Assessment of Cardiovascular Risk in Special Patient Populations

PhD Dissertation

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List of abbreviations

ADP	adenosine diphosphate	HDL	high density lipoprotein
AGE	advanced glycation end products	IGF-1	Insulin-like growth factor 1
ASA	acetylsalicylic acid	LDL	low density lipoprotein
AZA	Azathioprine	MET	metabolic equivalent
BMI	body mass index	MMF	Mycophenolate-mofetil
CAN	chronic allograft nephropathy	PA	physical activity
CHD	coronary heart disease	PAK	Pancreas after Kidney
CIT	cold ischemic time	PPP	platelet poor plasma
CKD	chronic kidney disease	PRED	Prednisolone
CN	carbamide nitrogen	PRP	platelet rich plasma
COX	cyclooxygenase	PV	plasma viscosity
CV	cardiovascular	RBC	red blood cell
CVD	cardiovascular disease	SCORE	Systemic Coronary Risk Evaluation
CSA	Cyclosporin A	SD	standard deviation
DGF	delayed graft function	SPK	Simultaneous Pancreas Kidney
DM	diabetes mellitus	SRAR	Steroid resistant acute rejection
ECG	electrocardiography	TAC	Tacrolimus
EF	ejection fraction	USRDS	United States Renal Data System
EI	elongation index	VO_{2max}	peak maximal oxygen consumption
ESC	European Society of Cardiology	WBV	whole blood viscosity
ESRD	end stage renal disease	WHO	World Health Organization
hct	hematocrit		

Introduction

Cardiovascular disease prevention is defined as an organized process at population and individual levels aimed at eradicating or minimizing health damage resulting from CVDs and their related complications. The population-level strategy was aimed at reducing the incidence of cardiovascular risk factors via long-term environmental and lifestyle changes targeting the population. The high-risk strategy targeting high-risk patients aims to manage existing CV risk factors with medication therapy, psychosocial care and dietary consultation on the individual level. Three levels of CV prevention can be distinguished. Primordial prevention encompasses the prevention of the development of CV risk factors via health preservation with promoting a physically active lifestyle, adequate healthy nutrition, maintaining the ideal bodyweight and facilitating and promoting the avoidance of smoking. Primary prevention is aimed at preventing the occurrence of the first cardiovascular event by treating CV risk factors with hypertension therapy, hyperlipidemia therapy, diabetes control, quitting smoking and lifestyle modification. Secondary prevention means preventing recurrent cardiovascular events with evidence-based medicine and the above mentioned risk factor management strategies, life-long lifestyle change and the combination of medication and non-medication therapy. In the present work, we examined CV risk in three specific patient populations. In the first group, we examined the effect of home- and center-based physical training program in elderly women with CV risk factors. In the second group, we examined the incidence of aspirin resistance in patients undergoing simultaneous pancreas- kidney transplantation. In the third group, we analyzed the CV mortality of patients undergoing kidney transplantation.

Impact of home- and center- based physical training program on cardio-metabolic health and IGF-1 level in elderly women

Introduction

Menopause is a critical state in the life of women generally accompanied by dysregulation in the cardio-metabolic profile resulted from critical changes in body composition such as excessive accumulation of fat at visceral level. Part of the cardio-metabolic health including physical performance, systolic/diastolic blood pressure, resting heart rate, fasting levels of plasma glucose and insulin level, abdominal visceral adipose tissue, weight, BMI, sedentary behavior are positively influenced by PA in elderly women. Elderly women who are physically active possess less risk of functional limitations and a higher health-related quality of life.

However, the effect of regular physical activity on certain parameters in elderly women, such as lipid- and IGF-1 levels are not unequivocal. Some investigations have proved that PA favorably modifies lipid parameters among elderly women, while others failed to demonstrate a significant effect.

Insulin-like growth factor -1 is a basic peptide composed of 70 amino acids, which is thought to play a central role in aging, cancer development, CV diseases, metabolism and muscle strength. A population-

based study examining the association of different IGF-1 levels with mortality, CVD, and cancer in the elderly has found a U-shaped relationship between IGF-1 level and fatal CV diseases, which means that both high and low levels of IGF-1 were associated with increased risk of CV mortality. Serum IGF-1 level is declining with age and postmenopausal women generally display even lower levels of IGF-1 compared to elderly men. Regular PA has several health preserving effects and it has been examined previously how it may modulate IGF-1 level. Some investigations have demonstrated a positive effect on IGF-1 levels especially in the case of resistance training, while aerobic exercise training had no considerable effect on IGF-1 concentrations.

Impaired hemorheological parameters have been identified as primary CV risk factors and associated with the early stage of atherogenic processes. Although the beneficial effects of regular PA on rheological parameters in healthy individuals have been revealed, available data among female patients with CV risk is relatively scarce.

Elderly women usually do not report only physical but also psychological and social changes during menopause that affect their global and CV health. General psychological-, and emotional well-being, and optimism are related to health promoting behaviors including healthy eating and lifestyle habits and self-care, supporting CV and overall health of elderly patients.

The aim of our study was to investigate whether 12 weeks of an applied home- and center- based physical training program is sufficient to change functional capacity, some important cardio-metabolic, hemorheological parameters, IGF-1 level and certain psychological items of elderly female patients with moderate to high CV risk.

