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

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PhD Thesis

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Table of contents

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 becomes 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 [1]. 425 million people worldwide, or 8.8% of adults in the 20-79 years age range of the population are estimated to have diabetes according to the 2017 report of the International Diabetes Federation. In addition, the global prevalence of IGT in this age group is 7.3% pointing to that at least one sixth of the adult population is affected by a disorder of the glucose metabolism [2]. A further major contributor to the challenge of diabetes is that 30-80% of people with this disease are undiagnosed. The main purpose in the diabetes-related scientific efforts is to prevent the development of complications and comorbidities of the disease that results an improvement in quality of life and a reduced risk of the premature death. 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. Almost all of diabetes related organ specific complications and the associated comorbid states are originated from micro- or macrovascular structural alterations. Besides the morphologic approach, it is important to focus on the exploration of functional disorders in the background of complications. By the end of the nineties it became clear that the less intensive treatment of hyperglycemia is responsible for the development of several complications in diabetes. Large prospective trials provided clear evidence that long-term hyperglycemia is associated with micro- and macrovascular complications in patients with diabetes [1, 2]. Later the detrimental role of recurrent hypoglycemic episodes and acute as well as chronic hyperglycemia was also proven in the development of severe disorders of several important organ systems [3, 4]. Moreover, in the last years the importance of the prediabetic metabolic state of type 2 diabetes in the development of the macrovascular complications became evident [3].

Prediabetes nowadays is regarded as a characteristic risk factor of cardiovascular morbidity and mortality [4]. Over 6.4 years, 6.9% of 9306 participants with biochemically confirmed

impaired glucose tolerance (IGT) experienced a cardiovascular event, and 2.6% of them had cardiovascular death in NAVIGATOR trial [5]. In a 23-year follow-up study on Japanese American men, the relative risks for sudden cardiac death were 2.22 in subjects with asymptomatic hyperglycemia, and 2.76 in diabetic patients [6]. Higher risk of sudden cardiac death was associated with borderline diabetes, diabetes with or without microvascular disease, compared to subjects without diabetes in a population-based case–control study of patients experienced out-of-hospital cardiac arrest due to heart disease [7]. Cardiovascular autonomic neuropathy (AN) is a risk marker of cardiovascular morbidity, and it causes a 3.65-fold increase in the relative risk of mortality [8]. 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 [QTd]) and may lead to sudden cardiac death. Prolongation of QT interval enhances increased myocardial electrical instability, predisposing diabetic subjects with AN to potentially fatal ventricular arrhythmias [9]. Prolonged QTc is more frequent in patients with IFG (30%) and with diabetes (42%) than in subjects with normal glucose tolerance (22%), and both IFG and diabetes increase the risk of prolonged QTc [10]. QTc interval duration was found to be significantly higher both during the day and night using ECG Holter recordings in patients with IGT compared to subjects with normal glucose tolerance [11]. IGT was confirmed in 15% of men and 23% of women with QTc prolongation (>440 ms) in the population-based Hisayama study in Japan [12]. In the clinical setting, the risk assessment of serious ventricular arrhythmias in individual patients is challenging since the prolongation of repolarization that manifests as QT interval prolongation on the ECG does not always correlate with subsequent development of ventricular arrhythmias [13]. Cardiac repolarization reserve may be reduced even without significant changes in the duration of cardiac repolarization; therefore, QT interval prolongation cannot reliably predict the development of ventricular arrhythmias [14]. 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 [15, 16]. Type 1 diabetes mellitus moderately lengthened ventricular repolarization, attenuated repolarization reserve, and enhanced the risk of sudden cardiac death in dogs [17], and similar mechanisms might also occur in patients suffering from prediabetic states.

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. Fasting and postprandial glucose as well as

HbA_1c are generally used in clinical studies and the everyday practice to express the glycemic control of the patients. However, the value of all three parameters is limited if the aim is to express the fluctuations of glucose levels. Thus the concept of glycemic variability (GV) was introduced to describe the inter-day or intra-day variations of glucose. The variability of blood or interstitial glucose as well as HbA_1c are characterized by several parameters to describe the deviations of the metabolic control [18]. This scientific activity received an impulse two decades ago when GV was proven to be associated with oxidative stress [19]. The results of the Verona Diabetes Study led to the conclusion that variability of fasting glucose is a predictor of the total, the cancer associated and the cardiovascular mortality in type 2 diabetic patients. The fact that higher GV is associated with increased frequency of hypoglycemia also strengthens the theory of increased mortality risk in patients with unstable glucose [20]. Keeping the strict balance of carbohydrate metabolism is a real challenge in a diabetic patient as several factors may influence the actual glucose values such as age, cognitive impairment or liver and kidney failure [21]. Glycemic stability is influenced by antidiabetic therapy, diet and body composition as well [22]. Reduced beta cell function is one of the most important risk factors of GV and an inverse relationship between residual C-peptide levels and GV has been shown in type 1 diabetic patients [23]. Beta-cell dysfunction might be associated with glycemic instability in type 2 diabetic patients as well [24]. In type 1 diabetic patients hyperglycemia is primarely related to the loss of endogenous insulin secretion while the impaired glucagon response to hypoglycemia explains the susceptibility to abnormally low glucose values [25]. These observations suggest the hypothesis of a more pronounced GV in type 1 than in type 2 diabetic subjects. 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. Regarding the less complex pathogenesis of type 1 than type 2 diabetes it is easier to elucidate the pathogenetic background of glycemic instability in type 1 diabetic patients. Thus exploration of the factors leading to increased GV in type 1 diabetes is an important aim of a clinical research. The potential association of AN with the development of higher GV 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 [26]. On the other hand, several manifestations of AN may lead to metabolic imbalance as frequently associated with postprandial hyperglycemia and recurrent hypoglycemic episodes [27].

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 in patients with IGT

- to measure the cardiovascular autonomic function in the same goup of patients with IGT

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

- to assess cardiovascular AN in the same group of type 1 diabetic patients

- 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 analyze potential relationships between GV and the measured parameters

- 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, 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%). Patients were excluded if they had excessive (> 5%) ectopic atrial or ventricular beats, were in a rhythm other than normal sinus, had repolarization abnormalities (i.e., early repolarization pattern, T wave inversion, and complete left bundle branch block or right bundle branch block), had a permanent pacemaker. The BMI of IGT patients was significantly higher ($P<0.05$) than among age- and

sex-matched healthy volunteers. Significant differences were seen between IGT and control groups in mean serum glucose (6.0±0.4 vs 5.2±0.4 mmol/L; P<0.0001), HbA_1c (5.9±0.4 vs 5.4±0.3%; P<0.0001), and serum glucose 120 min. level during OGTT (9.0±1.0 vs 5.5±1.3 mmol/L; P<0.0001).

2.2 The GV 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). They were non-obese (BMI: 22.3±0.8 kg/m^2) and their mean HbA_1c was 8.1±0.7%. All patients applied multiple daily injections of analogue insulins, their mean daily insulin dose was 42.8±2.9 U. Patients with a rhythm other than normal sinus, acute infection, thyroid disease or chronic alcohol consumption were excluded from the study.

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 [28]. These measurements provide a non-invasive, clinically relevant, reproducible and standardized gold-standard determination of autonomic function [29]. Three of these tests record the change of heart rate during specific manoeuvres while other two tests were designed to evaluate blood pressure changes [30]. 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.

3.1.1 Heart rate tests

Heart rate variation to deep breathing

Normally the heart rate is increased during inspiration and decreased by expiration. The patient was asked to breath deeply at six breath a minute rate (five seconds in and five seconds out). The result is expressed as the difference between maximum and minimum heart rates (beat/min) during the six breathing cycles.

