C/D Ratio in Long-Term Renal Function

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ABSTRACT

Background/Aims. Tacrolimus is an immunosuppressive drug. Its C0 concentration, commonly used for monitoring, does not always correspond to its pharmacologic effect. Thölking et al developed an indicator, the C/D ratio, that describes the drug's metabolism rate. Our purpose was to determine whether the points dividing the patients into fast, intermediate, and slow metabolizers that were assumed by those authors would be similar for long-term follow-up after renal transplantation (RTx).

Methods. We examined the C/D ratio in 571 patients at their most recent appointments—1 year and more after renal transplantation. The mean time after RTx was 84 months. We studied kidney function both at the most recent appointment and early after RTx.

Results. The median C/D ratio for our group was 1.68. Our observations revealed a negative correlation between the C/D ratio and creatinine concentration and a positive correlation between the C/D ratio and eGFR concentration long term after RTx. We formulated a C/D ratio cutoff point between an eGFR < and $\geq$ 60 mL/min/1.73 m^2 and came up with the value of 1.53. It was found that between the < 1.53 and $\geq$ 1.53 groups, there were significant differences in creatinine and eGFR concentrations at the most recent appointment, as well as differences in how creatinine and eGFR levels varied over time between RTx and the most recent observation.

Conclusions. The C/D ratio is useful for assessing the effect of the tacrolimus metabolism rate on long-term renal function. We propose the C/D ratio value of 1.53 as the cutoff point below which the ratio provides a negative prognosis for long-term renal function.

THE calcineurin inhibitor tacrolimus, in combination with mycophenolate mofetil and glucocorticosteroids, is a basis of immunosuppressive therapy following renal transplantation. The introduction of modern immunosuppression led to a reduction in the prevalence of acute renal transplant rejection, but chronic renal allograft failure remains a serious issue. Approximately 3% to 5% of renal allografts are lost annually after the first year following RTx [1,2]. There are various causes of long-term renal transplant failure, and 1 of them is calcineurin inhibitor nephrotoxicity. Tacrolimus has a narrow therapeutic window. An association has been observed between the drug's C0 concentration

(trough level) and its nephrotoxicity. [3–6]. Nevertheless, some authors have also noted nephrotoxicity among patients with low C0 concentrations of the drug [7]. Various factors are known to influence the drug's metabolism, such as hematocrit, serum albumin, age, sex, or body mass index.

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Cytochrome—mainly CYP 3A5—expression has a large impact on tacrolimus metabolism. Patients with a genotype containing the allele 1 – CYP3A5*1/*1 and CYP3A5*1/*3 are the so-called expressors producing the active form of the protein. Patients without the allele 1 – CYP3A5*3/*3 are nonexpressors producing the inactive form of the protein and requiring lower tacrolimus doses to reach the target blood concentration of the drug [8,9]. Approximately 95% of the white race have CYP3A5*3. Still, there exists a very substantial interindividual variability in respect of tacrolimus metabolism. The polymorphism of the enzyme's cofactors has some effect on the drug's metabolism as well. The interindividual variability is also affected by the presence of CYP3A5 in different organs, such as the liver, kidneys, or intestines, as the enzyme's expression levels may differ between them. For instance, CYP3A5 may demonstrate higher expression levels in renal tubules than the liver, thus reducing the drug's nephrotoxicity [10]. Its increased expression in the intestines may reduce the bioavailability of tacrolimus by up to 50% [11]. For these reasons, screening for cytochrome expression is not useful. Rather, the drug's metabolism rate should be studied. In 2014, Thölking et al introduced the C/D ratio, which is the tacrolimus blood C0 concentration expressed in ng/mL normalized by the daily dose of the drug expressed in mg. The same authors also introduced the C/D ratio cutoff point for fast and slow metabolizers. Additionally, they found that the tacrolimus metabolism rate had an effect on short- and long-term renal allograft function [12]. In our research we decided to discover whether the cutoff values for fast and slow metabolism arbitrarily determined in Thölking's article were indeed correct in drawing division lines between fast, intermediate, and slow tacrolimus metabolizers.

