Clinical significance of personalized tacrolimus dosing by adjusting to donor CYP3A-status in liver transplant recipients

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Aims
Donor’s CYP3A-status (CYP3A5 genotype and CYP3A4 expression) can provide prognostic information regarding tacrolimus-metabolizing capacity of the liver graft and initial tacrolimus dosing for therapeutic blood concentrations in liver transplants. The present work prospectively investigated whether CYP3A-status guided tacrolimus therapy has any potential clinical benefit for recipients in the early postoperative period.

Methods: The contribution of preliminary assaying of donor CYP3A-status to the optimization of initial tacrolimus therapy and to the reduction of adverse events (acute rejection, infection, nephrotoxicity) was investigated in 112 liver transplant recipients (CYPtest group) comparing to 101 control patients on tacrolimus concentration guided therapy.

Results: The time for achieving therapeutic tacrolimus concentration was significantly reduced, confirming potential benefit of initial tacrolimus therapy adjusted to donor’s CYP3A-status over classical clinical practice of tacrolimus concentration guided treatment (4 vs 8 days, P < 0.0001). Acute rejection episodes (3.6 vs 23.8%, P < 0.0001) and tacrolimus induced nephrotoxicity (8 vs 27%, P = 0.0004) were less frequent in CYPtest group than in control patients, whereas occurrence of infectious disease was not influenced by tacrolimus dosing strategy (3.6 vs 5.9% in CYPtest and control groups, P > 0.05). Acute rejection was often accompanied with tacrolimus blood concentrations lower than 10 ng mL⁻¹ (20/24 of control and 2/4 of CYPtest patients), while nephrotoxicity was associated with high tacrolimus concentrations (>20 ng mL⁻¹) in the first week after transplantation (13/27 of control and 2/9 of CYPtest patients).

Conclusion: CYP3A-status guided therapy significantly improved the risk of misdosing induced early adverse effects (acute rejection, nephrotoxicity).

KEYWORDS
acute rejection, CYP3A4 expression, CYP3A5 genotype, donor CYP3A-status, infection, liver transplant recipients, nephrotoxicity, tacrolimus

The authors confirm that the PIs for this paper are Katalin Monostory and László Kóbori, and that the PIs had direct clinical responsibility for patients.

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1 | INTRODUCTION

Liver transplantation is an accepted treatment option for patients with end-stage liver disease. Improvements in surgical techniques, anaesthesiology, intensive care and immunosuppression have led to increasing graft/patient survival, and further efforts are made to minimize the risk of graft dysfunction.1–4 Recipients’ drug therapy primarily focuses on immunosuppression and control of allograft rejection as well as on prevention of infections and avoidance of adverse effects of immunosuppressants. The cornerstone of maintenance immunosuppressive regimens is calcineurin inhibitor therapy, using tacrolimus, the most prominent agent.5,6 Tacrolimus displays a narrow therapeutic range and large pharmacokinetic variability among individuals; therefore, continuous monitoring of blood concentrations is essential for optimal therapeutic efficacy.7 Tacrolimus is a substrate of ABCB1 transporter and CYP3A enzymes; therefore, functional polymorphisms of these proteins are suggested to affect tacrolimus bioavailability. However, the association between various single nucleotide polymorphisms of ABCB1 and tacrolimus pharmacokinetics in liver transplantation is debated. Wei-lin et al. demonstrated the importance of polymorphisms of intestinal (recipient) ABCB1,9 whereas no appreciable influence of either liver (donor) or intestinal (recipient) ABCB1 genotypes on tacrolimus dose-requirement or concentration/dose ratios was found by other authors.9–12 In recipients, tacrolimus exposure appears to depend on the hepatic and intestinal tacrolimus-metabolizing capacity. The crucial enzymes in tacrolimus metabolism are CYP3A enzymes, and variability in CYP3A activities between individuals, from extremely slow to ultrafast is therefore of particular relevance in postoperative drug therapy.13 Genetic polymorphisms of CYP3A enzymes can basically contribute to interindividual variations that is further modulated by internal (age, hormonal status, disease) and environmental factors (comedication, nutrition), resulting in a transient switch into poor or extensive metabolizer phenotype due to phenoconversion.14,15 More than 100-fold interindividual variability was observed in hepatic CYP3A4 activity16; however, the clinical relevance of CYP3A4 genetic polymorphisms is considered to be low. The major underlying reason of interindividual variation is the inhibition or transcriptional induction of CYP3A4 due to concomitant drug treatment or environmental factors.17 In contrast to CYP3A4, CYP3A5 expression is highly polymorphic. The frequency of CYP3A5*3 allele, resulting in the absence of functional CYP3A5 enzyme, is 88–97% in white populations.13 Patients carrying functional CYP3A5*1 allele can metabolize tacrolimus at high rates; therefore, in liver transplantation, the association between tacrolimus pharmacokinetics and CYP3A5 genotypes of both the liver (donor) and the intestine (recipient) has been investigated. In full-size liver transplantation, tacrolimus concentration/dose ratios seem to be influenced by the donor CYP3A5 genotype rather than by the recipient in the early (<1 month after transplantation) and late postoperative period (> 1 month).9,10,18,19; however, some authors have suggested that both the donor and the recipient CYP3A5 genotypes are of major impact on tacrolimus clearance.12,20 In living-donor liver transplantation, due to the fact that the grafted liver regenerates its mass and tacrolimus clearance gradually increases with post-transplant time, the pharmacokinetics primarily affected by the intestinal (recipient) CYP3A5 genotype early after transplantation, and by the liver graft or by both the recipient and the donor genotypes at late postoperative time.21–25 Conditions as a consequence of non-balanced suboptimal immunosuppressive therapy can lead to allograft rejection episodes, whereas supraoptimal therapy can result in nephrotoxicity or increased susceptibility to infections.6,26 One of the causes, associated with these conditions, is tacrolimus-metabolizing capacity of the graft; therefore, any factor that can modulate tacrolimus blood concentrations influences the outcome of transplantation.17

