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Untargeted lipidomic profiling of plasma from patients with ST-elevation myocardial infarction

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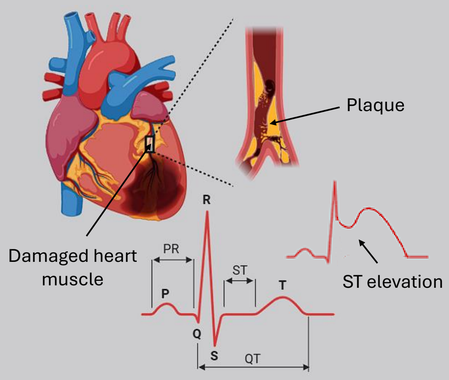
Introduction

Cardiovascular diseases (CVD) are the leading cause of death in developed and developing countries, **taking approximately 20.5 million lives each year**. However, according to a report by the World Heart Federation, **up to 80 % of premature heart attacks and strokes could be prevented** by the correct use of more specific diagnostic tools. Lipids are generally associated with atherosclerotic CVD development and pathology. Not only the commonly known cholesterol and TGs, but also **ceramides and phosphatidylcholines (PC) are related to CVD**. Ceramides and phosphatidylcholines play a significant role in inflammation connected to acute coronary syndromes.



Ceramides:

- are transported via lipoprotein particles
- accumulate in atherosclerotic plaques
- facilitate LDL vascular infiltration
- mediate the deactivation of protective NO production

Methods

We have analysed 18 plasma samples (**nine STEMI patients, nine controls**) using **LC-MS/MS** coupled to an **Orbitrap Exploris 480** system equipped with an **Accucore C30** column (150 × 2.1 mm; 2.6 µm). Samples were extracted with isopropanol, vortexed, and centrifuged (20 min, 21,000 × g, 4 °C). Data were processed and evaluated using **Compound Discoverer 3.3** software (Thermo Fisher Scientific, MA, USA) and R. Structural elucidation was performed in **SIRIUS** (University of Jena, Germany). The workflow is summarised in **Fig. 1**.

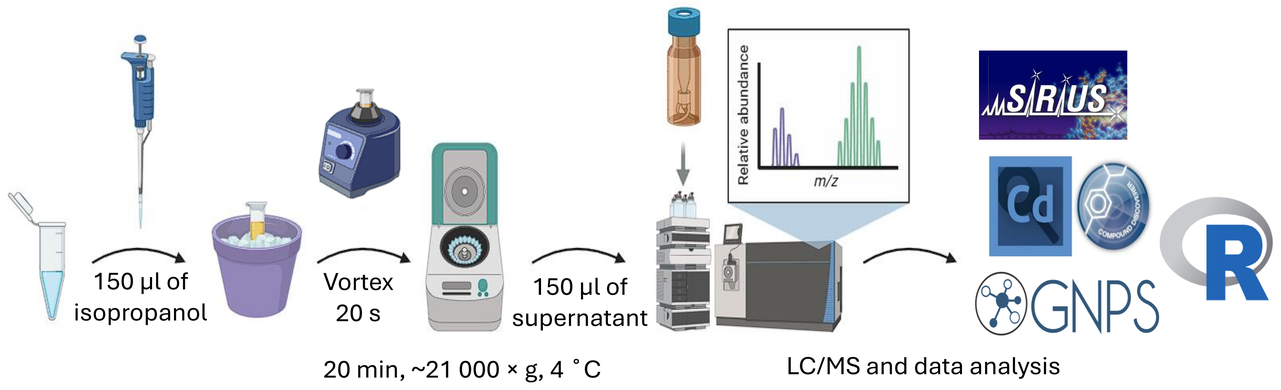


Fig.1. Sample preparation and analysis overview.

Results

By the untargeted lipidomic approach, 875 and 631 compounds in the positive and negative ionisation modes were detected after data processing and filtering based on the Peak Rating filter and *in-silico* comparison with MS/MS spectra in the databases.

Discriminant analysis between sample groups was performed using a differential analysis method, showing a volcano plot. Twenty-nine lipids were detected whose levels were elevated in the patient samples ($p < 0.05$). Blue data points correspond to the lipid class of sphingolipids, specifically ceramide and sphingomyelin compounds (**Fig. 2**). The statistical specification of these lipids is recorded in **Tab. 1**.

Violin plots were used to compare the relative concentrations of individual lipids between patient and control samples. The selected ceramides are illustrated in **Fig. 3**.

Using molecular networks (MNs) has proven to be a valuable tool for identifying the structures of unknown compounds. MNs offer a computational strategy emphasising the organisation and visualisation of numerous structurally related molecules based on their MS/MS or MS^1 spectra. The molecular network of phosphatidylcholines and their molecular weights is shown in the **Fig. 4**.