Methods

Sixty non-smoker female patients with moderate to high CV risk (mean age: 67.4±5 years) were enrolled into our study. They voluntarily agreed to participate in the study and then were randomly assigned either to the CV preventive training program or to the control group. Inclusion criteria were EF ≥55% and MET ≥5. Exclusion criteria were the following: previous CV events, heart failure, inducible myocardial ischemia and arrhythmias on an exercise stress test. Medication and drug therapy were not modified during the study. The investigation was approved by the Regional Ethics Committee of the University of Pécs (No. 5829) and a written informed consent was obtained from all subjects.

Study design

At baseline psychological test (SF-36 Questionnaire), resting ECG and echocardiography, treadmill-based exercise tolerance testing using the Bruce protocol, metabolic laboratory parameters (fasting glucose, hgbA1c, HDL-cholesterol, LDL-cholesterol, total-cholesterol, TG), IGF-1 level and hemorheological measurements (hematocrit, fibrinogen, plasma viscosity, whole blood viscosity, RBC aggregation and deformability) were performed. The patients participated in a 12-week physical training program lasting for 1 hour 3 times weekly. A daily walking program was implemented. For appropriate estimation of its efficiency, our patients were asked to wear a personalized activity tracker on their wrist.

The daily activity goal was to achieve 10,000 footsteps based on health experts' recommendation. Upon reaching week 12, all tests were repeated, with the exception of echocardiography.

IGF-1 measurements

IGF-1 levels were measured using Human IGF-1 Quantikine ELISA Kit (R&D Systems; Cat. No.: RD-DG100). The assay employs a quantitative sandwich immunoassay technique.

Hemorheological measurements

Hematocrit was measured by a micro hematocrit centrifuge (Haemofuge Heraeus Instrument, Germany) at room temperature. Plasma fibrinogen concentration was determined by Clauss method. WBV and PV were measured with a Hevimet 40 capillary viscometer (Hemorex Ltd., Budapest, Hungary). Plasma was collected after whole blood sample centrifugation for 10 minutes at 1500g. Measurements were performed at 37 °C. Red blood cell aggregation was measured using a LORCA (Laser assisted Optical Rotational Cell Analyzer; R&R Mechatronics, Hoorn, The Netherlands) aggregometer. Aggregation Index (AI), aggregation half time (t ½) and threshold shear rate (γ) were calculated. Red blood cell deformability was measured at 37°C using a LORCA ektacytometer.

Psychological surveys

SF-36 Questionnaire was applied to examine the psychological effects of the 12-week complex training program on the perception of health. It is a self-administered questionnaire measuring health over 8 dimensions (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health). Both groups rated their health status on a scale from 0 (worst health) to 100 (best health).

Statistical analysis

Data were shown as mean ± standard deviation (SD). Significance level was defined as $p<0.05$. To check differences in the interventional and in the control group we performed paired sample-t test. For testing how the two groups varied in time the interaction of time x group effect was applied. The normality was analyzed by Kolmogorov-Smirnov test. The nonparametric Wilcoxon Rank test was applied to analyze potential changes in psychological functioning and in the number of foot-steps, since these were ordinal variables. Data were shown as median and IQR.

Results - within groups

Home-based walking program resulted in a significant improvement in daily physical activity (4232 [3162-7219] vs 8455 [6757-11488] foot-steps) among our female patients ($p<0.001$). We did not register any adverse events during the trial.

The combined home-based and center based physical training program improved exercise capacity, described by the significantly increased metabolic equivalent (MET) (8.17±1.57 to 8.87±1.76) in the training group ($p=0.002$). Exercise capacity did not change in the control group.

Total cholesterol, LDL cholesterol, TG, HgbA1c, and fibrinogen level indicated a significant decrease during the investigated period ($p<0.05$). The other measured laboratory and hemorheological parameters did not show significant changes in the training group (Table 1).

Serum IGF-1 significantly increased in the training group (Table 1), while it decreased in the control group.

None of the laboratory and hemorheological parameters changed in the control group.

Table 1. Significant changes in metabolic laboratory parameters, IGF-1, and fibrinogen level after 12 weeks in the training group, n=30; values are baseline and 12 weeks (mean±SD). Level of significance: $p<0.05$

measured parameters	baseline	12 week	p value
$HgbA_{1C}$ (mmol/l)	6.24±0.67	6.06±0.58	0.007
total cholesterol (mmol/l)	5.17±1.13	4.77±1.12	0.042
LDL-cholesterol (mmol/l)	3.37±1.05	2.81±0.98	0.003
HDL-cholesterol (mmol/l)	1.46±0.39	1.51±0.46	ns
triglycerides (mmol/l)	1.68±0.71	1.28±0.71	0.002
IGF-1 (ng/ml)	59.68±27.37	66.79±22.74	0.006
Fibrinogen (g/l)	3.22±0.54	2.42±0.42	p<0.0001

Participants of the training group reported significantly fewer limitations in their everyday physical functioning ($p<0.05$). However, the other psychological items did not indicate significant changes following the training program. Participants in the control group did not report any changes in their psychological condition.

