Global metabolomic profiling of tumor tissue and paired serum samples identifies biomarkers for response to pre-surgical FOLFIRINOX treatment of human pancreatic cancer

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest carcinomas, with a 5-year survival rate of less than 10%. Current treatment protocols often employ neoadjuvant (treatment given before tumor removal) chemotherapy (NAT) for borderline resectable and locally advanced PDAC, including the use of FOLFIRINOX. The efficiency of these treatments is currently evaluated using imaging and carcinoma antigen 19-9 (CA 19-9, a common biomarker for PDAC). The clinical benefits of these methods are debateable due to the lack of sensitivity and specificity for assessing treatment response. In addition, relatively little is know about the metabolic response to NAT in PDAC patients. To address this need, global LC-MS metabolomics was applied to tumor tissue, and matched serum samples from patients treated with FOLFIRINOX (NAT) and a treatment-näive (no treatment given before tumor removal) (TN) group. The research goal was to identify differentially abundant metabolites (DAMs) which can be used as potential markers of treatment response. See **Figure 1** for graphical overview of the study.

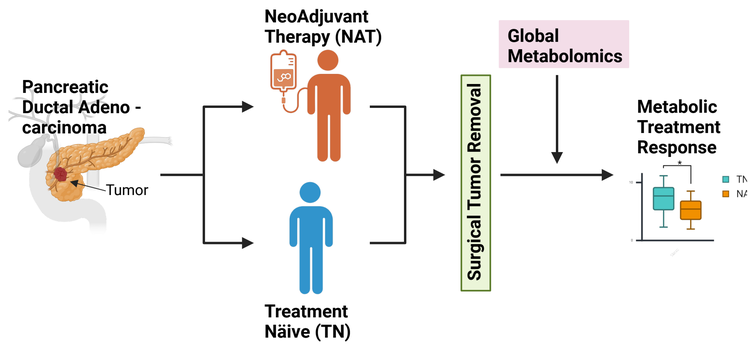

Figure 1: Graphical overview of the study.

METHODS

Patient and Sample information: The study includes pancreatic tissue samples and matched serum samples from 35 patients. Out of these patients 18 had no treatment given before tumor removal (TN = 18) and 17 got treated with FOLFIRINOX before tumor removal (NAT = 17). For more specific information about the study population see **Table 1**.

Category	Treatment-naïve (TN, n=18)		Neoadjuvantly-treated (NAT, n=17)	
Gender				
- Men	13	(72%)	07	(41%)
- Women	05	(28%)	10	(59%)
Age (years)	74.5	(50.0 - 84.0)	64.0	(51.0 - 75.0) *
BMI (kg/m²)	23.8	(20.7 - 39.6)	24.7	(17.3 - 34.8)
Comorbidity (all)	15/18	(83%)	08/17	(47%)
- diabetes	08		02	
- cardiovascular	08		02	
- hypertension	05		03	
- others	07		05	
Disease stage				
- primary resectable (PRPC)	18	(100%)	07	(35%)
- borderline resectable (BRPC)	00	-	10	(59%)
- locally advanced (LAPC)	00	-	01	(06%)
Resection type				
- PPPD	14	(78%)	16	(94%)
- TP	00	-	01	(06%)
- DP	04	(22%)	00	(00%)
Tumor size (mm)	37.0	(28.0 - 102.0)	35.0	(22.0 - 54.0)
TNM classification (8th edition)				
Tumor (T)				
- T1	00	-	00	-
- T2	05	(28%)	07	(41%)
- T3	13	(72%)	10	(59%)
- T4	00	-	00	-
Lymph node metastasis (N)				
- N0	04	(22%)	02	(09%)
- N1	05	(28%)	09	(41%)
- N2	09	(50%)	11	(50%)
Tumor regression grade				
- CAP 0	-	-	00	-
- CAP 1	-	-	00	-
- CAP 2	-	-	09	(53%)
- CAP 3	-	-	08	(47%)
Serum parameters				
- CA 19-9 preoperative (U/mL)	290.5	(11.0 - 11371.0)	56.0	(7.0 - 477.0)
- Bilirubin (µmol/L)	30.0	(5.0 - 341.0)	5.0	(3.0 - 24.0) *
- Albumin (mg/dL)	40.5	(34.0 - 45.0)	42.0	(24.0 - 44.0)
- C-reactive protein (mg/L)	4.3	(0.6 - 30.0)	2.4	(0.8 - 17.0) #
Adjuvant chemo (postoperative)	09/18	(50%)	16/17	(94%)
Overall survival (months)	19.1	(1.1 - 102.1)	16.4	(4.0 - 60.3)

Table 1: Clinical characteristics of the study population

Sample preparation: Serum metabolites were extracted by adding cold MeoH (LC-MS grade) in a 3:1 ratio (MeOH:Serum). Samples were then vortexed before centrifugation (10 min, 4°C, 21 100 RCF), aliquots of the supernatants was mixed together to create the pooled quality control (PQC). The remaining supernatants were placed in HPLC vials for analysis. Tissue metabolites were extracted using MeOH, water, MTBE and chloroform in a 15:13.5:15:15 µL/mg ratio. Middle phase was used for metabolomics analysis.

