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Tacrolimus concentration/dose ratio as a therapeutic drug monitoring strategy: the influence of gender and comedication

Odnos koncentracije i doze takrolimusa kao strategija terapijskog monitoringa leka: uticaj pola i komedikacije

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Abstract

Background/Aim. A combination of tacrolimus and other drugs such as corticosteroids has been commonly used immunosuppressive regimens. On the other hand, there is a growing body of evidence that male and female may differ in their response to the equal drug treatment. The aim of the study was to estimated the use of tacrolimus concentration/dose (C/D) ratio for the assessment of the influence of gender differences and comedication on tacrolimus exposure in renal transplant recipients. **Methods.** This prospective case series study included 54 patients, in which the unit of monitoring was outpatient examination (1,872) of the renal transplant patients. The patients were monitored in the period 2010–2014, starting one month after the transplantation. Tacrolimus trough concentrations (TTC) were measured by chemiluminescence microparticles immunoassay. **Results.** TTC and the tacrolimus C/D ratio were significantly lower in the females comparing with the males. Contrary to the males, in the females a significant increase of the tacrolimus daily dose (TDD) *per* body weight and TTC, along with the corticosteroid dose increase, was not accompanied by any significant changes in the tacrolimus C/D ratio; in different corticosteroid doses faster elimination of tacrolimus was found with the exception of the doses > 0.25 mg/kg. In the patients treated with proton pump inhibitors, mainly with pantoprazole TDD *per* body weight and TTC were significantly higher, while the tacrolimus C/D ratio was significantly lower compared to the patients without this treatment. In the patients treated with calcium channel blockers, TDD *per* body weight was significantly lower (particularly with amlodipine) while the tacrolimus C/D ratio was higher compared to the patients who were not treated by them. **Conclusion.** A lower tacrolimus exposure was detected in females in comparison to males. When gender differences were considered in the context of different corticosteroid doses, faster elimination of tacrolimus in the females was also seen, with the exception of the doses > 0.25 mg/kg. Tacrolimus exposure in the pantoprazole-treated patients was significantly less expressed, while in patients treated with CCB amplodipine the tacrolimus C/D ratio was significantly higher in comparison with the patients not treated with them.

Key words:
kidney transplantation; tacrolimus; immunosuppressive agents; drug therapy, combination; dose-response relationship, drug; sex.

Apstrakt

Uvod/Cilj. Kombinacija takrolimusa i drugih lekova kao što su kortikosteroidi, čest je imunosupresivni režim. S druge strane, raste broj dokaza da se muškarci i žene mogu razlikovati u odgovoru na lečenje istim lekom. Cilj ovog rada bio je da se proceni uticaj razlike među polovima i komedikacije na izloženost takrolimusu, uz pomoć odnosa koncentracije i doze (C/D odnos) takrolimusa kod bolesnika sa transplantiranim bubregom. **Metode.** Ispitivanje je sprovedeno kroz prospektivnu seriju od 54 bolesnika, gde je jedinica posmatranja bio kontrolni ambulantni pregled (1 872) bolesnika sa transplantiranim bubregom. Bolesnici su praćeni od 2010. do 2014. godine, a praćenje je započeto mesec dana nakon transplantacije. Minimalna koncentracija takrolimusa u krvi (*tacrolimus trough concentration* – TTC) merena je uz pomoć hemiluminiscentnog mikročestičnog imunoeseja. **Rezultati.** Odnos TTC i C/D bio je značajno niži kod žena nego kod muškara-

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ca. Za razliku od muškaraca, kod žena je nađeno značajno povećanje dnevne doze takrolimusa (TDD) po kg telesne težine i TTC, zajedno sa povećanjem doze kortikosteroida, koje nije bilo praćeno značajnim promenama odnosa C/D. Bolesnici koji su upotrebljavali inhibitore protonske pumpe (većinom pantoprazol), imali su značajno viši TDD po kg telesne težine i TTC, dok je odnos C/D bio značajno niži nego kod bolesnika bez ovog tretmana. Kod bolesnika koji su upotrebljavali blokatore kalcijumovih kanala (pogotovo amlodipina) TDD po kg telesne težine bio je značajno niži, dok je odnos C/D bio značajno viši nego kod bolesnika bez ovog tretmana. **Zaključak.** Rezultati pokazuju da su žene manje izložene takrolimusu nego muškarci. Kada su posmatrane polne razlike u odnosu na različite doze kortikosteroida, utvrđeno je brže eliminisanje takrolimusa kod žena, osim kada je doza kortikosteroida bila > 0,25 mg/kg. Izloženost takrolimusu u prisustvu pantoprazola bila je značajno manje izražena, dok je u prisustvu amlodipina bila značajno viša nego kod bolesnika koji nisu bili lečeni ovim lekovima.

Ključne reči:
transplantacija bubrega; takrolimus; imunosupresivi; lečenje kombinovanjem lekova; lekovi, odnos doza-reakcija; pol.

Introduction

Immunosuppressive therapy used to prevent liver, kidney or heart allograft rejection often includes tacrolimus, a calcineurin inhibitor. It is a potent agent, pharmacologically related to cyclosporine, but 10- to 200-fold more potent on a weight basis in T-cell immune function suppression. A combination of tacrolimus, mycophenolate mofetil and corticosteroids has been among the most commonly used immunosuppressive regimens, so far [1–4].

