Utilizing serum-derived lipidomics with protein biomarkers and machine learning for early detection of ovarian cancer in the symptomatic population

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Key Takeaways

- Ovarian cancer is lethal due to lack of robust biomarkers and vague symptoms that present at early stages.
- A robust, non-invasive, early detection diagnostic test for the symptomatic population will improve the poor prognosis of OC.
- We are developing a serum-based blood test to detect OC earlier in women with vague abdominal symptoms (VAS).
- Our novel machine learning (ML)-based multi-omic model achieves high AUCs in early-stage OC across independent, heterogeneous patient populations.
- ML + multi-omics shows improved performance over current methods, allowing for earlier cancer detection, shortening time to diagnosis, and improving patient outcomes.

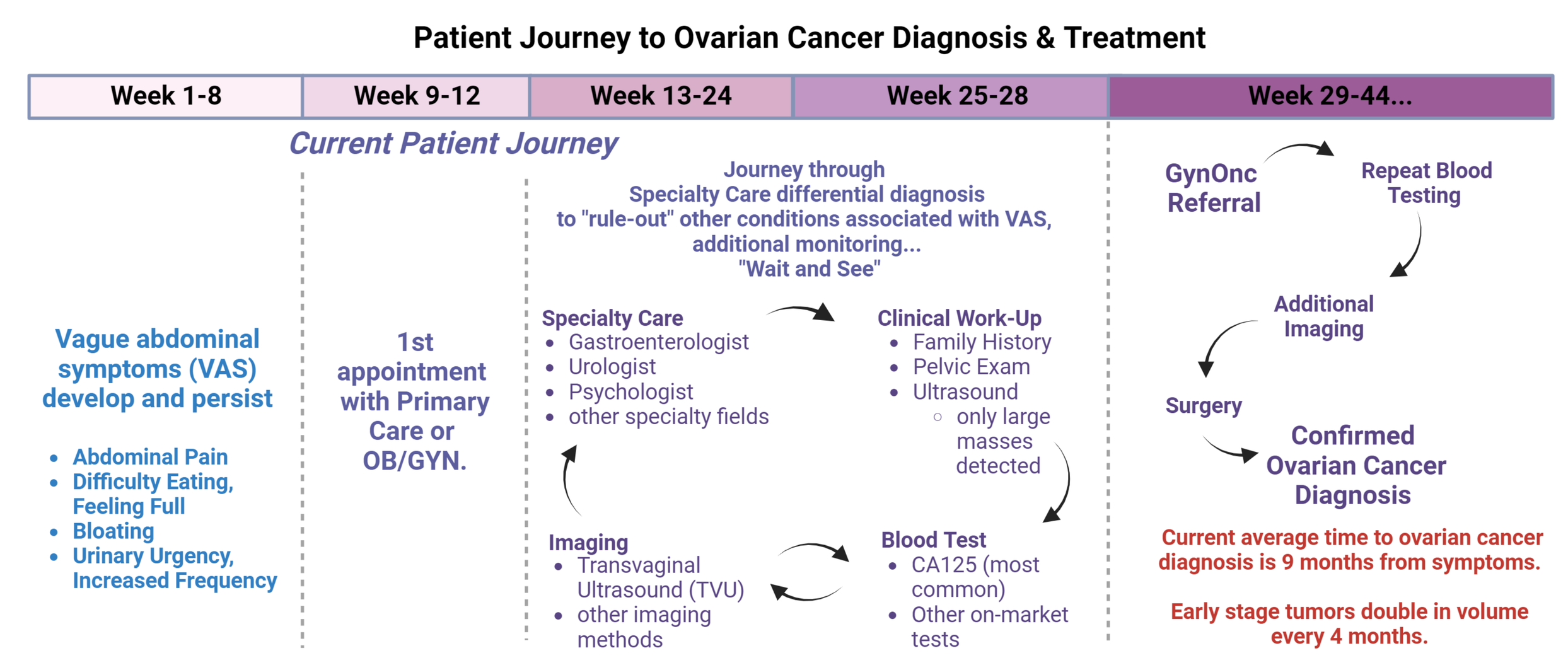
Current standard of care offers limited options for early-stage OC detection

- <50% diagnosed within 1 mo. of first doctor visit[1]
- Avg. time to OC diagnosis is 9 months in the U.S.[2]
- **>70% diagnosed with late-stage OC, 5-year survival 10-30%**[3]
- Lack of effective diagnostic tools available for early-stage OC
- **If OC is diagnosed at earlier stages, survival can jump to >90%**[4]