Drug-metabolizing capacity of the liver graft can be estimated by the evaluation of CYP-status. We have previously described a complex diagnostic system (CYPtest) that estimates hepatic CYP activities and drug-metabolizing capacity by combining CYP genotype and current CYP expression in leukocytes.16 CYP mRNA levels in leukocytes of those subjects who do not carry loss-of-function or gain-of-function alleles were proven to reflect hepatic CYP activities. CYP3A5 genotyping identifies the genetically determined CYP3A5 expresser or nonexpresser grafts, whereas CYP3A4 expression in donor leukocytes can estimate reduced or increased CYP3A4 activities in liver grafts. In liver transplant patients, it was

What is already known about this subject

- CYP3A enzymes are the main catalysts of tacrolimus metabolism.
- Donor’s CYP3A-status (CYP3A5 genotype and CYP3A4 expression) provides prognostic information regarding tacrolimus-metabolizing capacity of the liver graft.
- Recipients transplanted with low or high CYP3A4 expresser grafts or with grafts carrying CYP3A5*1 required substantial modification of the initial tacrolimus doses.

What this study adds

- Initial tacrolimus therapy adjusted to donor’s CYP3A-status has potential benefit over classical clinical practice of tacrolimus concentration guided treatment.
- CYP3A-status controlled therapeutic strategy facilitates rapid tacrolimus dose-finding in liver transplant recipients.
- CYP3A-status guided therapy improves the risk of mis-dosing induced early adverse effects (acute rejection, nephrotoxicity).
clearly demonstrated that CYP3A4 expression rates of donors combined with CYP3A5 genotypes influenced tacrolimus blood concentrations in recipients in the early post-transplant period. The recipients with liver grafts from low or high CYP3A4 expressers or with grafts carrying CYP3A5*1 required substantial modification of initial tacrolimus doses. The donor’s CYP3A-status (CYP3A5 genotype and CYP3A4 expression) is capable of identifying the risk of tacrolimus over- or under-exposure, and can provide significant information for appropriate initial dosage in the early period. Our present work aimed to prospectively investigate whether preliminary assaying of CYP3A-status of liver grafts and CYP3A-status guided tacrolimus therapy have potential clinical benefit for recipients (e.g. time for target tacrolimus concentration, hospitalization time), and whether personalized drug therapy can reduce the risk of misdosing induced adverse reactions (rejection episodes, infection, poor kidney function).

2 METHODS

2.1 Patients and study design

Adult patients (n = 226) who underwent liver transplantation were enrolled in this prospective, randomized-controlled study carried out at the Department of Transplantation and Surgery, Semmelweis University (Budapest, Hungary). Patients (n = 13) who were transplanted with a partial liver graft either from deceased or living donors or underwent re-transplantation were excluded from the study. CYPtest of donors and the study protocol were approved by the Hungarian Committee of Science and Ethics. Each recipient gave the informed consent to participate in the study. The patients’ demographic and clinical data (Table 1) as well as the details of tacrolimus therapy (dosage and predose blood concentrations) were recorded. Both the donors and the recipients belonged to the Caucasian (white) population. The post-transplant drug therapy except for tacrolimus was applied according to the conventional clinical protocol which included immunosuppressants and anti-inflammatory agents as well as prophylactic medications, such as antibiotics (sulfamethoxazole–trimethoprim, ciprofloxacin, meropenem), antiviral (ganciclovir, valganciclovir) and antifungal drugs (amphotericin B, fluconazole), acid-reducing agents (famotidine, pantoprazole), and, if necessary, analgesics/anaesthetics (propofol).

2.2 CYP3A-status of the liver grafts

The estimation of CYP3A-status of liver grafts in CYPtest group was assayed in donors’ peripheral blood samples obtained at the time of explantation. Genomic DNA and leukocytes were isolated from blood samples according to the methods described by Temesvári et al. CYP3A5 genotyping was carried out by hydrolysis single nucleotide polymorphism analysis for CYP3A5*3 using TaqMan probes (BioSearch Technologies, Novato, CA, USA) as previously described.

