

B7-H4 is over-expressed in early-stage ovarian cancer and is independent of CA125 expression

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Abstract

Objective. This study characterizes the expression of the novel biomarker B7-H4 in ovarian cancer tissue, normal ovaries, and benign ovarian tumors, and evaluates its relationship to CA125.

Methods. Ovarian tissue lysates from 251 patients with ovarian carcinoma were assessed for the levels of B7-H4 and CA125 by ELISA assays. For comparison, ovarian tissues from patients with benign ovarian tumors ($n=43$) and patients with normal ovaries ($n=32$) were tested. The marker concentrations were correlated with CA125 expression, clinicopathological variables, and patient outcome.

Results. Using a cut-off based on the 95th percentile of B7-H4 or CA125 concentration in the control group, B7-H4 was over-expressed in 48% of patients with stage I cancer, 55% of patients with stage II cancer, and 67% of patients with late stage cancer. CA125 was elevated in 31% patients with early stage cancer. B7-H4 was elevated in tumors of 30 patients with early stage cancer that were negative for CA125. The combination of B7-H4 and CA125 identified 56 early stage cancer patients (65%) as positive. Correlation of marker expression to clinical outcome showed that high B7-H4 levels were correlated with poor prognosis. However, the effect was not significant when outcome was adjusted for other clinicopathological variables.

Conclusion. B7-H4 expression was low in normal ovaries and in benign tumors while half of early stage and two-thirds of late stage cancers over-expressed B7-H4. The data are consistent with previous observations and support further investigation of B7-H4 in the detection of early stage ovarian cancer either alone, or in combination with CA125.

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Introduction

Despite advances in treatment, ovarian cancer mortality has been relatively constant for more than 30 years [1]. Most ovarian cancers (70–75%) are detected at late stages, where

patients have a predicted 5-year survival of only 15%. However, patients with ovarian cancer that is detected in stage I have a survival rate of 94% using currently available therapy [2]. Clearly, screening for ovarian cancer, to detect more cancers in early stages is an appealing approach to reduce mortality.

Improvement of early detection of ovarian cancer has been accomplished with transvaginal ultrasound (TVUS), measurement of serum CA125 levels, or a combination of the two methods. Among high-risk women, the sensitivity of transvaginal ultrasound for detecting early-stage disease was only 25% [3] and 31% in combination with CA125 [4]. In addition, TVUS

Abbreviations: AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; HR, hazard ratio; ROC, receiver operating characteristic; TMB, tetramethylbenzidine.

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screening has generally been associated with a high rate of false positive screens, leading to a large number of unnecessary surgical procedures [3–5]. The positive predictive value of CA125 in the most recently published PLCO (prostate, lung, colon, ovarian) trial was 3.7% [6]. At the time of conventional diagnosis, CA125 levels are elevated in only 50–60% of patients with stage I disease. Using longitudinal studies and newly developed algorithms, a rising value of CA125 might trigger detection of early stage ovarian cancer in a higher fraction of cases [7], but in approximately 20% of ovarian cancers, tissue levels of CA125 are low or absent even in late stages [8]. Additionally, the use of CA125 is limited because it is frequently elevated in pre-menopausal women or women with benign diseases such as endometriosis [9]. Greater sensitivity might be achieved by using multiple markers, provided that the specificity is not compromised. Therefore, novel biomarkers should not only complement CA125 but also demonstrate good tissue and cancer specificity.

Through genomic efforts, we have identified DD-O110 (known as B7-H4) as a novel biomarker that is over-expressed in ovarian cancer tissues when compared with normal tissues [10]. Immunohistochemical studies showed membranous staining in serous ovarian and breast cancer, confirming the tissue specificity and cell surface localization predicted by bioinformatic analysis [11,12]. B7-H4 expression is low in normal ovaries and other normal tissues [10,11]. B7-H4 is shed into serum and is elevated in serum samples from ovarian cancer patients, compared to healthy controls or women with benign gynecological diseases [13]. Although the function of B7-H4 in ovarian cancer is not yet known, a recent study has demonstrated that B7-H4 promotes malignant transformation of epithelial cells [10]. The over-expression of this cell surface protein may play a role in evading immune system surveillance as B7-H4 participates in the negative regulation of cell-mediated immunity in peripheral tissues [14–16].

