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The Serum Concentration of Human Kallikrein 10 Represents a Novel Biomarker for Ovarian Cancer Diagnosis and Prognosis[1]

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ABSTRACT

Human kallikrein 10 (*hK10*) is a secreted serine protease that is highly expressed in ovarian tissue. We hypothesized that *hK10* might represent a novel serological marker for ovarian cancer. We quantified by immunoassay, *hK10* in sera from 97 normal women (controls), 141 patients with benign gynecologic diseases, and 146 patients with ovarian cancer. We then examined the diagnostic and prognostic value of this measurement in ovarian cancer. We found that normal serum *hK10* ranged from 50 to 1040 ng/liter (mean = 439 ng/liter). *hK10* concentration is significantly elevated in serum of presurgical ovarian cancer patients (range: 106–11,746 ng/liter; mean = 1067 ng/liter) but not in serum of patients with benign gynecologic diseases (range: 120-1200 ng/liter; mean = 447 ng/liter). When a cutoff of 700 ng/liter was selected (diagnostic specificity = 90%), the diagnostic sensitivity for ovarian cancer is 54%. About 35% of CA125-negative ovarian cancer patients (CA125 < 23 kU/liter) were *hK10* positive at 90% specificity. In patients with stage I/II ovarian cancer, use of these two markers in combination results in a 21% increase in sensitivity, at 90% specificity, compared with CA125 alone. High serum *hK10* was strongly associated with serous epithelial type, late-stage, advanced grade, large residual tumor (>1 cm), suboptimal debulking, and no response to chemotherapy (all *P*s < 0.001). In univariate Cox survival analysis, high serum *hK10* is associated with increased risk for relapse and death (hazard ratio = 2.59 and 3.15, respectively, $P \leq 0.003$). This prognostic value remains significant for overall survival in the multivariate analysis. Kaplan-Meier survival curves demonstrated similar findings. Serum *hK10* represents a novel biomarker for ovarian cancer. We conclude that preoperative serum *hK10* concentration is a strong and independent unfavorable prognostic marker for ovarian cancer.

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy among North American women. Poor survival rates are mainly attributable to late diagnosis. Most patients at diagnosis have stage III/IV disease. The 5-year survival rate for stage III/IV ovarian cancer is only 25%, but for stage I/II disease, survival can be as high as 90%. CA125, the most widely used marker for ovarian cancer diagnosis, was discovered 20 years ago (1). The major disadvantages of this marker include poor sensitivity and specificity for ovarian cancer, especially for diagnosis of early-stage disease. In stage I/II ovarian cancer, 40–50% of patients are CA125 negative and high serum CA125 is also seen in many benign gynecologic diseases and other types of cancer (2). Therefore, there is an urgent need for new biomarkers for ovarian cancer.

Human kallikreins are secreted serine proteases, encoded by a group of genes tandemly localized on chromosome 19q13.3-4 (3). Initially, this gene family in humans was considered to have only three members, encoding for *hK1* (pancreatic/renal kallikrein), *hK2* (human glandular kallikrein), and *hK3* (widely known as prostate-specific antigen). Twelve new members have recently been identified, and they are designated as *hK4, hK5 . . . hK15*. Human kallikreins have diverse physiological functions such as skin shedding, activation of growth factors, activation of other proteases, digestion of growth factor binding proteins, and maintenance of neural plasticity. This family also contributes the best-known marker for prostate cancer, prostate-specific antigen (3).

hK10[3] (also known as the normal epithelial cell-specific 1, NES1) is one of the newly identified members of the kallikrein family. It was cloned by subtractive hybridization by virtue of its down-regulation in a radiation-transformed breast cancer cell line (4). The gene encoding for *hK10* (designated as *KLK10*) is 5.5 kb in length and is formed of five coding exons (5). Because the catalytic triad (histidine, aspartic acid, serine) specific for serine proteases is completely conserved in *hK10*, it is predicted to have trypsin-like serine protease activity. *KLK10* is down-regulated in breast and prostate cancer cell lines and in testicular carcinoma (6, 7). Overexpression of *KLK10* can suppress tumor growth in nude mice (6). These findings suggest that *hK10* may participate in cell growth control pathways.

