University of Szeged
Albert Szent-Györgyi Medical School
Doctoral School of Interdisciplinary Medicine

The assessment of neuropathic dysfunction in Type 1 diabetic patients: the role of metabolic and molecular/inflammatory mechanisms

PhD Thesis

Barbara Bordács MD.

Supervisors:
Tamás Várkonyi MD, Habil.
Krisztina Kupai MD, PhD

Szeged

Introduction

Diabetes mellitus is a chronic, non-communicable, complex metabolic disease that primarily affects carbohydrate metabolism, but its impact on protein and lipid metabolism can not be neglected. Diabetes is the most prevalent global cause of mortality and morbidity. It is estimated that around 537 million people have diabetes and the number is expected to increase constantly. Diabetes is characterized by hyperglycemia which is caused by absolute or relative insulin deficiency. In order to maintain glucohomeostasis, various hormones and neuropeptides interplay that are produced by the pancreas, liver, brain, intestine, adipose and muscle tissue. The central part is the endocrine pancreas, that secretes insulin and glucagon to sustain appropriate blood glucose levels.

Diabetes can be classified into 4 groups :

1.) Type 1 diabetes mellitus (T1DM),
2.) Type 2 diabetes mellitus (T2DM),
3.) Diabetes due to other causes (monogenic, neonatal, maturity-onset diabetes of the young, pancreas diseases,

drug-induced, endocrine disorders causing diabetes mellitus) and
4.) Gestational diabetes mellitus

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease, characterized by absolute insulin deficiency due to the autoimmune destruction of pancreatic beta cells.
There are many short-, and long-term complications attributed to diabetes mellitus. Long-term complications include micro-, and macrovascular damages. The latter includes cardiovascular diseases: coronary heart disease, atherosclerosis, peripheral arterial disease, stroke. The microvascular conditions include the classic triad: retinopathy, nephropathy and neuropathy

Polyneuropathies are the degenerations of peripheral and autonomic nerves due to different etiologies that are causing are causing different clinical symptoms Diabetic neuropathy severely impacts the quality of life of patients by causing lower extremity pain, leg ulceration, an increased need for amputations, and higher mortality rates in both T1DM and T2DM patients. There are many factors that contribute to neuropathic impairment including

glucotoxicity, activation of alternative metabolic pathways, the accumulation of advanced glycated end products (AGEs), oxidative stress, the activation of proinflammatory and prothrombotic mechanism. Additionally dyslipidaemia and lipid peroxidation play critical roles in the pathomechanism both in T2DM and T1DM.

Compared to the healthy population, patients with diabetes have a higher risk of cardiovascular complications and mortality, particularly due to the elevated levels of detrimental metabolic parameters that contribute to atherosclerosis Cardiovascular autonomic neuropathy (CAN) is the main reason for the high mortality. First the parasympathetic nervous system is concerned with silent ischaemia, tachycardia while resting, increased sympathetic tone, arrhythmias, abnormal adaptation, higher risk for cardiovascular events, while later the sympathetic nerves are injured, manifesting in orthostatic blood pressure drop on standing up causing dizziness, frailty, syncope.

Recent studies show that the process of NETosis plays an important role both in the pathogenesis and the complications of T1DM and T2DM. Neutrophil granulocytes are crucial parts of the innate immune system by phagocytosing and degrading pathogens (mainly bacteria and fungi) with their cytotoxic enzymes. During the process, they create neutrophl extracellular traps (NETs), that are weblike deoxyribonucleotid acids (DNAs) containing substances released by neutrophils. These include histones, granule proteins (neutrophil elastase [NE], proteinase 3 [PR3], myeloperoxidase [MPO]) and cytosolic proteins (actin, alpha-actinin, S100 calcium-binding proteins). In T1DM as the autoimmune destruction of beta-cells starts the neutrophiles take part in the process. Previous studies revealed, that in case of T1DM, the absolute number of neutrophils in the peripheral blood is decreased, while the NE, PR3 and PAD4 was significantly higher in neutrophils from T1DM patients, than in the control groups and correlated positively with autoantibodies against beta-cells.

