**SUPPLEMENTARY FILE: SGLT2-inhibitors modulate the Cardiac Autonomic Neuropathy and reduce the vaso-vagal syncope recurrence in patients with type 2 diabetes mellitus: the SCAN study**

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**RESULTS**

In our study, the patients under sodium-glucose transporter 2 inhibitors (SGLT2-I users) vs. those without SGLT2-I therapy (Non-SGLT2-I users) exhibited a significant difference regards the number of vasovagal syncope (VVS) recurring events (40 vs. 200 events; p<0.05) at follow-up end. However, in these patients (SGLT2-I users vs. Non-SGLT2-I users) and specifically only in those with VVS recurrence, we investigated the parameters of heart rate variability (HRV) and the 123I-metaiodobenzylguanidine (123I-MIBG) myocardial scintigraphy indexes, to stage the vagal tone and cardiac autonomic dysfunction only for the patients presenting vasovagal syncope (VVS) recurrence, as all causes, mixed, cardio-inhibitory and vasodepressor. However, we found as follows:

-for the patients with **all causes of syncope**, the SGLT2-I-users vs. Non-SGLT2-I users’ showed at baseline a significant difference regards all investigated parameters (p<0.05), at the exception of heart rate (HR), (p>0.05). **Supplementary** **table 1**. At 1 year of follow-up, for all causes of syncope, SGLT2-I-users vs. Non-SGLT2-I users’ patients showed a significant difference regards all investigated parameters (p<0.05). **Supplementary** **table 1**.

-For the patients with **mixed syncope**, SGLT2-I-users vs. Non-SGLT2-I users’ evidenced at baseline a significant difference regards all investigated parameters (p<0.05), at the exception of HR, and low frequency/high frequency ratio (LF/HF ratio), (p>0.05). **Table 3**. At 1 year of follow-up, SGLT2-I-users vs. Non-SGLT2-I users’ patients showed a significant difference regards all investigated parameters (p<0.05). **Supplementary** **table 1**.

-For the patients with the **cardio-inhibitory syncope**, SGLT2-I-users vs. Non-SGLT2-I users’ at baseline showed significant difference regards all investigated parameters (p<0.05), at the exception of HR, and LF (p>0.05). **Supplementary** **table 1**. At 1 year of follow-up, SGLT2-I-users vs. Non-SGLT2-I users’ patients evidenced significant difference regards all investigated parameters (p<0.05), at the exception of HR (p>0.05). **Supplementary** **table 1**.

-For the patients with the **vaso-depressor syncope**, SGLT2-I-users vs. Non-SGLT2-I users’ at baseline showed a significant difference regards all investigated parameters (p<0.05), at the exception of HR, and LF/HF ratio (p>0.05). Table 3. At 1 year of follow-up, SGLT2-I-users vs. Non-SGLT2-I users’ patients evidenced significant differences regards all investigated parameters (p<0.05). **Supplementary** **table 1**.

**Comparing in each cohort of study (SGLT2-I users and Non-SGLT2-I users’ patients) for the patients with VVS recurrence the data at 1 year of follow-up vs. baseline**, we noted that:

1. **for all causes of syncope**, the patients SLGT2-I users had a significant reduction of heart rate (HR, 60.2±9.4 vs. 75.2±11.3; p<0.05), and of high frequency (HF, 15.32±0.73 vs. 19.12±1.19; p<0.05), and a significant increase of low frequency (LF, 88.91±3.12 vs. 82.93±5.51; p<0.05), and of the LF/HF ratio (LF/HFr, 5.82±0.35 vs. 4.35±0.39; p<0.05). Thus, the SGLT2-I users evidenced a significant increase of the Heart to Mediastinum ratio (H/Mlate, 2.55±0.29 vs. 1.90±0.11; p<0.05), and a significant reduction of the Washout rate (WR, 29.17±6.55 vs. 47.45±7.58; p<0.05). On the contrary, these parameters did not reach the statistical significance in the cohort of Non-SLGT2-I users comparing the follow-up end vs. baseline condition (p>0.05). **Supplementary table 1**.

