#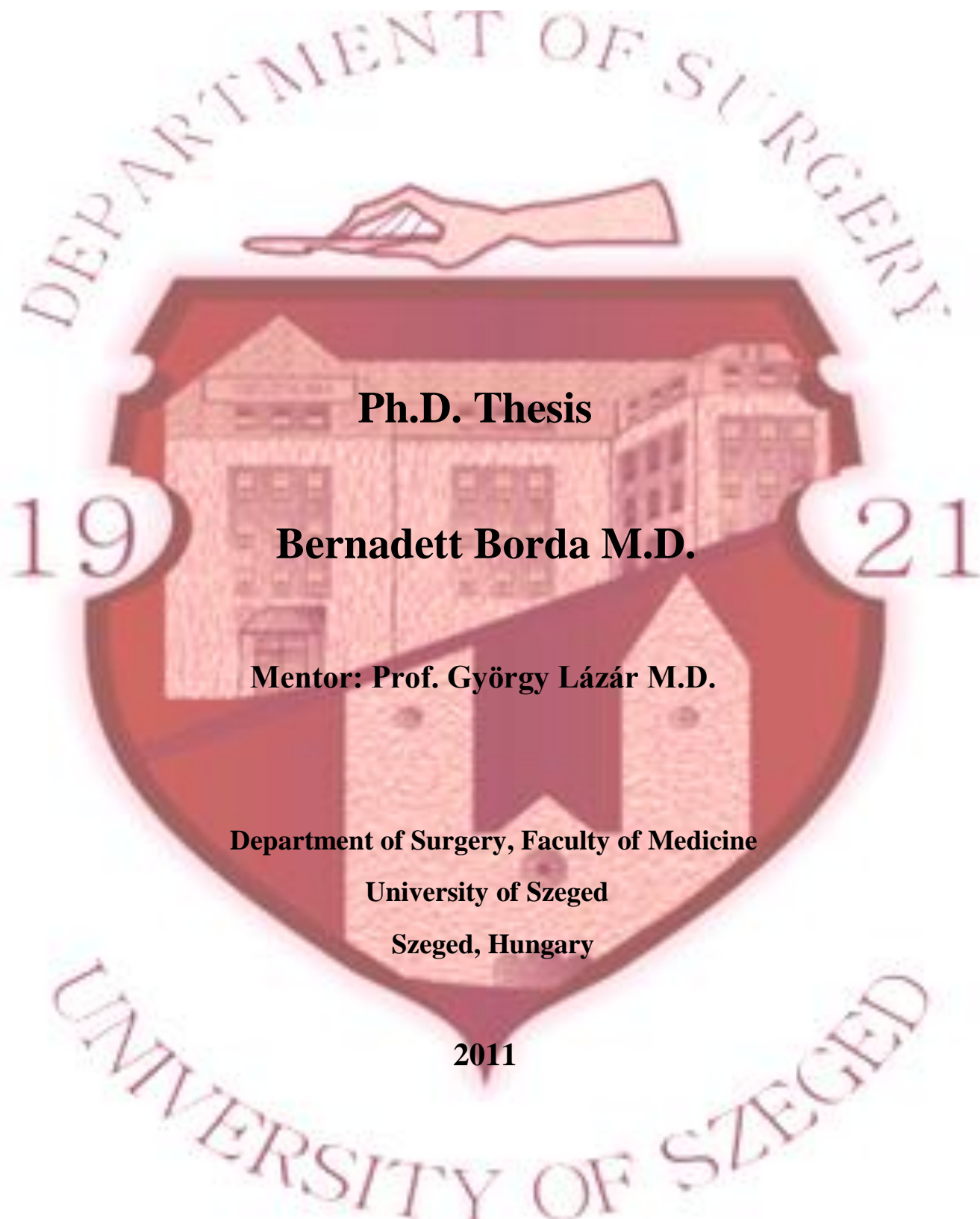 Risk factors of new onset diabetes mellitus and dyslipidemia – and effects on the function and histopathology of the allograft

Ph.D. Thesis

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LIST OF ARTICLES AND ABSTRACTS RELATED TO THE SUBJECT OF THE DISSERTATION

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A vesetranszplantáció után újonnan kialakult diabetes mellitus gyakorisága és hatása a graft működésére. *Hypertonia és Nephrologia* 2008; **12:** 21-25. **IF: 0.000**

II. **B. Borda**, E. Szederkényi, Cs. Lengyel, Z. Morvay, J. Eller, F. Marofka, V. Szabó, T. Takács, P. Szenohradszky, Z. Hódi, Gy. Lázár:

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III. **B. Borda,** Cs. Lengyel, E. Szederkényi, Z. Morvay, J. Eller, Gy. Lázár:

Post-transplant diabetes mellitus − risk factors and effects on the function and morphology of the allograft. *Acta Physiologica Hungarica* 2011; **IF: 1.226** accepted for publication

IV. **Bernadett Borda,** Csaba Lengyel, Edit Szederkényi, György Lázár:

Patients after kidney transplantation with post-transplant diabetes mellitus versus wit normal glucose metabolism - a case control study. *Experimental and Clinical Transplantation* 2011; **IF: 0.832** submitted

List of abstract related to the subject of the dissertation:

1. **Bernadett Borda**, György Lázár: Functional and histopathological changes in renal transplant patients with new-onset diabetes and dyslipidemia. Bilateral Cooperation of Doctoral Schools Timisoara-Szeged an European Model for Setting up a Doctoral Programme in the Mecical Field, *Fiziologia physiology* Timisoara, 2011; Suppl. ISSN 1223-2076, 6.

LIST OF ARTICLES NOT RELATED TO THE SUBJECT OF THE DISSERTATION

1. **Bernadett Borda,** Edit Szederkényi, Pál Szenohradszky, Zita Morvay, József Eller, György Lázár:

   Functional and histopathological changes in kidneys from marginal donors. *Annals of Transplantation* 2011; **IF: 0.975** submitted

2. E. Szederkényi, B. Iványi, Z. Morvay, P. Szenohradszky, **B. Borda,** F. Marofka, É. Kemény, Gy. Lázár: Treatment of Subclinical Injuries Detected by Protocol Biopsy Improves the Long-Term Kidney Allograft Function: A Single Center Prospective Randomized Clinical Trial. *Transplantation Proceedings* 2011; **43**:1239-1243. **IF: 0.993**

3. Tibor Takács, Attila Paszt, Zsolt Simonka, Szabolcs Ábrahám, **Bernadett Borda**, Aurél Ottlakán, Katalin Ormándi, Máté Lázár, András Vörös, Zsuzsanna Kahán, György Lázár

   Radioguided occult lesion localization versus wire-guided lumpectomy for the treatment of no palpable breast lesions**.** *Clinical Radiology* **IF: 1.765** submitted

ABBREVIATIONS

ACR: Acute cellular rejection

ANOVA: Analysis of Variance

ATG: Antithymocyte globulin

BMI: Body mass index

CG: Cockroft-Gault

CI: Confidence intervall

CIT: Cold ischemia time

CNI - tox: Calcineurin inhibitor toxicity

CNI: Calcineurin inhibitor

CsA: Cyclosporine-A

CVD: Cardiovascular disease

eGFR: Estimated glomerular filtration rate

EVR: Everolimus

HbA_{1C}:   Glycated hemoglobin

HDL: High-density lipoprotein

HLA: Human leucocyte antigen

IF/TA: Interstitial fibrosis/tubular atrophy

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

IS: Immunosuppression

LDL: Low-density lipoprotein

MAPK: Mitogen-activated protein kinase

MDRD: Modification of diet in renal disease

MMF: Mycophenolate mofetil

N: Normal

NODL: New onset dyslipidemia

NODM: New onset diabetes mellitus

OGTT: Oral glucose tolerance test

OR: Odds ration

PTDM: Post-transplant diabetes mellitus

S: Steroid

SD: Standard deviation

SRL: Sirolimus

Tac: Tacrolimus

TC: Total cholesterol

TG: Triglyceride

Tx: Transplantation

1   INTRODUCTION

## 1.1   THE HISTORY OF KIDNEY TRANSPLANTATION

The first successful experimental kidney transplantation was performed in 1902 by *Emerich Ullmann* in Vienna. The next important step was the development of the technique of vascular sutures by *Alexis Carrel*, who was awarded in 1912 by the Nobel Prize in Physiology or Medicine. The first human kidney transplantation was performed by *Jean Hamburger*. Together with René Kuss, Hamburger defined the precise methods and rules for conducting renal transplantation surgery, and is attributed with founding the medical discipline of nephrology. The first successful human kidney transplantation was performed in Boston on 23 December 1954 by *Joseph Murray* between the identical Herrick twins at the Peter Bent Brigham Hospital.

