN and 6-MMP [131]. This is strongly recommended in patients with inadequate response to therapy or toxicity. However, the monitoring of thiopurine metabolites does not replace the standard laboratory evaluation as described above.

6.3. Concentration-effect relationship

The appropriate timing of 6-TGN and 6-MMP measurements is 12–16 weeks after the initiation of azathioprine treatment, the timing when these metabolites have reached their steady state in patients with IBD. The target metabolite concentration has been studied and the recommended range is provided in the British Society of Gastroenterology guideline [130,131]. Briefly, the therapeutic range of 6-TGN, the main metabolite that has immunosuppressive effect, is 235–450 pmol per 8×10 [8] red blood cells (RBC), with concentrations below or above this range indicating sub-therapeutic and supra-therapeutic level, respectively. However, the interpretation of 6-TGN should be done while taking into account the level of 6-MMP, which is the metabolite of the TPMT pathway. The normal concentration of 6-MMP is < 5700 pmol per 8×10 [8] RBC, indicating appropriate metabolism of azathioprine to 6-TGN but not 6-MMP. Patients with 6-TGN within the therapeutic range but 6-MMP >5700 pmol per 8×10 [8] RBC should decrease their azathioprine dose. Patients with 6-MMP >5700 pmol per 8×10 [8] RBC despite of 6-TGN <235 pmol per 8×10 [8] RBC are considered as having a predominant methylation pathway, which results in less 6-TGN and more 6-MMP, which in turn increases the risk for reduced efficacy and hepatotoxicity. The solution is to reduce the azathioprine dose to 25–33% of the usual dose and to add allopurinol. Allopurinol in this case helps to shunt the metabolic pathway to produce more 6-TGN [131]. The above-mentioned recommendations come from a study in patients with IBD, but no data on organ transplantation are available.

Unlike IBD, TDM of thiopurine metabolites in the treatment of AIH is still controversial and no standard cutoff levels have been defined, although there is evidence to show that patients with biochemical remission have significantly higher mean 6-TGN concentrations compared to those who are not [132]. Clinicians may evaluate 6-TGN and 6-MMP concentration in selected patients with AIH who do not respond well to a standard dose of azathioprine or whenever they experience toxicity. In those cases it is reasonable to target the same therapeutic window as for IBD [133].

6.4. Inter-patient variability

The inter-patient variability of azathioprine is much larger than the intra-patient variability [134]. TPMT testing is now generally recommended before the start of azathioprine [131,135,136]. This can be done by either an enzyme activity assay or *TPMT* genotyping. Zarca et al. found that TPMT activity testing is more cost-effective compared with genotyping in the French population [137]. TPMT activity is measured in erythrocytes and therefore, a recent blood transfusion can interfere with this test [127]. On the other side, genotyping of *TPMT* can miss the rare variants that are not generally tested, and it does not quantify TPMT enzyme activity. *TMPT*2*, *TMPT*3A*, and *TPMT*3C* account for 90% of the low activity phenotype in patients of European descent and predict leukopenia. The starting dose of azathioprine should be adjusted according to TPMT activity. Patients with very low or absent TPMT activity (homozygous non-functional alleles) should avoid using azathioprine. Patients with intermediate activity or heterozygous deficiency should use a 50% lower dose than patients with normal TPMT activity to prevent leukopenia [135,138].

Another important genotype to determine before azathioprine initiation is nudix hydrolase 15 (*NUDT15*). This enzyme converts 6-TGN triphosphate (more cytotoxic) to 6-TGN monophosphate (less cytotoxic). A poor metabolizer phenotype of *NUDT15* is responsible for myelosuppression in the Asian population. Consequently, the genotyping of *NUDT15* is suggested before the starting of azathioprine in patients of Asian descent [131,135].

6.5. Analytic methods

The analytic methods by Lennard et al. and Dervieux et al. are the 2 most commonly used methods for the monitoring of thiopurine metabolites. The Dervieux method results in a 2.6-fold higher 6-TGN concentration compared with the method by Lennard [139]. The above-mentioned therapeutic ranges are derived from studies using the Lennard method. As a result, clinicians should be aware of the analytic method used in their laboratory when thiopurine metabolite concentrations are interpreted.

7. Conclusions

There is an important lack in our knowledge of the optimal strategy of immunosuppressant TDM for gastro-enterologic diseases and to a lesser extent, liver transplantation. Most of the evidence regarding the benefits of TDM comes from studies that were performed among kidney transplant recipients and these may not be extrapolated to other indications. We believe that optimal dosing of these agents may be achieved through the use of dosing algorithms (computerized dosing) which incorporate pharmacogenetic (*e.g. CYP3A4* and *CYP3A5*) and clinical data (*e.g.* age and body surface area) [51]. This is an active field of research in kidney transplantation and it is expected that the results of these studies will make their way into other fields of medicine.

More recently, the IPV of tacrolimus has been identified as a prognostic marker for adverse outcomes in kidney and liver transplantation. It is likely that this will be pursued further and that IPV (at least for tacrolimus) will be adopted into everyday clinical practice as an easy-to-use predictor for tacrolimus treatment. Automatic calculation of the IPV from a electronic patient file is possible and feasible.

The development of novel blood sampling techniques, such as dried blood spot monitoring, will facilitate more detailed pharmacokinetic sampling and home monitoring, and may allow for more personalized immunosuppression. In many centers, the COVID-19 pandemic has fueled remote monitoring of immunosuppressants and we feel that this may become the standard for selected groups of patients. The measurement of the intra-allograft/intra-lymphocytic immunosuppressant concentrations is a promising strategy as it may better correlate with an immunosuppressant's efficacy and toxicity than the whole blood concentration. However, these alternative strategies of TDM need to be much better investigated.

In conclusion, TDM is required for most immunosuppressants that are currently used in liver transplantation, IBD, and AIH. The knowledge of the pharmacokinetics, pharmacodynamics, and pharmacogenetics of these agents has greatly expanded over the last 20 years and we now have tools that allow for a further individualization of immunosuppressive therapy and the monitoring thereof.

Practical points

- Therapeutic drug monitoring (TDM) of tacrolimus and mammalian target of rapamycin inhibitors (mTORi) is standard of care after liver transplantation.
- TDM is increasingly used in liver transplantation and gastroenterology for mycophenolic acid (MPA) and azathioprine.
- For tacrolimus and mTORi the pre-dose concentration (C_0) is suitable for routine TDM.
- The MPA C_0 has a poor correlation with total exposure and LSS is the preferred method for TDM of MPA.
- In patients who have experienced drug-related toxicity or rejection despite the tacrolimus C_0 being within the therapeutic range, an (abbreviated) AUC measurement should be considered.
- Currently, pharmacodynamic biomarkers of immunosuppressive drug therapy are in the developmental stage and cannot be recommended for TDM in clinical practice yet.
- The use of novel analytic methods such as dried blood spot analysis is expected to increase in the next few years.

Research agenda

- The efficacy of dosing algorithms for tacrolimus should be studied further and comparing computerized-dosing to conventional (physician-guided dosing) are needed.
- The value of intracellular concentration of immunosuppressants (tacrolimus, mTORi, and MPA) requires further study to demonstrate the correlation with transplant outcomes.
- The benefit of TDM-guided over fixed-dosing MPA only exists in kidney transplantation and requires more studies in liver transplantation.
- The monitoring of azathioprine's metabolites needs further study to demonstrate the benefit in liver transplantation.
- Pharmacodynamic biomarkers should be further explored to be used as a real-time representative of patient's immune status, which could add values to TDM.

Declaration of competing interest

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