However, tacrolimus has a dose-dependent toxicity, as well as large intra- and inter-individual pharmacokinetic variability. Numerous factors which are supposed to contribute to the aforementioned are: gender, age, body mass index, albumin concentration, diarrhoea, corticosteroids and other comedication, food, hepatitis, diabetes, gene polymorphism, etc. [1, 2, 5, 6]. Additional reasons for tacrolimus pharmacokinetic variability include its poor dissolution, restricted absorption, strong affinity for erythrocytes (tacrolimus concentrations in whole blood is up to 30 times greater than in plasma) and hepatic impairment, which can be associated with a decrease of tacrolimus clearance and about 3-fold increase of its half-life [1, 2, 7].

Patient-tailored regimen requires the exploration of multiple clinical factors in order to determine their effects on tacrolimus pharmacokinetics [5, 6, 8–10]. For example, single-nucleotide polymorphism is found on the genes encoding for cytochrome P450 (CYP) 3A family, especially CYP 3A4 and CYP 3A5 members, responsible for the major route of tacrolimus metabolism, both in the liver and intestine. Moreover, new findings indicate that this also applies to P-glycoprotein (P-gp) efflux pump, as well. On the other hand, the inhibition and induction of CYP 3A-mediated metabolism of tacrolimus are regarded as the clinically most important drug-drug interaction mechanism. Drugs that inhibit this enzyme system, such as azoles, calcium channel blockers, macrolide, HIV-protease inhibitors, etc., may produce the increased tacrolimus blood concentrations [1, 7, 11, 12]. Quite the opposite, the inducers of CYP 3A may reduce its blood concentrations (carbamazepine, phenobarbital, nevirapin, rifampicin, St John's wort). Since oral prednisone is an integral component of most immunosuppressive regimens in solid organ transplantation, its potential interactions with tacrolimus are of special importance. It is mostly due to the common metabolic (CYP 3A) and transporter pathways (P-gp) of corticosteroids and tacrolimus [13].

There is a growing body of evidence that male and female may differ in their response to the equal drug treatment, as a result of their differences in drug pharmacokinetics [14, 15]. It is essential to understand these gender differences since they can result in a modified pharmacological response and may affect both drug effectiveness and safety. The research considering the difference in pharmacokinetic properties of tacrolimus between male and female patients is in progress [16–19].

Therapeutic drug monitoring (TDM) is very important for drugs with a narrow therapeutic index (NTI), for drugs with proven relationship between drug exposure, efficacy and adverse effects, and when samples for TDM are easily accessible. According to the revised European Medicines Agency Guideline on the Investigation of Bioequivalence, tacrolimus is NTI drug, with 90.00–111.11% acceptance criterion tightened for the area under the curve (AUC), while for C_{max}, 80.00–125.00% acceptance limits are still valid [20]. Tacrolimus is administered daily, divided in two doses, every 12 hours, and the dose adjustment is based on tacrolimus trough concentrations (TTC), which has been standard practice for many years [21]. Tacrolimus target levels in renal transplant recipients have been defined between 5 and 10 ng/mL without induction therapy, while with induction therapy between 7 and 10 ng/mL [7, 22, 23]. The importance of TDM can be seen from the fact that overexposure can be linked with significant tacrolimus toxicity [24], while underdosing is associated with an increased risk of kidney rejection [23, 25–27]. Since TTC are routinely monitored and the dose is adjusted based almost solely on trough measurements, the effects of the multiple factors affecting tacrolimus pharmacokinetics are not regarded in the consistent manner by various transplant centers. Therefore, trial and error approach to dosing is still a common everyday practice and needs a novel approach. On the other hand, although full dose interval area under the concentration-time curve (AUC_{0-12}) is generally considered the best marker for tacrolimus exposure, it has not been used as a routine method in the clinical settings, due to its complexity and the high cost of the procedure [28]. Quite recently, however, the tacrolimus concentration/dose (C/D) ratio, a relatively simply obtained TDM tool, has been suggested to be used to define tacrolimus exposure profile better [5].

The aim of the study was to estimate the use of tacrolimus C/D ratio for the assessment of the influence of gender differences and comedication on tacrolimus exposure in renal transplant recipients.

Methods

The study was designed as a prospective case series study, in which the unit of monitoring was outpatient examination recorded in the database of patients subjected to kidney transplantation in the Center for Solid Organ Transplantation of the Military Medical Academy, Belgrade, Serbia (the tertiary health care university hospital). The study group consisted of 54 patients subjected to renal transplantation. They were all monitored in the period from 2010 to 2014 (mean follow-up time was 636.70 ± 209.28 days), starting one month after the transplantation.

Transplantation protocol and concurrent medication

All the patients were treated in accordance with the established therapeutic protocol in the Center, as described in the earlier study [29]. After kidney transplantation, they were subjected to the triple-drug-therapy, including corticosteroids (methylprednisolone, prednisone), mycophenolate mofetil and tacrolimus (Prograf®, Fujisava, Japan). After renal transplantation, an induction therapy (anti-T lymphocyte globulin – ATG) was applied to 29 (53.7%) of our patients. ATG was administered intravenously (as a slow intravenous infusion) as a series of divided doses during the first post-transplant week (in a dose 2–4 mg/kg/day). On the day of transplantation, tacrolimus was introduced in the initial oral dose 0.1–0.3 mg/kg/day, divided into 12-h intervals [30]. The patients were given the dose of 500 mg of methylprednisolone, intravenously, on the day of the surgical intervention, before the transplantation itself; the next 2 days the dose was 250 mg/day, and then reduced to 125 mg/day in the following 2 days, followed by 3 days, in the dose of 1.5 mg/kg/day. During the second week after transplantation, the dose of 0.3 mg/kg/day of prednisone was administered orally; the same dosage was used until the end of the first month. The prednisone dose of 10 mg/day was prescribed until the end of the first year after transplantation, while 10 mg dose was recommended every other day, during the second year of treatment and later on. Mycophenolate mofetil was given orally, 1 g, twice daily, starting 2 days before the kidney transplantation. Three months after transplantation, mycophenolate mofetil dose was reduced to 500 mg, twice daily. After this dose reduction, mycophenolate mofetil was taken permanently.

