

Ph.D. THESIS

SUMMARY

SODIUM-GLUCOSE COTRANSPORTER-2 INHIBITORS BEYOND THE GLYCEMIC CONTROL

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I. INTRODUCTION - STUDY MOTIVATION

Diabetes mellitus (DM) is a chronic, non-communicable, metabolic, heterogeneous disease from the etiopathogenic, clinical, and therapeutic point of view, which requires a multifactorial treatment strategy, not just glycemic control. The severity of this disease is dictated by its increased prevalence worldwide. Often underdiagnosed, DM alters the quality of life, and accompanies many complications. Moreover, diabetes is a significant risk of developing CV disease, the leading cause of death in patients with DM.

The ideal treatment in type 2 diabetes (T2D) would consist of a minimum number of molecules that act on as many pathogenic mechanisms as possible, being effective in blood glucose control, without risk of hypoglycemia, without weight gain, with beneficial CV effects. Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are a new generation of drugs, called gliflozins, oral antihyperglycemic, which lower blood glucose by eliminating glucose through urine, independent of insulin secretion. Compared to other treatment molecules, SGLT2i offers the benefit of weight loss, lowers blood pressure, and has a reduced risk of hypoglycemia. At the same time, this class of drugs has proven CV protection in patients at increased risk and has slowed the progression of chronic kidney disease.

The present study aimed to observe and identify the pleiotropic side effects of SGLT2i (Dapagliflozin) treatment in patients with T2D.

In these patients, visceral (epicardiac, mediastinal, intra-abdominal) and subcutaneous adipose tissue, cardiac and renal function, fatty liver load, and degree of liver fibrosis, respectively, after initiation of Dapagliflozin treatment, were evaluated in dynamics to search for possible associations between all these factors and the evolution of metabolic parameters, respectively anthropometric, under treatment with SGLT2i.

II. LITERATURE REVIEW

DM is considered a heterogeneous syndrome characterized by hyperglycemia that results either from impaired insulin secretion, insulin resistance, or both. In the long term, this chronic hyperglycemia causes dysfunction or insufficiency of several organs. DM complications appear as a consequence of the disease and are closely linked to glycemic control, the more precarious it is, the more frequent and precocious the complications. DM represents a significant cause for the onset of atherosclerosis, the anatomical substrate that underlies the diabetic macroangiopathy clinically manifested by coronary heart disease, cerebrovascular disease, and peripheral arterial disease.

DM is a significant risk factor in the development of heart failure. In DM, heart failure (both systolic and diastolic) may develop in the presence of risk factors such as atherosclerosis, high blood pressure, age, long-standing DM, and renal dysfunction. Although diastolic dysfunction is rare in people who do not have diabetes, recent studies have shown that between 40 and 75% of people with T2D, without clinical signs of heart failure, have left ventricular dysfunction at cardiac ultrasounds screening, in the absence of risk factors. Diastolic dysfunction is a pathological condition preceding left heart failure, often asymptomatic and insufficiently diagnosed and characterized by altered relaxation and compliance of the left ventricular myocardium. The gold method for its diagnosis is transthoracic Doppler echocardiography at the mitral valve level.

For several decades, T2D therapy has focused on reducing blood sugar, based on the idea that intensive glycemic control will bring microvascular and macrovascular benefits. Clinical studies, on a large number of patients with DM, demonstrated the link between the glucose level expressed by HbA1c and the occurrence of chronic complications, highlighting, on the one hand, the beneficial effect of strict glycemic control on microangiopathic complications.

SGLT2i represents a new class of oral antidiabetic drugs with a unique mechanism of action, independent of insulin secretion. Through its unique mechanism of action, SGLT2i determines pleiotropic effects. Recent studies with cardiovascular targets have shown that SGLT2i reduces the risk of cardiovascular events, particularly heart failure, in patients with T2D and cardiovascular disease or multiple cardiovascular risk factors. The mechanisms

underlying these SGLT2i-induced cardiovascular benefits are independent of its effects on HbA1c, blood pressure, or cholesterol.

III. RESEARCH

1. CLINICAL TRIAL

1.1. MATERIALS

We performed a prospective observational study over one year, on 53 patients, in which their diabetologist decided to introduce 10 mg of Dapagliflozin in their therapy. They underwent a clinically, imaging, and biologically assessment before and after six months of SGLT2i therapy.