Heart rate response to Valsalva manoeuvre

During the strain period of Valsalva manoeuvre the blood pressure drops and the heart rate rises in physiologic conditions. Following the procedure the blood pressure rises and the heart rate slows. The patient was instructed to blow into a mouth-piece connected to a modified manometer and holding it at a pressure of 40 mm Hg for 15 seconds while an electrocardiogram was recorded continously. The Valsava ratio is calculated at the evaluation as the ratio of the longest R-R interval after the manouevre to the shortest R-R interval during the procedure.

Heart rate response to standing (30:15 ratio)

Following the position change from lying to standing the heart beat frequency is immediately increased which is maximal at the 15^{th} beat after standing up. Then a relative bradycardia occurs in healthy subjects with a maximum at about the 30^{th} beat. At the start of the test the patient was lying quietly while the heart rate was recorded continuously at the electrocardiogram. Then the patient was asked to stand up without interrupting the detection of the heart rate. The 30/15 ratio was expressed as the ratio of the longest R-R interval at around the 30^{th} beat to the shortest R-R interval at around the 15^{th} beat following standing up.

3.1.2 Blood pressure tests

Blood pressure response to standing (orthostatic hypotension)

In healthy subjects in case of standing pooling of blood in the lower extremities causes a minor fall in the blood pressure, which is rapidly corrected by peripheral vasoconstriction.

Severe postural hypotension is a characteristic sign of AN. This test is based on blood pressure determinations in lying position and after standing up. The postural fall is defined as the difference between systolic pressure after 10 minutes lying and systolic pressures at 1st, 5th and 10th minutes after standing up. The largest difference from the systolic pressure in lying is evaluated as blood pressure response to standing.

Blood pressure response to a sustained handgrip

During a sustained handgrip a sharp rise in diastolic blood pressure is expected in healthy subjects, due to a heart rate dependent increase in cardiac output with unchanged peripheral resistance. At the begining of the tests the maximal contractile capacity of the patient's hand was determined. Handgrip was then maintained at 30 % of that maximum up to three minutes. Blood pressure was determined before the test and a during the handgrip at one minute interval. The result was expressed as the difference between the highest diastolic blood pressure during handgrip and the diastolic pressure before the procedure began.

Finally each CRT was scored as 0 (normal), 1 (borderline) or 2 (abnormal) and by this method an autonomic score (0-10) was calculated to express the overall severity of AN. Age-corrected normal reference values were applied based on the definition of Ewing and the recommendations of the Toronto Neuropathy Expert Group [8, 31].

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 [32]. 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 2000 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 QTc interval using Bazett's ($QTc = QT/\sqrt{RR}$), Fridericia ($QTc = QT/[RR/1{,}000]1/3$), Framingham ($QTc = QT + [0.154 \times \{1{,}000 - RR\}]$) and the Hodges formulas ($QTc = QT + 1.75 \times [60{,}000/RR - 60]$), the QTd, the PQ and QRS intervals, the duration of terminal part of T waves (Tpeak − Tend) and the short-term variability of QT interval (STVQT). The RR and QT intervals, as well as duration of the T wave from the peak to the end (Tpeak − Tend) intervals were measured semi-automatically in 30 consecutive beats (minimum number of intervals needed for variability measurements) and were calculated as the average of 30 beats. The QT intervals were analyzed by conventional computerized QT measurement technique, all QT intervals were checked in a blinded manner by the same expert investigator of the team and fiducial cursor positions set by the software were manually corrected if needed [33]. QTc interval duration was defined as the mean duration of all QTc intervals measured. The PQ and QRS intervals were measured as the average of 15 consecutive beats. All measurements were carried out using limb lead II and in case of excessive noise in limb lead II, lead V5. To characterize the temporal instability of beat-to-beat heart rate (HR) and repolarization, Poincaré plots of the QT and RR intervals were constructed, where each QT and RR value is plotted against its former value. STVQT and STVRR 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. This 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 [34].

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 patient was continuously lying in a supine position. The images were acquired at a 1 frame per minute rate. The test was terminated after 120 minutes. 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. When the HTE of gastric emptying was longer than 120 minutes of study period, it was calculated by computerized extrapolation from the linear emptying curve of the test meal [35].

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). During this procedure a tiny flexible electrode was placed under the skin. The surface of the electrode contained glucose oxidase enzyme that catalysed a biochemical reaction in the presence of glucose. Following this process Oxygene transferred electrons to a receiving molecule and an electronic current was created. The magnitude of the current was converted into a glucose concentration. The electrode continuously measured the glucose levels of the interstitial fluid. 288 glucose readings were detected per 24 hours and the total period of continuous measurement was 6 days. The mathematical formulae of the applied methods of assessment for GV [36]:

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 GV

In order to identify potential factors impacting on GV, HbA_1c, 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 HbA_1c 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 as well as type 1 diabetic patients were done using the unpaired Student's t-test for normally distributed parameters (D'Agostino-Pearson test was used to assess normality of distribution). Linear regression test, Spearman correlation test and multiple regression analysis were applied to reveal correlations. 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

Comparison of the two groups (IGT patients vs controls) revealed no significant differences in HR, the PQ, QRS, QT and Tpeak − Tend intervals and the QTd. In order to reliably assess the duration of ventricular repolarization and to minimize the influence of changing HR on the QT interval, QTc interval was calculated by the Bazett's, Fridericia, Framingham and Hodges formulas. QTc values calculated with all the four formulas showed no significant differences between IGT patients and controls. Electrocardiographic parameters in study subjects are presented in Table 1. As it has been shown that T wave amplitude may affect STVQT [37], 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).

	Control	Patients with IGT
RR (ms)	900 ± 144	914 ± 163
PQ (ms)	161 ± 18	162 ± 24
QRS (ms)	94 ± 9	94 ± 8
QT (ms)	402 ± 39	411 ± 43
QTc (ms) Bazett	424 ± 19	431 ± 25
QTc (ms) Fridericia	416 ± 23	424 ± 27
QTc (ms) Framingham	417 ± 22	424 ± 26
QTc (ms) Hodges	416 ± 25	424 ± 29
QTd (ms)	42 ± 17	44 ± 13
$T_{peak} - T_{end}$ (ms)	86 ± 14	88 ± 23
T wave amplitude (μV)	220 ± 119	225 ± 120
STV_{RR} (ms)	18.5 ± 14.3	10.5 ± 6.7*
STV_{QT} (ms)	3.7 ± 0.7	5.0 ± 0.7**

Values are represented as mean ± SD. Values are considered statistically significantly different at P < 0.05 (*), P < 0.0001 (**) compared with the control group. n = 18 in each group.
IGT, impaired glucose tolerance; QTc, frequency corrected QT interval (calculated by the Bazett's, 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.

Table 1. Electrocardiographic parameters in patients with IGT and age-matched controls.

To characterize the instability of cardiac ventricular repolarization, the STVQT 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 [38]. 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 (ρ=−0.3152; P=0.203). As individual representative examples (Poincaré plots) illustrate (Figure 1) and grouped average data show (Table 1), STVQT was significantly increased by 36% in IGT patients compared to controls (5.0±0.7 ms vs 3.7±0.7 ms, P<0.0001). 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).

