Lipidomic Test for Early Diagnosis of Pancreatic Cancer: Transfer to Clinical Practice

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1. Overview

- LDPC test – patented lipidomic quantification method
- Method transfer – lipidomic differences between females and males analyzed at the University of Pardubice and at Lipidica
- Selection of biological material (serum and plasma) – lipidomic profiling of cancer patients and controls
- Prediction for high-risk patients
- Upcoming plans – clinical performance study according to the in vitro diagnostic regulation (IVDR)

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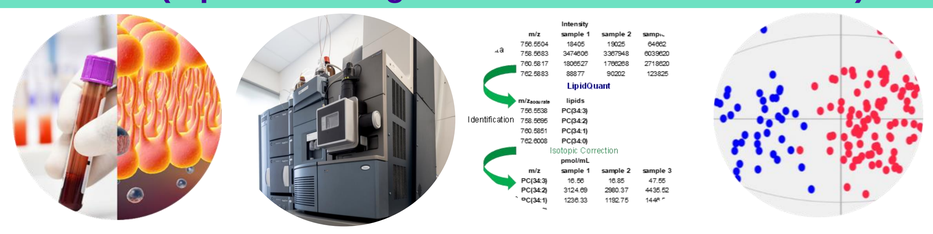
2. LDPC (Lipidomic Diagnostics of Pancreatic Cancer) test



Sample collection (isolation of plasma/serum)
Sample preparation (liquid-liquid extraction)
→ UHPSFC/MS analysis
→ Data processing (Laboratory-made software LipidQuant)
→ Multivariate data analysis

3. Analytical part of LDPC

Sample preparation (Folch extraction)

25 µL plasma/serum + 20 µL IS
→ + 2 mL $CHCl_3$ + 1 mL MeOH, ultrasonication
→ + 600 µL $(NH_4)_2CO_3$, shaking centrifugation
→ removal of aqueous phase, evaporation (N_2, 35 °C)
→ 500 µL $CHCl_3$: MeOH (1:1, *v/v*), dissolution

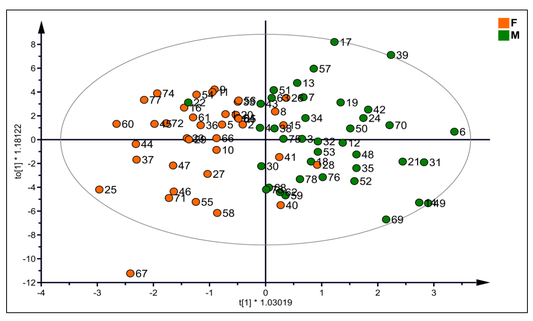
UHPSFC/MS method

- Lipid class separation – 2 internal standards per lipid class
- Acquity UPC^2 connected to Xevo G2-XS QTOF (Waters)
- Column: Viridis BEH (100 x 3 mm, 1.7 µm), column temperature 60 °C
- Modifier and make-up solvent: MeOH with 30 mM ammonium acetate and 1 % water
- Gradient: 0 min 1% modifier, 1.5 min 16% modifier, 4 min 51% modifier, 7 min 51% modifier. Total run time 8 min.
- Flow rate: 1.9 mL/min, Flow rate of make-up solvent: 0.25 mL/min, ABPR pressure: 1800 psi
- Positive ESI ion mode, mass range of *m/z* 300 – 1200, sensitivity mode, continuum mode, lock mass correction

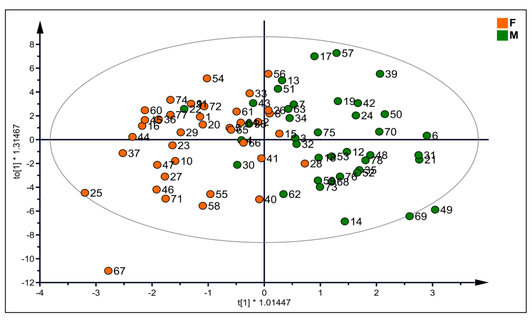
4. Method transfer

- Plasma samples from healthy controls – 39 females and 39 males
- Quantification of 158 lipids from 8 lipid classes
- Analysis at the University of Pardubice (Synapt G2-Si) and at Lipidica (Xevo G2-XS)
- Comparison of lipidomic profiles of females and males

OPLS-DA (University)



OPLS-DA (Lipidica)



Lipid classes	CE	Cer	MG	DG	TG	SM	PC	LPC
Females	=	↑	=	=	↓	↑	=	↓
Males	=	↓	=	=	↑	↓	=	↑

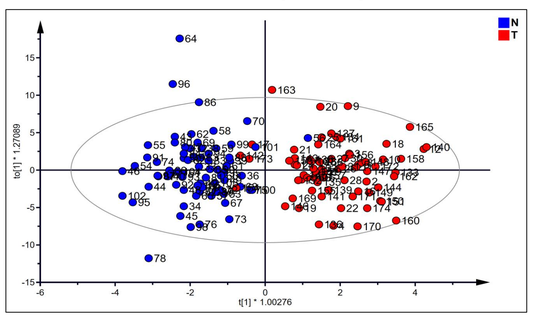
Conclusion

Successful transfer of the method from the university to the spin-off company Lipidica
→ comparable lipidomic profiles between females and males measured at two sites
→ same dysregulations of lipid classes