1.2. METHODS

Clinical data such as age, duration of diabetes, medical history as well as abdominal circumference, height, weight, blood pressure were obtained, and laboratory tests were performed during a one-day hospitalization.

For the evaluation of the patients' adipose tissue, a native CT scan was performed using available equipment Philips, MX 16. The images were subsequently processed using 3D Slicer digital analysis software version 481. For the selection of adipose tissue, a density threshold between -30 and -190 Hounsfield units was used. The epicardial (EPI), abdominal visceral fat at the 4th lumbar vertebra, and subcutaneous fat volumes were obtained. We corrected the results according to the patient's body surface. The thickness of the anteriorly EPI and mediastinal adiposity at the retrosternal level, the thickness of the left paravertebral abdominal visceral adipose tissue, at the level of the 4th lumbar vertebra, between the psoas muscle and the internal fascia of the musculature were measured, by an experienced radiologist.

Transthoracic cardiac ultrasound was performed in dynamics at the study inclusion, then at six months, respectively, at one year of treatment with SGLT2i, by a primary cardiologist, using Aloka SSD 4000 equipment. We performed screening for type 1 diastolic dysfunction (DD1) that we defined as a ratio $E/A < 1$ and $E/e' > 15$.

FibroScan® equipment (EchoSens, Paris, France), which measures the transient controlled vibration by the liver resistance and quantifies the fatty load with a controlled attenuation parameter (CAP), was used to assess the degree of fibrosis and hepatic steatosis. We performed this investigation on all patients included in the study, under fasting-state, by a gastroenterologist.

Statistical analysis was performed with SPSS version 20.0 (SPSS Inc. Chicago, IL, USA) and Microsoft Office Excel 2019. The Kolmogorov-Smirnov test was applied to see how the numerical variables are distributed. The central tendency and dispersion indicators of the numerical parameters were calculated. Continuous numerical variables are presented as mean \pm standard deviation and numeric variables with nonparametric distribution as median [interquartile range]. The qualitative/nominal variables are described as the number of individuals in the class and a percentage of the total subgroup.

To evaluate the differences between the indicators of the central tendency between the groups, we used: • t-student tests (paired), • ANOVA tests, • post-hoc tests (Bonferroni), • Friedman tests, • Mann-Whitney-U tests.

To test the statistical significance of the differences between percentages/proportions/prevalences, we used chi-square and chi-square tests for the trend. For the evaluation of intraoperative reproducibility, we calculated the correlation between the values of cardiac indices obtained in the same patient, by a cardiologist who repeated two times echocardiography, using the "intraclass correlation coefficient (ICC)" test and we considered an excellent result if the ICC was more high of 0.75. To compare the correlation between two diagnostic tests (epicardial fat measurement), we used the Bland Altman plot statistical test and the Pearson correlation coefficient.

The strength of the associations between the numerical variables analyzed was evaluated using the correlation coefficients: Pearson's and Spearman's, respectively. In the case of bivariate regressions, we calculated the coefficient of determination (R^2) to verify in

what proportion the independent variable variation generates the dependent variable variation.

In order to study the impact of numerical and continuous variables on dichotomous events, we built univariate and multivariate logistic regression models. The variation of the risk of occurrence of the dichotomous event was interpreted by the exponent means of B coefficient of the regression equation, an exponent that can be equivalent to the percentage change, in the relative value of the risk, relative to an increase with a unit of measure in the predictor scale. With the help of Nagelkerke' pseudo-coefficient of determination, we showed how the regression model built explains the occurrence of the dichotomous event.

To evaluate the force between a potential etiological factor and the occurrence of an event we calculated the relative risk, odds ratio, and correlation coefficient, and the statistical significance of the association between the exposure factor and the effect is described by the value of p and the 95% confidence interval.

In order to evaluate the predictive capacity of a positive diagnosis based on a value of a continuous variable, we performed "Receiver-Operating Characteristics" analyses. Predictive performance is described through sensitivity, specificity, and predictive values (positive and negative). The optimal threshold value of the predictor was considered equal to the Youden index. To analyze the statistical significance of the predictive capacity, we compared the area under the ROC curve of the model created with the non-discriminant (the area under the ROC curve = 0.5).

In the study, we calculated the 95% confidence interval and considered a p-value of less than 0.05 as significant for the statistical analyses.