Method	Overall Sens. | Spec.	Description/Application	Limitations
Ultrasound (TVU)	57% | 88%[5]	• Transvaginal ultrasound imaging used to visualize pelvic organs • Detects masses in cervix, uterus, fallopian tubes, and ovaries	• Small tumors not well detected until later stages • Difficulty distinguishing benign vs. malignant masses • Results vary by operator expertise[6]
CA125	79% | 78%[7]	• Blood test for CA125 protein, shed into bloodstream by ovarian cancer cells • Used as a tumor marker to detect ovarian cancer & monitor response to treatment[8]	• Elevated levels associated with benign & other malignant conditions, limited sensitivity in early-stage OC • Levels fluctuate (age, non-cancerous conditions)[8] • FDA cleared for disease monitoring post diagnosis only[9]
HE4	79% | 93%[3]	• Blood test for HE4 protein, secreted by epithelial ovarian cancer cells • Used as a tumor marker to detect ovarian cancer & monitor response to treatment[8]	• Elevated levels associated with benign & malignant conditions, limited sensitivity in early-stage OC[10] • Levels vary by smoker status, hormonal contraceptive use[11] • FDA cleared for disease monitoring post-diagnosis only, limited availability[9]
OVA1	92% | 50%[8]	• Blood test for CA125 + 4 biomarkers, integrates clinical information into algorithm • Distinguishes benign vs. malignant masses in women scheduled for surgery[12]	• Reduced sensitivity in premenopausal women with low-risk CA125, modest specificity, high false positive rate[12] • Dependency on menopausal state • FDA cleared for triaging adnexal mass already scheduled for surgery[13]
Overa	91% | 66%[8]	• Blood test for CA125 + 4 biomarkers, integrates clinical information into algorithm • Distinguishes benign vs. malignant masses in women scheduled for surgery[12]	• Reflex test to OVA1 • Modest overall specificity, high false positive rate • Reduced specificity for post-menopausal women[12] • FDA cleared for triaging adnexal mass already scheduled for surgery[13]
ROMA	74% | 93%[8]	• Blood test for CA125 + HE4 protein levels, integrates menopausal status • Classifies patients by risk • Distinguishes benign vs. malignant ovarian adnexal mass[8]	• Moderate overall sensitivity • Reduced sensitivity in pre-menopausal women[12] • Reduced sensitivity for early-stage OC • FDA cleared for triaging adnexal mass already scheduled for surgery[13]

Addressing diagnostic challenges in women with vague abdominal symptoms

- >80% individuals with early-stage OC present with VAS
- Many OC patients undergo gastrointestinal, general abdominal and/or urological evaluations first because symptoms overlap[4]
- While some advances are reported for asymptomatic individuals, the **biological complexity of the symptomatic population requires novel approaches**