The history of the Hungarian transplantation began in Szeged with the first kidney transplantation between siblings in 1962 performed by *Prof. András Németh* at the Department of Surgery, Medical University of Szeged. The organized kidney transplantation program was initiated in 1973 in Budapest headed by *Prof. Ferenc Perner*. The next center founded within the Hungarian program was the pioneer university in Szeged joining the kidney transplantation program in 1979. The head of the 1st Surgical Department of the Medical University was *Prof. Gábor Petri* in that year just as in 1962. *Ernő Csajbók* and *Pál Szenohradszky* performed the first two deceased donor kidney transplantations and established the Transplantation Unit within the Surgical Clinic (later Szeged Transplant Center). Later *Ferenc Marofka* and *Edit Szederkényi* joined the transplant team. In 2006, the first living donor kidney transplantation was performed using the new method of donor nephrectomy, the so called hand assisted laparoscopy, under the leadership of *Jr. Prof. György Lázár*.

1.2   RISK FACTORS OF CARDIOVASCULAR DISEASES

The leading causes of death in patients who died with a functioning allograft are cardiovascular diseases [1], which account for almost 40 percent of all deaths in this population. Renal transplantation (Tx) is the treatment modality of choice for virtually all suitable candidates with end-stage renal disease. Studies suggest that the survival advantage of transplantation may be largely attributed to the reduce in cardiovascular diseases (CVD). Obesity, impaired fasting glucose (IFG) or diabetes mellitus, hypertriglyceridemia or reduced high-density lipoprotein (HDL) level, hypertension, immunosuppressive (IS) therapy, cyclosporine-A (CsA)] and tacrolimus (Tac) and smoking are high-risk factors for the development of cardiovascular diseases [2,3,4].   The mechanism of action of calcineurin inhibitors (cycylosporine-A and tacrolimus) is that they block one limb of the activation, the calcineurin pathway *(Figure 1.*)
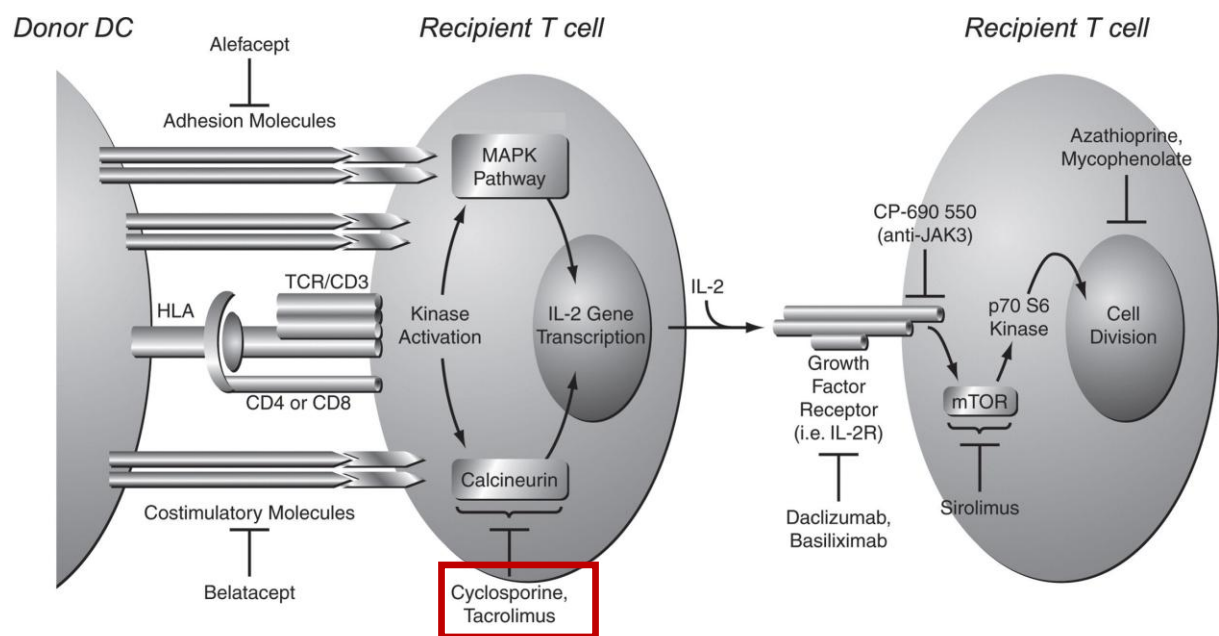
Figure 1. One pathway leads to activation of calcineurin and the other to activation of mitogen-activated protein kinases (MAPKs). The end result is IL-2 gene transcription. Cyclosporine-A and tacrolimus block one limb of the activation, the calcineurin pathway. The MAPK pathway is not completely blocked by either cyclosporine-A or tacrolimus and therefore T-cell signaling still occurs. *(Dianne B. McKay, Steven M. Steinberg Kidney Transplantion: A Guide to the Care of Kidney Transplant Recipients 2010)*

However, the impact of new onset diabetes mellitus (NODM)/ post-transplant diabetes mellitus (PTDM), and new onset dyslipidemia (NODL) developing after transplantation on the progression of histopathological changes attributable to interstitials fibrosis/tubular atrophy (IF/TA) in serial protocol biopsies during the first year after transplantation has not been described in details. Long-term renal transplant results have not changed considerably in the past two decades, even though the incidence of acute cellular rejection (ACR) and IF/TA in the first year has continuously decreased. IF/TA and ACR are still a major risk factor long-term allograft dysfunction. The protocol biopsies are generally evaluated by the Banff classification, a grading scale established by experienced nephro-pathologists and transplant clinicians [5,6].

1.3 DIAGNOSIS OF DIABETES AND DYSLIPIDEMIA

NODM or PTDM : According to the criteria of the American Diabetes Association, diabetes is present if the fasting blood glucose level is ≥ 7 mmol/L or if the blood glucose level measured 2-h following the oral administration of 75 g glucose (Oral glucose tolerance test, OGTT) is ≥ 11.1 mmol/L. IFG is defined as a fasting blood glucose level between 5.6 mmol/L and 6.9 mmol/L, whereas the normal value (N) for fasting blood glucose is <5.6 mmol/L or impaired glucose tolerance (IGT) (2-h values in the OGTT) between 7.8mmol/L and 11.0mmol/L. OGTT was performed in each patient. Patients with blood glucose level $\geq 11.$mmol/L were selected into the NODM group [7].

NODL: According to the recommendations of the World Health Organization, the normal level of total cholesterol (TC) is <5.17 mmol/L, the normal level of triglyceride (TG) is <1.69 mmol/L, the normal level of the low-density lipoprotein (LDL) is <1.03 mmol/L and the normal level of HDL is >1.3 in women and >1 in men. Patients who had higher values of TG, TC and LDL than normal were considered dyslipidemic [8].

Tissue sampling: Before the transplantation, a histological sample was taken from each kidney not yet implanted ("zero biopsy"). We performed a protocol biopsy 3 months, 1 year and 3 years after transplantation. We studied those patients whose "zero biopsy" was

intact, and in whom we could perform protocol biopsy 1 year after the transplantation. The ultrasound-guided protocol biopsy was performed (with the patient's prior consent) after the one-year fasting laboratory test. 16-G Tru-Cut needles and a biopsy gun were used to obtain the tissue cylinders.

Histological investigations: Morphological examinations included standard light microscopic stainings (H&E, PAS, trichrome and methenamine silver) and immunofluorescence analysis of the frozen sections with antibodies to HLA (human leucocyte antigen) class II antigens, complement 4d (C4d), C3, IgG, IgA and IgM. Embedding for electron microscopy was carried out in all cases and ultrastructural evaluation was performed optionally. Renal lesions were graded and diagnosed according to the 2003 modification of the Banff '97 classification (*Table 1*) [9]. IF/TA involved patients with grades II and III, and grade I was considered normal.