The other drugs were administered according to comorbidity. In order to control hypertension, calcium channel blockers (nifedipine, amlodipine), β adrenergic antagonists (propranolol, carvedilol, bisoprolol, atenolol, metoprolol, nebivolol) and/or diuretics (furosemide) were given. As a prophylaxis for peptic ulcers and surgical stress-related bleeding, H_2-antagonists (ranitidine) or proton pump inhibitors (pantoprazole, esomeprazole) were administered. The doses of all concomitant drugs were always within recommended therapeutic range. All the patients were also treated with cotrimoxazole (for *Pneumocystis jirovecii* prophylaxis) for 6 post-transplant months.

Clinical data

Physical examination, biochemical analyses (complete blood count, haematocrit, C-reactive protein test, creatinine blood test, blood urea nitrogen test, blood glucose level, sodium test, potasium test, blood calcium test, plasma protein test, albumin blood test, aspartate aminotransferase test, alanine aminotransferase test, blood sedimentation rate, urine test, including urine culture test and cytology exam of urine) and other medical examinations (blood pressure, color Doppler ultrasonography of the graft with an assessment of resistance index of its interlobular artery) were performed.

Therapeutic drug monitoring

TDM involved tacrolimus daily dose (TDD), TDD *per* body weight, TTC and the tacrolimus C/D ratio. The tacrolimus C/D ratio is the ratio between C_0 or TTC and 24-h dose (D) normalized by patient's weight (mg/kg/day) [5].

All these parameters were used to investigate the influence of comedication and gender differences on TDD adjustment.

TTC were measured by chemiluminescence microparticles immunoassay (CMIA) (ARCHITECT i1000SR Abbott Laboratories; Abbott Park, Illinois, USA). The whole blood samples were taken 12 h after the evening dose, 10 min before the morning dose.

Three days after the transplantation, tacrolimus dose was adjusted depending on the whole blood TTC. The target concentration range was from 5 to 10 ng/mL during the first month after the renal transplantation, as recommended [22, 31], although some authors recommend the lower range i.e. from 3 to 7 ng/mL [22, 32, 33]. After the first month of transplantation, TTC have been recommended target concentration range from 6 to 10 ng/mL in the renal transplant recipients. If the TTC was greater than 10 ng/mL, the TDD was reduced, while if the TTC was less than 6 ng/ml, the TDD was increased.

In accordance with the previous findings which showed that prednisone dosage was a significant covariate influencing tacrolimus parameters [34], the patients were divided into 3 groups according to corticosteroid doses per body weight: the group with the doses < 0.15 mg/kg, the group with the doses from 0.15 – 0.25 mg/kg and the group with the doses > 0.25 mg/kg.

Statistical analysis

The complete statistical analysis of data was done with the statistical software package, PASW Statistics 18. All variables were presented as frequency of certain categories, while statistical significance of differences were tested by the χ^2-square test. Continuous variables were summarized as means ($\bar{x}$) and standard deviations (SD). Continuous variables were compared using Student's *t*-test for independent samples or Mann-Whitney *U*-test. Two-way between-groups analysis of variance was used in order to analyze both individual as well as joint infuence of fixed factors on dependent

variables. The normality of the data was assessed using Kolmogorov-Smirnov test. Ratios between TDD *per* body weight, TTC and the tacrolimus C/D ratio were tested by Pearson's coefficient correlation. All the analyses were estimated at $p < 0.05$ level of the statistical significance.

Principles of ICH Good Clinical Practice were strictly followed and ethical approval No 01/31-01-13 from the Ethics Committee was obtained for the study protocol No.910-1.

Result

Demographic characteristics of renal transplant patients are presented in Table 1. A total of 54 patients was subjected to kidney transplantation, 34 (63%) males and 20 (37%) females; the average recipient age was 40.46 ± 11.38 years. Body height, body weight and body mass index were significantly higher in men.

The total number of 1,872 outpatient examinations was performed during this follow-up (Table 2). The average TDD, TTC and the tacrolimus C/D ratio in renal transplant patients were significantly lower in females comparing with males (Table 2).

A very strong correlation between TDD *per* body weight and the tacrolimus C/D ratio was shown ($r = -0.700$, $p < 0.0001$). The correlation between TDD *per* body weight and TTC, as well as between TTC and the tacrolimus C/D ratio was weak ($r = 0.218$, $r = 0.257$, respectively).

Renal transplant patients' biochemical analyses are shown in Table 1. All biochemical parameters, such as haematocrit, blood urea nitrogen, creatinine and proteinuria, were significantly higher in males in comparison with females.