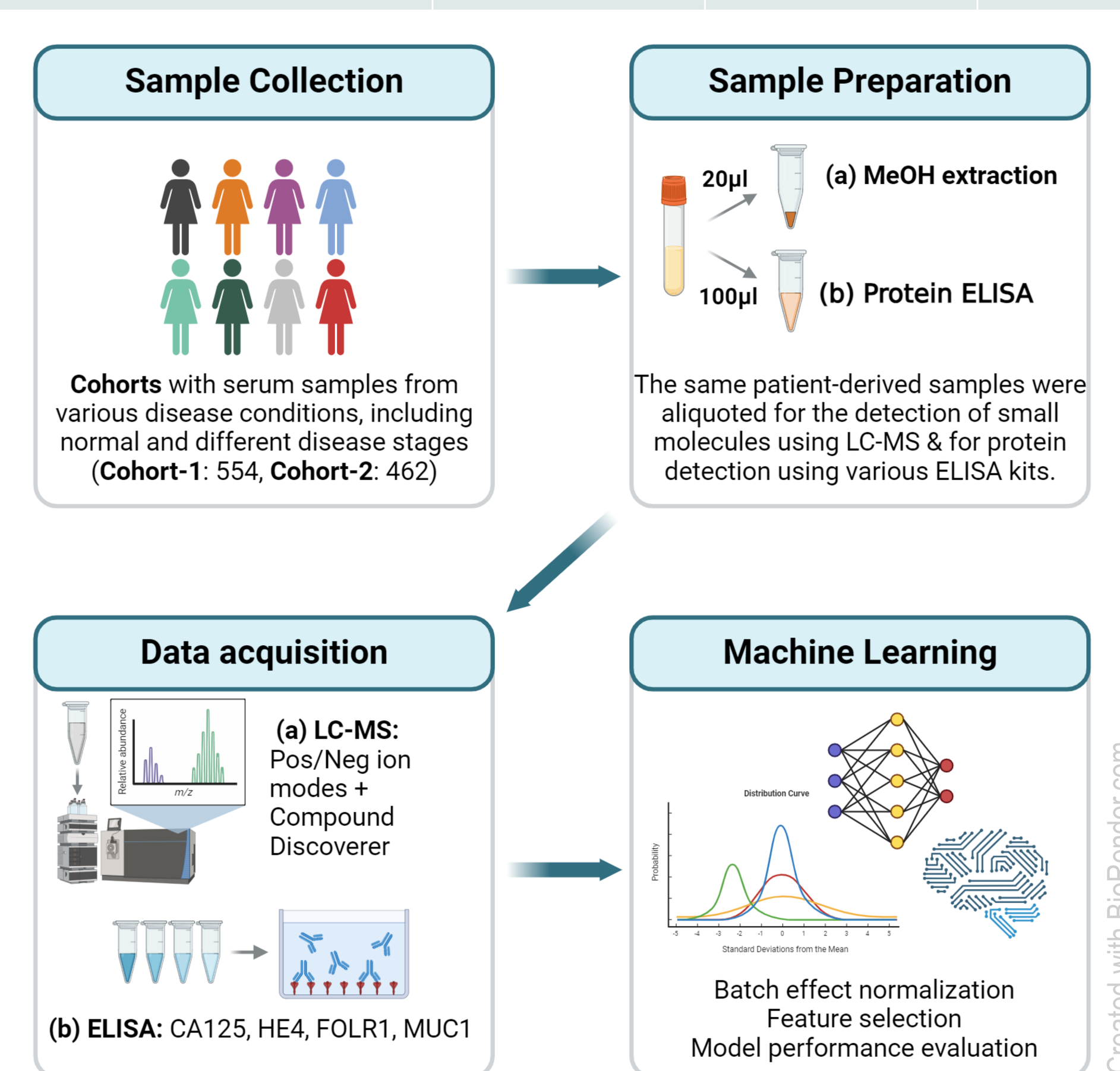

Patient Journey to Ovarian Cancer Diagnosis & Treatment
Week 1-8
Week 9-12
Week 13-24
Week 25-28
Week 29-44...
Current Patient Journey
Vague abdominal symptoms (VAS) develop and persist
Abdominal Pain
Difficulty Eating, Feeling Full
Bloating
Urinary Urgency, Increased Frequency
1st appointment with Primary Care or OB/GYN.
Journey through Specialty Care differential diagnosis to "rule-out" other conditions associated with VAS, additional monitoring... "Wait and See"
Specialty Care
Gastroenterologist
Urologist
Psychologist
other specialty fields
Clinical Work-Up
Family History
Pelvic Exam
Ultrasound
only large masses detected
Imaging
Transvaginal Ultrasound (TVU)
other imaging methods
Blood Test
CA125 (most common)
Other on-market tests
GynOnc Referral
Repeat Blood Testing
Additional Imaging
Surgery
Confirmed Ovarian Cancer Diagnosis
Current average time to ovarian cancer diagnosis is 9 months from symptoms.
Early stage tumors double in volume every 4 months.

- To address this, our study includes serum from healthy individuals (normal), early- and late-stage OC, and non-cancerous conditions that share symptoms common in OC:
  - Gyn: Endometriosis, fibroids, cysts, adnexal masses (benign)
  - Gastrointestinal (GI) disorders

