A longitudinal analysis of CA125 glycoforms in the monitoring and follow up of high grade serous ovarian cancer

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Abstract

1. Objective

Cancer antigen 125 (CA125) is generally considered the gold standard of biomarkers in the diagnosis and monitoring of high grade serous ovarian carcinoma (HGSC). We recently reported, that two CA125 glycoforms (CA125-STn and CA125-MGL) have a high specificity to HGSC and further hypothesized, that these cancer specific glycoforms are feasible candidates as biomarkers in HGSC treatment and follow up.

2. Methods

Our cohort consisted of 122 patients diagnosed with HGSC. Serum samples were collected longitudinally at the time of diagnosis, during treatment and follow up. Serum levels of CA125, CA125-STn and CA125-MGL were determined and compared or correlated with different end points (tumor load assessed intraoperatively, residual disease, treatment response, progression free survival).

3. Results

Serum CA125-STn levels at diagnosis differentiated patients with low tumor load and high tumor load (p=0,030), indicating a favorable detection of tumor volume. Similarly, the CA125-STn levels at diagnosis were significantly lower in patients with subsequent complete cytoreduction than in patients with suboptimal cytoreduction (p=0,025). Conventional CA125 did not differentiate these patients (p=0,363 and p=0,154). The CA125-STn nadir value predicted the progression free survival of patients. The detection of disease relapse was improved with CA125-STn, which presented higher fold increase in 80,0% of patients and earlier increase in 37,0% of patients.

4. Conclusions

CA125-STn showed promise as a useful biomarker in the monitoring and follow up of patients with HGSC utilizing a robust and affordable technique. Our findings are topical as a suitable indicator of tumor load facilitates patient selection in an era of new targeted therapies.

1. Introduction

Approximately a quarter of a million women were diagnosed with epithelial ovarian cancer (EOC) worldwide in 2012 [1]. Most of the patients are diagnosed with advanced disease (The International Federation of Gynecology and Obstetrics (FIGO) Committee on Gynecologic Oncology, stage III-IV) and a meager survival rate [2]. As metastatic cancer is generally a systemic disease [3], the majority of patients with advanced EOC develop recurrent disease regardless of optimal response to primary therapy.

Until recently, the prognosis of the most common and aggressive histological subtype of EOC, high grade serous ovarian carcinoma (HGSC), has remained poor [4]. The classical prognostic factors consist of cytoreductive surgery with no macroscopic residual tumor and response to platinum-taxane based chemotherapy [5]. Bevacizumab, a vascular endothelial growth factor inhibitor (VEGF-inhibitor), improves the progression free survival (PFS) especially in patients with poor prognosis [6,7]. Recent studies show that maintenance therapy with poly (ADP-ribose) polymerase (PARP) inhibitors improve significantly the PFS both after primary therapy [8] and in relapse [9]. As new treatment options like PARP inhibitors and immuno-oncologic drugs are implemented in the clinical setting, old practices such as refraining from early treatment of patients with asymptomatic recurrence have to be re-evaluated. Consequently, it is important to develop predictive and prognostic biomarkers that are useful in the monitoring of disease activity.

Cancer antigen 125 (CA125) is a validated biomarker in the diagnosis, monitoring and follow up of patients with HGSC [10–12] and according to wide consensus, the only serum marker that can be used in defining treatment response and disease progression together with radiologic assessment in clinical trial protocols [13]. The evaluation of the predictive and prognostic abilities of CA125 have attracted clinical interest and studies have shown that the nadir level and the time to serum CA125 normalization predict the PFS of HGSC patients [14,15]. In addition, it has been indicated that the preoperative CA125 level might predict optimal cytoreduction [16,17]; however, contradictory results have also been reported [18,19]. Further, CA125 has its limitations as a prognostic biomarker as small volumes of persistent disease might be present in up to 50% of patients regardless of the normalization of serum CA125, and the elevation of serum CA125 does not necessarily correlate with disease recurrence [12]. It is also of note, that CA125 is elevated in various benign conditions not related to HGSC [20–22].

CA125 is a mucin-type glycoprotein. The glycan structures on the surface of CA125 are heavily influenced by the oncogenic transformation of the cell [23,24]. Glycoforms of validated biomarkers have shown potential as prognostic markers during treatment and as sensitive markers in the detection of disease recurrence in other cancers [25,26]. We recently reported promising diagnostic potential of two CA125 glycoforms, recombinant human macrophage galactose-type lectin (CA125-MGL) and Sialyl-Thomsen-nouveau (CA125-STn), due to their high EOC specificity [27]. In the current longitudinal analysis, we present the prognostic potential of the glycoforms and the ability of the assays to detect disease relapse.