In this study, we analyzed B7-H4 expression in tissue lysates from ovarian cancer, benign tumors of the ovaries, and normal ovaries using a sensitive dual monoclonal antibody ELISA. In particular, a large number of early stage ovarian cancer tissues ($n=86$) were tested with the quantitative ELISA to investigate the significance of B7-H4 over-expression in early stage ovarian cancers seen in other studies [13]. Furthermore, this study evaluates, for the first time, the expression of B7-H4 and CA125 in ovarian tissue from the same patients and demonstrates that these two markers are expressed in an overlapping, but not identical set of patient tumor tissues. This characterization of B7-H4 expression in benign and malignant tissues supports further evaluation of B7-H4 as an important biomarker for ovarian cancer.

Materials and methods

Patients and tissue samples

All tissue samples were collected from patients and donors with appropriate informed consent. The study was carried out in accordance with the ethical standards of the Helsinki Declaration and was approved by the Institute of Obstetrics and Gynecology, University of Turin, Italy. Three hundred twenty-

six patients who underwent surgery at the Department of Gynecologic Oncology, University of Turin, were included in this study. Thirty two patients had normal ovaries and 43 patients had benign tumors of which 12 were diagnosed as serous cystadenomas, 4 as mucinous cystadenomas, 8 as fibroteratomas, 6 as mature teratomas, 3 as endometriosis, 2 as hemorrhagic cysts; 8 patients had benign ovarian tumors that were not further specified. Patients with normal ovaries and benign tumors were grouped together as a control group. Tumors of 251 women with primary ovarian cancer and complete clinical and pathological information documented at the time of surgery were selected. Histological examination, performed during intra-surgery frozen section analysis, allowed representative portions of each tumor, containing at least 80% tumor cells, to be selected for storage until analysis. Clinical and pathological information documented at the time of surgery included stage, grade and histology of tumors, debulking success, and residual tumor. Tumors were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. All early stage (stages I–II) ovarian cancer patients had pelvic and para-aortic lymph node sampling according to the guidelines of the European Organization for Research and Treatment of Cancer-Gynecological Cancer Group (EORTC-GCG).

Monoclonal antibody sandwich ELISA detection for B7-H4

The generation of specific mouse monoclonal antibodies (mAb) against recombinant B7-H4 has been described previously [10], as has the ELISA protocol for B7-H4 [13]. Briefly, 25 μ l of sample was added to high binding polystyrene plates coated with anti-B7-H4 mAb. The antigen was then detected with biotinylated detection mAb followed by horseradish peroxidase-conjugated streptavidin developed with TMB substrate. For calibration, standards of recombinant protein and two controls were run in parallel with the test samples on each plate.

Tissue lysates

Tumor tissues were frozen in liquid nitrogen immediately after surgery and stored at -80°C until extraction. The frozen tissue was pulverized on dry ice to a fine powder and 10 volumes of extraction buffer were added (50 mM Tris pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% NP-4, 1 mM PMSF, 1% aprotinin, and leupeptin). The suspension was incubated on ice and vortexed every 10 min. The mixture was centrifuged at $14,000\times g$ at 4°C and the supernatant (the tissue lysate) was collected and stored at -80°C until analysis. Protein concentration was determined with the bicinchoninic acid method, with albumin as standard (Pierce Chemical Co, Rockford IL).

CA125 measurement

CA125 levels were measured using the Immulite 2000 automated analyzer, according to the manufacturer's protocol (Diagnostic Products Corporation, Los Angeles, CA).

Statistical analysis

The MedCalc® Statistical Software (MedCalc Software, Mariakerke, Belgium), GraphPad Prism (GraphPad Software, San Diego, CA) and JMP Statistical Discovery Software v5.01 (SAS Institute, Cary, NC) were used for statistical analysis.

To determine the association between variables, Spearman correlation coefficients and associated p -values were determined. Generally, for all analyses a p -value <0.05 was considered significant. The 95% specificity was defined as the cut-point for B7-H4 and CA125 and used to categorize patients as B7-H4/CA125 positive or negative. The cut-point was determined as the 95th percentile of values in the control group (normal and benign) and was used to calculate sensitivity (true positive/[true positive+false negative]) of B7-H4 and CA125.

Univariate ROC analyses were performed for CA125 and B7-H4. The resulting area under the ROC curve (AUC) indicates the average sensitivity of a marker over the entire ROC curve. Multivariate ROC analysis combining CA125 and B7-H4 was facilitated by first performing multivariate logistic

regression and using the regression coefficients for the individual terms to generate a composite marker (CM) based on the equation $CM = (CA125 \text{ regression coefficient} \times CA125 \text{ value}) + (B7-H4 \text{ regression coefficient} \times B7-H4 \text{ value})$. Areas under the ROC curve and sensitivity and specificity at defined points of the curve were determined. The AUC for the composite marker was compared to the AUCs for B7-H4 and CA125 individually using the method of Hanley [17].