1.3. RESULTS

1.3.1. General characteristics of studied patients

Of the 53 patients who participated in the study, 32 (60.3%) were men. Their general characteristics were: mean age 57.6 ± 10.3 years, the diabetes duration ranged from 0 to 24 years, with a median of 7 years, mean BMI of 34.5 ± 4.7 kg/m², 43.4% of them had moderate or severe obesity, women exceeded the recommended abdominal circumference (waist) more than men ($p = 0.009$).

1.3.2. Imaging reproducibility

Analysis of the differences between the EPI fat values obtained by cardiac ultrasonography and cardiac computed tomography showed us that there is a significant but moderate association between them, the Pearson correlation coefficient being $r = 0.35$, $p = 0.009$. According to the Bland-Altman test, the average difference in the results of the two investigations was -0.72 ± 2.72 mm. The 95% upper and lower concordance limits (LOA) were 4.2 mm and -5.5 mm.

1.3.3. Analysis of metabolic and adiposity markers dynamics in the population of type 2 diabetes patients initiated on SGLT2i therapy

After six months of treatment with Dapagliflozin, the median value by which waist value was exceeded above the recommended guideline did not improve statistically significant except in men (20.5 [3; 53] mm versus 18 [-1; 46] mm, $p = 0.02$). The results suggest a significant improvement in weight in dynamics, both in women (initially 94.5 ± 15.3 kg versus 6 months 90.2 ± 15.7 kg, $p = 0.0002$, respectively initially versus 1 year 90.8 ± 16.1 kg, $p = 0.0007$; respectively 6 months versus 1 year, $p = 0.03$), as well as in men (initially 102.2 ± 15.8 kg versus 6 months 96.8 ± 13.6 kg, $p < 0.0001$, initially versus 1 year 97.9 ± 14.3 kg, $p < 0.0001$, respectively 6 months versus 1 year, $p = 0.01$). Factors involved in weight loss were: waist difference and initial weight.

Imaging of the adipose tissue revealed a significant reduction in EPI visceral fat after six months, in both genders, both in volume (35.6 ± 16.7 cm³ versus 19.7 ± 5.1 cm³, $p < 0.001$ in women; 39 ± 17.5 cm³ versus 21.4 ± 8.1 cm³, $p < 0.001$ in men), as well as posterior subcostal cardiac ultrasound-measured, as the distance of the hypoechoic space between the right ventricular wall and the liver (5 [5; 9] mm versus 4 [2; 6] mm, $p < 0.001$ in women; 5.8 [4; 10] mm versus 4 [0; 7] mm, $p < 0.001$ in men).

The EPI fat volume percentage reduction was negatively and significantly correlated with the exceedance of waist values above the recommendations according to sex, both before and after the treatment and with the percentage decrease of the abdominal fat volume at L4 level, respectively, with the Initial fat volume at the L4 level. It did not correlate with weight loss at six months, nor with hemoglobin A1c, age, or diabetes duration. We found positive and statistically significant associations between the decrease of the EPI adipose volume and the triglyceride values both the initial and at six months, respectively the initial volume of EPI fat and the initial left atrium volume.

The mediastinal fat did not decrease; on the contrary, it showed a statistically significant increase in men (24.8 ± 7.2 mm versus 29.2 ± 6.7 mm, $p = 0.01$).

In contrast to EPI visceral fat, abdominal visceral tissue measured both in thickness (68.5 ± 13.8 mm versus 68.7 ± 13.9 mm in women, and 59.3 ± 15.6 mm versus 62.2 ± 13.6 mm in men) as well as volume (women had 35.5 ± 27.7 cm³ versus 46 ± 17.4 cm³, and men 43.9 ± 29.6 cm³ versus 41.4 ± 19.1 cm³), there were no significant changes. The volume of abdominal fat at the level of the L4 vertebra was increasing in women, and decreasing in men, but did not reach statistical significance. In the multivariate regression analysis, we found a prediction model for the decrease of L4 visceral fat volumes with uric acid at six months, respectively initial waist as independent variables, with statistical significance ($R^2 = 0.32$, $p < 0.0001$).

