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Mass Spectrometry-Based Lipidomic Profiling for Screening Pancreatic Cancer: A Pilot Study

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Abstract - Background: Pancreatic cancer is among the most lethal malignancies, with a five-year survival rate of approximately 13%. This poor prognosis is mainly due to the absence of reliable strategies for early detection. Current diagnostic approaches depend largely on imaging techniques, which are often expensive, invasive, and insufficient for identifying disease at an early stage. Consequently, the development of a sensitive and specific non-invasive blood-based screening method could significantly improve early diagnosis and patient survival.

Methods: In this study, lipid concentrations in plasma and serum samples were analyzed using ultra-high-performance supercritical fluid chromatography coupled with mass spectrometry (UHPSFC-MS). Multivariate statistical analysis was applied to evaluate lipidomic patterns and to distinguish between different study groups.

Results: This pilot investigation included prospectively collected samples from patients diagnosed with Pancreatic ductal adenocarcinoma (PDAC; n = 177), healthy individuals (n = 218), and participants considered at high risk for pancreatic cancer (n = 93). Lipidomic profiling successfully differentiated PDAC patients from healthy controls with an accuracy exceeding 95%. The method also demonstrated strong capability for detecting early-stage cases and identifying individuals with low secretion of CA 19-9. The sensitivity of the lipidomic approach was approximately 30% higher than that of CA 19-9. In the high-risk cohort, the method achieved a specificity greater than 96% (95% CI: 89–99%), comparable to established imaging-based diagnostic strategies.

Conclusion: The findings from this pilot study highlight the potential of lipidomic profiling as a non-invasive blood-based screening approach for pancreatic cancer. The technique demonstrates improved diagnostic performance compared with conventional biomarkers and maintains high accuracy in early-stage disease as well as in high-risk populations. These results support the need for larger clinical trials to further validate lipidomic testing for early detection of PDAC.

I. INTRODUCTION

Pancreatic cancer is considered one of the most lethal forms of cancer, with an overall five-year survival rate of approximately 13%. Survival varies greatly depending on the stage of diagnosis, with reported rates of about 3% for metastatic disease, 17% for regional spread, and around 42% for localized tumors. The most prevalent histological type is Pancreatic ductal adenocarcinoma (PDAC), which represents more than 90% of pancreatic malignancies. In recent decades, both the incidence and mortality rates of pancreatic cancer have steadily increased, and projections suggest that it may become the second leading cause of cancer-related deaths by 2030.

Currently, the primary treatment options for PDAC involve a combination of surgical intervention and chemotherapy. However, fewer than 20% of patients are diagnosed at a stage where surgical resection is possible. This limitation is largely due to the fact that PDAC frequently develops without noticeable symptoms or presents with vague clinical manifestations, resulting in diagnosis at an advanced stage.

Several imaging techniques are routinely used for the detection and evaluation of pancreatic tumors, including endoscopic ultrasonography (EUS), magnetic resonance imaging (MRI), and computed tomography (CT). Although these modalities are effective diagnostic tools, they can be expensive, technically demanding, and sometimes uncomfortable or invasive for patients. In addition, exposure to radiation or contrast agents may pose risks, and certain patients may be unable to undergo these procedures due to allergies, implanted medical devices, or conditions such as claustrophobia.

Among currently available biomarkers, CA 19-9 is the only marker commonly used in the clinical management of pancreatic cancer. However, its diagnostic performance is limited because it shows low sensitivity for early-stage disease and lacks sufficient specificity for population screening. Other biomarker research approaches, including studies in genomics, transcriptomics, and proteomics, as well as the development of multi-marker diagnostic panels, have been explored extensively. Despite these efforts, no biomarker has yet achieved global clinical validation for the early detection of pancreatic cancer. Recently, a blood-based diagnostic test known as PancreaSure has been introduced in the United States, highlighting the growing interest in non-invasive screening strategies.

In this context, lipidomics has emerged as a promising area of research for biomarker discovery. Lipidomics focuses on the comprehensive study of lipid molecules within biological systems. Lipids play crucial roles in cellular structure, metabolism, and signaling pathways, and alterations in lipid metabolism have been observed in several cancers, including pancreatic cancer. Therefore, investigating lipid profiles in biological samples may provide valuable insights for early disease detection.