Global LC-MS Metabolomics Analysis: Samples were analyzed using the Ultimate 3000 HPLC coupled to Q-Exactive Orbitrap MS. Metabolite separation was achieved using XRs Diphenyl column with gradient separation, following Skogvold et al. 2021. All samples were analyzed in both positive and negative ionization mode. See **Figure 2** for the study workflow.

Data processing and statistical analysis: Compound Discoverer (3.3, Thermo Scientific) was used for peak integration, alignment , SERRF QC correction and many others. P-values were calculated using a two-tailed students t-test, and later adjusted using Benjamini-Hochberg correction procedure.

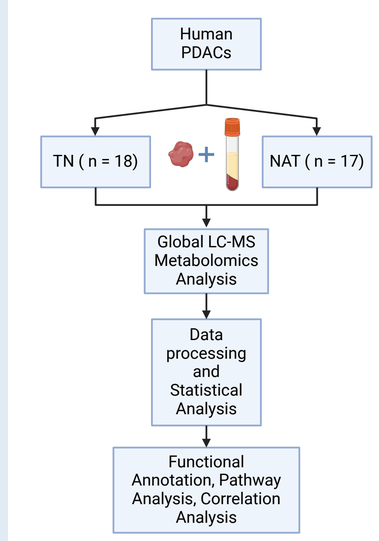

Figure 2: Study workflow

RESULTS

The PCA-plots showed clustering of the pooled quality controls (PQC) for both matrices in both ionization modes (**Figure 3**). Based on PC 1 and 2 the two groups (TN and NAT) could not be differentiated. After ensuring the analytical quality of the data, differentially abundant metabolites (DAMs) was found using differential analysis, as seen in **Figure 4**.

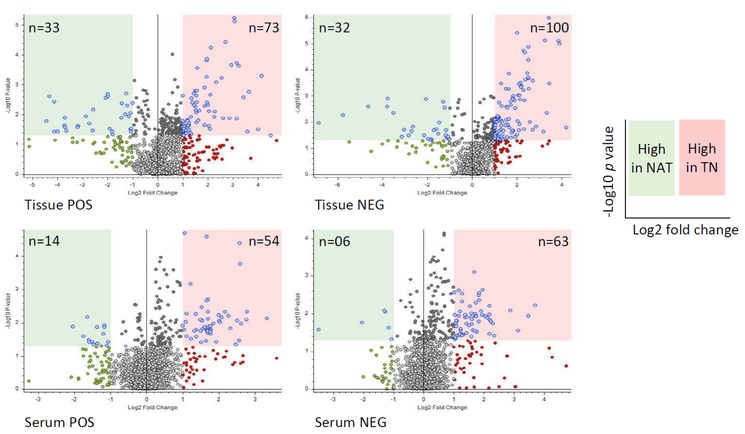

Figure 4: Volcano plots showing DAM distribution comparing NAT and TN PDAC in serum and tissue, both positive and negative ionization mode.