Results – between groups

The interaction of time x group effect revealed, that the training group significantly differed from the control group in four parameters including MET ($p=0.003$) (Figure 1A), LDL-cholesterol ($p=0.046$) (Figure 1B), triglyceride ($p=0.001$) (Figure 1C) and IGF-1 levels ($p<0.001$) (Figure 1D) after the intervention. The training group did not differ from the control group in the other investigated cardio-metabolic parameters.

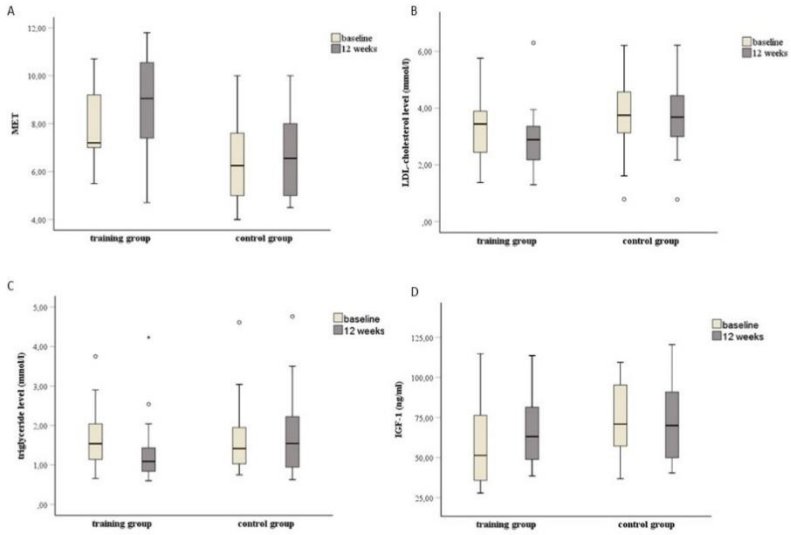

Figure 1. Box plots of cardio-metabolic parameters and IGF-1 level at baseline and after 12 weeks between the training-, and the control group. n=60. Levels of significance: p<0.05.
A. Significant difference in MET between the groups. p=0.003
B. Significant difference in LDL-cholesterol level between the groups. p= 0.046
C. Significant difference in triglyceride level between the groups. p= 0.001
D. Significant difference in IGF-1 level between the groups. p <0.001

The training group significantly differed from the control group in AI (p=0.037) and t1/2 (p=0.015), which are both describing parameters of RBC aggregation, as well as in PV (p=0.025) (Table 2). The other investigated hemorheological parameters did not differ between the two groups.

Psychological testing did not show significant differences between the training group and the control group.

Table 2. Significant changes in certain hemorheological parameters after 12 weeks between the groups, n=60; values 12 weeks (mean±SD). Levels of significance: p<0.05

measured parameters	training group	control group	p value
PV (mPas)	1.23±0,05	1.26±0.06	0.025
LORCA AI	66.31±4.94	70.05±4.51	0.037
LORCA t ½ (sec)	1.81±0.51	1.50±0.4	0.015

Discussion

In our study the home-, and center based physical training program resulted in a significantly improved functional capacity, metabolic laboratory-, (total-cholesterol, LDL-cholesterol, TG, HgbA1c), hemorheological parameters (fibrinogen, PV, RBC aggregation) IGF-1 level, and physical functionality. Maintenance of a physically active lifestyle is a great challenge especially for the elderly population and women are less likely to adhere physical training programs compared to men. We assumed that a combined, home-based walking and a center based training program fits well to the everyday life of the elderly ladies, and a center-based exercise program might be effective and enjoyable for this special

patient population. Most of the studies reported that the normal daily activity of healthy adults is only 4000 to 6000 steps and in older women it is even lower. Although in our study the elderly female patients could not completely fulfill the daily target of 10,000 steps, still the achieved significant improvement in daily PA (at baseline: 4232 [3162-7219]; after 12 weeks: 8455 [6757-11488] footsteps/day) is a great performance taking into accounts their age and co-morbidities.

After 12 weeks of our home- and center- based physical training program, we could demonstrate an average of 0.7 MET improvement in functional capacity. Furthermore, the training group significantly differed from the control group in MET level after the intervention, thus our results suggest that the training program significantly improved the functional capacity of our elderly female patients.

In our study we observed a significant decrease in the total cholesterol as well as in LDL cholesterol and TG levels in the training group, while in the control group no change could be observed in the metabolic parameters. In addition, the training group significantly differed from the control group in LDL cholesterol and triglyceride level after the training program, referring to the favorable effects of the home- and center-based training program on lipid status. Data in the literature regarding the effects of PA on lipid levels in different patient populations, including postmenopausal women, is controversial. On many occasions, lipid parameters have not been improved by increasing physical activity, while other studies have proved it to be affective to be effective. Examining our and the above described different training programs we may realize that those physical training programs were able to induce significant changes in lipid levels in elderly females which either contained resistance training or was intense and long enough or could effectively increase the daily physical activity level, like the present home- and center- based exercise program.

In accordance with previous studies following the home- and center- based training program HgbA1C significantly decreased among our elderly female patients, contributing significantly to the positive metabolic effects of PA. In the control group no change could be observed in the HgbA1C level.