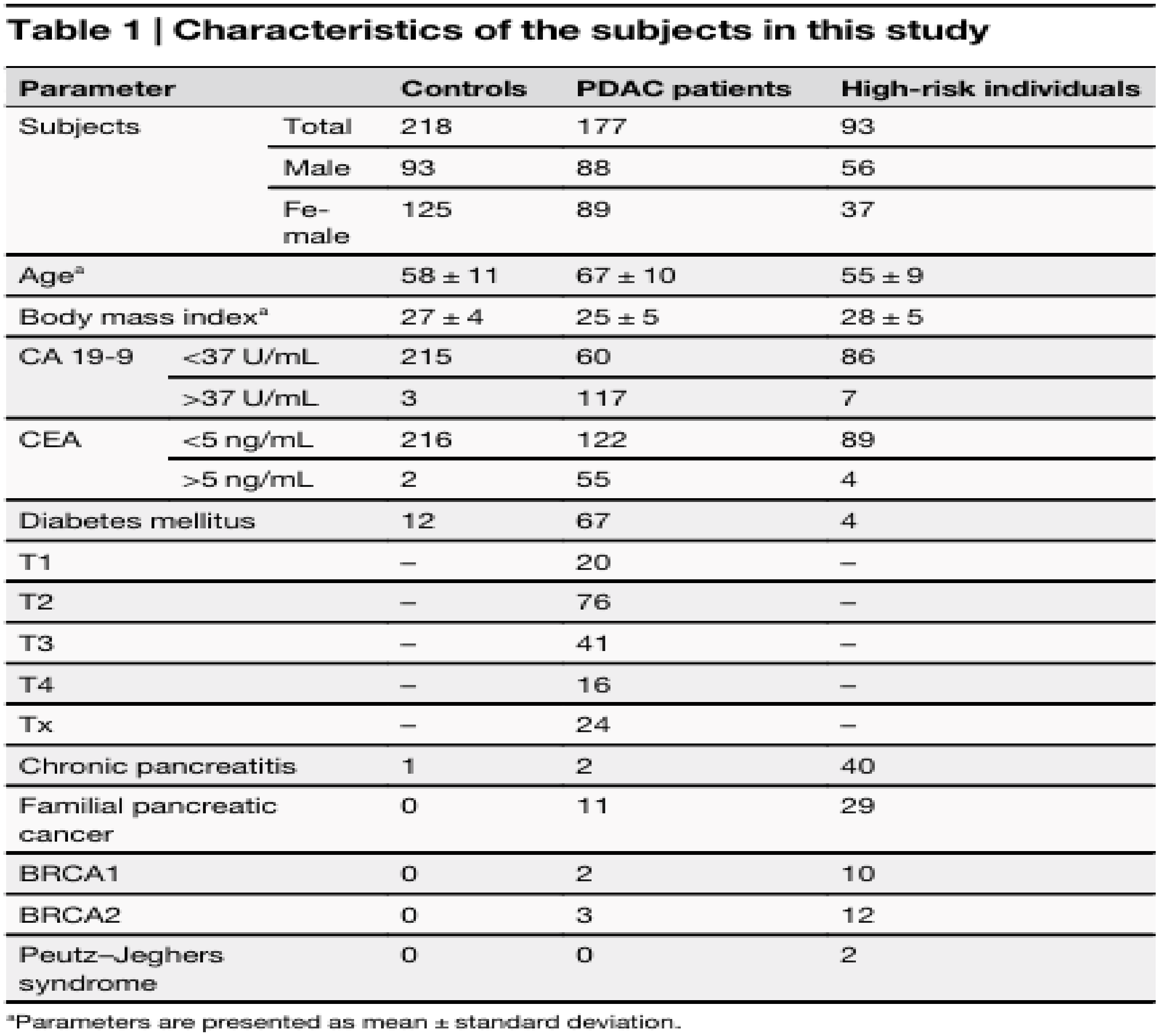


Table 1 | Characteristics of the subjects in this study

Parameter		Controls	PDAC patients	High-risk individuals
Subjects	Total	218	177	93
	Male	93	88	56
	Female	125	89	37
Age[a]		58 ± 11	67 ± 10	55 ± 9
Body mass index[a]		27 ± 4	25 ± 5	28 ± 5
CA 19-9	<37 U/mL	215	60	86
	>37 U/mL	3	117	7
CEA	<5 ng/mL	216	122	89
	>5 ng/mL	2	55	4
Diabetes mellitus		12	67	4
T1		–	20	–
T2		–	76	–
T3		–	41	–
T4		–	16	–
Tx		–	24	–
Chronic pancreatitis		1	2	40
Familial pancreatic cancer		0	11	29
BRCA1		0	2	10
BRCA2		0	3	12
Peutz–Jeghers syndrome		0	0	2

[a] Parameters are presented as mean ± standard deviation.

Prospective surveillance programs targeting individuals at elevated risk of pancreatic cancer have demonstrated improved detection of resectable tumors and better survival outcomes compared with patients diagnosed outside such monitoring programs. Because large-scale screening of the general population is not currently practical due to cost and logistical constraints, screening strategies are mainly recommended for high-risk individuals. In the general population, the lifetime risk of developing PDAC is approximately 1.5%. However, this risk increases significantly in individuals with factors such as obesity, smoking, excessive alcohol consumption, and certain dietary habits. Furthermore, individuals with hereditary cancer syndromes, familial pancreatic cancer, or hereditary pancreatitis are considered to have a substantially elevated risk.

Another group that may benefit from targeted screening includes individuals older than 50 years who are newly diagnosed with type 2 diabetes mellitus, particularly type 3c diabetes, as a small percentage of these individuals develop pancreatic cancer within a few years of diagnosis.

Although this group is not yet universally classified as high-risk, it represents a potentially important population for early detection efforts.

Despite advances in surgical techniques and systemic therapies, the prognosis of PDAC remains poor, primarily due to delayed diagnosis. Therefore, identifying reliable, non-invasive biomarkers capable of detecting pancreatic cancer at an early stage is a critical priority. Blood-based screening approaches with high sensitivity and specificity could significantly improve early detection and patient outcomes.

The present study expands upon our previous research demonstrating that lipidomic profiling can effectively differentiate patients with PDAC from healthy individuals with high diagnostic accuracy. In the first phase of this investigation, the methodology was optimized and limitations from earlier studies were addressed. In the second phase, the approach was applied to samples obtained from high-risk individuals, and the results were compared with imaging findings. The findings indicate that lipidomic profiling provides high sensitivity and specificity for detecting PDAC, including early-stage disease, and shows improved diagnostic performance compared with established biomarkers such as CA 19-9 and Carcinoembryonic antigen.

II.METHODS

Study Participants

This prospective study included three groups of participants consisting of both male and female subjects: healthy controls, patients diagnosed with Pancreatic ductal adenocarcinoma (PDAC), and individuals considered to be at high risk for developing pancreatic cancer. All participants were adults aged 18 years or older. Blood samples were obtained from each volunteer following an overnight fasting period.

Healthy controls were selected based on the absence of any previous cancer diagnosis throughout their lifetime, while other medical conditions were not considered exclusion criteria. Blood samples from PDAC patients were collected only after confirmation of the disease diagnosis and included individuals representing all tumor stages. Participants categorized as high-risk individuals did not have PDAC at the time of enrollment but had at least one recognized risk factor such as familial pancreatic cancer, chronic pancreatitis, or inherited genetic susceptibility syndromes.

In total, 488 participants were recruited for this investigation, including 218 healthy controls, 177 PDAC patients, and 93 high-risk individuals. Because this study was exploratory in nature, the sample size was determined by participant availability rather than by a predefined statistical power calculation. Clinical characteristics of all subjects are summarized in the supplementary dataset.

The study was conducted according to the ethical principles outlined in the Declaration of Helsinki and received approval from institutional ethics committees of participating medical centers in the

Czech Republic. Written informed consent was obtained from all participants prior to sample collection. Personal data were pseudonymized to maintain confidentiality.

III.SAMPLE PREPARATION

All biological samples were stored at −80 °C until lipid extraction was performed. Lipids were isolated using a modified version of the Folch extraction protocol. Briefly, 25 µL of EDTA-treated human plasma or serum was combined with 20 µL of an internal standard mixture and homogenized with 3 mL of chloroform and methanol in a 2:1 ratio. The mixture was sonicated for 15 minutes at 30 °C and subsequently allowed to cool to room temperature.

After cooling, 600 µL of 250 mM ammonium carbonate solution was added and the sample was agitated for five minutes. The mixture was centrifuged to separate the phases, and the aqueous layer was discarded. The organic fraction containing lipids was evaporated under a gentle nitrogen stream at 35 °C. The remaining residue was then dissolved in 500 µL of a chloroform–methanol mixture (1:1, v/v). Prior to analysis, the extract was diluted appropriately depending on the analytical method used for lipidomic measurements.

IV.LIPIDOMIC ANALYSIS

Quantitative lipidomic profiling was carried out using two complementary mass spectrometry-based analytical approaches: one incorporating chromatographic separation and the other relying on direct infusion analysis.

For chromatographic analysis, ultrahigh-performance supercritical fluid chromatography coupled with high-resolution mass spectrometry (UHPSFC-MS) was employed. This system was connected to a quadrupole time-of-flight mass spectrometer equipped with an electrospray ionization source operating in positive ion mode. Lipid class separation was performed using a BEH column maintained at 60 °C, with carbon dioxide serving as the primary mobile phase and methanol containing ammonium acetate and water acting as the modifier. Gradient elution was applied to achieve separation of lipid classes during an eight-minute analytical run.

The second approach involved flow injection analysis coupled with tandem mass spectrometry (FIA-MS/MS). In this technique, lipid extracts were directly introduced into the mass spectrometer without chromatographic separation. Specific precursor ion and neutral loss scanning strategies were used for lipid detection and quantification. Samples were delivered through a liquid chromatography system at controlled flow rates during injection, scanning, and washing phases. Measurements were also performed in positive electrospray ionization mode.

V.QUALITY CONTROL

To ensure analytical reliability and instrument stability, three types of quality control samples were analyzed throughout the experimental sequence. These included pooled human plasma (QC-P), pooled human serum (QC-S), and a certified reference plasma material known as NIST SRM1950.

The pooled samples were prepared by combining aliquots from previously analyzed plasma or serum samples to generate representative matrices.