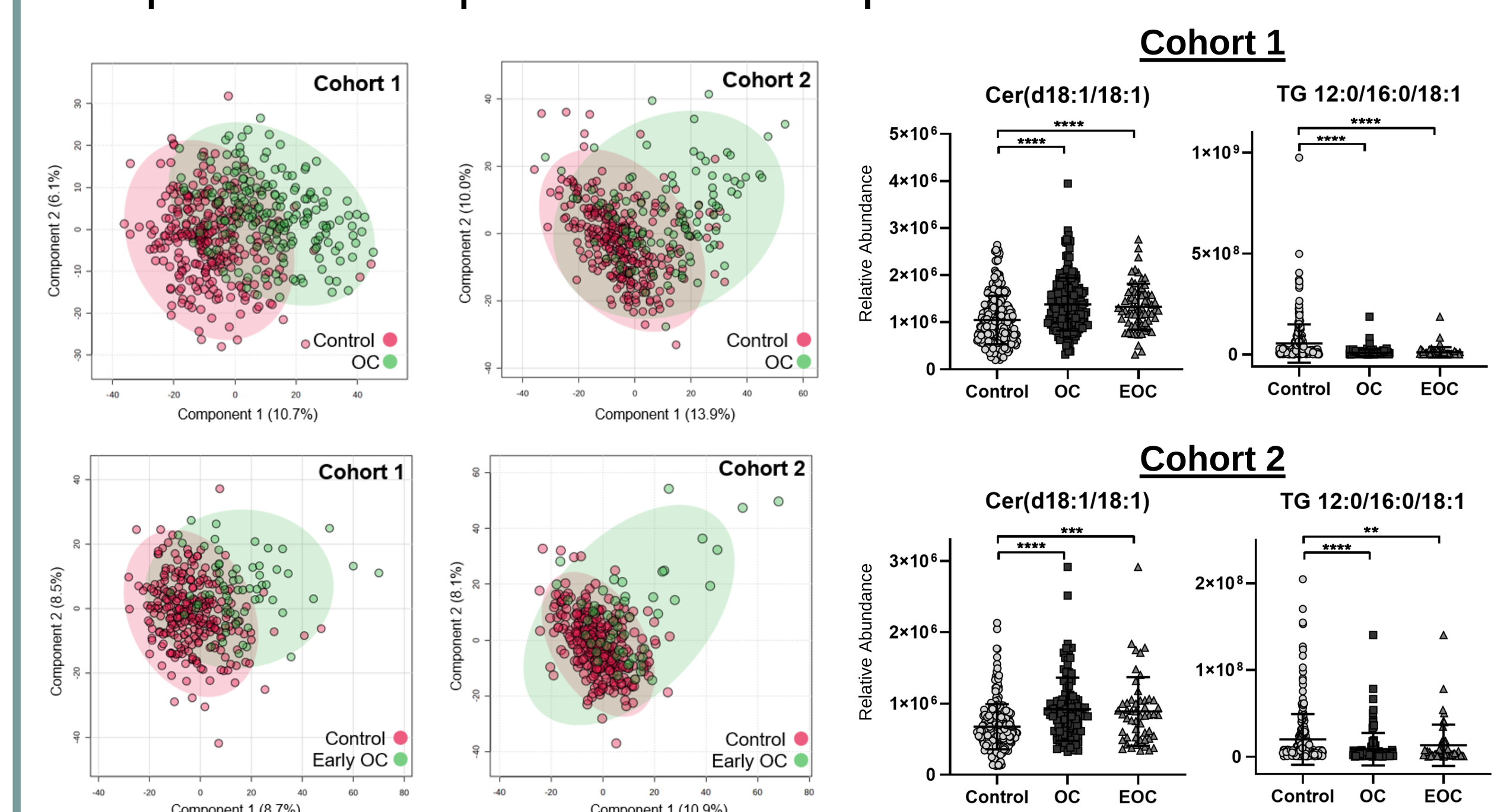
LC-MS for lipid detection + ELISA-based protein panel + Machine Learning

We conducted a multi-omics analysis of two independent, clinically annotated cohorts. Cohort 1 was obtained from the University of Colorado Gynecologic Tissue and Fluid Bank + commercial vendors. Cohort 2 specimens were collected from a **prospectively enrolled symptomatic population** through Manchester University NHS Foundation Trust + commercial vendors. Samples were blinded and cohorts were processed independently.

Diagnosis	Group	Cohort 1	Cohort 2	Combined
Cancer	All OC	218	109	327
	Early-stage OC	82	52	134
	Late-stage OC	136	57	193
	Borderline	25	20	45
Non-Cancer	All Controls	301	294	595
	Normal	82	208	290
	Benign	169	85	254
	GI Disorders	50	0	50
	Pre-cancer	0	1	1
Grand Totals		**544**	**423**	**967**

