THE INVOLVEMENT OF AUTONOMIC DYSFUNCTION IN THE PATHOGENESIS OF COMPLICATIONS IN PATIENTS WITH IMPAIRED GLUCOSE METABOLISM

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Summary of PhD Thesis

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Relevant publications

I. **Nyiraty Sz**, Pesei F, Orosz A, Coluzzi S, Vági OE, Lengyel Cs, Ábrahám Gy, Frontoni S, Kempler P, Várkonyi T.
Cardiovascular autonomic neuropathy and glucose variability in patients with longstanding type 1 diabetes. Is there an association?
Frontiers in Endocrinology 2018; 9:174. doi: 10.3389/fendo.2018.00174 **IF: 3.675**

II. **Nyiraty Sz,** Fehértemplomi K, Orosz A, Lengyel Cs, Ábrahám Gy, Kempler P, Várkonyi T.
Ismétlődő súlyos hypoglykaemiákhoz vezető neuropathia pancreatogen diabeteses betegben (esetismertetés) [Recurrent hypoglycemias caused by neuropathy in a patient with pancreatogenic diabetes. A case report]
Diabetologia Hungarica 2017; 25 (2): 117-124.

III. Orosz A, Baczkó I**, Nyiraty Sz,** Körei AE, Putz Zs, Takács R, Nemes A, Várkonyi T, Balogh L, Ábrahám G, Kempler P, Papp JG, Varró A, Lengyel Cs.
Increased short-term beat-to-beat QT interval variability in patients with impaired glucose tolerance.
Frontiers in Endocrinology 2017; 13;8:129. doi: 10.3389/fendo.2017.00129. **IF: 3.675**

IV. Várkonyi T, Körei A, Putz Zs, Martos T, Keresztes K, Lengyel Cs, **Nyiraty Sz**, Stirban A, Jermendy G, Kempler P.
Advances in the management of diabetic neuropathy.
Minerva Medica 2017; 108 (5): 419-437. **IF: 1.878**

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Other Publication

Németh BCs, Várkonyi T, Somogyvári F, Lengyel Cs, Fehértemplomi K, **Nyiraty Sz,** Kempler P, Mándi Y.
Relevance of α-defensins (HNP1-3) and defensin ß-1 in diabetes
World Journal of Gastroenterology 2014; 20 (27):9128-9137. **IF: 3.36**

Published Abstracts

Lengyel Cs, Takács R, **Nyiraty Sz**, Orosz A, Nemes A, Várkonyi T, Baczkó I, Wittmann T, Papp Gy, Varró A.
QT-variabilitás és vércukorszint: kimutatható-e összefüggés egészséges szénhidrát-anyagcseréjű egyénekben? [QT variability and blood glucose level: Is there a relationship in patients with normal carbohydrate metabolism?]
Orvosi Hetilap 2012; 153 (16):623-624.

Lengyel Cs, **Nyiraty Sz**, Németh N, Putz Zs, Orosz A, Takács R, Várkonyi T, Baczkó I., Wittmann T, Kempler P, Papp Gy, Varró A.
A rövid távú QT-variabilitás vizsgálata csökkent glukóztoleranciában [Short-term beat-to-beat QT interval variability in patients with impaired glucose tolerance]
Diabetologia Hungarica 2014; 22 (Suppl.2):96.

Várkonyi T, Fehértemplomi K, **Nyiraty Sz**., Orosz A, Szabó M, Vági OE, Lengyel Cs, Kempler P, Wittmann T.
Inzulinpumpával kezelt 1-es típusú diabeteses betegek autonóm funkciójának vizsgálata [Assessment of autonomic function in insulin pump-treated type 1 diabetic patients]
Diabetologia Hungarica 2014; 22 (Suppl.2):145-146.

Fehértemplomi K, **Nyiraty Sz**, Orosz A, Takács R, Lengyel Cs, Lázár M, Papós M, Pávics L, Várkonyi T, Wittmann T, Kempler P.
A kóros gyomorürülés kialakulásáért felelős tényezők vizsgálata 1-es típusú diabetesben [Assessment of factors responsible for impaired gastric emptying in type 1 diabetes]
Diabetologia Hungarica 2014; 22 (Suppl.2):27-28.

