Human Kallikrein 6 (hK6): A New Potential Serum Biomarker for Diagnosis and Prognosis of Ovarian Carcinoma

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Purpose: **The discovery of new ovarian cancer biomarkers that are suitable for early disease diagnosis and prognosis may ultimately lead to improved patient management and outcomes.**

Patients and Methods: **We measured, by immunoassay, human kallikrein 6 (hK6) concentration in serum of 97 apparently healthy women, 141 women with benign abdominal diseases, and 146 women with histologically proven primary ovarian carcinoma. We then calculated the diagnostic sensitivity and specificity of this test and examined the association of serum hK6 concentration with various clinicopathologic variables and patient survival.**

Results: **Serum hK6 concentration between normal and benign disease patients was not different (mean, 2.9 and 3.1 μg/L, respectively). However, hK6 in presurgical serum of ovarian cancer patients was highly elevated (mean, 6.8 μg/L; $P < .001$). Serum hK6 decreased after surgery (to a mean of 3.9 μg/L) in 68% of patients. The diagnostic sensitivity of serum hK6 at 90% and 95% specificity is 52% and 47%, respectively, in the whole patient population. For early stage disease (stage I or II), sensitivity is approximately 21% to 26%. When combined with CA-125, at 90% specificity, sensitivity increases to 72% (for all patients) and to 42% in stage I or II disease. Serum hK6 concentration correlates moderately with CA-125 and is higher in patients with late-stage, higher-grade disease and in patients with serous histotype. Preoperative serum hK6 concentration is a powerful predictor of disease-free and overall survival in both univariate and multivariate analyses.**

Conclusions: **Serum hK6 concentration seems to be a new biomarker for ovarian carcinoma and may have value for disease diagnosis and prognosis.**

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OVARIAN CANCER kills more women in North America than all other gynecological malignancies combined. The American Cancer Society estimates that 23,400 new cases of ovarian cancer will be diagnosed in 2001 and 13,900 deaths will result from the disease.[1] The high fatality-to-case ratio associated with ovarian cancer is partially caused by the lack of a recognizable pattern of symptoms in its early stages; 70% of women with ovarian cancer are diagnosed with advanced stage disease. This disease has a 5-year survival rate of 85% if diagnosed early (stage I or II carcinoma), but survival decreases to less than 20% in women presenting with stage III or IV disease.[2] Clearly, the development of new methods for early ovarian cancer diagnosis will likely contribute to improved patient outcomes.

The only well-validated ovarian cancer tumor marker, CA-125, was discovered about 20 years ago.[3,4] CA-125 has clinical value for disease monitoring, and it is used as an aid for the early detection of relapse and for assessing response to treatment.[5-7] CA-125 also has some prognostic value[8] and can aid in disease diagnosis.[4,9] More recently, the diagnostic value of CA-125 was shown to be improved by combination of markers, including CA-125 plus D-dimer[10] or CA-125 plus OVX1, LASA, CA 15–3, CA 72–4, and prostasin.[11-13] The application of CA-125 for screening asymptomatic individuals has been reported,[11,14-17] but its value is still under investigation.

The sequencing of the human genome has raised hopes that new cancer biomarkers may soon be discovered. By using whole-genome mining approaches, investigators have identified many candidate biomarkers for ovarian cancer diagnosis and prognosis.[13,18-20] It is now believed that the discovery of new biomarkers may ultimately lead to cancer-specific panels, which, when used with artificial network approaches, may bring about high specificity and sensitivity for cancer classification, diagnosis, and prognosis.[18-21]

The human kallikrein gene family consists of 15 genes, all tandemly localized on chromosome 19q13.4.[22,23] All genes encode for secreted serine proteases of relatively low molecular mass (approximately 30 kd). Among these kallikreins, prostate-specific antigen (PSA) is the best cancer marker.[24,25] In addition, human glandular kallikrein 2 (hK2) is an emerging prostate cancer marker.[26] Recently, we reported preliminarily that human kallikrein 6 (hK6) is a potential serological marker for ovarian carcinoma.[27] Indeed, many kallikreins seem to be disregulated in

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Table 1. Descriptive Statistics of Serum hK6 in Noncancer (healthy), Benign Disease, and Ovarian Cancer Patients

Variable	Mean ± SE	Range	Percentiles				
			5	25	50	75	95
Noncancer (n = 97), hK6 (μg/L)	2.94 ± 0.099	0.89 to 6.58	1.49	2.28	2.90	3.54	4.44
Benign disease (n = 141), hK6 (μg/L)	3.12 ± 0.074	1.30 to 6.16	1.99	2.50	3.00	3.60	4.88
Presurgical ovarian cancer (n = 146), hK6 (μg/L)	6.81 ± 0.57	1.30 to 38.00	2.19	3.12	4.40	7.15	25.06
Postsurgical ovarian cancer (n = 105), hK6 (μg/L)	3.87 ± 0.25	0.80 to 21.82	1.82	2.66	3.20	4.20	7.72

ovarian cancer, and their transcript levels seem to have either favorable or unfavorable prognostic value.[28-36] This article examines in detail the diagnostic and prognostic value of serum hK6 levels in ovarian carcinoma.

