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Evaluation of ovarian cancer remission markers HE4, MMP7 and Mesothelin by comparison to the established marker CA125

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INTRODUCTION

While over 80% of advanced stage ovarian epithelial cancer patients attain clinical remission with standard platinum/paclitaxel-based chemotherapy [1], the vast majority of them will relapse within two to five years [2,3]. It has become standard clinical practice to include CA125 testing in patient surveillance. Elevation in CA125 often precedes clinical evidence of relapse by imaging or physical exam [4-8]. Data from a large randomized clinical trial, however, demonstrate no survival advantage from CA125 screening [9]. These results are controversial, in part because the trial did not control for treatment after recurrence [10] or the role of more active drugs and regimens [11]. Recently, it has been shown that each week delay of intervention after first CA125 elevation correlated with a 3% increased chance of suboptimal resection at secondary cytoreductive surgery (SCS) [12]. HE4 has been shown to be effective for ovarian cancer detection [13,14] and received approval by the Food and Drug Administration as a recurrence monitoring marker. Limited information suggests that rising HE4 could detect a recurrence earlier than CA125 [14-16]. Mesothelin and MMP7 have been suggested as a marker for ovarian cancer diagnosis [17,18] or remission monitoring [14]. The goal of this study is to evaluate if any of the recently identified ovarian cancer tumor markers HE4, MMP7 and Mesothelin complements CA125 in monitoring disease status.

METHODS

Patients and serum specimens

Twenty-three patients were recruited from the offices of Pacific Gynecology Specialists (PGS), a private practice consisting of 6 gynecologic oncologist located on the Swedish

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Medical Center campus. Eligible patients included all women with ovarian and fallopian cancer who received both primary surgery and chemotherapy at Swedish Medical Center under the direction of PGS physicians during the years 2001-2008. Patients were approached by their treating physician or research nurse at their initial clinical visit. All but one patient entered clinical remission following surgery and chemotherapy. Clinical follow-up information (treatment regimen, response to treatment, clinical CA125 values, physical exam results, imaging results, date and type of recurrence, and date of death) as well as personal and family history were available through medical chart abstraction and a questionnaire completed at enrollment. Sixteen of the 23 women participated in the POCRC Surgical Donation Protocol at the time of their initial surgery, and they donated blood to the repository just prior to their surgery. These pre-treatment samples were available for marker measurement. At the time of a blood draw, each patient was required to have hemoglobin levels above 9 mg/dl, and platelet counts above 100,000 and no severe anemia or thrombocytopenia per her most recent clinical laboratory results. In some cases, this requirement reduced the number of blood draws. Characteristics of the 23 patients are listed in Table 1 and a timeline deputizing each patient's marker levels and clinical data is shown in Table S1. A total of 182 serum samples (mean samples per patient: 8, range: 3-24) were tested for each of the markers. Blood was processed and placed at −80°C within 4 hours of the collection time.

Evaluation of protein marker levels in serum samples

CA125 was measured using an in-house clinical assay based on Bio-Plex technology running on a Luminex platform using antibodies from Research Diagnostics (RDI-TRK4C29-X306 for capture and RDI-TRK4C29-X52 for detection) [19]. The assay is certified for clinical use in the State of Washington through the Clinical Laboratory Improvement Amendments (CLIA). Its performance is comparable to that of other clinical assays [19-21]. HE4 was measured on the Abbott ARCHITECT i1000SR platform using previously described antibodies [13]. Mesothelin was measured using a bead-based Luminex assay developed in-house with previously described antibodies (4H3 for capture and OV569 for detection) [22-25]. MMP7 was measured using a bead-based Luminex assay (R&D Systems; Cat. # LMP907). The mean fluorescence units measured in the Mesothelin and MMP7 assays were normalized by the averaged expression in four replicates of a normal human serum sample, resulting in relative fluorescence units (RFU). The sample consisted of a pool of 73 sera from high-risk participants without cancer [18,26].