Orosz A, Nyiraty Sz, Németh N, Putz Zs, Várkonyi T, Baczkó I, Kempler P, Papp JGy, Varró A, Lengyel Cs.
A QT-variabilitás vizsgálata csökkent glükóztoleranciában. [QT variability in patients with impaired glucose tolerance]
Cardiologia Hungarica 2014; 44 (Suppl.E):64.

Lengyel Cs, Orosz A, **Nyiraty Sz**, Nemeth N, Putz Zs, Takacs R, Nemes A, Várkonyi T, Baczko I, Abraham Gy, Kempler P, Papp JG, Varro A.
Short-term beat-to-beat QT-interval variability in patients with impaired glucose tolerance
Diabetologia 2015; 58 (1):506.

Nyiraty Sz, Fehértemplomi K, Pesei F, Orosz A, Lengyel Cs, Kempler P, Ábrahám Gy, Várkonyi T.
Inzulinpumpával kezelt 1-es típusú diabeteses betegek autonom idegrendszeri funkciójának hosszú távú követése [Long-term follow-up of autonomic function in insulin pump-treated type 1 diabetic patients]
Diabetologia Hungarica 2016; 24 (Suppl.1):63-64.

Pesei F, **Nyiraty Sz**, Fehértemplomi K, Orosz A, Lengyel Cs, Lázár M, Pávics L, Kempler P, Ábrahám Gy, Várkonyi T.
A lassult gyomormotilitás és a hypoglykaemia összefüggésének elemzése három eset kapcsán [Analysis of the relationships between slower gastric emptying and hypoglycemia from the observations of three cases]
Diabetologia Hungarica 2016; 24 (Suppl.1):70-71.

Várkonyi T, **Nyiraty Sz**, Coluzzi S, Fehértemplomi K, Pesei F, Orosz A, Lengyel Cs, Frontoni S, Kempler P, Ábrahám Gy.
Long-term follow-up of autonomic function in type 1 diabetic patients on insulin pump treatment.
Diabetologia 2016; 59 (1):465.

Orosz A, Baczkó I, **Nyiraty Sz**, Körei EA, Putz Zs, Domsik P, Nemes A, Várkonyi T, Balogh L, Ábrahám Gy, Kempler P, Papp GJ , Varró A, Lengyel Cs.
Analysis of repolarization parameters of ECG in prediabetic condition
*Sporttudományi Kaleidoszkó*p ISBN 978-963-306-499-3 2016; 12-14.

Lengyel Cs, Putz Zs, Orosz A, **Nyiraty Sz**, Körei A, Takács R, Baczkó I, Nemes A, Várkonyi T, Ábrahám Gy, Kempler P, Papp Gy, Varró A, Sepp R.
A QT szakasz variabilitását jellemző paraméterek vizsgálata csökkent glukóztoleranciában [Analysis of parameters characterizing QT variability in patients with impaired glucose tolerance]
Diabetologia Hungarica 2017; 25 (Suppl.2):62-64.

Nyiraty Sz, Pesei F, Orosz A, Coluzzi S, Lengyel Cs, Ábrahám Gy, Frontoni S, Kempler P, Várkonyi T.
Az autonóm neuropathia és a glukózvariabilitás kapcsolatának vizsgálata 1-es típusú diabetesben [Assessment of the relationship between autonomic neuropathy and glycemic variability in type 1 diabetes]
Diabetologia Hungarica 2017; 25 (Suppl.2):72-73.

Nyiraty Sz, Orosz A, Pesei F, Lengyel Cs, Ábrahám Gy, Kempler P, Várkonyi T.
A glükózvariabilitás okainak vizsgálata 1-es típusú diabetesben. [Assessment of the factors responsible for glycemic variability]
Magyar Belorvosi Archivum 2017; 70 (Suppl.1):40.

Várkonyi T, **Nyiraty Sz**, Pesei F, Orosz A, Lengyel Cs, Ábrahám Gy, Kempler P.
A hypoglykaemia gyakoriságáért felelős tényezők vizsgálata hosszú ideje fennálló 1-es típusú és inzulinnal kezelt 2-es típusú diabetesben [Assessment of the factors responsible for hypoglycemia in long standing type 1 and insulin-treated type 2 diabetes]
Diabetologia Hungarica 2017; 25 (Suppl.2):102-103.

Nyiraty Sz, Pesei F, Orosz A, Coluzzi S, Lengyel Cs, Ábrahám Gy, Frontoni S, Kempler P, Várkonyi T.
Analysis of the relationship between glycaemic variability and autonomic neuropathy in type 1 diabetes
Diabetologia 2017; 60 (Suppl.1):548.