2. Material and Methods

1. Study Population

Patients with suspected ovarian malignancies were recruited at the Department of Obstetrics and Gynecology at the Turku University Hospital, Finland, from 2009 to 2017. This prospective study was designed to evaluate the value of serum biomarkers in EOC monitoring and was approved by the Ethics Committee in the Hospital District of Southwest Finland (ETMK 53/180/2009). Patients diagnosed with HGSC and with at least three longitudinal serum samples during treatment were included (n = 122). The histopathological diagnoses were confirmed by a pathologist specialized in gynecologic pathology and the disease stage was determined according to the FIGO2014 guidelines.

The patients were treated with either primary debulking surgery (PDS, n = 55) or neoadjuvant chemotherapy (NACT, n = 67) followed by interval debulking surgery (IDS) by a team of experienced gynecologic oncologists (Table 1). The operating team systematically assessed and documented the disease spread and tumor volume in the peritoneal cavity and retroperitoneum with a standardized 16-part questionnaire during surgery. Each abdominal site and possible metastasis was included, and a recently validated disease dissemination score ranging from 0 to 21 was calculated (Table 2) [28]. We divided patients to a low tumor load group (score 0-11) and a high tumor load group (score 12-21) based on the disease dissemination score. The operating team also assessed the amount of residual tumor (Table 1). First line chemotherapy included carboplatin combined with paclitaxel. However, 15 patients received single-agent carboplatin because of frailty or a weakened general state of health. For one patient, gemcitabine was administered instead of a taxane as a result of severe allergic reactions to both paclitaxel and docetaxel. Bevacizumab was included in the first line therapy for 31 patients and continued as

maintenance therapy for 15 months or until progression. The treatment response was evaluated after primary therapy and was based on a clinical examination, a CT scan and the level of serum CA125 in accordance with the standard response evaluation criteria [13]. Disease recurrence was defined by radiologic and/or serologic criteria [13].

2. Sample collection and biomarker analyses

Serum samples were collected longitudinally from the time of diagnosis (baseline) until possible first recurrence. The baseline serum sample was drawn before debulking surgery and any oncological treatments. During first line treatment, samples were collected before each cycle of chemotherapy. Follow up samples were collected after initial treatment during control visits to the outpatient clinic approximately every 3-6 months.

The samples were collected into vacuum tubes with gel and clot activator. Samples were incubated at room temperature and centrifuged. Serum was aliquoted and stored in -70 °C. The CA125 values (U/ml) were determined with the ECLIA method (Modular E170 automatic analyzer, Roche Diagnostic GmbH, Mannheim, Germany). For some samples, the CA125 level was not originally determined. In these cases, the level of serum CA125 was manually determined with the EIA method (Fujirebio Diagnostics Inc., Malvern, PA, USA). These two methods have shown good correlation in previous studies [29].

The serum CA125 glycoform measurement with in-house time resolved fluorometry (TRF) CA125-MGL and CA125-STn immunoassays were performed in an identical manner to as described before [30,31]. In short, biotinylated capture Ov185 monoclonal antibody or Ov185 F(ab')2 (50 ng/30 µl /well) were immobilized to streptavidin-coated low-fluorescence microtiter wells (Kaivogen Oy, Turku, Finland) in the assay buffer. After washing, 25 µl of standard (OVCAR-3 cell line purified CA125) or diluted serum sample was added in triplicates and incubated. Samples were diluted in buffer solution. After washing, the captured CA125 antigen was incubated with the Eu+3-chelate-doped Fluoro-MaxTM polystyrene nanoparticles (NPs) (Seradyn Inc., Indianapolis, IN) conjugated with human lectin-MGL (1×10^7 /25 µl /well) and STn-mAb STn-NPs-conjugates (5×10^6/25 µl /well). After incubation, the wells were washed with wash buffer. The time-resolved fluorescence for Eu+3 was then measured from dry wells using VictorTM 1420 Multilabel counter.