At baseline low levels of IGF-1 were measured in our study. In the training group significant increase in IGF-1 levels could be observed but still remained below the average level of healthy middle-aged female adults. Moreover, the training group differed significantly from the control group in IGF-1 levels after the intervention, suggesting that the home- and center-based training program caused the beneficial changes in the IGF-1 levels. Based on previous results and our findings it seems that in the case of aerobic exercise training a more intensive PA level is needed to change IGF-1 level. The decrease in IGF-1 levels in the control group may be due to the lack of regular PA.

PA could be a promising method of improving hemorheological parameters in the elderly, but available data among postmenopausal women with CV risk is rare. Our results have revealed significant decrease in fibrinogen levels in the training group. The training group significantly differed from the control group in RBC aggregation (AI, t ½) and in PV, suggesting that the organized training program led to beneficial hemorheological changes.

Besides objective measurements SF36 questionnaire was applied to measure the psychological well-being of our patients. Physical functionality, which is the patients' subjective judgement of their physical state, has improved, meaning they have experienced fewer limitations during their everyday physical tasks, like shopping, walking or bathing. The better physical functionality was in accordance with the improved functional capacity measured by treadmill. However, no significant improvement could be measured in other examined psychological parameters (vitality, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, mental health).

Conclusion

The present study demonstrated a significant improvement in several cardio-metabolic parameters such as functional capacity, physical functioning, total-, and LDL cholesterol, TG, HgbA1c as well as IGF-1 levels, and certain hemorheological parameters (fibrinogen, PV, RBC aggregation) of elderly female patients with moderate to high CV risk after 12 weeks of complex home- and center-based training program.

Achieving significant changes in IGF-1 and lipid levels by a physical training program seems to be more difficult than in the case of other cardio-metabolic parameters. According to our findings and data in the literature in order to improve IGF-1 level and lipid parameters in elderly women physical training programs should either contain resistance training elements or be intensive enough or effectively increase the daily physical activity level and completely change sedentary lifestyle behavior.

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2. Acetylsalicylic acid resistance after simultaneous pancreas-kidney transplantation

Introduction

Simultaneous pancreas-kidney transplantation is one of the well-known treatment options in ESRD with type 1 diabetes mellitus. Since the first SPK transplant in 1966, surgical techniques as well as patients care systems have improved, both short and long-term results are better. SPK transplantation improved patients' quality of life in several aspects compared to patients treated with conservative therapy. The leading cause of death is CVD after SPK transplantation, which means that significant number of the patients die with functioning graft. For achieving good long-term results CV prevention is essential. According to the ESC prevention guideline, the key points are lifestyle changes (proper nutrition, regular exercise), and for remaining risk factors medication therapy, which includes antiplatelet therapy. Resistance to platelet aggregation inhibiting therapy is a well-known phenomenon. According to the literature, ineffective platelet aggregation inhibition occurs in 5-55% of the cases. The aim of the present study was to examine acetylsalicylic acid resistance in SPK patients as well as to identify the cause of this resistance.

Methods

The investigational period was from March 2009 to December 2013. 32 recipients were selected from the Department of Surgery in the University of Pecs (male: 22. female: 10, age: 47,4 ±8,6 years). Platelet aggregation measurements took place at the Hemorheological Research Laboratory of the 1st Department of Medicine at the University of Pecs. The research was approved by the Regional Ethics Committee of the University of Pecs (approval number: 4816) and a written informed consent was given by all participants.

Platelet aggregation measurements

Patients were routinely given 100 mg of ASA q.d. After 1 month blood samples were obtained from the cubital vein in 3.8% sodium-citrate tubes. Following multiple stages of centrifugation, platelet rich plasma (PRP), then platelet poor plasma (PPP) was separated. Platelet aggregation measurements were performed using turbidimetric Carat TX-4 (Carat Diagnostics Ltd., Budapest, Hungary) platelet aggregometer, which calculates the maximum of platelet aggregation curve via light transmission intensity changes between PRP and PPP samples. ASA therapy was considered effective as long as the maximum of platelet aggregation was under 40% respectively.

Measured factors

Laboratory tests were performed at the Department of Laboratory Medicine at the University of Pecs. Patients' antropometric parameters, tobacco use, medication therapy were assessed in an outpatient setting. The incidence of chronic allograft nephropathy and steroid resistant acute rejection was evaluated.

Statistical analysis

Linear logistic regression analysis was performed using the 21.0 version of IBM SPSS Statistics software (IBM Corporation, New York, United States).

Results

Our study revealed ASA resistance in 40.6% of the 32 transplant patients (13 patients). Linear logistic regression analysis with the resistance data and the above mentioned 24 investigational factors (Table 3) did not detect any statistically significant association between the variables.

Table 3. Factors which may influence ASA resistance

Age	Hemoglobin level	ACE Inhibitors
BMI	Hematocrit	Antilipid therapy
Smoking	Platelet count	Calcium channel blockers
Diabetes Mellitus	Triglyceride level	Everolimus, Sirolimus
Type of Transplantation	LDL-cholesterol level	Cyclosporine
CAN	serum glucose level	Methylprednisolone
SRAR	serum creatinine level	Mycophenolate Mofetil Mycophenolic acid

Discussion

In the present study we found very high rate of acetylsalicylic acid resistance after SPK transplantation (40.6%). We could not reveal association between ASA resistance and the examined parameters (age, smoking habits, antropometrical-, laboratory parameters, immunosuppression, transplant specific factors). Following SPK transplantation, the CV risk profile may further increase with the introduction of immunosuppressive therapy. The incidence of CVD can be further increased by ASA resistance, which may affect patients' long-term life expectancy. It has been also proved that ASA resistance contributes to the elevated incidence of cardio,- and cerebrovascular events after kidney transplantation. ASA resistance is a widely examined phenomenon and several previous studies demonstrated that numerous factors could play a role in its development such as smoking, female sex, higher age, elevated fibrinogen level, increased RBC aggregation, low hemoglobin level, elevated lipid levels, NSAIDs (ibuprofen, diclofenac). Further possible factors are patient non-compliance and increased heart rate. Genetic factors such as COX-1 gene polymorphism in platelet surface glycoprotein or COX-2 overexpression could also play an important role in the development of ASA resistance.