2003 MODIFICATION OF THE BANFF '97 CLASSIFICATION

1.	**NORMAL** (N)
2.	**CALCINEURIN INHIBITOR TOXICITY** (CNI-tox)
3.	**ACUTE CELLULAR REJECTION** (ACR) Types (Grades) **IA** – Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells) **IB –** Cases with significant interstitial infiltration (>25% of parenchyma affected) and foci of moderate tubulitis (>10 mononuclear cells/tubular cross section or group of 10 tubular cells) **IIA** – Cases with mild to moderate intimal arteritis (v1) **IIB** – Cases with severe intimal arteritis comprising >25% of the luminal area (v2) **III –** Cases with "transmural" arteritis and/or fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)
5.	**INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY (IF/TA)** **[Chronic allograft nephropathy]** Grades **Grade I** *Mild* interstitial fibrosis and tubular atrophy without (a) or with (b) specific changes suggesting chronic rejection **Grade II** *Moderate* interstitial fibrosis and tubular atrophy (a) or (b) **Grade III** *Severe* interstitial fibrosis and tubular atrophy and tubular loss (a) or (b)
6.	**PYELONEPHRITIS**
7.	**OTHER** – acute tubular necrosis, glomerulonephritis and BK polyomavirus nephritis

Table 1. Renal lesions were graded and diagnosed according to the 2003 modification of the Banff '97 classification.

1.4   AIMS OF STUDY

> ➢ To determine the prevalence of newly developed diabetes mellitus and dyslipidemia in patients having kidney transplantation in the Southern Great Plain, Hungary.

> ➢ To examine which factors (data of donors and recipients, applied immunosuppressants) influence the development of adverse events.

> ➢ To evaluate the effect of newly developed diabetes mellitus and dyslipidemia on the function and morphology of the allograft.

Previous studies have confirmed that diabetes mellitus and dyslipidemia have an adverse effect on the allograft, but it is not known whether there is significant difference between the function and morphology of the kidneys of checked-up patients with normal glucose and lipid metabolism compared to that of patients with abnormal glucose and lipid metabolism one year after the transplantation.

2   PATIENTS AND METHODS

2.1   INCLUSION CRITERIA

Our studies were performed in patients who had received a kidney transplant between January 1, 2004 and December 31, 2008 (n = 154 patients) in Study I or between January 1, 2005 and December 31, 2009 (n = 115 patients) in Study II at the Department of Surgery in the University of Szeged, Hungary. Patients who died during the study, were under eighteen, had not undergone primary cadaver kidney transplantation, had been diagnosed with diabetes and dyslipidemia before the transplantation, had shown a hypertensive kidney on their "zero biopsy" or had not consented to the protocol biopsy were excluded from the study.

The control group was formed by patients with normal glucose and lipid metabolism.
We enlisted the patients into 4 groups (N, IFG, IGT, and PTDM) in Study I and into 4 groups (N, NODM, NODL, NODM+NODL) in Study II. In Study II the NODM group involved other (IFG, IGT) abnormal glucose metabolism.

2.2   IMMUNOSUPPRESSIVE DRUGS ADMINISTERED 1 YEAR AFTER TRANSPLANTATION

The initial daily dose of *Tac* was 0.20 mg/kg in two portions, and then the target blood level was 10-15 ng/mL for 6 weeks and 5-10 ng/mL after week 6.

The initial dose of *CsA* was 8-10 mg/kg daily, in two portions, and then the target blood level was 1,300-1,600 ng/mL in month 1, 900-1300 ng/mL in months 2 and 3, 750-950 ng/mL in months 4 to 6 and 700 ng/mL afterwards (blood levels were determined two hours after administration).

The initial dose of *sirolimus (SRL)* was 0.1 mg/kg once daily; the target blood level was 10-15 ng/mL for 6 weeks and then 5-10 ng/mL.

In the case of *everolimus (EVR),* the initial dose was 0.02 mg/kg twice daily, and then the target level was 3-8 ng/mL.

500 mg *steroid (S)* was administered intravenously on day 0 in the operating room, immediately before restoring blood flow to the graft; 125 mg was administered intravenously on day 1, 32 mg orally on day 2, 24 mg orally on day 3, 16 mg orally on days 4 to 28, 12 mg orally during month 2, 8 mg orally during month 3, and 4 mg orally during months 4 to 6. The dose was further decreased in problem-free cases.

The daily dose of *mycophenolate mofetil (MMF)* was 2 g in two portions and was reduced by 50 or 75% in the event of gastrointestinal symptoms or leucopenia.

1.25 mg/kg *antithymocyte globulin (ATG)* was administered for 7 to 21 days.

Basiliximab was given on day zero within 2 hours prior to the operation 20 mg i.v. and 20 mg i.v. on day 4.

For the treatment of ACR, steroid in a daily dose of 500 mg was administered intravenously for 3 days, followed by 250 mg per day intravenously for 2 days.

2.3   RISK FACTORS

We examined the donor and the initial and the one-year after transplantation recipient data [age, gender, cold ischemia time (CIT), urine production in the last 24 hours, human leucocyte antigen (HLA)- mismatch, body weight, body height, body mass index (BMI) and glycated hemoglobin (HbA$_{1C}$)]. In 2008, the measurement of HbA$_{1C}$ was introduced. In diabetes mellitus, the measurement of HbA$_{1C}$ serves for the 2–3-month retrospective monitoring of carbohydrate metabolism.   Of the administered immunosuppressants, we studied the effects of the calcineurin inhibitors (CNI) cyclosporine-A and tacrolimus on glucose and lipid metabolism (*Table 2*). We examined the effect of lipids on glucose metabolism.

	+ MMF		- MMF		Total		+ MMF		- MMF		Total
	+S	-S	SRL/EVR	+S	-		+S	-S	SRL/EVR	+S	-
CsA	19	23	25	3	70	CsA	15	25	9	1	50
Tac	17	24	24	2	67	Tac	17	27	12	2	58
SRL/EVR	10	5	-	2	17	SRL/EVR	3	0	-	4	7
Total	46	52	49	7	154	Total	35	52	21	7	115

STUDY I.	STUDY II.

Table 2. Immunosuppressive therapy 1 year after transplantation in the groups of Studies I and II

CsA- cyclosporine – A; Tac – tacrolimus; SRL – sirolumus; EVR – everolimus; S – steroid; MMF – mycophenolte mophetil

2.4 FUNCTIONAL EXAMINATION OF THE ALLOGRAFT

Changes in renal function were assessed by the *serum creatinine* [µmol/L] level and the *estimated glomerular filtration rate* (eGFR) [mL/min/1.73m^2] in the different glucose metabolism groups. eGFR was calculated by using:

- **the Cockroft-Gault (CG) formula**

    eGFR = (140-age) x body weight/serum creatinine x 1.04 in women and 1.23 in men

- **the modification of diet in renal disease (MDRD) formula**

$$eGFR = 32{,}788 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ in women})$$

(in patients over 18 years)

2.5   HISTOPATHOLOGICAL EXAMINATION OF THE ALLOGRAFT

Before the transplantation, a histological sample was taken from each kidney not yet implanted ("zero hour" biopsy). We studied the one-year protocol biopsies of those patients who had a normal histology result of the zero biopsy.

2.6   STATISTICAL ANALYSIS

For the comparison of the mean values, t-test and one-way analysis of variance were used, as well as the Mann–Whitney and Kruskal–Wallis tests in cases of non-normality. The normal distribution of samples was tested by using the Kolmogorov–Smirnov test. Categorical data were analyzed by using chi-square and Fisher's exact tests. Temporal changes in renal function parameters were compared by using repeated measures of the analysis of variance (ANOVA). The multivariable dependence of NODM and NODL on both categorical and continuous data was analyzed by using logistic regression. SPSS version 15.0 (© 2007 SPSS Inc.) was used for statistical analys.

3 RESULTS

3.1 STUDY I – POST-TRANSPLANT DIABETES MELLITUS

3.1.1 RISK FACTORS OF THE PTDM

Of the initial and one-year after transplantation data relating to the donors and the recipients, BMI ($p = 0.003$), body weight ($p = 0.02$) and age of the recipients ($p = 0.005$) differed significantly in the control and the PTDM groups. The level of HbA_{1C} was $4.2 \pm 0.2\%$ in the N group, $4.9 \pm 0.5\%$ in IFG group, $5.2 \pm 0.9\%$ in patients with IGT and $10.4 \pm 3.6\%$ in the PTDM group. The difference between the HbA_{1C} levels was significant ($p < 0.001$) when comparing the N and the PTDM groups (*Table 3*).