PATIENTS AND METHODS

Patient Population

Included in this study were 97 apparently healthy women (ages 26 to 72 years; mean, 52 years; median, 49 years), 141 women with benign diseases (ages 21 to 76 years; mean, 46 years; median, 45 years), and 146 women with histologically proven primary ovarian carcinoma (ages 28 to 78 years; mean, 56 years; median, 57 years). Of the benign lesions, 50 were classified as endometriosis, 22 as mucinous cystadenomas, 26 as ovarian dermoid cysts, 10 as ovarian benign teratomas, 15 as corpus luteum, and 18 as serous cystadenomas. Malignant tumors were staged according to the International Federation of Gynecology and Obstetrics criteria. Histologic classification was based on the World Health Organization and International Federation of Gynecology and Obstetrics recommendations. The characteristics of the ovarian cancer patients in terms of stage, grade, histotype, residual tumor after surgery (debulking success) menopausal status, and response to chemotherapy are described later. Serum samples from all patients were collected before surgery, before initiation of therapy and stored at −80°C until analysis. For 105 ovarian cancer patients, serum was also available after surgery. This sample was obtained approximately 2 to 3 weeks after surgery.

Sera were obtained from four centers as follows: the Gynecologic Oncology Unit, University of Turin, Italy (20 cancers, 25 benigns, 40 controls); the Department of Obstetrics and Gynecology, University Hospital Groningen, the Netherlands (41 cancers, 30 benigns, 20 controls); the Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Leuven, Belgium (46 cancers, 22 benigns, 15 controls); and the Department of Clinical Chemistry, Helsinki University Central Hospital, Finland (39 cancers, 64 benigns, 22 controls). Our protocols have been approved by the review boards of all participating institutions.

All patients were treated with platinum-based chemotherapy, and response to treatment was assessed as described elsewhere.[36] Follow-up information was available for 131 of the ovarian cancer patients with a median follow-up of 25 months and a range of 1 to 106 months. Sixty-four (49%) of these patients relapsed and 28 (21%) died during the course of the follow-up period.

Analysis of hK6 and CA-125

CA-125 was measured with a commercially available automated immunoassay method (Immulite 2000, Diagnostic Products Corp, Los Angeles, CA). The upper limit of normal for this method is 23 KU/L. The concentration of hK6 was measured with a procedure developed in our laboratory, as described elsewhere.[37] The assay has a detection limit of 0.1 μg/L and a

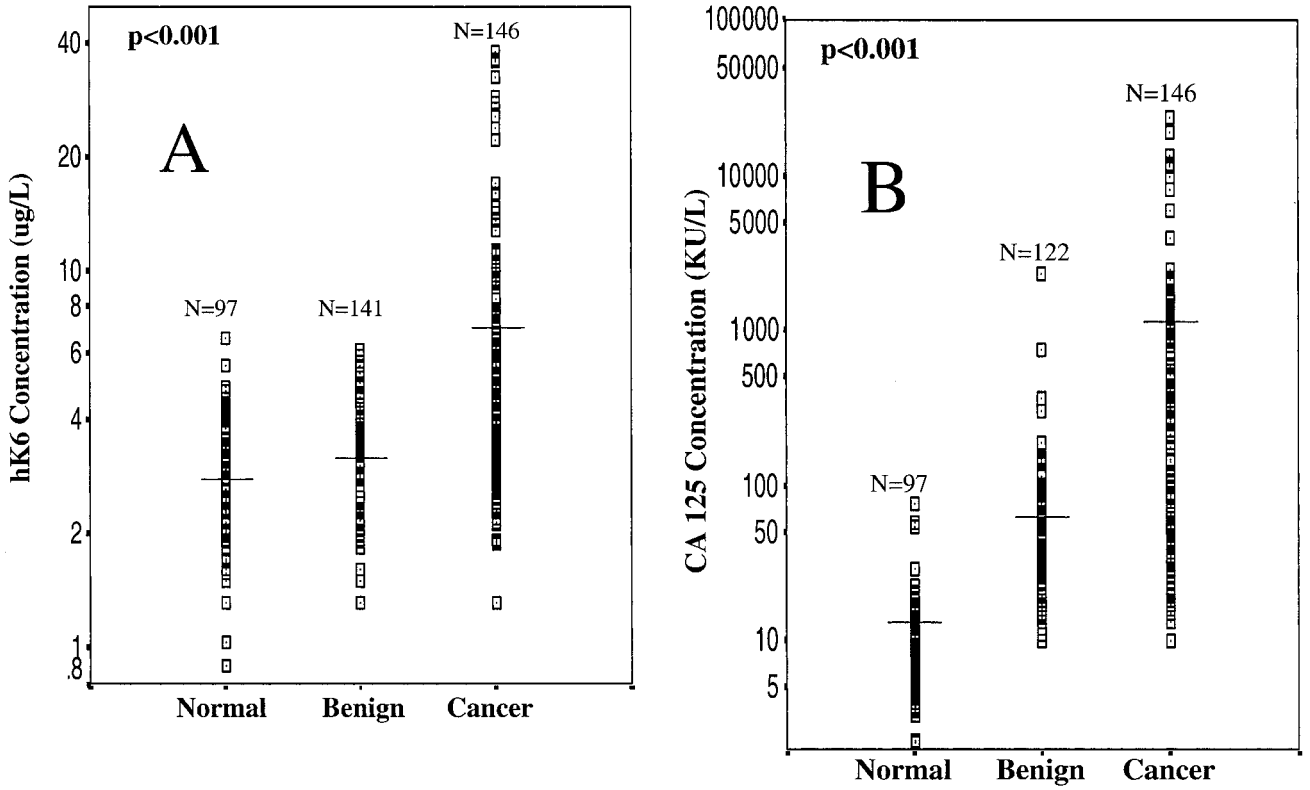

Fig 1. Distribution of serum hK6 (A) and CA-125 (B) concentration in normal, benign disease, and ovarian cancer patients. Horizontal lines represent mean values; N, number of patients per group. The *P* value calculated by analysis of variance represents comparison of the normal group with the ovarian cancer group.