2. **for mixed syncope**, SGLT2-I users had a significant reduction of HR (56.5±9.6 vs. 73.6±12.1; p<0.05), and of HF (15.27±0.71 vs. 19.07±1.82; p<0.05), and a significant increase of LF/HFr (5.86±0.25 vs. 4.62±0.48; p<0.05), and of H/Mlate (2.49±0.02 vs. 1.80±0.10; p<0.05). This cohort of patients showed a significant reduction of the WR (32.41±1.52 vs. 46.10±8.07; p<0.05).

In the Non-SLGT2-I users, we found a significant reduction of HF (17.02±0.13 vs. 18.37

1.23; p<0.05), with a significant increase of LF/HFr (5.04±0.25 vs. 4.53±0.47; p<0.05), and of H/Mlate (1.60±0.35 vs. 1.42±0.14; p<0.05). **Supplementary table 1**.

3. **for cardio-inhibitory syncope**, SGLT2-I users had a significant reduction of HR (56.3±5.5 vs. 72.7±6.7; p<0.05), and of HF (15.12±0.17 vs. 20.10±1.56; p<0.05), and a significant increase of LF/HFr (5.90±0.07 vs. 4.01±0.15; p<0.05), and of H/Mlate (2.53±0.28 vs. 1.84±0.13; p<0.05). This cohort of patients showed a significant reduction of the WR (41.75±6.55 vs. 59.10±6.59; p<0.05).

In the Non-SLGT2-I users, we found a significant reduction of HF (17.06±0.18 vs. 18.05

1.71; p<0.05), with a significant increase of the H/Mlate (1.72±0.30 vs. 1.56±0.18; p<0.05), and a significant reduction of WR (32.95±5.77 vs. 39.35±7.53; p<0.05). **Supplementary table 1**.

4. **for vaso-depressor syncope**, SGLT2-I users had a significant reduction of HR (56.7±9.9 vs. 75.8±11.4; p<0.05), and of HF (15.38±0.80 vs. 19.01±1.24; p<0.05), and a significant increase of LF/HFr (5.78±0.41 vs. 4.31±0.34; p<0.05), and of H/Mlate (2.38±0.32 vs. 1.95±0.09; p<0.05). This cohort of patients showed a significant reduction of the WR (26.69±4.59 vs. 46.19±6.11; p<0.05).

In the Non-SLGT2-I users, we found a significant reduction of HR (70.8±10.1 vs. 78.08±11.85; p<0.05) and of the HF (17.34±0.93 vs. 18.96±1.25; p<0.05). **Supplementary table 1**.

Finally, we tested the correlation between sympathetic system parameters (HR, LF/HFr, H/Mlate,

and WR) and study variables as C reactive protein (CRP, marker of inflammation) glycemia (glucose homeostasis), the left ventricle ejection fraction (LVEF, index of cardiac pump), the noradrenaline blood values (marker of sympathetic system overdrive), and the systolic blood pressure (hemodynamic index). For the glycemia we found a direct correlation with delta values of HR (R= 0.150; p 0.001), and a direct inverse correlation with delta values of LF/HFr (R= -0.111; p 0.006) and H/Mlate (R= -0.409, p0.001). **Supplementary table 2**. The CRP inversely correlated with delta values of LF/HFr (R=--0.119; p 0.004), H/Mlate (R= -0.129, p 0.001). **Supplementary table 2**. Finally, the noradrenaline blood values directly correlated with the delta values of HR (R= 0.948; p 0.003), and inversely with delta values of LF/HFr (R= -0.105; p 0.009) and H/Mlate (R= -0.185, p0.001). **Supplementary table 2**.