MATERIALS, PATIENTS AND METHODS
Patients

Our analysis covered 571 patients at a minimum 1 year after renal transplantation who were under the care of the Clinical Department of Nephrology, Transplantology, and Internal Medicine at Pomeranian Medical University in Szczecin and the Department of Nephrology of the Integrated Hospital in Szczecin. The average length of the post-transplantation period was 84 months (a minimum of 12 months and a maximum of 288 months). After kidney transplantation, all of the patients received triple immunosuppressive therapy with glucocorticosteroids, a calcineurin inhibitor (tacrolimus), and mycophenolate mofetil. Some of the patients were given the immediate-release formula of tacrolimus, which they received twice daily in 12-hour intervals, and the others were given the slow-release formula, which they received once a day. The concentrations and doses of tacrolimus were studied at the 2 most recent appointments held 1 to 2 months apart, then the C/D ratio was calculated based on these measurements. The ratio was obtained by dividing the drug's C0 concentration expressed in ng/mL by its daily dose expressed in mg. Moreover, the lowest creatinine concentration (NADIR Crea) and the highest eGFR concentration (ZENITH GFR), achieved up to 6 months after RTx, as well as the most recent creatinine and eGFR

concentrations, were studied. Also, the difference between the current creatinine and eGFR concentrations and the best values achieved up to 6 months after RTx were studied. Patient details are shown in Table 1.

Methods

Creatinine concentration was determined in blood serum using the immunoenzymatic method, while tacrolimus concentration was determined in whole blood at the Teaching Hospital's central laboratory. Only tacrolimus C0 (trough) levels were used for analysis. The GFR level was estimated using the CKD-EPI Formula with the help of the National Kidney Foundation calculator. Human involved in this study were treated in a manner in accordance with the Declaration of Helsinki and the Declaration of Istanbul. The local ethics committee of the Pomeranian Medical University in Szczecin, Poland, approved the protocol study—KB-0012/23/18 (2-5-18).

STATISTICAL ANALYSIS

We used Statistica 11 software (StatSoft, Tulsa, USA) for statistical analysis. The Shapiro-Wilk test was used to study the distribution. The Mann-Whitney U test was used to compare the 2 groups of non-normal distribution. Spearman's rank correlation test was used to study correlations. Not normally distributed data were shown as the median (minimum-maximum). P values were noticeable if $< .05$. The receiver operating characteristic (ROC) curve was drawn in order to determine the C/D ratio's predictive value for renal function defined as eGFR concentration.

Table 1. Patient Details

	N	Mean	SD
Last Crea (mg/dL)	571	1.56	0.86
Last eGFR (mL/min/1.73 m^2)	571	54	21.8
NADIR Crea (mg/dL)	571	1.33	0.5
ZENITH eGFR (mL/min/1.73 m^2)	571	63.7	22.3
Δ Crea (mg/dL)	571	0.22	0.68
Δ eGFR (mL/min/1.73 m^2)	571	9.1	18.8
Time after Tx (month)	571	83.9	59
TAC IR	191		
TAC SR	380		
Age (years)	571	46.6	12.6
CIT (h)	571	21	9
HLA mismatches			
A	571	1.21	0.7
B	571	1.3	0.7
DR	571	0.8	0.6
Rejection	571	0.16	0.36
Donor age (years)	571	45	12.3

Abbreviations: Δ Crea, difference between most recent crea and NADIR crea; ΔGFR, difference between most recent eGFR and ZENITH eGFR; CIT, cold ischemic time; Last Crea, mean creatinine value at 2 most recent appointment; Last eGFR, mean estimated glomerular filtration rate value (by CKD-EPI calculation) at 2 most recent appointments; NADIR crea, lowest creatinine concentration in first 6 months after renal transplantation; ZENITH eGFR, highest estimated glomerular filtration rate in first 6 months after renal transplantation (CKD-EPI calculation); TAC IR, immediate-release tacrolimus (received twice a day); TAC SR, slow-release tacrolimus (received once a day); time after Tx, time after renal transplantation.

