**Title page**

**Title**

Cyclosporine Metabolites’ Metabolic Ratios May Be Markers of Cardiovascular Disease in Kidney Transplant Recipients Treated with Cyclosporine A-Based Immunosuppression Regimens

**Authors**

Ewa HRYNIEWIECKA1,2, Jolanta ŻEGARSKA2, Dorota ŻOCHOWSKA2, Emilia SAMBOROWSKA3, Radosław JAŹWIEC3, Maciej KOSIERADZKI4, Sławomir NAZAREWSKI5, Michał DADLEZ3,6, Leszek PĄCZEK2,7

**Affiliations**

1Department of Clinical Nursing, Medical University of Warsaw, 27 Ciolka St, Warsaw, Poland; tel. 0048228360972; fax 0048228360972

2Department of Immunology, Transplant Medicine and Internal Diseases, Medical University of Warsaw, 59 Nowogrodzka St, Warsaw, Poland; tel. +48225021461; fax +48225022130

3 Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Science, 5a Pawinskiego St, Warsaw, Poland; tel. 0048225923476; fax 0048226584766

4Department of General and Transplantation Surgery, Medical University of Warsaw, 59 Nowogrodzka St, Warsaw, Poland; tel. 0048225021126; fax 0048225022155

5Department of General, Vascular and Transplant Surgery, Medical University of Warsaw, 1a Banacha St, Warsaw, Poland; tel. 0048225992467; fax 0048225991468

6 Institute of Genetics and Biotechnology, Biology Department, 5a Pawinskiego St, Warsaw, Poland; tel. 0048226596072

7Department of Bioinformatics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 5a Pawinskiego St, Warsaw, Poland; tel. 0048225921108; fax 0048225922190

**Corresponding author**

Ewa Hryniewiecka, Department of Immunology, Transplant Medicine and Internal Diseases, Medical University of Warsaw, 59 Nowogrodzka St, Warsaw, Poland; tel. +48225021461; fax +48225022130; ewa.hryniewiecka@wum.edu.pl

**Acknowledgments:**

This work was supported by Polish National Science Centre (grant no. 2013/09/B/NZ2/00275) and Polish National Centre of Research and Development (grant no. NR13014410).

NCN (grant nr 2013/09/B/NZ2/00275) i NCBiR (grant nr NR13014410)

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Keywords:**

Kidney transplantation, cyclosporine, cyclosporine metabolites, cardiovascular disease, arrhythmia, hypertriglyceridemia, hyperuricemia, obesity, overweight

**Abstract:**

Cardiovascular disease (CVD) remains one of the primary causes of death after kidney transplantation (KTX). Cyclosporine (CsA) metabolites may play a role in CVD. Metabolic ratio (MR) may be considered a measure of intraindividual differences of CsA metabolism. The study was aimed at analysis of associations of CVD with indices of CsA metabolism: MRs and dose-adjusted CsA concentrations (C/D and C/D/kg).

The study was performed in the Department of Immunology, Transplant Medicine, and Internal Diseases of the Medical University of Warsaw and involved 102 KTX recipients. Whole blood concentrations of cyclosporine A, AM1, AM9, AM4N, demethylcarboxylated (dMC-CsA), dihydroxylated (DiH-CsA), trihydroxylated (TriH-CsA) cyclosporine metabolites were determined by liquid chromatography coupled with tandem mass spectrometry.

Lower AM9/CsA were observed in diabetics. Patients with coronary disease and/or myocardial infarction had lower dMC-CsA/CsA and higher AM4N/CsA. Supraventricular arrhythmia was associated with higher AM1/CsA and AM4N/CsA. Hypertriglyceridemia (hTG) was associated with lower AM9/CsA, higher C/D and C/D/kg. Hyperuricemia and/or gout were associated with higher D/C and AM1/CsAMR. Decrease of AM9/CsA and AM4N and higher D/C were associated with overweight/obesity. Systolic blood pressure (BP) positively correlated with dMC-CsA/CsA and negatively with C/D/kg. Diastolic BP correlated positively with AM1/CsA, dMC-CsA/CsA, DiH-CsA/CsA and TriH-CsA/CsA.