Definition of marker elevation

Marker elevation was defined by two methods that are commonly used for disease monitoring with CA125: (1) Marker rises above a standard population threshold. (2) Marker rises above 2 x the lowest value (nadir) measured during the remission period. The definitions of disease progression by both methods are based on GCIG recommendations for disease monitoring using CA125 [27]. Due to the relative infrequency of blood draws, conditions were relaxed to require a single measurement above threshold to count as a positive marker increase.

Definition of response to chemotherapy

For all four markers, response to chemotherapy was measured as defined by the Gynecologic Cancer Intergroup (GCIG) for CA125: a 50% reduction in marker levels confirmed and maintained for at least 28 days [27].

RESULTS

In this study, marker behavior during remission and before recurrence was evaluated independently from whether the marker had dropped with surgery. A marker was required to have remission levels below threshold before a pre-recurrence rise was considered valid. All results are summarized in Supplemental Table S2 and charted in Supplemental Figure S1.

Determination of marker threshold

Relevant thresholds for using these markers for remission monitoring have not been established. There is an established threshold range for various commercial CA125 assays [27], however, no threshold has been determined yet for the CA125 assay used in this study. Thresholds for HE4 have been reported only recently but remain uncertain [28-31]. Given that HE4 serum values increase with age [32], separate HE4 thresholds were established for women below 50 years of age and women equal or above. The thresholds for all four markers were determined as the 95th percentile of expression in healthy controls. Control sera were obtained from 646 participants enrolled in an ongoing POCRC trial who were recruited from the general population and thus assumed to be healthy. Further sera were obtained from 122 POCRC participants who were either perceived healthy (73) or had benign ovarian and non-ovarian disease (49). The thresholds were established as follows (number of women in parentheses): CA125: 38 U/ml (637), HE4: premenopausal: 44 pmol/l (241), postmenopausal: 62 pmol/l (289), MMP7: 24 RFU/50 μl (635), Mesothelin: 20 RFU/ 50 μl (122).

Marker behavior

Of the 23 patients in the study, one was resistant to treatment (Table 1, #14). The remaining 22 patients achieved remission determined by normalization of CA125 levels, physical examination and/or imaging by computerized tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI) or ultrasound. Of those, 20 patients developed recurring disease with a median progression-free interval (PFI) of 18 months (range: 9-48 months). Two patients stayed in remission (Table 1, #3 and #7) and had all four markers below their threshold values during the remission period.

In the patient with persistent disease following chemotherapy and the presence of tumor confirmed by imaging results, CA125, HE4 and MMP7 dropped with surgery but remained above their respective thresholds thereafter. In this patient, Mesothelin dropped with initial treatment but remained below threshold, suggesting that it might not be associated with disease burden.

In the 20 cases with recurrence, 18 had a rise in CA125 resulting in physical examination and/or imaging (CT, PET or MRI) and the subsequent detection of a recurring mass. Patient #b had CA125 serum levels that were consistently in the normal range. Her disease progression was instead followed with the serum marker CA27-29. Patient #2's recurrence was detected by physical exam alone (see Table S1). Pre-surgical samples were available for 13 of the 20 cases. CA125 dropped with surgery in 10 of the 13 patients with available pre-surgical blood draw. An example graph is shown in Figure 1 where patient #13 shows a CA125 drop and the other three patients don't. CA125 was below threshold during remission and rose in 12 patients (Table 2). HE4 levels dropped with surgery in all 13 patients. HE4 levels were below threshold during remission and rose before or at recurrence in 10 patients (e.g. patient #13 in Figure 1). In a further four patients, HE4 remission levels oscillated around threshold (patient #8 in Figure 1) or remained above threshold during remission (patient #9 shown in Figure 1 and patients #6 and #15 shown in Figure S1) before elevating further before recurrence (shaded cells in Table 2). Mesothelin levels dropped in