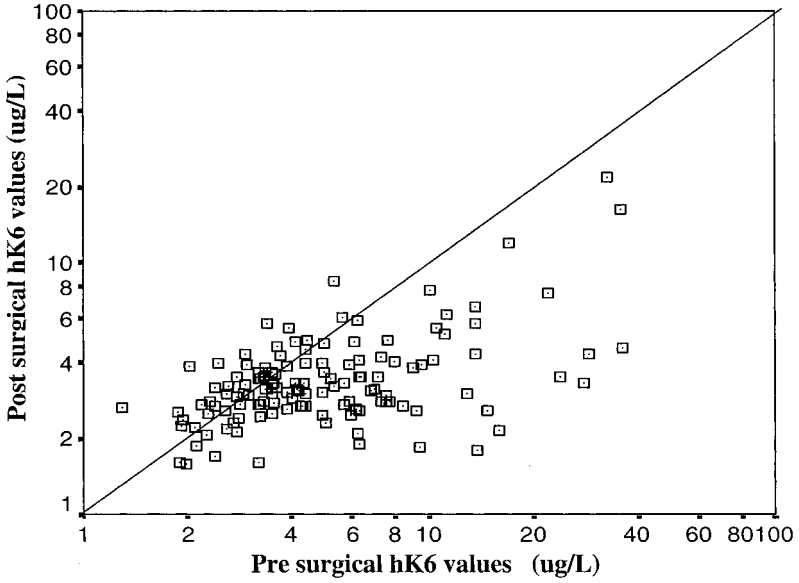

Fig 2. Changes of serum hK6 concentration after ovarian cancer surgery: 68% of patients demonstrated a decrease in the postsurgical serum, in comparison with the presurgical serum ($P < .001$ by the paired *t* test).

dynamic range up to 50 μg/L. Precision was less than 10% within the measurement range. Serum samples were analyzed in duplicate with inclusion of three quality control samples in every run.

Statistical Analysis

To analyze data, patients were divided into different groups according to clinical and pathologic parameters. The analyses of differences between log(hK6) serum concentration before and after surgery were performed with the paired *t* test.

Receiver operating characteristic (ROC) curves were constructed for hK6 and CA-125 serum concentration by plotting sensitivity versus 1-specificity, and the areas under the ROC curves (AUC) were calculated. The noncancer group included the normal individuals and the patients with benign disease. Correlations between different variables were assessed by the Pearson correlation coefficient on log-transformed data. Analysis of variance (ANOVA) was used to determine differences between two or more groups. These tests treated log(hK6) concentration in serum as a continuous variable. hK6 serum concentration was also classified as either hK6-positive ($>$ 4.2 or 4.4 μg/L) or hK6-negative ($\leq$ 4.2 or 4.4 μg/L). The relationship of this dichotomous variable with other clinicopathologic correlates was established with the χ^2 test or the Fisher's exact test, as appropriate.

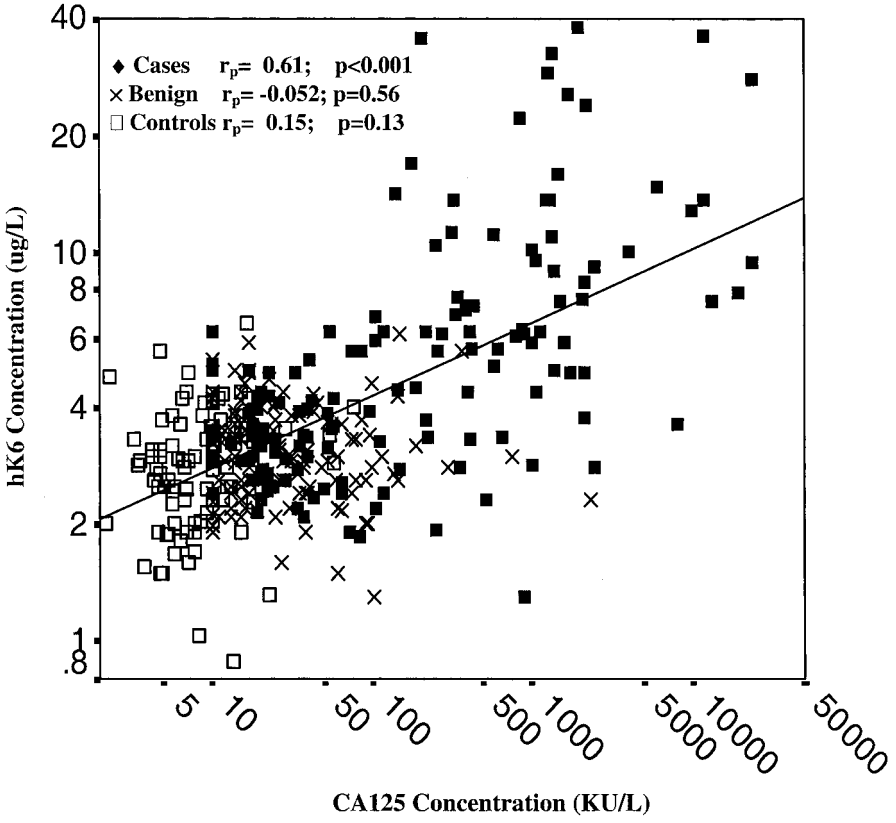

Fig 3. Correlation between serum hK6 and CA-125 concentration (n = 365). r_p, Pearson correlation coefficient.