Conclusion

Our study confirmed very high incidence of acetylsalicylic acid resistance following SPK transplantation. Our study found no significant correlation between the examined risk factors and ASA resistance. Nevertheless, the clarification of the background of ASA resistance would be an essential task in cardiovascular prevention of SPK patients.

3. Assessment of cardiovascular mortality in a single center cohort analysis of 593 patients after Kidney Transplantation

Introduction

Systematic medical data collection and analysis of transplant outcomes could be an important way to improve care of transplanted patients. In several countries systematic transplant data collection and transplant registries exist and provide beneficial information for decades successfully. However, there is no renal transplant registry in Hungary at present.

This is the first paper describing kidney transplant characteristics in the whole cohort, the influence of Romani ethnicity on graft survival and the assessment of CV mortality covering the past 25 years at our single center in Pécs, Hungary.

Methods

Patients, who were transplanted between 1993 and 2016 at the Department of Surgery, University of Pécs, were selected to load their data into our database. Recipient-, donor-, and transplant procedure data were registered. Recipient data included age, sex, ethnicity, body mass index, duration of dialysis and mortality. Donor data included age, sex, donor type and creatinine level at the time of donation. Transplant procedure-related data included cold ischemia time, graft function during hospitalization, early or delayed graft function, serum creatinine, serum carbamide nitrogen, and hemoglobin level and thrombocyte count at discharge. Patients were categorized as Romani or non-Romani-Europid by two transplant coordinators independently, who were in personal contact with all patients. This investigation was approved by the Regional Ethics Committee (license number: 6957) of the University of Pécs. The evaluation was blinded, using the patients' registry number to protect patient privacy.

Statistical analysis

Data are described as mean ± SD, median and interquartile range. Graft and patient survival were evaluated by Kaplan-Meier analysis with Log-Rank test. Correlations between variables were calculated by Spearman's rank correlation and chi-square test was applied in case of categorical values. To determine differences between groups independent sample t-test and analysis of variance (ANOVA) were applied. In case of the lack of normal distribution Mann-Whitney U and Kruskal-Wallis tests were carried out. Data were analyzed using SPSS version 23.0 statistical software. Recipients with functioning kidney grafts for more than 7.5 years (n=210) were compared to those with functioning grafts for less than 7.5 years (n=68) using multivariate analysis of graft survival between 1993 and 2007 (all transplants).

Results

Gender effects

There was a significant difference between female and male recipients in BMI ($p<0.001$), creatinine-, ($p<0.001$), CN level ($p=0.001$) and thrombocyte count at discharge ($p=0.009$). There was no significant difference between male and female recipients regarding recipient and donor age at transplantation, duration of dialysis, CIT, donor creatinine concentration at transplantation, recipient hemoglobin concentration and thrombocyte count at discharge, re-dialysis ratio, type of transplantation, Romani ethnicity and CV event ratio.

Primary graft function and cause of death

Primary non-function occurred in 2.7% of the recipients, whereas DGF developed in 12.2% of recipients (Figure 2A). There was no gender difference in primary graft function ($p=0.357$).

The leading cause of death after transplantation was CVD (54.2%), infections (14.5%) and malignant diseases (8.4%) (Figure 2B). The cause of mortality did not differ significantly between genders ($p=0.485$). There was no significant association between kidney transplant failure and CV mortality. We could not reveal higher creatinine level ($p=0.794$) or re-dialysis ratio ($p=0.726$) among patients with CV mortality compared to patients with other causes of mortality. Our results did not reveal an association between the duration of dialysis before transplantation and CV mortality ($p=0.250$).

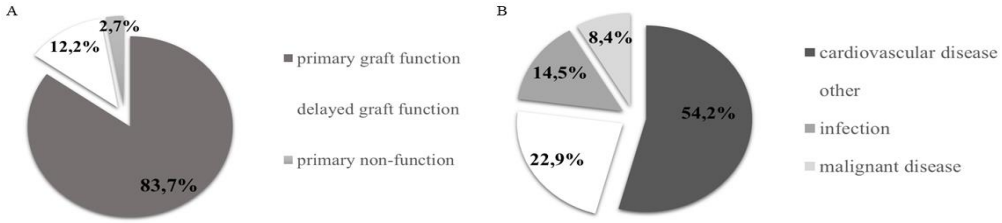
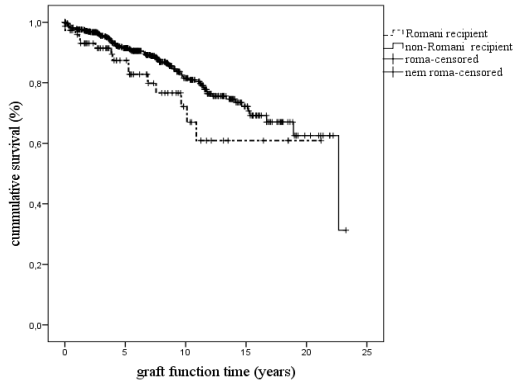