In the descriptive analysis of the patients, stratified by the predominance of the visceral tissue, compared to the subcutaneous tissue, we observed that 27.4% (14/51) of the patients presented a subcutaneous/visceral tissue ratio < 1 , all being men. In this subgroup, we observed a significant reduction in the EPI fat volume ($p = 0.002$) and an increase of the mediastinal fat thickness at six months ($p = 0.02$). The volume of fat volume at the L4 vertebra was decreasing in the subgroup with the predominance of visceral tissue (baseline 49.4 ± 32.9 cm³, after six months 43.2 ± 20.7 cm³, $p = 0.5$) and increasing in the subgroup with predominantly subcutaneous adipose tissue (34.1 ± 26.3 cm³ versus 43.8 ± 17.6 cm³, $p = 0.007$).

In the study of "abdominal hypertriglyceridemia," we included the patients who met two criteria: the initial value of triglycerides > 150 mg/dl and waist increased above the recommended value for each gender, respectively > 94 cm in men and > 80 cm in women. In these patients, the waist exceedance above recommendations, by gender, was a mean of 29.7 ± 13.4 cm initially, and at the six-month follow-up, there were no significant changes of 27.8 ± 13.6 cm ($p = 0.6$). As a percentage, women had $41.7 \pm 24.7\%$ ($p = 0.0002$) less of EPI fat volume and men had a similar decrease in volume, respectively $43 \pm 17.8\%$ ($p = 0.0001$). In this subgroup, women presented a median increase of 65.1% of the L4 fat volume compared to men, where the volume decreased by a median of 19% ($p = 0.04$).

1.3.4. The dynamics of cardiac function after dapagliflozin treatment

In the study of cardiac function were included 84.9% (45/53) of the total patients who participated in the research, who did not have valvulopathies or major myocardial structural changes and had a left ventricular ejection fraction over 40%. Of these, 53.3% (24/45) were men. In the study group, 73.3% (33/45) of the patients were diagnosed with DD1 based on two criteria: the E/A ratio < 1 and the E/e' ratio > 15 . After six months of Dapagliflozin treatment, the prevalence of DD1 was 71.1%, and after one year, only 24.4% (11/53) patients had DD1 ($p < 0.001$). To see to what extent the dynamics of the values of the serological and paraclinical markers influenced the outcome of DD1 remission at one year of treatment with SGLT2i, we performed univariate, then multivariate binary regressions. In the univariate binary regression analysis, we found associations with the improvement of liver stiffness, the increase of the GFR, and the decrease of HbA1c. In the univariate analysis, the EPI fat volume and the left atrium volume were associated with DD1 correction (Exp (β) = 1.09, $p = 0.02$, respectively Exp (β) = 1.06, $p = 0.04$).

According to a univariate logistic regression model, we observed that the difference in liver stiffness score after six months of treatment is a predictive factor, statistically significant (Exp (β) = 1.24, $p = 0.04$). The difference in the CAP score was not significantly

associated with DD1 remission (Exp (β) = 0.99; p = 0.4, R^2 = 0.000016). Weight loss after one year did not significantly influence DD1 correction (Exp (β) = 0.85; p = 0.08, R^2 = 0.02). Patient age was not statistically significantly associated with DD1 evolution (Exp (β) = 0.96; p = 0.3, R^2 = 0.0009). Patients who had a long history of diabetes had a higher risk of not remitting DD1 for each year of diabetes, Exp (β) = 0.81; p = 0.01. A diabetes duration \leq 8 years represents a statistically significant predictive factor for DD1 remission, with a sensitivity of 68.7% and a specificity of 81.8%, according to the ROC curve. After one year of treatment, DD1 recovered in a percentage of 75.6%, equally in women and men, only in 58% in patients who were treated with iSGLT2, compared with patients who needed to intensify the treatment, with an association of Dapagliflozin and saxagliptin (dipeptidyl-peptidase 4 inhibitors), in which remission was 100%, with statistical significance p = 0.04.

1.3.5. The impact of dapagliflozin on steatosis and hepatic fibrosis

In terms of liver steatosis severity, all patients had moderate (12%) to severe (88%) steatosis when included in the study. At the 6-month evaluation, the severity of liver steatosis by degrees was significantly improved (p = 0.01) as follows: mild steatosis (S1) 5.8%, moderate steatosis (S2) 25%, severe (S3) 69.2%. The mean CAP score decreased statistically significantly after Dapagliflozin treatment, in both genders (363.6 ± 31.7 dB/m versus 316.1 ± 52 dB/m, p = 0.001 in women, and 346.4 ± 54.3 dB/m versus 315.6 ± 54.1 dB/m, p = 0.02 in men). In multivariate analysis, the liver steatosis improvement was associated with age and EPI fat volume after six months of SGLT2i treatment.

