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ABSTRACT SUBMISSION FORM

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Preference: ☐ ***Oral presentation*** ☐ ***Poster***

Title **Improving analytical and annotation robustness in small molecule metabolomics using GCxGC-HRTOFMS**
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Improving analytical and annotation robustness in small molecule metabolomics using GCxGC-HRTOFMS

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Summary: *This presentation focus on the enhanced annotation and detectability capacity provided by high resolution information contained in multidimensional chromatography data. The presentation will focus on the combination of untargeted screening of small metabolites and small lipids in human blood serum samples from different form of colorectal cancers.*

Keywords: *Metabolomics, GCxGC-HRMS, Chemometrics*

1 Introduction

According to the world health organization (WHO), colorectal cancer (CRC) ranks as the third most frequently diagnosed cancer and the second leading cause of cancer-related deaths. The current endoscopic-based or stool-based diagnostic techniques are either highly invasive or lack sufficient sensitivity. Thus, there is a need for better screening approaches.

The rapid advancement of high throughput "omics" approaches, such as meta-genomics, transcriptomics, proteomics, metabolomics, lipidomics, microbiomics, and volatolomics, offer a potentially less invasive alternative than available techniques to develop novel biomarkers for CRC screening that could contribute to its clinical management.

For small molecules "omics" screening, gas chromatography coupled to mass spectrometry (GC-MS) represents a method of choice to screen biofluid samples. However, GC-MS is mostly used for targeted analysis due to the high complexity of the matrices. To tackle this limitation, comprehensive two-dimensional gas chromatography coupled to mass spectrometry provided increased separation capabilities, making it a method of choice for untargeted small molecule metabolomics.

In this study, we analyzed 64 human serum samples representing three different groups of colorectal cancer using cutting edge GC×GC–LR/HR-TOFMS techniques. We analyzed samples with two different specifically tailored sample preparation approaches for lipidomics (fatty acids) (25 µL serum) and metabolomics (50 µL serum). In-depth chemometric screening with supervised and unsupervised approaches and metabolic pathway analysis were applied on both datasets. This study was published and is available in open access [1].

2 Methods

In this study, we focused on three sample classes: advanced cancer – adenocarcinoma; cancer – adenoma; and control samples. The demographic information is included in the table below (Table 1).

Table 1: main demographic parameters for the study population.

	Adenocarcinoma	Adenoma	Control
Total no of participants (Female/male)	20 (9/11)	23 (12/11)	21 (9/12)
Female age (Mean ± SD)	66.50 ± 7.99	64.70 ± 8.69	65.81 ± 8.73
Male age (Mean ± SD)	69.45± 8.54	64.70 ± 8.69	65.81 ± 8.93
Location[1] (A/T/D/S/R/recto-sigmoid/splenic flexure)	(7/0/3/3/6/0/1)	(7/1/4/3/7/1/0)	-
BMI (Mean ± SD)	25.39± 3.66* missing 4 data	25.63± 4.28* missing 5 data	27.40± 3.89* missi 5 data
pTNM staging[2]			
Stage-0 (pTisN0M0)	1		
Stage -I (pT1N0M0 or pT1NxMx)	5	-	-
Stage -II (pT2N0Mx or pT2NxMx)	8		
Stage -III (pT3N0Mx or ypT3N0Mx)	6		
Alcohol (Yes/No)	10/10	10/13	12/9
Smoking (Yes/No)	5/15	4/19	3/18

Each serum was analyzed using two different analytical approaches. First, a lipidomics method focused on specifically on fatty acids and derivatives. Next, a more polyvalent MSTFA derivatization approach was developed to screen for more general metabolite families. The protocols are presented in Figure 1.

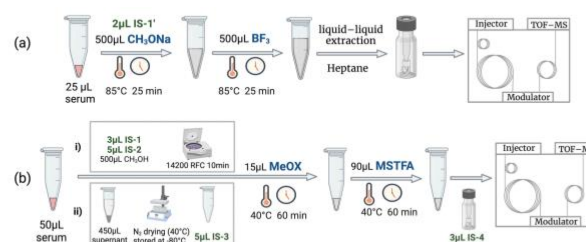

Fig 1. Sample preparation workflows

A general instrumental set up was used for both analytical workflows. The analysis was performed using a normal column set configuration on a Pegasus 4D (LECO corp). Each workflow has an optimized temperature program. For the identification confirmation, the same set up was installed in a GC-HRT 4D (LECO corp). The data acquisition was conducted following the mQACC guidelines.

For the data processing, the chromatograms were aligned and pre-processed. A PLS-DA model was created to identify group-specific features for both analytical workflows. All the method detailed can be found in Bhatt et al. [1].

3 Results and discussion

Using the lipidomics approach, we obtained a semi-targeted screening of 30 fatty acids compounds. From those compounds, 8 displayed statistically significant differences between the groups (Table 2). Based on these 8 compounds, we obtained a clear stratification based on cancer status. PUFA (ω-3) molecules are inversely associated with increased odds of CRC, while some PUFA (ω-6) analytes show a positive correlation.

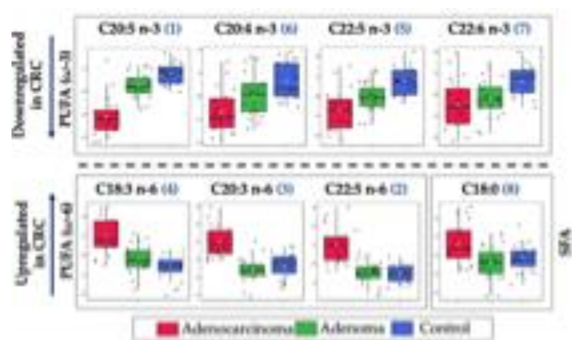

Fig 2: Normalized area for the 8 significant compounds listed in Table 2.

Moreover, these 8 markers were also providing clustering trends for adenocarcinoma stages (Figure 3). These results will require future validation.

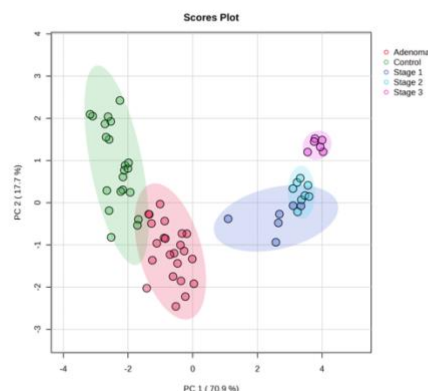

Fig 3: PCA score plot showing group clustering and including adenocarcinoma staging.

For the metabolomics profiling, 8 compounds were also identified as significant using the same data processing approach. However, the fold change and the resulting clustering were not as clear as for the lipidomics workflow.

4 Conclusions and perspectives

A Lipidomics study revealed that specific PUFA (ω-3) molecules are inversely associated with increased odds of CRC, while some PUFA (ω-6) analytes show a positive correlation. With the metabolomics approach, some proteogenic amino acids were identified but they do not lead to a clear stratification between groups. This unique study provides a more comprehensive insight into molecular-level changes associated with CRC and allows for a comparison of the efficiency of two different analytical approaches for CRC screening using the same serum samples and a single instrumentation. Moreover, method translation between large screening on LRMS system and targeted injection on HRMS provided an added value for specific annotation of the compounds of interest.

5 References

[1] Bhatt et al Int. J. Mol. Sci. 2023, 24(11), 9614; https://doi.org/10.3390/ijms24119614