3. Statistical analyses

The statistical analyses were performed with R (Version 3.3.3.) and IBM SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY: IBM Corp). The medians, 25th and 75th quartiles of serum biomarker values were calculated for each time point regardless of treatment regimen (NACT + PDS) and also for the NACT and PDS groups separately. We evaluated the normality of the biomarker value distributions visually and with the Shapiro-Wilks test. A logarithmic transformation was made to correct for the skewness of the data. Baseline and postoperative biomarker values were compared to different end points with the one-way ANOVA test. A nadir value was calculated for each biomarker and it was defined as the lowest serum biomarker value during treatment or within 3 months after treatment. Optimal nadir cut off values were determined from receiver operating characteristic (ROC) curves with the Youden index method separately for each assay. PFS was dichotomized for the ROC curves as progression vs no progression, and the median follow up time in the progress free group was 19,6 months. We evaluated the correlations of the nadir levels to PFS with Kaplan-Meier survival curves and statistical correlations were evaluated with the log rank test and the Cox's proportional hazards model. A subset of clinically relevant risk factors was included in the multivariate analysis in a stepwise selection procedure. We evaluated the ability of the biomarkers to detect recurrent disease from four longitudinal serum samples; a sample drawn at the time of relapse and three samples drawn at separate follow up visits preceding the detection of relapse. P < 0,05 was considered significant in all statistical analyses.

3. Results

1. The serum CA125-STn level at diagnosis is a useful indicator of tumor load and predicts the cytoreduction result in debulking surgery

The number of patients treated with PDS and NACT were comparable in the cohort (45,5% and 54,5%, Table 1) and the profiles of the biomarkers were similar during treatment (Supplemental Tables S1 and S2). The serum biomarker levels decreased most rapidly during the three initial cycles of chemotherapy regardless of the treatment strategy (PDS or NACT). At the end of primary therapy, the levels of conventionally measured CA125 and its glycoforms were lower in patients treated with PDS than NACT; however, the differences were not statistically significant. Thus, the PDS and NACT groups were combined for further analyses.

We detected a significant difference in the serum levels of CA125-MGL (p=0,026) and CA125-STn (p=0,030) at diagnosis in patients with low tumor load and high tumor load (Table 3).

Contrarily, the serum levels of conventional CA125 were indifferent to the disease dissemination score (p=0,363). Further, the serum CA125-MGL (p=0,022) and CA125-STn (p=0,025) levels at diagnosis were significantly lower in patients with subsequent complete cytoreduction (R0) than in patients with suboptimal cytoreduction (macroscopic residual disease >0mm) in debulking surgery (Table 3). In contrast, the conventional CA125 assay did not detect differences in the baseline samples between patients with subsequent complete and suboptimal cytoreduction (p = 0,154). We detected no significant correlations between baseline biomarker levels and treatment response, PFS or overall survival (OS).

2. CA125-STn nadir level predicts progression free survival

The follow up time of patients in this study ranged from 2,3 to 118,6 months, with a median of 21,5 months. Disease recurrence was detected in 67 (54,9%) patients during the follow up period. PFS ranged from 1,1 to 46,0 months, with a median of 12,8 months.

The median nadir values for the conventional CA125, CA125-MGL and CA125-STn assays were 12,00 U/ml (interquartile range: 19,50), 0,60 U/ml (IQR: 1,30) and 2,40 U/ml (IQR: 11,50), respectively. The optimal nadir cut off values for each biomarker were 33,00 U/ml, 0,60 U/ml and 0,80 U/ml for the conventional CA125, CA125-MGL and CA125-STn assays, respectively. In univariate analysis, only the serum CA125-STn nadir of < 0,80 U/ml was identified as a predictor of PFS (p = 0,020) (Table 4, Figure 1). In multivariate analysis, the CA125-STn nadir value < 0,80 U/ml showed a significant association with PFS (p = 0,040) (Table 4), while other factors, such as age, disease stage or residual tumor were not significantly associated with PFS. The biomarker nadir values did not predict overall survival in our cohort.

3. The CA125 glycoform assays improve the detection of recurrent HGSC

Preceding the detection of relapse, the serum CA125-STn levels increased earlier than the conventional CA125 levels in 37,0% (13/35) of patients (Figure 2A). Further, the fold increase of the serum CA125-STn levels was stronger than in the conventional serum CA125 levels in the majority of patients (80,0%, 28/35, Figure 2B) suggesting a generally clearer evidence of progression. Although the CA125-MGL assay did not show early increase compared to the conventional CA125 assay, it also performed with higher fold increase in 11,0% (4/35) of patients. The glycoform assays detected disease relapse with similar sensitivity to that of the conventional assay in 20,0% (7/35) of patients (Figure 2C). The results suggest that the glycoform

assays are useful indicators of disease relapse, as there were no patients in which the conventional CA125 assay outdid either of the glycoform assays.