	N (n=115) Mean ± SD	IFG (n=8) Mean ± SD	IGT (n=6) Mean ± SD	PTDM (n=25) Mean ± SD	p value
DONOR DATA					
Gender (female/male)	49/66	3/5	1/5	8/17	
Age (years)	43.63 ±12.31	47.2±9.7	45.5±5.4	45.52±8.54	**0.529**
Urine production in the last 24 hours	5.847±3.096	4.820±1.814	4.956±1.574	6.033±3.488	**0.454**
Creatinine (μmol/L)	91.69±38.98	97.98±44.5	94.46±38.67	95.96±42.91	**0.593**
CIT (h)	16±4	17±3	15±4	15±2	**0.104**
HLA mismatches	3.4±1.44	3.4±1.38	3.1±1.51	3.2±1.24	**0.458**
INITIAL RECIPIENT DATA					
Gender (female/male)	43/72	4/4	2/4	9/16	
Age (years)	**41.82±4.71**	49.02±6.78	48.03±3.47	**58.58±5.86**	**0.005**[*1]
Body weight (kg)	71.42±14.22	72.31±12.87	72.67±11.89	74.14±21.17	**0.258**
Body height (cm)	168.53±10.61	167.36±11.09	171.56±8.79	170.60±11.67	**0.427**
BMI (kg/m^2)	25±1.2	26±2.4	26±3.2	27±1.3	**0.357**
RECIPIENT DATA 1 YEAR AFTER TX					
Body weight (kg)	**74.42±14.22**	77. 31±12.87	80±10.45	**89.14±21.17**	**0.02**[*2]
HbA$_{1C}$ (%)	**4.2±0.2**	4.9±0. 5	5.2±0.9	**10.4±3.6**	**<0.001**[*]
BMI (kg/m^2)	**25±2.8**	26±3.8	25±4.2	**30±3.5**	**0.003**[*4]

Table 3. Donor, initial and 1-year after transplantation recipient data.

N- normal; IFG- impaired fasting glucose; IGT- Impaired glucose tolerance; PTDM- post-transplant diabetes mellitus; CIT- cold ischemia time; HLA- human leucocyte antigen; BMI-body mass index; HbA$_{1C}$ - glycated haemoglobin; SD- standard deviation

As regards the immunosuppressive therapy, the ratio of patients who developed PTDM was 8.6% in those taking CsA, and 26.8% in those on Tac; the difference was significant (p = 0,004) (*Table 4*).

	N	IFG	IGT	PTDM	Total
CsA	59 (84.3%)	3 (4.2%)	2 (2.8%)	6 (8.6%)*	70
Tac	43 (64.0%)	2 (2.9%)	4 (5.9%)	18 (26.8%)*	67
p value	0.25	0.31	0.53	0.004	

Table 4. Incidence of diabetes mellitus and impaired glucose tolerance in patients treated with cyclosporine-A or tacrolimus.* p < 0.004 indicated significant difference between the cyclosporine-A and tacrolius groups.
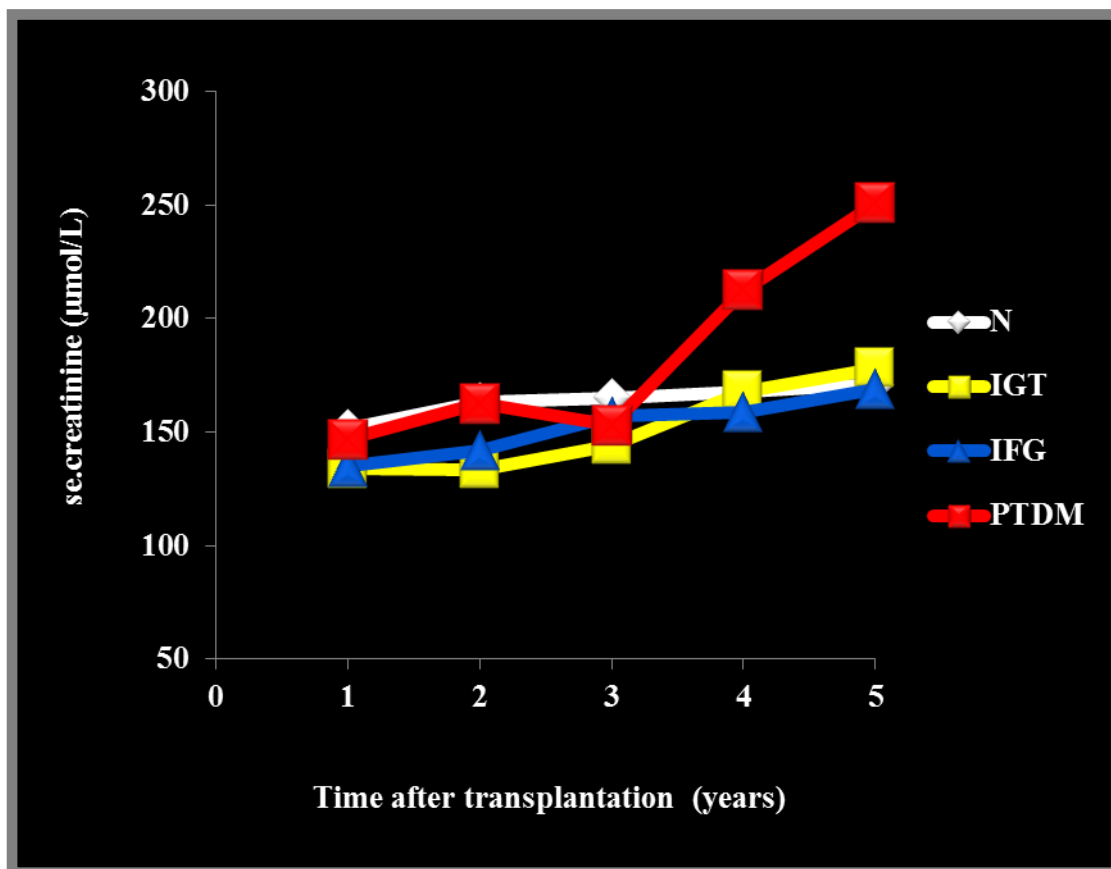
N – normal; IFG - impaired fasting glucose; IGT - impaired glucose tolerance; PTDM – post-transplant diabetes mellitus; CsA- cycylosporine – A; Tac- tacrolimus

In the case of combined CsA–S treatment, 10.4% of the patients developed diabetes, and there was a significant difference if we compared it to the group of patients taking CsA without steroids 4.5% (p = 0.044). The incidence of newly developed diabetes among patients on the Tac–S combination was 27.7%; compared to the incidence rate in patients receiving steroid-free immunosuppression 21.1%, which was not significantly different (p = 0.846). No significant difference, in regard to the development of diabetes, was found between the

normal and the PTDM groups in case of the combinations of MMF and Tac (p = 0.76), SRL and Tac (p = 0.53), and EVR and Tac (p = 0.43).

3.1.2 FUNCTIONAL CHANGES OF THE ALLOGRAFT

Studying the functional changes of the allograft one year after the transplantation, we found that the serum creatinine value was 151.56±44.38 μmol/L in normal patients and 158.80±49.74 μmol/L in the PTDM group. There was no significant difference between the normal and the PTDM groups (p = 0.54) (*Figure 2*).

Figure 2. Changes of serum creatinine level in the different glucose metabolism groups. N- normal; IGT- impaired glucose tolerance; IFG- impaired fasting glucose, PTDM- post-transplant diabetes mellitus

The $eGFR_{CG}$ (calculated by using the CG formula) in the N and PTDM groups was 57.97±20.25 and 52.95±17.89 mL/min/1.73m^2, respectively. We had similar results when calculating eGFR using the MDRD formula. The calculated $eGFR_{MDRD}$ (calculated by using the MDRD formula) in the N and PTDM groups was 52.28±18.99 and 49.54±18.04 mL/min/1.73m^2, respectively. No significant difference was found between the N and the PTDM groups when comparing the eGFR values ($p = 0.34$ with the CG and $p = 0.46$ with the MDRD formula) (*Figure 3*).