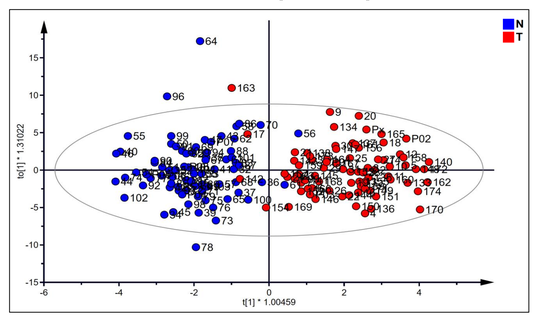
5. Selection of biological material

- Lipidomic profile of 74 healthy controls and 73 patients with pancreatic ductal adenocarcinoma (PDAC)
- Quantification of 170 lipids from 9 lipid classes
- Training set (127 samples) + validation set (20 samples)
- Comparison of statistical model for plasma and serum

OPLS-DA (Serum)



OPLS-DA (Plasma)



Training set

	Serum	Plasma
Sensitivity	94.5 %	94.5 %
Specificity	98.6 %	98.6 %
Accuracy	96.6 %	96.6 %

Validation set

	Serum	Plasma
Sensitivity	90 %	100 %
Specificity	100 %	90 %
Accuracy	95 %	95 %

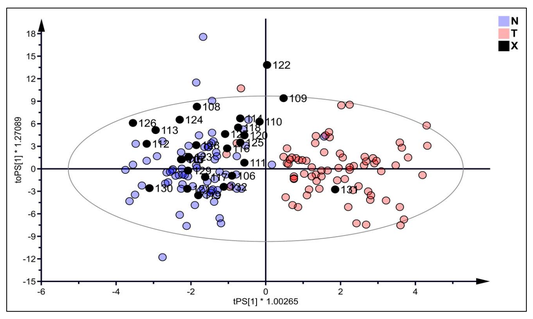
Conclusion

Lipidomic profiling of PDAC patients and healthy controls
→ same results when using serum or plasma
→ same model parameters for training set, comparable for validation set

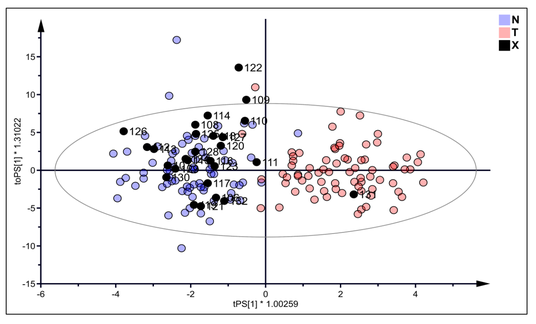
6. High-risk patients prediction

- Lipidomic profile of 74 healthy controls and 73 patients with PDAC
- 27 patients from high-risk group for PDAC, currently healthy according to endoscopic ultrasound (EUS) or magnetic resonance imaging (MRI) examination

OPLS-DA (Serum)



OPLS-DA (Plasma)



Conclusion

Prediction of the state of high-risk patients
→ same results for serum and plasma
→ all patients classified as healthy (except for one who will be further monitored by doctors)

7. Clinical performance study of the LDPC test

- The next step on the way to clinical practice
- Analysis of a larger cohort of samples + comparison with CA 19-9, CEA and EUS and/or MRI results according to the study protocol
- Determination of sensitivity and specificity of the test

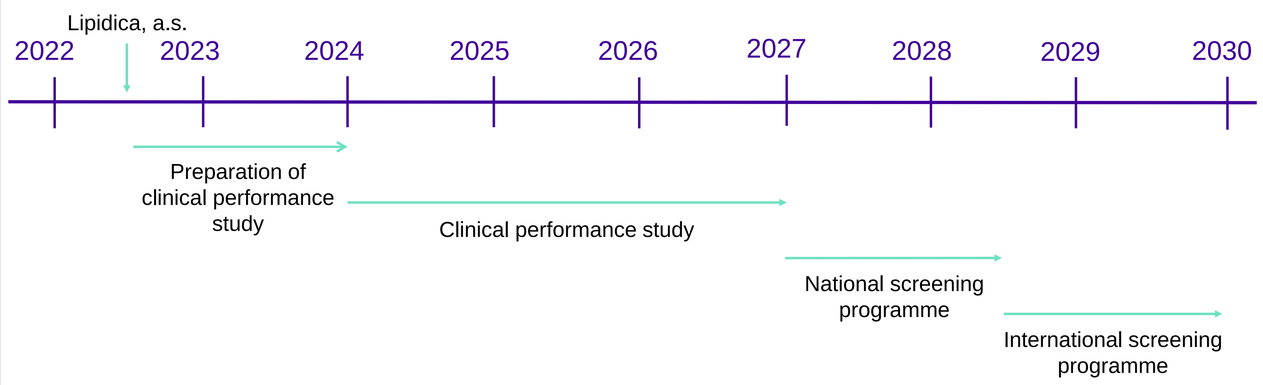
Inclusion criteria

1. Patients with newly diagnosed PDAC (resectable stage)
2. People at increased risk for PDAC
   A) People with ≥ 2 relatives from the same side of the family (at least one is first-degree relative) diagnosed with PDAC
   B) People diagnosed with genetic mutation – STK11, BRCA1, BRCA2, CDKN2A, APC, ATM, MLH1, MSH2, MSH6, PMS2, EPCAM, PALB2 or TP53
   C) Patients with hereditary pancreatitis

Exclusion criteria

1. History of cancer
2. Incurable malignancy
3. Vegan or vegetarian diet
4. Inability to undergo radical surgery for pancreatic cancer
5. Inability to undergo planned imaging examinations

8. Timeline



9. Summary

- Successful transfer of the method from the university to the spin-off company Lipidica
- Lipidomic profiling of PDAC patients and healthy controls – same model parameters for serum and plasma
- High-risk patients classified as healthy based on lipidomic profiling

Acknowledgements

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References

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[3] Sawhney M. S. et al. *Gastrointest. Endosc.* 95 (2022) 814