European Journal of Obstetrics and Gynecology 312 (2025) 114525

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.journals.elsevier.com/european-journal-of-obstetrics-and-gynecology-and-reproductive-biology

Full length article

Novel glycovariant biomarkers of CA125 and CA15-3 and their diagnostic performance across histotypes of ovarian cancer: A multi-cohort study in Sweden and Finland

Hanna Roos Alexander [a,*], Shruti Jain [b], Kamlesh Gidwani [b], Benjamin Ulfenborg [c], Ulrika Ottander [a], Eva Lundin [d], Johanna Hynninen [e], Shamima Afrin Ruma [b], Kaisa Huhtinen [f], Karin Sundfeldt [g], Kim Pettersson [b], Annika Idahl [a]

[a] Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, Umeå, Sweden
[b] Department of Life Technologies and FICAN West Cancer Centre, University of Turku, Finland
[c] Systems Biology Research Centre, School of Bioscience, University of Skövde, Sweden
[d] Department of Medical Biosciences, Pathology, Umeå University, Sweden
[e] Department of Obstetrics and Gynecology, Turku University Hospital and University of Turku, Turku, Finland
[f] Cancer Research Unit, Institute of Biomedicine and FICAN West Cancer Centre, University of Turku, Turku, Finland
[g] Department of Obstetrics and Gynecology, Sahlgrenska Centre for Cancer Research, Sahlgrenska University Hospital and Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Epithelial ovarian cancer
Biomarkers
Glycovariant
CA125
Diagnostics

ABSTRACT

Objective: To evaluate diagnostic accuracy of novel nanoparticle immunoassays across different histotypes of tubo-ovarian carcinoma (TOC) at diagnosis.

Method: This multicenter observational study consisted of consecutive patients (n = 1,312) having surgery due to suspected ovarian pathology. Serum were analyzed with Sialyl-Thomsen-nouveau (STn) antibody and Macrophage-Galactose-Lectin (MGL) for the detection of cancer antigen 125 (CA125) and 15-3 (CA15-3) glycoforms using CA125 enzyme immunoassay (EIA), CA15-3^{EIA} and HE4^{EIA} as references. Receiver operating characteristics (ROC) were applied and sensitivity at 75 % and 98 % specificity were calculated across histotypes.

Result: TOC was present in 596 patients and 716 had benign disease. CA125^{STn} showed higher sensitivity at 98 % specificity compared with CA125^{EIA} for high grade serous ovarian carcinoma (HGSC) (0.85 vs 0.62, $p < 0.001$), HGSC early-stage (0.66 vs 0.24, $p = 0.003$), and HGSC late-stage (0.90 vs 0.69, $p < 0.001$). CA15-3^{STn} showed higher sensitivity at 98 % specificity compared to CA125^{EIA} for mucinous ovarian carcinoma (0.50 vs 0.16, $p = 0.038$). No improvements were found for low grade serous carcinoma (LGSC), endometrioid and clear cell histotypes. The best performing combined biomarker test was CA125^{STn} + HE4^{EIA} with higher sensitivity at 98 % specificity for HGSC (0.93 vs 0.86, $p < 0.001$) and HGSC late-stage (0.97 vs 0.91$p < 0.001$) compared with CA125^{EIA} + HE4^{EIA}.

Conclusion: The STn glycovariants of CA125 and CA15-3 have improved sensitivity at high specificity for high grade serous and mucinous ovarian carcinoma and often perform better than the commonly used biomarker CA125^{EIA}.

Introduction

Detecting specific glycosylation patterns on a protein marker rather than the protein levels, as currently measured, could potentially increase the specificity and the sensitivity of the ovarian cancer (OC) biomarker assays [1]. Carcinomas originating in both the ovaries and the fallopian tubes are today included in epithelial derived OC, consequently the term tubo-ovarian carcinoma (TOC) has been suggested [2]. TOC is a heterogenous group of histological subtypes rather than a single disease entity. Each subtype has its own molecular and cellular profile, precursor lesion and spreading pattern as well as clinical presentation and prognosis [3]. The diagnostic performance of the tumor markers varies

* Corresponding author at: Universitetstorget 4, 901 87 Umeå, Sweden.
E-mail address: hanna.roos@umu.se (H.R. Alexander).

https://doi.org/10.1016/j.ejogrb.2025.114525
Received 19 March 2025; Received in revised form 28 May 2025; Accepted 8 June 2025
Available online 10 June 2025
0301-2115/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Table 1
Characteristics of the study population at the time of gynecological surgery.

	n	EOC n = 596	Benign disease n = 716
Cohorts, n (%)			
Gothenburg	404	213 (36)	191 (27)
Umeå	502	99 (17)	403 (56)
Turku	406	284 (48)	122 (17)
Age, median (range)		66 (22–90)	54 (16–94)
<51 years	363	69(12)	294 (41)
≥51 years	946	527 (88)	419 (59)
Clinical stage[a], n (%)			
Early stage		138 (23)	
Stage I		83 (14)	
Stage II		55 (9)	
Late stage		440 (74)	
Stage III		288 (48)	
Stage IV		152 (26)	
EOC, histotype, n (%)			
HGSC		408 (68)	
LGSC		52 (9)	
Mucinous		38 (6)	
Clearcell		29 (5)	
Endometrioid		51 (9)	
Carcinosarcoma		7 (1)	
Undifferentiated		11 (2)	
Benign diagnoses, n (%)			
Neoplastic tumor[b]			487 (66)
Endometriosis			83 (12)
Non-neoplastic tumor[c]			148 (21)

Abbreviations: EOC, epithelial ovarian cancer; HGSC, high grade serous carcinoma; LGSC, low grade serous carcinoma.

[a] Clinical stage according to FIGO classification. Stage classification missing, n = 18.

[b] Neoplastic tumor includes serous cystadenoma, mucinous cystadenoma, benign cystic teratoma, benign Brenner tumors, dermoid cysts, benign fibroma, benign thecomas.

[c] Non-neoplastic tumor includes follicular cyst, corpus luteal cyst.

across different histotypes [4,5], and performs best in high grade serous carcinoma (HGSC) which is usually detected at late stage. There is an intense interest to identify sensitive and specific biomarkers, that detect the whole panel of histological subtypes to allow early detection and diagnosis.

The two best biomarkers for early detection that are currently in clinical use, are cancer antigen 125 (CA125), the antibody-defined version of Mucin 16 (MUC 16), and human epididymis protein 4 (HE4) [6]. However, these two biomarkers show insufficient sensitivity and specificity for the detection of early-stage TOC, as well as specific histotypes [7]. The carcinoma antigen 15-3 (CA15-3), the antibody-defined version of Mucin 1 (MUC1), is primarily a tumor marker for breast cancer but is reported to be elevated in TOC as well [8,9]. Glycovariants of CA15-3 and CA125 have been reported to be elevated in ovarian cyst fluid [10].