Quality control extracts were injected regularly during the analysis to monitor signal stability and reproducibility. QC-P and QC-S samples were analyzed after every 20 sample injections, while the reference plasma standard was introduced after every 120 injections to facilitate potential inter-laboratory comparisons.

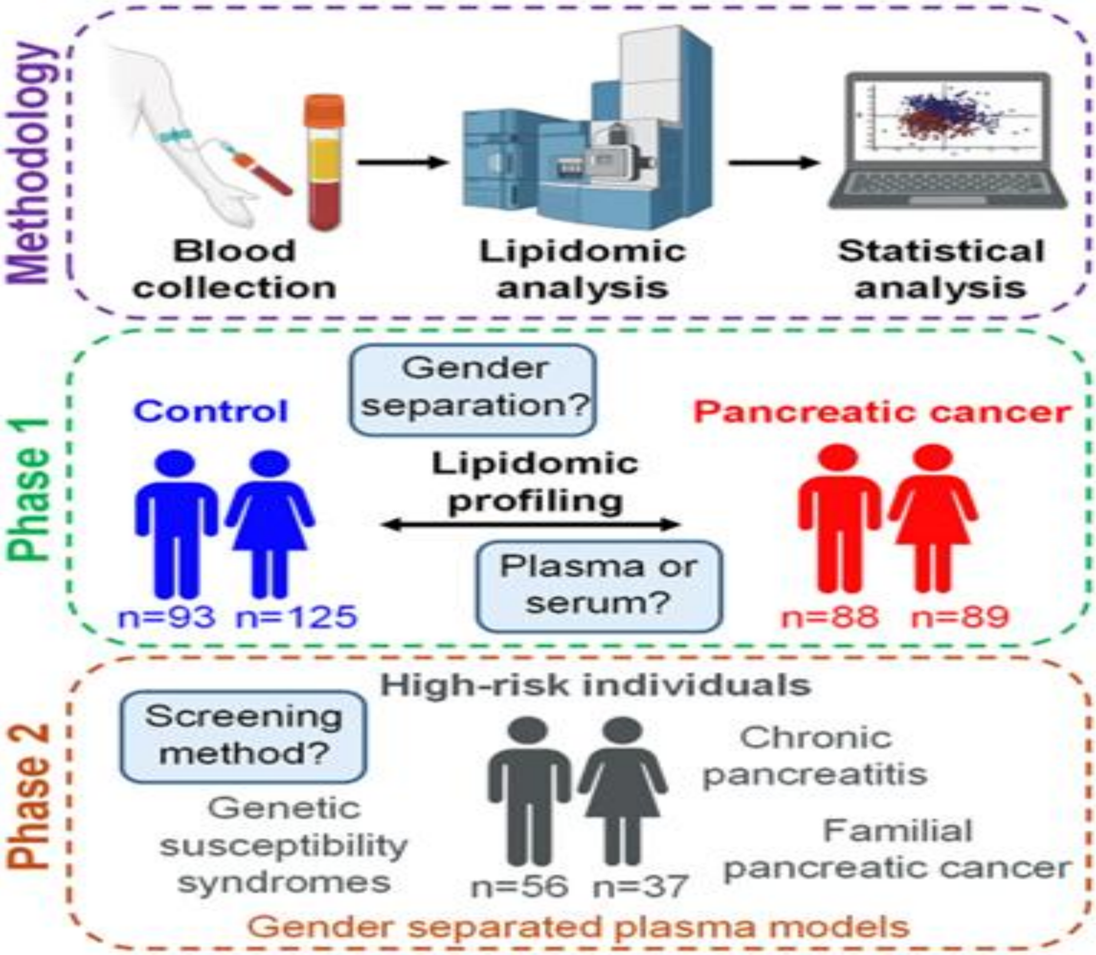


Fig. 1 | Overview of the study design. The figure illustrates the overall study workflow, including the methodology, primary research questions, and cohort structure across individual phases. A prospective sample collection involves 488 subjects, comprising 218 healthy controls, 177 PDAC patients, and 93 high-risk individuals.

VI.DATA PROCESSING

Raw UHPSFC-MS data underwent noise filtering, lock-mass correction, and conversion from continuum to centroid format. Signal intensities corresponding to lipid species were extracted using dedicated software tools, and lipid concentrations were calculated with specialized lipidomics software that also performed isotopic corrections.

For the FIA-MS/MS dataset, raw spectral files were processed using dedicated lipid identification software, and the resulting tables were manually verified against precursor ion and neutral loss spectra to ensure correct peak assignment. Final datasets containing lipid concentrations were assembled using statistical software.

Only lipid species detected in at least 75% of samples were retained for further analysis. Missing values were replaced using a small constant corresponding to a fraction of the minimum observed concentration for the respective lipid species.

VII.CONVENTIONAL BIOMARKER MEASUREMENTS

Concentrations of established pancreatic cancer biomarkers were determined to compare their diagnostic performance with lipidomic profiling. Levels of CA 19-9 were measured using a chemiluminescent microparticle immunoassay. A threshold value of 37 U/mL was used to define positive results.

Similarly, the concentration of Carcinoembryonic antigen (CEA) was determined using a two-step immunoassay based on antigen–antibody binding. A cutoff value of 5 ng/mL was applied to classify positive samples.

VIII.IMAGING EXAMINATION

Imaging procedures were performed to evaluate pancreatic morphology and detect suspicious lesions. Endoscopic ultrasonography was carried out under conscious sedation using a specialized ultrasound endoscope introduced through the patient's upper gastrointestinal tract. Images and video recordings were stored for further evaluation. When suspicious lesions were identified, fine-needle biopsy was performed to obtain tissue samples for cytological or histological examination.

Magnetic resonance imaging of the pancreas was also conducted following recommended guidelines for early pancreatic cancer detection. The protocol included diffusion-weighted imaging and cholangiographic sequences to visualize pancreatic ducts and cystic structures. For enhanced imaging, intravenous administration of a gadolinium-based contrast agent was performed.

Statistical Analysis

Statistical analysis of lipidomic data was conducted to determine differences between study groups. Comparisons of lipid concentrations were performed using Welch's t-test and fold-change calculations, with statistical significance defined as p-values below 0.05. To account for multiple testing, Bonferroni correction was applied.

Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic performance of the lipidomic models, and the corresponding area under the curve (AUC) values were calculated. Data visualization and statistical analyses were conducted using the R programming environment.

Multivariate statistical modeling was performed using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). These methods were used to identify lipid species contributing most strongly to the separation between PDAC patients and control groups. Model validation was performed using cross-validation techniques and permutation testing to evaluate robustness and prevent overfitting. Predictive performance was assessed using independent validation datasets.

IX.RESULTS

Study Design

The proposed strategy for the early identification of Pancreatic cancer relies on the quantification of lipid species in human plasma or serum followed by statistical interpretation using multivariate analytical techniques. In our previous work, lipidomic profiling demonstrated the ability to differentiate patients diagnosed with Pancreatic ductal adenocarcinoma (PDAC) from healthy individuals with sensitivity, specificity, and overall diagnostic accuracy greater than 94% in the training dataset and above 80% in an independent validation cohort.

Despite these promising results, several methodological questions remained unresolved and required clarification before the approach could be considered for clinical screening. To address these issues, a prospective cohort study including 488 participants was conducted (Table 1). The overall experimental workflow and analytical design are illustrated in Figure 1.