Kaplan-Meier progression-free survival (PFS) and overall survival (OS) curves were constructed to demonstrate the survival differences between the hK6-positive and hK6-negative patients. The log-rank test was used to examine the significance of the differences among the survival curves. The effect of serum log(hK6) concentration on patient OS and on progression of the disease was assessed with the hazards ratio, calculated by both univariate and multivariate Cox proportional hazards regression models. In the multivariate analysis, the clinical and pathologic variables that may affect survival, including stage of disease, tumor grade, residual tumor, and histologic type, were included in the model to adjust for their impact.

RESULTS

Serum hK6 Concentration in Cancer and Noncancer Patients

The mean, median, range, and selected percentiles of serum hK6 concentration among noncancer (normal; n = 97), benign disease (n = 141), presurgical (n = 146), and postsurgical (n = 105) ovarian cancer patients is shown in Table 1. The mean and median values between noncancer and benign disease patients were not statistically significant. Statistically significant associations between serum hK6 concentration and the subtypes of benign gynecologic diseases were not observed. A P value of .56 was calculated using ANOVA analysis and log(hK6) values. The mean and median hK6 values in presurgical ovarian cancer patients were significantly higher than those in the noncancer and benign groups ($P < .001$). The distribution of hK6 concentration in the three groups of patients is presented in Fig 1 along with the corresponding CA-125 values. Presurgical serum hK6 concentration is not different between normal and benign disease patients but is significantly elevated in a proportion of ovarian cancer patients. CA-125 values are progressively increased from normal to benign to cancer patients.

For dichotomous classification of this patient population as hK6-positive and hK6-negative, we selected the hK6 cutoffs of 4.2 μg/L (90% diagnostic specificity) and 4.4 μg/L (95% diagnostic specificity).

Changes of Serum hK6 Concentration After Surgery

For 105 patients with ovarian cancer, we had serum samples before and after surgery. As shown in Fig 2, 71 patients (68%) demonstrated a decrease in hK6 concentration after surgery, 21 (20%) had unchanged values, and 13 (12%) had higher hK6 serum levels after the operation. By using the paired t test on log(hK6) values, we found a strong and statistically significant difference of hK6 concentration before and after surgery (t value, 7.89; $P < .001$).

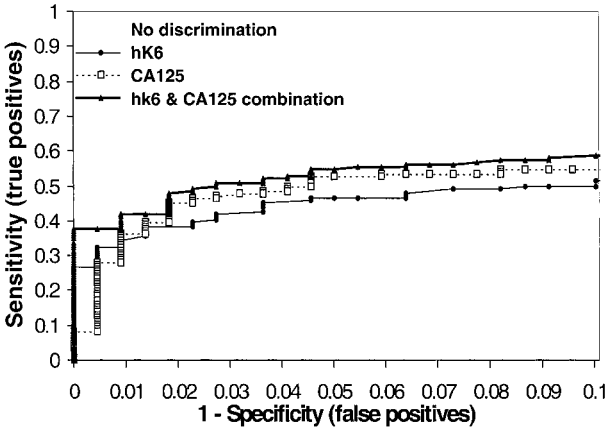

Fig 4. Receiver operating characteristic curves for serum hK6 and CA-125 concentration. The noncancer group included all normal patients and patients with benign disease.

Correlation Between Serum hK6 and CA-125 Concentration

The logarithmic plot of Fig 3 shows the correlation between serum log(hK6) and log(CA-125) concentration (Pearson correlation $r_p = 0.61$ for the cases, -0.052 for the benign subjects, and 0.153 for the control subjects). Although the correlation in cancer patients is significant ($P < .001$), there are still many samples with quite variable values. For example, at CA-125 levels of approximately 500 KU/L, hK6 concentration ranges from 2 to 40 μg/L, whereas samples with hK6 levels of approximately 6 μg/L may have CA-125 values ranging from 5 to more than 5,000 KU/L.

Diagnostic Sensitivity and Specificity of Serum hK6 Concentration

For this calculation, we considered various subgroups of patients, as shown in Table 2. In the noncancer group, we have included all patients who are either normal or have benign disease. When the whole patient group was analyzed, diagnostic sensitivity was around 52% at 90% specificity and 47% at 95% specificity. The ROC curve of Fig 4 indicates a slight diagnostic advantage of CA-125 in comparison with hK6. In the subgroup of patients with CA-125 more than 60 KU/L, the diagnostic sensitivity of hK6 is 71% and 65% at specificities of 90% and

Table 2. Comparison of Sensitivity and Specificity of Serum hK6 Concentration at Selected CutOff Points