All the patients were treated with corticosteroid therapy. The average corticosteroid daily dose (CDD) was 14.40 ± 5.85 mg or 0.22 ± 0.09 mg/kg. The males were subjected to significantly higher CDD, expressed in miligrams (males 15.16 ± 6.37; females 13.29 ± 4.82; $p < 0.0001$), but it turned out that comparing with females, it was a significantly lower dose when expressed in mg/kg *per* body weight (males 0.21 ± 0.09; females 0.23 ± 0.09; $p < 0.0001$). In the groups of patients who received less than 0.15 mg/kg and 0.15–0.25 mg/kg of corticosteroids, the female patients were treated with significantly higher TDD *per* body weight in comparison to the males (Table 3 and Figure 1). However, in the group of patients who received more than 0.25 mg/kg of corticosteroids, the male patients were treated with significantly higher TDD *per* body weight in comparison with the females. In the males, along with the prednisone dose increase (> 0.25:0.15–0.25, > 0.25:< 0.15 and 0.15–0.25:< 0.15 mg/kg) both TDD *per* body weight and TTC increased significantly, while the tacrolimus C/D ratio decreased significantly (Table 3). However, in the females this significant increase of TDD *per* body weight and TTC, along with the corticosteroid dose increase, was not accompanied by any significant changes in the tacrolimus C/D ratio.

Out of 1,872 outpatient examinations in 1,407 (75.2%) (888 examination including males and 519 including females) were registered treatment with proton pump inhibi-

Table 1

Demographic characteristics and biochemical analyses of renal transplant patients according to gender

Parameter	Male [n = 34 (63%)]	Female [n = 20 (37%)]	Total [n = 54 (100%)]
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Age, years	41.44 ± 11.73	38.80 ± 10.85	40.46 ± 11.38
Height, m	1.78 ± 0.06	1.65 ± 0.07**	1.74 ± 0.09
Weight, kg	72.38 ± 13.15	59.06 ± 8.91**	67.96 ± 13.47
Body mass index, kg/m^2	22.38 ± 3.31	19.97 ± 2.31**	21.49 ± 3.18
Haematocrit, L/L	0.40 ± 0.05	0.38 ± 0.05**	0.39 ± 0.05
Blood urea nitrogen, mmol/L	11.42 ± 18.58	8.17 ± 8.90**	10.03 ± 15.29
Creatinine, μmol/L	152.38 ± 55.13	108.54 ± 42.17**	133.64 ± 54.49
Proteinuria, g/24h	0.35 ± 0.30	0.19 ± 0.24**	0.30 ± 0.29

Statistically significant difference (males/females): **- $p < 0.01$; $\bar{x}$ - mean; SD – standard deviation.

Table 2

Average tacrolimus daily doses (TDD), TDD per body weight, tacrolimus through concentrations (TTC) and the tacrolimus concentration/dose (C/D) ratio in renal transplant patients: gender distribution

Parameter	Outpatient examinations by gender ($\bar{x} \pm SD$)		
	Male (n = 1,154; 61.6%)	Female (n = 718; 38.4%)	Total (n = 1,872; 100%)
Outpatient examinations *per* patient, n	33.94 ± 11.17	35.90 ± 10.78	34.67 ± 0.96
TDD, mg	5.56 ± 3.53	4.50 ± 2.31**	5.13 ± 3.13
TDD *per* body weight, mg/kg	0.075 ± 0.047	0.079 ± 0.041	0.077 ± 0.045
TTC, ng/mL	6.74 ± 2.31	6.26 ± 2.45**	6.54 ± 2.38
Tacrolimus C/D ratio, ng/mL/mg/kg/day	137.56 ± 102.50	100.45 ± 64.99**	121.78 ± 90.37

Statistically significant difference (males/females): **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

