ASSESSMENT OF NEURONAL DYSFUNCTION IN YOUNG PATIENTS WITH TYPE 1 DIABETES MELLITUS AND IN PATIENTS AFTER KIDNEY TRANSPLANTATION

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PhD Thesis

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Relevant publications

Full papers

I**. Vágvölgyi A,** Maróti Á, Szűcs M, Póczik C, Urbán-Pap D, Baczkó I, Nemes A, Csajbók É, Sepp K, Kempler P, Orosz A, Várkonyi T, Lengyel C. Peripheral and autonomic neuropathy status of young patients with type 1 diabetes mellitus at the time of transition from pediatric care to adult-oriented diabetes care. FRONTIERS IN ENDOCRINOLOGY 27;12:719953. DOI: 10.3389/fendo.2021.719953 **impact factor: 6.055** (2021)

II. **Vágvölgyi A,** Borda B, Orosz A, Szűcs M, Nemes A, Lázár G, Baczkó I, Kempler P, Várkonyi T, Lengyel C. Peripheral sensory and cardiovascular autonomic dysfunction in kidney transplant patients. DIABETES STOFFWECHSEL UND HERZ 31 : 3 pp. 42-50. , 9 p. **impact factor 0.18** (2022)

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Other publications

Kempler P, Gaál Z, Hermányi Z, **Vágvölgyi A,** Ságvári B, Kun E, Lengyel C. Mikor ellenőrzik betegeink hypoglykaemiás epizód lezajlását követően ismételten vércukorértéküket? METABOLIZMUS 17: pp. 1-7., 7 p. (2020)

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Abbreviations

ACE – angiotensin-converting enzyme

AN – autonomic neuropathy

ARB – angiotensin-receptor blocker

ASA – acetylsalicylic acid

Ao – aortic diameter

BMI – body mass index

BP – blood pressure

Ca – calcium

CAN – cardiovascular autonomic neuropathy

CGMS – continuous glucose monitoring system

CKD - chronic kidney disease

CPT – current perception threshold

CRT – cardiovascular reflex test

DCCT – Diabetes Care and Complications Trial

DPN – diabetic peripheral neuropathy

ECG – electrocardiogram

EDIC – Epidemiology of Diabetes Interventions and Complications

EF – ejection fraction

eGFR – estimated glomerular filtration rate

GGT – gamma-glutamyl transferase

GOT – glutamic oxaloacetic transaminase

GPT – glutamate pyruvate transaminase

HbA1c – haemoglobin A1c, glycated haemoglobin

HCT – health care transition

HR – heart rate

HRRDB – the heart rate response to deep breathing

ISPAD – International Society for Pediatric and Adolescent Diabetes

IVSd – interventricular septum thickness at end-diastole

KTx – kidney transplantation

LA – left atrial diameter

LH – left hallux

LR – left radius

LV – left ventricle

LVEDD – left ventricular end-diastolic diameter

LVEDV – left ventricular end-diastolic volume

LVESD – left ventricular end-systolic diameter

LVESV – left ventricular end-systolic volume

MCV – mean corpuscular volume

MCHC – mean corpuscular hemoglobin concentration

mTOR – mammalian target of rapamycin

NM2000 – CPT value of the median nerve at a stimulating frequency of 2000 Hz

NM250 – CPT value of the median nerve at a stimulating frequency of 250 Hz

NM5 – CPT value of the median nerve at a stimulating frequency of 5 Hz

NP2000 – CPT value of the peroneal nerve at a stimulating frequency of 2000 Hz

NP250 – CPT value of the peroneal nerve at a stimulating frequency of 250 Hz

NP5 – CPT value of the peroneal nerve at a stimulating frequency of 5 Hz

PAI – platelet aggregation inhibitor

PTDM – post-transplant diabetes mellitus

PW –left ventricular posterior wall thickness at end-diastole

QTc – QT interval corrected for heart rate

QTd – QT dispersion

RH – right hallux

RR – right radius

SBPRSU – the systolic blood pressure response from lying to standing up

SE – standard error

STV – beat-to-beat short-term temporal variability

STV_{QT} – beat-to-beat short-term temporal variability of the QT interval

STV_{RR} – beat-to beat short-term temporal variability of the RR interval

TIR – Time-In-Range

Tpeak-Tend – duration of the T wave from the peak to the end

T1DM – type 1 diabetes mellitus

T2DM – type 2 diabetes mellitus

VR – Valsalva ratio, heart rate response to Valsalva manoeuver

30/15 ratio – heart rate response to standing up

1.   Introduction and aims of the studies

Neuropathy is one of the most detrimental and diversified neurological conditions that upsets several physiological processes. It considerably impairs patients' quality of life and it is also associated with increased morbidity and mortality (1). Neuropathy is usually not an independent disease, but a symptom or group of symptoms associated with other diseases. The number of diseases leading to the development of nerve damage is several hundreds, and the majority of them are rare diseases. The diseases that can lead to the development of neuropathy include metabolic disorders, chronic alcoholic and non-alcoholic liver, chronic kidney diseases, haematological pathologies, exogenous intoxications, infections, systemic diseases, polyneuropathies arising from allergic reactions, and diseases with a genetic background. Regarding its prognostic and clinical significance, diabetic neuropathy must be highlighted (2). Cardiovascular autonomic neuropathy and peripheral sensory neuropathy were investigated in two delicate patient groups: one group at a designated time-period of their disease and the other group with multiple risk factors.

The first special patient group we examined was a young type 1 diabetic population at the time of transition from paediatric care to adult-oriented health care system. These patients are transferred to our clinic for further adult care from the age of 18, usually with a prominent exposure time to diabetes. This considerable number of years spent with diabetes itself justifies attention paid to assessing the neuropathic status of this unique population. The transition from adolescence to adulthood is a challenging time of physical, psychological, and social change. Young people with any form of disability, chronic disease or significant mental health problems face even greater challenges, since they also have to deal with important changes in the care they need and the way it is provided (3). During this sensitive period, at the time of transition patients move from childhood to adolescence and then to adulthood, while the composition of the care team is changing at the same time. The medical care of diabetic children and adolescents is complex and requires a great amount of financial resources, as well as regular access to specialised health services to prevent diabetes-related complications. The transition from paediatric to adult care adds further complexity to this management (4). Young adults are at high risk of dropping out of medical care, only to resurface in the medical system with diabetes-related complications (5-8). The age between 18 and 30 is a period of life in which individuals define their independence and personal identity and make important educational and professional decisions (9). For young diabetic patients this phase is even more complicated by the inflexibility of the continuous, day-to-day management of a chronic disease (4). Besides

the classic risk factors for neuropathy, such as age, height, hypertension, dyslipidaemia, obesity, duration of diabetes, hyperglycaemia, glycaemic variability, smoking, alcohol consumption, low vitamin D level (10), additional influencing factors can be assumed/ must be considered due to the specific nature of childhood and adolescent diabetes care. The assessment of neuropathic complications' presence in this multifactorial, constantly changing system is a challenging task. The prevalence of neuropathic lesions in young adult patients with type 1 diabetes mellitus (T1DM) at the time of transition from paediatric care to adult-oriented diabetes care is poorly studied. Comparative studies with healthy volunteers to assess the possible neuropathic condition of this special population and to identify the potential early screening needs have not been performed yet.

The prevalence of diabetes mellitus is very high among children and adolescents (11, 12), and type 1 diabetes mellitus accounts for more than 90% of all diabetes cases in childhood is (13). In Hungary, a nationwide, population-based database of children and adolescents was established between 2001 and 2016. The figures in this database highlight that the incidence of T1DM in Hungary has risen from 16/ 100,000 to 23/100,000 and the prevalence of T1DM from 114/ 100,000 to 209/100,000 with a male predominance (14). The complications of juvenile diabetes are similar to those of the maturity-onset type (e. g. retinopathy, diabetic kidney disease, hypertension, cardiovascular autonomic and peripheral sensory neuropathy) and they pose a significant challenge to health care systems (15, 16). The diagnosis of diabetic autonomic neuropathy can only be set up after every other condition has been excluded, but diabetic peripheral neuropathy has a well-defined diagnosis, which goes as follows: "symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvessel alterations as a result of chronic hyperglycaemia exposure (diabetes) and cardiovascular risk covariates" (17). Previous studies indicate that the prevalence of cardiovascular autonomic neuropathy (CAN) can be at least 20% in unselected type 1 and type 2 diabetic patients (18–20) but it is very low in newly diagnosed patients with T1DM (21–23). During the course of the disease, however, the prevalence of CAN increases significantly both in T1DM and in type 2 diabetes mellitus (T2DM). The Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study found at least 30% prevalence after 20 years of T1DM duration (24) and up to 60% after 15 years in T2DM (18, 23, 25, 26). CAN has been associated with an increased risk of cardiovascular morbidity and has also been proved to increase the relative risk of mortality 3.65 times (18, 20, 22). Even though clinical diabetic neuropathy is uncommon in the paediatric population, subclinical neuropathy (peripheral nerve function abnormalities without any clinical symptoms)

may already be present in a relatively high proportion of adolescents (27–30).