Pesei F, **Nyiraty Sz**, Tóth B, Fehértemplomi K, Orosz A, Lengyel Cs, Pávics L, Ábrahám Gy, Kempler P, Várkonyi T.
Az autonóm és szenzoros neuropathia, vaIamint a gyomorürüIés szerepe a visszatérő súlyos hypoglykaemia kialakulásában 1-es típusú diabetesben
Diabetologia Hungarica 2018; 26 (suppl. 1) 82-83.

Nyiraty Sz, Pesei F, Tóth B, Orosz A, Coluzzi S, Lengyel Cs, Pávics L, Ábrahám Gy, Frontoni S, Kempler P, Várkonyi T.
A glukózvariabiIitás, a hypoglykaemia-gyakoriság és az autonóm neuropathia összefüggése 1-es típusú diabetesben
Diabetologia Hungarica 2018; 26 (suppl. 1) 76-77.

Magony S, **Nyiraty Sz**, Pesei F, Tóth B, Orosz A, Lengyel Cs, Ábrahám Gy, Kempler P, Várkonyi T.
Korai szenzoros hyperaesthesia polycystás ovarium szindrómában szenvedő nőbetegekben
Diabetologia Hungarica 2018; 26 (suppl. 1) 67-69.

1 Introduction and aims of the study

Diabetes in all forms exerts an unacceptably high human, social and economic burden all over the world and become one of the largest global health challenges of the 21st century. This metabolic disorder is among the top 10 causes of death globally and together with the other three major noncommunicable diseases (cardiovascular disease, cancer and respiratory disease) account for over 80% of all premature deaths. Although several pathogenetic processes responsible for complications are explored in details in the last decades, the prevalence of the detrimental consequences of diabetes does not reduce. It is mandatory to seek continously new pathways and parameters which supply explanations about the destructive effect of hyperglycemia. Finding the appropriate methods for the characterization of the mediating pathways of hyperglycemia and the evaluation of the possible risk factors is a challenge as well. Prediabetes nowadays is regarded as a characteristic risk factor of cardiovascular morbidity and mortality. This increased risk is particularly explained by the fact that cardiac AN promotes ventricular repolarization disturbances (including heart rate-corrected QT [QTc] prolongation, increased QT dispersion) and may lead to sudden cardiac death. The short-term variability of the QT interval (STVQT) was introduced as an early and sensitive indicator of repolarization instability that more reliably predicted ventricular arrhythmias and sudden cardiac death than prolongation of repolarization in previous experimental and clinical studies.

There is no doubt in the relevant literature about the importance of the accurate characterisation of the glycemic exposure which is responsible for the cardiovascular complications in prediabetic and diabetic patients. Thus the concept of glycemic variability (GV) was introduced to describe the inter-day or intra-day variations of glucose. However, most of the previous studies about the detrimental effects of GV were conducted on type 2 diabetic patients and thus the possible differences in causes and consequences of GV between type 1 and type 2 diabetic patients have not been clearly analysed up to now. The potential association of AN with the development of higher GV in type 1 diabetic patients should be in the focus of the scientific interest as there is a considerable interplay between diabetic neuropathy and GV. On the one hand it is well-known that the oscillating glucose is more disadvantageous to endothelial function and oxidative stress than high mean glucose and play a crucial role in the development of neuronal damage. On the other hand, several manifestations of AN may lead to metabolic imbalance as frequently associated with postprandial hyperglycemia and recurrent hypoglycemic episodes.

Based on previous data and our preliminary assumptions discussed above our main goals were:

- to determine the beat-to-beat short-term variability of the QT interval for assessment of repolarization instability and possible proarrhythmic risk, together with cardiovascular autonomic function in patients with IGT.