We have demonstrated the association of coronary disease/myocardial infarction, supraventricular arrhythmia, hypertriglyceridemia, hyperuricemia/gout, overweight/obesity and elevated arterial BP with higher MRs of AM1, AM4N, dMC-CsA, DiH-CsA and TriH-CsA, and lower MRs of AM9, which may indicate deleterious and favourable effects of individual CsA metabolites on cardiovascular system and suggest engagement of specific enzymatic pathways.

**Introduction**

Cardiovascular disease (CVD) remains one of the primary causes of death after kidney transplantation (KTX) [1, 2]. It is estimated that cardiovascular causes (cardiac arrhythmia, cardiac arrest, heart failure, acute myocardial infarction and coronary disease) account for 28% of deaths after KTX and kidney transplant recipients have a 6.4% higher risk of deaths or major cardiovascular event compared with the general population [1, 3].

Observations and studies conducted since the introduction of cyclosporine A (CsA) to the clinical practice indicate the existence of an association between CsA therapy and cardiovascular morbidity and mortality [4, 5]. Despite many years of research, it has not been determined with certainty whether CsA metabolites play a role in the occurrence of nephrotoxic effects and cardiovascular morbidity.

It is suggested that due to variable metabolic activity resulting from genetic polymorphisms of cytochrome P450 CYP3A4, CYP3A5 and glycoproteine P, as well as various extragenetic factors, rate of formation of individual CsA metabolites can differ significantly between individuals [6-8]. Cyclosporine metabolites AM1, AM9 and AM4N are considered the first-generation metabolites formed during phase I metabolism and may be detected in the blood [9]. The main P450 enzyme metabolizing CsA is CYP3A4 which contributes to approximately 80% of phase I metabolism and leads to formation of AM1, AM9 and AM4N [10, 11]. Cytochrome CYP3A5 has been also shown to metabolize CsA contributing mainly to formation of AM9 [11]. Metabolic ratio (MR) has been proposed as a parameter calculated by dividing metabolite concentration by the parent drug concentration. It can be considered a measure of variability of concentrations of individual CsA metabolites [12]. The concentration to daily dose ratio or dose-adjusted concentration: C/D [ng/mL/mg/day] and dose/kg-adjusted concentration: C/D/kg [ng/mL/mg/kg/day] may be considered as a general measure of rate of cyclosporine metabolism [7, 13].

The study was aimed at analysis of associations of CVD with cyclosporine metabolites and indices of CsA metabolism: CsA metabolites’ metabolic ratios and dose-adjusted CsA concentrations.

**Materials and methods**

The study was performed in the outpatient clinic of the Department of Immunology, Transplant Medicine, and Internal Diseases of the Medical University of Warsaw (MUW). The study involved 102 KTX recipients from each of whom 2 mL of blood was collected during the routine outpatient visit. The study group consisted of 49 females (48%), mean age was 50.6 years (SD 12.4), median time from KTX was 116.5 months (IQR 59.3-170.8).

The collected blood specimens were analysed by liquid chromatography coupled with tandem mass spectrometry (LC-MS-MS) using Waters Acquity Ultra Performance Liquid Chromatograph coupled with Waters TQ-S triple-quadrupole mass spectrometer (Waters, Manchester, UK) as described previously [14]. We have determined whole blood concentrations of cyclosporine A, AM1, AM9, AM4N, demethylcarboxylated cyclosporine metabolites (dMC-CsA), dihydroxylated cyclosporine metabolites (DiH-CsA) and trihydroxylated cyclosporine metabolites (TriH-CsA). The mass spectrometer operated in the multiple-reaction monitoring (MRM)- positive electrospray ionization (ESI) mode. For all analytes mass spectrometer optimized settings were as follows: capillary voltage = 2.5 kV, desolvation temperature = 200ºC, cone gas flow = 150 L/h, desolvation gas flow = 800 L/h, source temperature = 150°C. The ion transitions were m/z 1219.876> 1202.658 and m/z 1219.876> 1184.812 for CsA, m/z 1223.8> 1206.5 for CsA-D4 (used as internal standard), m/z 1218.68> 425.6 and m/z 1218.68> 1182.85 for AM1, m/z 1218.68> 212.4 and m/z 1218.68> 449 for AM9, m/z 1188.64> 661.49 and m/z 1188.64> 1170.7 for AM4N, m/z 1251.865> 1234.839 for DiH-CsA, m/z 1267.86> 1250.83 for TriH-CsA, m/z 1249.88> 1232.85 for dMC-CsA .Only first was used as quantification transition. The calibration curves range were 0.5-1000ng/ml, 2-1500ng/ml and 0.2-100ng/ml for CsA, AM1 and AM4N, respectively. Concentration of AM9 was calculated using AM1 calibration curve, other CsA metabolites concentrations were quantified using CsA calibration curve.Clinical and laboratory data were extracted from patients’ medical records and included in dedicated database.