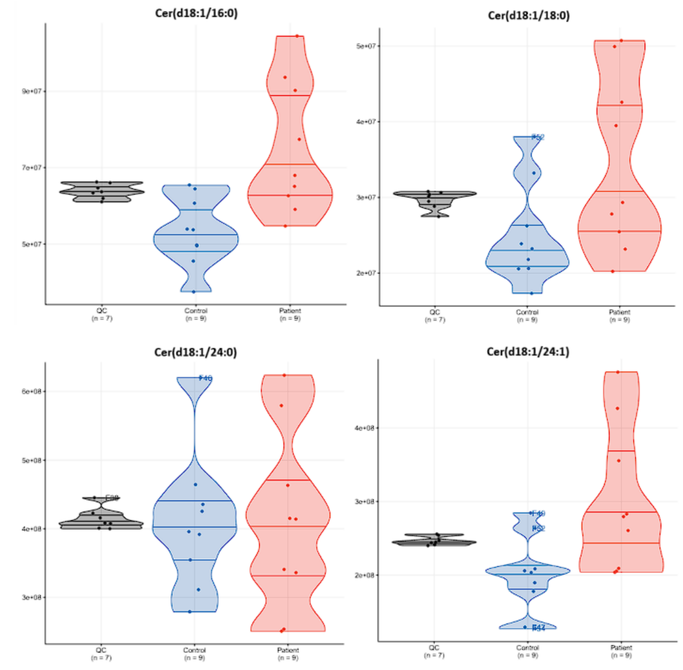


Fig. 3. **Violin plots** generated by the R programming language to **compare the relative concentrations of selected ceramides (Cer) in each group of samples**.

Lipidy	p-hodnota (t-test)	Míra změny (Fold change, K/P)
Cer 42:5;2O	0,032	−0,37
Cer 43:5;2O	7,5E−3	−0,63
Cer 18:1;2O/16:0	8,5E−4	−0,42
Cer 18:1;2O/18:0	0,032	−0,52
Cer 18:1;2O/24:1	1,7E−3	−0,49
Cer 18:1;2O/24:2	7,2E−3	−1,10
Cer 18:2;2O/23:0	0,030	−0,74
Cer 18:2;2O/24:1	6,9E−4	−0,77
Cer 18:2;2O/25:0	4,5E−3	−0,69
Hex3Cer 18:1;2O/24:1	5,4E−3	−0,28
PC 29:5	4,5E−3	−0,39
PC 16:0_22:5	4,6E−3	−0,34
PC 37:5	5,6E−4	−0,45
SM 34:2;2O	0,045	−0,22
SM 40:3;2O	0,041	−0,29
SM 42:3;2O	4,5E−3	−0,46
SM 42:4;2O	0,012	−0,55
SM 43:3;2O	4,1E−3	−0,64
SM 34:2;3O	8,7E−3	−0,37

Tab. 1. Statistically significant lipids separating patients and controls (positive mode, $p < 0.05$)

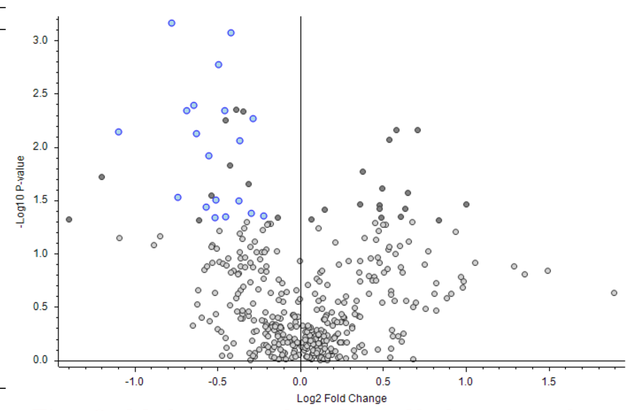


Fig. 2. **Volcano plot for all detected lipids in positive mode** after Peak Rating filter.

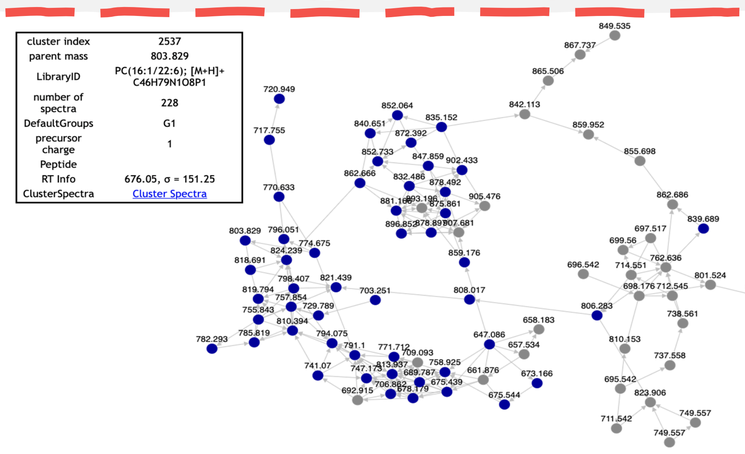


Fig. 4. **Molecular network of phosphatidylcholines** with their molecular weights. The molecular network was generated in **GNPS**.

Conclusion

Glycerophospholipids, primarily subclass **PC**, and sphingolipids, particularly **ceramides** and **sphingomyelins**, were identified as the **most discriminating lipid classes**. The most significantly elevated lipids in **STEMI patient samples** were evaluated as **Cer(d18:1/16:0)**, **Cer(d18:1/18:0)**, **Cer(d18:1/24:1)** and **Cer(d18:2/24:1)**. Our findings align with clinically validated **CERAM** (Mayo Clinic, USA) and **CERT2** (Zora Biosciences, Finland) tests, highlighting the potential of lipidomics in cardiovascular risk assessment. **To use comprehensive lipid profiles** for disease diagnosis **in clinical practice**, it is essential to **analyse multiple samples** and, ideally, **various diagnoses**.

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