hK10 is highly expressed in the ovary (4). Because *hK10* is a secreted protein, we hypothesized that its expression might be altered in ovarian cancer and that it may represent a novel biomarker for ovarian cancer. To examine this hypothesis, we first developed a highly sensitive and specific *hK10* immunoassay (8). With this method, we were able to quantify *hK10* in various biological fluids and tissue extracts (8). We found that *hK10* was highly elevated in ovarian tumor cytosols and that this elevation was associated with poor patient prognosis (9). In another preliminary investigation, *hK10* was found to be elevated in sera from ovarian cancer patients but not in normal healthy controls or patients with other types of cancer (10). In this study, we assess in detail the value of *hK10* as a serological biomarker for ovarian cancer diagnosis and prognosis.

MATERIALS AND METHODS

Production and Purification of Monoclonal Antibodies against *hK10*. Previously, we reported production and purification of human recombinant *hK10* from yeast and generation of polyclonal anti-*hK10* antibodies (8). To produce monoclonal antibodies against *hK10*, the splenocytes from the immunized mice were fused with the Sp2/0 myeloma cells using polyethylene glycol 1500. The fused cells were cultured in 96-well plates in DMEM (Life Technologies, Inc., Gaithersburg, MD) containing 20% FCS, 200 mM glutamine, 1% OPI (oxaloacetic acid, pyruvic acid, insulin), and 2% HAT (hypoxanthine,

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[3] The abbreviations used are: hK10, human kallikrein 10; OS, overall survival; PFS, progression-free survival; ROC, receiver operating characteristic; HR, hazard ratio.

aminopterin, thymidine; Sigma Chemical Co., St. Louis, MO) for selection at 37°C, 5% CO_2 for 10–14 days. The supernatants were collected and screened for positive clones, which were then expanded sequentially in 24-well plates and 6-well plates in complete media (reducing the FCS to 15% and changing the HAT to HT). Subsequently, isotyping was performed to isolate clones producing anti-*hK10* monoclonal antibodies of the IgG class. The monoclonals were then expanded in flasks to generate large amounts of supernatant in serum-free media (CD-1 media; Life Technologies, Inc.) containing 200 mM glutamine. Anti-*hK10* monoclonal antibodies were purified from the culture supernatant with a protein G column (Amersham Pharmacia Biotech, Piscataway, NJ) according to manufacturer's instructions.

Immunofluorometric Assay for *hK10*. By incorporating two monoclonal anti-*hK10* antibodies, a sandwich-type, one-step immunofluorometric assay for *hK10* was developed. White polystyrene microtiter plates were first coated with one monoclonal anti-*hK10* antibody (code B14) by incubating overnight 100 μl/500 ng/well of antibody diluted in a 50 mmol/liter Tris buffer (pH 7.80). The plates were then washed six times with washing buffer [containing 9 g/liter NaCl and 0.5 g/liter Tween 20 in 10 mmol/liter Tris buffer (pH 7.40)]. One hundred μl of *hK10* standards or samples were applied into each well. One hundred μl of another biotinylated monoclonal anti-*hK10* antibody (code 5D3) diluted 1000-fold in assay buffer [containing 60 g/liter BSA, 50 mmol/liter Tris (pH 7.80), 0.5 g/liter sodium azide, 2.5% normal mouse serum, 10% normal goat serum, and 1% bovine IgG) were also pipetted into each well (~50 ng of antibody/well). The mixture was then incubated for 2 h with shaking and washed with washing buffer for six times. Subsequently, the plates were incubated with 100 μl/well alkaline phosphatase-conjugated streptavidin (Jackson Immunoresearch) diluted 20,000-fold in a diluent containing 60 g/liter BSA, 50 mmol/liter Tris (pH 7.80), and 0.5 g/liter sodium azide for 15 min and washed as described above. Finally, 100 μl of 1 mM diflunisal phosphate diluted in substrate buffer [0.1 M Tris (pH 9.1), 0.1 M NaCl, and 1 mM $MgCl_2$) were added into each well and incubated for 10 min. One hundred μl of developing solution (1 M Tris base, 0.4 M NaOH, 2 mM $TbCl_3$, and 3 mM EDTA) were pipetted into each well and mixed for 1 min. The fluorescence was measured with a time-resolved fluorometer, the CyberFluor 615 Immunoanalyzer (MDS Nordion, Kanata, Ontario, Canada). More details have been published elsewhere (11).