Parameter	Cutoff	Sensitivity (%)	Specificity (%)
Total population (N = 384)	4.20	52	90
hK6 (μg/L), (97 controls; 141 benigns, 146 cancers)	4.40	47	95
CA-125 < 23 KU/L (N = 182)	4.30	17	90
hK6 (μg/L), (93 controls; 57 benigns, 32 cancers)	4.40	13	95
CA-125 23-60 KU/L (N = 73)	4.00	26	90
hK6 (μg/L), (3 controls; 40 benigns, 30 cancers)	4.20	15	95
CA-125 > 60 KU/L (N = 110)	4.50	71	90
hK6 (μg/L), (one control; 25 benigns, 84 cancers)	5.56	65	95

Table 3. Diagnostic Sensitivities for Ovarian Cancer with CA-125, hK6, and CA-125 or hK6 Analysis at 90% and 95% Specificity Cutoffs for Both Markers

	Sensitivity at 90% Specificity	Sensitivity at 95% Specificity
All patients with known stage (n = 124)		
CA-125	60	56
hK6	58	53
CA-125 or hK6, (97 controls; 141 benigns, 124 cancers)	72	69
Stage I/II patients (n = 43)		
CA-125	30	26
hK6	26	21
CA-125 or hK6, (97 controls; 141 benigns, 43 cancers)	42	37

Table 4. Relative risk* (RR) of Ovarian Cancer According to Quartiles of Serum hK6

Parameter	Quartiles (μg/L) 1 (0.89-2.60) n = 96	2 (2.61-3.29) n = 96	3 (3.30-4.27) n = 96	4 (4.28-38.00) n = 96
*hK6 unadjusted**				
RR	1.00	1.41	3.12	20.00
95% confidence intervals		0.71 to 2.79	1.43 to 6.85	7.70 to 48.46
P		.32	.003	< .001
hK6 adjusted†				
RR	1.00	1.21	2.31	5.33
95% confidence intervals		0.56 to 2.62	1.05 to 5.02	2.32 to 12.24
P		.62	.036	< .001

*Estimated from unconditional logistic regression models.
†Multivariate models were adjusted with the CA-125 quartiles.

95%, respectively. In the subgroup of patients with low CA-125 (< 23 KU/L), approximately 13% to 17% of patients will still have elevated hK6, at hK6 cutoffs of 4.4 (95% specificity) or 4.3 μg/L (90% specificity), respectively. In the subgroup of patients with slightly elevated CA-125 (23 to 60 KU/L), the diagnostic sensitivity of hK6 is 15% to 26% at specificities of 95% to 90%, respectively (Table 2). However, the two markers can be combined to improve sensitivity by developing a logistic regression model with the terms log(hK6) and log(CA-125). We derived the combination function, $f(x) = 3.95 \log(\text{hK6}) + 1.97 \log(\text{CA-125})$, and performed ROC analysis, which supported the added value of using both variables together, in a multivariate function.

In Table 3, we calculated the additional contribution of hK6 in identifying ovarian cancer patients by using CA-125 and hK6 alone and in combination at 90% and 95% specificity. Among all patients with known stage (N = 124), hK6 analysis increases the sensitivity of CA-125 by 12% or 13%, at 90% or 95% specificity cutoffs, respectively, for both markers. The addition of hK6 increases the sensitivity of CA-125 alone from 30% to 42%, or from 26% to 37%, at 90% or 95% specificity cutoffs for both markers, respectively, for ovarian cancer stages I or II.

Table 4 summarizes the relative risk (RR) of having ovarian cancer, based on serum hK6 concentration using the ovarian cancer and the combined control and benign groups. The RR increases exponentially with increasing hK6 concentration, reaching a value of 20 when hK6 is ≥ 4.3 μg/L. The RR is still substantial (RR = 5.3) in multivariate analysis, after adjusting for CA-125 levels.

Prognostic Value of Serum hK6

Higher ovarian cancer stage and grade are strongly associated with higher serum hK6 concentration (Fig 5 and Table 5). Furthermore, serous adenocarcinomas are more frequently associated with high serum hK6 concentration (positivity 68%) followed by endometrioid tumors (positivity 33%); mucinous

