Lipid profiling in the symptomatic population of women with OC: implications for early-stage detection

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Ovarian cancer (OC), the 5th leading cause of cancer-related deaths among women, and is often diagnosed at late stages due to vague abdominal symptoms (VAS) and a lack of effective diagnostic tools. Detection of late-stage OC occurs in 80% of patients when the five-year survival rate is <30%.

Gangliosides are lipids involved in cell signaling and regulation. Some gangliosides are present at low levels in healthy individuals but exhibit changes in expression in specific cancers. These tumor derived gangliosides (TMGs) are shed into circulation and accessible through liquid biopsy. Therefore, TMGs are emerging diagnostic biomarkers for early-stage cancer detection. In addition, altered lipid metabolism is a known cancer hallmark and other lipid classes are known to have potential as biomarkers. Therefore, we aimed to profile TMGs and other lipid levels by Liquid Chromatography coupled with Mass Spectrometry (LC-MS) in OC compared with controls, including clinical sub-populations representing symptomatic women.

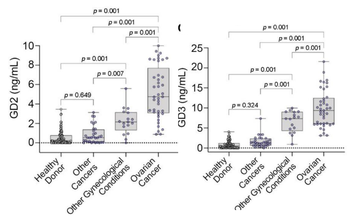
Current standard of care offers limited options for early-stage OC detection

- Most common path to diagnosis = TVU + CA-125[5]
- <50% diagnosed within 1 mo. of first doctor visit[7]
- Avg. time to OC diagnosis is 9 months in the U.S.[8]

Method	Overall Sens. | Spec.	Description/Application	Limitations
Ultrasound (TVU)	57% | 88%[1]	·Used to visualize pelvic organs ·Detects masses in cervix, uterus, fallopian tubes, ovaries	·Small tumors not well detected until later stages ·Difficulty distinguishing benign vs. malignant masses ·Results vary by operator expertise[2]
CA125	79% | 78%[3]	·Blood test for CA125 protein, shed into bloodstream by OC cells ·Used as a tumor marker to detect ovarian cancer & monitor response to treatment[4]	·Elevated levels associated with benign & other malignant conditions, limited sensitivity in early-stage OC ·Levels fluctuate (age, non-cancerous conditions)[4] ·FDA cleared for disease monitoring post diagnosis only[5]
HE4	79% | 93%[3]	·Blood test to measure HE4 protein secreted by epithelial OC cells ·Tumor marker to detect OC & monitor response to treatment[6]	·Elevated levels associated with benign & malignant conditions, limited sensitivity in early-stage OC[6] ·Levels vary by smoker status, hormonal contraceptive use[7] ·FDA cleared for disease monitoring post-diagnosis only, limited availability[5]
OVA1	92% | 50%[8]	·Blood test to measure CA125 + 4 biomarkers, integrates clinical information into algorithm ·Distinguishes benign vs. malignant masses in women scheduled for surgery[8]	·Reduced sensitivity in premenopausal women with low-risk CA125, modest specificity, high false positive rate[8] ·Dependency on menopausal state ·FDA cleared for triaging adnexal mass already scheduled for surgery[9]
Overa	91% | 66%[8]	·Blood test to measure CA125 + 4 biomarkers, integrates clinical information into algorithm, reflex test for OVA1 ·Distinguishes benign vs. malignant masses in women scheduled for surgery[8]	·Reflex test to OVA1 ·Modest overall specificity, high false positive rate ·Reduced specificity for post-menopausal women[8] ·FDA cleared for triaging adnexal mass already scheduled for surgery[9]
ROMA	74% | 93%[8]	·Blood test for CA125 + HE4 protein levels, integrates menopausal status ·Classifies patients by risk ·Distinguishes benign v. malignant ovarian adnexal mass[4]	·Moderate overall sensitivity ·Reduced sensitivity in pre-menopausal women[8] ·Reduced sensitivity for early-stage OC ·FDA cleared for triaging adnexal mass already scheduled for surgery[9]

GD2 and GD3 gangliosides have been previously implicated as diagnostic biomarkers for OC[10]

- Gangliosides = glycosphingolipids in the plasma membrane that can shed into circulation
- GD2 and GD3 gangliosides (detected by ELISA) were elevated in the serum of patients with OC
- A GD2+GD3+age model was superior to the standard of care (CA125) in diagnosing OC.



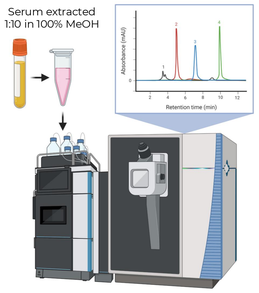
Addressing diagnostic challenges in the VAS population

- >80% individuals with early-stage OC present with VAS
- Many OC patients undergo gastrointestinal, general abdominal and/or urological evaluations first because symptoms overlap[11]
- While some advances have been reported for asymptomatic individuals, the **biological complexity of the symptomatic population requires novel approaches**
- To address this, our study included serum from healthy individuals (normal), both early- and late-stage OC, as well as a range of non-cancerous gyn conditions that share symptoms common to OC:
  - Endometriosis, fibroids, cysts, adnexal masses (benign)
  - Gastrointestinal (GI) disorders

UHPLC-HRMS/MS for lipid detection

We generated lipidomic profile data using UHPLC-HRMS/MS (Exploris 240, Thermo Scientific) from a clinically annotated cohort of serum samples.