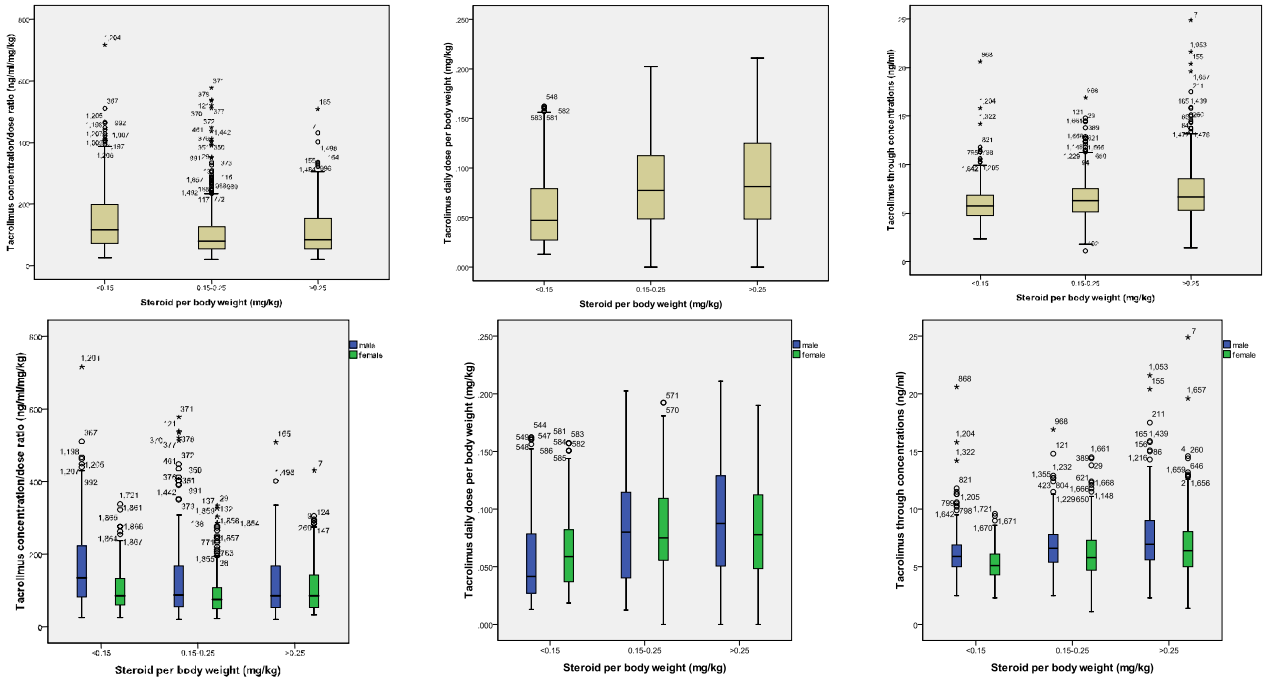

Fig. 1 – Impact of gender on distribution of tacrolimus daily doses *per* body weight, tacrolimus trough concentrations, and the tacrolimus concentration/dose ratio in renal transplant patients according to corticosteroid dose comedication.

Table 3

Gender distribution of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in renal transplant patients according to corticosteroid dose comedication

Gender	Corticosteroid dose (mg/kg)	TDD *per* body weight (mg/kg)	TTC (ng/mL)	Tacrolimus C/D ratio (ng/mL/mg/kg/day)
		x̄ ± SD	x̄ ± SD	x̄ ± SD
Male + Female	< 0.15	0.058 ± 0.038	5.95 ± 1.85	147.58 ± 99.83
	0.15–0.25	0.081 ± 0.042#**	6.44 ± 2.12#**	106.86 ± 83.44#**
	> 0.25	0.090 ± 0.049	7.26 ± 2.88	111.59 ± 76.63
Male/Female	< 0.15	0.057 ± 0.039/0.064 ± 0.035##**	6.17 ± 1.89/5.28 ± 1.54##**	161.35±105.31/106.52 ± 66.45##**
	0.15–0.25	0.080 ± 0.046/0.081 ± 0.039##	6.69 ± 2.01/6.12 ± 2.24##**	129.15 ± 109.36/93.61 ± 59.16##**
	> 0.25	0.091 ± 0.050/0.087 ± 0.047##*	7.57 ± 2.87/6.93 ± 2.86##**	116.08 ± 81.54/106.82 ± 70.92##
Male	< 0.15	0.057 ± 0.039	6.17 ± 1.89	161.35 ± 105.31
	0.15–0.25	0.080 ± 0.046#**	6.69 ± 2.01#**	129.15 ± 109.36#**
	> 0.25	0.091 ± 0.050	7.57 ± 2.87	116.08 ± 81.54
Female	< 0.15	0.064 ± 0.035	5.28 ± 1.54	106.52 ± 66.45
	0.15–0.25	0.081 ± 0.039#**	6.12 ± 2.24#**	93.61 ± 59.16#
	> 0.25	0.087 ± 0.047	6.93 ± 2.86	106.82 ± 70.92

Statistically significant difference: *- $p < 0.05$ and **- $p < 0.01$; #- < 0.15/0.15–0.25/>0.25; ##- males/females; x̄ – mean; SD – standard deviation.

tors. In these cases TDD *per* body weight and TTC were registered treatment with significantly higher compared to the patients without this treatment, while the tacrolimus C/D ratio was significantly lower in the renal transplant recipients whose treatment included one of the drugs from this group (Table 4). Considering gender, the ratio of these parameters was the same as in the whole patient population treated with proton pump inhibitors (Table 4). However, in males, TDD *per* body weight was significantly lower, while TTC and tacrolimus C/D ratio were significantly higher compared to female patients (Table 4). Considering various proton pump inhibitors, the patients who were treated with pantoprazole were given significantly higher TDD *per* body weight comparing with esomeprazole, while their TTC and the tacrolimus C/D ratio were significantly lower (Table 5).

Out of 1,872 outpatient examinations in 39.9% (551 including males and 195 including females) cases calcium channel blockers were registered. In these patients TDD *per* body weight was significantly lower compared to the patients without this treatment, while the tacrolimus C/D ratio was higher in the renal transplant recipients whose treatment included one of the drugs from this group (Table 4). Taking into account all the examined parameters (TDD *per* body weight, TTC and tacrolimus C/D ratio), when the comparison between the groups treated and not treated with calcium channel blockers was done, it turned out that there were no significant differences considering the males and the females (Table 6). In our patients, the tacrolimus C/D ratio was higher in the renal transplant recipients treated with amlodipine than in those treated with nifedipine (157.15 ± 118.11 *vs*

Table 4

Impact of comedication with/without proton pump inhibitors and calcium channel blockers on tacrolimus daily doses (TDD) *per* body weight tacrolimus trough concentrations (TTC), and tacrolimus concentration/dose (C/D) ratio in the renal transplant patients acording to gender