During Phase 1, the influence of biological variables such as gender and sample matrix on lipid concentrations was examined, as previous studies have reported inconsistent findings regarding these factors. In addition, the analytical workflow was enhanced using updated lipid quantification software and by expanding the lipid panel to include phosphatidylethanolamines (PE), which previously showed potential diagnostic relevance in a smaller pilot cohort.

Unsupervised principal component analysis (PCA) demonstrated partial clustering of samples according to gender, whereas minimal variation was observed between plasma and serum matrices. In contrast, clear separation was observed between lipidomic profiles of healthy individuals and PDAC patients. Furthermore, the tight clustering of quality control samples confirmed the stability and reproducibility of the analytical platform. Incorporating these methodological improvements significantly enhanced the discriminative capability of the lipidomic model and improved the accuracy of blind sample classification.

In Phase 2, the optimized analytical approach was applied to samples obtained from individuals considered to be at high risk for pancreatic cancer. These participants were enrolled through national surveillance programs aimed at early pancreatic cancer detection using regular imaging assessments. The diagnostic performance of lipidomic profiling was subsequently compared with conventional imaging techniques and established tumor biomarkers such as CA 19-9 and Carcinoembryonic antigen.

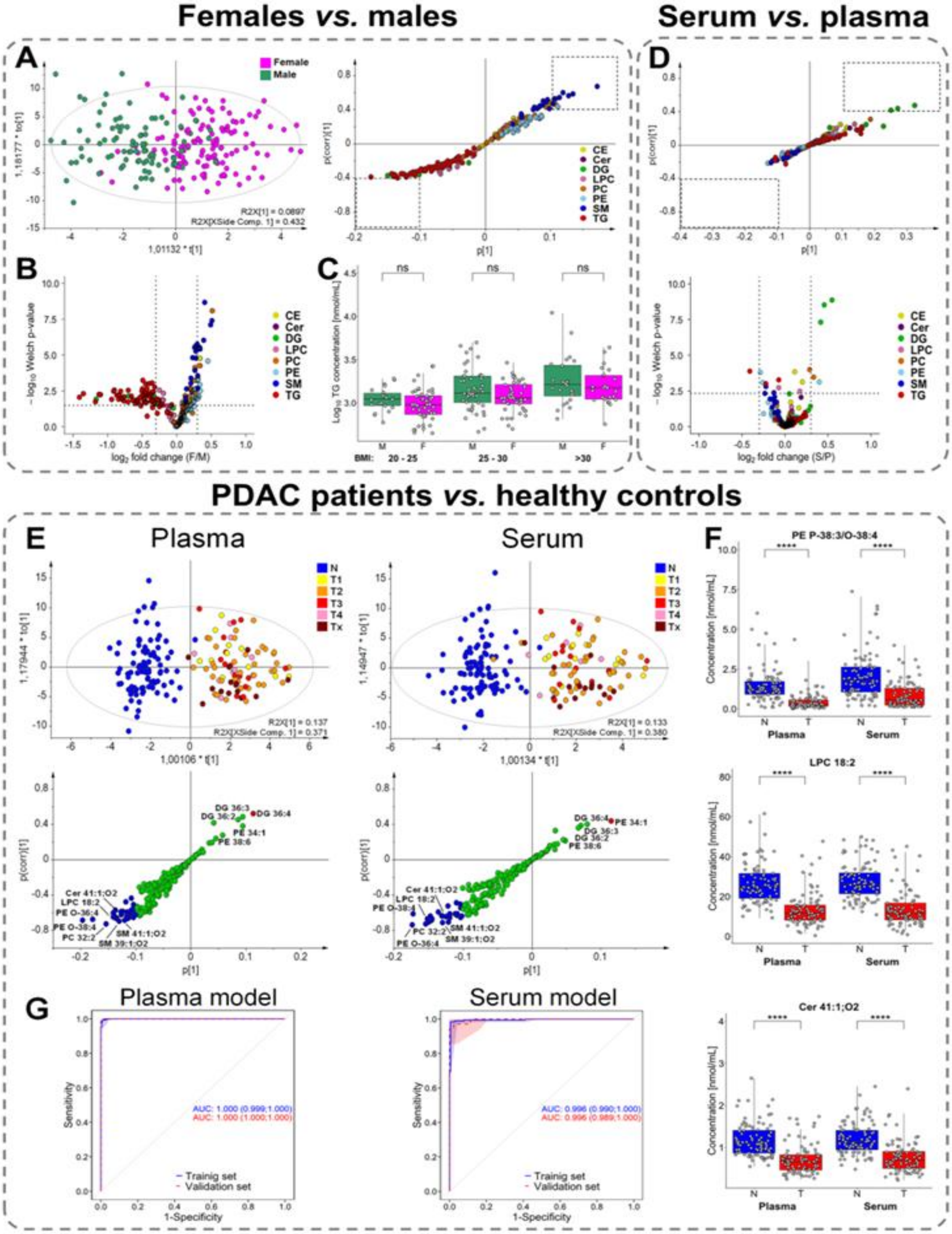


Figure 2 | Comparative analysis of lipid profiles obtained using UHPSFC/MS across different study groups.

Lipidomic profiles were evaluated across several biological and clinical comparisons, including females vs. males (plasma-based model), serum vs. plasma samples (female models), and patients with Pancreatic ductal adenocarcinoma (PDAC) compared with healthy individuals (male models).

(A) Orthogonal partial least squares discriminant analysis (OPLS-DA) score plots demonstrating group separation, along with corresponding S-plots identifying lipid species contributing most strongly to discrimination between groups.

(B) Volcano plot illustrating significantly altered lipid species based on $\log_2$-transformed fold change and $-\log_{10}$ p-values derived from a two-sided t-test.

(C) Relationship between body mass index (BMI) and the cumulative concentration of triacylglycerols. Statistical significance was evaluated using the Mann–Whitney U test, where p-values greater than 0.05 were considered non-significant (ns). BMI categories included 20–25 (22 males and 49 females), 25–30 (47 males and 46 females), and >30 (24 males and 25 females).

(D) Multivariate and univariate comparisons between serum and plasma lipid profiles. The S-plot derived from OPLS-DA highlights lipid species contributing most significantly to separation between the two biological matrices. The accompanying volcano plot displays $\log_2$ fold changes and $-\log_{10}$ p-values obtained from a two-sided t-test.

(E) Supervised OPLS-DA score plots showing cancer samples classified according to tumor stage, with color coding as follows: T1 (yellow), T2 (orange), T3 (red), T4 (rose), and Tx (brown), where tumor stage information was unavailable. The associated S-plots identify the most upregulated (red) and downregulated (blue) lipid species within both plasma and serum models.

(F) Box-plot representation of the most significantly altered lipid species, highlighting differences in lipid concentrations between healthy controls (N, blue; $n = 93$) and PDAC patients (T, red; $n = 88$). Statistical significance was determined using the Mann–Whitney U test, with **** indicating $p < 0.0001$.

(G) Receiver operating characteristic (ROC) curves with 95% confidence intervals illustrating the diagnostic performance of plasma- and serum-based models in both training and validation datasets. The curves represent combined male and female predictions derived from gender-specific lipidomic models.