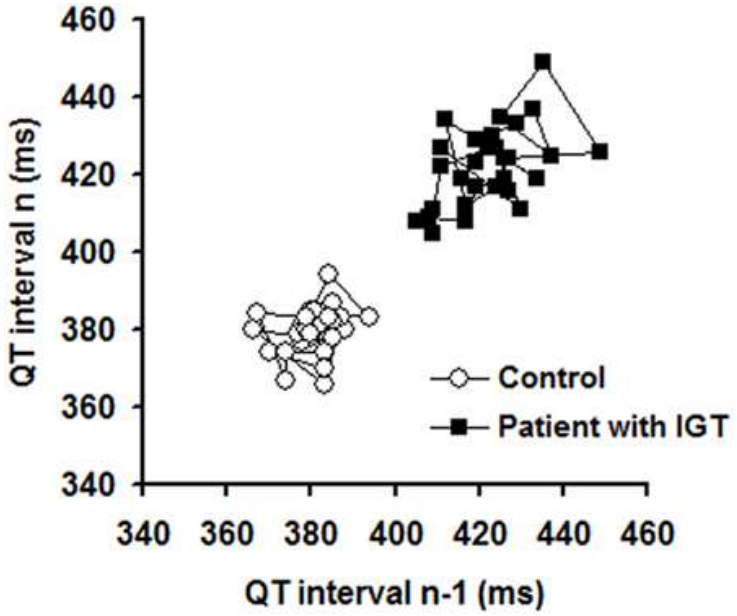

Figure 1. Representative Poincaré plots illustrating short-term temporal variability of the QT interval in a control individual and in a patient with impaired glucose tolerance (IGT).

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. Autonomic parameters of IGT patients and age-matched control subjects are shown in Table 2. Pearson correlation coefficient values indicated that neither laboratory data nor autonomic parameters correlated with STVQT, these data are presented in Table 3**.** However, 30/15 ratio had significant negative correlation with STVQT (r=–0.4729; P=0.048).

	Control	Patients with IGT
Heart rate (HR) variation during deep breathing (1/min)	16 ± 7	11 ± 8*
Valsalva ratio	1.7 ± 0.3	1.2 ± 0.1**
30/15 ratio	1.3 ± 0.3	1.2 ± 0.1
Systolic BP fall after standing up (mmHg)	8 ± 8	6 ± 7
Diastolic BP increase after sustained handgrip (mmHg)	11 ± 6	14 ± 6
AN score	2.4 ± 1.2	2.7 ± 1.3

Values are represented as mean ± SD. Values are considered statistically significantly different at P < 0.05 (*), P < 0.0001 (**) compared with the control group. n = 18 in each group.
IGT, impaired glucose tolerance; 30/15 ratio, immediate HR response to standing; BP, blood pressure; AN, autonomic neuropathy.

Table 2. AN parameters of IGT patients and age-matched control subjects.

	STV_{QT} in patients with IGT (ms)	
	Pearson *r*	*P* value (two-tailed)
HbA1c (%)	0.2708	0.277
OGTT 0 min (mmol/l)	0.2118	0.399
OGTT 120 min (mmol/l)	−0.1118	0.659
Heart rate (HR) variation during deep breathing (1/min)	−0.0379	0.881
Valsalva ratio	0.1101	0.664
30/15 ratio	−0.4729	0.048*
Systolic BP fall after standing up (mmHg)	−0.0163	0.949
Diastolic BP increase after sustained handgrip (mmHg)	−0.0685	0.787
AN score	−0.1353	0.593

Values are represented as Pearson correlation coefficient. Values are considered statistically significantly different at P < 0.05 (*).
STV_{QT}, beat-to-beat short-term temporal variability of the QT interval; IGT, impaired glucose tolerance; HbA1c, hemoglobin A1c; OGTT, oral glucose tolerance test; 30/15 ratio, immediate HR response to standing; BP, blood pressure; AN, autonomic neuropathy.

Table 3. Correlation of short-term QT interval variability (STVQT) with laboratory data and AN parameters in patients with IGT.

4.2 Results of the GV study

The mean values of the measured CRT and variability parameters in type 1 diabetic patients are listed in Table 4. 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 [36]. 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).

AN test	**type 1 diabetic patients (mean±SE)**
Heart rate response to deep breathing	23.9±2.6 (beats/min)
30/15 ratio	1.15±0.07
Valsalva ratio	1.72±0.13
Diastolic RR response to handgrip	16.2±2.7 (mm Hg)
Systolic RR response to standing up	7.6±1.4 (mm Hg)
AN score	2.1
GV parameter	
CONGA	8.09±0.4 mmol/L
SD	3.49±0.1 mmol/L
MAGE	6.12±0.2 mmol/L
MAG	2.25±0.2 mmol/L

AN autonomic neuropathy, RR blood pressure, CONGA overlapping net glycaemic action, SD standard deviation, MAGE Mean amplitude of glycemic excursions, MAG Mean absolute glucose

Table 4. Results of CRT-s and GV parameters in type 1 diabetic patients

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). The further analyses were done on the whole group (n=20). 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 (ρ =0.47, P<0.05; Figure 2) thus showing that higher GV expressed with SD was associated with more

severe cardiovascular AN in this group of type 1 diabetic patients.

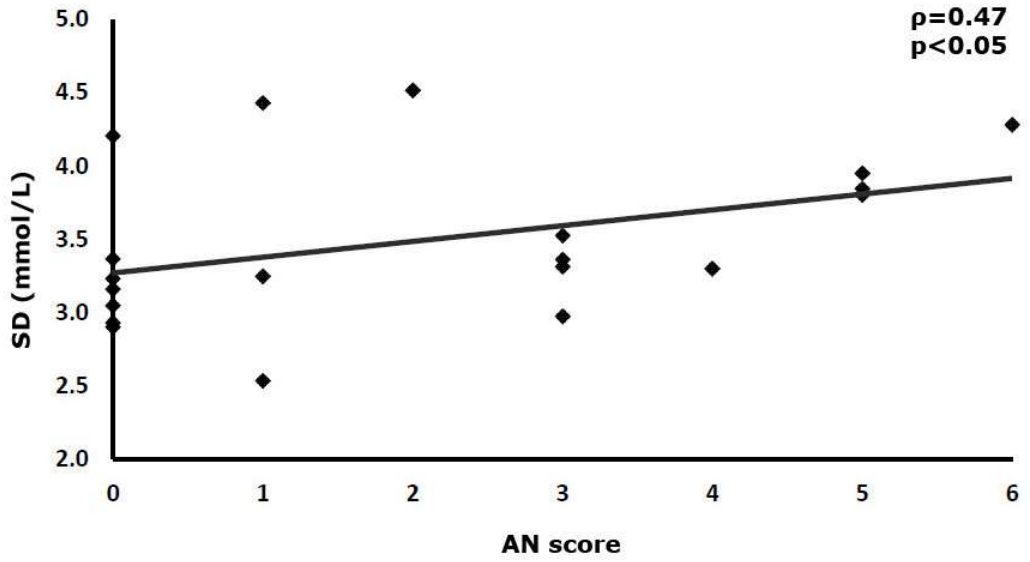

Figure 2. Correlation between standard deviation (SD) of continously measured interstitial glucose levels and autonomic neuropathy (AN) scores in type 1 diabetic patients

The statistical analysis revealed a further positive correlation between SD of the continuously measured glucose values and the systolic blood pressure response to standing (ρ=0.51, P<0.05; Figure 3). This observation reflects pronounced systolic fall of blood pressure due to sympathetic AN in the presence of GV characterized by higher SD.