calculated by using the CG formula
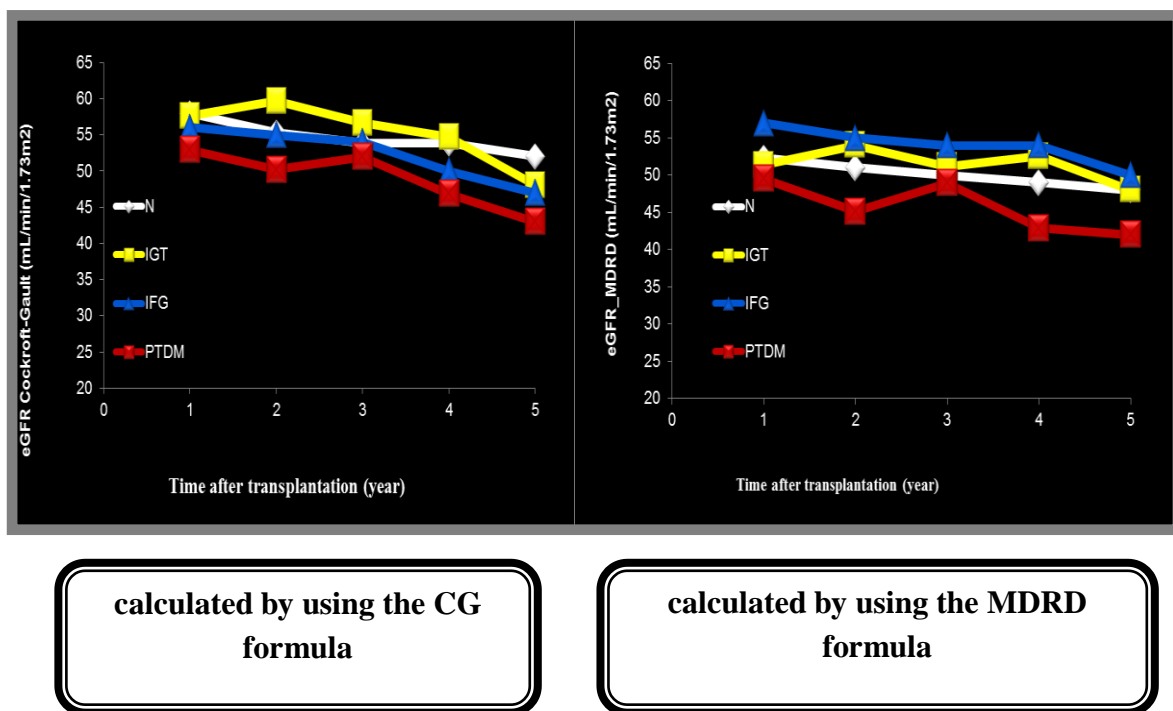
calculated by using the MDRD formula

Figure 3. Changes of eGFR in the different glucose metabolism groups.

N- normal; IGT- impaired glucose tolerance; IFG- impaired fasting glucose; PTDM- post-transplant diabetes mellitus; CG- Cockroft–Gault; MDRD- modification of diet in renal disease

The post-transplant serum creatinine and $eGFR_{CG}$ and $eGFR_{MDRD}$ levels in the groups with a different glucose metabolism status were compared by using the repeated measures ANOVA statistical analysis. $eGFR_{CG}$ and $eGFR_{MDRD}$ values did not seem to differ significantly in the

PTDM group, but there was a significant difference between the serum creatinine levels measured in the first and the fifth years (p = 0.0003). In the first year after Tx, the serum creatinine level was 158.80±49.74 μmol/L, whereas in the fifth year, it was 212.43±131.20 μmol/L. (*Table 5*).

			1 year after Tx Mean ± SD	5 years after Tx Mean ± SD	p value
eGFR (mL/min/1.73m^2)	Cockroft-Gault	N	57.97±20.25	53.86±17.68	0.324
		IFG	56.78±16.86	54.65±18.32	0.246
		IGT	57.58±14.20	54.70±22.13	0.297
		PTDM	52.95±17.89	49.87±22.32	0.423
	MDRD	N	52.28±18.99	46.96±18.93	0.264
		IFG	55.93±46.75	53.78±44.43	0.534
		IGT	51.34±14.51	52.50±27.61	0.817
		PTDM	49.54±18.04	45.87±24.19	0.334
Serum creatinine (μmol/L)		N	151.56±44.38	167.81±125.93	0.323
		IFG	135.95±38.32	159.64±58.28	0.427
		IGT	133.86±40.32	167.83±84.63	0.202
		PTDM	**158.80±49.74**	**212.43±131.20**	**0.0003***

Table 5. eGFR and serum creatinine levels at various post-transplantation times, *p=0.0003 denoting significant difference.

N- normal; IGT- impaired glucose tolerance; IFG- impaired fasting glucose; PTDM- post-transplant diabetes mellitus; eGFR- estimated glomerular filtration rate; MDRD- modification of diet in renal disease, Tx- transplantation; SD- standard deviation

3.1.3 HISTOPATHOLOGICAL CHANGES OF THE ALLOGRAFT

Regarding the morphological changes of the kidney, there was significant difference between the PTDM and the N groups in the frequency of severe IF/TA (p = 0.0004) and ACR (p = 0.001), whereas no significant difference could be demonstrated in the morphological signs of CNI- tox. (p = 0.075) between the two groups (*Table 6*).

	ACR	CNI-tox	IF/TA	Pyelonephritis	Other	Normal
N (n=77)	**13** **(16.8%)**	9 (11.6%)	**10** **(12.9%)**	2 (2.6%)	1 (1.3%)	**42** **(54.5%)**
IFG (n=8)	1 (12.5%)	1(12.5%)	2 (25%)	0 (0%)	2 (25%)	2 (25%)
IGT (n=5)	1 (20%)	0 (0%)	2 (40%)	0 (0%)	1 (20%)	1 (20%)
PTDM (n=25)	**6 (24%)**	3 (12%)	**13 (52%)**	1 (4%)	0 (0%)	**2 (8%)**
p value (compared N and NODM)	**0.001**	0.075	**0.0004**	0.453	0.287	**0.003**

Table 6. Protocol biopsy results in the different glucose metabolism groups. N vs. PTDM (ACR p = 0.001; IF/TA p = 0.0004; N p = 0.003)

N- normal; IGT- impaired glucose tolerance; IFG- impaired fasting glucose; PTDM- post-transplant diabetes mellitus; ACR- acute cellular rejection; CNI- tox- calcineurin inhibitor toxicity; IF/TA- interstitial fibrosis/tubular atrophy

According to the results of a stepwise logistic regression analysis of the data on the recipients (gender, age, serum creatinine, urine production in the last 24 hours) and donors (age, gender, serum creatinine, eGFR, BMI, body weight and IS), calcineurin inhibitors had the major effect on the morphological changes of the kidneys. Diabetes mellitus markedly increased the

risk of ACR and IF/TA (ACR: p = 0.025, odds ratio (OR) = 7.2, confidence interval (CI) = 1.276–40.960; IF/TA: p = 0.0004, OR = 24.9, CI = 4.5–138.219).

3.2 STUDY II – NEW ONSET DIABETES MELLITUS AND DYSLIPIDEMIA

3.2.1 RISK FACTORS OF NODM AND NODL

The difference between NODM and N patients was significant in regard to TG (p = 0.0001) and TC (p = 0.025), whereas it was not significant in regard to HDL (p = 0.307) and LDL (p = 0.510). We observed that triglyceride level increased drastically above a fasting blood glucose level of 10 mmol/L (*Figure 4*).
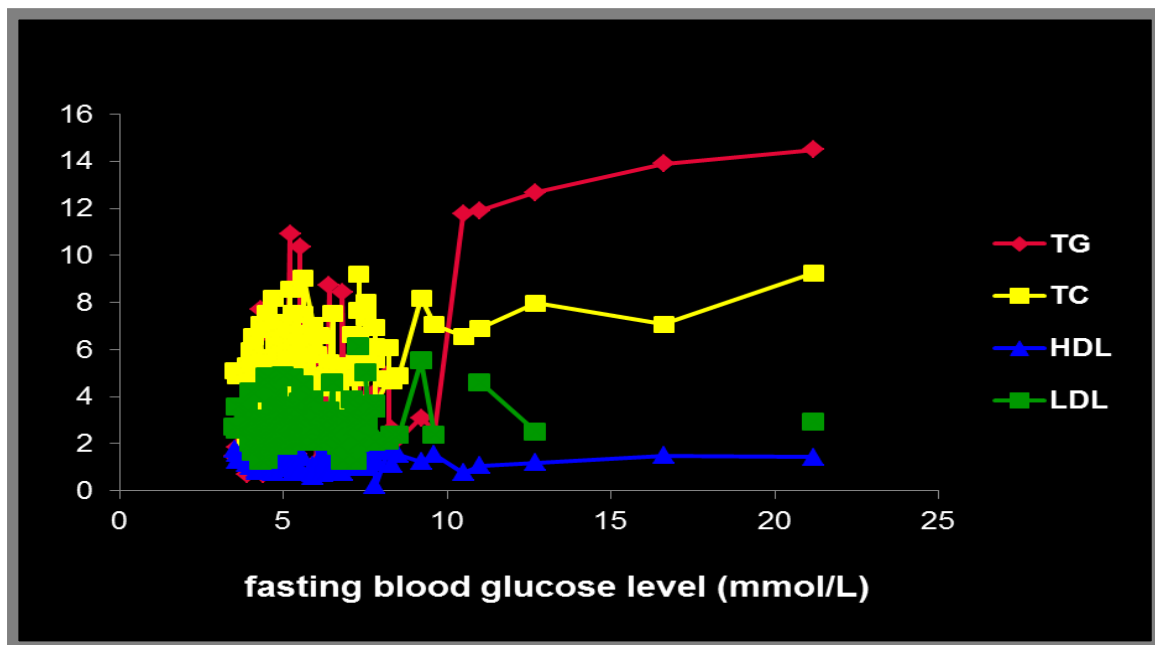
Figure 4. Changes of lipid levels with the increased fasting blood glucose level