Influence of Gender and Sample Matrix on the Lipidome

Samples obtained from healthy individuals were first analyzed to evaluate the potential effect of gender on lipid composition in both plasma and serum. The study included 93 male and 125 female participants with comparable age distribution and body mass index. A total of 190 lipid species belonging to 11 lipid subclasses were examined using both univariate statistical analysis and multivariate modeling.

Supervised orthogonal partial least squares discriminant analysis (OPLS-DA) demonstrated a clear distinction between lipid profiles of male and female participants. Visualization of the most altered lipid species using S-plots and volcano plots revealed several gender-associated differences. Females generally exhibited higher concentrations of sphingomyelins and ether-linked phosphatidylcholines with shorter fatty-acid chains. Conversely, males showed elevated levels of triacylglycerols and diacylglycerols containing shorter acyl chains.

Minor differences were also observed for other lipid classes. Slightly increased levels of phosphatidylethanolamines were detected in females, while lysophosphatidylcholines were marginally higher in males. In contrast, lipid classes such as cholesteryl esters, ceramides, and phosphatidylcholines showed similar concentrations across both genders.

Although certain lipid species were differentially expressed, no consistent pattern was identified regarding fatty-acid chain length or degree of unsaturation. Similar trends were observed in both plasma and serum datasets, confirming the reproducibility of these findings.

Because male participants displayed a slightly higher average body mass index, the relationship between body mass index and total triacylglycerol concentration was further examined. Statistical evaluation revealed that elevated triacylglycerol levels in males were not significantly associated with body mass index but rather reflected metabolic differences between genders. Nevertheless, increased triacylglycerol concentrations were correlated with higher body mass index values in both sexes, indicating that this lipid class may be influenced by lifestyle factors and therefore may not represent a reliable biomarker.

X.COMPARISON BETWEEN PLASMA AND SERUM MATRICES

To determine whether the biological matrix affects lipidomic measurements, lipid profiles obtained from plasma and serum samples were compared using the same group of healthy volunteers. Blood samples for both matrices were collected simultaneously to ensure consistency. To avoid bias introduced by gender-related differences, the analysis was performed separately for male and female participants.

The results indicated that most lipid classes showed comparable concentrations in plasma and serum. However, a small number of diacylglycerol species exhibited matrix-dependent differences, with serum samples demonstrating approximately 10–30% higher concentrations, particularly for DG species containing 36 carbon atoms.

For other lipid classes, no statistically significant differences were detected between the two matrices. Minor tendencies were observed, including slightly reduced levels of phosphatidylethanolamines and sphingomyelins in serum and modestly increased concentrations of certain lipids, but these variations were not statistically significant. Overall, the findings suggest that both plasma and serum are suitable matrices for lipidomic profiling in pancreatic cancer research.

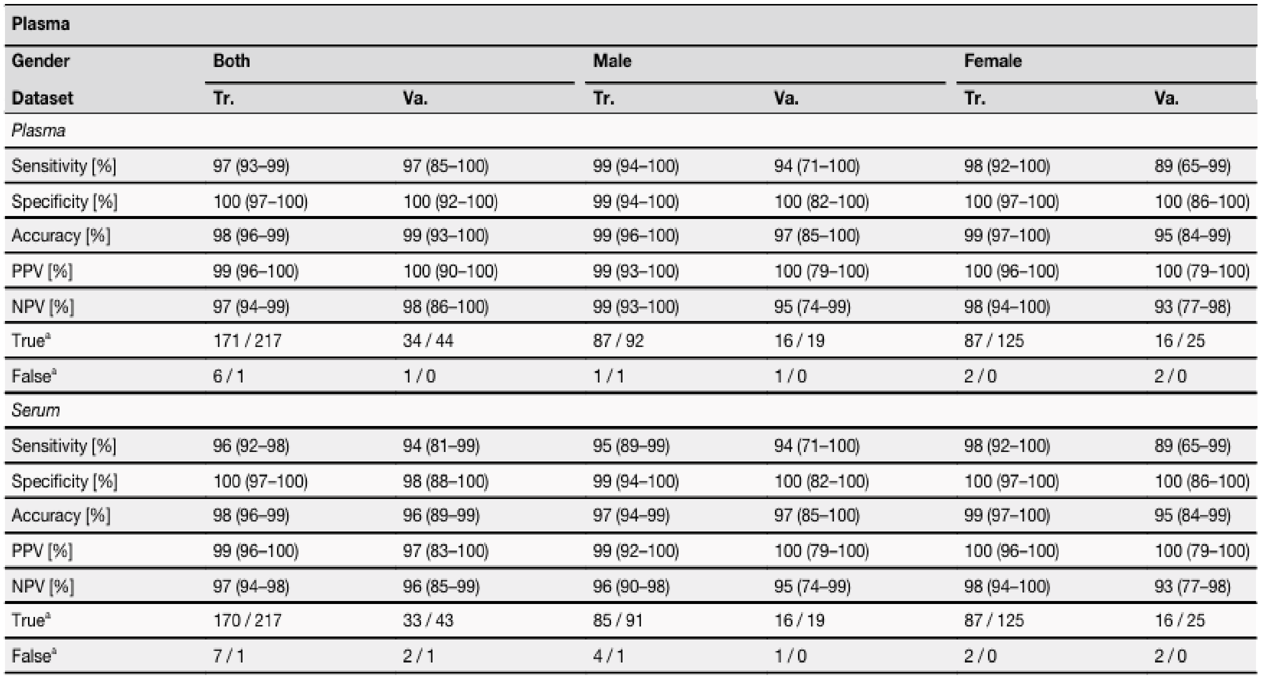
XI.LIPIDOMIC DIFFERENCES BETWEEN PDAC PATIENTS AND HEALTHY CONTROLS

Multivariate statistical analysis revealed pronounced differences between lipidomic signatures of PDAC patients and healthy individuals. OPLS-DA score plots demonstrated a clear separation of cancer samples from control samples in both plasma and serum models. When cancer samples were further classified according to tumor stage, lipidomic patterns remained distinguishable across different stages of disease progression.

The S-plots derived from OPLS-DA models identified lipid species that were most strongly upregulated or downregulated in PDAC samples. These findings were further confirmed through univariate statistical analysis. Box-plot visualization of the most significantly altered lipid species demonstrated substantial differences in concentration between healthy controls and PDAC patients.

To evaluate diagnostic performance, receiver operating characteristic (ROC) curves were generated using prediction scores obtained from the multivariate models. The analysis demonstrated strong diagnostic capability for both plasma-based and serum-based models in training and validation datasets, confirming the potential of lipidomic profiling as a reliable tool for distinguishing pancreatic cancer patients from healthy individuals.