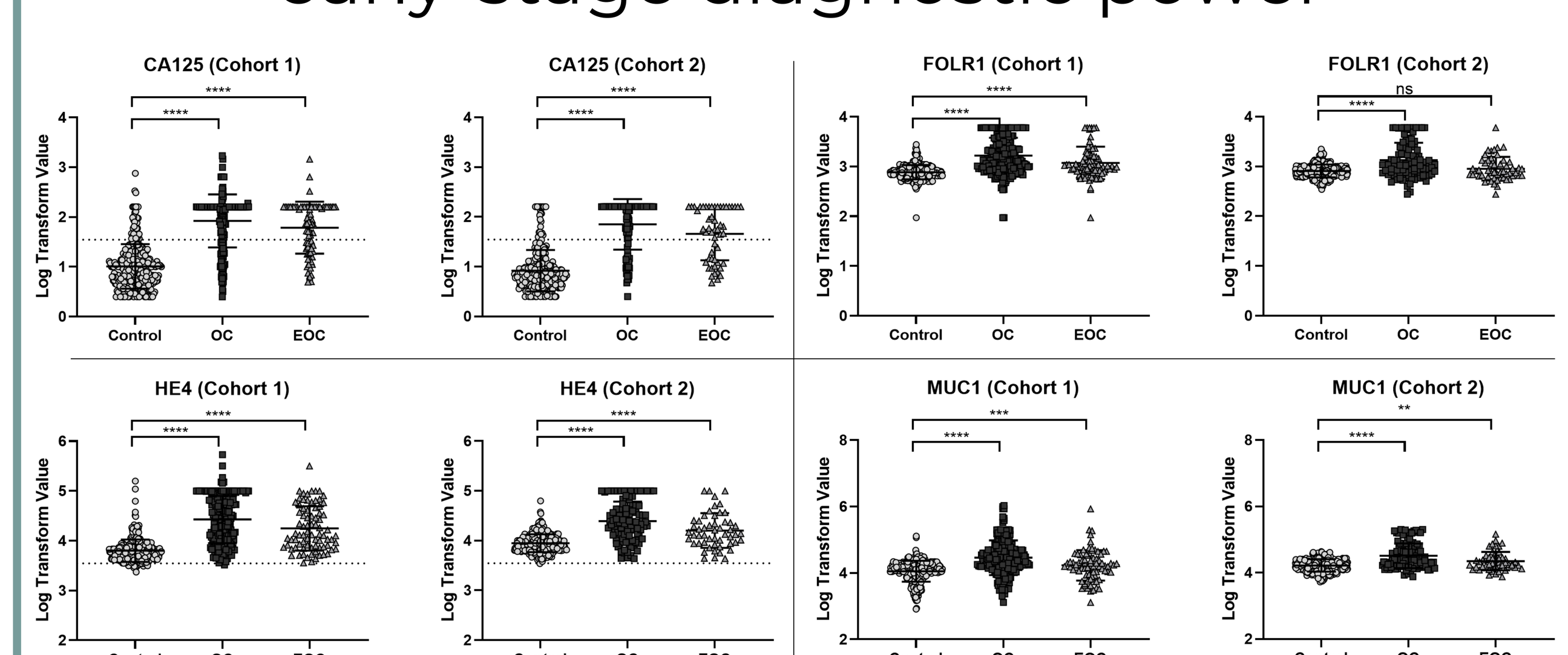

Sample Collection
Cohorts with serum samples from various disease conditions, including normal and different disease stages (Cohort-1: 554, Cohort-2: 462)
Sample Preparation
20µl
(a) MeOH extraction
100µl
(b) Protein ELISA
The same patient-derived samples were aliquoted for the detection of small molecules using LC-MS & for protein detection using various ELISA kits.
Data acquisition
(a) LC-MS: Pos/Neg ion modes + Compound Discoverer
(b) ELISA: CA125, HE4, FOLR1, MUC1
Machine Learning
Batch effect normalization
Feature selection
Model performance evaluation
Created with BioRender.com

Ovarian cancer serum shows a distinct lipidomic profile compared to controls



- PLSDA comparing controls v. all OC (top left) and controls v. early-stage OC (bottom left) show **clear lipid profile differences**.
- There are a range of significantly altered lipid classes in OC when compared to the diverse symptomatic population.
- Divergence is more clearly observed for specific lipid species (right), highlighting **individual lipids as potential biomarkers** for early-stage OC in the complex VAS population.