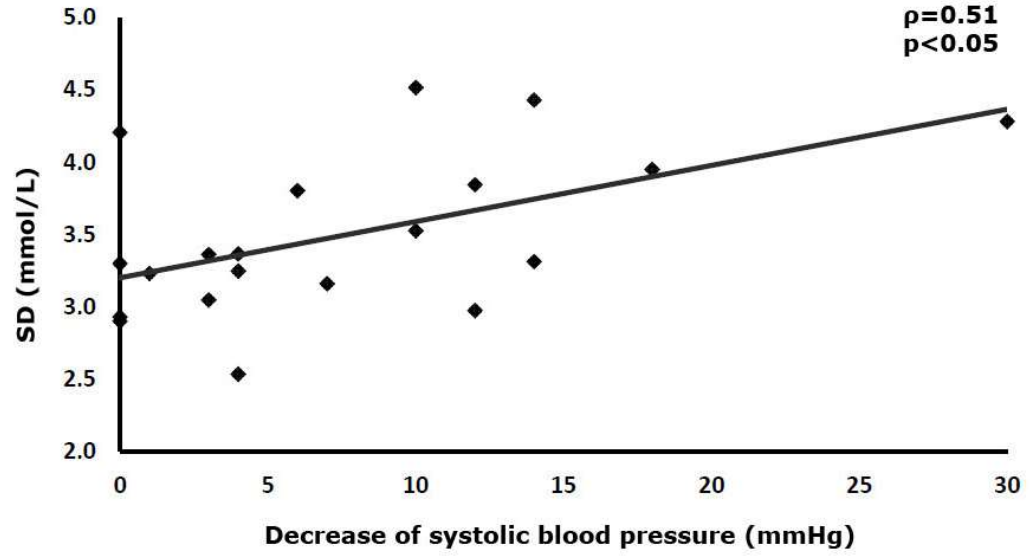

Figure 3. Correlation between standard deviation (SD) of continously measured interstitial glucose levels and orthostatic decrease of systolic blood pressure in type 1 diabetic patients

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 (ρ=0.62 P<0.01; Figure 4). Higher MAG values were associated with significantly lower results of the 30/15 ratio (heart rate response to standing).

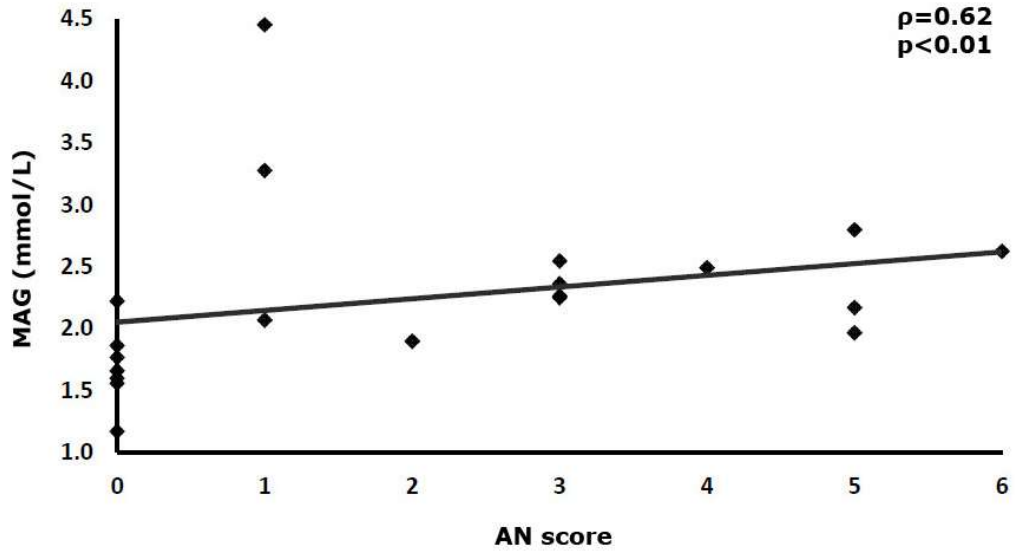

Figure 4. Correlation between the mean of absolute glucose (MAG) of continously measured interstitial glucose levels and the AN score in type 1 diabetic patients

The negative correlation coefficient (ρ=-0.5, P<0.05) reflects impaired cardiovascular autonomic function among patients with more severe GV (Figure 5).

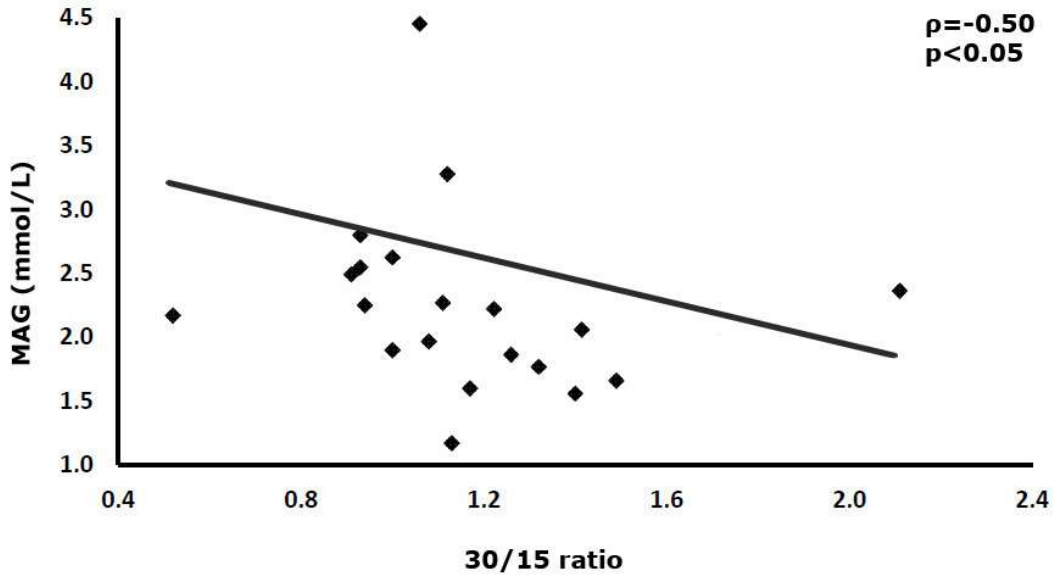

Figure 5. Correlation between the mean of absolute glucose (MAG) of continously measured interstitial glucose levels and the 30/15 ratio in type 1 diabetic patients

Similarly to SD, MAG also correlated positively with the level of orthostatic systolic blood pressure fall supporting the association between GV and sympathetic dysfunction (ρ=0.59 P<0.01; Figure 6). 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: ρ=0.62, P<0.01, MAG-AN score: ρ=0.51, P<0.05). When the correlations were adjusted for HbA_1c, age, and duration of diabetes at a multivariate analysis the relationship between SD and the systolic blood pressure response to standing remained significant (ρ=0.49, P<0.05).