five of the 13 patients. They were below threshold and elevated before recurrence in five patients (two with a drop and three without) but their rise was also detected by CA125 and/or HE4 (#9, #10, #12, #13 and #15, Table 2). Mesothelin levels had a smaller amplitude than the other markers (e.g. patient #9 in Figure 1), a steep pre-recurrence elevation being observed only in patients #8 and #9. Patient #8 saw Mesothelin rise only one month after recurrence (Figure 1). MMP7 levels dropped in four of the 13 patients. In four patients (one with a drop and three without), the marker was below threshold during remission and rose before recurrence. Three of these events were also detected by CA125 and/or HE4 (patients #9, #16 and #17). In patient #4, MMP7 was the only maker to elevate before recurrence. Marker graphs are shown in Figure 1 for four exemplary patients and in Figure S1 for all patients.

Advantage of HE4 over CA125 in the prediction of a clinical recurrence

Table 3 shows that five recurrences were detected by HE4 alone. This includes three patients with HE4 lead times of 4½ months (#13 shown in Figure 1) and around one month (#2 and #11 shown in Figure S1). This also includes two patients with HE4 at or above threshold during the entire remission period (#8 and #9 shown in Figure 1). A further two recurrences were detected earlier by HE4 than by CA125. These two patients also had HE4 levels consistently above threshold during remission. In six patients, the recurrence was detected by both markers at the same time. However in one of these six (#16), imaging was negative at the time of elevation of both markers resulting in the recurrence to be called three months later (Figure S1).

DISCUSSION

Elevation in CA125 expression levels often precedes clinical evidence of relapse by imaging or physical exam in roughly 80% of patients with ovarian cancer [4-8]. Ovarian cancer remission monitoring using CA125 is part of standard care as it is generally accepted that earlier detection of relapse leads to improve outcomes. This assumption has been challenged as a recent, large randomized study demonstrated no survival advantage and reduced quality of life with CA125 monitoring [9] although endpoint [10], altered drugs and regimens [11] or a more contemporary definition of CA125 elevation [35] could have changed this study's outcome. Despite the controversy, CA125 monitoring remains a part of standard clinical practice. The purpose of this study is investigate the ability of HE4, Mesothelin and MMP7 marker levels to complement CA125 in monitoring disease status in patients with ovarian cancer, remission and first recurrence.

Of the three markers in this study, HE4, and to a limited extent MMP7, have been previously investigated for the same purpose. In a study of 80 ovarian cancer patients, Allard *et al*. found HE4 to compare well to CA125 for recurrence detection, including patients where CA125 is not of utility [37]. Havrilesky *et al*. measured HE4 and MMP7 as part of a marker panel and found increased lead time of 1-15 months over CA125 [14]. In a study of 31 ovarian cancer patients, Anastasi *et al*. found HE4 to rise 5-8 months before CA125 [15]. Mesothelin has been previously associated with ovarian cancer [17,18,38] but has not been tested for disease monitoring.

In this study, a Mesothelin elevation before recurrence was seen in in four patients but this elevation was also detected by CA125 and/or HE4. This and the relatively low amplitude of expression makes Mesothelin less suited as a marker for ovarian cancer monitoring.

MMP7 displayed pre-recurrence elevation in one patient with a lead time of three weeks. This recurrence was neither preceded by CA125 nor HE4. There was also an association of elevated MMP7 at first clinical recurrence with shorter survival. Although patient numbers

were too small for statistical significance, the behavior of MMP7 in ovarian cancer monitoring warrants evaluation in a larger patient population.