Liver stiffness assessed by fibroscan was improved in both genders, but the median values did not reach statistical significance. Women showed a median score of 6.9 kPa versus 6.4 kPa (p = 0.3), while men had a median score of 7.4 kPa versus 7.3 kPa (p = 0.1). Regarding the degrees of severity, their initial distribution was as follows: F0/1 - 51%, F2 - 17%, F3 - 10.7%, F4 - 21.3%. To see how the fibrosis grades were redistributed into the cohort, we applied the Chi-Square test for trend and obtained a marginal statistical significance (p = 0.057), their distribution after six months of treatment being as follows: F0 / 1 - 60, 8%, F2 - 23.5%, F3 - 9.8%, F4 - 5.9%. Cases of severe hepatic fibrosis showed a percentage reduction of 15.4% (p = 0.8%, 95% CI -56.3% - 51.5%).

The AST value decreased with a median of 5 U/L (95% CI 1.6-7.3), minimum values - 9 U/L, respectively maximum 57 U/L. The ALT value decreased by a median of 9 U/L (95% CI 2.7-13), minimum -13 U/L, maximum 139 U/L. The APRI score improved compared to the initial value, with statistical significance (0.25 versus 0.2, p = 0.005).

To investigate the specific causal relationship between stiffness difference after SGLT2i as a dependent variable and differences in values between lipid fractions, EPI fat volumes, respectively L4 lumbar visceral fat, 6-month and 1-year weight differences after SGLT2i, waist difference, the CAP score and with the initial fasting glucose values, respectively HbA1c, as independent variables, we constructed univariate regression models. Of all the independent variables, only HDLc and LDLc differences influenced how stiffness changed after Dapagliflozin, at 20% (R^2 = 0.2) and 16% (R^2 = 0.16), respectively. Thus, an increase in the standard deviation of the HDLc difference will increase the stiffness difference by 0.45 (p = 0.001), and a standard deviation of the LDLc difference will increase it by 0.41 (p = 0.004).

1.4. DISCUSSIONS

Our data showed a weight reduction of mean 4.9 kg, more pronounced in men (with statistical significance), after six months of treatment, and just 4 kg after one year compared to baseline. We observed a significant increase in weight at a one-year follow-up compared to the 6-month follow-up. This weight decrease has not been influenced by age or diabetes duration, or by the SGLT2i impact on the A1c hemoglobin of the analyzed subjects. Instead, the number of centimeters they decreased in the abdominal circumference, the weight, and the degree of obesity they had when they were included in the study were associated factors with our weight target.

Our study has shown that dapagliflozin has led to a significant decrease in the EPI volume after only six months of treatment. The majority of patients (~75%) showed a reduction in EPI volume by >10%, 15% of them had an increase by >10% on the second CT scan assessment, while the rest of the subjects had less than 10% changes. This result observed in both genders was linked to the weight loss (analyzed as a dichotomous event), but not to the waist decrease. The percentage reduction in the abdominal visceral fat volume at L4 was inversely proportional to the percentage reduction in epicardial fat volume in 9% of patients.

The visceral adipose tissue volume at the level of the fourth lumbar vertebra did not decrease; on the contrary, the women in the study showed an increase in volume, with marginal statistical significance. The percentage of this fat volume directly correlated in dynamics, depending on age and how weight, waist, and obesity evolved after SGLT2i. Similar to other studies, in our group of patients evaluated, 73% were diagnosed with diastolic dysfunction. However, our research has screened in particular for DD1, which we considered to be of scientific interest precisely because it represents an alteration of the active relaxation of the left ventricle, without any myocardial structural impairment, a dysfunction that precedes systolic alteration. Our experiment showed an improvement in the diastolic function after six months of treatment, but especially after 48 weeks of therapy with dapagliflozin. From an initial percentage of 73%, at the last cardiac ultrasound evaluation, DD1 was present only in 24.4% of patients. This diastolic improvement could be attributed to the effect of improving the cardiac output, respectively, reduced plasma volume, followed by SGLT2 inhibition.