Figure 2 (A). Primary graft function and (B). Cause of death between 1993 and 2017. All transplants (n=593)

Characteristics by ethnicity

Donor age at transplantation was significantly lower in Romani recipients ($p=0.031$). There were significantly fewer living donors among Romani patients ($p=0.004$). Romani recipients had a significantly shorter graft survival ($p<0.05$) (Figure 3) and higher re-dialysis ratio ($p=0.026$). Mortality and CV event ratio did not differ between ethnicities. There was no difference between Romani and non-Romani Caucasian regarding donor creatinine concentration at transplantation, recipient age at transplantation, duration of dialysis, CIT, recipient's creatinine, CN, hemoglobin concentrations, and thrombocyte count at discharge.

Figure 3 . Graft survival time between 1993 and 2016 by ethnicity. All transplants (n=593).



Factors determining long term graft function: Comparison of short- and long-term graft survival

Recipient data with functioning kidney grafts for more than 7.5 years (n=210) were compared to those with a potential graft-survival of 7.5 years but cessation of graft function earlier (n=68) using multivariate analysis (Table 4). In order to ensure statistically correct design and accurate comparability for this analysis only data of those patients were included who had a graft survival of at least 7.5 years i.e. patients who were transplanted before 2007. Longer graft survival was associated with a significantly (5 years in average) younger donor age (p=0.004) and non-Romani ethnicity (p=0.02). However, there was no significant difference in recipient age, dialysis duration, CIT, recipient BMI, renal function at discharge and CV event rates.

Graft survival time had a significant negative correlation with duration of dialysis before transplantation (δ=-0.195; p=0.000022).

Table 4: Comparison of short- (<7.5 years) and long-term (>7.5 years) graft survival between 1993 and 2007 (n=278); values are mean±SD or median and IQR.

	Characteristics	<7.5 years graft functioning time (68)	>7.5 years graft functioning time (210)	p
Ethnicity	**Romani ethnicity** (%)	22.1% (15)	11% (23)	**0.02**
Donor factors	**Age at transplantation** (yrs)	49.05±12.63	43.74±12.30	**0.004**
	Creatinine at transplantation (umol/l)	82.74 (56-105)	89.95 (64-105)	0.632
	Living donors (%)	5.9% (4)	7.6% (16)	0.79
Recipient Factors	**Duration of dialysis** (months)	38.4 (12-36)	34.8 (12-48)	0.997
	Cold ischemic time (sec)	1256.2 (1051-1455)	1216.55 (1070-1470.5)	0.728
	Age at transplantation (yrs)	44±14.99	42.37±13.02	0.392
	BMI at transplantation(kg/m^2)	24.76±4.22	25.7±5.08	0.185
	Creatinine at discharge (umol/l)	223.57 (116.75-273.25)	187.96 (119-205)	0.615
	Carbamide nitrogen at discharge (umol/l)	21.14 (8.05-17.67)	17.5 (8.45-16.92)	0.312
	Hemoglobin at discharge (g/l)	102.05±8.77	105.86±22.63	0.286
	Thrombocyte count at discharge (G/l)	238.11±98.34	254.2±93.39	0.317

Factors determining long term graft function: Immunosuppressive-treatment during the observation period

The investigated first transplantation era (1993-1998) was characterized by PRED+CSA+AZA based immunosuppression. In 1998 a major change was the replacement of AZA with MMF. In 2000 Tac was introduced in selected younger patients but patients already on CSA were not converted to Tac.

In cases of good graft function (creatinine concentration <130 mmol/l and the absence of acute rejection), steroid therapy was stopped after 3 or 12 months. Recipients remaining on Medrol therapy had an inferior graft function in the long run. Also mortality was higher in patients receiving Medrol continuously than in patients who stopped taking steroids due to good graft function, ($p=0.001$). 84% ($n=72$) of patients received Medrol by the time of their death.

Mortality was higher ($p<0.003$) in CSA-, than in Tac-treated patients. However, CSA-patients were transplanted earlier and they were 8 years older in the CSA than in the TAC group.

Factors determining long term graft function: Results of 5-year quintiles analysis

The 25 years of observation (Fig 6A) was divided into 5 equal (5-year long) periods according to the time of transplantation. There was a significant increase in graft survival in all time-groups after 1998 compared to the first observation period (1993-1998). Azathioprine was replaced by mycophenolate mofetil (MMF) in 1998.