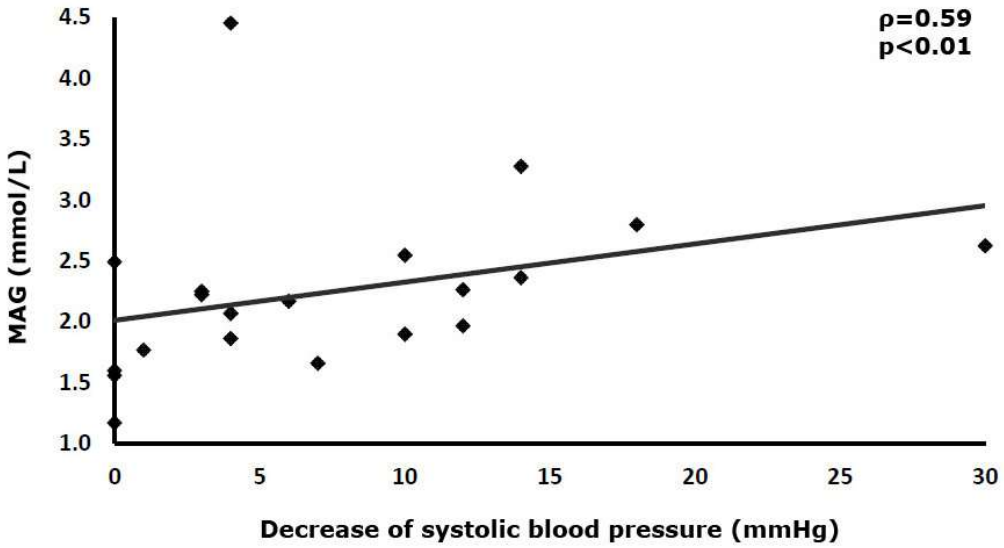

Figure 6. Correlation between the mean of absolute glucose (MAG) of continously measured interstitial glucose levels and orthostatic decrease of systolic blood pressure in type 1 diabetic patients

Higher HbA_1c levels were associated with increased GV as measured by CONGA (Figure 7) or MAG (Figure 8). This observation was proven by the positive statistical correlation between HbA_1c and CONGA (ρ=0.56, P<0.05) and MAG (ρ=0.45, P<0.05). No statistical correlations were found between age, duration of diabetes, gender, daily insulin dose or BMI of type 1 diabetic patients and various parameters of their GV. Finally, no associations were proven between glucose levels below 3.9 mmol/L and markers of GV or AN. Similarly lack of correlation was observed between the number of severe hypoglycemic episodes or hypoglycemia unawareness and the GV parameters or the severity of AN.

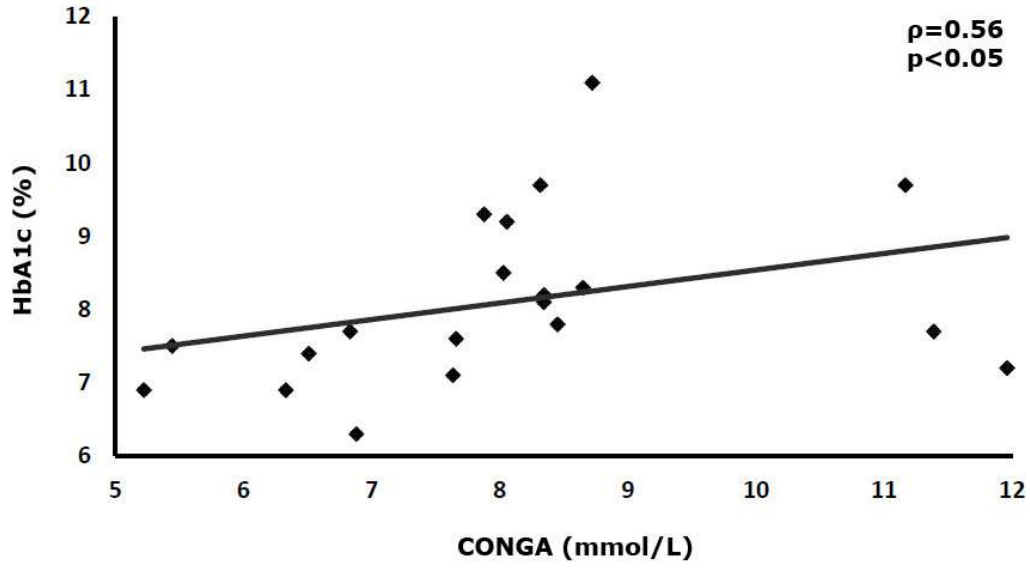

Figure 7. Correlation between the continuous overlapping net glycaemic action (CONGA) and HbA_1c in type 1 diabetic patients

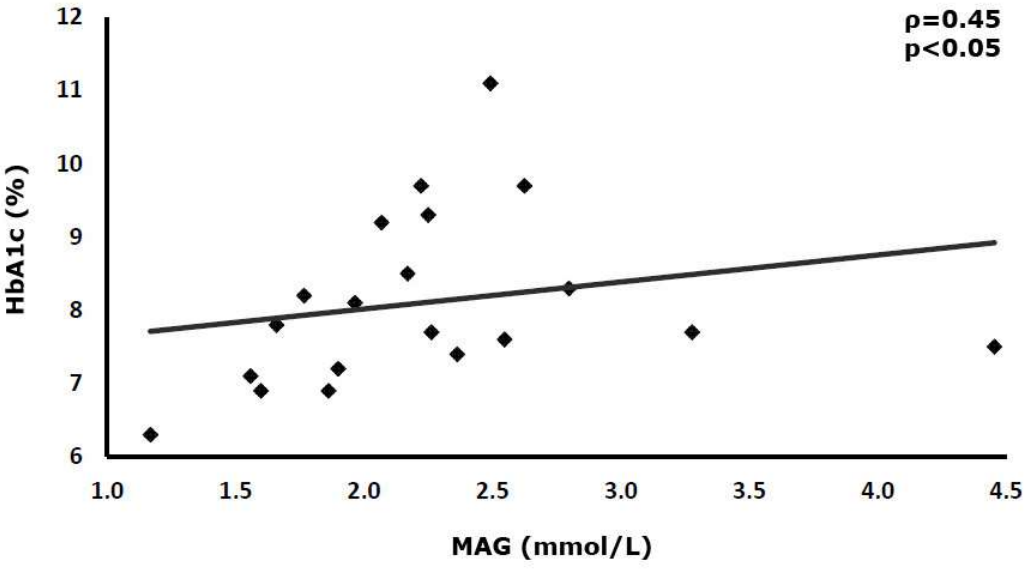

Figure 8. Correlation between the mean of absolute glucose (MAG) of continously measured interstitial glucose levels and HbA_1c in type 1 diabetic patients

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. At time of the acute pancreatitis diabetic ketoacidosis manifested and since then he has been treated with insulin. Previously diabetes was not discovered. A duodenum-preserved removal of the pancreas head due to pseudocyst was performed soon and then a multiple daily insulin treatment and a pancreatic enzyme substitution was indicated. Following these he did not attend the gastroenterology or diabetology departments in the next ten years. He injected the insulins but irregularly took his drugs and was not compliant with diet. 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. The attacks were frequent in the previous weeks and during these his tongue was bitten, defecation and tonic limb convulsions were present. He was unconscious for a few minutes and aura or tenebrosity was not observed. 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. At the admission to our Diabetes Unit he complained about intermittent diarrhoea, and symmetric bilateral sural numbness. He did not have further digestive complaints or classic hypoglycemic symptoms. His physical examination revealed asthenic appearence and a mobile subcutaneous node next to the umbilicus at the site of the insulin injections. His body weight was 57 kg with a height of 169 cm. Laboratory results: HbA_1c: 10.2%, fasting venous glucose: 33.4-11.5 mmol/L, urine protein: 6 mg/dl, ɣ glutamyl transferase (ɣGT): 109 U/L (high), Fe: 2.4 umol/L, htc: 32%, hgb: 110 g/L. 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. Results of examinations: echocardiography: telesystolic mitral valve prolapse, abdominal sonography: calcification of pancreas, gastroscopy: remains of food following 12 hours fasting, gastro-esophageal reflux disease, esophageal varicosity grade I., duodenogastric biliary reflux, antrum gastritis, duodenitis. Histology: no signs of adult celiac disease. Intraepithelial lymphocyte number (IEL): <30/100 enterocyte (normal). Ophthalmology: background retinopathy. The CRT-s revealed very severe AN at admission (Table 5). The peripheral sensory function was

assessed with a Neurometer and the CPT values were normal despite of the established severe autonomic disorder (Table 6).