The most significant benefit regarding DD1 remission was in patients who required during the study of diabetic treatment intensification with an association of SGLT2i and DPP4i. In these patients, DD1 remission was statistical significance in a 100% rate, while in the group of patients treated only with SGLT2i, the remission rate was 58%. It is considered that the association of the inhibitors SGLT2 and DPP4 is beneficial to the diastolic function probably because the SGLT2 inhibitors neutralize specific effects of the DPP4 inhibitors on the cardiovascular system and due to improved glycemic control

In the study that we conducted, most patients presented moderate to severe liver steatosis that was strongly associated with their age. After six months of therapy with dapagliflozin, liver fat has improved statistically for both genders. No association between CAP score and the other imaging indices of adipose tissue was found. However, if we analyze the CAP score, as a dichotomous event, we observe that it was influenced by the subjects' age and by the remaining epicardial fat value after the treatment with dapagliflozin. Older patients with a higher reduction in epicardial fat were more likely to improve liver steatosis as well.

The stiffness score decreased, but did not reach statistical significance. Analyzed by the degree of fibrosis, the initial data of the patients indicated a prevalence of 49% of liver fibrosis in our study, divided into severity degrees as follows: 17% - F2, 10% - F3, 22% - F4. After treatment with dapagliflozin, only 5.9% had severe fibrosis (F4), patients with moderate fibrosis (F3) remained stable, and 23.5% of subjects had mild fibrosis (F2). The liver stiffness was reduced by a median of 0.4 kPa in women, respectively 2.4 kPa in men. Regarding fibrosis indexes APRI, respectively FIB-4, a statistically significant reduction was also observed in women. Overall, the APRI score in the studied group was a median of 0.25 initially, with the same median for both genders, then, at the 24-week assessment, the median of APRI in the group was 0.20, without significant statistical differences between women (0.18) and men (0.21). The FIB-4 index was generally reduced from 1 ± 0.4 to 0.8 ± 0.3 with marginal statistical significance.

Patients who had higher A1c hemoglobin before dapagliflozin showed a significantly higher reduction in stiffness in about 13% of the analyzed patients. Similarly, the difference in epicardial fat volume increased directly with the difference of stiffness score, an association that was observed in 9% of cases. The higher the GFR value after SGLT2i, the greater the differences between the two assessments were in ~10% of patients. In 15% of patients, the higher the value of ALT, the higher the difference of stiffness was.

The specific causal relationships with statistical significance, between stiffness differences and the differences of HDLc and LDLc respectively, were underlined in the linear regression analysis model which explains in a 20% rate the simultaneous increase in the HDL-cholesterol and stiffness difference, and in a 16% rate the concomitant increase in the difference of LDLc and liver stiffness. The improvement of liver steatosis was not associated with the weight loss of patients, which suggests the existence of other mechanisms through which SGLT2i influences the reduction of liver fat.

SGLT2i have proved to be effective, not only as an antihyperglycemic treatment but also in weight loss, lowering blood pressure levels, respectively, reducing the risk of hospitalization for heart failure and renal protection.

IV. CONCLUSIONS AND PERSONAL CONTRIBUTION

- patients lose weight after six months by a mean of 4.9 kg, but they regain 0.9 kg of the lost weight after one year; Patients who had triglyceride values >150 mg/DL and who exceeded the waist measurements compared to the recommendations by gender had a predominantly subcutaneous fat and showed a weight increase of 1.3 kg at one year versus six months of therapy
- men are more prone than women to abdominal obesity reduction expressed by the waist
- the overall weight loss is higher in men than in women
- patients with a higher body mass index achieve a more significant weight loss
- patients with a subcutaneous/visceral adipose tissue ratio below 1 (all were men in this research) have recorded a steady waist
- the EPI volume decreased significantly after six months in both men and women, specifically from 35.6 cm³ to 19.7 cm³ in women and from 39 cm³ to 21,4 cm³ in men
- the epicardial fat measured by ultrasound has gradually decreased from a range of 5-9 mm to 2-6 mm in women and 4-10 mm to 0-7 mm in men after six months, with a general mean difference in measurements at one year of -2.8 mm
- mediastinal fat showed an upward trend in both genders, statistically significant from 24.8 mm to 29.2 mm in men; the pattern was similar irrespective of the predominant adipose tissue type
- men had stable values of abdominal fat at the L4 level, as compared to women who showed an increasing trend in volume from 35.5 cm³ to 46 cm³
- the L4 fat volume was lower in dynamics but statistically insignificant in patients with the predominance of the visceral fat and more significantly increased in those with a predominant subcutaneous adipose tissue
- The EPI volume decreased by a mean of 39.5% irrespective of age, diabetes duration, or how the HbA1c and weight values were changed. This reduction was higher for 9% of patients with a lower percentage drop in the fat volume at L4
- the decrease in the EPI volume depends 28% on the initial volumes of the epicardial fat and the left atrium
- dynamics of the triglyceride values influence the dynamics of the epicardial fat volume in 75-79% cases
- the value of the uric acid directly influenced the difference in the fat volume at the level L4 after treatment and the initial waist; the more the waist at initiation and the 6-month uric acid increased, the more the L4 volume decreased
- we concluded that the higher the uric acid difference, the lower the percentage reduction of L4 adipose tissue volume
- men in the hypertriglyceridemic waist group showed a decrease in the fat volume at L4 vertebrae by a median of 19%, unlike women who showed a statistically significant increase by a median of 65%