Follow-up information and complete information on age, stage, grade and histotype were available for 233 patients. For survival analysis, the time until relapse (either local recurrence or distant metastasis) or death was used to calculate the progression-free survival (PFS) time. At discontinuation of follow-up, 88 patients were still alive and were censored in all survival analyses. The prognostic abilities of B7-H4 and CA125 were tested via expression of the markers both as continuous variables and as binary categorical variables. The median value of B7-H4 and CA125 in the subpopulation of patients who did not have an event during the follow-up period was chosen for the binary categorization into “non-risk” and “at risk” groups based on the biomarkers. Kaplan–Meier product-limit survival analyses were performed on the categorically expressed markers, and the log rank test was utilized to examine the significance of the differences of survival distributions between groups [18]. Univariate and multivariate Cox proportional hazards regression models [19] were developed and both the continuous and categorical variables were evaluated. Multivariate Cox models were employed to provide estimates of the effect of clinicopathological factors on the prognostic capabilities of B7-H4 and CA125. For all Cox regressions, the Hazard Ratio (HR), the confidence interval for the HR and the significance of the HR were calculated.

Results

Three hundred twenty-six patients were included in this study and detailed patient information is listed in Table 1. The median age of the control group was 49 years (range 24–78 years) and median age of the cancer patients was 57.5 years (range 19–99 years). Patients were monitored for survival and recurrence for up to 150 months.

The expression levels for both B7-H4 and CA125 span over a wide dynamic range (5 log scales), with the highest expression of both markers found in cancer patients. As shown in Fig. 1, the range of B7-H4 expression levels in normal ovaries and benign ovarian tumors was narrower than levels of CA125 in the same tissues.

To categorize patients as B7-H4 or CA125 positive or negative, the 95th percentile of B7-H4 or CA125 concentration in the control group was determined. For all analyses, expression in normal ovaries and benign ovarian tumors was used to define ‘control’. In clinical settings, cancer more often needs to be distinguished from benign pelvic masses than from normal ovaries; therefore expression in benign tumors was an important parameter of this study.

The 95th percentile of all controls was 426 pg per mg of total protein for B7-H4 and 3301 U/mg for CA125, respectively.

B7-H4 and CA125 expression in ovarian cancer of different histological types

Highest median levels of B7-H4 were observed in patients with undifferentiated ovarian tumors, with 75.8% of cancers showing over-expression. B7-H4 was also over-expressed in the majority of serous, endometrioid and clear cell cancer. Expression in mucinous and non-epithelial cancers was lower (Table 1 and Fig. 2A). In agreement with recent studies [20], the

Table 1
Clinical and pathological characteristics associated with B7-H4 levels

Variable	Total number of patients ^a	B7-H4 negative* (%)	B7-H4 positive* (%)	p-value (χ^2 test)
<i>Stage</i>				
I	66	34 (51.5)	32 (48.4)	0.0076
II	20	9 (45)	11 (55)	
III	146	47 (32.2)	99 (67.8)	
IV	19	5 (26.3)	14 (73.7)	
<i>Grade</i>				
I	37	21 (56.8)	16 (43.2)	0.0053
II	45	18 (40.0)	27 (60.0)	
III	143	38 (26.6)	105 (73.4)	
<i>Histotype</i>				
Serous	109	34 (31.2)	75 (68.8)	0.0002
Endometrioid	44	17 (38.6)	27 (61.4)	
Clear Cell	17	5 (29.4)	12 (70.6)	
Mucinous	19	9 (47.3)	10 (52.6)	
Undifferentiated	33	8 (24.2)	25 (75.8)	
Non-epithelial	29	22 (75.9)	7 (24.1)	
<i>Age</i>				
Up to 45 years	41	18 (43.9)	23 (56.1)	0.45
46–55 years	74	25 (33.8)	49 (66.2)	
Above 56	134	45 (33.6)	89 (66.4)	
<i>Outcome</i>				
No evidence of disease	86	40 (46.5)	46 (53.4)	0.045
Alive with disease	30	8 (27.6)	22 (72.4)	
Dead	117	37 (31.6)	80 (68.4)	

* using the 95th-percentile of normal and benign as cut-off point (426 pg/mg total protein).