Gender	Comedication	TDD *per* body weight (mg/kg) $\bar{x} \pm SD$	TTC (ng/ml) $\bar{x} \pm SD$	Tacrolimus C/D ratio (ng/ml/mg/kg/day) $\bar{x} \pm SD$
Both	PPT	0.080 ± 0.045/0.066 ± 0.044**	6.66 ± 2.52/6.16 ± 1.83**	115.70 ± 86.24/140.09 ± 99.70**
Both	CCB	0.074 ± 0.043/0.078 ± 0.046*	6.62 ± 2.35/6.49 ± 2.40	136.00 ± 107.90/112.87 ± 76.11**
Males/Females	With PPI	0.078 ± 0.046/0.085 ± 0.041**	6.78 ± 2.42/6.46 ± 2.68**	130.44 ± 98.03/92.35 ± 55.86**
	With CCB	0.072 ± 0.045/0.080 ± 0.037**	6.71 ± 2.33/6.37 ± 2.41*	147.70 ± 116.94/103.96 ± 68.71**
Males/Females	Without PPI	0.065 ± 0.051/0.066 ± 0.038	6.52 ± 1.81/5.85 ± 1.80**	166.23 ±114.74/117.93 ± 78.61**
	Without CCB	0.078 ± 0.050/0.078 ± 0.043	6.77 ± 2.30/6.23 ± 2.46**	127.79 ± 85.33/99.33 ± 63.78**
Males	With/without PPI	0.078 ± 0.046/0.065 ± 0.051**	6.78 ± 2.42/6.52 ± 1.81	130.44 ± 98.03/166.23±114.74**
	With/without CCB	0.072 ± 0.045/0.078 ± 0.050	6.71 ± 2.33/6.77 ± 2.30	147.70 ± 116.94/127.79 ±85.33
Females	With/without PPI	0.085 ± 0.041/0.066 ± 0.038**	6.46 ± 2.68/5.85 ± 1.80**	92.35 ± 55.86/117.93 ± 78.61**
	With/without CCB	0.080 ± 0.037/0.078 ± 0.043	6.37 ± 2.41/6.23 ± 2.46	103.96 ± 68.71/99.33 ± 63.78

Statistically significant difference: *- $p < 0.05$ and **- $p < 0.01$; The same for: with/without comedication; $\bar{x}$ – mean; SD – standard deviation; PPI – proton pump inhibitors; CCB – calcium channel blockers.

Table 5

Relationship of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in the renal transplant patients without/with proton pump inhibitors pantoprazole or esomeprazole comedication

Proton pump inhibitors	TDD *per* body weight (mg/kg) $\bar{x} \pm SD$	TTC (ng/mL) $\bar{x} \pm SD$	Tacrolimus C/D ratio (ng/mL/mg/kg/day) $\bar{x} \pm SD$
Without	0.066 ± 0.044	6.16 ± 1.83	140.09 ± 99.70
Pantoprazole	0.081 ± 0.043**	6.62 ± 2.57**	112.27 ± 84.26**
Esomeprazole	0.075 ± 0.053	6.93 ± 2.08	145.81 ± 97.09

Statistically significant difference: **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

Table 6

Relationship of tacrolimus daily doses (TDD) *per* body weight, tacrolimus trough concentrations (TTC), and the tacrolimus concentration/dose (C/D) ratio in the renal transplant patients without/with calcium channel blockers amlodipine or nifedipine, comedication

Calcium channel blockers	TDD *per* body weight (mg/kg)	TTC (ng/mL)	Tacrolimus C/D ratio (ng/mL/mg/kg/day)
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
Without	0.079 ± 0.046	6.50 ± 2.40	112.85 ± 76.32
Amlodipine	0.066 ± 0.043**	6.68 ± 2.62**	157.15 ± 118.11**
Nifedipine	0.078 ± 0.043	6.57 ± 2.08	118.76 ± 93.67

Statistically significant difference: **- $p < 0.01$; $\bar{x}$ – mean; SD – standard deviation.

118.76 ± 93.67; $p < 0.001$), while TDD *per* body weight was lower in the patients treated with amlodipine than with nifedipine (0.066 ± 0.043 vs 0.078 ± 0.043; $p < 0.001$) (Table 6).

The influence of gender and comedication on TTC, as well as the tacrolimus C/D ratio was investigated by using two-way between-groups analysis of variance. When the dependent variable was TTC, statistically significant individual influence of gender, comedication with proton pump inhibitors and corticosteroid groups were established ($p = 0.002$; $p = 0.003$; $p < 0.0001$, respectively), while their joint influence was not significant. On the other hand, when tacrolimus C/D ratio was considered, individual influence of all already mentioned independent variables was also significant ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$, respectively). Whenever the influence of corticosteroid groups associated with any other investigated independent variables was estimated, a significant influence on the tacrolimus C/D ratio was found (with gender, $p = 0.001$; with proton pump inhibitors, $p = 0.044$; with calcium channel blockers, $p < 0.0001$; with gender + proton pump inhibitors, $p = 0.005$; with gender + calcium channel blockers, $p = 0.001$; proton pump inhibitors + calcium channel blockers, $p < 0.0001$).

Calculated the tacrolimus C/D ratio, which corresponded to the tacrolimus target concentration range from 6 to 10 ng/mL, was 130.98 ± 97.11 ng/mL/mg/kg. In the patients with TTC

over therapeutic range (> 10 ng/mL) calculated tacrolimus C/D ratio was 174.36 ± 118.57 and in the patients with subtherapeutic concentration range (< 6 ng/mL) the tacrolimus C/D ratio was 104.46 ± 72.23.