Table 2 | Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) (with 95% confidence interval) for the lipidomic profiling method in the training and validation sets separated by matrix and gender



Plasma						
Gender	**Both**		**Male**		**Female**	
Dataset	**Tr.**	**Va.**	**Tr.**	**Va.**	**Tr.**	**Va.**
Plasma						
Sensitivity [%]	97 (93–99)	97 (85–100)	99 (94–100)	94 (71–100)	98 (92–100)	89 (65–99)
Specificity [%]	100 (97–100)	100 (92–100)	99 (94–100)	100 (82–100)	100 (97–100)	100 (86–100)
Accuracy [%]	98 (96–99)	99 (93–100)	99 (96–100)	97 (85–100)	99 (97–100)	95 (84–99)
PPV [%]	99 (96–100)	100 (90–100)	99 (93–100)	100 (79–100)	100 (96–100)	100 (79–100)
NPV [%]	97 (94–99)	98 (86–100)	99 (93–100)	95 (74–99)	98 (94–100)	93 (77–98)
True[a]	171 / 217	34 / 44	87 / 92	16 / 19	87 / 125	16 / 25
False[a]	6 / 1	1 / 0	1 / 1	1 / 0	2 / 0	2 / 0
Serum						
Sensitivity [%]	96 (92–98)	94 (81–99)	95 (89–99)	94 (71–100)	98 (92–100)	89 (65–99)
Specificity [%]	100 (97–100)	98 (88–100)	99 (94–100)	100 (82–100)	100 (97–100)	100 (86–100)
Accuracy [%]	98 (96–99)	96 (89–99)	97 (94–99)	97 (85–100)	99 (97–100)	95 (84–99)
PPV [%]	99 (96–100)	97 (83–100)	99 (92–100)	100 (79–100)	100 (96–100)	100 (79–100)
NPV [%]	97 (94–98)	96 (85–99)	96 (90–98)	95 (74–99)	98 (94–100)	93 (77–98)
True[a]	170 / 217	33 / 43	85 / 91	16 / 19	87 / 125	16 / 25
False[a]	7 / 1	2 / 1	4 / 1	1 / 0	2 / 0	2 / 0

Concentrations of lipids were measured by UHPSFC/MS method.

[a] Number of samples is determined as true (true positive/true negative) and false (false negative/false positive).

Diagnostic Performance of Lipidomic Profiling

Among the developed classification models, the gender-specific plasma model demonstrated the highest performance in distinguishing healthy individuals from patients with Pancreatic ductal adenocarcinoma (PDAC). Consequently, this model was used for all subsequent analyses. The diagnostic capability of the lipidomic profiling approach was further compared with established tumor biomarkers, including CA 19-9 and Carcinoembryonic antigen (CEA), using the same sample set.

CA 19-9 is widely applied in clinical practice for the diagnosis of pancreatic cancer in symptomatic patients and for monitoring treatment response, whereas CEA is mainly used to evaluate disease progression and therapeutic outcomes in PDAC. Because lipidomic analysis requires prior model development, the dataset was divided into training and validation subsets before evaluation. For CA 19-9 and CEA, commonly accepted clinical cutoff values were applied (37 U/mL for CA 19-9 and 5 ng/mL for CEA), and the data were divided into identical training and validation subsets to ensure consistency with the lipidomic model.

The lipidomic profiling approach demonstrated excellent diagnostic accuracy. In the training dataset, the model achieved an accuracy of 99% (95% confidence interval [CI]: 97–100%), while the validation dataset showed an accuracy of 96% (95% CI: 89–99%). Specificity reached 100% in both datasets (95% CI: 97–100% for the training set and 92–100% for the validation set). Sensitivity was also high, reaching 98% (95% CI: 95–100%) in the training dataset and 92% (95% CI: 77–98%) in the validation dataset.

In contrast, although CA 19-9 and CEA exhibited similarly high specificity levels exceeding 98%, their sensitivity was substantially lower. In the training dataset, sensitivity was 66% (95% CI: 59–73%) for CA 19-9 and 31% (95% CI: 24–38%) for CEA. In the validation dataset, sensitivity decreased to 60% (95% CI: 42–76%) for CA 19-9 and 20% (95% CI: 8–37%) for CEA. These findings demonstrate that lipidomic profiling provides superior diagnostic sensitivity compared with conventional biomarkers.

A key challenge in pancreatic cancer diagnostics is the detection of early-stage tumors (T1 and T2). Both CA 19-9 and CEA show markedly reduced sensitivity in these early stages. In contrast, lipidomic profiling maintained relatively consistent sensitivity across all tumor stages, highlighting its potential as an effective tool for early disease detection. Stage-specific diagnostic performance data are summarized in the supplementary materials.

XII.LIPIDOMIC ANALYSIS IN HIGH-RISK INDIVIDUALS

Although lipidomic profiling effectively differentiates PDAC patients from healthy controls, lipid patterns in individuals with elevated cancer risk may be influenced by additional biological or metabolic factors. Therefore, validating the diagnostic model in a high-risk population is essential.

A total of 93 samples from high-risk individuals were analyzed as blinded samples using predictive models constructed from healthy controls and PDAC patients. The validation cohort consisted of 93 control samples and 88 tumor samples for males, and 125 control samples and 89 tumor samples for females. Although slight differences existed between subgroups regarding age distribution and prevalence of diabetes mellitus, earlier investigations demonstrated that these factors did not significantly affect the lipid classes analyzed. Therefore, these differences were not considered to represent a limitation of the study.

Visual inspection of prediction results indicated that lipidomic profiles of high-risk individuals without pancreatic cancer were generally similar to those observed in healthy participants. This observation was further supported by box-plot comparisons of key lipid concentrations.

Prediction analysis classified 85 samples as negative, seven samples within a borderline confidence interval, and one sample as positive. Applying a strict classification threshold of 0.5 resulted in 89 correct predictions and four incorrect classifications, corresponding to a specificity of 96% (95% CI: 89–99%) when compared with imaging-based diagnostic outcomes obtained through endoscopic ultrasonography or magnetic resonance imaging.

All participants underwent at least one imaging examination at the time of blood sample collection and were subsequently invited for annual follow-up evaluations. However, not all individuals continued participation throughout the surveillance period. Imaging results revealed several pancreatic abnormalities, including intraductal papillary mucinous neoplasms (IPMNs), benign cysts, unspecified cystic lesions, and cases of chronic pancreatitis. Importantly, none of the monitored individuals developed pancreatic cancer during the study period, and all were classified as PDAC-negative according to imaging findings.

The biomarkers CA 19-9 and CEA were also measured in the high-risk cohort, demonstrating specificities of 93% (95% CI: 85–97%) and 96% (95% CI: 89–99%), respectively. Notably, positive findings identified by lipidomic profiling did not coincide with those detected using CA 19-9 or CEA, indicating that the lipidomic method may capture distinct metabolic alterations.

High-risk participants were categorized based on their inclusion criteria, including chronic pancreatitis, familial pancreatic cancer, mutations in BRCA1 or BRCA2 genes, and Peutz–Jeghers syndrome. The lipidomic model did not demonstrate increased misclassification within any particular subgroup.

Follow-up blood samples obtained after one year were available for 23 high-risk individuals. In most cases, prediction scores remained similar to the original results and continued to fall below the diagnostic threshold, supporting the stability of the method. However, four individuals exhibited prediction scores that shifted from clearly negative values to a borderline confidence range. Although imaging examinations did not detect abnormalities in these cases, continued long-term monitoring is recommended to determine whether such metabolic alterations may represent early disease-related changes.

Ongoing collaboration with national surveillance programs dedicated to early pancreatic cancer detection continues, and participants remain under annual monitoring through imaging-based follow-up protocols.