**Compliance with Ethical Standards**

The study protocol was approved by the MUW Ethical Committee. Before the study procedures all patients have given their written informed consent. All procedures performed were in accordance with the ethical standards of the MUW Ethical Committee and with the 1964 Helsinki declaration and its later amendments. This article does not contain any studies with animal performed by any of the authors.

**Statistical analysis**

All calculations were performed using IBM SPSS software (IBM Corp., Armonk, NY, USA). Shapiro-Wilk and Bartlett’s tests were used for identification of normally distributed variables for continuous and categorical variables respectively. Variables with normal distribution are presented as mean and standard deviation (SD) and were analysed using Pearson correlation coefficient, Student’s t-test; not-normally distributed variables are presented as median and interquartile range (IQR) and were analysed using Spearman correlation coefficient and U Mann-Whitney test. Multivariable analyses were performed by logistic regression and included independent variables: patients’ age and gender, eGFR, time from KTX, steroid therapy, pMPA therapy and CsA concentration or daily dose. The differences were considered statistically significant when p<0.05.

**Results**

Demographic, clinical and laboratory data of the study participants are summarized in table 1. Analysis of associations between dose-adjusted and dose/kg-adjusted CsA concentrations has revealed negative correlations of AM9/CsA with C/D (r=-0.32, p=0.01) and of dMC-CsA/CsA (r=-0.5, p=0.03).

We have observed lower AM9/CsA in patients with diabetes mellitus (DM) (61.5 [IGR: 49.2-71.4] vs 74.8 [IQR: 60.1-90.1], p=0.03) and it was confirmed in multivariable analysis (β=-0.04, OR=0.96, 97.5% CI 0.93-0.99, p-0.01) (Figure 1A).

Patients with coronary disease (CD) and/or myocardial infarction (MI) had lower dMC-CsA/CsA ratios (0.53 [IQR: 0.43-0.6] vs 0.84 [IQR: 0.62-1.14], p=0.02). In multivariate analysis CD/MI was associated with higher AM4N/CsA (β=0.83, OR=2.3, 97.5% CI 1.1-5.3, p=0.03).

Supraventricular arrhythmia (SVA) was associated with higher AM1/CsA (471.6 [IQR: 460.8-483.7] vs 389.1 [IQR: 317.0-461.6], p=0.046) and AM4N/CsA (3.9 [IQR: 2.5-4.3] vs 2.8 [IQR: 2.1-3.6], p=0.03) which was confirmed in multivariate analysis (β=1.24, OR=3.5, 97.5% CI 1.6-10.4, p=0.007).

Hypertriglyceridemia (hTG) was associated with lower AM9/CsA (61.3 [IQR: 52.2-74.9] vs 74.3 [IQR: 61.8-94.6], p=0.008) which was confirmed in multivariate analysis (β=-0.02, OR=0.98, 97.5% CI 0.95-0.99, p=0.04). There was also a negative correlation of TG levels with AM9/CsA (β=-5.6, p=0.0003) (Figure 1C). hTG was associated with lower metabolism rate expressed by higher dose-adjusted CsA concentrations: C/D (53.7 ng/mL/mg/day [IQR: 38.9-67.2] vs 32.6 ng/mL/mg/day [IQR: 29.0-41.2], p=0.000001) and C/D/kg (0.65 ng/mL/mg/kg/day [IQR: 0.54-0.84] vs 0.49 ng/mL/mg/kg/day [IQR: 0.45-0.59], p=0.008). It was confirmed in multivariate analysis for C/D (β=0.11, OR=1.12, 97.5% CI 1.05-1.21, p=0.002) and C/D/kg (β=3.9, OR=51, 97.5% CI 2.5-2609, p=0.03).