^a =total number of patients in this study was 251 patients but some patients had missing grade or outcome information.

highest CA125 expression levels were observed in patients with serous, endometrioid and undifferentiated ovarian cancer (Fig. 2B). Again, using the 95th percentile of the control group as the cut-off, 36%, 32%, and 24% of these cancer histotypes were defined as positive, respectively. CA125 positive expression in clear cell, mucinous and non-epithelial cancer types was 17%, 26%, and 10%, respectively. In the group of women with benign tumors, CA125 was over-expressed in one woman with teratoma, three patients with cystadenoma and one woman with unspecified benign tumor; with only one of those patients having elevated B7-H4 levels. B7-H4 was elevated in one other woman with serous cystadenoma and one woman with hemorrhagic cysts.

B7-H4 expression did not correlate with age of patients but was similar in all age groups (Table 1) indicating that the expression is not influenced by menopausal status.

Correlation of B7-H4 and CA125 expression with stage

The median levels of B7-H4 increased with stage (Fig. 1A) and grade (data not shown). For early stage cancers, 48.5% of patients with stage I and 55% of patients with stage II had B7-H4 values higher than the 95th percentile of normal ovaries and

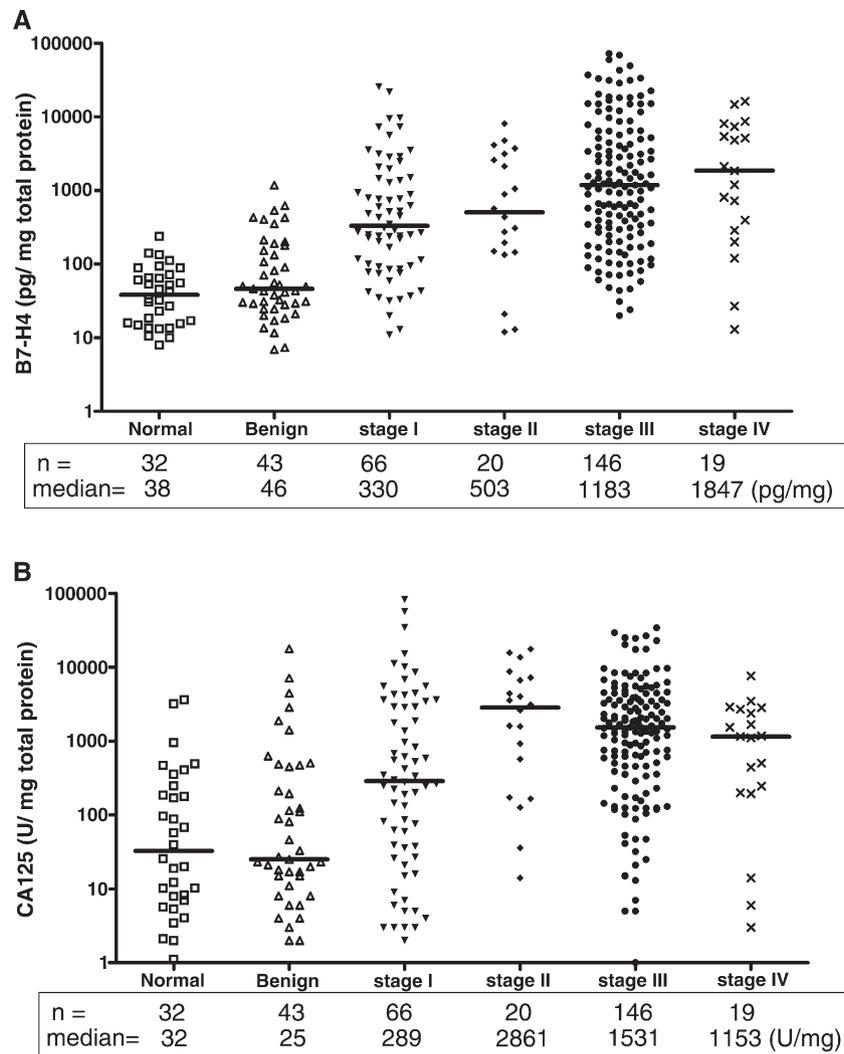


Fig. 1. Expression of B7-H4 (A) and CA125 (B) in ovarian tissue lysates. Concentrations in normal ovaries and benign tumors are compared to ovaries with cancer of different stages. The scatter plot displays the median values as horizontal lines. The actual median values and number of patients (n) are listed.

benign tumors (Table 1). Median level of expression and percentage of positive patients were even higher (67%) in patients with late stage disease (Fig. 1A). The increase of median values for B7-H4 expression in early stage vs. late stage was significant ($p=0.0016$). When all non-epithelial cancers ($n=29$) were removed from the analysis, the percentage of positive cases increased from 50% to 55% in early stage cancer and from 67% to 71% in late stage cancer.