Many glycoproteins undergo aberrant glycosylation during the malignant process which occurs in many types of human cancers [11]. Lectins are the most widely used biorecognition agent for glycans but have poor binding strength. There are now results suggesting that detection of tumor specific glycan structures on glycoprotein biomarkers in serum samples is feasible, by using lectin coated fluorescent lanthanide nanoparticles for detection [12] (Jain et al 2024, manuscript). The diagnostic performance of the method in the different TOC histological subtypes is not known.

The aim of the present study was to evaluate the diagnostic accuracy of the nanoparticle aided detection of CA125 and CA15-3 glycoforms across different histotypes of TOC at the time of diagnosis.

Methods

Study design

A cross sectional multicenter observational study was performed at three universities: Gothenburg and Umeå in Sweden, and Turku in Finland.

Study population

At the University hospital of Umeå, serum samples were collected 1990–2016, at the Sahlgrenska hospital of Gothenburg 2016–2019, and at the University hospital of Turku 2009–2019. The recruitment and sampling processes were similar at all study centers. Consecutive patients undergoing surgery due to suspected ovarian pathology were included after informed consent. Serum were drawn prior to surgery and any neoadjuvant chemotherapy.

Clinical stage and morphological classification

Diagnoses were validated by histopathological report review by senior consultant gynecologic pathologists at each study center, and adapted to the 2014 WHO pathologic classification of gynecologic tumors [13]. Cases classified before 2014 with a serous carcinoma grade 2 diagnosis, were re-classified as either HGSC or LGSC after reassessment of tumor slides to avoid/diminish the risk of misclassification. Samples with metastases to the ovary with primary origin of cancer elsewhere and borderline ovarian tumors were excluded from the analysis. Women with carcinoma with the primary site in the ovary C56.9, fallopian tube C57.0 and peritoneum C48.1–2 were included and classified as TOC. The stage classification according to FIGO 2014 was retrieved by manual review of the medical records.

The reference serum were drawn from women with benign ovarian diseases undergoing gynecologic surgery.

Collection of samples

At the University hospital of Umeå, Sweden, samples were centrifuged for 1500xg in 15 min, and the serum was added to kryo-pipes which were frozen into −80 degrees C within one hour in standard freezers. At Sahlgrenska University hospital of Gothenburg, Sweden and Turku University Hospital, Finland the samples were processed similarly as above according to standard protocol.

Laboratory assays

Materials

OVCAR-3 cell line purified CA125, anti-CA125 (Ov185) mab, anti-CA15-3 (Ma552) mAb and STn1242 mAb were provided by Fujirebio Diagnostics (Gothenburg, Sweden). Yellow streptavidin-coated low-fluorescence microtiter plates, wash buffer, and assay buffer were obtained from Uniogen Oy (Turku, Finland). Recombinant human lectin MGL-Fc was purchased from Sino Biologicals Inc. (Beijing, China). Europium (III)-chelate doped Fluoro-Max™ polystyrene nanoparticles (95 nm diameter, 30,000 chelates per particle, Eu^{+3}-NP) were from Seradyn Inc. (Indianapolis, IN).

Glycovariant assays

The cohort was tested using three biomarkers: $CA125^{STn}$, $CA15\text{-}3^{STn}$, and $CA125^{MGL}$.

The glycovariant (GV) immunoassays detect CA125 and CA15-3 carrying STn or MGL-binding glycans using a dual-antibody/lectin approach. For capture, biotinylated anti-CA125 (Ov185 Fab2) or anti-CA15-3 (Ma552 Fab2) immobilizes total CA125/CA15-3 on streptavidin-coated wells. For detection, glycosylation-specific probes (STn antibody or MGL lectin) conjugated to Eu^{+3}-NPs bind to captured GVs, enabling quantification via time-resolved fluorescence (TRF). Assay details were adapted from prior studies [10,14]. Briefly, capture

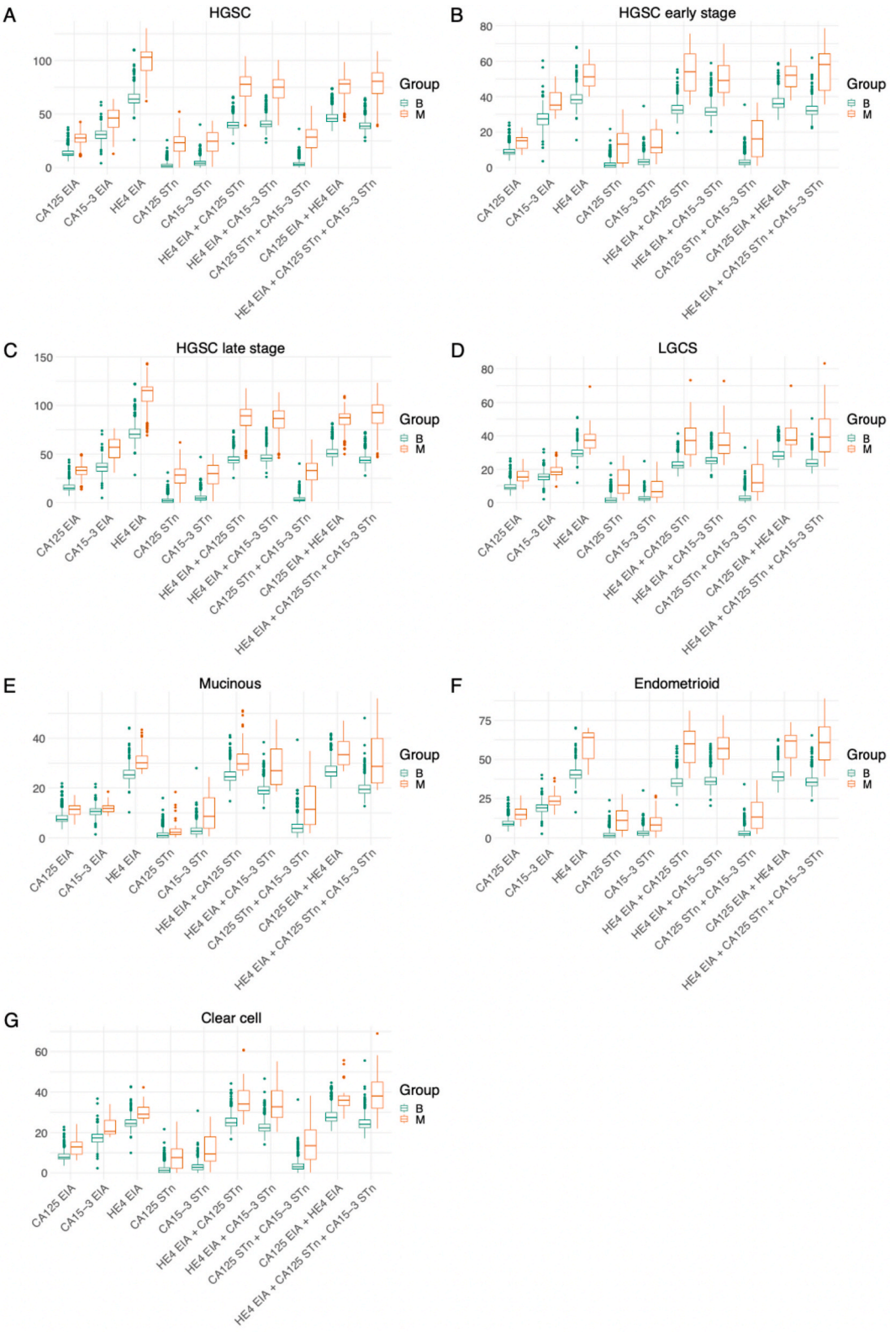

Fig. 1. A-G. Predictions from logistic regression models divided by histotype (panel A-G). Regression models for assays and combinations are shown on X. The Y axis represents predicted values from each model where assays are weighted by their coefficients. Samples from women with benign ovarian pathology are indicated in green and malignant pathology in brown.