HE4 displayed several advantages over CA125 for predicting a recurrence. Confirming similar results cited above, we observed greater lead time than for CA125 (Table 2) and in five cases HE4 was the only marker which was elevated before recurrence (Table 3). In addition, HE4 proved to be valuable even in cases where CA125 was elevating at the same time. Patient #16, for example, showed simultaneous elevation of CA125 and HE4. However, at the time of marker elevation the patient's CT results were negative and clinical recurrence was therefore called only three months later at a positive imaging result. Results from this patient suggest HE4 may be more sensitive than imaging for confirming elevated CA125 elevations in some patients. Compared with imaging additional benefits for measuring HE4 levels as a confirmatory test include low cost, convenience and lack of ionizing radiation. Another patient's recurrence was detected not by rising CA125 but by physical exam. Her CA125 level rose only after the physician called the recurrence. However, HE4 was already elevated at that time. An unexpected observation in patient #3 was a steady rise of HE4 to and above threshold in the last year of the study period during which both, imaging results and CA125 remained negative. This could indicate tumor recurrence and will need to be confirmed or ruled out by future medical data abstraction.

A very different behavior of HE4 was seen in four patients where marker levels during remission were consistently at or above threshold (Patients #8 and #9 in Figure 1, Patients #6, 8, 9 and 15 in Figure S1) and further rose before recurrence. This finding was unexpected. HE4 serum levels are related to progression of disease stage [39] and hence to tumor burden. A failure of HE4 levels to normalize at the completion of primary therapy could be related to persistent disease not detected by CA125 nor by physical exam or CT imaging. These patients may represent a high risk group who could potentially benefit from additional treatment or more intensive monitoring. Confirmation of this HE4 behavior in a larger number of patients is therefore warranted.

These and previous findings demonstrate the usefulness of HE4 in supporting or surpassing CA215 as a remission monitoring marker and speak in favor of adding HE4 alongside CA125 in settings where biochemical monitoring of ovarian cancer disease status is deemed clinically appropriate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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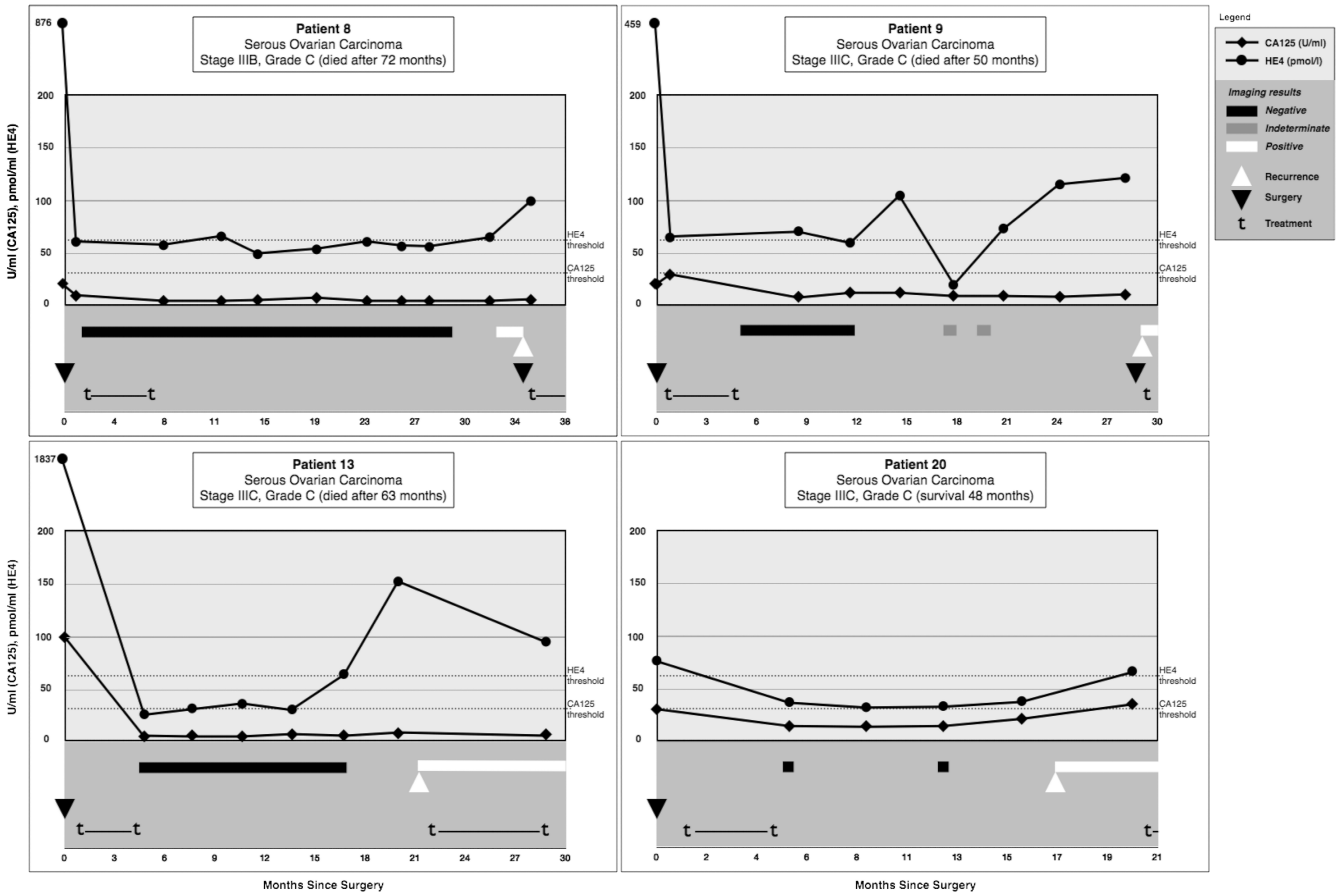