For CYP3A4 expression, total RNA was extracted from leukocytes, RNA (3 μg) was reverse transcribed into single-stranded cDNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA), and then real-time polymerase chain reaction with human cDNA was performed using KAPA Probe Fast qPCR Mastermix kit (KAPA Biosystems, Cape Town, South Africa) and TaqMan probes for CYP3A4 and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Microsynth AG, Balgach, Switzerland). The quantity of CYP3A4 mRNA relative to that of GAPDH was determined. Three categories of CYP3A4 expression were applied to describe low, normal and high expressers. The cut-off values for the CYP3A4 mRNA levels in leukocytes have been previously established on the basis of the cut-off values for the hepatic CYP3A4 activities (nifedipine oxidation or midazolam 1- and 4-hydroxylation). Low expressers displayed a CYP3A4/GAPDH ratio <10⁻⁶, normal expressers a ratio between 10⁻⁶ and 10⁻⁴, and high expressers a ratio >10⁻⁴.

### Table 1

| Recipients’ data | CYPTest group | Control group |
|------------------|---------------|---------------|
| Number           | 112           | 101           |
| Sex: male/female (%) | 62/50 (55.4%/44.6%) | 64/37 (63.4%/36.6%) |
| Age at the time of transplantation (y) | | |
| Median (range)   | 50.5 (42, 56) | 49 (36, 53) |
| Bodyweight (kg, median (range)) | 75.5 (65, 87.6) | 76 (65, 84) |
| Primary liver disease | | |
| 1. Acute liver failure | | |
| Drug-induced      | -             | 2 (2.0%)      |
| Mushroom poisoning| -             | 1 (1%)        |
| Other             | 1 (0.9%)      | 2 (2.0%)      |
| 2. Chronic liver disease | | |
| Hepatitis C       | 46 (41.4%)    | 38 (37.6%)    |
| Hepatitis B       | 6 (5.4%)      | 4 (4.0%)      |
| Alcohol-related liver disease | | |
| Primary sclerosing cholangitis | 16 (14.3%) | 9 (8.9%) |
| Primary biliary cirrhosis | 3 (2.7%) | 5 (5.0%) |
| Autoimmune hepatitis | 4 (3.6%) | 3 (3.0%) |
| Congenital fibrosis | 3 (2.7%) | 3 (3.0%) |
| Cryptogenic cirrhosis | 3 (2.7%) | 3 (3.0%) |
| Tumour: hepatocellular carcinoma | 4 (3.6%) | 2 (2.0%) |
| Tumour: others   | 2 (1.8%)      | 4 (4.0%)      |
| Wilson’s disease  | 1 (0.9%)      | 7 (6.9%)      |
| Others           | 5 (4.5%)      | -             |

Primary patients (n = 13) who were transplanted with a partial liver graft either from deceased or living donors or underwent re-transplantation were excluded from the study. CYPtest of donors and the study protocol were approved by the Hungarian Committee of Science and Ethics. Each recipient gave the informed consent to participate in the study. The patients’ demographic and clinical data (Table 1) as well as the details of tacrolimus therapy (dosage and predose blood concentrations) were recorded. Both the donors and the recipients belonged to the Caucasian (white) population. The post-transplant drug therapy except for tacrolimus was applied according to the conventional clinical protocol which included immunosuppressants and anti-inflammatory agents as well as prophylactic medications, such as antibiotics (sulfamethoxazole–trimethoprim, ciprofloxacin, meropenem), antiviral (ganciclovir, valganciclovir) and antifungal drugs (amphotericin B, fluconazole), acid-reducing agents (famotidine, pantoprazole), and, if necessary, analgesics/anaesthetics (propofol).
2.3 | Immunosuppressant therapy and drug monitoring

Tacrolimus therapy was started 6 hours after transplantation and tacrolimus (Prograf, Astellas Pharma) was given twice a day. The daily dose was defined as the sum of the morning dose, given after blood sampling for trough blood concentration measurement, and the evening dose administered after 12 hours. In CYPTest group (n = 112), the patients' tacrolimus therapy was guided by the donors' CYP3A-status:27; (i) patients transplanted with CYP3A5 expresser grafts carrying CYP3A5*1/*3 or CYP3A5*1/*1 genotype received 0.2 mg kg\(^{-1}\) bodyweight of tacrolimus; (ii) whereas for those with CYP3A5 non-expressor grafts (CYP3A5*3/*3), tacrolimus therapy was adjusted to the donors' CYP3A4 expression. Normal dose (0.1 mg kg\(^{-1}\) bodyweight) was given to the patients transplanted with grafts expressing CYP3A4 at normal level (10\(^{-6}\) to 10\(^{-4}\)), reduced dose (0.05 mg kg\(^{-1}\) bodyweight) was administered to the recipients with grafts expressing CYP3A4 at low level (<10\(^{-6}\)), whereas increased dose (0.2 mg kg\(^{-1}\) bodyweight) was targeted in patients with high CYP3A4 expresser liver grafts (>10\(^{-4}\)). In the control group (n = 101), the initial tacrolimus dose was adjusted to the recipients' bodyweight (0.1 mg kg\(^{-1}\)) and thereafter controlled by the predose tacrolimus blood concentrations (C\(_{0}\)) according to the conventional clinical protocol. Oral tacrolimus dosage was adjusted to a target therapeutic window in the blood concentration range of 10–15 ng mL\(^{-1}\). For control patients, an algorithm of (target C\(_{0}\)/current C\(_{0}\) × current dose) for calculation of daily tacrolimus dose was applied. The immunosuppressant therapy based on tacrolimus was applied in combination with mycophenolate mofetil and steroid (methylprednisolone). Mycophenolate mofetil was applied at the daily dose of 2 g at the early postoperative period, whereas the initial methylprednisolone dose of 1 g was administered at the time of operation, and the subsequent doses were gradually tapering (500–250–125–32 mg day\(^{-1}\)) to a maintenance daily dose of 32 mg in the first week and of 20 mg day\(^{-1}\) thereafter.