Diabetic peripheral neuropathy (DPN) can affect both small and large nerve fibers, and DPN is associated with neuropathic pain, foot ulceration, gangrene (31). According to some studies, the prevalence of DPN may be as high as 7–11% in young patients (8–21 years old) with T1DM (32, 33). Several risk factors of DPN have been identified in young T1DM population, namely poor glycaemic control, large glycaemic variability, older age, pubertal stage, longer diabetes duration, smoking, increased diastolic blood pressure, obesity, increased LDL cholesterol and triglyceride, as well as lower HDL cholesterol levels (33–40). However, genetic predisposition has also been suggested to play a role since children with good metabolic control and short diabetes duration have also been diagnosed with subclinical DPN (32, 36, 41). The transition of adolescents and young adults with chronic diseases from paediatric to adult health care is supposed to be a well-prepared and systematic process (42, 43), but lifelong diabetes management skills still pose significant challenges to young T1DM patients (34). If the transition is structured and professionally guided (planning, transfer assistance and integration), it can actually improve disease-specific measures, quality of life and self-care skills as proved by a recent review by Schmidt et al. (45). Several studies demonstrated an improvement in the glycaemic control of young T1DM patients following a structured transition program from paediatric to adult diabetes care (46– 49). On the other hand, the prevalence of neuropathic lesions has never been investigated in this population at the time of transition, so we set out to assess it with a view to define potential earlier screening needs. Apart from serving as important feedback to paediatric diabetes care, our results can also be used as a remarkable baseline reference point for further follow up in adult diabetes care.

In addition to diabetes, neuropathy can also be caused by a number of other conditions already mentioned, and it can also occur as a side effect of the medical therapies applied. The other target group studied by our neuropathy laboratory was a group of patients with multiple risk factors following kidney transplantation. Kidney transplantation (KTx) is the preferred treatment for virtually all suitable candidates with end-stage renal kidney failure. Chronic kidney disease (CKD) is common worldwide (50) and it is associated with an elevated risk of mortality due to cardiovascular disease (51). The risk of sudden cardiac death proportionally increases as kidney function deteriorates (52), which cannot be attributed to coronary artery disease-related risk factors alone (51, 53). Within the group of CKD patients, kidney transplantation patients constitute a special subpopulation. CKD may be accompanied by peripheral sensory neural impairment, cardiovascular autonomic dysfunction and cardiac repolarization abnormalities, all of which increase the risk of sudden cardiac arrest.

The high rate of cardiovascular mortality among CKD patients can be attributable to orthostatic and intradialytic hypotension, reduced heart rate variability or impaired spontaneous baroreflex sensitivity as well as cardiovascular autonomic dysfunction due to resistant hypertension, which are all complications of kidney dysfunction (54). The impaired baroreceptor, cardiopulmonary and chemoreceptor reflex function, activation of the renal afferents, accelerated renin-angiotensin-aldosterone system activity (54) and cardiovascular structural remodelling may also be held responsible for the high mortality rate in CKD (55). Krishnan and Kiernan reviewed (56) the potential neurologic dysfunctions induced by-uremic toxins such as urea, creatinine, parathyroid hormone, myoinositol and β2-microglobulin (57). As a result of these toxins, hydroelectrolytic changes in small nerve fibres elicit expansion or shrinkage of the endoneurial space, but further studies are needed to shed light on the exact pathogenesis of neuronal damage (56). Some studies have found a significant improvement in some neuropathic symptoms and electrophysiological indices in pancreas and kidney transplant patients, mostly in case of advanced neuropathy (58–60). The goal of our study was to broaden the knowledge we have of cardiovascular autonomic and sensory peripheral neuropathy, as well as cardiac repolarization abnormalities after KTx, and to compare the data we collected to that of age- and gender-matched healthy controls.

2. Study populations

2.1. Study population of the transition study

Young patients with type 1 diabetes mellitus were eligible for the study at the time of health care transition from the Department of Paediatrics and Paediatric Health Centre to the Department of Medicine at the University of Szeged, Hungary. The measurements were performed from September 2019 to February 2020 as the first neuropathic status assessment in adult care. The exclusion criteria included cases of pernicious anaemia, alcoholism, chronic hepatitis, uraemia, exposure to chemical agents, nerve compression, and chemotherapy treatment.

We studied 29 young patients with T1DM [mean ± SD; age: 22.4 ± 2.9 years; body mass index (BMI): 22.8 ± 3.0 kg/m2; haemoglobin A1c (HbA1c): 8.5 ± 2.1%, diabetes duration: 12.2 ± 5.8 years; 13 men/16 women]. A total of 30 age-matched healthy volunteers (age: 21.5 ± 1.6 years; BMI: 22.3 ± 3.7 kg/m2; HbA1c: 5.3 ± 0.3%; 12 men/18 women) were enrolled in the

study as controls. Relevant clinical data of T1DM patients and control subjects are presented in **Table 1.**

All T1DM patients and healthy controls successfully underwent the following examinations: 4 standard Ewing tests for evaluation of the cardiovascular autonomic neuropathy and a complex peripheral neuronal testing with Neurometer®, Neuropad®-test, Tiptherm®, Monofilament® and Rydel-Seiffer tuning fork. Neuropathic complaints were assessed with a questionnaire.

Table 1. Clinical data of T1DM patients and control subjects.

	T1DM patients (n=29)	Controls (n=30)	p value
Duration of diabetes (years)	12.2 ± 5.8		
Age (year)	22.4 ± 2.9	21.5 ± 1.6	0.115
Weight (kg)	66 ± 13	68 ± 14	0.622
Height (cm)	170 ± 11	174 ± 9	0.113
BMI (kg/m^2)	22.8 ± 3.0	22.3 ± 3.7	0.58
Waist-to-hip ratio	0.79 ± 0.07	0.80 ± 0.14	0.891
Male sex (%)	13 (44.8)	12 (40.0)	0.795
Systolic BP (mmHg)	127 ± 25	121 ± 13	0.253
Diastolic BP (mmHg)	80 ± 9	74 ± 8	*0.003*
Hypertension (%)	6 (20.7)	0 (0.0)	*0.011*
Hypercholesterolemia (%)	2 (6.9)	0 (0.0)	0.237
History of smoking (%)	9 (31)	4 (13.3)	0.125
Alcohol consumption (%)	8 (27.6)	13 (43.3)	0.279

The data are presented as mean ± SD. BMI, Body mass index; BP, blood pressure.

2.2 Study population of the kidney transplant study

The transplant study comprised 23 KTx patients (mean ± SD;-age: 50.4 ± 6.46 years, 11 men/12 women) who have regular care at the Department of Surgery, University of Szeged, Hungary. Inclusion criteria for the KTx patients were: first cadaver kidney transplantation, age of men: 18-55, age of women: 18-60. The mean time that elapsed since the transplantation in the KTx group was 8.55 years. The control group consisted of 19 age- and gender-matched healthy volunteers (age: 49.3 ± 7.31 years, 9 men/10 women; **Table 2.**).

Table 2. Clinical data of KTx patients and control subjects.

	KTx patients (n=23)	Controls (n=19)	p value
Age (year)	50.4 ± 6.5	49.3 ± 7.3	0.633
Weight (kg)	79.5 ± 14.0	78.8 ± 18.8	0.913
Height (cm)	170.5 ± 9.7	173.1 ± 11.0	0.258
Body mass index (kg/m^2)	27.4 ± 4.2	26.1 ± 4.7	0.428
Male sex, n (%)	11 (48)	9 (47)	0.977
Systolic BP (mmHg)	144.4 ± 16.7	133.3 ± 19.1	0.095
Diastolic BP (mmHg)	85.0 ± 9.1	82.2 ± 11.3	0.519
History of smoking, n (%)	9 (39)	2 (11)	*0.030*
Alcohol consumption, n (%)	1 (4)	4 (21)	0.126
Hypertension, n (%)	19 (83)	4 (21)	*<0.001*
Hypercholesterolaemia, n (%)	7 (30)	3 (16)	0.268
Diabetes mellitus, n (%)	8 (35)	0 (0)	*0.002*
β-blocker, n (%)	9 (39)	2 (11)	*0.030*
ACE inhibitor or ARB, n (%)	14 (61)	2 (11)	*0.0003*
Ca-antagonist, n (%)	11 (48)	1 (5)	*0.001*
Antidiabetics but not insulin, n (%)	4 (17)	0 (0)	*0.043*
Insulin, n (%)	2 (9)	0 (0)	0.162
Statin, n (%)	4 (17)	2 (11)	0.530
Diuretics, n (%)	9 (39)	0 (0)	*0.001*
ASA/PAI, n (%)	4 (17)	3 (16)	0.893
Steroid, n (%)	13 (57)	0 (0)	*<0.001*
Tacrolimus, n (%)	20 (87)	0 (0)	*<0.001*
Everolimus, n (%)	2 (9)	0 (0)	0.162
Cyclosporine, n (%)	2 (9)	0 (0)	0.162
Mycophenolate mofetil, n (%)	18 (78)	0 (0)	*<0.001*

The data are presented as mean ± SD. BP, blood pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; Ca, calcium; ASA, acetylsalicylic acid; PAI, platelet aggregation inhibitor.