- to determine parameters of GV in patiens with long-standing type 1 diabetes

- to assess cardiovascular AN in the same group of diabetic patients

- to analyze potential relationships between GV and cardiovascular AN

- to investigate further possible pathogenetic factors and indicators of GV including HbA1c, BMI, gender, age, daily insulin dose, diabetes duration and frequency of hypoglycemia in the group of type 1 diabetic patients

- to demonstrate the diagnostic steps and the therapeutic options of life-threatening GV in a patient with long-standing diabetes due to chronic pancreatitis

2 Patients

2.1 The short-term QT interval variability study

We studied 18 IGT patients with the age of 63 ± 11 years. Their descriptive parameters at the study procedures: body mass index (BMI): 31±6 kg/m^2, fasting glucose: 6.0±0.4 mmol/L, 120 min postload glucose: 9.0±1.0 mmol/L, hemoglobin A1c (HbA_1c): 5.9± 04%; mean±SD. 18 healthy controls were enrolled into the study (age: 56±9 years, BMI: 27±5 kg/m^2, fasting glucose: 5.2±0.4 mmol/l, 120 min postload glucose: 5.5±1.3 mmol/L, HbA_1c: 5.4±0.3%).

2.2 The glycemic variability study

20 middle-aged type 1 diabetic patients with long-standing disease were involved in the study (age: 39.5±3.4 years, duration of diabetes: 17.5±3.4 years; mean±SE). They were non-

obese (BMI: 22.3±0.8 kg/m^2) and their mean HbA_1c was 8.1±0.7%.

2.3 The patient in the case report

The male patient had a diabetes due to chronic pancreatitis. His diabetes was discovered 13 years before. He was 45 years old, his BMI was 20 kg/m^2. Laboratory parameters at admission: HbA_1c: 10.2%, fasting glucose 33.4-11.5 mmol/L.

3 Methods

3.1 Assessment of AN

Cardiovascular consequences of AN were detected in order to characterize the presence and severity of the neuronal dysfunction. The five standard cardiovascular reflex tests (CRT) were applied in all patients. These measurements provide a non-invasive, clinically relevant, reproducible and standardized gold-standard determination of autonomic function. Three of these tests record the change of heart rate during specific manoeuvres while other two tests were designed to evaluate blood pressure changes. Most of the tests aiming to detect changes in heart rate are used primarily (but not exclusively) for the assessment of parasympathetic innervation while the blood pressure responses predominantly reflect the impairment of sympathetic functions. The heart rate changes were analyzed during deep breathing, in positions of lying and standing up (30/15 ratio) and during and after of Valsalva manoeuvre. Systolic blood pressure was determined in response from lying to standing up, and diastolic pressure change was measured during a sustained handgrip.

3.2 Assessment of peripheral sensory neuropathy by application of Neurometer

The peripheral sensory function was studied with a Neurometer (Neurotron Incorporated, Baltimore, MD, USA). This device is suitable for the quantification of the function of different nerve fibres and provides a simple, non-invasive and quantitative measure of peripheral sensory function. Low voltage, electric sine wave stimulation was applied transcutaneously and the current perception threshold (CPT) values were determined. In our study the median and peroneal nerves were tested. The surface electrodes with 1 cm diameter were placed on the terminal phalanx of the index and the great toe. The electrodes were fixed only on intact skin

surface, because wounds or scars would have been disturbed the peripheral sensations. The amplitude of the delivered stimuli was between 0.01 and 9.99 mA. The stimulus was initially increased until a sensation was reported and than short stimuli (2-5 s) were applied at progressively lower amplitudes until a minimal threshold for consistant detection was determined. The CPT values of the upper and lower limbs were detected at three different stimulating frequencies (2 kHz, 250 Hz, 5 Hz).

3.3 Determination of QT interval variability

Before the ECG recording, all IGT patients and controls were at rest, in the supine position for 10 minutes. Then, 12-lead ECG-s were continuously recorded for 5 minutes at rest, also in the supine position to obtain signals with the least amount of motion artifact. In all leads, the ECG signals were digitized at 2,000 Hz sampling rate with a multichannel data acquisition system (Cardiosys-A01 software, MDE Heidelberg GMBH, Heidelberg, Germany) connected to a personal computer and stored for later off-line analysis. Out of the repolarization parameters, we analyzed the frequency corrected QT interval (QTc) and the short-term variability of QT interval (STVQT).

3.4 Scintigraphic gastric emptying

At the start of the test after an overnight fast the patient ingested a breakfast containing a bread roll, 200 ml of water and 2 hard-boiled eggs labeled with 40 MBq 99 mTc human serum albumin macroaggregates in a sitting position. Following the breakfast, the patients were continuously lying in a supine position. Generation of time-activity curves over the whole stomach as a region of interest made it possible to analyze the quantitative characteristics of gastric emptying. Calculation of the scintigraphic gastric half-emptying time (HTE) provided a numeric parameter characterizing the postprandial stomach motility.