Transplant-related parameters improved over time (Fig 6B-F). CIT was the longest in the first two quintiles (1993-1998, 1998-2003) (Fig 6B.), it had a decreasing tendency (Fig 6B) and it was significantly shorter in the last period (2013-2017) vs. all other periods ($p<0.001$) (Fig 6B). There was a significant association between CIT and the presence of CAN ($p=0.00054$), creatinine concentration at discharge ($\delta=0.142$; $p=0.042$) and longer CIT was significantly associated with higher mortality ($p=0.00298$). Recipient creatinine concentration at discharge was significantly higher in the first period than in the second ($p=0.042$) and the fourth (2008-2013) ($p=0.039$) periods (Fig 6C). Recipient CN concentration at discharge was highest in the first period and lowest in the last period ($p=0.012$) (Fig 6D). Recipient BMI was significantly higher in the last period than in all other periods ($p<0.05$) (Fig 6E). Donor creatinine concentration at transplantation was significantly higher in the second period than in the last two periods ($p=0.006$) (Fig 6F).

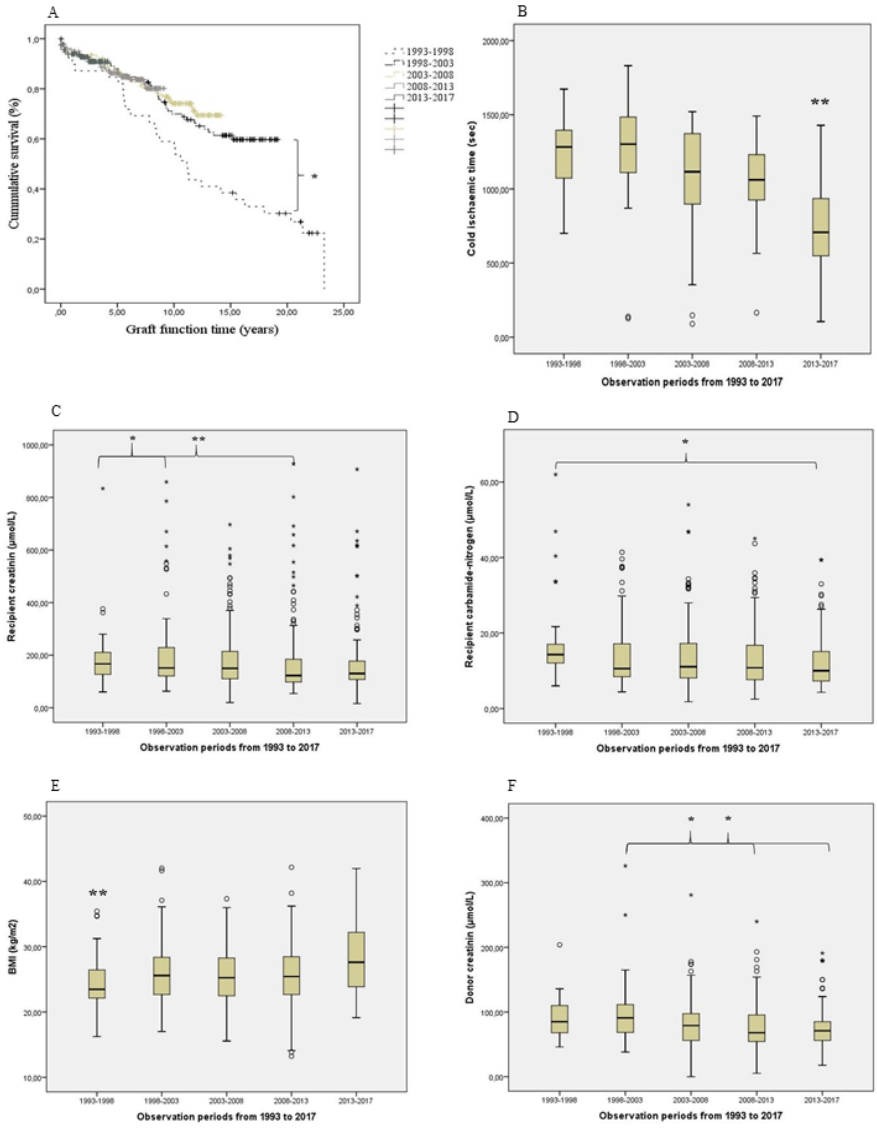


Figure 6. Factors determining long term graft function between 1993 and 2007 (n=593).
(A) Graft survival between (comparison of 5 year intervals according to time of transplantation). *: p =0.012.
(B) Cold ischemia time in the 5-year periods. **: p <0.001 vs. all previous quintiles.
(C) Differences in recipient creatinine concentration at discharge among the 5-year periods. *: p =0.042. **: p= 0.039.
(D) Differences in recipient carbamide nitrogen concentration at discharge among the 5-year periods. *: p = 0.012.
(E) Differences in recipient BMI among the 5-year periods. **: p value <0.05.
(F) Differences in donor creatinine concentration at transplantation among the 5-year periods. *: p = 0.006.

Discussion

In this retrospective cohort we assessed and analyzed the outcome of kidney transplantation over 25 years at a Hungarian single center. The main purpose of this report was to gain insight into transplant activity and long-term outcomes from South-Hungary. The main findings of the present study are that mortality of transplanted patients was similar to that of the general population and was not in connection with transplant-related factors, and that graft survival significantly increased at the turn of the century.