antibodies (50 ng/25 μl/well in assay buffer) were immobilized for 60 min (RT). After washing, 25 μL of standard or diluted serum (1:10) in assay buffer (300 mM NaCl, 100 mM $CaCl_2$, 5 μg/ml MAK33 poly blocker) was added in triplicate and incubated for 60 min at RT with shaking. A tracer (25 μL of Eu^{+3}-NPs coated with MGL lectin or anti-STn mAb in assay buffer) was added and incubated for 1 h at RT with shaking. Wells were washed six times, and TRF of Eu^{+3} was measured (λex: 340 nm; λem: 615 nm) using the Hidex Sense Multi-Technology

Table 2
Single biomarker analyses across different EOC histotypes in the multi-cohort study on nanoparticle aided detection $CA125^{STn}$ and $CA15.3^{STn.}$

			75 % specificity			98 % specificity		
Histotype	EOC	Benign	SN	p-value[a] vs ref.		SN	p-value[a] vs ref.	
Biomarker	n	n		$CA125^{EIA}$	$HE4^{EIA}$		$CA125^{EIA}$	$HE4^{EIA}$
HGSC	408	716						
$CA125^{EIA}$			0.95	Ref		0.62	Ref	
$HE4^{EIA}$			0.97		Ref	0.84		Ref
$CA125^{STn}$			0.93	0.136	0.005	0.85	<0.001	0.640
$CA15.3^{STn}$			0.92	0.046	0.001	0.79	<0.001	0.129
HGSC es	41	716						
$CA125^{EIA}$			0.83	Ref		0.24	Ref	
$HE4^{EIA}$			0.88		Ref	0.56		Ref
$CA125^{STn}$			0.73	0.697	0.406	0.66	0.003	0.697
$CA15.3^{STn}$			0.83	1	0.943	0.54	0.040	1
HGSC ls	349	716						
$CA125^{EIA}$			0.98	Ref		0.69	Ref	
$HE4^{EIA}$			0.98		Ref	0.88		Ref
$CA125^{STn}$			0.97	0.295	0.266	0.90	<0.001	0.730
$CA15.3^{STn}$			0.95	0.040	0.040	0.84	<0.001	0.209
LGSC	52	716						
$CA125^{EIA}$			0.83	Ref		0.31	Ref	
$HE4^{EIA}$			0.85		Ref	0.48		Ref
$CA125^{STn}$			0.83	1	1	0.52	0.065	0.987
$CA15.3^{STn}$			0.71	0.344	0.262	0.40	0.566	0.680
Mucinous	38	716						
$CA125^{EIA}$			0.76	Ref		0.16	Ref	
$HE4^{EIA}$			0.76		Ref	0.29		Ref
$CA125^{STn}$			0.53	0.112	0.088	0.16	1	0.219
$CA15.3^{STn}$			0.68	0.617	0.677	0.50	0.038	0.088
Endometrioid	51	716						
$CA125^{EIA}$			0.84	Ref		0.31	Ref	
$HE4^{EIA}$			0.92		Ref	0.73		Ref
$CA125^{STn}$			0.84	1	0.461	0.55	0.051	0.170
$CA15.3^{STn}$			0.80	0.991	0.325	0.45	0.355	0.023
Clear cell	29	716						
$CA125^{EIA}$			0.72	Ref		0.21	Ref	
$HE4^{EIA}$			0.76		Ref	0.34		Ref
$CA125^{STn}$			0.72	1	1	0.48	0.324	0.791
$CA15.3^{STn}$			0.83	0.865	1	0.52	0.230	0.865

Abbreviations: EIA, enzyme immunoassay; EOC, epithelial ovarian cancer; es, early stage; HGSC, high grade serous carcinoma; LGSC, low grade serous carcinoma; ls, late stage; ref, reference; SN, sensitivity; STn, Sialyl-Thomsen-nouveau.

[a] p-value, statistical significance was assessed with McNemar test. p-values were corrected for multiple testing with Benjamini-Hochberg correction and results considered significant <0.05.

Microplate Reader (Hidex Oy, Turku, Finland).

For CA125 GVs (both MGL and STn), purified CA125 from OVCAR-3 cell line was used to prepare standards (2–500 U/mL). For $CA15\text{-}3^{STn}$ standards, ascites from a TOC patient with 300 U/mL CA15-3 was used. Standards and samples were added in triplicate, and the coefficient of variance (CV%) was calculated. Intra- and inter-assay CVs were <10 % and <15 %, respectively, validated using low/high controls per plate.

Reference Assays:

Conventional EIAs for CA125, CA15-3, and HE4 were used as references. Fujirebio CanAg CA125 EIA, CanAg CA15-3 EIA, and CanAg HE4 EIA kits were run according to the manufacturer's instructions. CA125 and HE4 were chosen as reference biomarkers due to their widespread use in clinical TOC diagnosis, while CA15-3, primarily used for breast cancer, is also reported as elevated in TOC.

Statistical analyses

Results from the glycovariant and conventional enzyme

Table 3
Combined biomarker analyses across different EOC histotypes in the multi-cohort study on nanoparticle aided detection $CA125^{STn}$ and $CA15.3^{STn}$.

Subgroup	EOC n	Benign n	75 % specificity SN	75 % specificity p-value[a]	98 % specificity SN	98 % specificity p-value[a]
HGSC	408	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.97	Ref	0.86	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.98	0.516	0.93	<0.001
$CA15.3^{STn}$ + $HE4^{EIA}$			0.98	0.445	0.92	<0.001
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.98	0.512	0.93	<0.001
HGSC, early stage	41	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.93	Ref	0.61	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.93	1	0.76	0.257
$CA15.3^{STn}$ + $HE4^{EIA}$			0.95	0.861	0.71	0.598
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.93	1	0.78	0.122
HGSC, late stage	349	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.99	Ref	0.91	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.99	1	0.97	<0.001
$CA15.3^{STn}$ + $HE4^{EIA}$			0.99	1	0.96	0.001
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.99	1	0.97	<0.001
LGSC	52	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.83	Ref	0.46	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.85	0.496	0.63	0.065
$CA15.3^{STn}$ + $HE4^{EIA}$			0.85	0.602	0.54	0.571
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.83	0.334	0.63	0.065
Mucinous	38	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.74	Ref	0.29	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.76	1	0.29	1
$CA15.3^{STn}$ + $HE4^{EIA}$			0.89	0.111	0.50	0.073
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.87	0.219	0.50	0.073
Endometrioid	51	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.94	Ref	0.73	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.96	1	0.76	0.904
$CA15.3^{STn}$ + $HE4^{EIA}$			0.96	1	0.76	0.674
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.96	1	0.8	0.303
Clear cell	29	716				
$CA125^{EIA}$ + $HE4^{EIA}$			0.86	Ref	0.28	Ref
$CA125^{STn}$ + $HE4^{EIA}$			0.86	1	0.48	0.514
$CA15.3^{STn}$ + $HE4^{EIA}$			0.90	1	0.66	0.111
$CA15.3^{STn}$ + $CA125^{STn}$ + $HE4^{EIA}$			0.93	0.974	0.62	0.142