Patient Population. Included in this study were 97 apparently healthy women (ages 26–72 years; mean = 52 years, median = 49 years), 141 women with benign gynecologic diseases (ages 21–76 years; mean = 46 years, median = 45 years), and 146 patients with histologically confirmed primary ovarian carcinoma (ages 28–78 years; mean = 56 years, median = 57 years). Of the benign lesions, 50 were classified as endometriosis, 22 as mucinous cystadenomas, 10 as benign ovarian teratomas, 26 as ovarian dermoid cysts, 15 as corpus luteum, and 18 as serous cystadenomas. Tumors were staged according to the International Federation of Gynecology and Obstetrics criteria. Histological classification was based on the WHO and International Federation of Gynecology and Obstetrics recommendations. The characteristics of the ovarian cancer patients included stage, grade, histological type, postsurgery residual tumor, debulking success and response to chemotherapy. Serum samples from all patients were collected presurgically, before initiation of therapy, and stored at −80°C until analysis. For 105 ovarian cancer patients, serum was also available 2–3 weeks after surgery.

Sera were obtained from the following four centers: The Gynecologic Oncology Unit, University of Turin, Turin, Italy; the Department of Obstetrics and Gynecology, University Hospital Groningen, Groningen, the Netherlands; the Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Leuven, Belgium; and the Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland. Our protocols have been approved by the review boards of all participating institutions.

Patients were monitored for survival and disease progression for a median duration of 25 months (range: 1–106 months). Follow-up information was available for 131 of the ovarian cancer patients. Sixty-four (49%) of these relapsed and 28 (21%) died during the course of the follow-up period. All patients were treated with platinum-based chemotherapy and response to treatment was assessed as described elsewhere (12).

Measurement of CA125. CA125 was measured with a commercially available automated immunoassay method (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). The upper limit of normal for this method is 23 kU/liter.

Statistical Analysis. The nonparametric Mann-Whitney *U* test was used to determine differences between two groups, and the nonparametric Kruskal-Wallis test was used for the analysis of differences among more than two groups. These tests treated *hK10* concentration in serum as a continuous variable. The analyses of differences between *hK10* serum concentration before and after surgery were performed with the nonparametric McNemar test. The binomial distribution was used to compute the significance level of the McNemar test. Unconditional logistic regression models were developed to evaluate the ability of *hK10* levels to predict presence of ovarian cancer. ROC curves were constructed for serum *hK10* and CA 125 by plotting sensitivity *versus* (1-specificity), and the areas under the ROC curves were calculated. Correlation between different variables was assessed by Spearman correlation coefficient. *hK10* serum concentration was also classified as either *hK10* positive or *hK10* negative. The relationship of this dichotomous variable to other clinicopathological correlates was established with the χ^2 test or the Fisher's exact test, as appropriate.

The impact of serum *hK10* concentration on patient OS and on progression of the disease (PFS) was assessed with the HR, calculated by both univariate and multivariate Cox proportional hazards regression models. In the multivariate analysis, the clinical and pathological variables that may affect survival, including stage of disease, tumor grade, residual tumor, and histological type, were adjusted. Kaplan-Meier PFS and OS curves were also constructed to demonstrate the survival differences between the *hK10*-positive and *hK10*-negative patients. The log rank test was used to examine the significance of the differences among the survival curves.

RESULTS

Immunofluorometric Assay for *hK10*. With two newly developed *hK10*-specific monoclonal antibodies, a noncompetitive, one-step immunoassay for *hK10* was established. Compared with the previously reported assay (10), the sensitivity of this assay is improved (detection limit of 0.02 μg/liter). The assay has no detectable cross-reactivity with other kallikreins (data not shown). Other assay characteristics were similar to those reported previously (8).