- The prevalence of type 1 diastolic dysfunction after six months of SGLT inhibition was 71.1% and only 24.4% after one year compared to the initial 73.3% prevalence at the beginning of the study
- DD1 remission was associated with the improvement of liver stiffness at a 74% rate, with the increase of GFR in 75% of cases and a 78% rate with the decrease of HbA1c. In a lower percentage it was associated with the EPI volume and the left atrium volume
- A reduction of stiffness score of 1 kPa increases the probability of DD1 remission by 24%
- A difference of more than 0.4 kPa in the stiffness score predicts DD1 correction with a sensitivity of 71% and specificity of 70%
- The dynamics of A1c hemoglobin increased the probability of DD1 correction by 85% for each percentage reduction by 1%. As the SGLT2i decreased HbA1c by a mean of 0.7% in the DD1 subgroup analysis, the impact of A1c in this situation was 37%
- by lowering the GFR by 1ml/min after six months of SGLT2i, we expect to decrease the probability of DD1 remission by 9%
- Age, BMI, or patient weight after one year did not influence the diastolic dysfunction outcome. Instead, the diabetes duration decreases the likelihood of remittance for each year of age. We can predict with a sensitivity of 68.7% and specificity of 81.8% the remission of DD1 in patients with a T2D history of ≤ 8 years
- after one year of treatment the diastolic dysfunction was remitted in 75.6% cases, equally regardless of gender; the percentage of DD1 remission was 58% for those treated only with Dapagliflozin and 100% for those treated with an association of SGLT2i and DPP4i
- liver steatosis improves significantly after just six months of SGLT2i in both genders (363 dB/m versus 316 dB/m in women, 346 dB/m versus 315 dB/m in men)
- the improvement of hepatic steatosis was more frequent in older patients, who had a lower value of the EPI volume after six months, than in younger patients
- individuals with severe liver stiffness have shown a 15.4% reduction in the stiffness score; overall, after the SGLT2i therapy we observed a stiffness score of 6.9 versus 6.4 kPa in women, with a mean difference of 0.4 kPa and 7.4 versus 7.3 kPa in men, with a mean difference of 2.4 kPa, without statistical significance
- the APRI score reduced from 0.25 to 0.20 statistically significant, similar in both sexes
- the difference between the total cholesterol values significantly linked to the improvement of liver stiffness
- there are positive associations between the difference of the stiffness and the level of initial A1c hemoglobin, the ALT value, the difference of EPI volume and the LDL-cholesterol
- there is an inverse relationship between the difference between GFR and the stiffness' difference

The results of this research support the recommendations of the authorities to include SGLT2i even before metformin in patients with T2D known with or at high risk of developing cardiovascular disease, heart failure, and chronic kidney disease. Based on these results, we propose the introduction of SGLT2i as the first treatment option in the future national treatment guide of the T2D.

The study has shown that the treatment with Dapagliflozin has differentiated benefits on adipose tissue, depending on its location. Thus the epicardial fat, steatosis, and liver fibrosis have decreased significantly without the visceral adipose tissue reduced from other regions (subchapters 1.3.3, 1.3.5). In other words, the weight loss for patients treated with SGLT2i is not generalized, but due to the reduction in specific volumes of ectopic fat.

The results obtained have demonstrated, for the first time, the beneficial effect of Dapagliflozin on the diastolic type 1 dysfunction, at the same time as reducing the epicardial adipose tissue in patients with T2D independently of the glycemic control (sub-chapter 1.3.4.1).