3. Methods

3.1 12-lead electrocardiogram (ECG)

Both kidney transplant patients and controls were examined in the supine position after 5 minutes of rest. The 12-lead ECG was performed continuously for 10 minutes in the same position to avoid motion artefacts as much as possible. The ECG signal digitisation was performed with a multichannel data acquisition system (CAR03-IA, Cardiosys EXTRA software, MDE Heidelberg GmbH, Heidelberg, Germany), the sampling rate was 2,000 Hz and the data were stored for later analysis.

All data acquired from with kidney transplant patients (KTx) and control subjects were appropriate for analysis, no data were excluded on the basis of exclusion criteria, which are the following: several ($>5\%$) ectopic atrial or ventricular beats, non-sinus rhythm, abnormal repolarization (i.e. early repolarization, T-wave inversion, complete bundle branch block), permanent pacemaker activity, acute metabolic disorder, significant artefacts in the ECG signal recording, high amount of food intake during the last 3 hours, alcohol or caffeine consumption or smoking during the last 10 hours.

Repolarization was analysed based on the following parameters: frequency corrected QT interval (QTc) using Bazett ($QTc = QT/\sqrt{RR}$), Fridericia ($QTc = QT/[RR/1{,}000]1/3$), Framingham ($QTc = QT + [0.154 \times \{1{,}000 - RR\}]$) and Hodges formulae ($QTc = QT + 1.75 \times [60{,}000/RR - 60]$), QT dispersion (QTd), terminal T-wave duration ($T_{peak} - T_{end}$), frequency corrected T_{peak}-T_{end} interval using Bazett and Fridericia formulae and short-term variability of QT intervals (STV_{QT}).

The RR and QT intervals, as well as the duration of the T wave from the peak to the end ($T_{peak} - T_{end}$) intervals were measured semi-automatically from 30 consecutive beats (minimum number of intervals needed for variability measurements) and were calculated as the average of 30 beats. Conventional computerised QT measurement technique was used for the analysis of QT intervals; blinded QT interval checking and if necessary, manual repositioning of the automatically set fiducial cursor were performed by the same investigator of the team (61). The duration of QTc interval was determined as the average of the measured QTc intervals. PQ and QRS intervals were determined as the average of the measured intervals of 15 consecutive beats. The measurements were performed in lead II or lead V5 if significant noise was present in the former.

Poincaré plot analysis of the QT and RR intervals was performed to determine the temporal instability of the beat-to-beat heart rate (HR) and repolarization. Each QT and RR value was plotted against its former value. STV_{QT} and STV_{RR} were calculated using the following formula: $STV = \Sigma|Dn+1 - Dn| (30x\sqrt{2})^{-1}$, where D represents the duration of the QT and RR intervals. The calculation defines the STV as the mean distance of points perpendicular to the line of identity in the Poincaré plot and relies on previous mathematical analysis (62).

3.2 Cardiovascular autonomic function testing

Autonomic neuropathy (AN), neuronal dysfunction and consequent cardiovascular changes were characterized with Ewing's five standard cardiovascular reflex tests (CRT) (63) in the kidney transplant study. Meanwhile, Körei et al. (64) confirmed that the handgrip test should no longer be part of cardiovascular autonomic testing being highly dependent on hypertensive status and baseline diastolic blood pressure. Based on this finding the handgrip test was not assessed in the study of the young T1DM patients at the time of transition. The Ewing-tests are the gold standards of the determination of autonomic dysfunction; they provide non-invasive, clinically relevant, standardised and reproducible data of autonomic functions. Reflex tests were performed by measuring the blood pressure and obtaining continuous 6-lead ECG signals. Then the signals were digitised with a multichannel data acquisition system (Cardiosys-A01 software, MDE Heidelberg GMBH, Heidelberg, Germany), the sampling rate was 2 kHz and the data were stored for later analysis.

Three of five tests record heart rate changes when performing specific activities, while the rest measures changes in blood pressure. Tests, which record heart rate changes predominantly, reflect changes in parasympathetic function, while those based on blood pressure responses primarily describe sympathetic function disturbances (65). Heart rate changes were measured during deep inhalation and exhalation, in lying and standing positions with 30/15 ratio, and during and after Valsalva manoeuvre (66). Systolic blood pressure changes were measured after standing up from a lying position, while diastolic changes were recorded during gripping with the hand for 3 minutes. CRT-s were scored separately: 0 (normal), 1 (borderline), 2 (abnormal). The overall autonomic score was calculated from the sum of each test results to characterise the severity of AN.

3.2.1 Heart rate response to deep breathing (HRRDB)

Physiologically, the heart rate increases on inhalation and decreases on exhalation. Patients were instructed to take deep breaths at a rate of six breaths per minute (inhale for five seconds and exhale for five seconds). The difference between the measured maximum and minimum heart rates (beats/min) was calculated during six cycles of breathing.

3.2.2 Heart rate response to standing up (30/15 ratio)

Normally, the heart rate increases promptly after standing up from a lying position and at about the 15th heartbeat after standing up, it reaches a peak. After that, relative bradycardia presents in healthy individuals with the lowest rate at around the 30th beat. Patients were lying supine at the beginning of the test, then they were asked to stand up while the ECG was recorded continuously. The ratio of the longest R-R interval (around beat 30th) and the shortest R-R interval (around beat 15th) was calculated, and recorded as the 30/15 ratio.

3.2.3 Heart rate response to the Valsalva manoeuvre (Valsalva-ratio [VR])

In healthy individuals, the blood pressure decreases and the heart rate increases during the holding period of Valsalva manoeuvre. After the manoeuvre, the blood pressure increases and the heart rate decreases. Subjects were asked to exhale into a specific manometer through a mouthpiece and hold their breath at 40 mmHg for 15 seconds. During that, ECG was continuously recorded. The ratio of the longest R-R interval following the test and the shortest R-R interval during the manoeuvre was calculated and recorded as the Valsalva-ratio.

3.2.4 Systolic blood pressure response to positional change (from lying to standing up, SBPRSU)

Normally, in a standing position, the redistribution of blood to the lower limbs is immediately compensated by vasoconstriction in the peripheral vessels. Marked orthostatic hypotension is an important feature of cardiovascular consequences of neuropathy. The test is performed by measuring the blood pressure in a lying position and after standing up. An orthostatic drop in blood pressure is determined with systolic blood pressure measurements. 10 minutes after lying supine and at 1, 5 and 10 minutes after standing up. The difference between the values measured is noted and the largest difference is recorded as the response to standing up.

3.2.5   Diastolic blood pressure response during sustained handgrip

Changes in diastolic blood pressure were measured during sustained handgrip. At first, patients were asked to clamp a hand-held dynamometer with their dominant hand exerting full force so that we could determine the maximal grasping force, and then they were instructed to maintain the grasp for 3 minutes at a constant, 30% force level. Blood pressure was measured once every minute on the contralateral, relaxed upper limb and the maximally increased diastolic blood pressure was recorded as the response to sustained handgrip.

3.3   Sensory nerve testing

3.3.1   Neurometer®

Sensory function of the peripheral nerves was examined with a Neurometer device (NM-01/CPT Neurometer, MDE Heidelberg GmbH, Heidelberg, Germany). The equipment allows for non-invasive, simple testing and provides a possibility for the quantitative analysis of sensory nerve function in different types of nerve fibres (67). Transcutaneous, low voltage, sine-wave electrical stimulation was applied and the current perception threshold (CPT) was determined. This study tested the median and the peroneal nerves. The 1 cm diameter surface electrodes were positioned on the distal phalanx of the index finger and that of the hallux. The electrodes were fastened to intact skin surfaces to avoid peripheral sensory disturbance caused by scars and wounds. The amplitude range of the applied stimuli was 0.01 to 9.99 mA. At the beginning of the test, current intensity was gradually increased until the patient indicated sensation. Then short (2-5 sec) stimulations were applied at progressively lower intensities until the minimal intensity of consistent sensation was determined. CPT intensities were determined at three different stimulation frequencies (2000 Hz, 250 Hz, 5 Hz) for both the upper and the lower limbs.