3.5 Characterization of GV

GV was calculated from the results of a continuous glucose measurement (CGM) system (iPro 2 with Enlite sensor, Medtronic Minimed Inc). The mathematical formulae of the applied methods of assessment for glucose variability:

Standard deviation (SD): It expresses how much variation or dispersion there is from the average.

Mean amplitude of glycemic excursions (MAGE): The calculation of the MAGE is obtained by measuring the arithmetic mean of the differences between consecutive peaks and nadirs provided that the differences are greater than one SD of the mean glucose value.

Continuous overlapping net glycemic action (CONGA): CONGA is calculated by determining the difference between values at different set intervals, and the difference is then applied to the CONGA formula.

Mean absolute glucose (MAG): MAG calculates the sum of the differences between successive glucose values divided by the total time measured in hours.

3.6 Analysis of possible pathogenetic factors of glucose variability

In order to identify potential factors impacting on GV, HbA1c, body mass index (BMI), age, diabetes duration and daily insulin dose of patients were explored. Different categories of hypoglycemia were also characterized. Measured hypoglycemia was defined when blood glucose was below 3.9 mmol/L detected by CGMS. Severe hypoglycemia was categorized if serious cognitive impairment requiring assistance from another person associated with blood glucose lower than 3.9 mmol/L occured. Hypoglycemia unawaraness was established if a measured hypoglycemia was not recognized by the patient.

3.7 Verification of the glycemic state in IGT patients and control subjects

Fasting venous blood samples were obtained from each patient and controls for the determination of serum glucose and hemoglobin A1c (HbA1c) levels. Oral glucose tolerance test (OGTT) was carried out with 75 g glucose to confirm the diagnosis of IGT according to the World Health Organization recommendation (120 minute value in 7.8–11.0 mmol/l range).

3.8 Statistical methods

Comparisons between IGT patients and controls for the QT interval variability study were done using the unpaired Student's t-test for normally distributed parameters (D'Agostino-Pearson test was used to assess normality of distribution) and linear regression for revealing

correlations. Statistical analyses between different parameters of glucose variability and the diabetic patient's characteristics were performed with the Spearman correlation tests and multiple regression analysis. CRT-s and CGMS parameters were expressed as mean values ± standard error (SD). Statistical significance was defined by $P < 0.05$ level. The statistical analyses were performed using the SigmaStat 4.0 Systat Software and Statistica 12 packages.

4 Results

4.1 Results of the QT interval variability study

QTc values calculated with all the four formulas showed no significant differences between IGT patients and controls. As it has been shown that T wave amplitude may affect STVQT, we have also compared the T wave amplitudes in both groups. T wave amplitudes did not differ significantly between IGT patients and control subjects (225±120 vs 220±119 μV, P=0.882). To characterize the instability of cardiac ventricular repolarization, the short-term beat-to-beat variability of the QT interval was calculated in IGT patients and age-matched controls. Since it is reasonable to assume that STVQT can be, at least in part, influenced by the short-term variability of the RR interval, the STVRR was also calculated in both groups. Patients with IGT exhibited a significantly lower STVRR compared to controls (10.5±6.7 vs 18.5±14.3 ms, P=0.0373). No significant correlation was found between STVQT and STVRR values in IGT patients (r=−0.3152; P=0.203). Standard CRT-s indicated significant deteriorations in Valsalva ratio (P<0.0001) and the heart rate responses to deep breathing among IGT subjects compared to controls (P=0.033). However, no significant differences in 30/15 ratio, systolic blood pressure response after standing up, diastolic blood pressure response after sustained handgrip, and AN score were detected between the two groups. 30/15 ratio had significant negative correlation with STVQT (r =–0.4729; P=0.048).