RESULTS

The Shapiro-Wilk test was used to determine the distribution of the C/D ratio. The distribution was not normal. The median value as 1.68 (with Min-Max 0.2 to 13.4, Fig 1). Using Spearman's test, a negative correlation was identified between the C/D ratio and creatinine concentration at the most recent appointments: $P < .05$, R = -0.17 (Fig 2), and a positive correlation was found between the C/D ratio and eGFR values at the 2 most recent appointments: $P < .05$, R = 0.11 (Fig 3). Moreover, a positive correlation was observed between the C/D ratio and the time after RTx expressed in months: $P < .05$, R = 0.17 (Fig 4). Because Spearman's rank correlation test shows a low value of R, it is rather the trend. In order to determine the C/D ratio cutoff point that divided the ratio's values into favorable and unfavorable for long-term renal function, the ROC curve was plotted, and the C/D ratio cutoff point was determined at a level that separated GFR concentrations greater than or equal to 60 mL/min/1.73 m^2 from GFR concentrations below that value. This particular GFR value was assumed in this study because the mean GFR level in our group was 54 mL/min/1.73 m^2 and because CKD is classified based on the eGFR levels, with an eGFR concentration of 60 mL/min/1.73 m^2 used to separate mild from moderate renal injuries. With such an eGFR level, the C/D ratio cutoff point was 1.53 (Fig 5). The study group patients were divided into those with a C/D ratio below and those over and equal to 1.53. A statistically noticeable correlation was identified between them in respect of creatinine and eGFR levels at the most recent appointments ($P = .002$ and $P = .002$, respectively). In the C/D ratio $\geq$ 1.53 group, the time after renal transplantation was noticeably longer ($P = .002$). We found that between the C/D ratio < 1.53 and $\geq$ 1.53 groups, there were significant differences revealed by the Δ check for creatinine and GFR between early into the post-transplantation period and the most recent observations. The increase in creatinine concentrations and the

Fig. 1. Histogram of the distribution of the C/D ratio (ng/ml/mg). The patients showed a not-normal distribution. Median value − 1,68.

drop in GFR levels at the time of the observation were significantly greater in the C/D ratio < 1.53 group ($P = .02$ for Δ crea and $P = .01$ for Δ GFR). We found no difference in the C/D values or renal function between the group receiving immediate-release tacrolimus and those receiving slow-release tacrolimus. We found that between the C/D ratio $\leq$ 1.53 and $\geq$ 1.53 groups, there were significant differences of daily dose and concentration of tacrolimus (Table 2). We compared the groups with C/D ratio above and below 1.53 by such data as: episodes of acute rejection and HLA mismatches, cold ischemic time, number of transplantation, age of recipients and donors; we did not find any differences between them.

DISCUSSION

Immunosuppressive therapy prevents both acute and chronic renal allograft rejection. Tacrolimus is the preferred drug in the standard triple immunosuppressive therapy and will continue to be used in the years to come. Because of its narrow therapeutic window and interindividual pharmacokinetic variability, it is necessary that therapies with this drug should be individualized in order to avoid overdosage or underdosage. In daily medical practice, the C0 concentration measurement is the only indicator of the drug's metabolism. However, the C0 level and the dose of the drug do not always correlate with the pharmacologic effect. Hence, there is a need for new therapy monitoring methods. One of these could be the C/D ratio developed by Thölking et al. These authors used values lower and greater than the mean value in order to divide their patients into 3 groups of the so-called fast, intermediate, and slow tacrolimus metabolizers. The C/D ratio values for these groups were set at <1.05, 1.05 to 1.54, and $\geq$ 1.54. Does this division reflect the actual existence of 3 groups demonstrating varying tacrolimus metabolism rates affecting renal function? In our research, the study group differed noticeably from the 1 examined by Thölking et al. Ours included far more patients and with different times after RTx−12 months and more. It was less homogenous, but it allowed for observations of the effects that the ratio had on long-term renal allograft function. In our studies, we identified a negative creatinine-C/D ratio correlation and a positive eGFR-C/D ratio correlation (the ratio, as well as creatinine and eGFR concentrations, were determined on the basis of measurements from 2 most recent appointments) for the whole group. This meant that the tacrolimus metabolism rate had an effect on long-term renal allograft function. At each distribution point (Fig 2), a higher ratio was more favorable for long-term renal function. In that case, are the C/D ratio points determined by Thölking et al justified? According to those authors' results, a negative effect on renal function (in a 2-year follow-up) at 1, 2, 3, 6, 12, and 24 months was exerted by both fast and intermediate metabolism. Favorable effects throughout the follow-up period were only achieved with a C/D ratio $\geq$ 1.54. Intermediate metabolizers only started to demonstrate favorable effects on renal
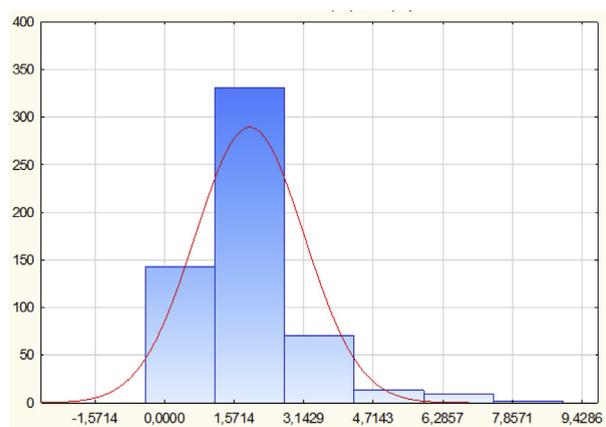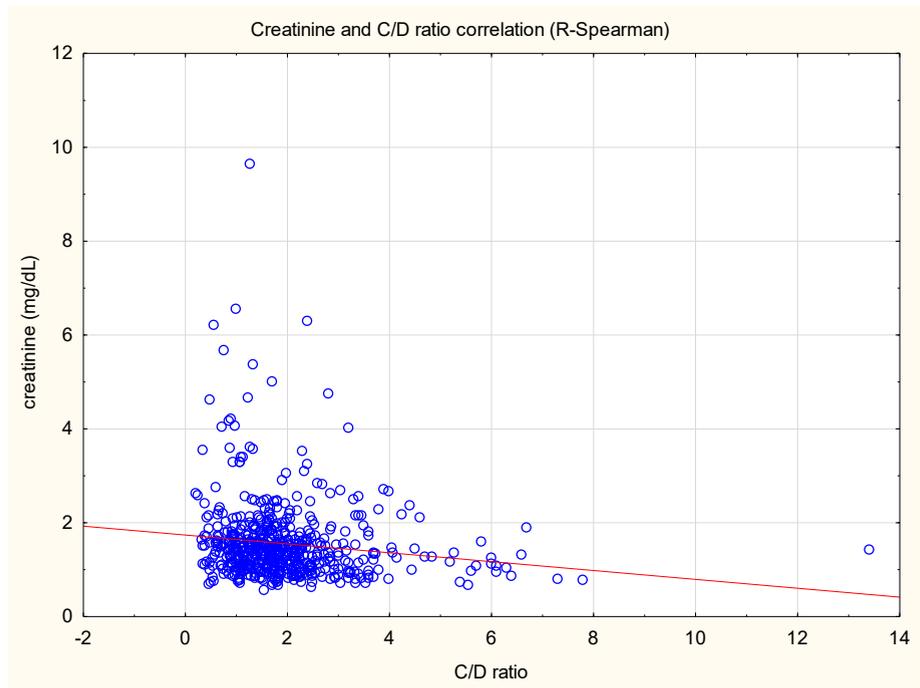
Fig. 2. Negative correlation between creatinine concentration and the C/D ratio.