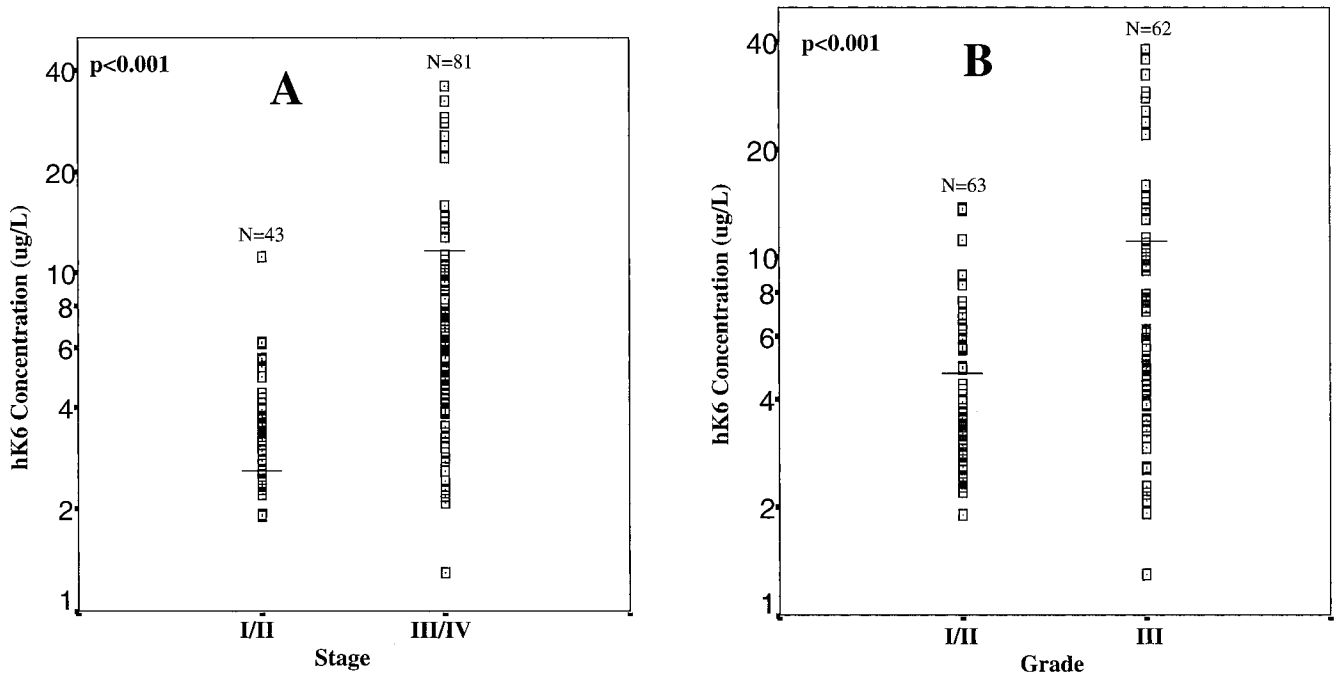

Fig 5. Distribution of serum hK6 concentration in ovarian cancer patients of stages I/II and III/IV (A) and grades I/II and III (B). N, number of patients per group; horizontal lines indicate mean values. *P* < .001 by analysis of variance.

Table 5. Relationship Between hK6 Status and Other Variables in Ovarian Cancer Patients*

Variable	No. of Patients	No. of Patients (%) hK6-Negative No.	hK6-Negative %	hK6-Positive No.	hK6-Positive %	P
Stage						
I	32	27	84.4	5	15.6	
II	11	8	72.7	3	27.3	
III	73	18	24.7	55	75.3	
IV	8	3	37.5	5	62.5	< .001†
x	22					
Grade						
G1	39	31	79.5	8	20.5	
G2	24	7	29.2	17	70.8	
G3	62	19	30.6	43	69.4	< .001†
x	21					
Histotype						
Serous	74	24	32.4	50	67.6	
Endometrioid	15	10	66.7	5	33.3	
Mucinous	22	20	90.9	2	9.1	
Others	27	17	63.0	10	37.0	< .001†
x	8					
Residual tumor (cm)						
0	76	52	68.4	24	31.6	
1–2	17	3	17.6	14	82.4	
> 2	35	6	17.1	29	82.9	< .001†
x	18					
Debulking success						
SO	49	9	18.4	40	81.6	
OD	81	53	65.4	28	34.6	< .001‡
x	16					
Menopausal status						
Pre/peri	39	24	61.5	15	38.5	
Post	103	46	44.7	57	55.3	.091‡
x	4					
Response to CTX						
NC/PD	21	4	19.0	17	81.0	
CR/PR	107	61	57.0	46	43.0	< .001‡
NE	18					

Abbreviations: x, status unknown; SO, suboptimal debulking (> 1 cm); OD, optimal debulking (0 to 1 cm); CTX, chemotherapy; NC, no change; PD, progressive disease; CR, complete response; PR, partial response; NE, not evaluated.

*hK6 cutoff = 4.4 μg/L (median).

†χ^2 test.

‡Fisher's exact test.

tumors are rarely associated with high serum hK6 (9%). Furthermore, high serum hK6 concentration is associated with the presence of residual tumor, suboptimal debulking, and poor response to chemotherapy. All of these associations were highly significant ($P < .001$).

In univariate Cox analysis, serum hK6 concentration is associated with shorter PFS and OS (Table 6). These associations remained statistically significant in the multivariate analysis. The multivariate Cox model was adjusted for hK6 status, CA-125 status, clinical stage, histological type, grade, and residual tumor size. The prognostic value of CA-125 was no longer statistically significant in the multivariate analysis. In addition to presurgical serum hK6, stage of disease was the only other parameter that was associated with both PFS and OS in multivariate analysis.

Similar data were obtained with Kaplan-Meier survival analysis (Fig 6). Patients with high presurgical serum hK6 have much shorter PFS and OS than patients with low preoperative hK6 levels. Although all patients with high serum hK6 relapsed by 6 years, more than 50% of patients with low preoperative serum hK6 were still in remission.

DISCUSSION

The discovery of new ovarian cancer biomarkers for early diagnosis, prognosis, monitoring, and prediction of therapeutic response may contribute to improved clinical outcomes. The only well-accepted ovarian cancer biomarker, CA-125, was discovered 20 years ago. A number of other potential ovarian cancer biomarkers have been identified, but their clinical value is not established.[1,10-13,20,38] This article describes a novel ovarian cancer biomarker, hK6, a member of the expanded human kallikrein gene family.