In patients with hyperuricemia and/or gout we have observed higher dose-adjusted CsA concentrations D/C (60.5 ng/mL/mg/day [IQR: 43.4-71.4] vs 37.2 ng/mL/mg/day [29.1-48.0], p=0.001), which was confirmed in multivariate analysis (β=0.04, OR=1.04, 97.5% CI 1.01-1.08, p=0.02). Multivariate analysis has revealed association of higher AM1/CsA with hyperuricemia and/or gout (β=0.006, OR=1.08, 97.5% CI 1.002-1.01, p=0.01).

Decrease of AM9/CsA was associated with overweight and obesity (51.0 [IQR: 46.8-61.4] vs71.4 [IQR: 59.6-84.4], p=0.001) (Figure 1D) with similar trend for AM4N/CsA (2.32 [IQR: 1.95-2.8] vs 2.83 [IQR: 2.23-3.7], p=0.054), and it was confirmed in multivariate analysis (β=-0.06, OR=0.04, 97.5% CI 0.89-0.98, p=0.004 and β=-0.85, OR=0.43, 97.5% CI 0.18-0.86, p=0.03, respectively). There was also negative correlation of AM9/CsA with BMI (r=-0,39, p=0.00001) confirmed in multivariate analysis (β=-.07, p=0.003) (Figure 1B), which revealed also a positive correlation of dMC-CsA/CsA with BMI (β=4.9, p=0.003). In patients with obesity/overweight we have observed higher dose-adjusted CsA concentrations D/C (63.7 ng/mL/mg/day [IQR: 48.9-73.5] vs 40.0 ng/mL/mg/day [IQR: 30.7-55.9], p=0.02) and it was confirmed in multivariate analysis (β=0.06, OR=1.07, 97.5% CI 1.01-1.13, p=0.02).

Using multivariable analysis we have found positive correlation of systolic blood pressure (SBP) with dMC-CsA/CsA (β=9.8, p=0.04), and negative correlation with dose/kg-adjusted CsA concentration C/D/kg (β=-21.3, p=0.03). Diastolic blood pressure (DBP) correlated positively with AM1/CsA (r=0.22, p=0.03 and β=0.02, p=0.03), dMC-CsA/CsA (β=11.3, p=0.0005), DiH-CsA/CsA (r=0.34, p=0.001 and β=0.12, p=0.0006) and TriH-CsA/CsA (β=2.2, p=0.002).

There were no statistically significant differences nor correlations for arterial hypertension, peripheral artery disease, stroke, ventricular arrhythmia, hypercholesterolemia and CVD in general.

**Discussion**

The first interesting finding was the general lack of associations between dose-adjusted (C/D) and dose/kg-adjusted (C/D/kg) CsA concentrations with metabolic ratios of individual cyclosporine metabolites. If we assume that C/D and C/D/kg ratios are a general measure of the rate of CsA metabolism, then their higher values should occur in so called “poor metabolizers”. Those subjects should have lower MR values. However, the inverse relationship was demonstrated only for AM9/CsA and C/D, and dMC-CsA/CsA. This probably means that the greater metabolic clearance of the drug does not fully reflect the extend of the formation of individual metabolites. Thus, C/D and C/D/kg ratios cannot be used as a surrogate for direct measurements of individual CsA metabolite concentrations.