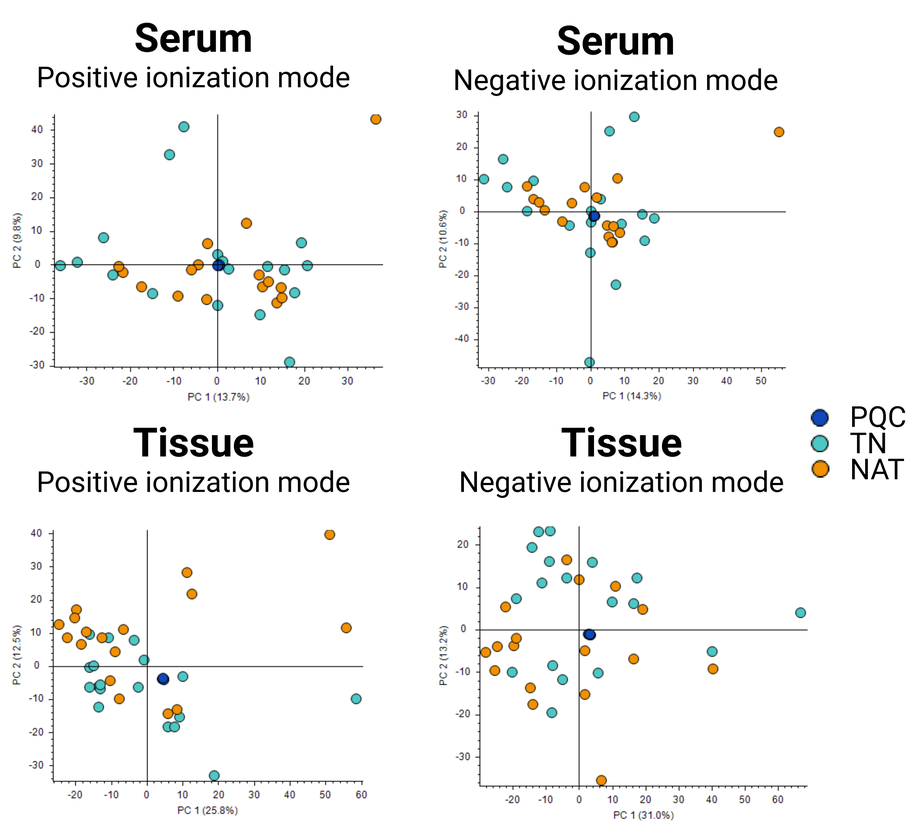

Figure 3: Principal Component Analysis (PCA). PCA score plot for serum and tissue samples, including PQC samples. TN, treatment naive; NAT, neoadjuvant therapy; PQC, pooled quality control.

Figure 6: Box plot of CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; BHB, 3-hydroxybutyric acid; GCDC, glycochenodeoxycholate; Citrulline, in both tissue and serum. * p-value < 0.05.

As seen from **Figure 4**, there were many DAMs differentiating the NAT and TN groups. Among the DAMs, 45 was considered level 1 and 2 of identification (Schymanski et al. 2014). See **Figure 5** for example of two level 1 metabolites. Out of all the DAMs, 4 were overlapping between the serum and tissue data (**Figure 6**), with similar trends. Out of these 4, GCDC was the only one with higher peak areas in the NAT group compared to the TN group. The diagnostic performance of these markers was evaluated using multivariate ROC curves compared to CA 19-9, see **Figure 7**.

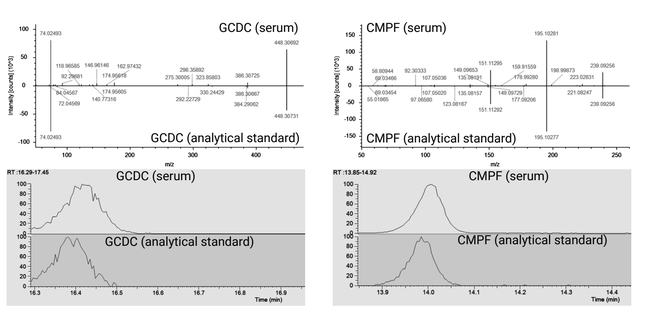

Figure 5: Matching MSMS spectra and chromatographic peak obtained from serum sample with analytical standards.

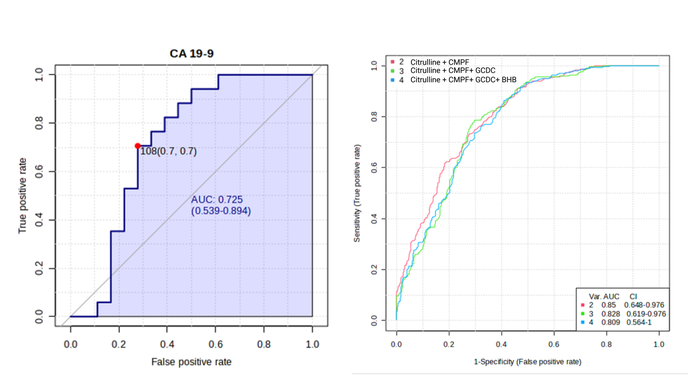

Figure 7: ROC analysis of CA19-9 and multivariate ROC curves combining CMPF, BHB, GCDC and citrulline.

The multivariate ROC model combining all 4 overlapping DAMs had a higher AUC (0.873) compared to the commonly used PDAC biomarker CA 19-9 (AUC = 0.805). This implies that the combination of these 4 DAMs give higher diagnostic prediction accuracy, and is more specific and sensitive compared to CA 19-9 alone.

CONCLUSION

This is the first study to use global metabolomics to investigate the treatment response of neoadjuvant therapy (FOLFIRINOX) in PDAC patients. Four overlapping DAMs between serum and tissue were observed. The NAT group showed higher abundance of GCDC and lower abundances of CMPF, 3-HBA and citrulline compared to the TN group, in both serum and tissue. Combining these 4 metabolites yielded a higher accuracy in diagnostic prediction compared to CA 19-9. A metabolite panel composed of these 4 metabolites could potentially be used for early monitoring of FOLFIRINOX treatment response in PDAC patients.

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Schymanski EL, Environmental Science & Technology **2014** *48* (4), 2097-2098 DOI: 10.1021/es5002105
Skogvold HB, Journal of Proteome Research **2021** *20* (8), 4010-4021 DOI: 10.1021/acs.jproteome.1c00326