allograft function at month 6 after RTx. As the C/D ratio across the entire group had an effect on renal function, we decided to find the cutoff point that would have an effect on long-term prognosis. For this purpose, the ROC curve was plotted, and the cutoff point was set at a GFR greater than or equal to and a GFR lower than 60 mL/min/1.73 m^2. This

GFR value was assumed in this study because the mean GFR level in our group was 54 mL/min/1.73 m^2 and because CKD is classified based on the eGFR levels, with an eGFR concentration of 60 mL/min/1.73 m^2 used to separate mild from moderate renal injuries. With such an eGFR level, the C/D ratio cutoff point was 1.53. A C/D ratio of 1.53 is a
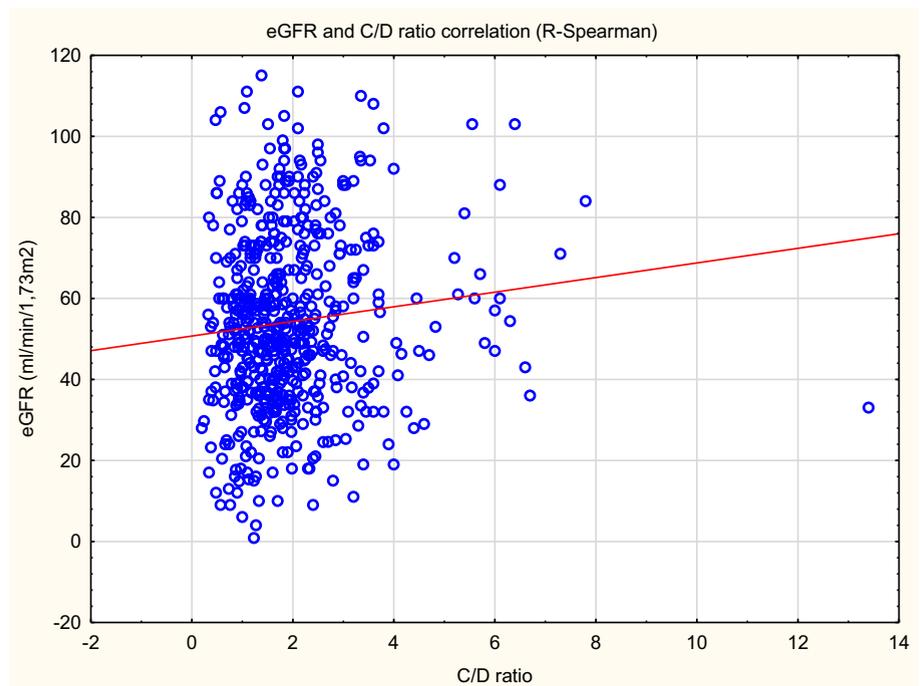