CA125 levels increased with stage as well, with highest median level in stage II cancer patients (Fig. 1B). Since CA125 was highly expressed in two women with normal ovaries and in women with benign tumors, the 95th percentile of controls was higher than the median level of CA125 in the cancer patients. In good agreement with published data on serum levels [8], CA125 was over-expressed in only 25% of tumor tissues from patients with stage I cancer while CA125 expression was high in most patients with late stage cancer (Fig. 1B). Removing all non-epithelial cancers from the analysis slightly increased the median level in all stages but not the percentage of CA125-positive cases.

Correlation between B7-H4 and CA125 expression

Expression of B7-H4 and CA125 in cancer of all stages correlated weakly, even though the correlation was statistically significant (Spearman $r=0.33$, $p<0.001$). When CA125 and B7-H4 expression was considered, 75% of tumors were positive for one or both markers. In the ROC analysis, the area under the curve for the combined marker was 0.91. In early stage cancer (stages I and II), no correlation between the expression of B7-H4 and CA125 was observed (Spearman $r=0.15$, $p=0.18$). CA125 was elevated in tumors from 26 patients (31%) with early stage cancer, while B7-H4 was elevated in 44 (52%) of these tumors. Of these 44 cases, 14 patients also had high CA125 levels and 30 patients were negative for CA125. Using fixed cut-offs for both markers and considering a positive result when either cut-off is exceeded and a negative result only when both values are below the cut-off, the combination of B7-H4 plus CA125 led to 56 positive (65%) and 30 negative results in early stage cancers (Fig. 3). Using a logistic regression approach [17] to calculate the area under the curve, the AUC increased