4. Discussion

In this study, we demonstrated for the first time that the CA125 glycoform, CA125-STn, is a prognostic biomarker in patients with HGSC and outperforms the conventional CA125 assay as an indicator of tumor load. A high serum CA125-STn level at diagnosis was associated with larger tumor volume and residual disease after cytoreduction. Further, the CA125-STn nadir predicted PFS and the CA125 glycoform assays detected disease relapse more sensitively than the conventional CA125 assay. We recently observed, that the CA125-MGL assay showed potential in the differential diagnostics of epithelial ovarian cancer [27]. However, the CA125-STn assay appeared superior in treatment monitoring and follow up of HGSC based on the current study.

The serum level of conventionally measured CA125 at diagnosis did not predict the following cytoreduction result in our cohort. This result is in line with previous studies on preoperative CA125 levels [16,18,19]. Although it has been demonstrated that a preoperative serum CA125 value of > 500 U/ml is a risk factor of suboptimal cytoreduction, there have not been evidence that the preoperative CA125 value adds accuracy to the estimation of operability performed with a CT scan [17]. Thus, the preoperative serum level of conventionally measured CA125 is not a feasible tool for clinical decision making. In the current study, the baseline level of CA125-STn predicted optimal cytoreduction and showed good association with tumor load assessed during surgery, indicating a beneficial correlation to disease burden. However, a definite cut off value was not determined. Our next study will focus on operability assessment in more extensive cohorts.

The nadir value of conventionally measured CA125 has been reported to be an independent prognostic factor of PFS [14,32,33]. This was not seen in our cohort; however, it might be due to differences in cohorts and/or treatment regimens (e.g. chemotherapy and maintenance therapy). However, a CA125-STn nadir level of > 0,80 U/ml performed as an independent predictor of disease recurrence in our cohort. This finding is of clinical interest as patients with suboptimal response are identified better and can be directed to individual treatment strategies.

In large randomized clinical trials, CA125 has been reported to be both supportive to [34] and in poor concordance with [35] radiologic response evaluation. The studies advising against routine measurement of CA125 in HGSC follow up have been made in an era before novel targeted therapies

[36]. Today, due to new therapy options, clinical trials of patients with an asymptomatic CA125 progression are a field of interest. Well-designed trials of targeted therapies or immune checkpoint blockade in patients with a low tumor burden are needed [37]. Biomarkers detecting early relapse are of critical importance in the selection of patients benefiting from these new therapies. Our results indicate, that the CA125-STn assay might be a helpful tool in patient selection.

The strengths of this study are the prospective study setting, standardized therapy regimen and evaluation criteria. In addition, the sample analyses were performed by the same investigator in an identical manner. A limitation of the study is that some patients lacked serum samples at one or several time points. However, we did not detect any discrepancies in the behavior of the biomarkers in the longitudinal sample analyses. Another limitation is that the residual tumor volume was evaluated exclusively by the surgeon and not confirmed by imaging studies.

In conclusion, the CA125-STn assay showed promise as a novel prognostic biomarker in HGSC. Specifically, it could be useful in the selection of patients to drug trials focusing on patients with an early, asymptomatic recurrence. Importantly, the assay is robust and inexpensive. A commercial CA125-STn assay made on the present research method would not differ from an ordinary sandwich-based biomarker assay (e.g. the conventional CA125 assay). The CA125-MGL assay did not offer further improvement to the conventional or the CA125-STn assay. Our findings need confirmation in studies with larger and more heterogeneous cohorts.

Conflict of interest statement

Kim Pettersson and Kamlesh Gidwani have a pending patent application for the CA125-MGL assay. Other authors do not have any conflicts of interest to disclose.

Author contribution section

Liina Salminen: Conceptualization, Formal analysis, Writing - Original Draft. **Nimrah Nadeem:** Investigation. **Shruti Jain:** Investigation. **Seija Grènman:** Funding acquisition, Writing - Review & Editing. **Olli Carpén:** Resources. **Sakari Hietanen:** Investigation. **Sinikka Oksa:** Investigation **Urpo Lamminmäki:** Methodology. **Kim Pettersson:** Methodology, Resources, Writing - Review & Editing. **Kamlesh Gidwani:** Methodology, Writing - Review & Editing. **Kaisa Huhtinen:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Johanna Hynninen:** Conceptualization, Methodology, Writing - Review & Editing, Project administration

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Legends:

Table 1. Patient characteristics.

Table 2. Disease dissemination score, values range from 0 to 21. The operating team assessed the metastatic status of the peritoneal cavity and retroperitoneum during surgery.

Table 3. Serum biomarker levels at baseline (median, 25th and 75th quartiles) and their correlation to the dissemination score and residual disease at cytoreductive surgery (p-values).

Table 4. Adjusted hazard ratios (HR) with 95% confidence intervals (CIs) of PFS by different clinical variables of HGSC patients using the log rank test and the Cox's proportional hazards model.