It is well established that CsA therapy is associated with cardiovascular disease including high incidence of arterial hypertension [15, 16]. Histopathological changes affecting cardiac muscle both in atria and ventricles, such as degenerative changes, myocardial fibrosis and myofibrils’ disorganization, were also described [17-19]. Searching for the causes of this complications has led to the identification of certain mechanism that link CsA to adverse effects on the cardiovascular system [5]. In this context the effect of CsA metabolites was also suspected, however small number of studies were carried out to clarify this issue and they have concentrated mainly on histopathological issues or unfavourable influence of parent drug cyclosporine A (18, 20). The majority of studies analysed mechanism of nephrotoxicity and vasoconstriction pointing at cyclosporine, AM4N, AM1 and AM9 as having the highest nephrotoxic potency [21]. Tubular and mesangial cells viability and proliferation are inhibited mainly by cyclosporine, AM9, AM1A, AM1c, AM1c9 AM4N, AM1 and AM19 [22, 23].

Our analyses revealed significant associations of higher AM4N/CsA MR with coronary disease/myocardial infarction and supraventricular arrhythmia. This may suggest both the deleterious effect of AM4N on coronary arteries and the promotion of atherosclerotic processes, as well cardiomyocyte damage also at the level of atria. Interestingly, the occurrence of obesity/overweight and higher BMI was accompanied by lower metabolic ratios of AM4N/CsA and AM9/CsA. In contrast, in obese/overweight KTX recipients higher dMC-CsA/CsA and dose-adjusted CsA concentrations were observed. Differences in the direction of relationships between MRs of individual metabolites and dose-adjusted CsA concentrations emphasize the variability of the proportion of studied metabolites with respect to general rate of CsA metabolic clearance.

Particularly noteworthy is the observation that higher MR AM9/CsA are associated with absence of diabetes mellitus, hypertriglyceridemia, overweight/obesity and lower BMI. Akhlaghi et al. have described lower MR of AM1, AM9, AM1c, AM1c9 and AM19 in patients with diabetes mellitus suggesting that it may be the consequence of reduced hepatic biotransformation in diabetic patients [24]. However, it can be also hypothesised that shifting the balance in the formations of individual CsA metabolites towards AM9 may contribute to the reduction of synthesis of other more harmful CsA metabolites. Thus, the increased formation of AM9 would indicate a favourable cyclosporine metabolic profile in an individual KTX recipient. It may be also important that in the formation of AM9 is involved CYP3A5 isoenzyme, while other cyclosporine metabolites are mainly produced by CYP3A4 [11]. The constellation of activity of these two isoenzymes in a given patients may translate into a final metabolic profile of cyclosporine. In the study group we have performed the analysis of genetic polymorphisms of CYP3A4 and CYP3A5 associated with their enzymatic activity, but the incidence of individual genotypes was too low to allow the analysis of their relationships with CVD. Negative correlation of AM9/CsA with higher triglycerides blood levels may be also the consequence of altered CsA metabolism in patients with dyslipidaemia. It was found that hypercholesterolemia inhibits CsA metabolism and leads to higher C/D AUC [25]. We have not observed relationship between hypercholesterolemia or total cholesterol blood levels. However, in our study group hypertriglyceridemia was associated with higher dose-adjusted and dose/kg-adjusted CsA concentrations. It may suggest that similarly to cholesterol triglycerides could inhibit cyclosporine metabolism.

Similarly to AM4N/CsA MR, increased AM1/CsA ratios were observed in patients with supraventricular arrhythmia, hyperuricemia/gout and higher DBP values suggesting unfavourable effect of this metabolite on cardiovascular system. Higher dMC-CsA/CsA MR was found in overweight/obese patients and in relationship with higher SPB and DBP. The only parameter that correlated with DiH-CsA/CsA and TriH-CsA/CsA metabolic ratio was DBP. This relationship was independent of kidney graft function even though the effect of DiH-CsA and TriH-CsA on eGFR values in KTX patients was observed [26]. It has confirmed our previous preliminary results [27]. This may suggest a similar mechanism that contributes to rise of blood pressure and worsening of renal function in the presence of elevated DiH-CsA and TriH-CsA. We cannot exclude that those adverse effects are associated with endothelin-mediated vascular dysfunction observed during 21-day cyclosporine treatment in rats [28]. The importance of those and maybe other metabolites in inducing a rise in arterial pressure indicated the existence of a negative correlation between dose/kg-adjusted CsA con centration and SBP.