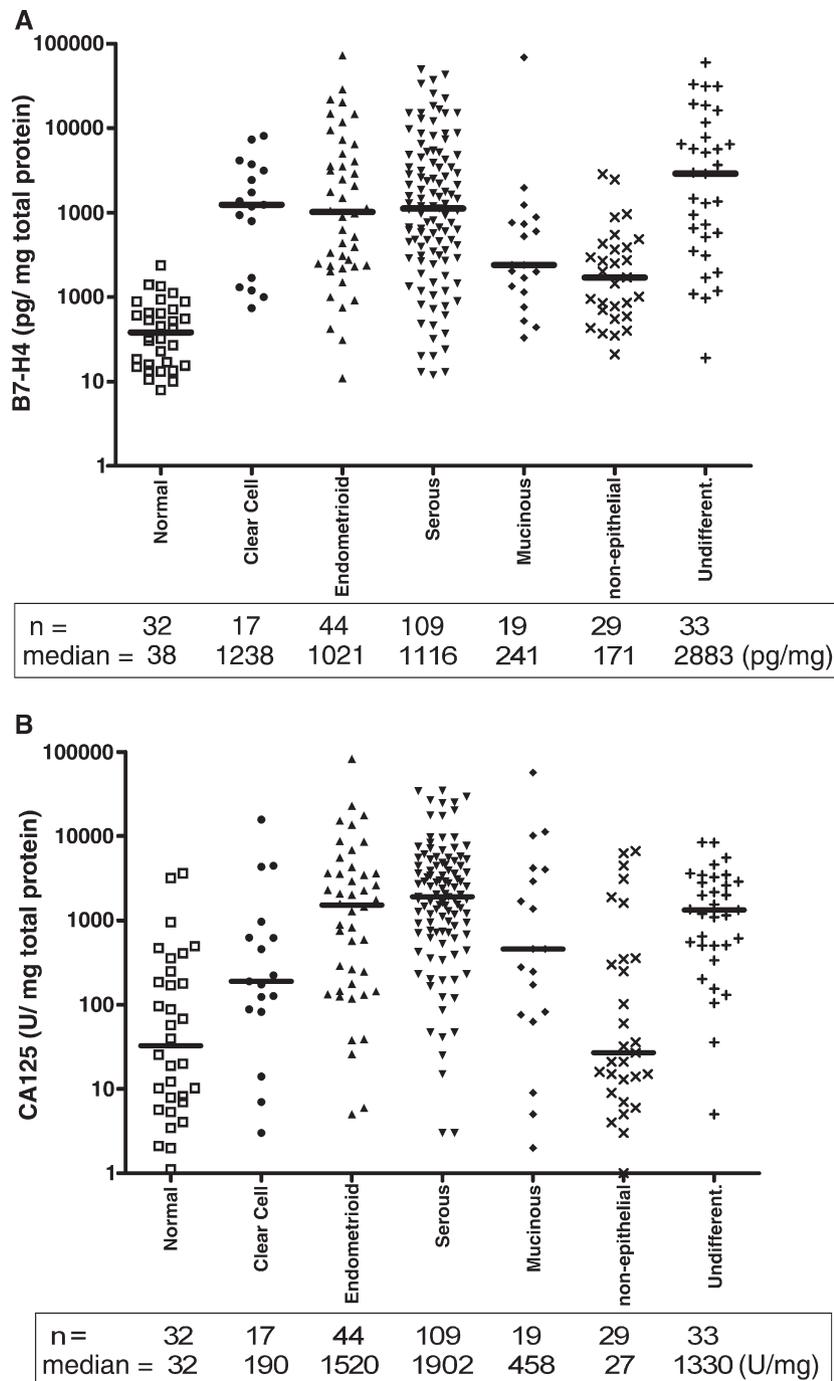


Fig. 2. Expression of B7-H4 (A) and CA125 (B) in normal ovaries and ovarian cancer of different histological types. The results for median values and number of patients (n) are listed.

from 0.83 for B7-H4 and 0.73 for CA125 alone to 0.87 for the composite marker. These results demonstrate that the combination of B7-H4 and CA125 increased the detection of early stage ovarian cancer in tissue lysates over the use of B7-H4 (p -value=0.002) or CA125 alone (p <0.0001).

Detection of patients with mucinous ovarian cancer is particularly difficult because most markers are not elevated in this type of cancer. In our study, B7-H4 was over-expressed in 52.6% of patients with mucinous cancer and two more patients were positive if B7-H4 and CA125 were used in combination

(total 63%), further suggesting that B7-H4 expression may complement CA125 expression. However, at this point we cannot exclude that some of the mucinous cases had mixed histologic differentiation and may include endometrioid or serous components that expressed B7-H4 and/or CA125.

Correlation of B7-H4 level with outcome

Complete clinicopathological and outcome information and marker values for CA125 and B7-H4 were available for 233

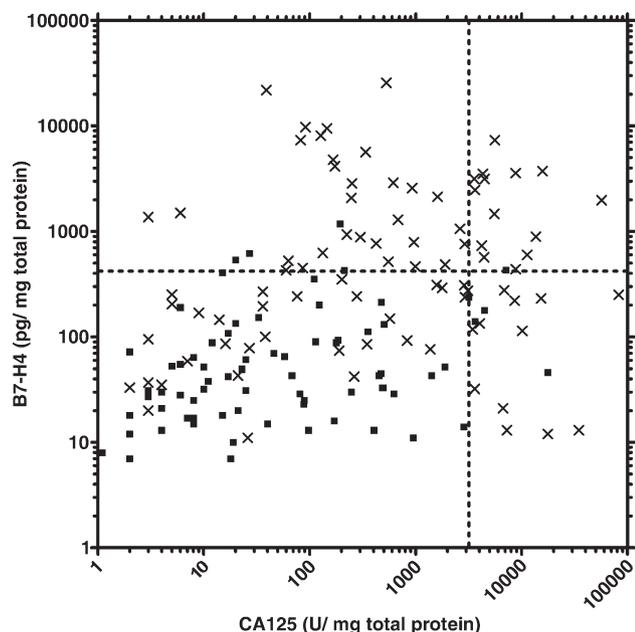


Fig. 3. Composite analysis of B7-H4 (y-axis) and CA125 (x-axis) levels in patients with early stage ovarian cancer (x) and patients with benign tumors or normal ovaries (■). The dotted line indicates the 95th percentile of normal and benign controls for B7-H4 (426 pg/mg total protein) and CA125 (3220 U/mg total protein).

patients who were monitored for survival and recurrence for up to 150 months. One hundred seventeen patients died within this period, 30 survived but suffered recurrence, and 86 showed no evidence of disease after treatment. As shown in Table 1, patients who remained disease-free were more likely to have low expression of B7-H4 at the time of surgery than patients who later suffered recurrence or eventually died.