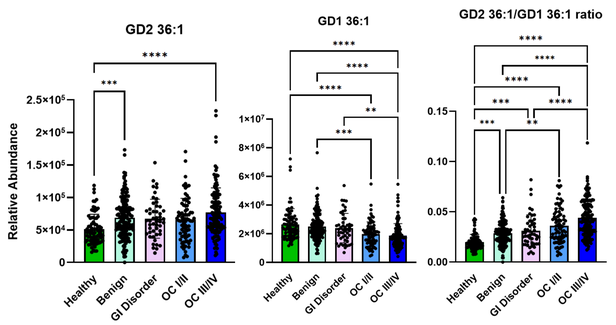
Cohort Sample Breakdown	
Normal	82
Benign	169
GI Disorder	50
Early-stage (I/II) OC	82
Late-stage (III/IV) OC	135
Total	**518**



Lipid Class	# Detected
Carnitine (Car)	28
Ceramide (Cer)	250
Cardiolipin (CL)	20
Diacylglycerol (DG)	102
Fatty Acid (FA)	49
FA Derivative	133
Ganglioside	129
Glycosphingolipid (GSL)	95
LysoP-lipids (LPX)	178
Monoacylglycerol (MG)	12
Phosphatidic acid (PA)	47
Phosphatidylcholine (PC)	770
Phosphatidylethanolamine (PE)	169
Phosphatidylglycerol (PG)	64
Phosphatidylinositol (PI)	89
Phosphatidylserine (PS)	77
Sphingomyelin (SM)	251
Sterol + Vitamin	101
Triacylglycerol (TG)	213
Other	242

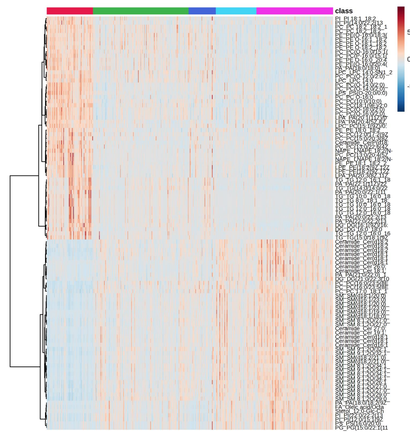
Ganglioside retention times confirmed with ganglioside-specific fragment moieties. Gangliosides and fatty acids were assigned using isotopologue distributions using MAVEN[12]. Other lipids detected using CompoundDiscoverer (Thermo Scientific)

Gangliosides are altered in OC compared to a complex VAS population



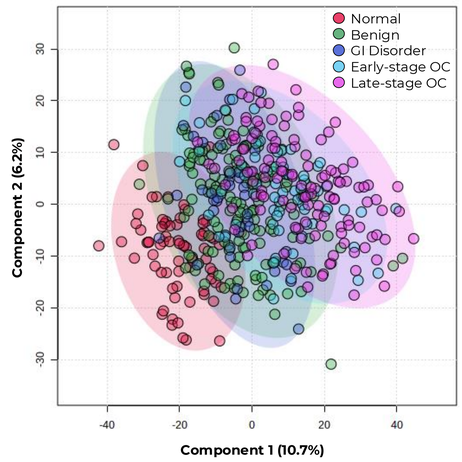
- LC-MS analysis shows **gangliosides are significantly altered in both early- and late-stage OC** when compared against a complex population of controls, including healthy individuals, those with benign adnexal masses, and those diagnosed with GI disorders.
- Interestingly, in the case of GD2 and GD1, taking the ratio of GD2 36:1 to GD1 36:1 resulted in a dramatically increased signal compared to those species alone.

Global lipid profiles are altered in OC compared to a complex VAS population



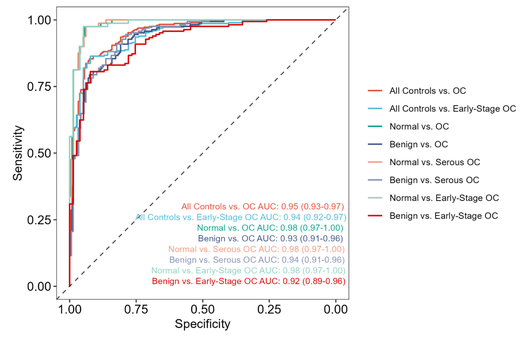
- As aberrant lipid metabolism has been identified as a cancer hallmark and various disease states display unique lipid profiles, we also interrogated the wider lipidome.
- While gangliosides are altered in OC, the heatmap (top 50 lipid species by ANOVA) includes other classes such as ceramides, triglycerides, phospholipids, etc.
- **Early- and late-stage OC show clear lipidomic differences** compared to normal, benign, and GI disorders.
- This highlights the potential diagnostic value of lipids in OC.

Lipidomics is a powerful tool for separating cancer from non-cancer



- PLSDA scores plot showing separation of normal, benign, GI disorder, early-stage OC, and late-stage OC.
- Lipidomics analysis shows clear progression from normal to benign & GI disorders to cancer, allowing us to **distinguish cancer from non-cancer in the complex population representing VAS.**

The power of multi-omics: Lipids enhance clinical performance when combined with select protein biomarkers



A multi-omics + machine-learning approach (<50 features, a mix of gangliosides, lipids, + protein biomarkers) achieves the highest accuracy in distinguishing early-stage OC from non-OC controls. **This represents a significant advancement in the early detection of ovarian cancer for symptomatic women.**

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