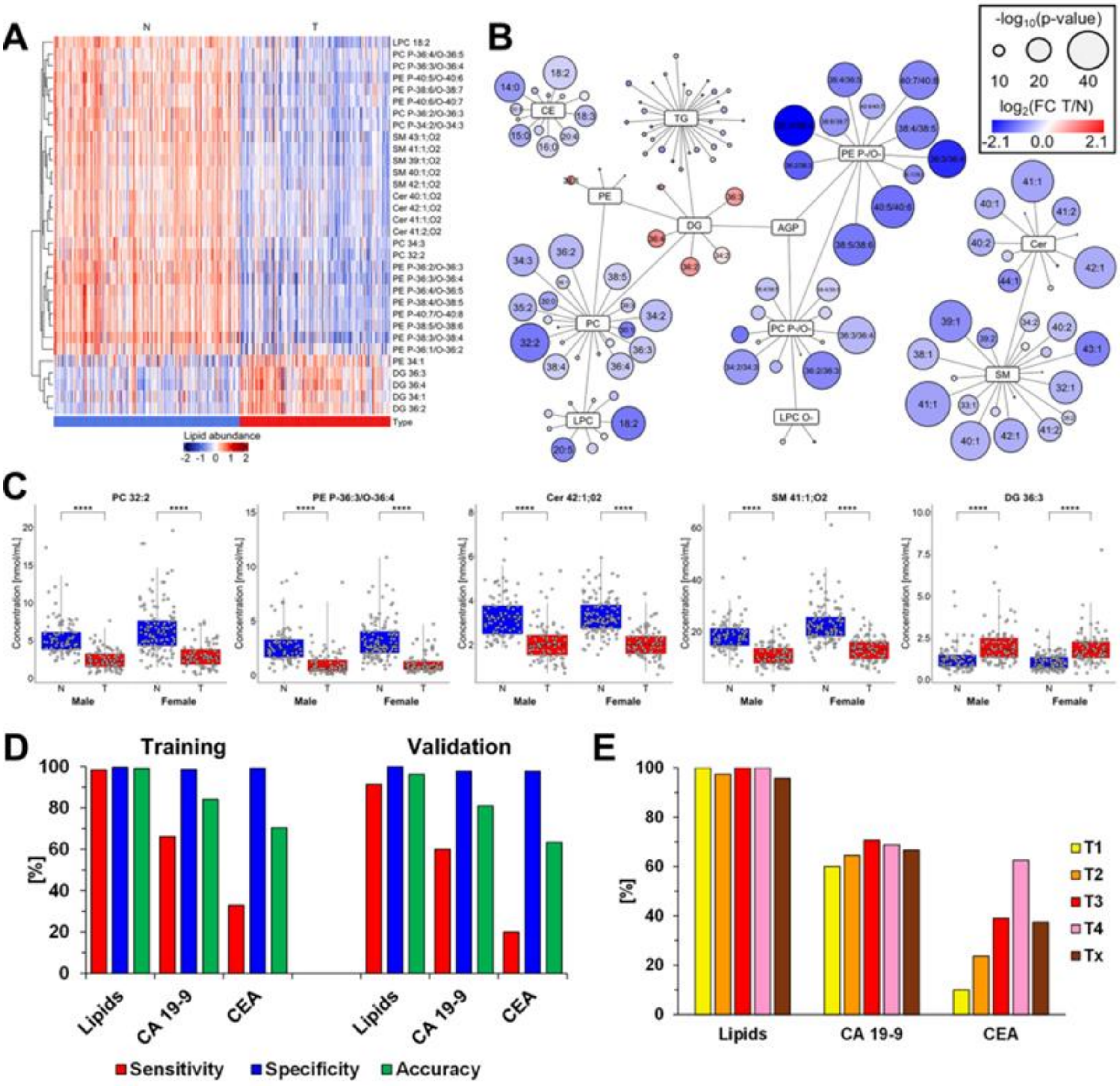


Figure 3 | Plasma lipidomic alterations associated with Pancreatic ductal adenocarcinoma (PDAC)

Lipidomic profiling results comparing PDAC patients (T) with healthy control subjects (N) using plasma-based analytical models.

(A) Heatmap illustrating the most significantly altered lipid species, displaying their concentration patterns across individual study samples.

(B) Network representation of identified lipid species. In this visualization, node size reflects the statistical significance level determined by a two-sided t-test, while the intensity of red or blue coloration represents the magnitude and direction of fold change between tumor and control groups (T/N).

(C) Box-plot analysis highlighting the most dysregulated lipid species and demonstrating concentration differences between PDAC patients (T, red) and healthy controls (N, blue). Statistical significance was evaluated using the Mann–Whitney U test; significance levels are indicated by asterisks, with **** corresponding to $p < 0.0001$. Study populations consisted of 93 control and 88 tumor samples for males, and 125 control and 89 tumor samples for females.

(D) Comparative diagnostic performance of lipidomic profiling and conventional tumor biomarkers, including CA 19-9 and Carcinoembryonic antigen (CEA). Sensitivity (red), specificity (blue), and overall accuracy (green) are presented for both training and validation datasets.

(E) Sensitivity analysis of individual diagnostic approaches according to tumor stage. Tumor stages are represented by color coding: T1 (yellow), T2 (orange), T3 (red), T4 (rose), and Tx (brown; stage not specified). Performance comparisons are shown for lipidomic profiling, CA 19-9, and CEA within the training dataset.

XIII.DISCUSSION

Early detection plays a critical role in improving the clinical outcomes of patients diagnosed with Pancreatic ductal adenocarcinoma (PDAC). Identifying the disease at an early stage significantly increases the likelihood of successful treatment and improved survival rates. In the present study, we demonstrate that a blood-based lipidomic analysis can effectively detect PDAC with a high level of diagnostic accuracy. The results indicate that alterations in circulating lipid molecules provide a promising biomarker signature that can differentiate cancer patients from healthy individuals.

Physiological and biological variables are frequently discussed in lipidomics research because they can influence lipid metabolism and potentially affect biomarker discovery. Such factors may introduce variability in lipid profiles, which could lead to misleading conclusions if not properly addressed during model development. Our findings showed strong agreement with previously reported observations regarding gender-related variations in lipid composition. Earlier studies conducted by Tabassum et al. summarized evidence from multiple investigations and proposed biological mechanisms explaining these gender-dependent lipid differences. Similarly, results from Sales et al. reported comparable trends, although some discrepancies were observed in the dysregulation patterns of phosphatidylethanolamine plasmalogens and in the statistical significance of several lipid classes.

Among the investigated lipid categories, sphingomyelins displayed the most pronounced gender-associated variations. These molecules also represented one of the most strongly altered lipid groups when comparing healthy individuals with PDAC patients. The observed influence of gender on lipid metabolism confirmed the necessity of incorporating gender-specific models during lipidomic analysis. By applying separate predictive models for male and female cohorts, the potential confounding effects related to biological sex were minimized, thereby improving the robustness and reliability of the diagnostic model.

Overall, the findings highlight the importance of considering demographic and biological variables during biomarker discovery studies. Integrating such factors into analytical models can enhance the predictive performance of lipidomic profiling and improve its potential utility as a non-invasive screening tool for pancreatic cancer.

In comparison with conventional tumor biomarkers, lipidomic profiling demonstrated significantly improved diagnostic performance. The method showed approximately 30% higher sensitivity compared with the widely used biomarker CA 19-9. Furthermore, when applied to individuals considered at high risk for pancreatic cancer, the lipidomic approach achieved a specificity of 96% (95% confidence interval: 89–99%) when evaluated against imaging-based diagnostic techniques such as endoscopic ultrasonography and magnetic resonance imaging. These findings highlight the strong potential of lipidomic profiling as a screening strategy. The approach is minimally invasive, relatively cost-effective, suitable for high-throughput analysis, and capable of delivering high diagnostic accuracy—features that are essential for population-level screening tools.