The blood samples for drug assay were taken from the patients before the morning dose (C\(_{0}\), 12-h postdose trough concentrations), and tacrolimus concentrations were assayed routinely (every day in the first week and every second day from the second week). Tacrolimus doses administered to the control patients were modified if the exposure was out of the target range of 10–15 ng mL\(^{-1}\). The blood concentrations were measured using enzyme immunoassay techniques for tacrolimus (TACR Flex Dimension Dade Behring Inc., Newark, DE). The assay range for tacrolimus was 1.1–33.6 ng mL\(^{-1}\). The intra- and interday variability for the quantification was <10%. Predose concentrations were calculated by dividing the C\(_{0}\) by the corresponding 24-hour dose on a mg/kg bodyweight basis.

2.4 | Graft function and tacrolimus-related adverse events

The primary endpoint was the time from the initiation of tacrolimus therapy until the first day of at least 3 consecutive days with tacrolimus C\(_{0}\) within the target range (time to achieve target C\(_{0}\)). The proportion of patients achieving the target tacrolimus concentration was recorded as well. Furthermore, biochemical parameters for graft function (serum aspartate transaminase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, total and direct bilirubin, prothrombin time, albumin) and signs for any tacrolimus-related adverse effects, such as nephrotoxicity, infection and rejection, were recorded. Early graft function abnormalities were diagnosed as initial poor function28 as primary non-function.29,30 Acute kidney injury manifested in deterioration of renal function was defined by an increase in serum blood urea nitrogen and creatinine levels and by a reduction of estimated glomerular filtration rate. Renal impairment due to surgery-related events (in patients having long, complicated surgical procedure with substantial blood loss), hypotension, septic ischaemia (patients with haemodynamic instability associated with an increase in CRP level and a positive blood/urine culture) or hepatorenal syndrome (in patients with pretransplant poor kidney function) was not considered to be tacrolimus induced kidney dysfunction. Apart from these complications, tacrolimus related nephrotoxicity was considered if an initial increase in the serum creatinine level was >0.5 mg/dL above the pretransplant baseline.31 For the diagnosis of acute rejection, abnormal liver function parameters (elevation of hepatic enzymes in serum—transaminases, alkaline phosphatase and γ-glutamyl transferase—and/or of serum bilirubin concentration) were always confirmed by histopathological evaluation of liver biopsy samples.32 Banff schema was applied for grading of rejection.33

2.5 | Statistical analysis

Statistical analysis of biochemical and clinical parameters as well as of tacrolimus blood concentrations was carried out using GraphPad Instat (v3.05, GraphPad Software, San Diego, CA, USA). Parameter distributions were analysed by Kolmogorov–Smirnov test. Between-group differences were calculated by Mann–Whitney U-test. The benefit of donor CYP3A-status guided tacrolimus therapy over the classical dosing was also evaluated by comparing the ratio of patients with therapeutic tacrolimus concentrations and the length of intensive care and hospital stay in CYPTest group with those in control group. The frequencies of early allograft dysfunction and tacrolimus-related adverse events (poor kidney function, infection, rejection episodes) were also compared. The differences between the CYPTest and control groups were calculated by Fisher’s exact test. A 2-tailed P-value <0.05 was considered to be statistically significant.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the
common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

3 | RESULTS

3.1 | Potential benefit of assaying donor’s CYP3A-status in initial tacrolimus therapy

