Imaging and TOF-SIMS examination on urinary EXTRACELLULAR VESICLES from patients with type 1 diabetes to determine symptoms of diabetic kidney disease

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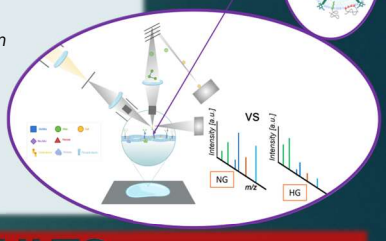


INTRODUCTION

ToF-SIMS (Time of Flight - Secondary Ion Mass Spectrometry) is a direct and non-destructive technique for analyzing molecules with a mass m<1000 Da, such as lipids. ToF-SIMS allows for qualitative semi-native testing without isolation, fixation or labelling. An example that can be measured is nanometric **extracellular vesicles (EVs),** which are becoming increasingly important in diagnosis, treatment and pharmacotherapy. These vesicles undergo changes in their composition and structure, such as **amino acid and lipid profile**, as well as **transported cargo**, which can provide useful information about the onset or presence of disease in the biological system, such as **Chronic Kidney Disease (CKD)**. This disease, in which **kidney damage** is observed, remains a significant clinical issue in both type 1 (T1DM) and type 2 (T2DM) diabetes. There is a need for early markers of kidney damage in these diseases, and one proposed is nanometric urine EVs (uEVs).

In this study, we used ToF-SIMS to compare and evaluate changes in the lipid and amino acids composition of uEVs in urine of people with well-controlled T1DM and healthy people using ToF-SIMS.

WORKFLOW

RECRUITMENT OF PATIENTS WITH TYPE 1 DIABETES (T1D) AND CONTROL

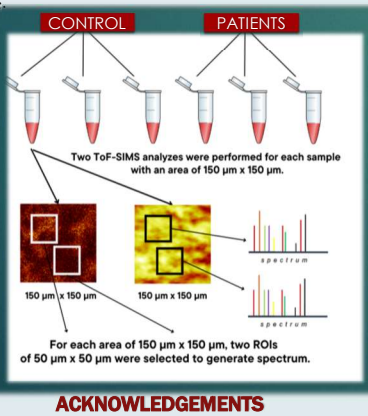
UEVs were collected from **33 patients** with T1DM who had good metabolic control (15 years duration, using personal insulin pumps and HbA1C ~7%) and **13 healthy individuals**. The patients were recruited from the Department of Metabolic Diseases at Jagiellonian University Medical College.

uEVs ISOLATION

The uEVs were concentrated and purified using low-pressure filtration, pelleted by ultracentrifugation, and suspended in a PBS solution.

ToF-SIMS ANALYSIS

The ToF-SIMS (ION-ToF GmbH, Münster, Germany) with a Bi_3^+ LMIG, as a primary ion source was used. Two biological replicates were conducted for an individual **EV** sample. Data were recorded in the mass range of 0-900 Da for positive ions, collected from three different area in size 150 × 150 μm², for one sample.



ACKNOWLEDGEMENTS

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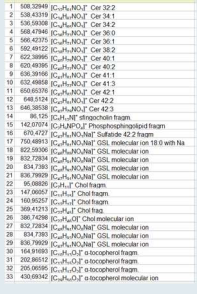
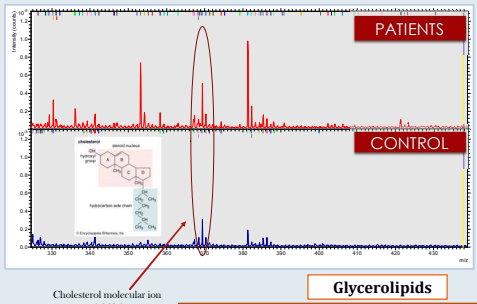
RESULTS

I STEP – list of characteristic peaks for lipids and amino acids to comparison



II STEP – checking the presence of peaks in spectra



Cholesterol molecular ion m/z 369,35.

III STEP
intensity comparison of characteristic peaks and statistical analysis

We perform statistical analysis by calculating mean values and intensity standard deviations for selected peaks in each group of samples. We determined the significance of differences between the mean values for the groups using Tukey's test and one-way ANOVA. The significance level for the tests was $p \leq 0.05$.

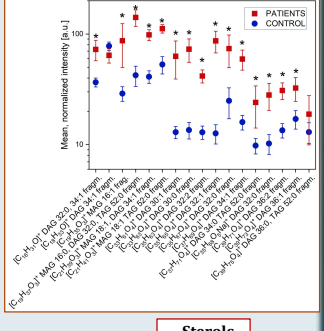
Table 1. Demonstrated differences in the percentages of all analyzed lipids and amino acids.

Group	CONTROL [%]	PATIENTS [%]
All lipids (6 groups)	98.0	58.3
Amino acids	1.9	41.7

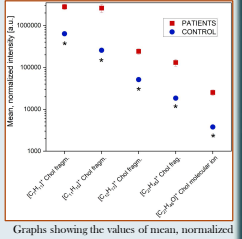
Table 2. Tested differences in the percentages of individual lipid groups.

Lipid group	CONTROL [%]	PATIENTS [%]
Fatty acids	95.22	74.08
Glycerophospholipids	0.90	4.67
Glycerolipids	0.03	0.29
Sphingolipids	0.13	15.95
Prenols	3.10	0.85
Sterols	0.63	4.17

Glycerolipids



Sterols



Graphs showing the values of mean, normalized intensities for all identified, characteristic peaks in positive ionization. Statistically significant differences are *p<0.05

CONCLUSION

- Results have shown that patients with T1DM without CKD exhibit alterations in the content of **specific lipid groups** and changes in the **percentage composition of individual amino acids** in the uEVs compared to the control group.
- Urinary Extracellular Vesicles can provide valuable insights into the **pathological processes** occurring in the body of a patient with T1DM, but further research is necessary to determine whether uEVs could serve as a marker for diabetic kidney disease.