In the 2010s, a new technique, called continous glucose monitoring (CGM) was developed in which glucose levels in the interstitial space in the subcutis are detected by an electrode placed under the skin. These devices supply real-time glucose readings, trend arrows indicating the direction and rate of glucose change, and customizable alerts for impending hypoglycemia or hyperglycemia. The most important CGM metrics are time in range (TIR), time above range (TAR) and time below range (TBR). The device is known to significantly improve carbohydrate metabolism by reducing HbA1c levels and also decreasing long-term complications. This improvement in glycemic control is crucial, as hyperglycemia is known to adversely affect lipid metabolism, leading to dyslipidemia, which is a risk factor for cardiovascular disease

Objectives and methods:

The aim of the thesis was to evaluate the effects of CGM on both microvascular and macrovascular complications of diabetes, as well as on lipid metabolism, that remained insufficiently characterized until now in T1DM. We aimed to investigate the potential associations between various

components of complex metabolic control and diabetic neuropathy in individuals with T1DM in a comprehensive clinical study. This trial assessed autonomic and peripheral sensory nerve functions, inflammatory/NETosis biomarkers, as well as metabolic parameters including glycemic and lipid profiles. Particular emphasis was placed on the potential of CGM to enhance metabolic and inflammatory regulation and to preserve neuronal function. Furthermore, we systematically analyzed key CGM-derived metrics in our T1DM cohort to explore their correlations with cardiovascular risk factors.

- Patients were recruited in a diabetology outpatient clinic (Department of Medicine, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary). The inclusion criteria were a diagnosis of T1DM and an age between 18–65 years. During the visits, blood sampling, neuropathy tests, and the collection of CGM data were performed. Patients were divided into two groups based on whether they used CGM or not. A total of 61 patients were studied. In total, 24 patients used CGM sensors and 37 did not. A total of 19 of the CGM user patients had been

wearing sensors for more than 4 years while 5 of them used sensors for between 6 months and 4 years. All measurements involved in the thesis were conducted with prior approval from the relevant ethics committee (67/2022-SZTE, approval date: 7 JUNE 2023).

- The metabolic state was evaluated by measuring TG, HDL, LDL and total cholesterol, HBA_{1c} levels. The reference ranges for these parameters in our laboratory are: TG: <1.70 mmol/L, HDL: >1.40 mmol/L, LDL <3.0 mmol/L, and total cholesterol: <5.20 mmol/L.
- Citrullinated histone H3 (cit-H3), PAD4 and NE levels of 29 T1DM and 9 healthy individuals from human serum regarding NETosis markers were measured with enzyme-linked immunosorbent assay (ELISA). The unit of measurement is pg/mg for citrullinated histone H3, ng/mg for PAD4 and pg/mg for NE.
- We collected electronical reports from the patients at the same time with the laboratory testing of the blood samples. Examinating all the values, we used TIR, TAR and TBR data for finding correlations.
- Peripheral sensory nerve function was assessed by a Neurometer (MSB Ltd, Balatonfüred, Hungary) and a

calibrated tuning fork applied to the upper and lower extremities.

Autonomic function was characterized with Ewing's standard cardiovascular reflex tests in the study. Parasympathetic dysfunction was examined by measuring deep breathing test, Valsalva ratio (VR), and 30:15 ratio, while sympathetic nervous system impairment was tested by orthostatic blood pressure drop on standing up. As handgrip test is becoming a marker of hypertension and its complications more and more, we did not use in our evaluation.

Results

A total of 61 patients (age: 42.5 ± 1.8 years, DM duration: 22.8 ± 1.6 years, BMI: 25.3 ± 0.9, HbA1c: 8.1 ± 0.2%; mean ± SE) were studied. In total, 24 patients used CGM sensors (age: 35.7 ± 2.5, DM duration: 20.0 ± 1.8, BMI: 24.5 ± 0.8, HbA1C: 7.5 ± 0.8%) and 37 did not (age: 45.9 ± 1.8, DM duration: 25.0 ± 1.9, BMI: 26.5 ± 0.8, HbA1C: 8.3 ± 0.3%).

The mean sensor parameter values of the patients were close to the target range (TIR: 70.4±2.5%, TAR: 24.6±2.3%, TBR: 4.6±1.2%, mean±SE).

Metabolic parameters were 7.5±0.8 % for HbA1c, 0.9 ± 0.1 mmol/L for TG, 4.7 ± 0.2 mmol/L for cholesterol, 2.6 ± 0.1 mmol/L for LDL, 1.7 ± 0.1 mmol/L for HDL (mean±SE).