The traditional ovarian cancer biomarker, CA-125, falls short of being able to diagnose early ovarian cancer effectively.[39] In addition to its low sensitivity for early disease, CA-125 also suffers from low specificity; that is, elevated levels are seen in many benign gynecological diseases.[39] At present, it is widely

Table 6. Univariate and Multivariate Analysis of Serum hK6 in Relation to Progression-Free and Overall Survival

Variable	Progression-Free Survival			Overall Survival		
	HR	95% CI	*P*	HR	95% CI	*P*
Univariate Analysis						
hK6						
Negative	1.00			1.00		
Positive	4.10	2.28 to 7.36	< .001	3.15	1.36 to 7.29	.007
log(hK6)						
As continuous variable	7.42	3.49 to 15.77	< .001	8.61	2.93 to 25.97	< .001
CA-125						
Negative†	1.00			1.00	1.03 to 5.42	
Positive†	2.52	1.45 to 4.38	.001	2.36	1.000 to 1.003	.041
log(CA-125)						
As continuous variable	1.67	1.26 to 2.21	< .001	1.80	1.18 to 2.74	.005
Grading (ordinal)	2.50	1.71 to 3.64	< .001	2.34	1.21 to 1.41	< .001
Residual tumor (ordinal)	1.23	1.13 to 1.34	< .001	1.31	1.44 to 12.53	< .001
Histologic type*	2.49	1.37 to 4.54	.003	4.25		< .008
Multivariate Analysis						
hK6						
Negative	1.00			1.00		
Positive	4.86	1.10 to 21.47	.036	5.08	1.07 to 23.69	.038
log(hK6)						
As continuous variable	2.67	0.45 to 15.69	.27	14.54	0.55 to 378.5	.11
CA-125						
Negative†	1.00			1.00		
Positive†	2.86	0.69 to 11.74	.14	2.17	0.38 to 63.17	.38
log(CA-125)						
As continuous variable	0.94	0.53 to 1.67	.85	0.80	0.29 to 2.16	.66
Stage of disease (ordinal)	2.54	1.37 to 4.69	.003	6.34	2.27 to 17.7	< .001
Grading (ordinal)	1.63	0.94 to 2.82	.078	1.56	0.66 to 3.68	.31
Residual tumor (ordinal)	1.09	0.42 to 2.26	.15	1.01	0.80 to 1.24	.98
Histologic type*	1.08	0.75 to 1.56	.65	1.18	0.94 to 1.31	.18

Abbreviations: HR, hazard ratio estimated from Cox proportional hazard regression model; 95% CI, confidence interval of the estimated HR.

*Serous versus others.

†Cutoff = 98 KU/L (95% specificity; 53% sensitivity; 48th percentile).

accepted that no single cancer biomarker will provide all of the necessary information for optimal cancer diagnosis and management. The current trend is to focus on the identification of multiple biomarkers that can be used in combination. Such approaches have already been shown to have clinical potential in ovarian cancer.[11-13] Other issues related to ovarian cancer screening by using biomarkers as well as other modalities have been addressed in excellent recent reviews and editorials.[16,38-40]

Serum hK6 represents a novel biomarker for ovarian carcinoma. This biomarker is more specific for ovarian cancer than CA-125 because elevations were not seen in benign diseases (Fig 1). The diagnostic sensitivity of hK6 is slightly less than the diagnostic sensitivity of CA-125 at the same specificity cutoff levels (Table 3 and Fig 4). However, hK6 can increase the sensitivity of CA-125 at all stages of the disease, including stage I or II disease (Table 3). As a result of the moderate correlation between hK6 and CA-125 (Fig 3), there are still patients with normal CA-125 who have elevated hK6 levels (Table 2). Thus, CA-125 and hK6 could be used in combination to increase the diagnostic sensitivity of each of the biomarkers alone, using the derived combination function $f(x) = 3.95 \log(\text{hK6}) + 1.97 \log(\text{CA-125})$. Clearly, and as discussed by Jacobs et al,[16] the sensitivity and specificity of CA126, hK6, and their combination still does not meet the criteria for using these markers in a population screening setting.

Similar to CA-125, hK6 is more frequently elevated in serous ovarian carcinoma than in endometrioid and mucinous carcinomas (Table 5). Serum hK6 is also more frequently elevated in late-stage and higher-grade disease. Serum hK6 is a powerful prognostic indicator of patient outcomes. Patients with preoperative hK6 above 4.4 μg/L have significantly worse prognosis than patients with low preoperative hK6 (Table 6 and Fig 6). Serum hK6 is a more powerful prognostic factor than serum CA-125. The prognostic value of CA-125 disappears in multivariate analysis, whereas serum hK6 is an independent prognostic indicator, as shown in the multivariate analysis.

The data of Table 5 regarding response to chemotherapy and the Kaplan-Meier curves allow us to comment as follows: (1) Virtually all patients with high presurgical hK6 relapse within 6 years, and most die (Fig 6); (2) 81% of patients who do not respond to chemotherapy have high presurgical hK6. Thus, presurgically high hK6 identifies patients who are refractory to chemotherapy and who are destined to relapse and die. These patients should be good candidates for clinical trials of other treatments, instead of chemotherapy. More targeted clinical studies to address these issues are warranted.