Serum *hK10* Concentration in Noncancer and Ovarian Cancer Patients. The distributions of serum *hK10* in normal controls, patients with benign gynecologic diseases, and presurgical ovarian cancer patients are shown in Fig. 1. Serum *hK10* concentration is similar in normal controls and patients with benign gynecologic diseases (means and medians were 439 and 409 and 447 and 414 ng/liter for the two groups, respectively). However, serum *hK10* concentration in presurgical ovarian cancer patients (mean = 1607 ng/liter and median = 756 ng/liter) is significantly elevated (Mann-Whitney test; $P < 0.001$). The distribution of serum CA125 concentration among normal, benign disease, and ovarian cancer patient groups is also shown in Fig. 1. Unlike CA125, which is elevated in benign gynecologic diseases and ovarian cancer, serum *hK10* concentration is only elevated in ovarian cancer patients.

Serum *hK10* Concentration before and after Surgery. To determine whether ovarian tumor tissue is responsible for the elevation of serum *hK10* in ovarian cancer patients, serum concentrations were compared before and after surgery. In ~70% of the patients, postsurgical *hK10* was dramatically reduced ($P < 0.001$ by the McNemar test).

Correlation between Serum *hK10* and CA125 Concentration. With Spearman correlation analysis, a weak correlation ($r_s = 0.40$) between serum *hK10* and CA125 was observed. Clearly, in many samples, there was a great variability between *hK10* and CA125 values.

Diagnostic Sensitivity and Specificity of Serum *hK10* for Ovarian Cancer. The sensitivity of serum *hK10* for ovarian cancer diagnosis at 90% specificity is shown in Table 1. ROC curves for *hK10* and CA125, as well as their combination were also constructed. The areas under the curve (and their 95% confidence intervals) were 0.80

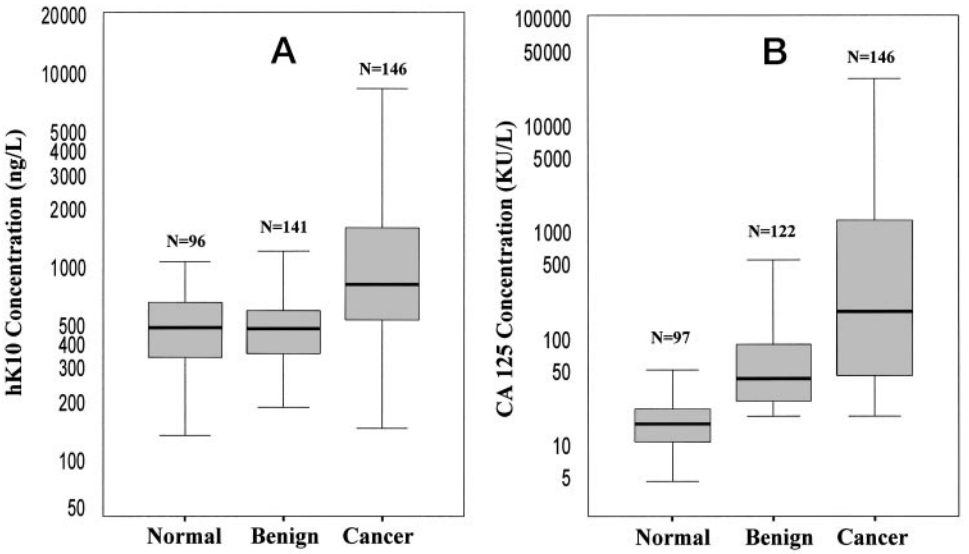

Fig. 1. Distribution of serum *hK10* (*A*) and CA125 (*B*) in normal controls, patients with benign gynecologic diseases, and patients with ovarian cancer. The *boxplots* display the 10th, 25th, 50th (*bold horizontal line* is the median), 75th, and 90th percentiles. *P*s calculated by the Mann-Whitney test were found to be <0.001 between *hK10* concentrations in cancer *versus* normal, <0.001 between cancer *versus* benign, and 0.97 between normal *versus* benign. For CA125, *P*s were <0.001 between cancer *versus* normal, cancer *versus* benign, and normal *versus* benign groups.