3.3.2   Neuropad®

Sudomotor dysfunction, which frequently occurs in autonomic neuropathy, was examined with Neuropad® screening tests for all patients and controls (68). The test can detect neuropathy with very high sensitivity (69) on the basis of the fact that nerve fibre impairment in the distal extremities not only affects sensation, but perspiration as well, thus extreme dryness of the feet may occur. The adhesive pad of the kit contains blue anhydrous cobalt II chloride

salt, which reacts and changes to pink when it absorbs water. The tests were performed at room temperature (23°C) following 10 minutes of rest after patients took off their shoes and socks. The pads were placed on the soles on both sides between the heads of the first and second metatarsi. The colour change was read at 10 minutes after adhesion. Total decolouration to pink was considered normal, a mixed pink and blue colour was evaluated as pending, while a total blue colour was deemed pathological.

3.3.3 128-Hz Rydel-Seiffer graduated tuning fork

The 128-Hz Rydel-Seiffer graduated tuning fork was used to evaluate the sense of vibration at the distal end of the radius and at the level of the halluces. Results of the tuning fork examination were compared to age-dependent normal values published by Martina et al. in 1998 (70). On a scale of 1-8, the normal range was 7-8, borderline was 6 and pathological was 1-5, implying an impaired sense of vibration.

3.3.4 Semmes-Weinstein Monofilament Test®

The Semmes-Weinstein Monofilament Test® using a 10 g monofilament is a simple method for the objective screening of a diabetic foot for protective sensation loss (71). The test was performed under calm and quiet circumstances, and the tested individuals were blinded for the place and way of application of the filament. Five regions of the sole were examined in all candidates: hallux, first metatarsus, second metatarsus, heads of the third and the fifth metatarsus. Unaffected sensation in at least 4 regions was considered normal, while 0-3 was defined as pathological.

3.3.5 Tiptherm®

The Tiptherm® (Tip-Therm GmbH, Düsseldorf, FRG) device can be used for the early diagnosis of polyneuropathy of a symmetrical pattern. It is a pen-shaped instrument with flat sides, which tests temperature sensitivity of the skin (72). It contains a 14 mm diameter plastic cylinder and a 14 mm diameter metal cylinder on each end separately. The examiner touches the skin of the patient randomly with one end for 1 second on both hands and feet. The patient has to report which touch was colder (73). In case of normal temperature sensation (<10°C), the individual can differentiate between the two subjective sensations elicited by the flat surfaces of the Tiptherm®, while subjects with impaired temperature sensation cannot distinguish between the two ends.

3.4 Questionnaire for the evaluation of neuropathic complaints

Neuropathic complaints were assessed with a questionnaire. Every individual had to make a statement about the presence or absence of burning, pinprick sensations, numbness, tingling, hypoesthesia, hyperesthesia, and also about the intensity of these symptoms and frequency of their occurrence.

3.5 Laboratory data

Venous blood samples were collected from the subjects for the determination of glucose (mmol/l), haemoglobin A1c (HbA1c), white blood cell count (G/l), red blood cell count (T/l), haemoglobin (g/l) , haematocrit (%), MCV (fl), MCHC (g/l), thrombocyte (G/l), blood urea nitrogen (mmol/l), creatinine (μmol/l), eGFR (ml/min/1.73m^2), uric acid (μmol/l), C-reactive protein (mg/l), calcium and corrected calcium (mmol/l), phosphate (mmol/l), sodium (mmol/l), potassium (mmol/l), total protein (g/l), albumin (g/l), cholesterol (mmol/l), triglyceride (mmol/l), HDL-cholesterol (mmol/l), LDL-cholesterol (mmol/l), GOT (U/l), GPT (U/l), GGT (U/l), total bilirubin (μmol/l), direct/conjugated bilirubin (μmol/l), alkaline phosphatase (U/l). The following parameters were determined from urine: total protein, albumin, creatinine, nitrite, pH, protein, glucose, ketone body, urobilinogen, bilirubin, white blood cell, red blood cell and the urine sediment test. Due to logistical reasons, collection of blood and urine samples was not possible from all patients and controls.

3.6 Echocardiographic examination

Transthoracic echocardiography was performed in T1DM patients and their controls to determine standard morphological and functional parameters. The subjects underwent a complete two-dimensional (2D) transthoracic echocardiographic study using a Toshiba Artida imaging system (Toshiba Medical Systems, Tokyo, Japan) with a PST-30SBP phased-array transducer (1–5 MHz). The images were obtained in accordance with the recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging (74). In all cases, left ventricular (LV) dimensions, volumes and ejection fraction (EF), and left atrial (LA) dimensions were measured, and complete 2D Doppler studies were performed. For purely logistical reasons, not all T1DM patients and controls were subjected to echocardiography.

4. Statistical analysis

Statistical data were reported as the mean ± SD; with frequencies (n) and percentages (%), when appropriate. Pearson's chi-squared test or Fisher's exact test was used to analyse categorical data, whereas independent samples t-test was used in case of continuous data. The connections between the continuous or ordinal variables were examined by Pearson's and Spearman's correlation analysis. Power analysis for the transition study was performed using the software G* Power (Version 3.1.9.2) for power-and-sample size calculation (University of Düsseldorf, Germany). The calculated sample size was 28, working with an effect size d = 0.8, alpha as Type I error of 0.05, and a power value of 0.95. Statistical tests were performed using R statistical software (R version 3.6.0 in the transplant study, version 3.6.2 in the transition study, https://www.r-project.org/), values of p<0.05 were considered significant.

5. Ethics statement

Both studies were carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The transition study was approved by the Hungarian Medical Research Council (approval No. 31891-5/2019/EÜIG), the transplant study was approved by the institutional Human Research Ethical Committee (approval No. 128/2018-SZTE). All subjects have given written informed consent to the study.

6. Results

6.1 Results of the transition study

6.1.1 Clinical and laboratory data of young patients with type 1 diabetes mellitus and control subjects

The mean duration of type 1 diabetes was 12.2 ± 5.8 years. Age, weight, height, body mass index, and waist-to-hip ratio did not differ significantly between control subjects and young diabetic patients. The mean systolic blood pressure did not differ significantly between T1DM patients and control subjects; however, T1DM patients had higher diastolic blood pressure (80 ± 9 vs. 74 ± 8 mmHg; p = 0.003; **Figure 1**).
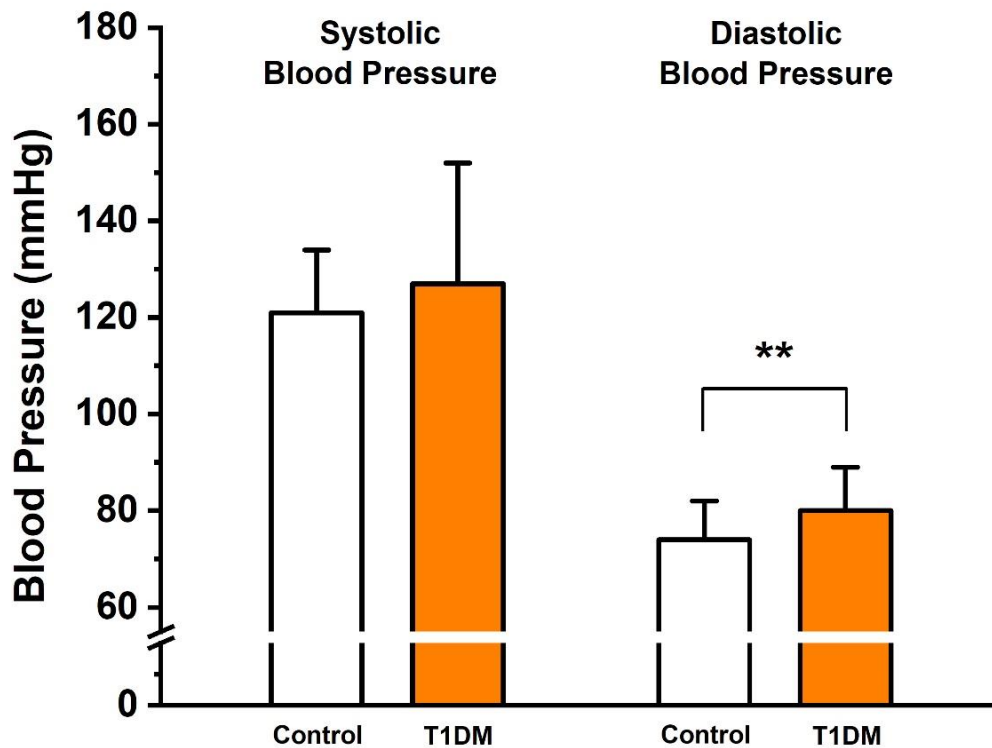
Figure 1. Mean systolic and diastolic blood pressure in the control group and in type 1 diabetes mellitus (T1DM) patients.
**p<0.01 vs. control group; n=30 and 29 individuals in the control and T1DM groups, respectively.