Of 112 donors, 111 carried 1 or 2 CYP3A5*3 alleles (22 donors with CYP3A5*1/*3 and 89 donors with CYP3A5*3/*3 genotype; Table 2), displaying allele frequency (89.3%) similar to that in Caucasian white populations (88–97%). Liver grafts with wild-type CYP3A5 allele (CYP3A5*1) were considered to be extensive tacrolimus-metabolizers (20.5%) requiring increased tacrolimus dose (0.2 mg kg\(^{-1}\) bodyweight) in recipients, whereas CYP3A5 nonexpresser grafts (CYP3A5*3/*3) displayed poor, normal or extensive tacrolimus metabolism depending on their CYP3A4 activities. Hepatic CYP3A4 activities of the CYP3A5 nonexpresser liver grafts were estimated on the basis of CYP3A4 mRNA levels in donors’ leukocytes (Table 2). The classical clinical protocol suggests initial tacrolimus dose of 0.1 mg kg\(^{-1}\) bodyweight. However, patients transplanted with liver grafts carrying CYP3A5*3/*3 and expressing CYP3A4 at low level (31.3%) required reduced tacrolimus dose (daily dose of 0.05 mg kg\(^{-1}\) bodyweight), whereas for those recipients who were transplanted with grafts from high CYP3A4 expresser donors (8.9%) increased tacrolimus dose (0.2 mg kg\(^{-1}\) bodyweight) was targeted. The tacrolimus blood concentrations in patients of the control and CYPtest groups achieved the target therapeutic range in the 20-day post-transplantation period, although with different rates (Figure 1A and B). Despite the fact that modified initial tacrolimus dose was administered to more than half of the patients (n = 68) in the CYPtest group, the patients’ blood concentrations achieved the target therapeutic range (10–15 ng mL\(^{-1}\)) much faster than those control recipients who received 0.1 mg kg\(^{-1}\) initial tacrolimus dose and were on blood concentration guided therapy thereafter (CYPtest group: 4.56 ± 2.07 days, 95%CI = 4.19–4.93; control group: 8.33 ± 3.55 days, 95%CI = 7.63–9.01; P < 0.0001; Figure 1C). By the eighth day,

| CYP3A5 genotype | CYP3A4 expression |
|-----------------|-------------------|
| CYP3A5 genotype | Low expresser | Normal expresser | High expresser |
| *1/*1           | 1 (0.9%)       | -                | -              |
| *1/*3           | 9 (8.0%)       | 9 (8.0%)        | 4 (3.6%)       |
| *3/*3           | 35 (31.3%)     | 44 (39.3%)      | 10 (8.9%)      |

**TABLE 2** CYP3A-status of the liver grafts in CYPtest group (n = 112)

**FIGURE 1** Tacrolimus blood concentrations in patients on concentration-guided therapy (control) and on donors CYP3A-status guided therapy (CYPtest). Day-by-day tacrolimus C\(_0\) concentrations (individual data and median with trend curve) are presented for all patients in the control (A) and CYPtest (B) groups. The range of 10–15 ng mL\(^{-1}\) indicates the target C\(_0\) range. (C) The time to achieve the target concentration (median, range) in CYPtest group significantly differed from that in control group (***P < 0.0001). (D) The ratios of patients with target tacrolimus concentrations in control and CYPtest groups were compared in the 20-day post-transplant period.
most of the patients (97.5%) in CYPtest group displayed target concentration of tacrolimus, whereas the therapeutic blood concentration was detected merely in 59% of the recipients in the control group (Figure 1D). Tacrolimus is metabolized by both the hepatic and the intestinal CYP3A enzymes; therefore, the combined impact of the donor and the recipient CYP3A5 genotypes on tacrolimus blood concentration was also investigated (Figure 2). The tacrolimus concentration normalized by dose and the bodyweight \([\text{Co}/(\text{dose/bodweight})]\) was significantly lower in patients transplanted with CYP3A5 expresser graft (carrying CYP3A5*1) than in those with CYP3A5 non-expresser graft (CYP3A5*3/*3). However, the recipients’ CYP3A5 genotype seemed to have no significant effect on tacrolimus concentration.

3.2 Clinical benefit of CYP3A-status guided tacrolimus therapy

Biochemical and clinical parameters regarding liver graft and kidney functions as well as adverse reactions (acute rejection and infection episodes) were followed in the first 3 weeks after transplantation (Table 3). All patients were alive in the early postoperative follow-up period. Biochemical parameters indicating liver function quickly returned to normal values both in CYPtest and control groups. The incidence of initial poor function in CYPtest group was similar to that in control group (8.9 and 8.9%, respectively), whereas recipients with primary nonfunction graft did not occur in the groups of the present study. The incidence of initial poor function of the allograft after transplantation was close to the lowest range of previously described incidence data (8.7–24.7%).

![FIGURE 2](image)

During the first 3 weeks after transplantation, some adverse events were more frequent in control patients on tacrolimus concentration guided therapy than in the patients on CYP3A-status guided therapy. Infections (bacterial, viral or fungal) and acute rejection are the early complications of inappropriate immunosuppression. Due to the prophylactic drug therapy, infection occurred only in 3.6 and 5.9% of the patients in CYPtest and control groups, respectively; however, the differences in this complication between the 2 groups were found