Test	At admission	Follow-up	Normal value
Heart rate response to deep breathing	4/min.	1/min.	≥15/min.
Valsalva ratio	1.015	1.025	≥1.21
30/15 ratio	1.012	1.005	≥1.04
Orthostatic systolic BP fall	16 mm Hg	38 mm Hg	≤10 mm Hg
Diastolic BP elevation at handgrip	7 mm Hg	1 mm Hg	≥16 mm Hg
Overall score	8	9	≤2

Table 5. Results of the CRT-s of the patient with recurrent hypoglycemias at admission and 6 months later

Frequency (Hz)	Patient's results (mA)	Normal values (mA)
Median nerve		
2000 Hz	3.67	1.2-3.98
250 Hz	0.84	0.22-1.89
5 Hz	0.41	0.16-1.0
Peroneal nerve		
2000 Hz	1.67	1.79-5.23
250 Hz	1.52	0.44-2.80
5 Hz	1.10	0.18-1.70

Table 6. Current perception thresholds (CPT) of the patient with recurrent hypoglycemias at admission

Although the patient didn't have digestive symptoms, we measured the scintigraphic gastric emptying due to the presence of the AN. The HTE of radioiodine labelled test meal revealed an extremely slow gastric motility (HTE: 487.6 min, normal range: ≤ 67.6 min). This phenomenon gave evidence about the etiology of the recurrent life-threatening postprandial hypoglycemic episodes. 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 or sudden fall of glucose after all breakfasts and most of the dinners explaining the etiology of the unconsciousness (Figure 9). All of the hypoglycemic episodes were followed by hyperglycemic intervals in accordance with the Somogyi phenomenon.

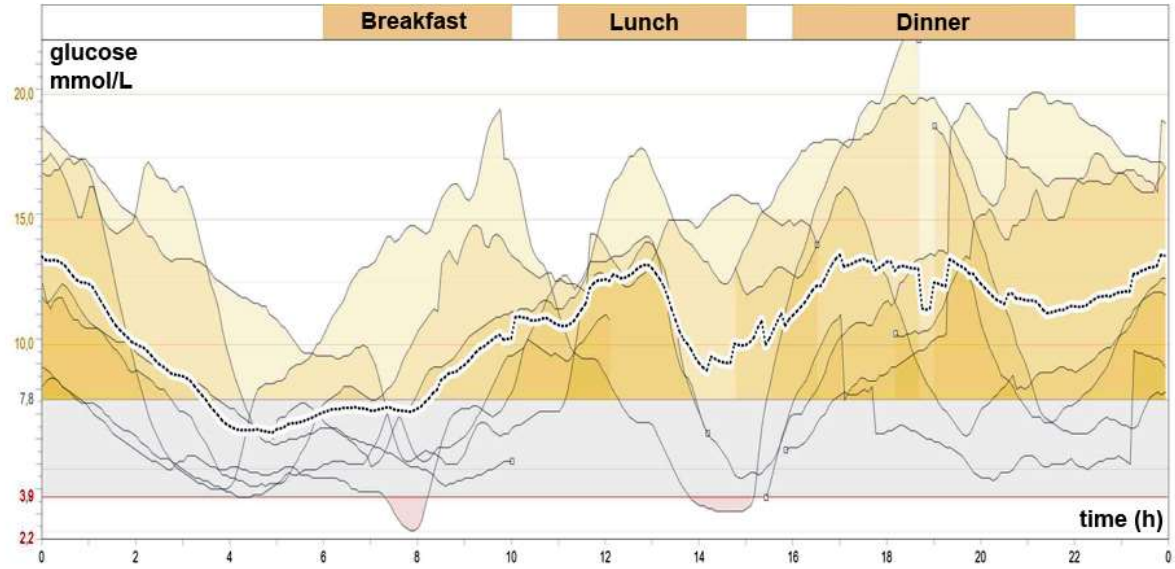

Figure 9. Interstitial glucose levels of the patient with recurrent hypoglycemias on 6 consecutive days after admission

5 Discussion

Cardiac autonomic dysfunction may be present in prediabetes as well and lead to repolarization disturbances and may increase the risk of ventricular arrhythmias and sudden cardiac death. 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. Patients with prediabetic conditions or diabetes have higher risk for sudden cardiovascular death. Cardiac AN and instability of cardiac repolarization, detected by QTc prolongation or increased QTd, contribute to the increased risk for sudden cardiac death. Prolonged QTc was related to a progressive worsening of glucose tolerance after adjustment for possible confounding factors in elderly women with IGT or diabetes [39]. Impairment of cardiac parasympathetic and sympathetic innervation as well as QT interval prolongation may play a partial role in the pathogenic mechanism of sudden unexpected death in diabetic patients. Cardiovascular adaptation mechanisms, including

baroreflex sensitivity and HR variability, are also impaired in diabetic AN that may further increase the risk for arrhythmia development [40]. However, decreased repolarization capacity and increased arrhythmia susceptibility is not necessarily preceded by significant changes in the duration of cardiac repolarization, and in these cases, cardiac repolarization reserve may be reduced without manifest QT interval prolongation [14]. Importantly, a wide range of non-cardiovascular drugs or even dietary constituents with only mild repolarization blocking effects can increase the risk for serious ventricular arrhythmias and sudden cardiac death in patients with impaired repolarization reserve. Therefore, in this clinical setting, the prediction of lethal ventricular arrhythmias is especially challenging. STVQT has been suggested as an early and sensitive indicator of temporal repolarization instability based on previous experimental and clinical studies [16, 41, 42]. Our study is the first to indicate that patients with IGT, a prediabetic condition, have repolarization instability indicated by elevated beat-to-beat STVQT. This study was not designed to assess the exact mechanisms responsible for repolarization disturbances in patients with IGT; but however, several possible mechanisms may be considered. Compelling recent evidence suggests a direct link between type 2 ryanodine receptor (RyR2) dysfunction in the endo/sarcoplasmic reticulum leading to altered intracellular calcium homeostasis, glucose intolerance, and impaired insulin secretion in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) [43, 44]. The known RYR2 mutations identified in these CPVT patients were previously linked to reduced binding affinity of calstabin2 to the RyR2 channel resulting in intracellular Ca^{2+} leak [45, 46]. In knock-in mouse models where these CPVT-linked mutations leading to RyR2-mediated Ca^{2+} leak were reconstituted, mitochondrial dysfunction and blunted ATP production with concomitantly increased sarcolemmal KATP channel function (reversible by the KATP blocker glibenclamide) were found in pancreatic β-cells to cause reduced insulin secretion and consequently, IGT. In addition to causing altered glucose metabolism and providing triggers for cardiac arrhythmias, the RyR2-mediated Ca^{2+} leak - by depleting Ca^{2+} stores - may also contribute to arrhythmia substrate creation via reduced IKs current, i.e., decreased Ca^{2+}-dependent IKs activation and consequently, impaired repolarization reserve [47]. Interestingly, and in accordance with this mechanism, reduced IKs density, impaired repolarization reserve, and increased risk for sudden cardiac death were described in diabetic dogs [17]. Although there is no doubt that RyR2 channel dysfunction is directly linked to heart failure [48], cardiac arrhythmia development [49], IGT, and reduced insulin release [50], however, further clinical studies are needed to determine whether RYR2 mutations leading to leaky RyR2 channels are frequently present in patients diagnosed with IGT in general. Repolarization instability can be a long-standing risk factor for cardiovascular