At the time of transition, 10 young T1DM patients received multiple injections and 19 T1DM patients were on insulin pump therapy. Only two of the T1DM individuals used a continuous glucose monitoring system (CGMS), both as augmentation of their insulin pump therapy. In terms of multiple injections or pump therapy, none of the T1DM patients' therapy has changed over the past year following their transition to adult-oriented diabetes care. However, since then nine more patients have started to use CGMS. Out of 29 T1DM patients, two had hypercholesterolaemia and six patients were receiving antihypertensive medications (two were taking beta-blockers, five patients were taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and three T1DM patients were taking Ca^{2+} channel blockers).

Regarding the laboratory parameters, the average serum glucose and HbA1c values were significantly higher in young patients with type 1 diabetes mellitus compared to healthy controls. The serum magnesium, albumin, and creatinine levels were significantly lower among

T1DM patients, and significantly higher serum alkaline phosphatase level and eGFR were detected in T1DM patients compared to controls. Other laboratory parameters (lipid, liver and blood test profile) did not show any significant differences (**Table 3**).

Two-dimensional echocardiography has not revealed any significant differences between the two groups. Relevant results are shown in **Table 4**.

Table 3. Laboratory data of T1DM patients and control subjects.

	T1DM patients	Controls	p value
Glucose (mmol/L)	8.4 ± 5.6 (n=18)	4.6 ± 0.6 (n=21)	***0.010***
HbA1c (%)	8.5 ± 2.1 (n=23)	5.3 ± 0.3 (n=21)	***< 0.001***
Blood urea nitrogen (mmol/L)	4.4 ± 1.2 (n=21)	4.3 ± 1.0 (n=22)	0.839
Corrected calcium (mmol/L)	2.3 ± 0.1 (n=13)	2.3 ± 0.1 (n=20)	0.373
Magnesium (mmol/L)	0.8 ± 0.1 (n=11)	0.9 ± 0.1 (n=19)	***0.031***
Sodium (mmol/L)	139.5 ± 2.2 (n=22)	140.1 ± 3.0 (n=21)	0.431
Potassium (mmol/L)	4.2 ± 0.3 (n=22)	4.3 ± 0.4 (n=21)	0.776
Blood urea nitrogen (mmol/L)	4.4 ± 1.2 (n=21)	4.3 ± 1.0 (n=22)	0.839
Creatinine (μmol/L)	70.7 ± 11.8 (n=21)	79.9 ± 16.6 (n=21)	***0.047***
eGFR (mL/min/1.73m^2)	113.9 ± 22.0 (n=21)	100.8 ± 17.8 (n=21)	***0.040***
Cholesterol (mmol/L)	4.7 ± 1.4 (n=22)	4.3 ± 0.9 (n=21)	0.284
Triglyceride (mmol/L)	1.3 ± 1.1 (n=22)	1.0 ± 0.5 (n=21)	0.323
HDL-cholesterol (mmol/L)	1.6 ± 0.3 (n=21)	1.7 ± 0.4 (n=21)	0.491
LDL-cholesterol (mmol/L)	2.2 ± 0.6 (n=17)	2.2 ± 0.7 (n=21)	0.876
Total protein (g/l)	74.1 ± 4.4 (n=14)	73.9 ± 8.5 (n=21)	0.93
Albumin (g/l)	47.7 ± 5.1 (n=19)	51.4 ± 3.3 (n=21)	***0.012***
Alkaline phosphatase (U/L)	97.8 ± 44.5 (n=21)	63.8 ± 13.8 (n=22)	***0.003***
Urine pH	6.11 ± 0.8 (n=13)	6.3 ± 0.8 (n=21)	0.493

The data are presented as mean ± SD. HbA1c, haemoglobin A1c, eGFR, estimated glomerular filtration rate.

Table 4. Echocardiographic parameters of T1DM patients and control subjects.

	T1DM patients (n=24)	Controls (n=15)	p value
Ao (mm)	27.2 ± 3.0	27.2 ± 2.6	0.978
LA (mm)	36.1 ± 4.4	36.7 ± 4.3	0.672
LVEDD (mm)	44.9 ± 4.8	46.7 ± 4.0	0.217
LVESD (mm)	27.9 ± 2.7	28.9 ± 3.5	0.347
IVSd (mm)	8.2 ± 1.0	8.7 ± 0.9	0.099
PW (mm)	8.3 ± 1.1	8.6 ± 0.8	0.261
LVEDV (ml)	100.0 ± 15.5	101.7 ± 23.2	0.800
LVESV (ml)	31.3 ± 6.1	33.1 ± 12.0	0.592
EF (%)	69.4 ± 3.3	68.5 ± 4.3	0.491

The data are presented as mean ± SD. Ao, aortic diameter, LA, left atrial diameter, LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVSd, interventricular septum thickness at end-diastole; PW, left ventricular posterior wall thickness at end-diastole, LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction.

6.1.2 Cardiovascular autonomic function of young patients with type 1 diabetes mellitus and control subjects

Standard cardiovascular reflex tests did not indicate any significant deteriorations in heart rate responses to deep breathing (HRRDB), standing up (30/15 ratio) and the Valsalva manoeuvre (Valsalva ratio, VR) or in the systolic blood pressure response to positional change (from lying to standing up, SBPRSU) in young T1DM subjects compared to controls (**Table 5**).

Table 5. Results of cardiovascular autonomic function tests in T1DM patients and controls.

	T1DM patients (n=29)	Controls (n=30)	p value
HRRDB (1/min)	32 ± 11	32 ± 9	0.877
30/15 ratio	1.2 ± 0.3	1.1 ± 0.2	0.171
VR	2.2 ± 0.4	2.3 ± 0.3	0.149
SBPRSU (mmHg)	5 ± 6	3 ± 4	0.272

The data are presented as mean ± SD. HRRDB, heart rate response to deep breathing; 30/15 ratio, heart rate response to standing up; VR, Valsalva-ratio, heart rate response to the Valsalva manoeuvre; SBPRSU, systolic blood pressure response to positional change (from lying to standing up).
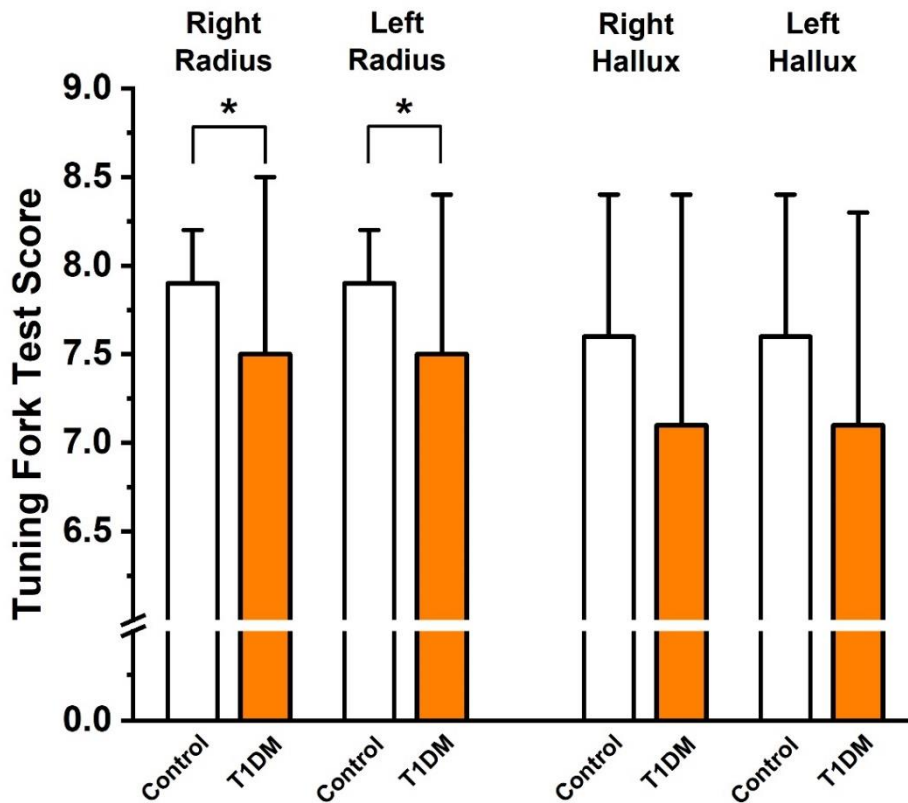
6.1.3 Peripheral sensory function in young T1DM patients and control subjects

No significant differences were detected with Neurometer® (**Table 6**), Neuropad®-test and Semmes-Weinstein Monofilament Test® between the two groups. The vibrational sense on the hallux was intact; however, on the radius on both sides, the vibrational sensing was significantly impaired in the T1DM group compared to the controls with 128 Hz Rydel-Seiffer graduated tuning fork test (**Figure 2**). The Tiptherm®-test also identified significant temperature sensitivity impairment in T1DM patients (11 sensing failures vs. 1, $p < 0.001$). In addition, neuropathic complaints were significantly more frequent present in the T1DM patient group than in the controls (9 vs. 0, $p < 0.01$).