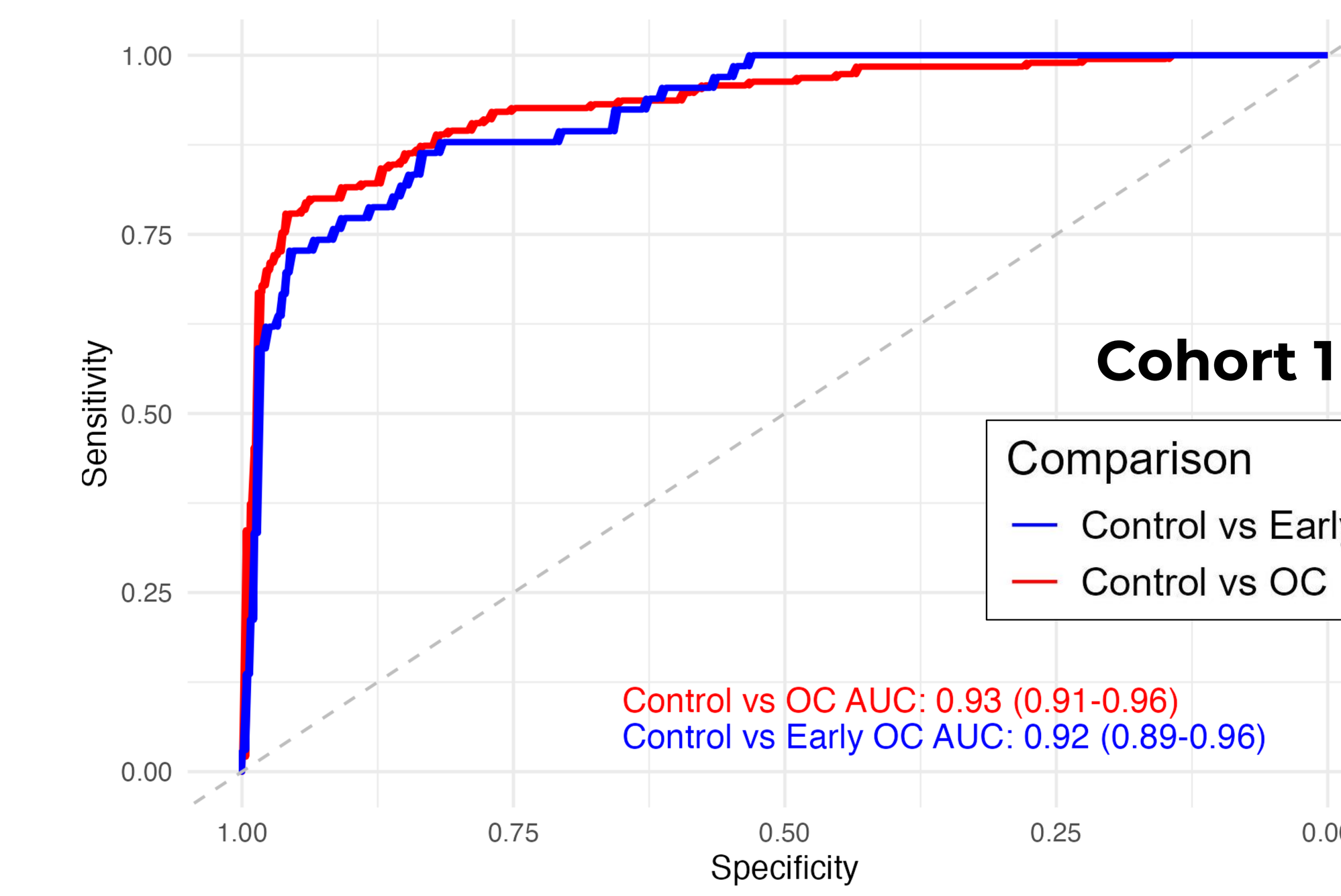
Protein biomarkers differ in OC but lack early-stage diagnostic power