4.2 Results of the glucose variability study

The CRT mean values of the patient group reflected a moderate autonomic impairment while all measured mean GV parameters of the patients were higher than the previously published reference values in healthy subjects. As a next step of the analysis, the patients were divided into two groups: patients with AN scores 0–1 (n = 10) and patients with AN scores 2–10 (n = 10). The GV parameters were compared and no significant difference was proven

between the groups with a tendency of higher GV parameters in the AN group (CONGA: 7.6±0.55 vs 8.5±0.56 mmol/L, P=0.235; SD: 3.3±0.15 vs 3.67±0.18 mmol/L, P= 0.129, MAGE: 5.9±0.4 vs 6.2±0.16 mmol/L, P= 0.678; MAG: 2.16±0.3 vs 2.33±0.09 mmol/L, P=0.06; patients without AN vs patients with AN, mean±SE). The AN scores calculated from the CRT-s expressing the overall severity of cardiovascular AN correlated positively with the SD of continuously measured interstitial glucose levels (r=0.47, P<0.05). The statistical analysis revealed a further positive correlation between SD of the continuously measured glucose values and the systolic blood pressure response to standing (r=0.51, P<0.05). The relationship between GV and AN was further strengthened by the fact that MAG, a marker of GV correlated positively with the AN scores of the patients (r=0.62 P<0.01). Higher MAG values were associated with significantly lower results of the 30/15 ratio (heart rate response to standing). The negative correlation coefficient (r=-0.5, P<0.05) reflects impaired cardiovascular autonomic function among patients with more severe GV. Similarly to SD, MAG also correlated positively with the level of orthostatic systolic blood pressure fall supporting the association between GV and sympathetic dysfunction (r=0.59 P<0.01). When AN scores were also calculated after the exclusion of the handgrip tests similarly significant correlations were found between SD, MAG and AN scores (SD-AN score: r=0.62, P<0.01, MAG-AN score: r=0.51, P<0.05). When the correlations were adjusted for HbA1c, age, and duration of diabetes at a multivariate analysis the relationship between SD and the systolic blood pressure response to standing remained significant (r=0.49, p<0.05). Higher HbA1c levels were associated with increased GV as measured by CONGA or MAG. This observation was proven by the positive statistical correlation between HbA1c and CONGA (r=0.56, P<0.05) and MAG (r=0.45, P<0.05).

4.3 The diagnostic steps of life-threatening glycemic instability in our patient with diabetes due to chronic pancreatitis

The 45 year-old patient was a chronic alcoholic consumer by his previous history. In 2000 he had acute pancreatitis and later chronic pancreatitis developed. Before his admission to our ward he was urgently transported to the Department of Traumatology with head injury associated with severe recurrent convulsions and transient unconsciousness. After the wound treatment diagnostic steps were done to explore a possible epilepsy on Clinic of Neurology. Cranial CT scans, EEG and carotid-vertebral arterial Doppler ultrasound were performed without finding any morphologic disorder. Due to the convulsions gabapentin treatment was

initiated and a transmission to our Internal Medicine Department was indicated for the therapy of an extreme glucose fluctuation. The daily glucose profiles revealed extreme instability, the values were frequently abnormally low following breakfast and two severe hypoglycemic episodes were also reported at that time. The CRT-s revealed very severe AN at admission. The peripheral sensory function was assessed with a Neurometer and the CPT values were normal despite of the established severe autonomic disorder. We measured the scintigraphic gastric emptying due to the presence of the AN. The half time of the emptying (HTE) of radioiodine labelled test meal revealed an extremely slow gastric motility (HTE: 487.6 min, normal range: ≤ 67.6 min). In order to explore the daily glycemic state of the patient in details CGMS was applied on 6 consecutive days. The CGMS detected a regularly recurrent trend of postprandial hypoglycemia after all breakfasts and most of the dinners explaining the etiology of the unconsciousness. All of the hypoglycemic eoisodes were followed by hyperglycemic intervals in accordance with the Somogyi phenomenon.

5 Discussion

In our study, we show for the first time that beat-to-beat STVQT, an early and sensitive parameter of repolarization instability, is increased even before QTc prolongation or enhanced QTd could be detected in patients with IGT. clinical studies. Our study is the first to indicate that patients with IGT, a prediabetic condition, have repolarization instability indicated by elevated beat-to-beat STVQT. Relative sympathetic predominance was observed in CRT-s during IGT, as sympathetic parameters (systolic BP fall after standing up and diastolic BP increase after sustained handgrip) were unchanged, whereas two of three parasympathetic parameters measured (HR variation and Valsalva ratio) were significantly decreased. In addition, a significant negative correlation was seen between the values measured in the third parasympathetic test (30/15 ratio) and STVQT in our study. The significantly lower STVRR values also represent this parasympathetic dysinnervation and subsequent relative sympathetic predominance in patients with IGT. Putz et al. described a mainly subclinical, asymptomatic small-fiber neuropathy, and mild impairment of cardiovascular autonomic function in IGT subjects. Similarly to our findings, HR variation and Valsalva ratio were decreased, whereas 30:15 ratio was unchanged among the tests evaluating parasympathetic activity; however, sympathetic function was also mildly impaired in patients with IGT.