morbidity and mortality in prediabetic states and during development of diabetes. However, the role of additional cardiovascular risk factors cannot be excluded in early prediabetic conditions. Early sympathetic nerve dysfunction and insulin resistance may also play a role in the development of decreased coronary flow reserve in patients with normoglycemia [51]. In this regard, increased QT interval variability associated with sympathetic dysinnervation was observed in patients with type 2 diabetes in the supine position and the QT variability was further elevated in the context of sympathetic activation upon standing [52]. 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. Sympathetic predominance acutely evoked by graded head-up tilt test resulted in similar changes, such as decreased variance of HR and increased variance of repolarization duration [53, 54]. The prevalence of distal symmetric polyneuropathy that may result in weakness, sensory loss, pain, autonomic dysfunction, gait impairment, falls, and disability has been reported to be 11% in patients with IGT [55]. It is known that IGT is present in about 40% of patients with idiopathic peripheral neuropathy and abnormal microvascular endothelial dysfunction is common in both patient groups [56]. It has long been known that IGT is associated with AN and a shift is observed in sympathovagal balance to sympathetic overactivity [57]. Prevalence of parasympathetic dysfunction was 25%, whereas the prevalence of sympathetic dysfunction was 6% in 268 patients with IGT in the Finnish Diabetes Prevention Study [58]. Abnormal sinus arrhythmia test (55 vs 33%; P=0.004) and abnormal Valsalva maneuver (34 vs 7%; P=0.004) were significantly more frequent in patients with IGT than in control subjects; however, the frequency of abnormal postural test was not different in these two groups (P=0.334) [59]. Insulin resistance was associated with global autonomic dysfunction and an increased LF/HF (low frequency/high frequency) ratio indicating sympathetic overactivity [60]. However, the autonomic dysfunction was less significant in IGT patients than in diabetic subjects. IGT induced decrease in parasympathetic modulation (decreased HF power and 30/15 ratio) and a shift toward augmented sympathetic tone (increased LF/HF ratio) were also confirmed in an epidemiological study [61]. Putz et al. described a mainly subclinical, asymptomatic small-fiber neuropathy, and mild impairment of

cardiovascular autonomic function in IGT subjects [57]. 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. Moreover, these IGT patients also had abnormal circadian blood pressure regulation and increased diastolic blood pressure [62]. Abnormal HR recovery was more common in patients with IFG (42%) and diabetes (50%) than in participants with normal glucose tolerance (31%) in a population-based Italian study; the relative risks were 1.34 (95% confidence intervals = 1.2–1.5) and 1.61 (95% CI = 1.35–1.92), respectively. Fasting plasma glucose found to be an independent predictor of abnormal HR recovery ($P<0.0003$) even after adjustments for other confounders [63]. Moreover, impaired glucose regulation significantly ($P<0.006$) correlated with adrenergic autonomic dysfunction when age, an important confounder, was removed from the model [64]. The self-assessment of autonomic symptoms by patients with IGT and early diabetes correlated to the degree of autonomic dysfunction defined by abnormal 30:15 ratio and reduced quantitative sudomotor axon reflex test sweat volume [65]. Further clinical studies are warranted and needed to evaluate whether there is a direct link between the increased STVQT detected in our study and increased risk for sudden cardiac death in patients with IGT, preferably in a large patient cohort.

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 in the literature. 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 GV, while patterns of hypoglycemia were not associated with AN or GV.

These results supplement the published observations in the literature. Previously altered motor and sensory axonal functions were found in patients with type 1 diabetes in the presence of high MAGE, an important marker of GV acquired from continuous interstitial glucose monitoring [66]. In another study, type 1 diabetic patients with painful neuropathy had a higher mean glucose, a greater M-value, and more glycemic excursions compared with the painless group [67]. Interestingly, in DCCT, conducted on type 1 diabetic patients, GV calculated from the 7-point daily glucose profiles was not associated with retinopathy, nephropathy or cardiovascular AN. The discrepant findings in DCCT with our study might be explained by different methods: the 7-point daily glucose profile is less sufficient to express GV than CGM applied in our study. Moreover, 30/15 ratio from the CRT-s was not performed in DCCT while

we used it [68]. Although GV often occurs in type 1 diabetic patients, the possible associations of this metabolic imbalance and neuropathy were more frequently analyzed in type 2 diabetic patients [69, 70]. GV was identified as a risk factor of macrovascular complications as well as cardiovascular and malignant diseases but only in type 2 diabetic patients [71, 72]. The connection between AN and hyper/ or hypoglycemia is well documented. Autonomic nerve dysfunction is related to decreased hypoglycemia awareness leading to late realization and non-efficient management of abnormally low glucose [73]. Impaired counter-regulatory response of epinephrine, norepinephrine to hypoglycemia was found in AN explaining the clear association between the occurence of severe hypoglycemia and advanced cardiovascular AN [74, 75]. Finally, hypoglycemia-associated autonomic failure has been described [76]. On the other hand, parasympathetic autonomic dysfunction has been shown to be associated with postprandial hyperglycemia among newly diagnosed type 2 diabetic patients [77]. Slower gastric emptying mainly as a consequence of a parasympathetic involvement develops during the progression of diabetic neuronal damage [78]. Gastroparesis in AN is considered as the underlying mechanism in patients with unexplained periods of hypoglycaemia followed by hyperglycemia [79, 80]. Abnormal gastrointestinal peptide release due to autonomic dysfunction (including pancreatic polypeptide, motilin) cause additional motility and secretory dysfunctions that result abnormal carbohydrate absorption [81]. In our study several parameters of GV acquired from continuous interstitial glucose measurement associated with results of CRT-s and HbA_1c of type 1 diabetic patients with long-standing disease. Continuous interstitial glucose detection provides a more detailed glucose time series than the self-monitored capillary glucose sampling or the variabiliy of HbA1c. This method ensures the calculation of at least 10 parameters that describe glycemic stability of diabetic patients [36]. The mean values of GV parameters reflected serious glycemic instability in this group of patients with long-standing type 1 diabetes (Table 4). SD as the most widely applied parameter that shows a linear relationship with mean glucose [22] correlated with the overall severity of cardiovascular AN and the systolic blood pressure response to standing. 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 GV 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 were both altered if GV was enhanced. The statistical correlation between the measured GV and cardiovascular autonomic functions allows a conclusion that there might be a causal relationship betveen glycemic instability and AN. The original correlations between AN score and GV indeces were reproduced, when handgrip tests were excluded from the calculation of the AN scores of the patients. The low sensitivity and specificity of the handgrip test in the diagnosis of cardiovascular AN and its high dependency on hypertensive status and baseline diastolic BP were proven before [82]. The similar significant correlations without handgrip tests support the hypothesis that handgrip has a low value in the measurement of AN and strengthens the reproducibility of the observed correlations between autonomic function and GV. The relationship between AN and GV was proven, although the mean severity of AN was moderate of the patients, while the mean GV was severe (Table 4). Several factors may also impact GV, including duration of diabetes, age, BMI or HbA_1c. When we analyzed the effects of these parameters on GV separately no correlations were found between the variables. However, when the correlations between AN and GV were adjusted for age, diabetes duration, HbA_1c and BMI at a multivariate analysis, the relationship between SD and the systolic blood pressure response to standing remained significant ($\rho=0.49$, $P<0.05$), the further correlations lost their significance. It can be concluded that most of the associations of AN and GV are influenced by the longer duration of diabetes, higher age, HbA_1c or BMI. By the fact that the correlation between SD and orthostatic hypotension remained significant after adjustment for confounding variables, it is noteworthy to stress the concept that the relationship of advanced AN and severe variability of glucose is independent from other factors in type 1 diabetic patients with long term disease. Orthostatic hypotension is a characteristic sign of the late progressive stages of AN [8] and primarily refers to the impairment of the sympathetic autonomic function. Orthostatic blood pressure response was among the two tests associated with a high risk of severe hypoglycemia in the EURODIAB IDDM Complications Study [75]. In our study, SD and MAG both correlated with orthostatic systolic blood pressure fall that underlines the association of sympathetic dysfunction and severe GV. All of these observations point to the coexistence of advanced AN manifested in abnormal sympathetic function and increased GV in type 1 diabetic patients.