Table 1 *Diagnostic sensitivity of serum hK10 (at 90% specificity) for ovarian cancer in different patient groups, classified by CA125 values or disease stage*

Patient group or test	Sensitivity, %
Test, *hK10* alone	
All patients (N = 146 with cancer)	54
Patients with CA125 < 23 kU/liter	35
Patients with CA125 23–60 kU/liter	12
Patients with CA125 > 60 kU/liter	65
Patients with known stage (N = 124)	
Test, CA125 or *hK10*	
CA125 alone	60
hK10 alone	55
CA125 + *hK10*	73
Stage I/II patients (N = 43)	
Test, CA125 or *hK10*	
CA125 alone	30
hK10 alone	35
CA125 + *hK10*	51

(0.73–0.85) for *hK10*, 0.79 (0.73–0.86) for CA125, and 0.84 (0.78–0.89) or their combination. The overall diagnostic sensitivities of *hK10* and CA125 are similar, whereas their combination results in the highest area under the ROC curves.

Diagnostic Sensitivity and Specificity of Serum *hK10* in Combination with Serum CA125. The study population was divided into three groups, according to their CA125 values, and the sensitivity of *hK10* for detecting ovarian cancer (at 90% specificity) was calculated (Table 1). Approximately 35% of ovarian cancer patients with normal serum CA125 values were found to be *hK10* positive at 90% specificity. Similarly, 12% of ovarian cancer patients with slightly elevated serum CA125 were found to be *hK10* positive at 90% specificity. On the other hand, 65% of ovarian cancer patients with high serum CA125 were also *hK10* positive. These data demonstrate that *hK10* can identify a significant proportion of ovarian cancer patients who are missed by CA125 analysis alone. When *hK10* was combined with CA125, the achieved sensitivity (73%) was superior to *hK10* (55%) or CA125 (60%) alone, at 90% specificity cutoffs for both markers (Table 1). Importantly, in patients with stage I/II ovarian cancer, sensitivity with the two markers is 51%, compared with 30% for CA125 and 35% for *hK10* alone (Table 1).

Prognostic Value of Serum *hK10* in Ovarian Cancer. A cutoff of 843 ng/liter (95th percentile of the *hK10* concentration in normals) was selected to categorize patients as *hK10* positive and *hK10* negative. The relationship between serum *hK10* concentration and various clinicopathological characteristics of ovarian cancer was examined by χ^2 test or Fisher's exact test, where appropriate. As shown in Table 2, *hK10* positivity is strongly associated with late stage disease (stage III/IV), advanced grade (grade 3), serous histological type, large residual tumor (>1 cm), suboptimal debulking, and no response to chemotherapy (all *P*s < 0.001).

The impact of preoperative serum *hK10* concentration on ovarian cancer survival was also examined with the Cox proportional hazards regression model. The results of univariate and multivariate analysis are presented in Table 3. In univariate analysis, *hK10*-positive patients

Table 2 *Relationship between hK10 status and other variables in ovarian cancer patients*

Variable	Patients	No. of patients (%)		*P*
		hK10 negative[a]	*hK10* positive	
Stage				
I	32	30 (93.8)	2 (6.3)	
II	11	9 (81.8)	2 (18.2)	<0.001[b]
III	73	35 (47.9)	38 (52.1)	
IV	8	4 (50.0)	4 (50.0)	
X[c]	22			
Grade				
G1	39	34 (87.2)	5 (12.8)	
G2	24	17 (70.8)	7 (29.2)	<0.001[b]
G3	62	27 (43.5)	35 (56.5)	
X	21			
Histotype				
Serous	74	38 (51.4)	36 (48.6)	
Endometrioid	15	13 (86.7)	2 (13.2)	<0.001[b]
Mucinous	22	21 (95.5)	1 (4.5)	
Others	27	20 (74.1)	7 (25.9)	
X	8			
Residual tumor (cm)				
0	76	63 (82.9)	13 (17.1)	
1–2	17	7 (41.2)	10 (58.8)	<0.001[b]
>2	35	10 (28.6)	25 (71.4)	
X	18			
Debulking success[d]				
SO	49	16 (32.7)	33 (67.3)	<0.001[e]
OD	81	66 (81.5)	15 (18.5)	
X	16			
Response to CTX[f]				
NC/PD	21	7 (33.3)	14 (66.7)	<0.001[e]
CR/PR	107	74 (69.2)	33 (30.8)	
NE	18			

[a] Cutoff = 843 ng/liter (95th percentile of normals).
[b] χ^2 test.
[c] X, status unknown; NC, no change; PD, progressive disease; CR, complete response; PR, partial response; NE, not evaluated; CTX, chemotherapy.
[d] OD, optimal debulking (0–1 cm); SD, suboptimal debulking (>1 cm).
[e] Fisher's exact test.