Table 6. Peripheral sensory function testing with Neurometer® assessing current sensation threshold at the median and peroneal nerves at three different stimulating frequencies (2000 Hz, 250 Hz, 5 Hz).

	T1DM patients (n=29)	Controls (n=30)	p value
NM2000	188 ± 93	166 ± 86	0.353
NM250	85 ± 78	56 ± 38	0.078
NM5	50 ± 53	34 ± 28	0.154
NP2000	266 ± 122	270 ± 102	0.898
NP250	158 ± 104	121 ± 67	0.105
NP5	95 ± 76	84 ± 45	0.525

The data are presented as mean ± SD. NM2000, current perception threshold (CPT) value of the median nerve at a stimulating frequency of 2000 Hz; NM250, CPT value of the median nerve at a stimulating frequency of 250 H; NM5, CPT value of the median nerve at a stimulating frequency of 5 Hz; NP2000, CPT value of the peroneal nerve at a stimulating frequency of 2000 Hz; NP250, CPT value of the peroneal nerve at a stimulating frequency of 250 Hz; NP5, CPT value of the peroneal nerve at a stimulating frequency of 5 Hz.

Figure 2. Peripheral sensory function testing using the 128-Hz Rydel-Seiffer graduated tuning fork on the distal end of the right (RR), left (LR) radius and the right (RH) and left (LH) hallux in the control group and in type 1 diabetes mellitus (T1DM) patients.
*p<0.05 vs. control group; n=30 and 29 individuals in the control and T1DM groups, respectively.

6.1.4 Correlations between studied parameters

The duration of diabetes (12.2 ± 5.8 years) did not correlate with the results of the cardiovascular reflex tests (HRRDB: r: −0.225, p = 0.242; 30/15 ratio: r: −0.099, p=0.610; VR: r: −0.138, p=0.475, SBPRSU: r: 0.128, p=0.507), the HbA1c level (r: 0.163, p=0.458), or the diastolic blood pressure in T1DM patients (r: 0.309, p=0.103). In addition, no correlation was found between DM duration and the results of the vibration testing performed by Rydel-Seiffer graduated tuning fork on the right (r: −0.158, p = 0.415) and left (r: −0.162, p = 0.403) radius. In the whole study population (diabetic and control subjects together), a significant correlation was found between the HbA1c level and the Valsalva ratio (n = 44, r: −0.483, p = 0.001) and diastolic blood pressure (n = 44, r: 0.352, p = 0.019). Furthermore, a borderline relationship was found between the results of the Tiptherm®-tests and the HbA1c level (normal Tiptherm®-tests: n = 35, HbA1c: 6.6 ± 1.9% vs. abnormal Tiptherm®-tests n = 8, HbA1c: 8.9 ± 2.7%, p = 0.051).

6.2 Results of the kidney transplant study
6.2.1 Clinical and laboratory data of KTx patients and control subjects

The frequency of smoking, hypertension and diabetes mellitus was higher in the KTx group compared to controls. The patients in the KTx group took more different kinds of drugs than the subjects in the control group (**Table 2**).

The analysis of the laboratory parameters of the KTx group revealed anaemia and hypalbuminaemia. The kidney function described with creatinine and urea was significantly impaired in KTx patients (**Table 7**). LDL-cholesterol was significantly lower in the KTx group compared to controls.

Table 7. Laboratory data of KTx patients and control subjects.

	KTx patients (n=23)	Controls (n=19)	p value
Haemoglobin (g/l)	129.7 ± 19.2	142.4 ± 12.7	*0.015*
Haematocrit (%)	39.2 ± 4.6	41.9 ± 3.5	*0.042*
Glucose (mmol/l)	5.7 ± 2.1	5.0 ± 0.5	0.136
Blood urea nitrogen (mmol/l)	11.4 ± 5.7	5.4 ± 2.4	*<0.001*
Creatinine (μmol/l)	162.2 ± 93.9	79.3 ± 17.3	*<0.001*
eGFR (ml/min/1.73m^2)	47.7 ± 22.4	89.4 ± 15.6	*<0.001*
Uric acid (μmol/l)	332.8 ± 73.6	270.9 ± 17.3	*0.015*
C-reactive protein (mg/l)	4.3 ± 5.2	0.90 ± 2.7	*0.012*
Albumin (g/l)	46.1 ± 3.3	49.8 ± 3.1	*<0.001*
Cholesterol (mmol/l)	5.2 ± 1.1	5.6 ± 0.8	0.123
Triglyceride (mmol/l)	2.0 ± 1.0	1.6 ± 1.1	0.215
HDL-cholesterol (mmol/l)	1.4 ± 0.3	1.6 ± 0.4	0.089
LDL-cholesterol (mmol/l)	2.8 ± 0.8	3.4 ± 0.70	*0.032*

The data are presented as mean ± SD. BP, blood pressure; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker; Ca, calcium; ASA, acetylsalicylic acid; PAI, platelet aggregation inhibitor; eGFR, estimated glomerular filtration rate.

6.2.2   Cardiovascular autonomic function in KTx patients and control subjects

Significant impairment was found in KTx patients' heart rate response to deep breathing, standing up, and systolic blood pressure response to standing up. The AN score was also significantly higher among KTx patients vs. controls (**Table 8**).

In the KTx group, the heart rate response to deep breathing showed a positive correlation with the eGFR (p=0.007, r=0.549). A significant negative correlation between the serum creatinine level and the Valsalva-ratio was detected with the separate KTx group analysis (p=0.04, r=-0.432). Time elapsed since transplantation correlated positively only with systolic blood pressure (p=0.024, r=0.468) in the KTx group.

Table 8. Autonomic neuropathy (AN) parameters of KTx patients and age-matched control subjects.

	KTx patients (n=23)	Controls (n=19)	p value
Heart rate (HR) variation during deep breathing (beats/min)	16.7 ± 5.9	21.21 ± 6.9	*0.031*
Valsalva ratio	1.6 ± 0.2	1.6 ± 0.3	0.338
30/15 ratio	1.1 ± 0.2	1.20 ± 0.2	*0.007*
Systolic BP fall after standing up (mmHg)	12.3 ± 13.7	4.6 ± 6.1	*0.022*
Diastolic BP increase after sustained handgrip (mmHg)	18.2 ± 8.5	17.1 ± 14.7	0.763
AN score	2.4 ± 1.6	1.4 ± 1.2	*0.020*

The data are presented as mean ± SD. 30/15 ratio, immediate HR response to standing; BP, blood pressure; AN, autonomic neuropathy.

6.2.3   Peripheral sensory function in KTx patients and control subjects

Significant differences were demonstrated in the peripheral sensory function of the peroneal and median nerves in all three frequencies tested with Neurometer®. At the median nerve, testing revealed increased thresholds in KTx patients versus controls at all tested frequencies. The peroneal nerve parameters were also significantly elevated in KTx patients.

With the application of Neuropad®-test, Tiptherm®, Monofilament® and Rydel-Seiffer tuning folk no further significant differences were identified (**Table 9**).

A significant positive correlation (p=0.025, r=0.46) was found between plasma glucose and the CPT value of the peroneal nerve at a stimulating frequency of 5 Hz in KTx patients. There was no correlation between the plasma glucose and the remaining measured parameters.

Table 9. CPT values of the upper and lower limbs at three different stimulating frequencies (2000 Hz, 250 Hz, 5 Hz) in KTx patients and age-matched control subjects.

	KTx patients **(n=23)**	**Controls** **(n=19)**	**p value**
NM2000	278.3 ± 84.4	157.8 ± 61.5	***<0.001***
NM250	112.2 ± 91.1	48.0 ± 42.6	***0.005***
NM5	78.0 ± 71.2	29.0 ± 23.1	***0.004***
NP2000	453.4 ± 175.9	288.6 ± 98.2	***<0.001***
NP250	262.3 ± 168.9	156.8 ± 82.9	***0.013***
NP5	142.5 ± 129.4	82.0 ± 59.0	0.053

The data are presented as mean ± SD. NM2000, CPT value of the median nerve at a stimulating frequency of 2000 Hz; NM250, CPT value of the median nerve at a stimulating frequency of 250 Hz; NM5, CPT value of the median nerve at a stimulating frequency of 5 Hz; NP2000, CPT value of the peroneal nerve at a stimulating frequency of 2000 Hz; NP250, CPT value of the peroneal nerve at a stimulating frequency of 250 Hz; NP5, CPT value of the peroneal nerve at a stimulating frequency of 5 Hz.

6.2.4   ECG repolarisation parameters in KTx patients and control subjects

Comparison of the two groups (KTx patients vs. control) revealed no significant differences in heart rate, the PQ, QRS and QT intervals and the QT dispersion. Furthermore, significant differences in the QTc values calculated with the Bazett, Fridericia, Framingham and Hodges formulae and the beat-to-beat short-term temporal variability of the QT interval were not identified. However, a significant decrease in the beat-to beat short-term temporal variability of the RR interval (STV_{RR}) in KTx patients compared to healthy controls was detected. The duration of the T wave from the peak to the end (T_{peak}-T_{end}) was also significantly decreased in the KTx group compared to the control group. The frequency corrected T_{peak}-T_{end} intervals showed no significant differences between KTx patients and controls (**Table 10**).