Despite these encouraging results, several limitations should be considered. First, the study primarily focused on differentiating PDAC patients from healthy individuals and high-risk subjects. Other cancer types were not included in the analysis; therefore, the specificity of the lipidomic signature for pancreatic cancer compared with other malignancies remains to be determined. Future investigations should incorporate additional cancer cohorts to further evaluate disease specificity.

Another limitation relates to the potential influence of medications on lipid metabolism. In this study, medication use was not included as an exclusion criterion, and its effects on lipidomic profiles were not systematically analyzed. Although certain pharmaceutical compounds are known to alter lipid metabolism, no extreme outliers were detected in the current dataset. The high predictive performance observed in the classification models suggests that commonly prescribed medications may not significantly affect the diagnostic capability of the method. Nevertheless, further studies will be required to evaluate the influence of pharmacological treatments and other environmental factors on lipidomic signatures.

Additional limitations include the retrospective nature of sample analysis and the relatively limited number of samples available for certain study groups, particularly among high-risk individuals. Moreover, the duration of follow-up for participants was relatively short. Continued monitoring of these individuals is currently ongoing, with follow-up examinations performed annually. Long-term observation will provide further insights into the predictive potential of lipidomic biomarkers for early pancreatic cancer detection.

To address these limitations and validate the current findings, a large multicenter clinical trial has been initiated and registered at ClinicalTrials.gov NCT6549725. This study involves collaboration among fourteen clinical centers in the Czech Republic and aims to evaluate the diagnostic performance of the lipidomic method in a larger population of high-risk individuals as well as patients with surgically resectable PDAC. The trial will also directly compare lipidomic results with imaging-based diagnostic approaches.

XIV.DATA AVAILABILITY

The lipidomic datasets generated during this study are provided as supplementary materials. Data obtained from ultrahigh-performance supercritical fluid chromatography–mass spectrometry analyses are available in Supplementary Data files 2 and 3, while lipid concentration data derived from flow injection analysis tandem mass spectrometry measurements are included in Supplementary Data file 4. All figures presented in the study were generated using the datasets provided in these supplementary materials.

The raw experimental data supporting the findings of this research are publicly accessible through the Zenodo data repository. Additional information required for independent reanalysis of the datasets can be obtained from the corresponding author upon reasonable request.

REFERENCES

[1] National Cancer Institute. Cancer Stat Facts: Pancreatic Cancer. https://seer.cancer.gov/statfacts/html/pancreas.html (2025).

[2] Pishvaian, M. J. & Brody, J. R. Therapeutic implications of molecular subtyping for pancreatic cancer. Oncology 31, 159–168 (2017).

[3] Rahib, L. et al. Projecting Cancer Incidence and Deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 74, 2913–2921 (2014).

[4] Ilic, M. & Ilic, I. Epidemiology of pancreatic cancer. World J. Gastroenterol. 22, 9694–9705 (2016). Vincent, A., Herman, J., Schulick, R., Hruban, R. H. & Goggins, M. Pancreatic cancer. Lancet 378, 607–620 (2011).

[5] Yang, J. S. et al. Early screening and diagnosis strategies of pancreatic cancer: a comprehensive review. Cancer Commun. 41, 1257–1274 (2021).

[6] Singhi, A. D., Koay, E. J., Chari, S. T. & Maitra, A. Early detection of pancreatic cancer: opportunities and challenges. Gastroenterology 156, 2024–2040 (2019).

[7] Root, A., Allen, P., Tempst, P. & Yu, K. Protein biomarkers for early detection of pancreatic ductal adenocarcinoma: progress and challenges. Cancers 10, 67 (2018).

[8] Goggins, M. Molecular markers of early pancreatic cancer. J. Clin. Oncol. 23, 4524–4531 (2005).

[9] Pereira, S. P. et al. Early detection of pancreatic cancer. Lancet Gastroenterol. 5, 698–710 (2020).

[10] Makohon-Moore, A. & Iacobuzio-Donahue, C. A. Pancreatic cancer biology and genetics from an evolutionary perspective. Nat. Rev. Cancer 16, 553–565 (2016).

[11] Halbrook, C. J., Lyssiotis, C. A., di Magliano, M. P. & Maitra, A. Pancreatic cancer: advances and challenges. Cell 186, 1729–1754 (2023).

[12] Cohen, J. D. et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science359,926–930(2018).

[13] Reese, K. L., Pantel, K. & Smit, D. J. Multibiomarker panels in liquid biopsy for early detection of pancreatic cancer—a comprehensive review. J. Exp. Clin. Cancer. Res. 43, 250 (2024).

[14] Cohen, J. D. et al. Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. Proc. Natl. Acad. Sci. USA 114, 10202–10207 (2017).

[15] Polanco,P.M.etal. Confirmatory clinical validation of a serum-based biomarker signature for detection of early-stage pancreatic ductal adenocarcinoma. Curr. Oncol. 32, 638 (2025).

[16] Sagini, K., Urbanelli, L., Buratta, S., Emiliani, C. & Llorente, A. Lipid biomarkers in liquid biopsies: novel opportunities for cancer diagnosis. Pharmaceutics 15, 437 (2023).

[17] Wolrab, D.,Jirásko, R., Chocholoušková, M., Peterka, O.&Holčapek, M. Oncolipidomics: mass spectrometric quantitation of lipids in cancer research. Trac-Trend Anal. Chem. 120, 115480 (2019).

[18] Canto,M.I. et al. Risk of neoplastic progression in individuals at high risk for pancreatic cancer undergoing long-term surveillance. Gastroenterology 155, 740–751 (2018).

[19] Dbouk,M.etal. The multicenter cancer of pancreas screening study: impact on stage and survival. J. Clin. Oncol. 40, 3257–3266 (2022).

[20] Mazer,B.L.etal. Screening for pancreatic cancer has the potential to save lives, but is it practical?. Expert Rev. Gastroentrol. 17, 555–574 (2023).

[21] Calderwood, A. H. et al. American Society for Gastrointestinal Endoscopy guideline on screening for pancreatic cancer in individuals with genetic susceptibility: methodology and review of evidence. Gastrointest. Endosc. 95, 827–854 (2022).

[22] McGuigan, A. et al. Pancreatic cancer: a review of clinical diagnosis, epidemiology, treatment and outcomes. World J. Gastroenterol. 24, 4846–4861 (2018).

[23] Klein,A.P. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat. Rev. Gastro. Hepat. 18, 493–502 (2021).

[24] Vaněk, P., Urban, O., Zoundjiekpon, V. & Falt, P. Current screening strategies for pancreatic cancer. Biomedicines 10, 2056 (2022).

[25] Hart, P.A.etal.Type3c(pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. Lancet Gastroenterol. 1, 226–237 (2016).

[26] Sharma, A. et al. Model to determine risk of pancreatic cancer in patients with new-onset diabetes. Gastroenterology 155, 730–739 (2018).

[27] Ansari, D. et al. Pancreatic cancer: yesterday, today and tomorrow. Future Oncol. 12, 1929–1946 (2016).