Table 3 *Univariate and multivariate analysis of hK10 with regard to PFS and OS*

Variable	PFS			OS		
	HR[a]	95% CI[b]	*P*	HR[a]	95% CI[b]	*P*
Univariate analysis						
hK10						
Negative	1.00			1.00		
Positive	2.59	1.57–7008	<0.001	3.15	1.48–6.71	0.003
As a continuous variable	1.002	1.001–1.003	<0.001	1.002	1.001–1.004	<0.001
CA125						
Negative[c]	1.00			1.00		
Positive[c]	2.52	1.45–4.38	0.001	2.36	1.03–5.42	0.041
As a continuous variable	1.001	1.000–1.002	<0.001	1.001	1.000–1.003	0.018
Stage of disease (ordinal)	2.81	1.93–4.07	<0.001	3.07	2.05–4.60	<0.001
Grading (ordinal)	2.50	1.71–3.64	<0.001	2.34	1.53–3.58	<0.001
Residual tumor (ordinal)	1.23	1.13–1.34	<0.001	1.31	1.21–1.41	<0.001
Histological type[d]	2.49	1.37–4.54	0.003	7005	1.44–12.53	0.008
Multivariate analysis						
hK10						
Negative	1.00			1.00		
Positive	1.31	0.65–2.62	0.45	3.43	1.23–5.54	0.018
As a continuous variable	1.001	1.00–1.002	0.15	1.002	1.000–1.004	0.031
CA125						
Negative[c]	1.00			1.00		
Positive[c]	1.12	0.44–2.87	0.79	1.22	0.28–5.34	0.78
Stage of disease (ordinal)	2.08	1.13–3.83	0.017	4.062	1.53–10.72	0.005
Grading (ordinal)	1.82	1.13–2.94	0.013	1.43	0.74–2.74	0.27
Residual tumor (ordinal)	1.10	0.97–1.25	0.12	1.28	0.61–2.66	0.51
Histological type[d]	1.03	0.43–2.47	0.94	4.16	0.51–34.1	0.18

[a] HR estimated from Cox proportional hazards regression model.
[b] Confidence interval of the estimated HR.
[c] Cutoff = 98 kU/liter (95% specificity; 53% sensitivity; 48th percentile).
[d] Serous *versus* others.

had a significantly increased risk for relapse (HR = 2.59) and death (HR = 3.15) compared with *hK10*-negative patients ($P \leq 0.003$). Similar results were obtained when *hK10* was considered as a continuous variable. In addition, high CA125, late-stage disease, high tumor grade, large residual tumor, and serous histological type were all associated with poor PFS and OS. In multivariate analysis, only *hK10* status (for OS), stage of disease, and grade (for PFS) remained significant. To further demonstrate the prognostic significance of serum *hK10*, Kaplan-Meier survival curves were also constructed (Fig. 2). *hK10*-positive patients were much more likely to relapse and die than *hK10*-negative patients ($P \leq 0.005$).

DISCUSSION

The traditional ovarian cancer biomarker, CA125, is not efficient for diagnosis of early ovarian cancer (13, 14). In addition to its low sensitivity for early disease, CA125 also suffers from low specificity *i.e.*, elevated levels are seen in many benign abdominal diseases (14). It is conceivable that no single cancer biomarker will provide all of the necessary information for optimal cancer diagnosis and management. Current efforts focus on the identification of groups of biomarkers that can be used in combination. Such approaches have already shown to have clinical potential in ovarian cancer (15). Other issues related to ovarian cancer screening, by using biomarkers in combination with ultrasonography, have been addressed in many recent reports and editorials (16, 17).