Disscusion

Corticosteroid dose, comedication use and patients' gender are known to be among the numerous factors that have been identified as contributors to a large tacrolimus intra- and inter-individual pharmacokinetic variability. Due to this and to the fact that tacrolimus is the NTI drug, the use of TDM, in conjunction with clinical assessment of the patients, is particularly important. The results of this study demonstrate the relevance of TDM in renal transplant recipients who are normally subjected to numerous drugs with a potential to interact with tacrolimus, but in the context of clinical covariates, such as a patient gender. TDD, TDD *per* body weight, TTC and tacrolimus C/D ratio were chosen to be used as TDM tools.

Tacrolimus is well-known to be primarily metabolised in the intestine and liver, by the CYP 3A family, especially CYP 3A4 and CYP 3A5 members, and is a substrate for P-gp efflux pump [2, 4]. Some drugs that are substrates of CYP 3A4, including tacrolimus, show a higher clearance in women than in men, and that the difference persists after correcting some physiologic factors, such as body weight [35, 36]. According to our results, TDD *per* body weight was not significantly different between the genders, but the average TTC and tacrolimus C/D ratio in renal transplant patients were significantly lower in the females comparing with the males. Stratta et al. [5] suggested the tacrolimus C/D ratio as an alternative to the classic methods for evaluating tacrolimus exposure. Therefore, our results indicate a lower tacrolimus exposure in the females than in the males, which is in accordance with the previous findings of significantly lower values of tacrolimus AUC in female, as well as a longer mean $t_{1/2}$ in male patients compared with female ones [16]. The fact that total clearance of some substrates for CYP 3A are faster in females compared with males can be, at least partly, attributed to a higher hepatic CYP 3A4 content in females [37, 38].

According to the KDIGO clinical practice guidelines, the first-line agents for patients subjected to renal transplantation should include basiliximab induction, for low-risk patients, and an anti-thymocyte globulin for high-risk patients, in conjunction with maintenance immunosuppression, including tacrolimus, mycophenolate and steroids [39]. Since CYP 3A is responsible for > 90% of tacrolimus metabolic elimination, the inhibition or induction of CYP 3A4 will lead to clinically important drug interactions [34].

In our study, the patients treated with corticosteroid doses higher than 0.15 mg/kg had higher TTC and TDD *per* body weight, while their tacrolimus C/D ratio was lower in comparison to patients treated with doses lower than 0.15 mg/kg. Anglicheau et al. [34], similarly to our results, demonstrated that the higher the dose of steroids, the higher the dose of tacrolimus was needed to achieve target blood concentrations. Moreover, the higher the steroid dose was given, the lower tacrolimus C/D ratio was found. Since corticosteroids share metabolic CYP 3A and transporter P-gp pathways with tacrolimus, they are potential sites for pharmacokinetic interactions between these drugs. Generaly, corticosteroids are substrate or inducers of CYP 3A enzymes, but they can also act as their inhibitors [13, 34]. Higher TTC and TDD *per* body weight, as well as a lower tacrolimus C/D ratio in our patients treated with higher corticosteroid doses can be explained by corticosteroid induction of CYP 3A and P-gp pathways. Although tacrolimus interactions with corticosteroids are obviously of significant importance [1, 5, 7, 40], there are no enough data from clinical trials concerning their importance in kidney transplantation. However, the recently performed study indicated that corticosteroid withdrawal protocol profoundly affected tacrolimus levels and dosing [41]. Namely, a mean tacrolimus dose necessary to maintain similar TTC was higher in the group which was receiving corticosteroids during the whole study, compared to the group with an early steroid withdrawal (seven days after transplantation).

Studies investigating gender differences concerning corticosteroid treatment in renal transplant recipients have had conflicting results. Most of the data indicate that females generally have higher metabolism and clearance of drugs than males, owing to the higher activity of CYP 3A4 [42]. Considering corticosteroids, for example, the total clearance of methylprednisolone itself was 55% higher in females than in males [43]. On the other hand, corticosteroid IC_{50}, drug concentration which inhibits 50% of the maximum lymphocyte proliferation, as the indicator of immunosuppressive effects, is lower in females than in males, as far as prednisone and methylprednisolone are concerned [44]. Therefore, in comparison with males, immunosuppressive effect is achieved by lower blood steroid concentrations in females. Our results showed that although the female patients received a lower total daily steroid dose, if it is expressed in miligrams *per* kg of body weight, they were actually treated with significantly higher doses than the males. However, tacrolimus parameters monitored in this study (TTC, TDD *per* body weight and the tacrolimus C/D ratio) indicate that administration of significantly higher corticosteroid doses in the females was associated with faster metabolism of tacrolimus in comparison to the males. According to our results, this was not the case only with the group of patients treated with the highest corticosteroid doses (more than 0.25 mg/kg).