Serum hK6 likely originates from tumor cells because postoperatively, the levels are significantly decreased (Fig 2). In our previous study, which examined the prognostic value of hK6 analysis in ovarian tumor extracts, we verified the overexpres-

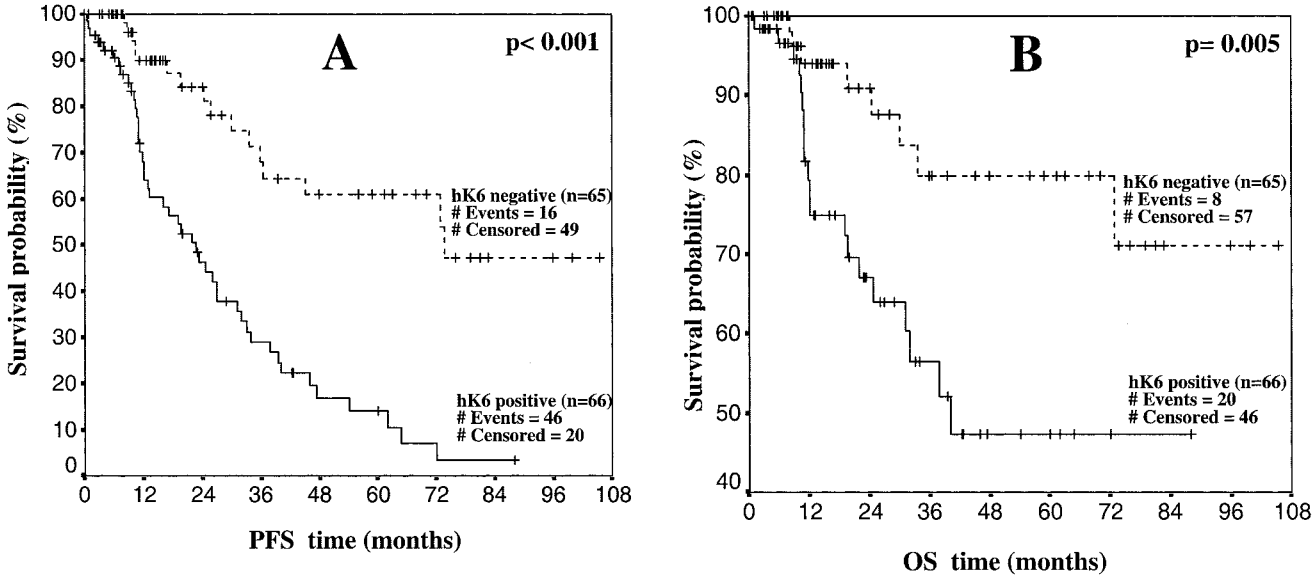

Fig 6. Kaplan-Meier survival curves describing progression-free survival (PFS) (A) and overall survival (OS) (B) in patients who are either hK6-negative or hK6-positive. $P < .001$ by the log-rank test.

sion of hK6 in tumor cells by immunohistochemistry and further provided evidence that intratumor hK6 concentration is also a strong prognostic indicator.[41] Interestingly, many other members of the human kallikrein gene family, including the enzymes hK4, hK5, hK7, hK8, hK9, and hK10, have already been shown to have prognostic significance in ovarian cancer.[28-36] Serine proteases not belonging to the kallikrein family have also been shown to have prognostic significance in ovarian cancer, including trypsin, prostasin, hepsin, and testisin.[13,42-44] It has been known for years that many other proteolytic enzymes have prognostic value in diverse cancers.[45,46] The biologic mechanisms of proteolytic enzyme involvement in cancer prognosis include their ability to degrade extracellular matrix, thus facilitating invasion and metastasis.[46-49] It seems likely that multiple members of the human kallikrein gene family are disregulated in ovarian cancer. It is thus possible that other members of this family will emerge as potential ovarian cancer biomarkers. If these proteases are involved in cancer progression, they may be suitable candidates as therapeutic targets. These possibilities merit further investigation.

In this article, we did not address the issues of ovarian cancer monitoring by measuring serum hK6 concentration or the possible elevations of serum hK6 in other cancers. In our previous preliminary investigation,[27] we showed examples of ovarian cancer patient monitoring with serum hK6. A more detailed study will be necessary to address the issue of monitoring patients whose tumors do not produce CA-125 but do still secrete hK6. As indicated in Table 2, such patients do exist. We have also shown[27] that serum hK6 is not elevated significantly in breast, thyroid, testicular, gastrointestinal, prostate, and lung cancer. Thus, serum hK6 seems to be a specific biomarker of ovarian cancer.

Table 5 shows preliminarily that presurgical serum hK6 concentration may be a predictor of response to chemotherapy in ovarian cancer patients. Among the nonresponders, 81% had elevated presurgical hK6 concentration, whereas 19% of these patients had low hK6 concentration. Among the patients who had either complete or partial response to chemotherapy, 57% had low preoperative hK6 concentration ($P < .001$). It will be interesting to conduct clinical studies to evaluate the value of serum hK6 concentration in predicting response to treatment, including chemotherapy.

In conclusion, we show evidence that serum hK6 concentration represents a novel biomarker for ovarian carcinoma, which has potential utility as a diagnostic, prognostic, and predictive tool. The combination of hK6 and CA-125 improves the diagnostic sensitivity of ovarian cancer at all stages, including early-stage disease. The current availability of a simple and reliable immunoassay for measuring serum hK6 concentration[37] will facilitate further studies to establish the clinical usefulness of serum hK6 analysis for the management of patients with ovarian carcinoma.

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