Abbreviations: EIA, enzyme immunoassay; EOC, epithelial ovarian cancer; HGSC, high grade serous carcinoma; LGSC, low grade serous carcinoma; ref, reference; SN, sensitivity; STn, Sialyl-Thomsen-nouveau.

[a] p-value, statistical significance was assessed with McNemar test. p-values were corrected for multiple testing with Benjamini-Hochberg correction and results considered significant <0.05.

immunoassays were imported into R version 4.2.3 (R Core Team, 2023). The data was log2-transformed and used to fit logistic regression models with individual assays, or combinations thereof, as predictors and sample group as response variable (benign vs malignant). Thus, each assay combination reported is a separate logistic regression model with the assays used as predictors. Analyses were performed using subsets of all samples in the pooled study population defined by sample characteristics, i.e. a priori chosen histotype and tumor stage subgroups. Combinations of biomarkers were tested for benefits (diagnostic performance) and only combinations with the highest diagnostic accuracy are reported.

Logistic regression models were evaluated by leave-one-out cross-validation (LOOCV), where all samples in the data except one were used to fit the model, the response predicted for the left-out sample. This was repeated such that each sample was left out once. Results reported on model performance, including area under the curve (AUC) and partial area under the curve (pAUC) for specificity 1–0.9, 95 % confidence intervals (CI), sensitivity (SN) and specificity (SP), were based on the LOOCV analysis.

The statistical significance of differences between sensitivities at predefined specificities (75 %, 98 %) were assessed with the McNemar test. These were chosen from what is reasonable considering a diagnostic situation in a cohort with symptomatic women (75 %) or a pre-symptomatic screening situation (98 %). P-values were corrected for multiple testing with Benjamini-Hochberg correction, results considered significant where false discovery rate (FDR) was <0.05.

Results

Study population

The study population consisted of 1,312 participants from Gothenburg (n = 404, 31 %), Umeå (n = 502, 38 %) and Turku (n = 406, 31 %), of which 596 were diagnosed with TOC. The mean age at TOC diagnosis was 66 years (range 22–90), 138 women were diagnosed in early stage (stage I and II) and 440 women in late stage (stage III and IV). In 18 women, no data on stage were available. The most common histotype was HGSC (n = 408, 68 %). The mean age of women with benign diagnoses was 54 (range 16–94). The study population is described in Table 1.

Diagnostic performance of glycovariant assays

The day-to-day variation of the control samples for the glycovariant assays was very low and the CV was below 20 % within the three sample replicates. The distribution of the biomarkers in malignant disease and benign controls per histotype is shown in Fig. 1A-G. In all tested histotypes at 98 % specificity, $CA125^{STn}$ and $CA15\text{-}3^{STn}$ performed equally or better than $CA125^{EIA}$ or $HE4^{EIA}$ as single biomarker tests (Table 2). Similar results were observed when biomarker combinations were tested. Adding $CA15\text{-}3^{STn}$ to the combination of $CA125^{STn}$ + $HE4^{EIA}$, the best performing single biomarkers, did not significantly improve diagnostic sensitivity further (Table 3).

Sensitivity at 75 % specificity was not improved with glycovariants compared with $CA125^{EIA}$ or $HE4^{EIA}$ (Table 2, Table S1). Glycovariant $CA125^{MGL}$ performed less well than the conventional biomarkers, therefore $CA125^{MGL}$ results will not be further reported. Improvements in sensitivity at 98 % specificity will be described for each histotype in the following sections.

Area under the curve (AUC) is presented for single (Fig. 2A-2G) and combined (Fig. 3A-3G) biomarker tests across histotypes. Additionally, AUC and pAUC with 95 % CI is presented in supplementary table S2, indicating the largest benefit for the glycovariant biomarkers at high specificity represented by pAUC.