In our study, in the renal transplant recipients who were treated with proton pump inhibitors, TDD *per* body weight and TTC were significantly higher compared to the patients without this treatment, while the tacrolimus C/D ratio was significantly lower in those whose treatment included one of the drugs from this group. Considering gender, our results showed that the males treated with one of these drugs were given lower doses of tacrolimus in comparison to the females, while their tacrolimus C/D ratio was higher in comparison with the females. Therefore, it can be concluded that males are slower metabolisers of tacrolimus when they are comedicated with proton pump inhibitors, in comparison with the females. Proton pump inhibitors are well-known to be metabolised by cytochrome CYP 3A4, as well as by CYP 2C19 [45–47]. Their typical representative, omeprazole is a com-

petitive inhibitor of CYP 3A4-mediated tacrolimus metabolism, especially in poor metabolisers for CYP 2C19 [48]. In the patients with CYP 2C19 gene mutations, proton pump inhibitors tend to be metabolised by CYP 3A4, and, therefore, such patients have a higher risk of interactions between proton pump inhibitors and tacrolimus. Moreover, both tacrolimus and most of the proton pump inhibitors are substrates for P-gp drug transporter, while proton pump inhibitors also act as P-gp inhibitor [49–51]. In the paper concerning our previous study, in patients subjected to kidney transplantation, a significant correlation between the increase of TTC and omeprazole application was shown [29]. However, most of the patients in the present study were treated with pantoprazole, which is only marginally metabolised *via* CYP 2C19 and CYP 3A4 [37, 39] and is a not substrate for P-gp [52]. The fact that the patients treated with pantoprazole in comparison with the ones not treated with this drug also showed increased TTC and a significantly decreased tacrolimus C/D ratio is probably in accordance with the aforementioned findings. On the other hand, Takahashi et al. [52] found that the tacrolimus C/D ratio was markedly higher during transplant recipient treatment with omeprazole in comparison with those treated with ranitidine and rabeprazole. Although we can only speculate on the influence of pantoprazole on the tacrolimus metabolism at the moment, it can be concluded that tacrolimus exposure in these patients was less prominent when compared with the patients not treated with this proton pump inhibitor.

Tacrolimus is known to lead to adverse events in the patients with calcium channel blockers comedication [32, 53, 54]. Drug interactions between calcium channel blockers (diltiazem, verapamil and nifedipine) and tacrolimus, both competitive substrates of CYP 3A4 and CYP 3A5 system, as well as P-gp, can result in the rapid TTC increase [53]. The potential of calcium channel blockers for interactions with tacrolimus is thought to be mediated through their common metabolism by the CYP 3A system, as well as by the P-gp efflux mediated transport. The decrease of the tacrolimus clearance by this partial competitive inhibition of the metabolic pathways can lead to the significantly elevated tacrolimus blood level and the related toxicity [32, 54]. Moreover, diltiazem is a potent mechanism-based inhibitor of CYP 3A, whose metabolite becomes as a result of its N-demethylation by this enzyme. Inactivation of CYP 3A occurs by binding this metabolite tightly and irreversibly [55]. The P-gp pump can be inhibited by blocking drug binding sites with calcium channel blockers. As a result, the efflux of tacrolimus in the intestinal lumen is reduced, and increased TTC appears [56, 57]. Although TTC did not differ between the groups, TDD *per* body weight was significantly lower in the calcium channel blocker treated group, compared to the patients from the non-treated group, while the tacrolimus C/D ratio was significantly higher in the renal transplant recipients whose treatment included one of the drugs from this group (amlodipine and nifedipine). This is in accordance with the suggestion of Stratta et al. [5] who stated that when taking into account targeted tacrolimus concentration, the higher the tacrolimus C/D ratio, the slower the metabolic efficiency (requiring low tacrolimus dose). This reduced metabolic efficacy was obviously caused by its interaction with calcium channel blockers. This was actually shown in healthy subjects evaluated for tacrolimus-amlodipine interactions, in whom amlodipine significantly increased tacrolimus blood exposure in CYP 3A5*1 carriers [58]. On the other hand, amlodipine significantly increased tacrolimus blood levels in CYP 3A4*1 carriers, but decreased it in CYP 3A4*3 homozigote carriers [58]. In renal transplant recipients included in our study, amlodipine exerted this effect, while this was not the case with nifedipine. Namely, the examined tacrolimus parameters in the nifedipine-treated group were not significantly different from the parameters in the group not treated with calcium channel blockers. The differences in the examined tacrolimus parameters concerning genders also indicated faster metabolism of this immunosuppressive drug in females comparing to males in the calcium channel blocker treated group.

Two-way between-groups analysis of variance pointed out that the tacrolimus C/D ratio is more sensitive parameter than TTC, taking into accounts the influence of all the examined variables on tacrolimus exposure. However, since the variability of the tacrolimus C/D ratio was rather large, both parameters should be evaluated in clinical studies in order to define rational tacrolimus dosing approach.

The target concentration intervention (TCI) approach as an alternative conceptual strategy to TDM enables evaluation of pharmacotherapy by comparing the clinical outcomes associated with different target concentrations [59].

Conclusion

According to the results of our study, the renal transplant recipients showed lower tacrolimus exposure in the females than in the males. When gender differences were considered in the context of different comedications, a faster elimination of tacrolimus in the females was also seen, with the exception of the highest corticosteroid doses (> 0.25 mg/kg). As far as the influence of corticosteroid dose on tacrolimus exposure is concerned, if a higher steroid dose was given, the lower tacrolimus C/D ratio was found. It can also be concluded that tacrolimus exposure in the proton pump treated patients, mainly with pantoprazole, was significantly less prominent in comparison with the patients not treated with them. On the other hand, a reduced elimination efficacy of tacrolimus in the patients treated with chalcium channel blockers, predominantly with amlodipine, was probably caused by interactions with these drugs.

According to the findings of this study, together with TTC, the tacrolimus C/D ratio would enable better estimation of the infuence of additional factors, like gender and comedication, on tacrolimus exposure in the patients subjected to renal transplantation. Therefore, further study should be done in order to define the target tacrolimus C/D ratio and associated clinical endpoints for rational dose individualization in the real clinical settings.

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