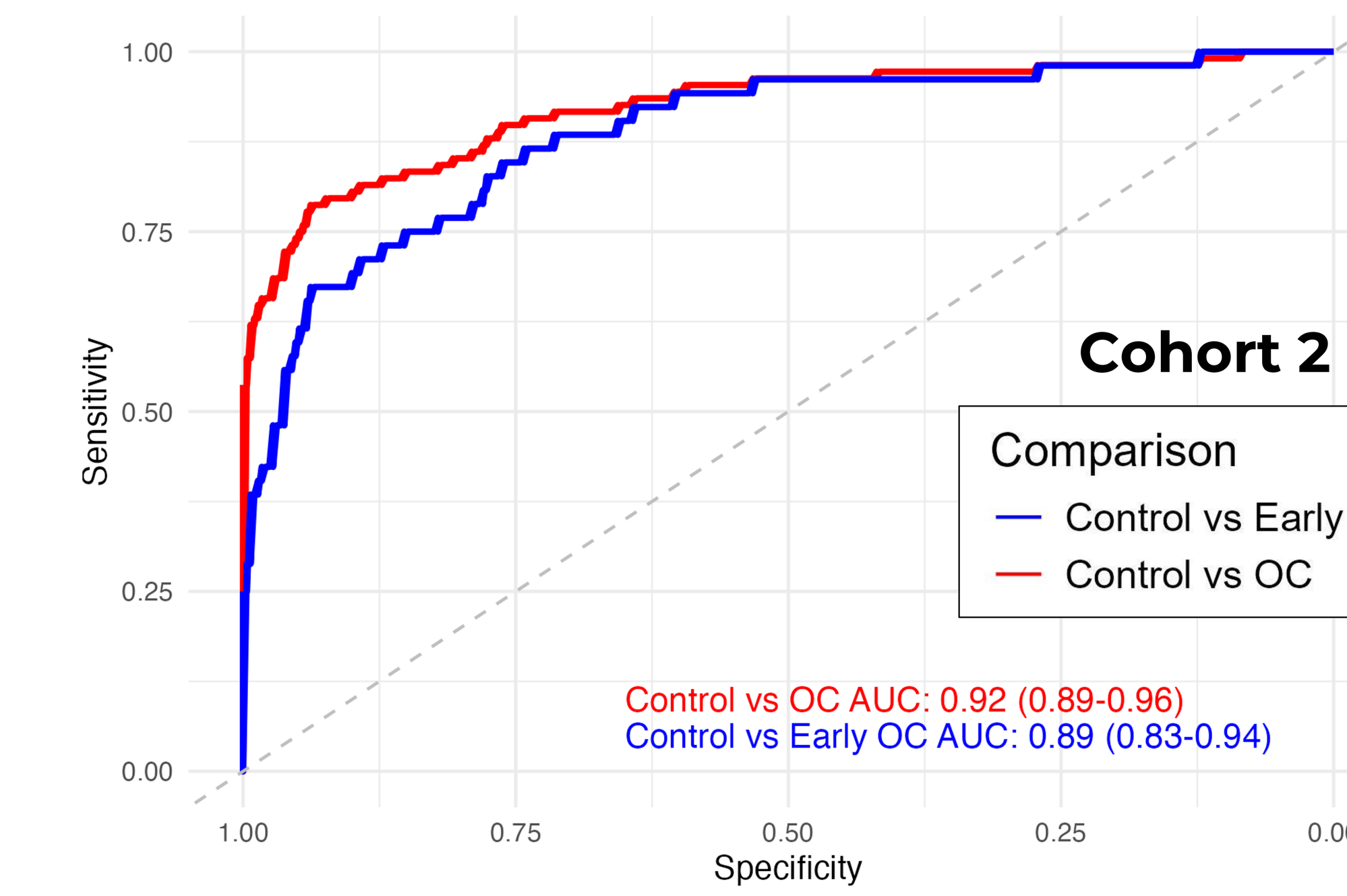


- Levels of CA125 and HE4 (used in clinical practice) with FOLR1 and MUC1 (promising diagnostic/therapeutic targets) were elevated in OC and early-stage OC.
- However, wide ranges and modest differences highlight the limitations of using these proteins as stand-alone biomarkers.