TG- triglyceride; TC- total cholesterol; HDL- high-density lipoprotein; LDL- low-density lipoprotein

When evaluating the donor data, no significant difference was found; however, in regard to the initial recipient data, there was a significant difference in age when comparing N and NODM patients (p = 0.004), N and NODL patients (p = 0.002) and N and NODL+NODM patients (p = 0.0001). The difference in BMI was significant when comparing N and NODM+NODL patients (p = 0.003). One year after transplantation, there was a significant difference in BMI between the N and NODM groups (p = 0.03), the N and NODL groups (p = 0.02) and the N and NODM+NODL groups (p = 0.001). HbA$_{1C}$ was 4.2±0.2% in the N group and 9.4±1.6% in the NODM group, which is a significant difference (p = 0.005). This value in the NODM+NODL group (11±0.5%) was significantly different from that of the N group (p = 0.0001) (*Table 7*).

	N	NODM	p value	NODL	p value	NODM& NODL	p value
DONOR DATA							
Age (year)	44.63±7.31	45.27±9.74	0.781	45.54±5.47	0.529	46.27±6.24	0.428
Urine production in the last 24 hours	5.847±3.096	4.820±1.814	0.674	4.956±1.574	0.454	5.652±3.785	0.315
Creatinine (μmol/L)	91.69±38.98	93.96±44.52	0.542	92.54±42.91	0.593	94.23±40.67	0.125
CIT (h)	16±4	17±3	0.248	15±4	0.845	17±2	0.534
RECIPIENT PRIMARY DATA							
Age (year)	**41.82±2.71**	**51.03±3.47**	**0.004**	**53.58±4.86**	**0.002**	**58.78±2.92**	**0.0001**
BMI (kg/m2)	**25±1.2**	26±3.4	0.678	26±2.8	0.328	29±3.2	**0.003**
RECIPIENT DATA ONE YEAR AFTER TX							
BMI (kg/m2)	**25±2.5**	**28±3.2**	**0.03**	**29±2.7**	**0.02**	**30±2.3**	**0.001**
HbA$_{1c}$ (%)	**4.2±0.2**	**9.4±1.6**	**0.005**	4.7±0. 5	0.749	**11±0.5**	**0.0001**

Table 7. Donor, initial and 1-year after transplantation recipient data.

N- normal; NODM- new onset diabetes mellitus; NODL- new onset dyslipidemia; CIT- cold ischemia time; BMI- body mass index; HbA$_{1C}$ - glycated hemoglobin; Tx- transplantation

According to the logistic regression analysis performed, an increase of in BMI by one resulted in a 12 percent increase in the risk of developing NODM, and an increase in BMI by one led to a 26% increase in the risk of developing NODL, whereas the administration of cyclosporine-A resulted in an increase of 28% in the risk of developing NODL (*Table 8*).

	NODM		NODL	
	p value	OR	p value	OR
Cyclosporin-A	0.076	0.319	**0.004**	**1.288**
Tacrolimus	**0.005**	**1.259**	0.330	0.600
BMI	**0.03**	**1.128**	**0.005**	**1.261**

Table 8. Results of logistic regression analysis in the NODM and NODL groups.

NODM- new onset diabetes mellitus; NODL- new onset dyslipidemia; OR- odds ratio; BMI- body mass index

When assessing the effect of CNIs (and, more specifically CsA and Tac) on glucose and lipid metabolisms, we found that those taking Tac developed diabetes significantly more frequently than those taking cyclosporine-A (p = 0.005), whereas the development of dyslipidemia was significantly more frequent in those taking CsA than in those taking Tac (p = 0.001) (*Table 9*).

		N	NODM*	NODL	NODM*&NODL
		(n=53)	(n=20)	(n=25)	(n=17)
CNI	CsA (n=50)	27(54%)	**6(12%)***	**15(30%)***	7(14%)
	Tac (n=58)	21(36%)	**14(24%)***	**9(16%)***	10(17%)
CNI free		5(72%)	0(0%)	1(14%)	0(0%)

Table 9. Calcineurin inhibitors and the incidence of new onset diabetes mellitus and dyslipidemia *1 p = 0.005; *2 p = 0.001 (*NODM and other abnormal glucose metabolism IFG/IGT)

N- normal; NODM- new onset diabetes mellitus; NODL- new onset dyslipidemia; CNI-calcineurin inhibitor; CsA- cyclosporine- A; Tac- tacrolimus

In regard to the effect of CsA and Tac on lipids, we found that TG was 3.02±1.51 mmol/L in those taking CsA and 2.15±1.57 mmol/L in those taking Tac, which is a significant difference (p = 0.004). The difference was proved to be significant also when assessing TC: it was 5.43±1.23 mmol/L in those receiving CsA-based immunosuppression and 4.42±1.31 mmol/L in patients taking Tac (p = 0.001). No significant difference in LDL and HDL levels was found between the two groups (*Table 10*).

		CNI		CNI free	p value
		CsA	Tac		
TG	(mmol/L)	3.02 ± 1.51	2.15 ± 1.57	2.18 ± 1.07	**0.004**
TC		5.43 ± 1.23	4.42 ± 1.31	4.75 ± 1.40	**0.001**
LDL		2.93 ± 0.83	2.94 ± 0.98	2.84 ± 0.82	0.18
HDL		1.37 ± 0.38	1.25 ±0.38	1.33 ± 0.39	0.093

Table 10. The effect of calcineurin inhibitors on lipids.

CNI- calcineurin inhibitor; CsA- cyclosporine-A; Tac- tacrolimus; TG- triglyceride; TC-total cholesterol; LDL- low-density lipoprotein; HDL- high-density lipoprotein

3.2.2 FUNCTIONAL CHANGES OF THE ALLOGRAFT

In regard to the function of the allograft, we found that there was significant difference in the serum creatinine level between the NODM+NODL group and the N group (p = 0.02) one year after the transplantation. The difference was not significant when comparing the NODM group and the NODL group with the N group. A similar result was obtained when assessing $eGFR_{CG}$: the difference between NODM+NODL patients and N patients was significant (p=0.004) (*Table 11*).

	N	NODM	p value	NODL	p value	NODM&NODL	p value
Serum creatinine (μmol/L)	**141.00** **±47.58**	147.00 ±55.84	0.594	155.00 ±57.22	0.154	**173.00** **±67.47**	**0.02**
eGFR$_{CG}$ (mL/min/1.73m^2)	**56.47** **±15.78**	51.81 ±17.33	0.764	52.61 ±17.95	0.642	**42.52** **±18.54**	**0.004**

Table 11. Serum creatinine and eGFR$_{CG}$ one year after transplantation in the different glucose and lipid metabolisms groups.

N- normal; NODM- new onset diabetes mellitus; NODL- new onset dyslipidemia; eGFR$_{CG}$- estimated glomerular filtration rate Cockroft-Gault

3.2.3 HISTOPATHOLOGICAL CHANGES OF THE ALLOGRAFT

When studying the morphological changes of the kidney, there was significant difference between the N and the NODM groups in ACR (6% vs. 25%, p = 0.004) and IF/TA (4% vs. 55%, p = 0.0002). In case of IF/TA, the difference between N and NODL patients was significant (4% vs. 36%, p = 0.003). Between the N and the NODM+NODL groups, the difference in IF/TA (4% vs. 41%, p = 0.0001) and ACR (6% vs. 24%, p = 0.03) was proved to be significant (*Table 12, Figure 5*).