Table 10. Electrocardiographic parameters in patients with KTx and age-matched control subjects.

	KTx Patients (n=23)	Controls (n=19)	p value
HR (1/min)	73 ± 10	68 ± 10	0.124
RR (ms)	844 ± 125	905 ± 129	0.127
PQ (ms)	158 ± 22	163 ± 26	0.501
QRS (ms)	100 ± 13	102 ± 12	0.738
QT (ms)	407 ± 39	416 ± 30	0.404
QTc (ms) Bazett	445 ± 26	439 ± 17	0.390
QTc (ms) Fridericia	432 ± 27	431 ± 17	0.929
QTc (ms) Framingham	432 ± 27	431 ± 16	0.935
QT (ms) Hodges	430 ± 27	430 ± 17	0.969
QTd (ms)	42 ± 12	42 ± 8	0.812
T$_{peak}$-T$_{end}$ (ms)	93 ± 16	102 ± 11	***0.032***
T$_{peak}$-T$_{end}$ (ms) Bazett	101 ± 17	108 ± 11	0.133
T$_{peak}$-T$_{end}$ (ms) Fridericia	99 ± 16	106 ± 11	0.075
STV$_{RR}$ (ms)	9.5 ± 8.9	15.1 ± 8.6	***0.044***
STV$_{QT}$ (ms)	4.5 ± 0.9	4.1 ± 1.1	0.321

Values are presented as mean ± SD. HR, heart rate; QTc, frequency corrected QT interval (calculated by the Bazett, Fridericia, Framingham and Hodges formulas); QTd, QT dispersion; T$_{peak}$-T$_{end}$, duration of the T wave from the peak to the end; STV$_{RR}$, beat-to-beat short-term temporal variability of the RR interval; STV$_{QT}$, beat-to-beat short-term temporal variability of the QT interval.

7. Discussion

7.1.1. Discussion of the transition study

Our study did not detect cardiovascular autonomic neuropathy or cardiac morphological disorders in this young T1DM population at the time of transition from paediatric care to adult-oriented health care system. Nevertheless, although both cardiovascular autonomic and cardiac conditions were physiological, we did find peripheral sensory neurological impairments with the 128 Hz Rydel-Seiffer graduated tuning fork test and the Tiptherm®-test. The young T1DM patient group also had more severe neuropathic complaints than the controls. The relatively low rate of diabetic peripheral neuropathy (DPN) in the young T1DM patient group suggests appropriate glycaemic control (31, 34, 38, 75, 76) and good paediatric diabetes care.

Based on the previous findings of Barkai et al. in 1998 (34), who detected some early symptoms and signs of peripheral sensory neuropathy in 23% of children with T1DM, our preliminary expectations exceeded the actual rate of DPN impairment in our T1DM group at the time of transition. In 1993, the DCCT (Diabetes Control Complications Trial) Research group reported that intensive insulin therapy delays the onset and slow the progression of DPN by 60% (77). Since then, the therapeutic approaches and modalities of diabetes (insulin pump therapy, continuous blood glucose monitoring, application of new generation analogue insulins) have improved considerably. In our T1DM population, 19 of 29 patients used an insulin pump at the time of the neuropathic assessment, two of them with CGMS. Since Time-In-Range (TIR) monitoring has only recently become widely available and adolescents are also known to comply poorly with sensor therapy, we used the HbA1c level of our patients to assess therapeutic efficacy. After the transition of the T1DM patients, in the adult-oriented diabetes care in the terms of multiple injections or pump therapy, none of the patients' therapy has changed over the past year; however, since then nine more patients started to use CGMS.

According to the ISPAD (International Society for Pediatric and Adolescent Diabetes) 2018 Clinical Practice Consensus Guidelines, screening for peripheral neuropathy should commence at the age of 11 years with 2 to 5 years of diabetes duration and it should be performed annually thereafter (78). The Society recommends the assessment of sensation, vibration and reflexes in the feet for peripheral neuropathy, as well as orthostatic tests and heart rate variability for cardiac autonomic neuropathy (78). Our study also confirms a need for early screening and better risk factor management as we found the prevalence of DPN to be significantly higher among T1DM subjects than in the control group. We did have an unusual finding in the T1DM group though, which was reduced vibration sensation in the upper limb and not in the lower one (**Figure 2**). Reduced vibration sense itself is not a surprising finding

in young T1DM patients as diabetic length-dependent peripheral neuropathy tends to present as large-fiber dysfunction first. However, peripheral neuropathies may affect different types of nerve fibers to different degrees. In diabetes mellitus the most commonly affected nerves are the sensory nerves in the lower limb, and the first symptoms usually present at the terminal of the longest nerves (79). One of the reasons why diabetic peripheral sensory neuropathy is so difficult to diagnose, especially in the early stage, is that peripheral neuropathies may affect different nerve fibers to a different degree. Novel approaches and strategies discussed in recently published reviews may help to improve diagnostic testing (80, 81).

Sensory function in the hands is rarely tested in peripheral sensorimotor neuropathy studies as most of these investigations tend to focus on the lower extremities (82–86). However, vibration perception is relatively easy to assess with a 128 Hz tuning fork, thus this test could be used to detect diabetic peripheral sensory neuropathy at an early stage (89). Normative values of vibration perception thresholds in finger pulps and metatarsal heads have already been published in healthy children and adolescents (87), as well as in adults (88). The study of Abraham et al. (89) reported reduced vibration perception both in the fingers and the toes of T1DM and T2DM patients; however, the difference between cases and control was significant only in the toes. The study of Ising et al. (90) also confirms that impaired vibrotactile sensation is more commonly detected in the feet of children and adolescents with type 1 diabetes than in the hands. However, in few cases of this study, concurrent impaired vibrotactile sense was found in the hand and one subject presented with impaired vibrotactile sense in the hand without having impaired sense in the foot at the same time. Due to the relative paucity of data on this issue, and given the unusual nature of our present findings on reduced vibration sensation in the upper but not in the lower limbs in young T1DM patients, further studies are warranted to confirm and/or extend these results.

Recent guidelines (23, 91) recommend assessing the cardiovascular autonomic function upon diagnosis in type 2 diabetes and within 5 years of diagnosis in type 1 diabetes followed by annual tests. Risk factors increasing the likelihood of developing CAN are mainly the lack of proper glycaemic control in T1DM and, in addition, dyslipidaemia, hypertension and obesity in T2DM. Other predisposing factors are glycaemic variability (92), oxidative stress, aging-related neuronal dysfunction, inflammation and certain genetic biomarkers (20, 23). Therefore, the intensive glycaemic control has to be emphasised as early as possible in T1DM to prevent or delay the development of CAN, and beside glycaemic control, multifactorial interventions (e.g., lifestyle modification, pharmacological therapy) might be effective in T2DM (23, 24, 93). The EURODIAB IDDM Study and the EURODIAB Prospective Study clearly prove that apart

from glycaemic control, the classic cardiovascular risk factors (high triglyceride level, body mass index, smoking, and hypertension) are all risk factors of autonomic neuropathy as well (36, 94). T1DM and T2DM share the two most common risk factors of CAN, namely the lack of proper glycaemic control and the duration of diabetes; however, the signs and symptoms appear later in T1DM than in T2DM. This is probably the result of metabolic abnormalities (dysglycaemia), oxidative stress and autonomic neuronal dysfunction, which have already been present prior to the diagnosis of T2DM (95–97). The pathogenesis of type 2 diabetes is more complex than in type 1 diabetes, and the abovementioned cardiovascular risk factors are much more frequently present, which explains the higher prevalence of autonomic neuropathy in type 2 diabetic patients. The mean duration of diabetes in our study group was 12.2 ± 5.8 years, which can definitely foretell the presence of cardiovascular autonomic neuropathy. Our study population of T1DM patients might have been protected as a result of their young age, efficient paediatric diabetes care and ideal body weight achieved via sport.

Among the young T1DM patients, we found significantly higher comorbidity with hypertension (in six diabetic patients) with a significantly increased diastolic blood pressure without detectable cardiac morphologic differences or cardiovascular autonomic neuropathy compared to controls. Additionally, in the whole observed population (diabetic and control subjects together), a significant correlation was found between HbA1c level and diastolic blood pressure. This latter connection has already been described in previous studies on diastolic blood pressure in childhood diabetes (98–100).

Poorly controlled and chronically treated diabetic patients are known to frequently develop hypomagnesemia (101, 102). Together with several previous studies, our present study also detected a lower serum magnesium level in young T1DM patients (103–109). According to Rodrigues et al. (110) there might be a correlation between reduced levels of magnesium and poor glycaemic control in patients with T1DM, which probably contributes to the early development of cardiovascular complications.