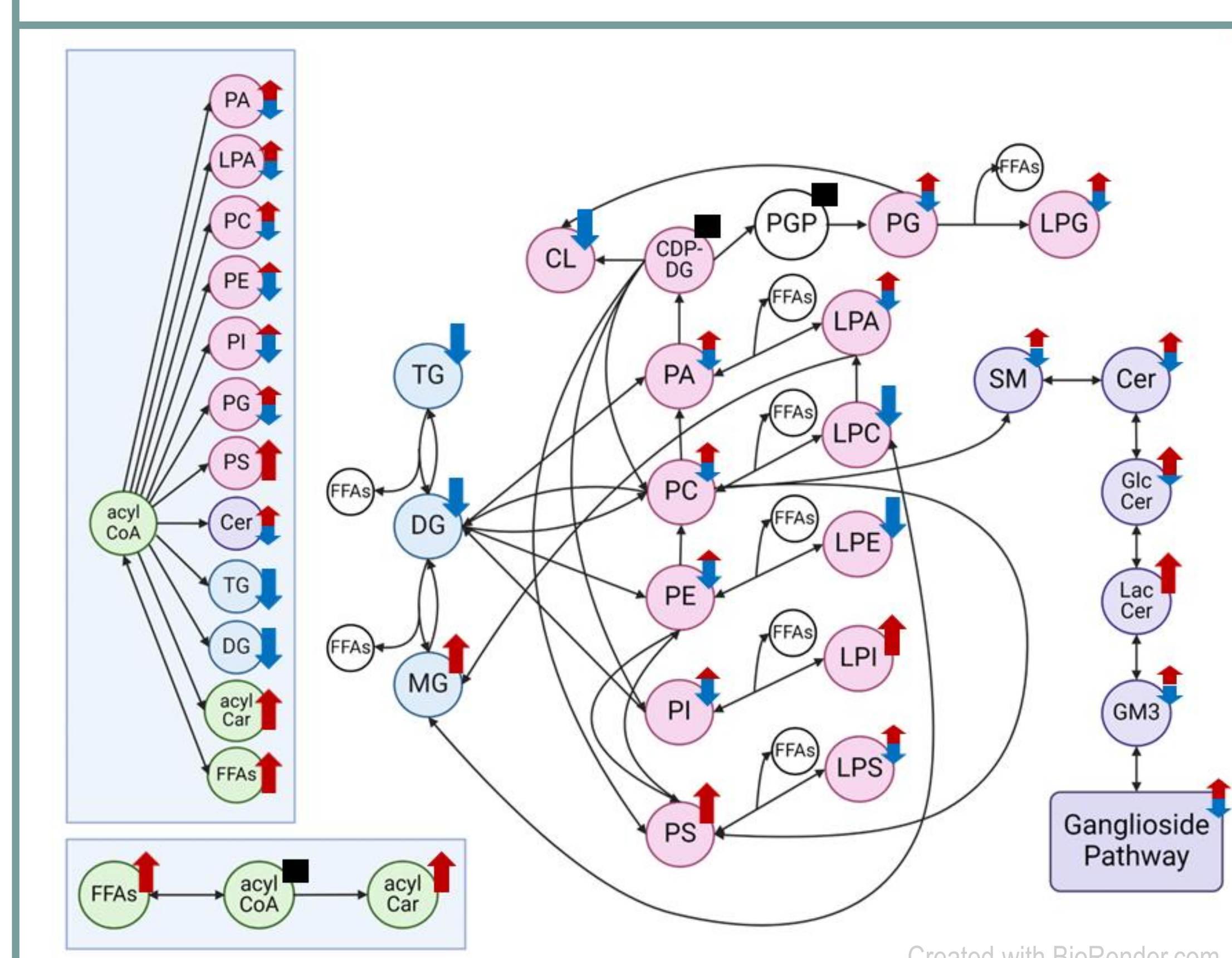
Multi-omic modeling distinguishes OC & early-stage OC in the VAS population

- Wide distributions and subtle alterations in lipid and protein biomarkers illustrate the need for **combinatorial approaches**.
- Machine learning-based modeling was performed to assess multi-omics as a tool to distinguish OC from VAS controls.





- For Cohort 1, the top-performing model showed **AUCs of 93% for controls vs. OC, and 92% for controls vs. early-stage OC** (top).
- The model demonstrates high performance when tested on Cohort 2 as an independent hold-out set, achieving AUCs of 92% for controls vs. OC and 89% for controls vs. early-stage OC. (bottom) This performance is underscored by the heterogenous nature of the cohorts and prospective collection in the complex intended use population.
- **These data illustrate the power of a multi-omic approach leveraging lipid and protein profiling to distinguish OC.**



Multi-omic modeling distinguishes OC & early-stage OC in the VAS population

- Lipid metabolism is a highly dynamic and deeply interconnected network of species.
- When comparing normal and OC serum, class level alterations in the lipid profile can be observed:
  - Increases in LacCer, LPI, PS, MG, acyl-Cer, FFAs
  - Decreases in LPC, LPE, TG, DG
  - A mix of increased and decreased species in gangliosides, Cer, SM, LPS, PI, PE, PC, PA
- This illustrates the potential of specific lipid classes and individual species for their utility as diagnostic biomarkers in a clinical diagnostic assay.

Increased
Decreased
Both increased + decreased
Not detected