Beside the cardiac repolarization disturbance in IGT patients, autonomic dysfunction was

abnormal in our studies in diabetic patients with glycemic instability. Our observations on type 1 diabetic patients prove associations between cardiovascular AN and higher GV for the first time. The positive and negative significant statistical associations found in our study indicate increasing values of MAG and SD in the presence of more severe AN reflected by two CRT-s or the AN score. We have also found higher HbA1c in the presence of increased glucose variability, while patterns of hypoglycemia were not associated with AN or glucose variability. The mean values of GV parameters reflected serious glycemic instability in this group of patients with type 1 diabetes. The SD of interstitial glucose values is higher in the presence of more severe AN and if blood pressure fall is more pronounced to standing. The orthostatic hypotension is a characteristic late symptom of advanced neuropathy and reflects a sympathetic dysfunction. This sympathetic impairment is frequently associated with altered norepinephrine levels which might explain an abnormal counterregulatory response to hypoglycemia. The other glucose variability marker, MAG reflected more unstable glucose in our study, if the cumulative autonomic score was higher and two reflex tests were more abnormal. MAG is a summation of all absolute changes in glucose, divided by the time elapsed during the measurements. One of the two reflex tests that correlated with MAG was a ratio of the heart rate responses to standing reflecting mainly the parasympathetic function, while the other, the orthostatic systolic blood pressure to standing supplied information on sympathetic function. Thus parasympathetic and sympathetic dsyfunctions are both altered if GV is enhanced. The relationship between AN and GV was clearly proven, although the mean severity of AN was moderate of the patients, while GV was severe. Several factors may also impact GV, particularly longer duration of diabetes is associated with higher GV. However, when the correlations were adjusted for HbA_1c, age, BMI and diabetes duration at a multivariate analysis, the relationship between SD and the systolic blood pressure response to standing remained significant ($r=0.49$, $p<0.05$). Although the moderate sample size may explain why only the correlation between SD and orthostatic hypotension remained significant after adjustment for confounding variables, it is noteworthy to stress the concept that in the presence of advanced AN a severe variability of glucose is observed in type 1 diabetic patients. Orthostatic hypotension is a characteristic sign of the late progressive stages of AN and primarily refers to the impairment of the sympathetic autonomic function. In our study, SD and MAG both correlated with orthostatic systolic blood pressure fall that supports the relationship of sympathetic dysfunction and GV.

As an interesting finding, we detected two correlations between markers of GV and HbA_1c. The general approach of the literature to the possible relationship of GV and HbA1c is that these parameters reflect different patterns of carbohydrate metabolism: parameters of GV

calculated from CGMS describe both hypo-and hyperglycemic episodes for a short-term period, while HbA_1c reflects mean blood glucose and primarily driven by the extent of hyperglycemia. The associations between HbA1c and GV as well as GV and AN allow conclusions that the higher HbA1c is responsible for AN and AN leads to GV but it also could be assumed that GV manifests in higher HbA1c that results AN.

The patient described in our case report was transported urgently to the department of neurology due to his convulsions and as his symptoms were not realized as signs of hypoglycemia. There were no prodromal classic symptoms of hypoglycemia. Moreover unconsciousness was the first sign of hypoglycemia due to his hypoglycemia unawareness and the abnormal counterregulatory response caused by the severe autonomic neuropathy.

6 Conclusions and new findings

1. We could prove at first that short-term QT interval variability is higher in patients with IGT.

2. The elevated temporal STVQT and concomitant cardiac AN may serve as early indicators of the increased instability of cardiac repolarization and elevated risk for sudden cardiac death in patients with prediabetic states.

3. Our studies on patients with long-standing type 1 diabetes established at first that increased GV is in a close relationship with advanced AN and might be manifested in higher HbA1c.

4. The dominance of the sympathetic dysfunction in patients with increased GV was proven.

5. These data draw attention to consider the possible presence of cardiovascular autonomic impairment in case of glycemic instability of type 1 diabetic patients.

6. The patient's case reminds that in the presence of advanced AN severe hypoglycemia might be responsible for recurrent convulsions and unconsciousness in diabetic patients even without classic prodromal symptoms.

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