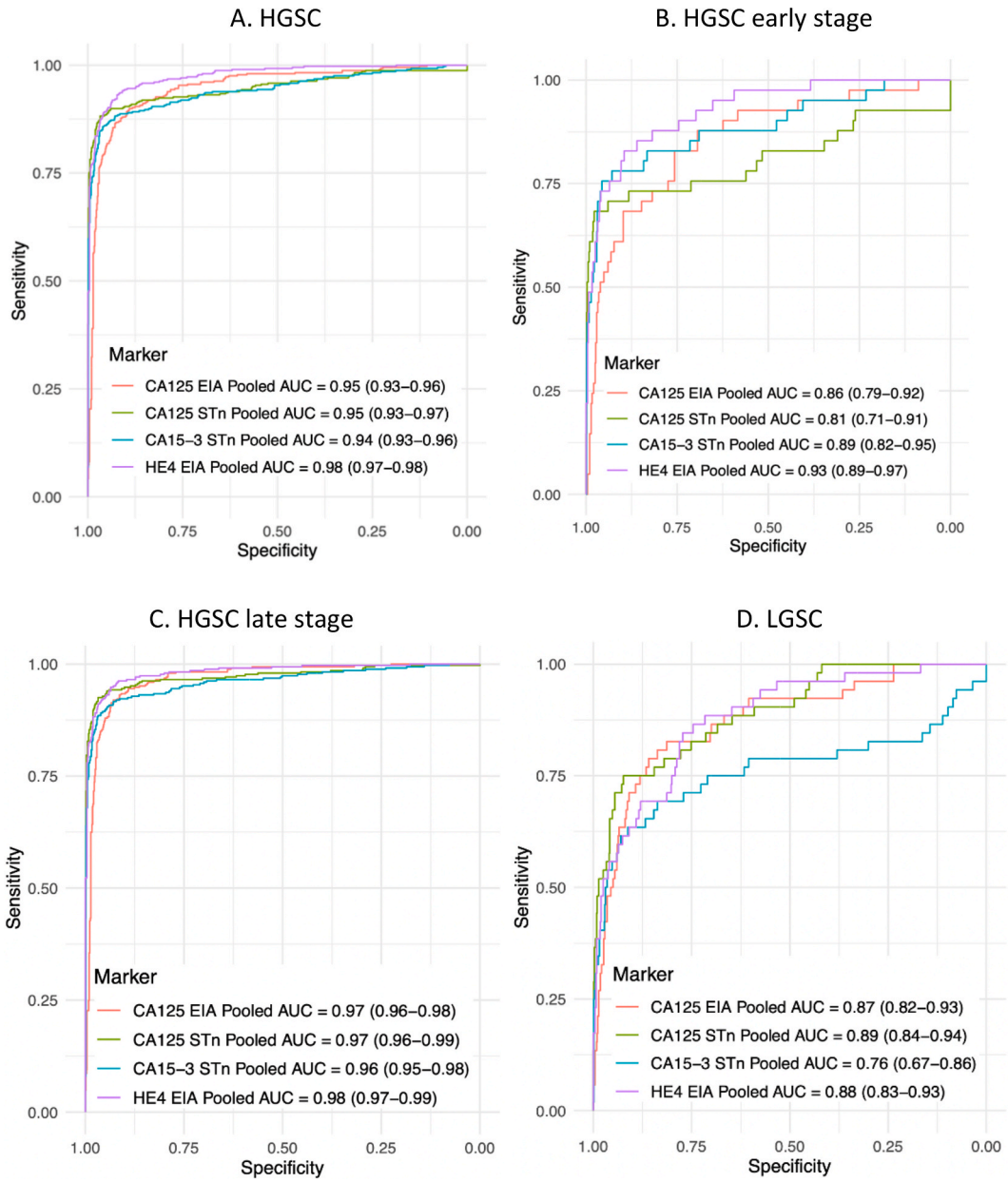

Fig. 2. A-G. ROC-curves across different histotypes. Glycovariant biomarkers and EIA markers as well as 95 % CI for the different biomarkers. Abbreviations: HGSC, high grade serous cancer; LGCS, low grade serous cancer.

High grade serous ovarian carcinoma

Sensitivity of $CA125^{STn}$ was better than $CA125^{EIA}$ (0.85 vs 0.62, $p < 0.001$) at 98 % specificity (Table 2). Combining $CA125^{STn}$ with $HE4^{EIA}$ showed higher diagnostic sensitivity compared with the combination of $CA125^{EIA}$ + $HE4^{EIA}$ (0.93 vs 0.86, $p < 0.001$) (Table 3).

High grade serous ovarian carcinoma, early-stage

The $CA125^{STn}$ glycovariants presented higher sensitivity than $CA125^{EIA}$ (0.66 vs 0.24, $p = 0.003$) at 98 % specificity (Table 2).

High grade serous ovarian carcinoma, late-stage

$CA125^{STn}$ exhibited higher diagnostic sensitivity than $CA125^{EIA}$ (0.90 vs 0.69, $p < 0.001$) at 98 % specificity (Table 2). $CA125^{STn}$ in combination with $HE4^{EIA}$ improved sensitivity compared to $CA125^{EIA}$ + $HE4^{EIA}$ (0.97 vs 0.91, $p < 0.001$) (Table 3).

Mucinous ovarian carcinoma

Conventional $HE4^{EIA}$ and $CA125^{EIA}$ presented lower diagnostic performance in this histotype compared to other histotypes. However, sensitivity of $CA15\text{-}3^{STn}$ was better than $CA125^{EIA}$ (0.50 vs 0.16, $p = 0.038$) at 98 % specificity in mucinous OC (Table 2). Combining $CA15\text{-}3^{STn}$ and $HE4^{EIA}$ tended to improve sensitivity compared with the combination $CA125^{EIA}$ + $HE4^{EIA}$ (0.50 vs 0.29, $p = 0.073$) (Table 3).

Low grade serous-, endometrioid-, clear cell- ovarian carcinoma

The STn glycovariant assays did not perform better than the $CA125^{EIA}$ or $HE4^{EIA}$ assays individually or in combination in these histotypes (Table 2) (Table 3).

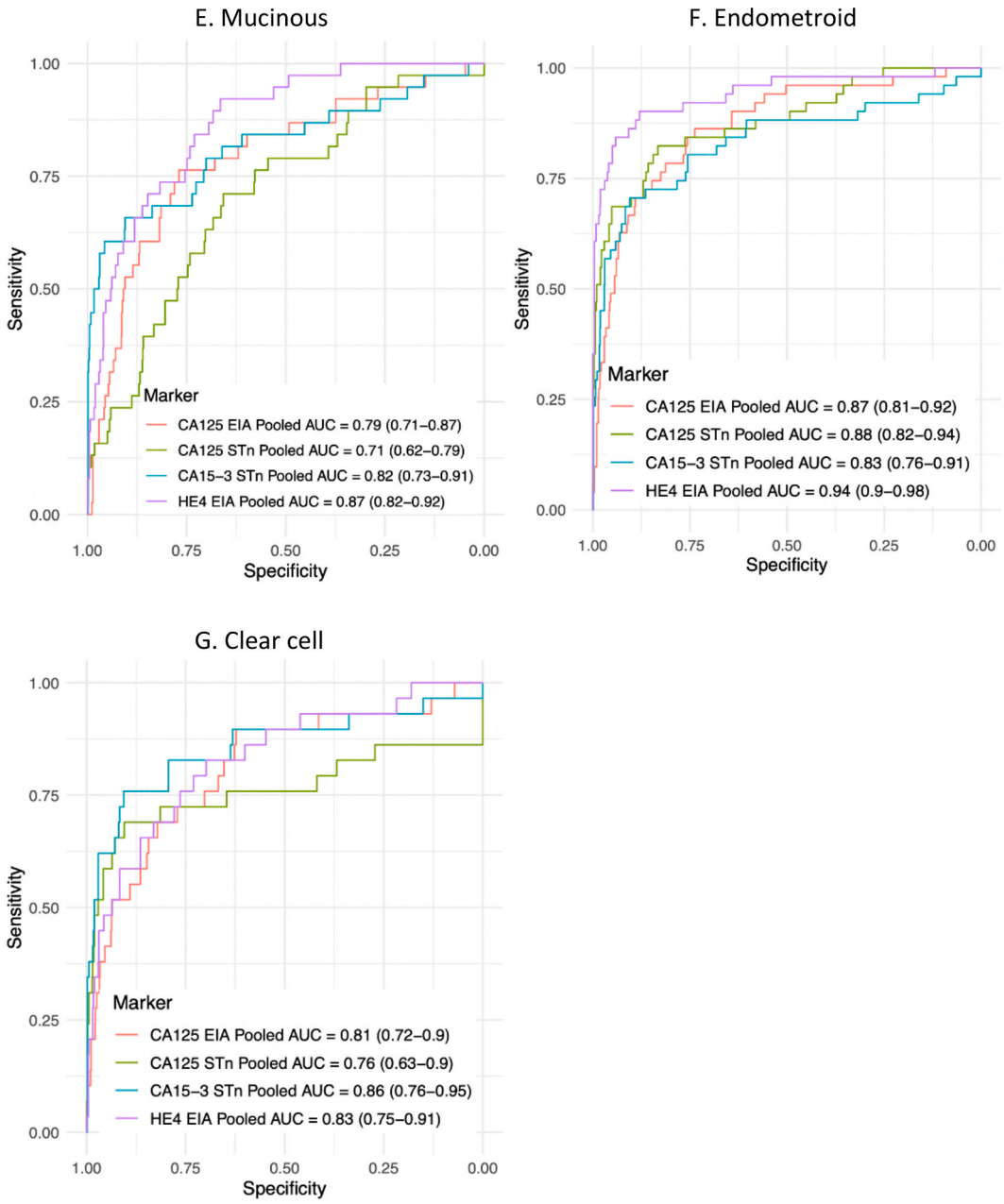

Fig. 2. (*continued*).