**Discussion**

In our study, the SGLT2-I users vs. Non-SGLT2-I users with VVS recurrence exhibited a significant difference regards the vaso-vagal tone, the sympathetic axis, and the parameters of autonomic function. Specifically, in these cohorts we evaluated the autonomic dysfunction by the HR, the ECG Holter parameters of vasovagal tone (LF, HF, and the LF/HF ratio), and the 123I-MIBG indexes of cardiac sympathetic function and innervation (Heart to Mediastinum ratio, and the Washout rate). Notably, in the SGLT2-I users vs. non-SLGT2-I users’ patients with all causes of VVS recurrence we found a significant difference, at baseline and at follow-up end, about all investigated parameters of cardiac autonomic dysfunction (CAN). The same trend was specifically observed for the mixed, cardio-inhibitory, and vasodepressor syncope. We did not find a significant difference regards the HR at baseline and at the follow-up end in the cohorts with cardio-inhibitory VVS recurrence. Notably, comparing the follow-up end vs. baseline condition parameters in the cohorts of patients with VVS recurrence, we found in the SGLT2-I users a significant amelioration of the HRV parameters, and of 123I-MIBG indexes (p<0.05). This was observed for all causes of VVS, mixed VVS, cardio-inhibitory VVS, and vasodepressor VVS recurrence (p<0.05). On the contrary, we did not found a significant recover of sympathetic system dysfunction in the Non-SGLT2-I users regards all causes of VVS recurrence (p>0.05). The same trend was seen for the WR in the mixed VVS, and for both parameters of cardiac denervation in the cohort of Non-SGLT2-I users with vasodepressor VVS (p>0.05). Finally, but not less relevant, the glycemia directly correlated with the increase of HR, and inversely with the delta values (amelioration) of the LF/HFr and H/Mlate at follow-up end (p<0.05). Similarly, an inverse correlation was found for the CRP (inflammation) and serum noradrenaline (over-sympathetic system drive) with the delta values of the LF/HFr, and the H/Mlate (p<0.05).

Taken together, these results might confirm a more severe autonomic dysfunction and cardiac denervation in non-SLGT2-I users vs. SLGT2i-users with VVS recurrence. These abnormalities were evidenced at baseline, and persisted in the non-SGLT2-I users at follow-up end. In detail, this trend was confirmed for all causes of VVS recurrence, and for any form of VVS recurrence at 1 year of follow-up. Notably, only the HR did not reach the statistical significant difference comparing the study cohorts with cardio-inhibitory VVS recurrence at 1 year of follow-up (p>0.05). In addition, the worse glycemic control, the over-inflammation and the over-sympathetic stimulation (as seen in Non-SGLT2-I users vs. SGLT2-I users), linked to highest values of HR, and more severe dysfunction of vasovagal and sympathetic system at follow-up end. However, comparing the follow-up end vs. baseline parameters in any cohort of patients with VVS, we might suggest that the SGLT2-I could reduce the vasovagal tone and the over-sympathetic drive in patients with type 2 diabetes mellitus (T2DM), (**1**). Parallely, this linked to worse glycemic control, with over-inflammation and over-sympathetic stimulation in Non-SGLT2-I users vs. SGLT2-I users. In this setting, we evaluated the vaso-vagal tone in VVS patients with T2DM by the HRV parameters, as the LF, HF, and LF/HFr (**2**). Indeed, the LF is a marker of baroreceptor activity, while the HF is a parameter modulated by the parasympathetic nervous system and by the changes of respiration phases and blood pressure (**2, 3**). Moreover, the LF/HF ratio is an index of the complex interaction between vagal and sympathetic tone, and a valuable marker to stage the degree and severity of the CAN, and to predict the VVS recurrence in T2DM patients (**2**, **3**). Notably, parallel to the dysfunction of vasovagal tone, the Non-SGLT2-I users vs. SGLT2-I users showed a more significant stage of CAN. Notably, both the cohorts of patients had the tendency to increase the Heart to Mediastinum ratio (H/Mlate) values, and to reduce the Washout rate (WR), which are both indexes of cardiac innervation (**4**). Despite this, the SGLT2-I users vs. Non-SGLT2-I users evidenced a significant difference about the H/Mlate and WR values at follow-up end (p<0.05). However, this could confirm in the T2DM patients a significant and chronic myocardial denervation that, linked to the over-sympathetic system activation, could be a main determinant cause of the VVS recurrence at follow-up end (**4**). Therefore, we might conclude that in the T2DM patients with VVS recurrence, the over stimulation of the vagal tone is a negative player together with chronic and impaired cardiac innervation, and sympathetic system over activation. To date, this complex vagal/sympathetic axis could be investigated by the HRV and MIBG parameters in T2DM with VVS recurrence at follow-up. In this setting, the SGLT2-I are drugs that could modulate the sympathetic system (**5**, **6**), via the control/regulation of the CAN. Thus, SGLT2-I could modulate the significant cardiac denervation in the T2DM with VVS. Moreover, we might speculate that these effects might lead to the reduction of VVS recurrence at 1 year of follow-up in the SGLT2-I users vs. Non-SGLT2-I users’ patients. Future studies will be designed to fully evaluate the CAN and VVS recurrence in T2DM, and the regulative/ameliorative effects played by the SGLT2-I at long-term follow-up and in a larger sample of patients.