Serum *hK10* is a novel biomarker for ovarian carcinoma. This biomarker is more specific for ovarian cancer than CA125 because elevations were not seen in benign diseases commonly associated with elevated CA125 levels (Fig. 1). The diagnostic sensitivity of *hK10* is comparable with the diagnostic sensitivity of CA125 at the same specificity cutoffs (Table 1). Furthermore, *hK10* can increase the sensitivity of CA125 at all stages of the disease, including stage I/II disease (Table 1). As a result of the weak correlation between *hK10* and CA125, there are still many patients with normal CA125 who have elevated hK10 levels (Table 1). Thus, CA125 and *hK10* can be combined to increase the diagnostic sensitivity of each of the biomarkers alone.

hK10, like CA125, is more frequently elevated in serous ovarian carcinoma than in endometrioid and mucinous carcinomas (Table 2). Serum *hK10* concentration is also more frequently elevated in late stage and higher grade disease. Serum *hK10* is a powerful predictor of patient outcomes. Patients with preoperative *hK10* concentration

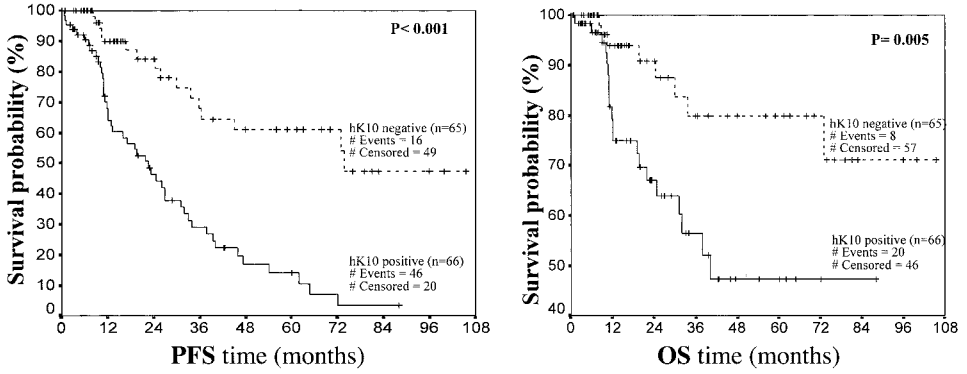

Fig. 2. Kaplan-Meier survival curves for ovarian cancer patients who are either serum *hK10* positive or *hK10* negative. *N* = number of patients. For discussion, see text.

above the 95th percentile of normals (843 ng/liter) have significantly worse prognosis than patients with low preoperative *hK10* (Table 3 and Fig. 2). Serum *hK10* concentration is a more powerful prognostic indicator that serum CA125. The prognostic value of CA125 disappears in multivariate analysis, whereas serum *hK10* is an independent prognostic indicator of OS, as shown in the multivariate analysis.

The data of Table 3 regarding response to chemotherapy and the Kaplan-Meier curves of Fig. 2 suggest that serum *hK10* analysis may assist physicians in selecting therapeutic options for the following reasons: (*a*) virtually all patients with high presurgical *hK10* relapse within 6 years and most die (Fig. 2). (*b*) Sixty-seven percent of patients who do not respond to chemotherapy have high presurgical *hK10* (Table 2). Thus, presurgically high *hK10* is most frequently associated with patients who are refractory to current chemotherapeutic schemes and who are destined to relapse and die. These patients should be good candidates for clinical trials evaluating alternative treatments. More targeted clinical studies to address these issues are warranted.

Serum *hK10* likely originates from tumor cells because postoperatively the levels are significantly decreased. In our previous study, examining the prognostic value of *hK10* analysis in ovarian tumor extracts, we verified the overexpression of *hK10* in tumor cells by immunohistochemistry and additionally provided evidence that intratumor *hK10* concentration is also a strong predictor of poor prognosis (9). Interestingly, many other members of the human kallikrein gene family, including KLK4 (mRNA), KLK5 (mRNA), hK6 (protein), KLK7 (mRNA), KLK8 (mRNA), KLK9 (mRNA), and hK11 (protein), have already been shown to have prognostic value in ovarian cancer (18). Serine proteases not belonging to the kallikrein family have also been shown to have prognostic value in ovarian cancer, including trypsin, prostasin, hepsin, and testisin (18). It has been known for years that many other proteolytic enzymes have prognostic value in many different cancers (19–22). The biological mechanisms of proteolytic enzyme involvement in cancer prognosis include their ability to degrade extracellular matrix, thus facilitating invasion and metastasis (23, 24). 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 protease family may 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 additional investigation.

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