Discussion

This cross-sectional study examined the diagnostic accuracy of glycovariants of CA125 and CA15-3 compared with $CA125^{EIA}$ and $HE4^{EIA}$ across different histotypes of tubo-ovarian carcinoma (TOC) at diagnosis. Highest sensitivity was provided by $CA125^{STn}$ for HGSC at high specificity. In mucinous OC, $CA15\text{-}3^{STn}$ was the best performing biomarker. For LGSC, endometrioid or clear cell OC the single glycovariant markers did not significantly improve the sensitivity nor decrease it. The combination $HE4^{EIA}$ + STn glycovariants performed significantly better than any single biomarker alone or the combination of $HE4^{EIA}$ + $CA125^{EIA}$, regardless of histotype. The glycovariant combinations performed best in participants with HGSC. For the smaller histotype subgroups the sensitivity of $CA125^{STn}$ and $CA15\text{-}3^{STn}$ is often numerically higher at 98 % specificity compared with $CA125^{EIA}$ or $HE4^{EIA}$ but the differences are not significant.

The strengths of the study include the heterogeneous study population representative of patients having surgical procedures for suspected ovarian pathology at three different university hospitals. Samples from patients with OC and benign ovarian lesions were processed together in the same batches and analyzed simultaneously. Laboratory staff members were blinded to case- control status, reference standard and index test, decreasing the risk of bias. All histopathological diagnoses were confirmed and validated by experienced senior consultant gynecological pathologists. The mean age of the women with TOC in our study is similar to the mean age for TOC in the Swedish population. Even though Benjamini-Hochberg correction for multiple comparisons was applied, false positive results cannot be ruled out. Despite being relatively large, a limitation of this study is the sample size for the less common histotypes, reducing power to detect improvements in these subgroups; negative findings should be interpreted with caution. Interpretation could have been enhanced by data on ultrasound and menopausal data, which were unavailable.

Historically, OC have been classified and treated as one disease but

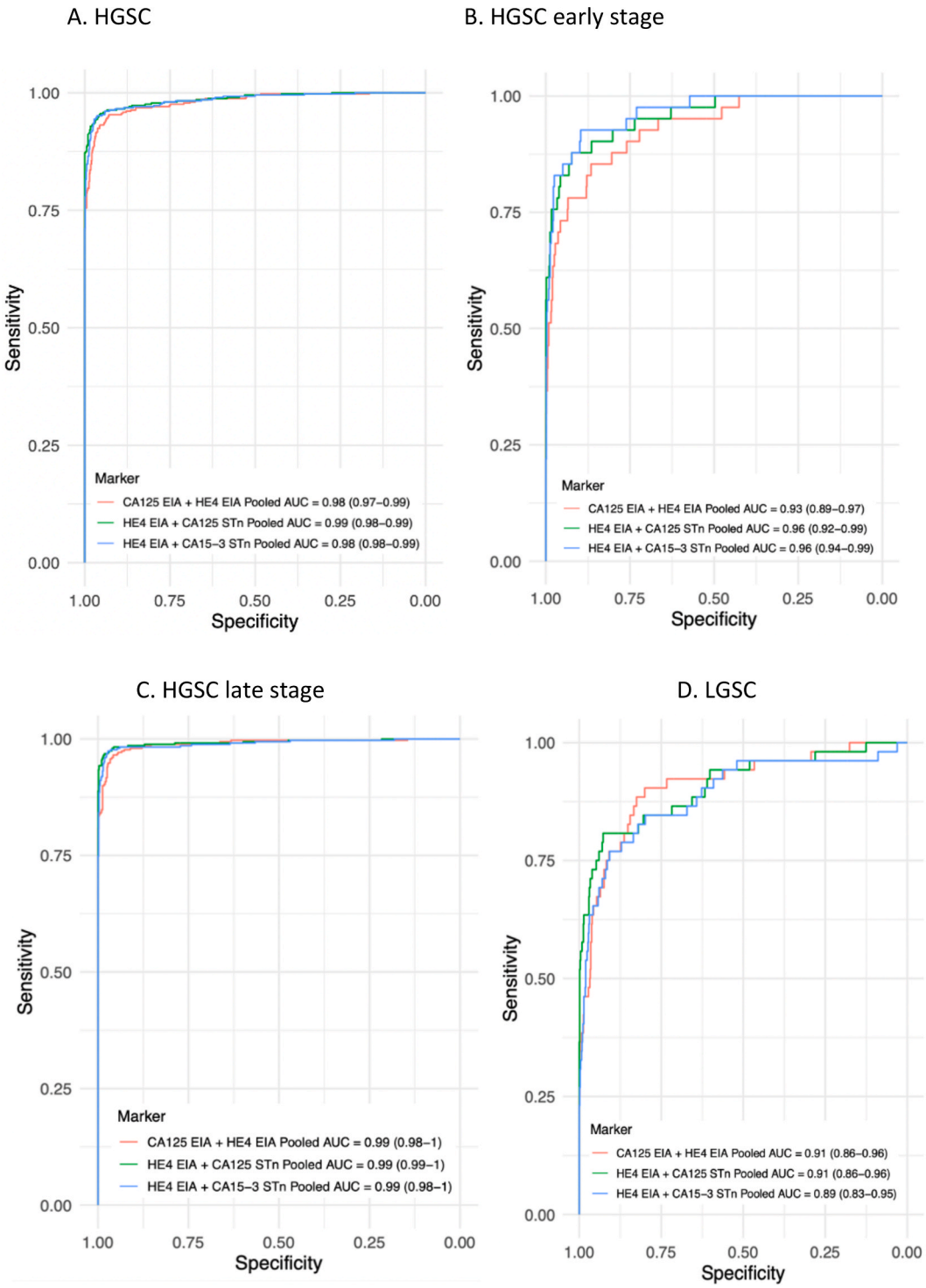

Fig. 3. A-G. ROC-curves across different histotypes. Combination glycovariant biomarkers and 95 % CI for the different biomarkers. Abbreviations: HGSC, high grade serous cancer; LGCS, low grade serous cancer.

over the last two decades there has been a growing understanding that the different histologic types of OC also represent different diseases [15,16]. Considering that different histotypes have different origin, molecular profiles, behavior, and clinical management [15], it is plausible that the different histotypes are also best detected with different biomarkers.