	ACR	IF/TA	CNI-tox	Pyelonephritis	Other	N
N	3(6%)	2(4%)	10(19%)	4(7%)	5(9%)	29(55%)
NODM *	5(25%)[1]	11(55%)[2]	1(5%)	0(0%)	1(5%)	2(10%)
NODL	4(16%)	9(36%)[3]	3(12%)	1(4%)	3(12%)	5(20%)
NODM&NODL	4(24%)[4]	7(41%)[5]	1(6%)	2(12%)	1(6%)	2(12%)
TOTAL	16 (14%)	29(25%)	15(13%)	7(6%)	10(9%)	38(33%)

Table 12. Protocol biopsy results in the different glucose and lipid metabolism groups   *1 N vs. NODM ACR p = 0.004; *2 N vs. NODM IF/TA p = 0.0002; *3 N vs. NODL IF/TA p = 0.003; *4N vs. NODM+NODL ACR p = 0.03; *5 N vs. NODM+NODL IF/TA p = 0.0001 (*NODM and other abnormal glucose metabolism)

N- normal; NODM- new onset diabetes mellitus; NODL- new onset dyslipidemia; ACR- acute cellular rejection; IF/TA- interstitial fibrosis and tubular atrophy; CNI-tox- calcineurin inhibitor toxicity
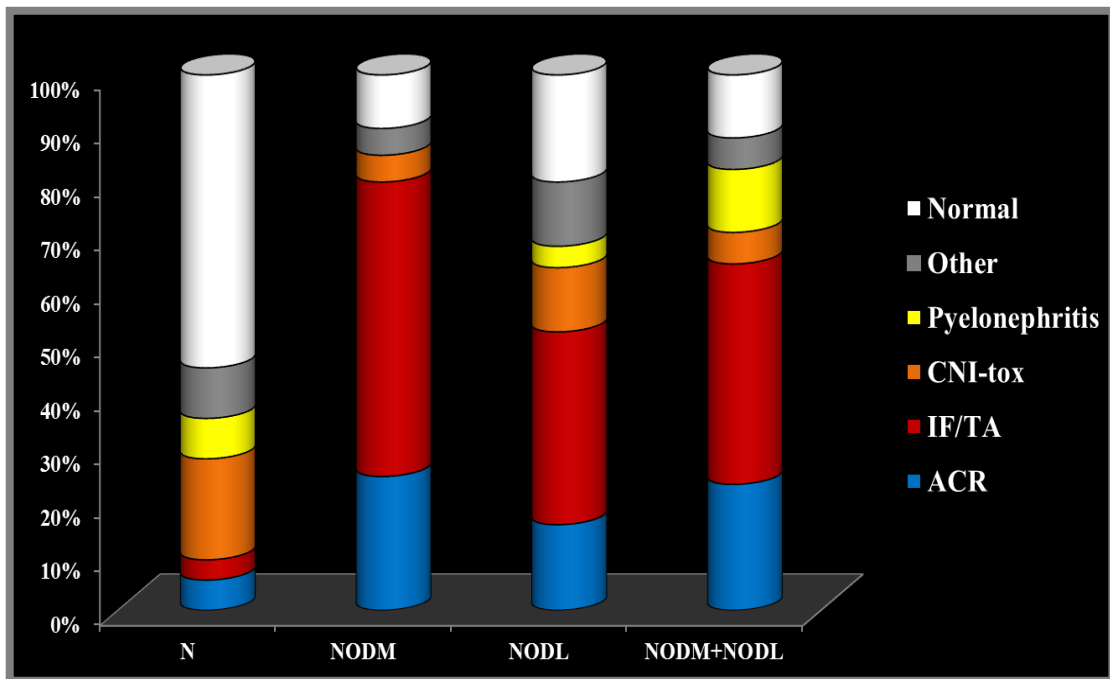
Figure 5. Protocol biopsy results.

N- normal (group); NODM- new onset diabetes mellitus (group); NODL- new onset dyslipidemia (group); ACR-acute cellular rejection; IF/TA- interstitial fibrosis and tubular atrophy; CNI-tox- calcineurin inhibitor toxicity

4   DISCUSSION

New-onset diabetes mellitus and dyslipidemia are serious and frequently observed complications following renal transplantation. Kidney transplant recipients who develop NODM and NODL are at an increased risk of developing fatal and nonfatal cardiovascular events and other adverse outcome including infection, reduced patient survival, graft rejection and accelerated graft loss. Identification of high-risk patients and implementation of measures to reduce the development of NODM and NODL may improve both long-term patient and graft outcome. Studies suggest that the survival advantage of transplantation may be largely attributed to the reduction in cardiovascular diseases associate with the improvement in renal function following a successful renal transplant. In a retrospective analysis of the United States Renal Data System comprising the data of more than 60,000 adult primary kidney transplant recipients transplanted between 1995 and 2000 and more than 66,000 adult patients on the waiting list over the same time period, Meier–Kriesche et al. [10] have demonstrated progressive decrease in cardiovascular death rates by renal transplant vintage for both diabetic and non-diabetic recipients of both living and deceased donor transplants. The cardiovascular disease death rate among transplant recipients was higher one year after transplantation. Renal transplantation ameliorates cardiovascular disease risk including impaired glucose tolerance or diabetes mellitus, hypertension and dyslipidemia that are derived, partially, from immunosuppressive medications such as calcineurin inhibitors.

In our study, the incidence of new-onset diabetes mellitus was 17%, whereas that of new-onset dyslipidemia was 22% in the patient population. We assessed the effects of NODM and NODL on each other, and we found that NODM, total cholesterol level and triglyceride level mutually affect the development of each other. In the studies by Valderhang et al. [11], the incidence of diabetes has been 17% and 14%, respectively.

Diabetic and dyslipidemic patients are continuously at elevated risk for developing CVD and other diabetic and dyslipidemic complications. This is also true for those developing new-onset diabetes and dyslipidemia after transplantation, who very rapidly develop comparable risks for CVD and death. Kamar et al. [12] and Schiel et al. [13] have found similar results,

namely that the risk factors of NODM include not only the immunosuppressive agents but also family history and age, body weight and BMI of the recipient, and these play at least the same importance in the development of diabetes [13,14,15,16]. Immunosuppressive therapy, the age of the recipient and body mass index had a significant effect on the development of NODM and NODL. Other clinical trials have also found these factors to be significant [17,18,19].

Obesity is a potentially detrimental condition due to its associated comorbid conditions including hyperinsulinemia and insulin resistance, diabetes mellitus and dyslipidemia. Obesity defined as BMI $\geq$ 30kg/m^2 has a reported prevalence of 9.5–29% on transplantation [19]. Studies reporting on renal transplant recipients have shown that high BMI at transplant is a significant independent predictor of congestive heart failure and atrial fibrillation. In two large historic cohort studies using the United Renal Data System Registry database, Abbott et al. [18] and Lentine et al. [17] demonstrated that a BMI $\geq$ 30 predicted a 43–59% relative risk in the increase of congestive heart failure compared to a BMI of < 30. In the post-transplant setting, excessive weight gain or obesity may become a problem for many patients. Patients on prednisone therapy may overeat as they often experience constant hunger or craving for sweets. In addition, the release from pre-transplant dietary restriction and habitual physical inactivity can result in rapid post-transplant weight gain. Management of post-transplant obesity includes lifestyle and dietary modifications. Steroid reduction or withdrawal must be balanced against the risk of allograft rejection and graft loss. The use of pharmacological agents for weight reduction in the post-transplant period is currently not recommended due to the unknown potential drug to drug interactions.