Internationally, the main cause of mortality after renal transplantation is CVD (30-40%), similarly to USRDS reports. In our study the leading cause of death after renal transplantation was CVD, however, the prevalence (54%) was higher than in the US. This difference is most likely attributable to a similar

difference in the general populations: according to the Hungarian Central Statistical Office database CV mortality in the general population is 55.2%. According to the WHO, the prevalence of CV death within the general population was 34% world-wide, and 23.4% in the United States in 2015. CV mortality had no association with graft function (creatinine level or re-dialysis ratio), CSA vs. Tac based immunosuppression or with dialysis duration before transplantation. Although, graft survival time correlated negatively with the duration of dialysis before transplantation, we found no association between the duration of dialysis and CV mortality, thus, the high CV mortality in our cohort was not due to inferior dialysis. Thus, CV mortality of kidney-transplanted patients seems to correlate with CV-mortality in the general population.

Our results support international findings, as Romani recipients had a significantly shorter graft survival time and higher prevalence of graft loss. However, we found no significant difference in mortality rates between ethnicities. There were significantly fewer living donors in the background of inferior graft survival of Romani recipients. Although higher prevalence of CVD and CV mortality has been demonstrated earlier in the general Romani vs. the majority population, our data do not support this in the transplanted population as a possible cause of the inferior outcomes. Better medical surveillance and better compliance of transplanted Romani patients than the general Romani population might be one reason.

Our results confirm previous investigations, as shorter graft survival was associated with significantly older donor age and had a significant negative correlation with the duration of dialysis.

Continuous Medrol therapy was a prognostic factor for mortality, however this was not associated with cardiovascular morbidity or re-dialysis. Tac-treated patients were significantly (8 years) younger and thus had a lower mortality and graft failure. CV event ratio was similar in the CSA and Tac groups, this observation supports the above conclusion, that CV mortality was primarily determined by general CV mortality in Hungary.

Graft survival time was shorter before 1998 (in the first observation period (1993-1998) compared to all other periods. The observation that graft survival increased significantly after 1998 is corroborated by the slight decrease in discharge creatinine and the significant decrease in CN after the first observation period. The most likely explanation for the increase in graft survival and function is the replacement of AZA with MMF in 1998. Further possible factors that may have contributed to the significant increase in graft survival following 1998 are the decrease in CIT and donor creatinine.

In our experience, the steady and substantial decrease in CIT since 1998 was not translated into better graft survival. However, we observed a significant positive correlation between CIT and the presence of CAN and a significant positive correlation between CIT and discharge creatinine level. Moreover, CIT positively correlated with mortality, as it was also demonstrated by others.

In our study, recipients' BMI was lowest in the first, and highest in the last observation period – remaining within the reference range, suggesting that most patients were not obese in our cohort at the

time of transplantation. Interestingly, the significant increase in recipient BMI after 2013 did not influence graft survival – at least its effect was not obvious due to the short follow-up time.

Conclusion

In our single center cohort CVD was the most common cause of mortality, followed by infections and malignant disorders. CV mortality had no association with graft function or CSA vs. Tac based immunosuppression. Possible cause of the higher CV mortality in our cohort is the generally high CV mortality in Hungary. Our study revealed that elderly donor age and Romani ethnicity was associated with shorter graft survival time, which is concordant with international findings. We did not observe higher CV event ratio among Romani recipients, contrary to that in the general Romani population in Hungary probably due to better health-care in the transplant program. Improved graft survival time in our transplant population after 1998 might be explained mainly by changes in immunosuppressive therapy. Our experience supports that longer CIT could increase the risk of graft failure and mortality.

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Summary of the new scientific results

1. Impact of home- and center- based physical training program on cardio-metabolic health and IGF-1 level in elderly women

1) The originality of our study was the use of a structured home-based walking program and center-based aerobic training program in elderly postmenopausal women, which is different compared to other studies that focused only on structured exercise program (aerobic and resistance).
2) The home-, and center-based training program resulted in a significant improvement in certain cardio-metabolic parameters (physical functionality, total cholesterol, LDL cholesterol, triglyceride, HgbA1c) IGF-1 levels, hemorheological parameters (fibrinogen, PV, RBC aggregation) and a psychological item (physical functioning) among our patients.
3) Summarizing our research and data in the literature in order to improve IGF-1 level and lipid parameters in elderly postmenopausal women physical training programs should either contain resistance training elements or be intensive enough or effectively increase the daily physical activity level and completely change sedentary lifestyle behavior.

2. Aspirin resistance after simultaneous pancreas-kidney transplantation

1) Previously, no study have confirmed the very high prevalence of ASA resistance after SPK transplantation.
2) The incidence of cardiovascular disease increases after SPK transplantation, and their appearance may further increase by the occurrence of ASA resistance, which could affect long-term patient outcomes.
3) Based on our results, the authors presume, that the control measurements of antiplatelet therapy after SPK transplantation could be clinically useful.

3. Assessment of cardiovascular mortality in a single center cohort analysis of 593 patients after Kidney Transplantation

1) This is the first comprehensive report to characterize the full Pécs cohort of renal transplantations that form a basis for further studies.
2) In our study CVD is the leading cause of death, however, the prevalence (54%) was higher than world-wide (34%) and in the United States (23.4%). This difference is most likely attributable to a similar difference in the general populations in Hungary (55.2%).
3) This is the first analysis of transplantation outcomes in patients with Romani ethnicity in south Hungary.
4) We found that Romani ethnicity was associated with shorter graft function time, which is concordant with international findings. However, our results did not reveal higher CV event

ratio among Romani patients, contrary to that in the general Romani population in Hungary, probably due to better medical-care in the transplant program.

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