| TABLE 3 | Clinical data of liver transplant patients in the early postoperative period |
|----------|--------------------------------------------------|
|          | CYPtest group | Control group | P-value |
| Time to achieve normal serum concentrations (d)** |          |            |        |
| AST      | 3 (2–4)       | 3 (2–5)      | Ns      |
| ALT      | 4 (2–7)       | 5 (2–9)      | Ns      |
| Prothrombin time | 1 (1–2)       | 1 (1–3)      | Ns      |
| Bilirubin | 1 (1–3)       | 1 (1–4)      | Ns      |
| Graft function (number of patients) |          |            | Ns      |
| Normal   | 102           | 92          |        |
| Initial poor function | 10         | 9          |        |
| Kidney injury (number of patients) |          |            | 0.0004  |
| Normal kidney function | 103       | 74          |        |
| Acute renal impairment | 9        | 27          |        |
| Adverse reaction |          |            | <0.0001 |
| Acute rejection | 4        | 24          |        |
| Infection | 4        | 6           | Ns      |
| Intensive care (d)** | 7 (5–9)     | 7 (5–10)    | Ns      |
| Hospital stay (d)** | 22 (18–29) | 24 (21–32) | 0.0174  |

*Values are expressed as median (range). AST, serum aspartate transaminase; ALT serum alanine transaminase; Ns, not significant.
to be statistically not significant (Table 3). The incidence of acute rejection confirmed by biopsy was significantly higher in control patients than in CYPtest recipients (23.8 vs 3.6%, OR: 8.4, 95%CI = 2.806–25.241, P < 0.0001; Table 3). The patients with a diagnosis of acute rejection mostly displayed tacrolimus blood concentrations under the range of therapeutic level (<10 ng mL\(^{-1}\)) for 2–5 days in the first week (20/24 and 2/4 patients in control and CYPtest groups, respectively). On the basis of tacrolimus concentration normalized by the daily dose and bodyweight, 17 of 24 control patients with acute rejection were most likely to be transplanted with liver grafts from CYP3A5 expresser donors (carrying wild-type CYP3A5*1 allele) or from CYP3A5 nonexpresser donors expressing CYP3A4 at high level (Figure 3). These recipients generally require higher daily dose of tacrolimus (0.2 mg kg\(^{-1}\) bodyweight) than those patients transplanted with grafts from normal or low CYP3A4 expresser donors (0.1 and 0.05 mg kg\(^{-1}\) bodyweight, respectively).\(^{27}\) Seven of 24 control patients were presumably transplanted with grafts expressing CYP3A4 at normal level (Figure 3), whereas all CYPtest patients with acute rejection (n = 4) were transplanted with grafts assayed to be normal CYP3A3 expressers carrying CYP3A5*3/*3.

Tacrolimus-related nephrotoxicity occurred more frequently in control patients than in the CYPtest group (27 vs 8%, OR: 4.18, 95%CI = 1.855–9.401, P = 0.0004; Table 3). Since the risk of nephrotoxicity is considered to be high at the tacrolimus trough concentration above 20–25 ng mL\(^{-1}\),\(^{36}\) the association between impaired renal function and exaggerated tacrolimus blood concentration was investigated. In approximately 50% of the control patients with acute renal impairment (13/27), renal dysfunction was accompanied with exaggerated tacrolimus blood concentrations (>20 ng mL\(^{-1}\)) at least in 2 days of the first week after transplantation. Exaggerated tacrolimus concentrations in the first postoperative week were also observed in the control patients without renal dysfunction; however, the ratio (10.8%) was significantly less (8/74, P = 0.0001). In CYPtest group, tacrolimus blood concentrations >20 ng mL\(^{-1}\) rarely occurred (5/112), and its prevalence in patients with and without acute renal dysfunction (2/9 and 3/103, respectively) was different from that in control patients (13/27 and 8/74, respectively).

The hospital stay after liver transplant surgery is generally 20–30 days at the Department of Transplantation and Surgery of Semmelweiss University (Budapest, Hungary). The length of average hospital stay of recipients on CYP3A-status guided tacrolimus therapy was 22 days which was moderately, but significantly, less than that of the patients on tacrolimus concentration guided therapy (24 days, P = 0.0174). However, the length of intensive care was the same (7 days) for patients in CYPtest and control groups.

![FIGURE 3](Image)

**FIGURE 3** Tacrolimus blood concentrations normalized by the dose and the bodyweight in patients with a diagnosis of acute rejection. The horizontal lane represents the cut-off tacrolimus levels between the patients assumed (control) or proved (CYPtest) to be transplanted with grafts from CYP3A5 expressers/CYP3A4 high expresser donors and CYP3A4 normal expressers.

4 | DISCUSSION

Liver transplantation is considered to be the only efficient, long-term therapeutic solution for patients with end-stage liver disease or acute liver failure. After transplantation, lifelong immunosuppressant therapy with the mainstay calcineurin inhibitor tacrolimus is essential for graft and patient survival. Because of the narrow therapeutic range and wide interindividual pharmacokinetic variability, permanent monitoring of tacrolimus blood concentrations as well as of liver graft function is required for the reduction of drug-related toxicity and early identification of graft injury, and eventually for the improvement of graft/patient survival rates.\(^{4,6,7}\) Therefore, in the early postoperative period, the knowledge of tacrolimus-metabolizing capacity of the liver graft can facilitate fast optimization of immunosuppressive therapy resulting in therapeutic tacrolimus concentrations and reducing the risk of over- and underdosing.