It is the first study revealing associations between cardiovascular disease and individual cyclosporine A metabolites and their metabolic ratios. We have pointed to specific cyclosporine metabolites, such as AM1, AM9, AM4N, dMC-CsA, DiH-CsA and TriH-CsA, which may play significant role in the development of cardiovascular complications after KTX. The observed relationships may also suggest the contribution of P450 isoenzymes activity to the development of CVD. Due to frequency if occurrence of individual gene polymorphism determining enzymatic activity of P450, the study group turned to be too small to test this hypothesis and it is the main weakness of the study.

In conclusion, we have demonstrated the association of coronary disease/myocardial infarction, supraventricular arrhythmia, hypertriglyceridemia, hyperuricemia/gout, overweight/obesity and elevated arterial blood pressure with higher metabolic ratios of AM1, AM4N, dMC-CsA, DiH-CsA and TriH-CsA, and lower metabolic ratios of AM9, which may indicate deleterious and favourable effects of individual cyclosporine metabolites on cardiovascular system and suggest engagement of specific enzymatic pathways.

**References**

1. System USRD. 2017 Annual Data Report. Epidemiology of kidney disease in United States 2017

2. Stoumpos S, Jardine AG, Mark PB. Cardiovascular morbidity and mortality after kidney transplantation. Transpl Int. 2015;28:10-21.

3. Lam N, Kim J, Knoll GA, McArthur E, Lentine KL, Naylor KL, Li AH, Shariff SZ, Ribic CM, Garg AX. The risk of cardiovascular disease is not increasing over time despite aging and higher comorbidity burden of kidney transplant recipients. Transplantation. 2017;101(3):588-596.

4. Laurés A, Gómez E, Baltar J, Alvarez-Grande J. Risk factors for cardiovascular disease during the first 2 years after renal transplantation. Transpl Proc. 2005;37:3778-3781.

5. Chakkera H, Sharif A, Kaplan B. Negative cardiovascular consequences of small molecule immunosuppressants. Clin Pharm Therap. 2017;102(2):269-276.

6. Zheng S, Tasnif Y, Hebert MF, Davis CL, Shitara Y, Calamia JC, Lin YS, Shen DD, Thummel KE. CYP3A5 gene variation influences cyclosporine A metabolite formation and renal cyclosporine disposition. Transplantation. 2013;95(6):821-827.

7. Lunde I, Bremer S, Midtvedt K, Mohebi B, Dahl M, Bergan S, Åsberg A, Christensen H. The influence of CYP3A, PPARA, and POR genetic variants on the pharmacokinetics of tacrolimus and cyclosporine in renal transplant recipients. Eur J Clin Pharmacol. 2014;70:685-693.

8. Dai Y, Iwanaga K, Lin YS, Hebert MF, Davis CL, Huang W, Kharasch ED, Thummel KE. In vitro metabolism of cyclosporine A by human kidney CYP3A5. Biochem Pharmacol. 2004;68(9):1889-1902.

9. Christians U, Sewing KF. Cyclosporine metabolism in transplant patients. Pharmac Ther. 1993;57:291-345.

10. Combalbert J, Fabre I, Fabre G, Dalet I, Derancourt J, Cano JP, Maurel P. Metabolism of cyclosporin A. IV. Purification and identification of the rifampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. Drug Metab Dispos. 1989;17(2):197-207.

11. Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fischer V, Tyndale R, Inaba T, Kalow W, Gelboin HV. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. J Biol Chem. 1989;264(18):10388-10395.

12. Brozmanova H, Grundmanna M, Safarcık K, Jegorovc A. High-performance liquid chromatographic method for therapeutic drug monitoring of cyclosporine A and its two metabolites in renal transplant patients. J Chromatogr B. 2000;749:93-100.

13. Elens L, van Schaik RH, Panin N, Meyer M, Wallemacq P, Lison D, Mourad M, Haufroid V. Effect of a new functional CYP3A4 polymorphism on calcineurin inhibitors’ dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenomics. 2011;12(10):1383-1396.