Nevertheless, at the time of health care transition we could only detect peripheral sensory neurological impairments in our young T1DM population, but no signs of cardiovascular autonomic neuropathy or cardiac morphological differences were present. However, peripheral sensory neurological impairments with several modalities were detected among young type 1 diabetic patients. In any case, our results may be considered as important feedback to paediatric diabetes care and a remarkable baseline reference point for further follow-up in adult diabetes care.

7.1.2. Limitations of the transition study

To extend the study population in the future by involving more young T1DM patients following their entrance to the adult healthcare system is planned. The present participants will be followed up annually to assess the progress of their neuropathic state and monitor their medications, as the latter might also influence the test results. The listed medicines might also have an impact on the test results. The low number of T1DM patients on oral antihypertensive therapy did not allow us to perform a statistical analysis on that. Logistical reasons prevented us from performing the echocardiographic examinations and collecting blood and urine samples from all patients and controls.

7.2.1. Discussion of the kidney transplant study

A definitive impairment of cardiovascular autonomic and peripheral sensory neuronal function was detected in patients after kidney transplantation in comparison to healthy volunteers in our study. Cardiovascular events cause the majority of deaths in kidney transplant patients (111). Cardiovascular autonomic neuropathy in diabetic patients is a risk factor of earlier mortality, as the impairment of the parasympathetic and sympathetic function leads to ventricular repolarization disturbances (112). Chronic renal failure can also contribute to the development of cardiac arrhythmias and sudden cardiac death (113). We found 3 of the 5 cardiovascular reflex tests to be abnormal in KTx patients, which suggests considerable impairment of the parasympathetic and sympathetic functions. Apart from that, the increased risk of cardiac events can also be attributed to the imbalance between the two systems in these patients. The deterioration of both functions suggests a long-standing pathological process with late autonomic damage in KTx patients. The significant effect of the renal morbidity on the cardiovascular autonomic function might be explained by our observations as the heart rate response to deep breathing was more altered if the eGFR was lower and heart rate response to Valsalva manoeuvre was more abnormal if the serum creatinine level was higher. The role of long-standing exposure is shown by the positive association between the systolic blood pressure and the time that elapsed since transplantations. In case of longer duration, higher systolic blood pressure was found.

Instability of cardiac repolarization, accompanied with an increased risk for sudden cardiac arrest, has not been found during the analysis of 12 ECG parameters in KTx patients compared to controls.

The peripheral sensory tests with Neurometer® point towards hypaesthesia in the KTx patients compared to controls both on upper and on lower limbs. Advanced stages of diabetic neuropathy typically manifest as hypaesthesia along the median and peroneal nerves (114). This finding points to the possible alterations of limb sensations in KTx patients that explains several symptoms and impaired protective mechanisms against different injuries. The pathological background of this autonomic and peripheral sensory neuropathy in KTx patients is highly complex. Uraemic neuropathy as a peripheral neuronal dysfunction is a common complication of uraemia in patients with CKD, especially with a GFR below 12 ml/min/1.73m^2. The estimated prevalence of peripheral neuropathy in this case is 50–70% (115, 116, 117). The pathogenesis of uraemic peripheral neuropathy includes the accumulation of uremic toxins (118, 119), hyperkalaemia (119, 116), thiamine deficiency and accumulation of slowly dialyzed neurotoxic molecules, such as methylguanidine, myoinositol, phenol derivatives, guanidinosuccinic acid and parathyroid hormone (120). The accumulation of the advanced glycation end-products contributes to diabetic, but also to non-diabetic renal diseases (119) and also might be responsible for neuropathy. The progress of uraemic neuropathy can be prevented with kidney replacement therapy. Previous studies revealed that precisely performed dialysis therapy improved early uraemic peripheral neuropathy (121). Following the kidney transplantation clinical recovery of the neuropathy has been documented (122, 123).

The prevalence of autonomic neuropathy in uraemic patients has been estimated more than 60% (124). Uraemic autonomic disorders (125) can be attributed to accumulated toxins (126), lack of neurotrophic factors (127), anaemia due to erythropoietin deficiency (119, 128, 129) and advanced arterial calcification (130). The haemoglobin and haematocrit levels of our study patients turned out to be lower than those of our controls.

The neuropathy in KTx patients may also be the result of the immunosuppressive drugs applied. One of the most widely used immunosuppressive agents following kidney transplantation is tacrolimus, a calcineurin-inhibiting drug. There are just small series of patients or case reports that have described tacrolimus-related development of peripheral neuropathy: chronic sensorimotor polyneuropathy was found in 2 cases after tacrolimus use following renal transplantation (131). The use of tacrolimus still needs to be closely monitored for symptoms of peripheral neuropathy (131). Incidence rates for paraesthesia and peripheral neuropathy have been reported as high as 40% (132). Tacrolimus-associated neuropathy has also been reported following the transplantation of organs other than the kidneys (133, 134, 135). In addition, tacrolimus-related optic neuropathy has also surfaced in the literature (136). The vast majority (87%) of our KTx patients received tacrolimus as part of their

immunosuppressive treatment. The reversible inhibitor of inosine monophosphate dehydrogenase, mycophenolate mofetil was administered in 78% of the KTx patients. Mycophenolate mofetil-induced peripheral neuropathy has also been detected recently according to the treatment of membranous glomerulonephropathy (137).

Nine per cent of our KTx patients were treated with the mammalian target of rapamycin (mTOR) inhibitor everolimus, the neuropathogenic effects of which are yet not well-known. So far there has only been one case report published about an everolimus-induced posterior reversible encephalopathy syndrome and bilateral optic neuropathy following kidney transplantation (138).

Our results may also be due to preexisting/post-transplant diabetes mellitus (PTDM) as 35% of our KTx subjects had diabetes. Immunosuppressive therapy is frequently accompanied by post-transplant diabetes with an incidence rate of 10-30% (139). In renal transplant patients, the risk of graft loss and mortality increases if the patient suffers from PTDM (140). Steroid-induced diabetes and consequent neuropathy must also be considered as 57% of our KTx patients received steroid therapy. A transient, steroid induced carbohydrate metabolism disturbance may also contribute to the neuropathological findings in this special patient group.

A significant positive correlation was observed between plasma glucose and the CPT value of the peroneal nerve at a stimulating frequency of 5 Hz in our KTx patients. This association reveals a possible relationship between lower limb small fibre dysfunction and high glucose levels in this group. Further correlations were not found between the metabolic and neuronal abnormalities supporting the fact that other pathogenetic factors than hyperglycaemia play important roles in the development of neuropathy. Type 1 and type 2 diabetic patients with a history of smoking, high blood pressure and hypertriglyceridaemia are often found to have cardiovascular autonomic and peripheral neuropathy (141, 142). The KTx patients in our study also showed a high prevalence of smoking and hypertension, which supports the potential aetiological role of these factors.

7.2.2. Limitations of the kidney transplant study

The main limitation of our study is its cross-sectional data acquisition method, since a follow-up design could yield further useful data on the progressive nature of neuropathy in chronic kidney diseases. Also, we would like to conduct another study where KTx patients would be compared to healthy controls and parallely to non-KTx subjects with chronic kidney insufficiency.

In summary, our study found that chronic renal disease following kidney transplantation is associated with extended cardiovascular autonomic dysfunction and upper as well as lower limb sensory neuropathy. The development of these conditions is probably linked to disease duration, kidney function and several cardiovascular risk factors, but the possible role of transplantation in the pathogenesis needs to be elucidated in further clinical studies.

8. Conclusions and new findings

1. Regarding the results of the 128 Hz Rydel-Seiffer graduated tuning fork test, the vibrational sense on the radius was significantly impaired in the young type 1 diabetic group compared to the controls. The Tiptherm®-test also identified significant temperature sensitivity impairment in type 1 diabetic patients. Further sensory nerve testing tests did not reveal any significant difference in the young diabetic population compared to controls. Neuropathic complaints were significantly more frequent in the young type 1 diabetic patient group than in controls.

2. Higher comorbidity with hypertension and significantly increased diastolic blood pressure without detectable cardiac morphologic differences or cardiovascular autonomic neuropathy was proven in young type 1 diabetic patients compared to controls.

3. A significant correlation was found between the HbA1c level and diastolic blood pressure in the observed population (diabetic and control subjects together).

4. In the kidney transplant study, 60% of the cardiovascular reflex tests were abnormal in kidney transplant patients in comparison with healthy controls, reflecting a common alteration of parasympathetic and sympathetic functions.

5. Instability of cardiac repolarization, accompanied by an increased risk of sudden cardiac arrest, has not been found in kidney transplant patients compared to controls.

6. Significant differences were demonstrated in the peripheral sensory function of the peroneal and median nerves in all three nerve fibre types tested with a quantitative analysis reflecting hypaesthetic conditions in kidney transplant patients.

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