The major role of CYP3A5 in tacrolimus clearance and the association between CYP3A5*1 allele and blood concentration or dose-requirement of tacrolimus in transplant patients were consistently demonstrated.\(^{7,27,37}\) The Clinical Pharmacogenetics Implementation Consortium provided dosing recommendations based on CYP3A5 genotype; notably for CYP3A5 expressers, the recommended starting dose was twice as high as the standard dose.\(^{38}\) In CYPtest group of the present study, for the patients transplanted with CYP3A5 expresser grafts (carrying CYP3A5*1/*3 or CYP3A5*1/*1), the initial tacrolimus dosage followed the double-dose strategy to reach the target concentration, in accordance with the Clinical Pharmacogenetics Implementation Consortium proposal. Besides the assessment of dose-requirement, genotyping for CYP3A5 was reported to facilitate rapid dose-finding and improved achievement of target tacrolimus concentration in kidney transplant patients.\(^{39,40}\) For refining genotype-based tacrolimus dosing, CYP3A4*22 resulting in reduced CYP3A4 expression has recently been suggested to be integrated with CYP3A5 genotype information.\(^{7,41,42}\) Nonetheless, variability in tacrolimus dose-requirement appears to be far from that is explainable by CYP3A5*1 and CYP3A4*22 (<20%).\(^{43}\) The impact of other CYP3A4...
genetic polymorphisms on tacrolimus pharmacokinetics is, however, disrupted, because the nongenetic factors can mask variability in CYP3A4 expression or activity attributed to genetic factors. The current approach regarding the estimation of hepatic CYP3A4 activity from leukocyte CYP3A4 expression seems to enable an accurate picture of tacrolimus-metabolizing capacity in CYP3A5 non-expressers (with CYP3A5*3/*3). For liver transplant recipients, the donor CYP3A-status (CYP3A5 genotype and CYP3A4 expression) was demonstrated to provide prognostic information regarding the initial tacrolimus dosing for therapeutic blood concentration. In the CYPtest group of the current study, the time for achieving target tacrolimus concentration was significantly reduced, confirming potential benefit of initial tacrolimus therapy adjusted to the donor's CYP3A-status over classical clinical practice of tacrolimus concentration guided treatment (4 days in CYPtest group vs 8 days in control group). Fast individualization of tacrolimus therapy may contribute to amelioration of tacrolimus misdosing induced consequences, such as acute rejection, infections or nephrotoxicity in the early postoperative period, which may have long-term impact on graft function as well as on the overall outcome of liver transplantation.

A number of clinical studies evaluated the impact of intestinal CYP3A5 polymorphism (recipient CYP3A5 genotype) on tacrolimus pharmacokinetics. Several trials involving mainly living donor liver transplant recipients demonstrated important consequences of recipients' CYP3A5 genotype on tacrolimus concentration/dose ratios and on dose requirements in the first postoperative month, whereas other studies emphasized the contribution of the donor (hepatic) CYP3A5 polymorphism to the variations in tacrolimus dose requirement over recipient (intestinal) CYP3A5 genotype. Our findings confirmed an important role of CYP3A5 expression (the presence of CYP3A5*3 allele) of the liver graft in tacrolimus concentrations normalized by the dose and bodyweight; however, recipients' CYP3A5 genotype failed to significantly modify dose-adjusted tacrolimus trough levels. Nongenetic factors, such as comedication induced transcription of CYP3A genes or inhibition of CYP3A activity, can transiently alter tacrolimus metabolizing capacity. The steroid methylprednisolone known to induce CYP3A expression and to increase the clearance of calcineurin inhibitors was administered to the patients at relatively high doses (tapering from 1000 mg of methylprednisolone to 32 mg/d in the first week, 20 mg/d thereafter) in the first postoperative period. Since the same dosing protocol was applied for each patient, we assumed that similar CYP3A induction profiles were developed in the control and CYPtest groups. The antifungal fluconazole is a weak CYP3A inhibitor that may decrease tacrolimus clearance; however, it was applied against fungal infection in 1 patient in the control group and in another patient in the CYPtest group, and no tacrolimus related adverse effect was observed as a consequence of fluconazole coadministration.