To further investigate survival, the patient data were used for the Kaplan-Meier analyses (Fig. 4) and univariate and multivariate Cox regression analyses (Table 2). The best B7-H4 cut-point for separating patients with different outcomes in Kaplan-Meier analyses was the median value (500 pg/mg total protein) of B7-H4 in the subpopulation of patients who did not have an event during the follow-up period. However, none of the CA125 cut-points tested showed statistically significant separation between different outcomes. As Fig. 4 shows, the probability of progression-free survival (PFS) was higher in patients with low expression of B7-H4. The Hazard Ratio (HR) from univariate analysis of B7-H4 was significant (HR=1.47 [95% CI: 1.04–2.09], $p=0.028$), while high CA125 expression was not correlated with poor outcome (Table 2). However, staging was the most powerful predictive variable in the uni- and multivariate analysis. The prognostic value of B7-H4 was attenuated and rendered insignificant when staging was included in the multivariate analysis. Since B7-H4 was over-expressed in many early stage cancers, the prognostic value of B7-H4 was assessed in the sub-population of patients with stage I and II cancer and was found not to be significantly associated with outcome in the Cox Regression analysis (data not shown).

Discussion

This study describes, for the first time, quantitative measurement of B7-H4 (DD-O110) expression in normal and benign tumor ovarian tissue and in a wide array of ovarian cancers of various histological types and a large number of early stage ovarian cancers. Previous studies measuring mRNA level [10], using IHC [11] or ELISA of tissue lysate [13] have shown that B7-H4 is absent or present at very low levels in all normal tissues. Here we demonstrate that B7-H4 is expressed in very low levels in normal ovaries and in benign ovarian tumors when compared to ovarian cancer tissues. This study also shows that B7-H4 is over-expressed in 50% of all early stage cancers and that the expression pattern is distinct from the expression pattern of CA125. These data confirm earlier results seen in serum samples, where in multivariate logistic regression analyses, B7-H4 was additive to CA125, especially in the

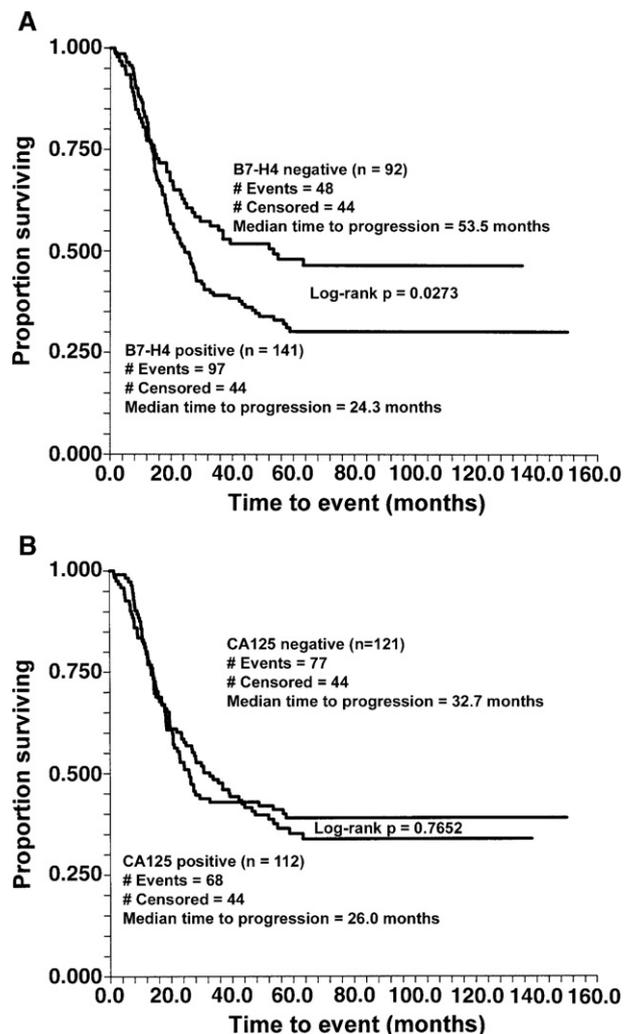


Fig. 4. Kaplan-Meier survival curves for B7-H4 (A) and CA125 (B) in patients with ovarian cancer of all stages. The patients were dichotomized into two groups based on the median value of B7-H4 (500 pg/mg total protein) and CA125 (1565 U/mg total protein) of the subpopulation of patients who did not have an event during the follow-up period.

Table 2
Univariate and multivariate analysis of prognostic value of B7-H4 for progression-free survival (PFS) using Cox regression analysis

Variable	PFS (progression-free survival)		
	HR	95% CI	<i>p</i>
<i>Univariate analysis</i>			
B7-H4 positive*	1.47	1.04–2.09	0.028
B7-H4 (continuous)	1.00	1.00–1.00	0.226
Stage (ordinal)			
II vs. I	1.66	0.62–4.43	0.309
III vs. I	6.77	3.72–12.3	<0.0001
IV vs. I	10.23	4.76–22.0	<0.0001
CA125 positive**	0.95	0.69–1.32	0.765
Age (years)	1.00	0.99–1.02	0.430
<i>Multivariate analysis</i>			
B7-H4 positive*	1.02	0.71–1.46	0.920
Stage (ordinal)			
II vs. I	1.65	0.62–4.43	0.317
III vs. I	6.72	3.67–12.3	<0.0001
IV vs. I	10.1	4.66–22.0	<0.0001
CA125 positive**	0.96	0.69–1.34	0.815
Age (years)	1.00	0.99–1.02	0.553

* Based on cutpoint of 500 pg/mg total protein (median of patients not experiencing event).