The biomarkers tested here are glycoproteins that physiologically serve certain roles, partly being surface markers, partly being of intracellular origin [17,18]. Alterations in the N-linked or O-linked glycosylation pattern of regulatory proteins or cellular receptors lead to many diseases, including cancer [19]. These alterations give rise to micro- and macro-heterogeneity in tumor cells [20]. It is thus not surprising that no single biomarker, but a panel of biomarkers is needed to cover the spectrum of different histotypes in TOC [5]. The improved sensitivity of $CA125^{STn}$ in HGSC likely reflects the overexpression of STn-glycosylated CA125 in this subtype, while $CA15\text{-}3^{STn}$'s utility in

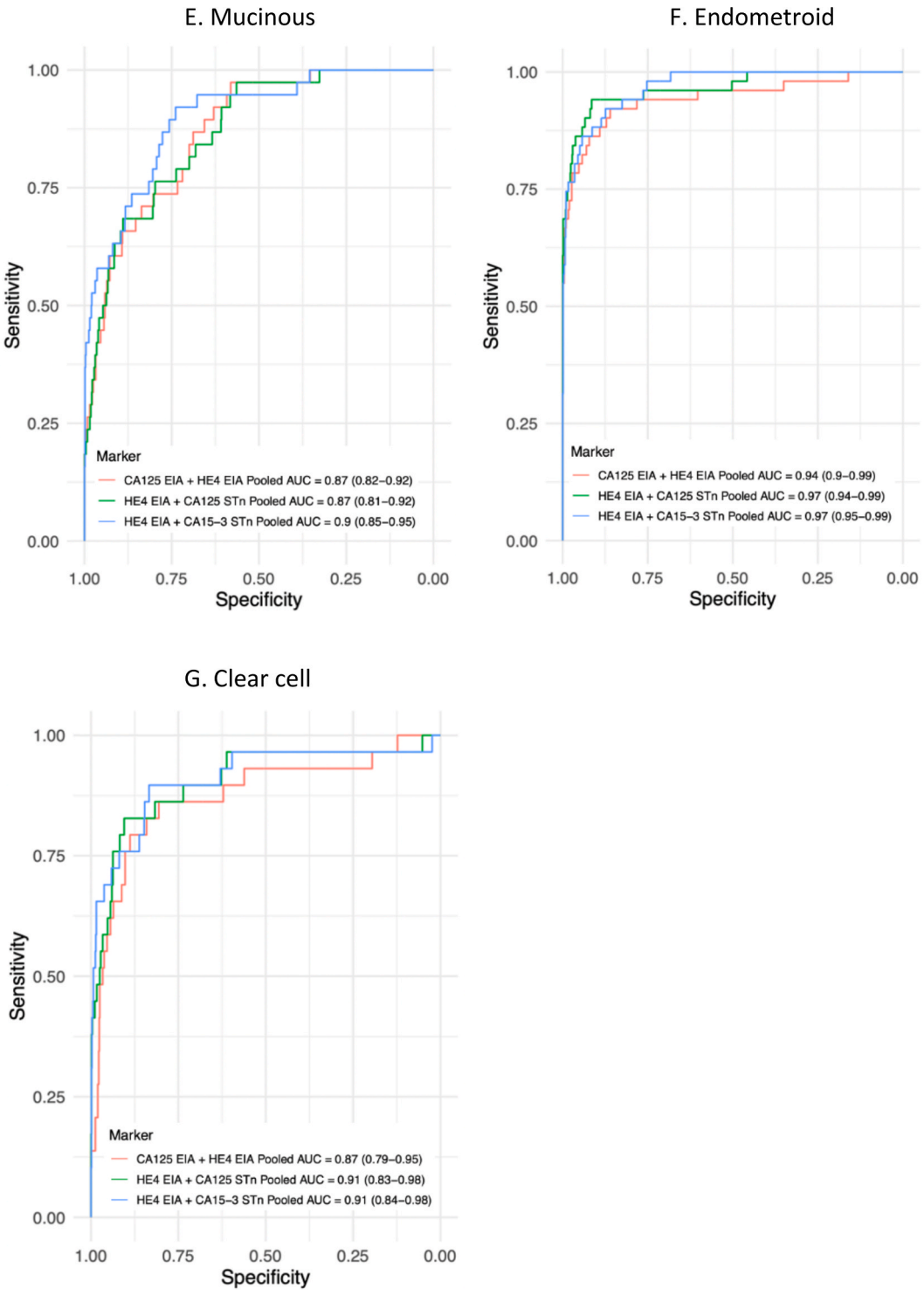

Fig. 3. (*continued*).

mucinous carcinomas aligns with their gastrointestinal-like mucin profiles enriched in STn glycans [21]. Moreover, targeting the glycan structures that are aberrantly formed during glycosylation in malignant compared with benign disease, [22] and the increased binding-strength afforded by nanoparticles coated with lectins with high glycan specificity is beneficial [23,24]. The improved performance in OC diagnosis has been described earlier using paired samples of serum and ovarian cyst fluid [10].

High sensitivity >75 % and especially high specificity >98 % are important in a screening setting to overcome and reduce the number of false positive results and unnecessary further examinations or invasive procedures [25]. In this cohort, $CA125^{STn}$ had highest sensitivity at high specificity in early stage HGSC, but still not sufficient for screening. However, the superior discriminative performance of the biomarker combination $CA125^{STn}$ + $HE4^{EIA}$ to detect early stage HGSC warrants further testing in asymptomatic women. In a screening setting a substantially lower prevalence of TOC, TOC at early stages, and controls without benign lesions might change performance. The results of the present study point to the need of further refinement of panels of tumor markers especially developed for early detection of TOC considering the

diversity of histotypes, tumor biology and tumor cell origin. Moreover, validation in retrospective longitudinal and prospective screening trials will be necessary before these assays can be adopted for population screening.

Conclusion

This study suggests that the STn glycovariant of CA125 and CA15-3 afford improved diagnostic sensitivity at high specificity in high grade serous and mucinous OC. For evaluation of the diagnostic performance of the glycovariant tests in less prevalent histotypes, larger cohorts are needed.

Ethics statement

The current study was approved by the Regional Ethical Review authority in Umeå (Dnr. 2017–376-31), the Ethical Review authority in Gothenburg (Dnr 201–15) and the Ethics Committee in the Hospital District of Southwest Finland (ETMK 53/180/2009).

Data availability statement

Data and blood samples used in the present study were extracted from biobanks in Umeå, Gothenburg and Turku after an application to the Swedish and Finnish Ethical Review Authority (https://etikprovningsmyndigheten.se and https://www.varha.fi/en/about-varha/scientific-research/ethics-committee respectively). The data cannot be shared publicly because the individual-level data contain potentially identifying and sensitive patient information and cannot be published due to legislative and ethical review restrictions.