When studying the effects of the *immunosuppressive agents* tacrolimus and cyclosporine-A on glucose and lipid metabolisms, the development of diabetes has been found to be significantly more frequent in those taking tacrolimus (24% vs. 12%), whereas those taking cyclosporine-A developed dyslipidemia significantly more frequently (30% vs. 16%) [8,21]. In the PTDM group of Study I, patients taking tacrolimus developed diabetes more frequently (Tac vs. CsA: 26.8% vs. 8.6%). In a study by Vincenti et al., the incidence of diabetes has been 33.6% with Tac and 26% with CsA treatment [20], whereas Heisel et al. have reported that the incidence of NODM is 16.5% in case of tacrolimus and 9.8% in case of cyclosporine-A [21]. When comparing cyclosporine-A and tacrolimus therapies, the differences in

triglyceride and total cholesterol have been significant (p = 0.004 and p = 0.001, respectively), but no significant difference has been found in HDL and LDL values. Badiou et al. have had a similar result: triglyceride and total cholesterol levels are significantly higher in patients taking cyclosporine-A than in those receiving tacrolimus therapy (6.14±1.37 vs. 5.28±1.32 mmol/L and 28% vs. 8%, respectively) [8]. Mycophenolate mofetil and sirolimus have not affected the development of diabetes [22]. In the PTDM group of Study I, patients taking tacrolimus developed diabetes more frequently (Tac vs. CsA; 26.8% vs. 8.6%) [24,25,26,27]. We found a significant difference between combined CsA–S treatment and S-free CsA treatment in regard to the development of diabetes, whereas the difference was not significant between combined Tac–S treatment and S-free Tac treatment [28]. Greater steroid exposure was associated with the development of glucose metabolism abnormalities in the CsA-group but not in the tacrolimus-treated patients. It may suggest that steroid played a major role in the development of diabetes in the CsA-treated patients. With tacrolimus, a reduction in steroid dose did not appear to affect the glycemic status, probably because tacrolimus had an impact on insulin secretion [24].

Treatments of diabetes mellitus

Lifestyle modification fails to achieve adequate glycemic control, therefore, medical intervention is recommended. Orally administered agents can be used either alone or in combination with other oral agents or insulin. Although oral hypoglycemic agents may be effective in many patients with corticosteroids or CsA- or Tac-induced NODM, insulin therapy may ultimately be necessary in up to 25% of patients, particularly in the early post-transplant period.

Metformin is the preferred agent for overweight patients; its use should be avoided with impaired allograft function due to the possibility of lactic acidosis. Care should also be taken when sulfonylurea derivatives are prescribed to patients with impaired allograft function or to elderly patients due to increased risk of hypoglycemia. "Non-sulfonylureatic" meglitinides are insulin secretagogues with a mechanism of action similar to that of sulfonylureas. Nonetheless, they have a more rapid onset and shorter duration of action and seemingly lower risks for hypoglycemia and lower amount of weight gain [29,30]. Thiazolidinedione derivatives are insulin sensitizers that may allow for a reduction in insulin requirement. The incidence of peripheral edema in increased when thiazolidinedione derivatives are used in

combination with insulin [28]. When added to sulfonylureas, thiazolidinedione derivatives and/or metformin, it results in additional lowering of HbA_{1C} by approximately 0.5–1%.

Treatments of the dyslipidemia

Besides the well-established efficacy and safety of the use of statins in transplant recipients, clinicians should remain vigilant to the potential drug to drug interactions in transplant patients who often require multiple medications. The use of statins in the presence of calcineurin inhibitors, particularly cyclosporine-A, often results in a severalfold increase in statin blood level and an increased risk for myopathy and rhabdomyolysis [29]. In addition to their lipid lowering effect, statins may offer protection against CVD via their antiproliferative and anti-inflammatory properties and ability to reduce circulating endothelin-1, C-reactive protein levels, systolic and diastolic blood pressure and pulse. Other classes of lipid lowering agents include fibric acid derivatives, nicotinic acid and ezetimibe [30] Ezetimibe and statin combination therapy can significantly improve cholesterol control due to their complementary mechanisms of action [31]. No significant drug to drug interaction between ezetimibe and calcineurin inhibitor has been reported.

Functional changes

One year after transplantation, no significant difference could be shown between diabetic patients and normal patients in regard to their serum creatinine and eGFR levels. The difference in regard to the allograft function was significant only between the NODM+NODL group and the N group, which proves that if both conditions are present, it may lead to the functional impairment of the graft even one year after transplantation. When comparing the first and the fifth post-transplantation years, we found a significant difference between the two groups (N-PTDM) in the serum creatinine but not in the $eGFR_{CG}$ and $eGFR_{MDRD}$ values [19].

We reached a different result in regard to the *histopathological changes* of the kidney. In the one-year protocol biopsies, we observed that the difference between the NODL and the N groups was significant only in regard to IF/TA, whereas both IF/TA and ACR differed significantly in the NODM and the NODM+NODL groups, when compared to the normal

group [30,31,32,33,34]. In the study of Arif et al., diabetic and normal patients have differed significantly in IF/TA ($p < 0.001$) [35].

Our own clinical study proved that one year after transplantation the allograft function had already been impaired if both medical conditions (NODM and NODL) were present; however, in regard to morphology, a single condition (NODM or NODL) was sufficient to lead to histological changes in the kidney. One year after kidney transplantation diabetic nephropathy (especially mesangial matrix increase and arteriola hyalinosis) did not develop, however, permanent hyperglycemia might result in morphological changes of the allograft. In IF/TA, hyperglycemia led to fibrogenesis by decreasing the number of functioning nephrons. It may be confirmed that the disruption of glucose and lipid homeostases severely damages the allograft, and this, without timely recognition and treatment, may not only lead to irreversible damages in the allograft, but it may increase the cardiovascular risks as well.

The cardiovascular risk of kidney transplant patients may be decreased, and the long-term survival of the graft may be increased by the timely recognition and treatment of diabetes and dyslipidemia. Thorough risk assessment should have an important role in choosing the immunosuppressive therapy. If the risk of diabetes is already high before the transplantation, we should avoid the use of tacrolimus, whereas in the case of dyslipidemia, cyclosporine-A should not be given to the patient. It is important to check the glucose and lipid metabolisms regularly by measuring the fasting blood glucose level, the level of $HbA_{1C,}$ total cholesterol. triglycerid, HDL and LDL every three months, because it allows for timely treatment, if needed. Aggressive treatment has been shown to reduce the risk for developing diabetic and dyslipidemic complications, it is recommended to quit smoking, decrease alcohol consumption, increase physical activity and reach the ideal body weight. Later, in case of worsening glucose metabolism, a reduction in the dose of the calcineurin inhibitor and even a switch to a calcineurin inhibitor-free combination may be considered. The success of treatment of diabetes and dyslipidemia is enhanced by frequent contact between patients and their physicians. If the target values cannot be achieved, blood glucose and lipid levels should be set with the help of a consultant internist.

Protocol biopsy may make it possible to recognize the harmful effects of NODM and NODL on the graft earlier; thus, with the timely initiation of treatment, the graft may be preserved in

the long term, and the risks of diabetes may be decreased. With the help of the protocol biopsy, an individually tailored immunosuppressive therapy can be set up for each patient.

The immunosuppressive therapy and the intensity of care provided to transplant recipients should be tailored to their needs, but in general, it is recommended that after a gradual reduction in the frequency of visits from 2 times per month during the fourth month to 1 occassion per month in the 6th month, this monthly schedule should be maintained until the end of the first year. In the next year, visits should be organized every 1 to 2 months and thereafter every 3 to 4 months as long as the transplant in functioning. Follow-up can take place at the clinic of the transplantation center, with a community nephrologist and a diabetologist experienced in the care of transplant recipients.

Considering the aforementioned findings, we might not only be able to preserve allograft function but also increase the survival of the patients.

5   SUMMERY AND NEW FINDINGS

➢ **Our clinical study proved that one year after transplantation the allograft function had already been impaired if both medical conditions (NODM & NODL) were present; however, in regard to morphology, a single condition (NODM or NODL) was sufficient to lead to histological changes in the kidney.**

➢ The prevalence of diabetes mellitus was 17%, whereas that of dyslipidemia was 22% one year after transplantation in our study. The development of diabetes and dyslipidemia was significantly influenced by the age and body mass index of the recipient and the immunosuppressant therapy.

➢ The prevalence of diabetes was significantly increased in patients taking tacrolimus, and that of dyslipidemia was significantly increased in patients taking cyclosporine-A. The triglyceride and total cholesterol levels of patients taking cycylosporine-A and tacrolimus were significantly different.

➢ One year after kidney transplantation, measuring the function of the allografts did not reveal any difference between patients having diabetes or dyslipidemia and patients with normal glucose and lipid metabolisms. However, this difference was significant if a patient had both factors (diabetes and dyslipidemia).

➢ Analyzing glucose metabolism, serum creatinine level was significantly different in the post-transplantation diabetes mellitus group one and five years after kidney transplantation.

➢ Evaluating the morphology of the allograft showed that one year after transplantation, there were significant differences in interstitial fibrosis/tubular atrophy and acute cellular rejection in normal patients vs. diabetic patients and normal patients vs. patients having diabetes&dyslipidemia.

6 ACKNOWLEDGEMENTS

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