Figure 1.

Graphs from four patients representing the range of CA125 and HE4 marker behavior from surgery to recurrence. The graphs focus on the clinically relevant expression range of 0-200 U/ml of CA125. HE4 expression units were in the same numeric range. Patient #8 had consistently normal CA125 levels. HE4 dropped with surgery, was above threshold during remission and further rose two months before recurrence. During remission, HE4 hovered around threshold. Patient #9 had no rising CA125 before recurrence but HE4 was above threshold during remission, dropped midway and rose eight months before recurrence. Patient #13's recurrence was detected by HE4 with a 4½ month lead time but not by CA125. In Patient #20 neither marker was above 2x threshold before surgery but CA125 and HE4 dropped to 50%, remained below threshold during remission and finally elevated, however three months after recurrence. Figure S1 shows the graphs of all 23 patients.

Table 1

Patient Characteristics.

Age is age at surgery, women below 50 are considered pre-menopausal; Residual disease of 2 cm or less is considered an optimal resection; First clinical recurrence: Imaging includes CT, MRI and PET, PE: physical examination; PFI - Progression-free interval. All patients received 6 cycles of a Taxane/ Platin, patient 14 received 4 cycles; Diagnostic draw indicates that a pre-surgical blood draw was available.

Pt	Age	Histology	Stage	Grade	Other initial treatment	Chemo-therapy Response	Residual Disease	1st clinical Recurrence through	Deceased	PFI (months)	Survival (months)	Total # of Draws	Diagnostic Draw
1	35	Serous Ovarian Cancer	IVA	B	Radiation	Recurrent	< 1 cm	Imaging	•	48	108	6	•
2	66	Serous Ovarian Carcinoma, primary looks endometrioid	IIIC	C	Gemcitabine	Recurrent	none	PE	•	23	84	9	•
3	60	Occult Fallopian Tube Cancer	IIC	C			none				46	24	•
4	63	Serous Ovarian Carcinoma	IIIC	C		Recurrent	≥ 2 cm	Imaging	•	19	40	6	•
5	60	Serous Ovarian Carcinoma	IVA	B		Recurrent	< 1 cm	Imaging		24	91	9	
6	86	Serous Ovarian Carcinoma	IIIC	B		Recurrent	< 1 cm	CA125	•	28	96	13	•
7	51	Serous Ovarian Carcinoma	IIIC	C	CTI 2103		< 1 cm				83	15	•
8	76	Serous Ovarian Carcinoma	IIIB	C	Gemcitabine	Recurrent	none	Imaging	•	34	72	11	•
9	70	Serous Ovarian Carcinoma	IIIC	B		Recurrent	< 1 cm	Imaging	•	29	50	10	•
10	48	Serous Ovarian Carcinoma	IIIC	C		Recurrent	1-2 cm	Imaging	•	16	44	7	•
11	51	Serous Ovarian Carcinoma	IIIC	C		Recurrent	< 1 cm	Imaging	•	9	11	4	•