CRediT authorship contribution statement

Hanna Roos Alexander: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Shruti Jain:** Writing – review & editing, Methodology, Investigation. **Kamlesh Gidwani:** Validation, Methodology, Investigation. **Benjamin Ulfenborg:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Ulrika Ottander:** Writing – review & editing, Resources, Investigation. **Eva Lundin:** Writing – review & editing, Validation, Resources, Investigation. **Johanna Hynninen:** Writing – review & editing, Validation, Resources. **Shamima Afrin Ruma:** Methodology, Investigation. **Kaisa Huhtinen:** Writing – review & editing, Validation, Formal analysis, Data curation. **Karin Sundfeldt:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Kim Pettersson:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Annika Idahl:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Funding

This study was founded by the Nordic cancer union (KPe, R241-A15062), the Cancer research foundation of northern Sweden (AId, AMP 18–917), Sjöbergsstiftelsen (KSu, 2024-983), the Swedish Cancer Foundation (KSu, CAN 2018/384), Cancera (KSu), the Swedish state under the agreement between the Swedish government and the county council, the ALF-agreement (KSu, ALFGBG-965552, AId, RV-7004584) and The European Union's Horizon 2020 research and innovation program (JHy and KHu, 667,403). The funders had no role in the study design nor the decision to publish the results.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kim Petterson is the co-inventor of an invention relating to the determination of the CA125 MGL glycovariant. Kaisa Huhtinen is currently working at Uniogen Oy, where glycovariant immunoassays for cancer diagnostics are developed

Acknowledgment

We are grateful for the willingness of the participants to donate samples for the research conducted here.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejogrb.2025.114525.

References

[1] Christiansen MN, Chik J, Lee L, Anugraham M, Abrahams JL, Packer NH. Cell surface protein glycosylation in cancer. Proteomics 2014;14(4–5):525–46.
[2] Kurman RJ. Origin and molecular pathogenesis of ovarian high-grade serous carcinoma. Ann Oncol 2013;24(Suppl 10):x16–21.
[3] Chakraborty S, Shenoy PS, Mehrotra M, Phadte P, Singh P, Rekhi B, Ray P. Through the looking glass updated insights on ovarian cancer diagnostics. Diagnostics (Basel) 2023;13(4).
[4] Lertkhachonsuk AA, Buranawongtrakoon S, Lekskul N, Rermluk N, Wee-Stekly WW, Charakorn C. Serum CA19-9, CA-125 and CEA as tumor markers for mucinous ovarian tumors. J Obstet Gynaecol Res 2020;46(11):2287–91.
[5] Zhang L, Chen Y, Wang K. Comparison of CA125, HE4, and ROMA index for ovarian cancer diagnosis. Curr Probl Cancer 2019;43(2):135–44.
[6] Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res 2019;12(1):28.
[7] Nebgen DR, Lu KH, Bast Jr RC. Novel approaches to ovarian cancer screening. Curr Oncol Rep 2019;21(8):75.
[8] Jeschke U, Wiest I, Schumacher AL, Kupka M, Rack B, Stahn R, Karsten U, Mayr D, Friese K, Dian D. Determination of MUC1 in sera of ovarian cancer patients and in sera of patients with benign changes of the ovaries with CA15-3, CA27.29, and PankoMab. Anticancer Res 2012;32(5):2185–9.
[9] Van Elssen CH, Frings PW, Bot FJ, Van de Vijver KK, Huls MB, Meek B, Hupperets P, Germeraad WT, Bos GM. Expression of aberrantly glycosylated Mucin-1 in ovarian cancer. Histopathology 2010;57(4):597–606.
[10] Jain S, Nadeem N, Ulfenborg B, Makela M, Ruma SA, Terava J, Huhtinen K, Leivo J, Kristjansdottir B, Pettersson K, Sundfeldt K, Gidwani K. Diagnostic potential of nanoparticle aided assays for MUC16 and MUC1 glycovariants in ovarian cancer. Int J Cancer 2022;151(7):1175–84.
[11] Kirwan A, Utratna M, O'Dwyer ME, Joshi L, Kilcoyne M. Glycosylation-based serum biomarkers for cancer diagnostics and prognostics. Biomed Res Int 2015;2015:490531.
[12] Chen K, Gentry-Maharaj A, Burnell M, Steentoft C, Marcos-Silva L, Mandel U, Jacobs I, Dawnay A, Menon U, Blixt O. Microarray glycoprofiling of CA125 improves differential diagnosis of ovarian cancer. J Proteome Res 2013;12(3):1408–18.
[13] Kurman R.J. C.M., Herrington C.S., Young R.H., (ed). WHO Classification of Tumours of Female Reproductive Organs. 4th ed (2014).
[14] Gidwani K, Huhtinen K, Kekki H, van Vliet S, Hynninen J, Koivuviita N, Perheentupa A, Poutanen M, Auranen A, Grenman S, Lamminmaki U, Carpen O, van Kooyk Y, Pettersson K. A Nanoparticle-lectin immunoassay improves discrimination of serum CA125 from malignant and benign sources. Clin Chem 2016;62(10):1390–400.
[15] Kobel M, Kang EY. The evolution of ovarian carcinoma subclassification. Cancers (Basel) 2022;14(2).
[16] Kobel M, Kalloger SE, Boyd N, McKinney S, Mehl E, Palmer C, Leung S, Bowen NJ, Ionescu DN, Rajput A, Prentice LM, Miller D, Santos J, Swenerton K, Gilks CB, Huntsman D. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med 2008;5(12):e232.
[17] Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and ovarian cancer: a comprehensive review. Cancers (Basel) 2020;12(12).
[18] Anastasi E, Farina A, Granato T, Colaiacovo F, Pucci B, Tartaglione S, Angeloni A. Recent insight about HE4 role in ovarian cancer oncogenesis. Int J Mol Sci 2023;24(13).
[19] Shen H, Lee CY, Chen CH. Protein glycosylation as biomarkers in gynecologic cancers. Diagnostics (Basel) 2022;12(12).
[20] Bangarh R, Khatana C, Kaur S, Sharma A, Kaushal A, Siwal SS, Tuli HS, Dhama K, Thakur VK, Saini RV, Saini AK. Aberrant protein glycosylation: Implications on diagnosis and Immunotherapy. Biotechnol Adv 2023;66:108149.

[21] Ricardo S, Marcos-Silva L, Pereira D, Pinto R, Almeida R, Söderberg O, Mandel U, Clausen H, Felix A, Lunet N, David L. Detection of glyco-mucin profiles improves specificity of MUC16 and MUC1 biomarkers in ovarian serous tumours. Mol Oncol 2015;9(2):503–12.
[22] Chen H, Deng Z, Huang C, Wu H, Zhao X, Li Y. Mass spectrometric profiling reveals association of N-glycan patterns with epithelial ovarian cancer progression. Tumour Biol 2017;39(7):1010428317716249.
[23] Silva MLS. Lectin biosensors in cancer glycan biomarker detection. Adv Clin Chem 2019;93:1–61.
[24] Gidwani K, Kekki H, Terava J, Soukka T, Sundfeldt K, Pettersson K. Nanoparticle-aided glycovariant assays to bridge biomarker performance and ctDNA results. Mol Aspects Med 2020;72:100831.
[25] Ain QU, Muhammad S, Hai Y, Peiling L. The role of urine and serum biomarkers in the early detection of ovarian epithelial tumours. J Obstet Gynaecol 2022;42(8):3441–9.