Substantial speed-up of reaching target concentration of tacrolimus was not the only benefit of immunosuppressant therapy fine-tuned according to the donor's CYP3A-status. In the present study, significant decrease in acute rejection episodes was observed in the patients on CYP3A-status guided tacrolimus therapy comparing to the control recipients on tacrolimus blood concentration guided dosing. Acute rejection has been demonstrated to be associated with low predose tacrolimus concentration (<10 ng mL\(^{-1}\)) both in kidney and in liver transplant recipients. The fact that subtherapeutic tacrolimus levels were measured in most of the control patients with a diagnosis of acute rejection (83%) in the first week after transplantation highlights the prognostic value of donor CYP3A-status and the importance of assessing tacrolimus-metabolizing capacity of liver grafts. Furthermore, 71% of the control patients with rejection episodes were assumed to be transplanted with liver grafts from CYP3A5 expresser donors or from donors with CYP3A5*3/*3 genotype expressing CYP3A4 at high level. Although the early acute rejection is generally reversible with escalation of immunosuppression or with high-dose steroid pulse therapy, and does not influence long-term graft or patient survival, most of these rejection episodes with transient graft dysfunction are avoidable with preliminary assessing of donor's CYP3A-status contributing to the fast recovery of patients. It should be mentioned that 2 of the 4 patients with rejection in CYPtest group displayed low tacrolimus concentrations (<10 ng mL\(^{-1}\)) in the first week after transplantation despite the fact that they were normal CYP3A4 expressers carrying CYP3A5*3/3. This suggests that even if the donor CYP3A-status guided therapy improves the individualization of tacrolimus treatment, careful monitoring of tacrolimus blood concentration is an important tool for reducing the incidence of adverse events.

The most frequent adverse effect of calcineurin inhibitors is nephrotoxicity often called as the Achilles heel of transplantation. Although the nephrotoxicity potential of tacrolimus is lower than that of ciclosporin, inappropriate dosing of tacrolimus can also induce kidney injury. In the early post-transplant period, acute renal impairment is associated with high mortality; therefore, reduction of the potential risk factors evoking renal injury is essential. The incidence of acute renal failure in the early post-transplant period is higher than 40%, and the calcineurin inhibitor induced kidney injury can compromise long-term survival of liver transplant recipients. Although total calcineurin inhibitor-free immunosuppressive therapy seems to be hardly acceptable for liver transplant patients, because of insufficient immunosuppression and high rates of acute, steroid-resistant rejection, CYP3A-status guided tacrolimus therapy might be an appropriate solution for reduction of tacrolimus induced nephrotoxicity in the early postoperative period. In the CYPtest group on tacrolimus therapy adjusted to the donor's CYP3A-status, tacrolimus associated acute kidney injury was less frequent than in control patients on tacrolimus concentration guided treatment. Furthermore, in control patients, exaggerated tacrolimus concentrations (>20 ng mL\(^{-1}\)) in the first postoperative week were frequently accompanied with acute renal injury. It was clearly demonstrated that the donor's CYP3A-status influenced the systemic tacrolimus exposure of recipients; however, it can be issued that the additional effect of recipient's CYP3A activity on tacrolimus nephrotoxicity may be attributed to the intrarenal metabolism and local exposure rather than the consequence of systemic tacrolimus concentration. Although significant correlation has been previously demonstrated between increasing risk of nephrotoxicity and...
increasing systemic exposure to tacrolimus (predose concentration). It is a limitation of the present study that the only information of renal tacrolimus metabolism was the recipient's CYP3A5 genotype, and no CYP3A4 expression data were available.

In the early post-transplant period, infections are common complications associated with immunosuppressive therapy; however, appropriate prophylactic drug therapy (antibacterial, antiviral, and antifungal) can successfully reduce the infection episodes. For prevention of graft rejection, increased tacrolimus doses are generally recommended that were proved to increase the susceptibility to infectious diseases. The donor CYP3A-status controlled tacrolimus therapy, however, did not ameliorate the risk of infection episodes in the present study, which may be explained by risk factors other than immunosuppression, e.g. prolonged ventilation and intensive care stay.

In conclusion, donor CYP3A-status, taking CYP3A5 genotype and CYP3A4 expression into account, can provide information on tacrolimus-metabolizing capacity of liver graft that can contribute to rapid optimization of tacrolimus therapy in the early postoperative period. However, it should be emphasized that CYP3A-assaying is an additional tool for rapid dose-finding and lowering the number of attempts of dose adaptation, and cannot replace careful monitoring of therapeutic tacrolimus concentrations. Furthermore, CYP3A-status controlled treatment of recipients significantly improved misdosing associated acute rejection and nephrotoxicity that may contribute to the avoidance of some late complications. Incorporating tailored initial tacrolimus therapy adjusted to the donor's CYP3A-status into the routine clinical practice can improve recipients' recovery and can ameliorate graft and patient survival.

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COMPETING INTERESTS
There are no competing interests to declare.

CONTRIBUTORS
N.C. collecting patients' clinical data, manuscript writing. Á.K. CYP3A5 genotyping of donors. M.D. contributed new analytical tools. F.F. assaying CYP3A4 expression. A.M. isolation of DNA from recipient samples. K.T. collecting blood samples, isolation of DNA and RNA from donor leukocytes. M.T. collecting patient clinical data. E.S. measuring blood levels of tacrolimus. L.B. patient care. Z.G. patient care. L.K. research design, coordination of the clinical part. K.M. research design, coordination of CYPtest and other laboratory testing, interpretation of data, manuscript writing.

DATA AVAILABILITY STATEMENT
The data supporting the findings of the present study are not publicly available; however on reasonable request, the data are available from the corresponding author.

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