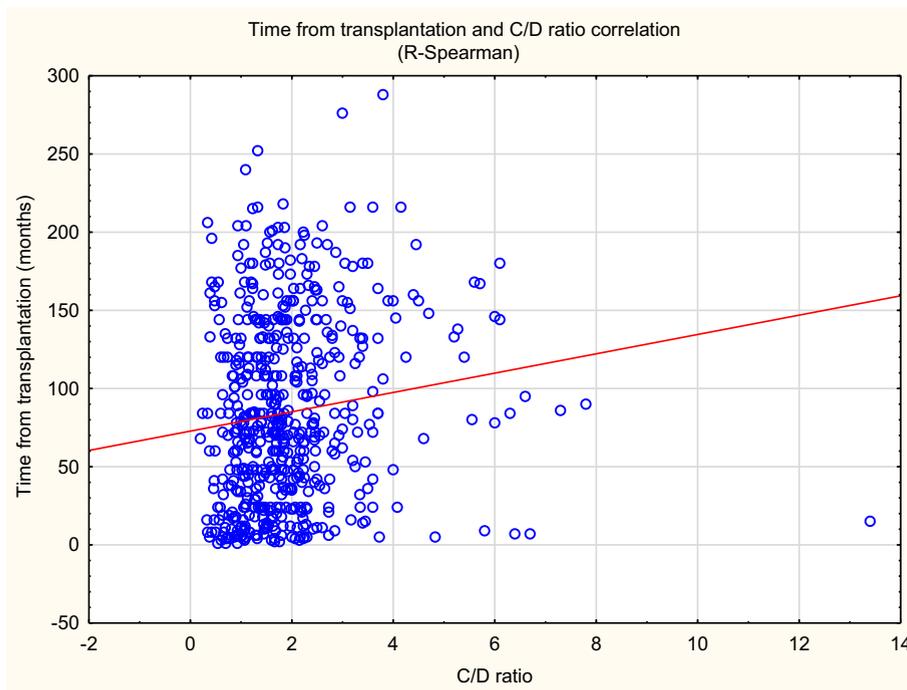**Fig. 3.** Positive correlation between the C/D ratio and eGFR concentration.

Fig. 4. Positive correlation between the C/D ratio and the time after RTx expressed in months.

prognostic factor for long-term renal allograft function. This value is close to the 1 proposed by Thölking et al as dividing intermediate and slow metabolizers. There is no point in forming 3 patient groups (of slow, intermediate, and fast metabolizers) instead of only 2–1 of slow and 1 of fast metabolizers. In our group of patients, the time after kidney transplantation was over 24 months for 465, over 5 years for 356, and over 10 years for as many as 169 of them. A C/D ratio of 1.53 can be assumed to be a prognostic factor for a truly long-term renal allograft function. Moreover, we found a positive correlation between the C/D ratio value and the time after renal transplantation. This correlation may be pointing to the existence of 2 mechanisms. The first may be that of tacrolimus metabolism slowing down following renal transplantation. Evidence to that may be found in the much lower mean C/D ratio in Thölking's group, $P = 1.29$. His ratio value was calculated on the basis of measurements made at 1, 3, and 6 months after RTx. It could primarily be the effect of glucocorticosteroids, as it has been proven that they are responsible for accelerating tacrolimus metabolism [13]. After renal transplantation, the dose of glucocorticosteroids is tapered; in some patients, its administration is discontinued completely in favor of double immunosuppressive therapy (single cases in our group). Also, the interactions between this and other drugs administered early in the post-transplantation period should be considered.
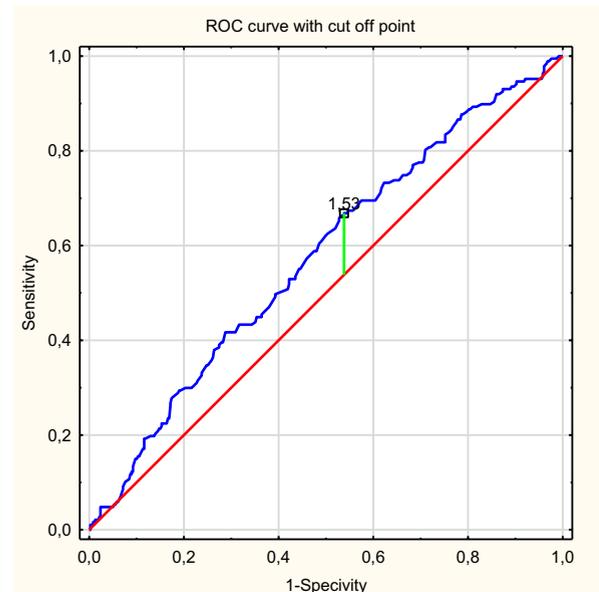