** Based on cutpoint of 1565 u/mg total protein (median of patients not experiencing event).

detection of early stage cancer [13]. Although quantitative measurement of B7-H4 expression in tissue lysates is not a preferred test in a clinical setting, data presented in this study are consistent with our previous data, and support the application of B7-H4 as an important serum biomarker for early stage ovarian cancer.

Although CA125 is an important serum marker for ovarian cancer, its use is limited due to its frequent elevation in women with benign diseases, and also because it is elevated in only half of women with early stage ovarian cancer [8]. It is likely that no single cancer biomarker can provide all of the necessary information for optimal cancer diagnosis and management. Therefore, recent efforts focus on the identification of new biomarkers that complement CA125. Since detection of early stage cancer is one of the key unmet clinical needs in ovarian cancer, markers indicative of early genetic changes, or subtle changes related to tumor surveillance or the immune system, will have a greater chance to detect cancer early [21]. The function of B7-H4 in ovarian cancer is not yet clear, but initial reports indicate that the over-expression of B7-H4 in cancer cells may play a role in evading immune system surveillance [22]. Our data showing higher B7-H4 expression in patients with poor outcome could indicate that the expression of B7-H4 facilitates cancer aggressiveness. The inhibition of T-cell response may contribute to an escape from immune attack and would also explain the need for early expression of B7-H4 during cancer progression.

In benign tumors, the immune system surveillance might not be required and therefore B7-H4 is not over-expressed. It is important to consider that the majority of pelvic masses (80%) are benign with cystic, solid, or mixed characteristics with a

favorable prognosis, while only 20% are malignant tumors [23]. Considering that most benign tumors are found in women of fertile age, the enucleation of the mass and preservation of the ovary is the preferred method of treatment. However, accurate diagnosis of the suspicious mass, whether it is malignant or benign, is often difficult. The evaluation of biopsy samples for one or more markers that are highly expressed in aggressive cancer, but are present in only low levels in benign tumors could increase the accuracy of traditional histological assessment, and may assist in determining the most appropriate treatment.

We showed recently that B7-H4 is elevated in serum of ovarian cancer patients but not in healthy women or patients with benign gynecological diseases [13]. Low levels of B7-H4 and CA125 in serum of patients with pelvic masses may indicate that the mass is benign, while the elevation of either one of the markers could suggest a greater likelihood of malignancy and would warrant more thorough diagnostic procedures and faster treatment of the patient.

Recent studies have shown that tissue markers, such as the kallikreins, may be useful as diagnostic and prognostic markers and might play a role in identifying patients with especially aggressive tumors [24–27]. Using IHC staining of tissues, other molecules such as osteopontin, claudin 3, MUC1, mesothelin, hK6 and hK10, prostasin, DF3, vascular endothelial growth factor, HE4, and CA19-9 have been evaluated as potential ovarian cancer markers that complement CA125 expression [20]. All evaluated markers complemented CA125 to some degree, but when expression in normal tissues was considered, MES and HE4 showed the greatest specificity. The concentration of tumor markers detected in tissue lysates does not necessarily reflect the amount of marker that will be detectable in serum. However, the aforementioned study [20] also showed that low or undetectable expression of CA125 in surgical specimens of epithelial ovarian cancers was associated with low levels of serum CA125 in pre-operative serum specimens.

Another study showed that serum mesothelin complements serum CA125, and that a combination of the two markers improved the ROC curve relative to either marker alone [28]. Similarly, studies that combined results of serum CA125 with levels of CA15.3, CA72-4, and macrophage colony-stimulating factor showed that efficiently combining information of marker levels significantly increased preoperative early-stage sensitivity while maintaining specificity [29].

As more biomarkers are discovered and validated, efforts will focus on combining all promising markers into a panel that can increase the sensitivity and specificity of detection of early stage ovarian cancers [30]. The increase in overall diagnostic accuracy conferred by such a panel could eventually allow earlier detection of ovarian cancer, which in turn, would have a significant impact on the survival of patients with this devastating disease.

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