12	60	Serous Ovarian Carcinoma	IVA	C		Recurrent	< 1 cm	Imaging	•	11	25	4	•
13	63	Serous Ovarian Carcinoma	IIIC	C		Recurrent	< 1 cm	Imaging	•	21	63	11	•
14	61	Serous Ovarian Carcinoma	IIIC	C		Progressive	< 1 cm				64	8	•
15	70	Serous Ovarian Carcinoma	IIIA	C		Recurrent	< 1 cm	Imaging	•	9	16	4	•
16	52	Serous Ovarian Carcinoma	IIIC	C		Recurrent	< 1 cm	Imaging	•	17	28	4	
17	56	Ovarian Cancer	IIIC	C		Recurrent	none	Imaging		39	68	9	
18	37	Serous Ovarian Carcinoma	IIIC	A		Recurrent	none	Imaging		18	66	5	
19	41	Serous Ovarian Carcinoma	IIIC	C		Recurrent	< 1 cm	Imaging	•	14	37	5	•
20	51	Serous Ovarian Carcinoma	IIIC	C		Recurrent	≥ 2 cm	Imaging		17	48	6	•
21	56	Serous Ovarian Carcinoma	IIIC	C	Avastin	Recurrent	< 1 cm	Imaging		17	47	4	
22	57	Serous Ovarian Carcinoma	IIIC	C	Avastin	Recurrent	< 1 cm	Imaging		18	42	3	
23	48	Serous Ovarian Cancer,	IIIC	C	Avastin	Recurrent	< 1 cm	Imaging		17	39	5	

Pt	Age	Histology	Stage	Grade	Other initial treatment	Chemo-therapy Response	Residual Disease	1st clinical Recurrence through	Deceased	PFI (months)	Survival (months)	Total # of Draws	Diagnostic Draw
		complex adnexal mass											

Table 2

Marker elevation prior to recurrence and lead time in days, by patient.
Numbers indicate lead time in days for marker elevation (doubling the remission nadir or above threshold) before first clinical recurrence in 20 patients with complete clinical remission. No values mean no elevation prior to recurrence. Shaded cells indicate that HE4 was consistently at or above threshold during remission.

Patient	CA125	HE4	Meso.	MMP7
1	12	12		
2		25		
4				20
5	35			
6	28	572		
8		682		
9		631	631	36
10	2	2	2	
11		44		
12	0	0	0	
13		137	38	
15	0	178	0	
16	95	95		95
17	227	0		0
18	97			
19	10	10		
20				
21	69			
22				
23	84	84		

Table 3

Number of patients with ovarian cancer recurrence first detected by elevation of either HE4 or CA125 or both and percent of recurrences detected by each marker. HE4 detects 14 recurrences and CA125 detects 11. Neither marker elevates in 3 patients.

<table>
<tr><th>HE4 alone</th><th>HE4 earlier than CA125</th><th>Both at same time</th><th>CA125 earlier than HE4</th><th>CA125 alone</th><th>None</th></tr>
<tr><td>5</td><td>2</td><td>6</td><td>1</td><td>3</td><td>3</td></tr>
<tr><td>25%</td><td>10%</td><td>30%</td><td>5%</td><td>15%</td><td rowspan="4">15%</td></tr>
<tr><td></td><td colspan="4">60%</td></tr>
<tr><td colspan="4">70%</td><td></td></tr>
<tr><td colspan="5">85%</td></tr>
</table>