- Among the metabolic values, positive correlations were found between HbA1c and TAR ($r=0.56$, $p<0.05$) and between TG and TAR ($r=0.53$, $p<0.05$). The autonomic tests revealed correlations between TAR and orthostatic blood pressure drop on standing up ($r=0.57$, $p<0.05$) and between TBR and 30:15 ratio ($r=0.53$, $p<0.05$).
- A significant positive correlation was found between HbA1c and TG levels in the overall group of T1DM patients ($r = 0.28$, $p = 0.045$). The mean lipid parameters were within the normal range. However, neither the HbA1c nor the lipid parameters showed a significant correlation with the cardiovascular autonomic or peripheral sensory function ($p > 0.05$ in all cases).

For further analysis of the metabolic differences, the diabetic group was divided into two subgroups based on CGM usage.

24 patients wore CGM, their parameters were: HbA1c: 7.5 ± 0.8 %, TG: 0.9±0.1 mmol/L, cholesterol: 4.7 ± 0.2, LDL: 2.6 ± 0.1 mmol/L, HDL: 1.7 ± 0.07 mmol/L. Among the 37 CGM non-users: HbA1c: 8.3 ± 0.3 %, TG: 1.2±0.1 mmol/L, cholesterol: 4.9 ± 0.1, LDL: 2.9 ± 0.1 mmol/L, HDL: 1.4 ± 0.076 mmol/L.

- CGM users exhibited significantly different metabolic parameters, including lower TG (0.9 ± 0.1 vs. 1.2 ± 0.1 mmol/L, $p = 0.034$) and HbA1C (7.5 ± 0.2% vs. 8.3 ± 0.3%, $p = 0.04$) levels, as well as higher HDL cholesterol levels (1.7 ± 0.1 vs. 1.5 ± 0.1 mmol/L, $p = 0.02$), compared to non-users.
- The evaluation of the cardiovascular autonomic reflex tests revealed a significant difference in VR values between CGM users and non-users (1.38 ± 0.06 vs. 1.27 ± 0.04, $p = 0.045$). No significant differences were observed in the results of the remaining three tests although results were more physiological in the CGM group.

- Using the Neurometer, we found a significant difference in large sensory nerve fiber function between CGM users and non-users, specifically in the perception thresholds at the median nerve during 2000 Hz stimulation (224.4 ± 21.2 vs. 290.6 ± 17.7, p = 0.01). No significant differences were observed in the results of further tests on the median and peroneal nerves between the CGM users and non-users.
- Calibrated tuning fork tests revealed significant differences in the vibration sense between CGM users and non-users at the right radius (7.4 ± 0.1 vs. 7.1 ± 0.1, p = 0.01) and the right hallux (7.2 ± 0.2 vs. 6.1 ± 0.3, p = 0.005). However, five out of six CPT values were higher, indicating some degree of hypesthesia among non-CGM users.

  We evaluated plasma cit-H3, PAD4 and NE regarding NETosis markers in 29 T1DM (age: 40.9±1,7, HbA1c: 7.6± 0.18 [means±SE]) patients and 9 healthy individuals with normal HbA1c and lipid parameteres (age: 32.3±2.6 [mean±SE]).

- The values for cit-H3 showed a significant difference between T1DM and healthy individuals: 0.21±0.03 vs. 0.27±0.01 (mean±SE), p=0.003, while there was no significant difference between CGM-user and non-user group.

  The level of cit- H3 showed no significant correlations with any of the metabolic parameters, including HbA1c, total cholesterol, LDL, HDL and TG.
- Regarding PAD4, there was no significant differences neither between the T1DM and control, nor the CGM-user and non-user groups.

  After further analysation, PAD4 and TG levels showed a strong positive correlation (r=0.49, p=0.006, while HbA1c, total cholesterol, LDL and HDL values showed no significant correlations with PAD4.

- Serum NE levels were higher in the control group, but the difference was not significant. The CGM and non- CGM groups did not differ statistically significantly. The NE of our T1DM patients showed no relevant correlations with HbA1C, TG, LDL, HDL or total cholesterol.