14. Hryniewiecka E, Zegarska J, Zochowska D, Samborowska E, Jazwiec R, Kosieradzki M, Nazarewski S, Dadlez M, Paczek L. Cardiovascular disease in kidney transplantation and its association with blood concentrations of cyclosporine and cyclosporine metabolites. Transpl Proc. 2018;145(4):247-254.

15. Textor S, Canzanello VJ, Taler SJ. Cyclosporine-induced hypertension after transplantation. Mayo Clin Proc. 1994;23:2614-2622.

16. Taler S, Textor SC, Canzarello VJ, Schwartz L. Cyclosporin-induced hypertension. Incidence, pathogenesis and management. Drug Safety. 1999;20(Suppl. 8):437-449.

17. Rezzani R, Rodella L, Dessy C, Daneau G, Bianchi R, Feron O. Changes in Hsp90 expression determine the effects of cyclosporine A on the NO pathway in rat myocardium. FEBS Lett. 2003;552:125-129.

18. Jurado F, Bellon JM, Pareja JA, Golitsin A, Millan L, Pasqual G, Bujan J. Effects of ischaemia reperfusion and cyclosporin A on cardiac muscle ultrastructure. Histol Histopatol. 1998;13:761-774.

19. Bianchi R, Rodella L, Rezzani R. Cyclosporine A up-regulates expression of matrix metalloproteinase 2 and vascular endothelial growth factor in rat heart. Int Immunopharmacol. 2003;3:423-433.

20. Rezzani R. Cyclosporine A and adverse effects on organs: histochemical studies. Progress Histochem Cytochem. 2004;39:85-128.

21. Sadeg N, Pham-Huy C, Rucay P, Righenzi S, Halle-Pannenko O, Claude JR, Bismuth H, Duc HT. In vitro and in vivo comparative studies on immunosuppressive properties of cyclosporines A, C, D and metabolites M1, M17 and M21. Immunopharmacol Immunotoxicol. 1993;15(2-3):163-77.

22. Bowers L. Studies of cyclosporine and metabolite toxicity in renal and hepatocyte culture systems. Transpl Proc. 1990;22(3):1135-1136.

23. Radeke H, Christians U, Bleck JS, Sewing KF, Resch K. Additive and synergistic effects of cyclosporine metabolites on glomerular mesangial cells. Kidney Int. 1991;39(6):1255-66.

24. Akhlaghi F, Dostalek M, Falck P, Mendonza AE, Amundsen R, Gohh RY, Asberg A. The concentration of cyclosporine metabolites is significantly lower in kidney transplant recipients with diabetes mellitus. Ther Drug Monit. 2012;34(1):38-45.

25. Prueksaritanont T, Koike M, Hoener BA, Benet LZ. Transport and metabolism of cyclosporine in isolated rat hepatocytes. The effects of lipids. Biochem Pharmacol. 1992;43(9):1997-2006.

26. Hryniewiecka E, Żegarska J, Żochowska D, Jaźwiec R, Borowiec A, Samborowska E, Tszyrsznic W, Dadlez M, Pączek L. Hydroxylated, hydroxymethylated, dihydroxylated, and trihydroxylated cyclosporine metabolites can be nephrotoxic in kidney transplant recipients. Transpl Proc. 2016;48:1551-1555.

27. Hryniewiecka E, Zegarska J, Zochowska D, Samborowska E, Jazwiec R, Kosieradzki M, Nazarewski S, Dadlez M, Paczek L. Cardiovascular disease in kidney transplantation and its association with blood concentrations of cyclosporine and cyclosporine metabolites. Transpl Proc. 2018;in press.

28. Ping N, Mi YN, Liu DZ, Zhang S, Chen JG, Cao YX. H2S prevents cyclosporine A-induced vasomotor alteration in rats. Cardiovasc Toxicol. 2017;17(3):287-296.

Figure 1. Associations of AM9/CsA with clinical and laboratory parameters. A. Lower AM9/CsA levels in patients with diabetes mellitus; B. Negative correlation of AM9/CsA with body mass index; C. Negative correlation of AM9/CsA with blood triglyceryde levels; D. Lower AM9/CsA in patients with overweight/obesity. BMI, body mass index; DM, diabetes mellitus; TG, triglycerydes.