**Tables’ legend.**

**Supplementary table 1**. In this table the parameters of vaso-vagal and sympathetic tone dysfunction at baseline and at 1 year of follow-up in patients that evidenced all causes of syncope recurrence (A), vaso-depressor syncope (B), cardio-inhibitory syncope (C), and mixed syncope (D). LF: low frequency; HF: high frequency; 123I-MIBG: 123I-metaiodobenzylguanidine. \* is for statistical significant (p<0.05).

**Supplementary table 2**. In this table the Correlation analysis for study variables and delta (Δ)values of heart rate, LF/HFr, Heart to Mediastinum ratio, and Washout rate. CRP: C reactive protein; LF/HFr: low frequency to high frequency ratio; LVEF: left ventricle ejection fraction; \* is for statistical significant (p<0.05).

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**Supplementary table 1.** Parameters of vaso-vagal and sympathetic tone dysfunction at baseline and at 1 year of follow-up in patients that evidenced all causes of syncope recurrence (A), vaso-depressor syncope (B), cardio-inhibitory syncope (C), and mixed syncope (D).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **BASELINE** |  | **At 1 year** |  |  |
|  | **SLGT2i-users** | **Non-SLGT2i users** | **SLGT2i-users** | **Non-SLGT2i users** | **P value** |
| **A) ALL CAUSES OF SYNCOPE** |  |  |  |  |  |
| **Autonomic system parameters** |  |  |  |  |  |
| Heart rate, bpm | 75.2±11.3 | 79.1±12.2 | 60.2±9.4 | 72.3±8.2 | 0.071;0.001\*\* |
| **ECG Holter parameters** |  |  |  |  |  |
| LF, normalized units | 82.93±5.51 | 82.98±4.53 | 88.91±3.12 | 85.09±4.13 | 0.001\*; 0.001\*\* |
| HF, normalized units | 19.12±1.19 | 18.65±1.29 | 15.32±0.73 | 17.19±0.70 | 0.014\*; ; 0.001\*\* |
| LF/HF ratio | 4.35±0.39 | 4.47±0.43 | 5.82±0.35 | 4.96±0.32 | 0.104; 0.001\*\* |
| **123I-MIBG myocardial scintigraphy parameters** |  |  |  |  |  |
| Heart to Mediastinum ratio | 1.90±0.11 | 1.89±0.54 | 2.55±0.29 | 2.05±0.52 | 0.020\*;0.001\*\* |
| Washout rate (%) | 47.45±7.58 | 42.61±9.81 | 29.17±6.55 | 40.66±10.92 | 0.045\*;0.001\*\* |
|  |  |  |  |  |  |
| **B) MIXED SYNCOPE** |  |  |  |  |  |
| **Autonomic system parameters** |  |  |  |  |  |
| Heart rate | 73.6±12.1 | 79.3±11.4 | 56.5±9.6 | 76.4±10.1 | 0.146; 0.043\*\* |
| **ECG Holter parameters** |  |  |  |  |  |
| LF, normalized units | 87.43±2.69 | 82.68±4.76 | 89.30±1.99 | 85.87±3.92 | 0.001\*; 0.007\*\* |
| HF, normalized units | 19.07±1.82 | 18.37±1.23 | 15.27±0.71 | 17.02±0.13 | 0.012\*; 0.001\*\* |
| LF/HF ratio | 4.62±0.45 | 4.53±0.47 | 5.86±0.25 | 5.04±0.25 | 0.543; 0.001\*\* |
| **123I-MIBG myocardial scintigraphy parameters** |  |  |  |  |  |
| Heart to Mediastinum ratio | 1.80±0.10 | 1.42±0.14 | 2.49±0.02 | 1.60±0.35 | 0.001\*; 0.001\*\* |
| Washout rate (%) | 46.10±8.07 | 51.77±8.41 | 32.41±1.52 | 48.35±1.31 | 0.049\*; 0.001\*\* |
|  |  |  |  |  |  |
| **C) CARDIO-INHIBITORY SYNCOPE** |  |  |  |  |  |
| **Autonomic system parameters** |  |  |  |  |  |
| Heart rate | 72.7±6.7 | 78.3±13.4 | 56.3±5.5 | 76.2±8.2 | 0.250; 0.061 |
| **ECG Holter parameters** |  |  |  |  |  |
| LF, normalized units | 79.95±3.09 | 84.11±3.56 | 89.31±2.01 | 83.25±3.80 | 0.547; 0.001\*\* |
| HF, normalized units | 20.10±1.56 | 18.05±1.71 | 15.12±0.17 | 17.06±0.18 | 0.001\*; 0.001\*\* |
| LF/HF ratio | 4.01±0.15 | 4.68±0.37 | 5.90±0.07 | 4.88±0.25 | 0.002\*; 0.001\*\*  |
| **123I-MIBG myocardial scintigraphy parameters** |  |  |  |  |  |
| Heart to Mediastinum ratio | 1.84±0.13 | 1.56±0.18 | 2.53±0.28 | 1.72±0.30 | 0.012\*; 0.004\*\* |
| Washout rate (%) | 59.10±6.59 | 39.35±7.53 | 41.75±6.55 | 32.95±5.77 | 0.004\*; 0.012\* |
|  |  |  |  |  |  |
| **D) VASO-DEPRESSOR SYNCOPE** |  |  |  |  |  |
| **Autonomic system parameters** |  |  |  |  |  |
| Heart rate | 75.84±11.45 | 78.08±11.85 | 56.7±9.9 | 70.8±10.1 | 0.389; 0.001\*\* |
| **ECG Holter parameters** |  |  |  |  |  |
| LF, normalized units | 81.67±5.69 | 83.07±4.53 | 88.71±3.89 | 85.04±4.19 | 0.003\*; 0.001\*\* |
| HF, normalized units | 19.01±1.24 | 18.96±1.25 | 15.38±0.80 | 17.34±0.93 | 0.0016\*; 0.001\*\* |
| LF/HF ratio | 4.31±0.34 | 4.39±0.39 | 5.78±0.41 | 4.92±0.37 | 0.301; 0.001\*\* |
| **123I-MIBG myocardial scintigraphy parameters** |  |  |  |  |  |
| Heart to Mediastinum ratio | 1.95±0.09 | 2.23±0.46 | 2.38±0.32 | 2.07±0.37 | 0.002\*; 0.012\*\* |
| Washout rate (%) | 46.19±6.11 | 41.40±8.43 | 26.69±4.59 | 37.26±8.89 | 0.007\*; 0.001\*\* |