Fig. 5. The ROC curve and the C/D ratio cut-off point for a GFR concentration greater than or equal to 60 mL/min/1.73 m2, and lower.

Table 2. The Mean Value of Daily Dose and Concentration of Tacrolimus in Group With C/D Ratio Below and Above 1.53

	C/D Ratio < 1.53		C/D Ratio ≥ 1.53		
	Mean	SD	Mean	SD	P
Daily dose of tacrolimus (mg)	6.48	3.78	3.07	1.3	< .05
Concentration of tacrolimus (ng/mL)	6.13	2.78	7.1	2.5	< .05

In a Mann-Whitney *U* test, we found between the C/D ratio < 1.53 and ≥ 1.53 groups there were significant differences of daily dose and concentration of tacrolimus.

Abbreviation: SD, standard deviation.

The second mechanism that may be responsible for the correlation discussed here is that the active renal allograft functions longer in greater C/D ratio patients. In order to find out exactly what the mechanism behind this correlation is, the C/D ratio in each patient should be determined repeatedly, prospectively from the time of transplantation, over a prolonged follow-up period. When we divided the study group into patients with a C/D ratio < and ≥ 1.53, we found that at the 2 most recent appointments, the first group demonstrated worse renal function defined as either creatinine concentration or eGFR concentration. The C/D ratio did not have an effect on NADIR creatinine or ZENITH eGFR. This could mean that the ratio is an indicator of long-term renal allograft function. Although tacrolimus nephrotoxicity is the greatest at the very beginning (as the doses are the largest), creatinine concentration grows only when renal injuries have accumulated and become considerable. This is why creatinine concentration has long been considered to be an insensitive marker of low-degree injuries. Changes in creatinine and eGFR concentrations were studied over time (since early into the post-transplantation period until the most recent observation). It was revealed that the C/D ratio < 1.53 group showed a significantly greater increase in creatinine concentration and a significantly greater drop in the eGFR value when compared to the C/D ratio ≥ 1.53 group, which allowed us to set the cutoff point at this particular value. A group of patients whose fast tacrolimus metabolism has a negative effect on renal function can be distinguished. For this group, modifications to the immunosuppressive therapy could be considered, such as the administration of mammalian target of rapamycin inhibitors accompanied by reduced tacrolimus doses or the administration of new immunosuppressive drugs−costimulation blockers (bellatacept). As the mechanisms responsible for the development of calcineurin inhibitor nephrotoxicity are well known, methods that diminish those mechanisms can be used in this group of patients, such as: inhibition of the renin-angiotensin-aldosterone system, administration of calcium channel blockers for the purpose of dilating the afferent glomerular arterioles, or application of antioxidants or known CYP3A5 inhibitors [14,15].

CONCLUSION

The C/D ratio, an indicator of the tacrolimus metabolism rate developed by Thölking et al, is a useful marker of long-term renal allograft function. It allows for a subgroup of patients particularly exposed to the nephrotoxic effects of tacrolimus to be singled out. On the basis of our research outcomes, we can recommend using the C/D ratio of 1.53 to divide patients into fast and slow metabolizers. Using the C/D ratio of 1.05 in order to distinguish between the groups of slow and intermediate metabolizers is not advisable. As the ratio values keep changing over time following kidney transplantation, there is a need for prospective long-term research focused on multiple determinations of the ratio in particular patients.

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