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 HbA_1c 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. HbA_1c shows slow fluctuations in average glycemia taking place over months, while GV parameters describe amplitudes only or amplitudes and timings of glycemic excursions of much shorter interval [83]. Thus GV markers seem to describe the glucose profile of type 1 diabetic patients with long-standing disease more accurately and identify earlier any worsening of glycemic control than HbA_1c tests. One explanation of our finding is that the patients in this study migh have spent more time in hyperglycemia than hypoglycemia if their variability markers were high. The hypoglycemic episodes were frequently followed by hyperglycemia and these intervals might have added to the 'purely' hyperglycemic episodes. The mean value of the HbA_1c of the patients was over the target (8.1%) supporting the longer standing hyperglycemia. Analyzing the variability markers separately, MAG is relatively weakly associated with hypoglycemia and reflects more hyperglycemia. On the other hand, calculation of MAG includes a timing component and is not a purely amplitude describing parameter that might explain the parallel change with HbA_1c [84]. The other parameter, CONGA is calculated from the difference between a current and earlier glucose measurements and expresses the SD of these differences reflecting definitely the timing of variability. These markers incorporating a time-dependent description of GV might have parallel kinetics with the change of HbA_1c [85]. The associations between HbA_1c and GV as well as GV and AN allow conclusions that the higher HbA_1c is responsible for AN and AN leads to GV but it also could be assumed that GV manifests in higher HbA_1c that results AN.

The relatively low number of patients might be considered as a limitation of our study. Nevertheless, a similar or even smaller number of diabetic patients were recruited in other studies on the same field. [73, 74, 86, 87]. It should be added that according to the protocol of our study, AN and CGMS assessments were performed only in those cases in which both were indicated as a part of a medical management. The lack of the assessment of the symptoms of AN are among the limitations although these would predict the possible presence of autonomic dysfunction. A further limitation is that not all but two of four GV parameters correlated with the AN tests. One of these parameters is the most frequently applied standard GV marker, the SD by which our data are comparable with further studies. Due to our study design it is not possible to differentiate whether GV induces neuropathy or the glycemic instability is a consequence of neuropathy. Beside this lack of the proven causal relationship our data draw attention to consider the possible presence of cardiovascular autonomic impairment in case of

glycemic instability of type 1 diabetic patients.

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. The chronic alcohol intake impaired the glucose producing capacity of the liver resulting less effective blood glucose correction in case of hypoglycemia. Previous studies revealed the inverse correlation between C peptide level and GV. In our patient the exocrine disease reached the Langerhans islets and led to decreased endogenous insulin secretion. This loss of insulin secretory capacity particularly explains the unstable glucose levels. The CRT-s established a very serious AN, as the measured autonomic score was 8 (the maxium score is 10). Analyzing the CRT-s separetely revealed that all tests of the patients had an abnormal result and both the parasympathetic and sympathecic functions were affected (Table 5). This severe neuropathy is the result of the common harmful effects of diabetes and chronic alcohol consumption. The data of the literature prove that in the presence of only one abnormal test there was no association with the increased frequency of severe hypoglycemia but in case of two altered tests the risk of severe hypoglycemias is increased by 1.7 times higher [75]. An interesting aspect of the case is the normal peripheral sensory function on the upper and lower limbs beside the severe AN (Table 6). Previous studies support our observation that it is not evident to develop the same severity of damage on different neuronal systems - although the etiology is similar [88]. Seriously delayed gastric emptying is a well-known finding in patients with AN, mainly in case of parasympathetic dysfunction. [89]. The emptying of the isotope-labeled test meal was extremely long in our patient as it was 7 times longer than in the healthy controls. The patient didn't have any digestive complaint although the seriously delayed stomach emptying. This means that gastroparesis can be found in patients with hypoglycemic episodes even without any gastrointestinal symptoms in the presence of severe AN [78]. The continuous observation of the interstitial glucose levels have proven the recurrent daily hypoglycemic episodes after breakfasts and dinners. This method had a prominent role in the exploration of the repetitive nature of the hypoglycemic episodes. The post-breakfast hypoglycemias were the most severe that explain the repeated unconsciousness with convulsions. The regular morning hypoglycemic episodes were followed by hyperglycemic peaks late in the morning and at noon as parts of Somogyi phenomenon. The patient injected rapid acting human regular insulins before breakfast, noon and dinner and an intermediate acting NPH insulin at bedtime. Although the patient consumpted all the meals in

the morning, the abnormal handling of the glucose to the duodenum due to the gastroparesis delayed the absorption and led to extremely low glucose levels. Moreover, the glucose absorption was also further altered by the insufficent digestive enzyme production of the pancreas as a result of the untreated chronic pancreatitis. The compliance of the patient was very low as he didn't attend the outpatient check-ups and didn't take the prescribed pancreatic enzyme substitution. This behavior might have been associated with his cognitive dysfunction induced by the recurrent hypoglycemias. His insulin treatment was adjusted in order to attenuate the GV by the initiation of a long-acting insulin analogue which was titrated up to the highest required dose. At the same time his preprandial bolus insulin doses were reduced to the lowest effective level. This 'more basis – less bolus' concept dimished the slow elevations of the glucose level and prevented the hypoglycemias. Oral pancreatic enzyme substitution was applied to ensure the more physiologic carbohydrate digestion and absorption. Low fiber containing and small portions of meals were adviced. In order to treat neuropathy the antioxidant alfa lipoic acid was administered parenterally and later orally which has an established effect on autonomic neuronal dysfunction by data of the literature [90]. Metoclopramid was also injected to achieve an enhanced gastric motility, decreased pyloric sphincter tone and an attanuation of nausea. Applying these treatments resulted that hypoglycemia was not realized in the next 6 months and the emptying of the stomach was 2 times faster than at the initial test. The results of CRT-s did not change during the 6-month treatment period (Table 5). The repeated CGM prove that his glucose levels were mainly still not in the normal range but the recurrent hypoglycemic episodes disappeared and an overall repairment of the glycemic control is achieved. The patient reported a characteristic beneficial effect on his quality of life.

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 observed disturbance of cardiac repolarization is associated with the relative dominance of sympathetic autonomic function in IGT.

3. The elevated temporal short-term QT variability and the concomitant cardiovascular autonomic neuropathy may serve as early indicators of the increased instability of cardiac repolarization and elevated risk for sudden cardiac death in patients with prediabetic states.

4. Our studies on patients with long-standing type 1 diabetes established at first that increased glycemic variability is in a close relationship with advanced autonomic neuropathy and might be manifested in higher HbA1c.

5. The altered sympathetic function in type 1 diabetic patients with increased glycemic variability was proven.

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

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

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