Conclusions

- A clear positive correlation was found between HbA1C and fasting TG levels among our participants. This relationship suggests that poor glycemic control is associated with elevated TG levels in T1DM patients as well. Alternatively, the presence of hypertriglyceridaemia may coinside with higher HbA1c levels.
- Upon analyzing CGM parameters, a significant positive correlation was identified between TAR and TG levels, as well as between TAR and HbA1c.
- Our findings highlight that elevated TG levels are not exclusively a sign of insulin resistance but are also associated with the worse glycemic control in insulin-dependent patients.
- Our findings revealed that patients not using CGM exhibited significantly higher HbA1c and fasting TG levels, whereas CGM users demonstrated higher HDL levels. CGM not only advantageous for improving HbA1c levels, but also several other lipid parameters.
- The calibrated tuning fork test indicated a better vibratory sensory function on the upper and lower extremities in patients wearing CGM. Similarly, Neurometer

measurements revealed lower perception thresholds within the normal range at 2000 Hz stimulating frequency on the median nerve in CGM users suggesting a better sensory condition in these subjects. Both methods assess the functionality of the large sensory nerve fibres which were found to be in a more physiologic state in CGM users.

- The application of CGM in individuals with T1DM has shown promise in mitigating the progression and severity of autonomic neuropathy through enhanced glycemic management.
- Our findings demonstrate significant positive correlations between TAR and orthostatic blood pressure changes upon standing, as well as between TBR and the 30:15 ratio, indicating that hyperglycemia adversely affects the sympathetic function, while hypoglycemia impacts the parasympathetic regulation.
- It arises that continuous glucose monitoring is beneficial for preventing both micro-, and macrovascular complications.

- Regarding NETosis, with our finding it arises that PAD4 is a possible macrovascular risk factor in T1DM by having an influence on lipid metabolism, especially TG levels.

List of publications linked to the thesis

MTMT identification: 10083307

1. **Bordács B.,** Várkonyi Á., Valkusz Z., Nyiraty S., Pósa A., Menyhárt A., Lengyel C., Kempler P., Kupai K., Várkonyi T. **Comprehensive Assessment of Neuropathy and Metabolic Parameters in Type 1 Diabetic Patients with or Without Using Continuous Glucose Sensors.** Int J Mol Sci. 2025 Feb 26;26(5):2062. **IF:4,9, D/Q rank: Q1**
2. **Bordács B.,** Várkonyi Á., Valkusz Z., Nyiraty S., Pósa A., Menyhárt A., Lengyel C., Kempler P., Kupai K., Várkonyi T. **Keep the balance: the multiple effects of continuous glucose monitoring on the management of type 1 diabetes.** Diabetes, Stoffwechsel und Herz, Band 34, 3/2025, p:155. **IF:0.9, D/Q rank: Q4**

Other publications:

3. Nemes A.; **Bordács B.;** Ambrus N.; Lengyel C. **Simultaneous Assessment of Left Ventricular Volumes and Aortic Valve Annular Dimensions by Three-Dimensional Speckle-Tracking Echocardiography in Healthy Adults from the MAGYAR-Healthy Study—Is**

There a Relationship? Life 2025, 15, 742. **IF: 3.2, D/Q-rank: Q1**

4. Nemes A.; **Bordács B**.; Ambrus N.; Lengyel C. **Longitudinal Systolic Excursion of the Mitral Annular Plane and Left Ventricular Rotational Mechanics Are Associated in Healthy Adults—Three-Dimensional Speckle-Tracking Echocardiography-Derived Insights from the MAGYAR-Healthy Study.** J. Clin. Med. 2025, 14, 3201. **IF: 3.0, D/Q-rank: Q1**
5. Nemes A, **Bordács B,** Ambrus N, Lengyel C. **Complex Associations Between Systolic Left Atrial and Left Ventricular Deformations in Healthy Adults-Detailed Analysis from the Three-Dimensional Speckle-Tracking Echocardiographic MAGYAR-Healthy Study.** Life (Basel). 2025 Feb 12;15(2):287. **IF: 3.2, D/Q-rank: Q1**
6. Dobra G, Gyukity-Sebestyen E, Bukva M, Boroczky T, Nyiraty S, **Bordacs B,** Varkonyi T, Kocsis A, Szabo Z, Kecskemeti G, Polgar TF, Szell M, Buzas K. P**roteomic profiling of serum small extracellular vesicles predicts post-COVID syndrome development.** Clin Immunol. 2025 May 24;278:110532. **IF: 4.5, D/Q rank: Q2.**