Figure 1. Kaplan-Meier curves of progression free survival of patients with serum biomarker nadir values below and exceeding the optimal nadir cut offs. The CA125-STn nadir value < 0,80 U/ml was significantly correlated with PFS (log rank test, p=0,020)

Figure 2. Relative serum biomarker values during early HGSC progression after response to primary therapy (time in months, x-axis). A.) Patients with earlier CA125-STn elevation compared to the conventional CA125 assay. B.) Patients with higher in fold elevation of CA125-STn compared to the conventional CA125 assay. C.) Patients with similar progression profiles regardless of assay.

Tables

Table 1. Patient characteristics.

Characteristic	**N / median**	**% / range**
Age (years)	66,0	38,0 – 82,0
Treatment		
PDS	56	45,5 %
NACT	67	54,5 %
FIGO stage		
I	2	1,6%
II	3	2,5%
III	77	63,1%
IV	40	32,8%
Chemotherapy		
Carboplatin + paclitaxel	105	86,1%
Single-agent carboplatin	15	12,3%
Other chemotherapy	1	0,8%
Unknown	1	0,8%
Residual disease		
0mm	37	30,3%
1mm – 10mm	48	38,3%
> 10mm	37	30,3%
Treatment response		
Complete	66	54,1%
Partial	31	25,4%
Progressive	22	18,0%
Unknown	3	2,5%

Table 2. Disease dissemination score, values range from 0 to 21. The operating team assessed the metastatic status of the peritoneal cavity and retroperitoneum during surgery.

	Points			
Anatomic location	**0**	**1**	**2**	**3**
Pelvic carcinomatosis	No	Yes		
Subdiaphragmatic surface carcinomatosis	No	Yes		
Carcinomatosis around the peritoneal cavity	No	Yes		
Small bowel mesentery carcinomatosis	No	Yes		
Small bowel mesentery retraction	No	Yes		
Large bowel mesentery carcinomatosis	No	Yes		
Small bowel serosa carcinomatosis	No	Yes		
Large bowel serosa carcinomatosis	No	Yes		
Invasion to bowel mucosa	No	Yes		
Largest omental nodule, cm	-	< 2	2 - 5	> 5
Largest right ovary nodule, cm	-	< 10	≥ 10	
Largest left ovary nodule, cm	-	< 10	≥ 10	
Pelvic lymph node metastasis, suspected	No	Yes		
Para-aortic lymph node metastasis, suspected	No	Yes		
Spleen metastasis	No	Yes		
Invasion to abdominal wall	No	Yes		
Invasion to liver surface	No	Yes		

Table 3. Serum biomarker levels at baseline (median, 25th and 75th quartiles) and their correlation to the dissemination score and residual disease at cytoreductive surgery (p-values).

	CA125 (U/ml)	**CA125-MGL (U/ml)**	**CA125-STn (U/ml)**
Low tumor load	840,0 (440,0 – 2070,0)	69,4 (26,7 – 141,3)	243,9 (62,1 – 1048,6)
High tumor load	1024,5 (521,3 – 1846,8)	137,1 (51,6 – 211,3)	748,4 (258,6 – 1493,0)
P - value	**0,363**	**0,026***	**0,030***
Residual disease, 0mm	839,5 (362,0 – 1592,0)	54,6 (16,5 – 139,4)	219,7 (21,7 – 938,0)
Residual disease, >0mm	918,0 (462,0 – 2323,8)	102,4 (33,8 – 206,9)	515,9 (170,8 – 1479,5)
P - value	**0,154**	**0,022***	**0,025***

Table 4. Adjusted hazard ratios (HR) with 95% confidence intervals (CIs) of PFS by different clinical variables of HGSC patients using the log rank test and the Cox's proportional hazards model.

Clinical variable	**Univariate p value**	**Multivariate p value**	**Multivariate HR**	**(95% CI)**
Elderly (age>65)	0,20	0,23	1,36	0,82 – 2,27
Disease stage (FIGO 2014):				
III vs IV	0,38	0,16	1,44	0,86 – 2,43
Residual tumor:				
1 – 10mm	0,80	0,44	0,78	0,41 – 1,48
>10mm	0,35	0,99	1,01	0,48 – 2,10
Conventional CA125 nadir > 33,00 U/ml	0,15	0,47	1,28	0,66 – 2,49
CA125-MGL nadir > 0,60 U/ml	0,13	0,79	1,09	0,59 – 2,02
CA125-STn nadir > 0,80 U/ml	0,02*	0,04*	2,68	1,05 – 7,14