**Supplementary table 2. Correlation analysis for study variables and delta values of heart rate, LF/HFr, Heart to Mediastinum ratio, and Washout rate.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **HEART RATE (Δ values)** |  |  | **LF/HFr (Δ values)** |  |  |
| **Study variables** | **R value** | **P value** | **Study variables** | **R value** | **P value** |
| CRP | 0.069 | 0.094 | CRP | -0.119 | 0.004\* |
| Glycemia | 0.150 | 0.001\* | Glycemia | -0.111 | 0.006\* |
| LVEF | -0.027 | 0.503 | LVEF | -0.052 | 0.202 |
| Noradrenaline | 0.948 | 0.003\* | Noradrenaline | -0.105 | 0.009\* |
| Systolic blood pressure | 0.059 | 0.148 | Systolic blood pressure | -0.051 | 0.209 |
|  |  |  |  |  |  |
| **Heart to Mediastinum ratio (Δ values)** |  |  | **Washout rate (Δ values)** |  |  |
| **Study variables** | **R value** | **P value** | **Study variables** | **R value** | **P value** |
| CRP | -0.129 | 0.001\* | CRP | 0.042 | 0.553 |
| Glycemia | -0.409 | 0.001\* | Glycemia | 0.029 | 0.679 |
| LVEF | 0.108 | 0.055 | LVEF | 0.024 | 0.736 |
| Noradrenaline | -0.185 | 0.001\* | Noradrenaline | -0.020 | 0.782 |
| Systolic blood pressure | -0.033 | 0.551 | Systolic blood pressure | -0.048 | 0.497 |