

## **A Multiparametric Serum Kallikrein Panel for Diagnosis of Non – Small Cell Lung Carcinoma**

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**Abstract Purpose:** Human tissue kallikreins are a family of 15 secreted serine proteases. We have previously shown that the expression of several tissue kallikreins is significantly altered at the transcriptional level in lung cancer. Here, we examined the clinical value of 11 members of the tissue kallikrein family as potential biomarkers for lung cancer diagnosis.

**Experimental Design:** Serum specimens from 51 patients with non – small cell lung cancer (NSCLC) and from 50 healthy volunteers were collected. Samples were analyzed for 11 kallikreins (KLK1, KLK4-8, and KLK10-14) by specific ELISA. Data were statistically compared and receiver operating characteristic curves were constructed for each kallikrein and for various combinations.

**Results:** Compared with sera from normal subjects, sera of patients with NSCLC had lower levels of KLK5, KLK7, KLK8, KLK10, and KLK12, and higher levels of KLK11, KLK13, and KLK14. Expression of KLK11 and KLK12 was positively correlated with stage. With the exception of KLK5, expression of kallikreins was independent of smoking status and gender. KLK11, KLK12, KLK13, and KLK14 were associated with higher risk of NSCLC as determined by univariate analysis and confirmed by multivariate analysis. The receiver operating characteristic curve of KLK4, KLK8, KLK10, KLK11, KLK12, KLK13, and KLK14 combined exhibited an area under the curve of 0.90 (95% confidence interval, 0.87-0.97).

**Conclusions:** We propose a multiparametric panel of kallikrein markers for lung cancer diagnosis with relatively good accuracy. This model requires validation with a larger series and may be further improved by addition of other biomarkers.

Lung cancer is the leading cause of cancer-related mortality worldwide in both men and women. According to WHO, two histologically distinct subtypes of lung cancer, that is, small-cell (SCLC) and non – small cell lung cancer (NSCLC), are recognized, depending on cell types. NSCLC accounts for

~ 80% of all cases and, in turn, is composed of three major histologic subtypes of adenocarcinoma, squamous cell carcinoma (SCC), and large-cell carcinoma (1). Patient survival from lung cancer depends mainly on cell type and stage of disease at presentation. When lung cancer is diagnosed at a localized stage, the 5-year survival is ~ 50%, whereas this rate drops to ~ 5% when diagnosis is made at the time of lymph node involvement or metastasis (2).

To supplement advances in surgical treatment, many studies have focused on the development of diagnostic methods, including new serum tumor markers. Thus far, a number of such biomarkers have been reported for NSCLC, including SCC antigen, carcinoembryonic antigen, neuron-specific enolase, cytokeratin 19 fragment (CYFRA 21-1), cancer antigen 125 (CA-125), and tissue polypeptide antigen. However, the expression of these antigens does not seem to be sufficiently sensitive and specific to be reliable for the diagnosis of the majority of lung malignancies.

Proteases may represent good diagnostic/prognostic biomarkers, as they are involved in cancer progression (3). Human tissue kallikreins are a family of 15 highly conserved serine proteases (KLK1-KLK15) encoded by the largest contiguous cluster of protease genes in the human genome. Due to their protease activity and expression in many tissues and cell types, kallikreins have been implicated in a wide range of physiologic functions, ranging from regulation of cell growth to tissue remodeling and skin desquamation. Kallikreins may also be directly or indirectly involved in cancer pathogenesis by

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promoting angiogenesis and degradation of extracellular matrix proteins (4). Accumulating evidence indicates that many members of the kallikrein family are differentially expressed in cancer, primarily in hormone-related malignancies, at the mRNA and protein levels. Furthermore, many members of the kallikrein family have been reported to be promising diagnostic/prognostic biomarkers for several cancer types, including breast, prostate, ovarian, and testicular carcinomas. In addition to prostate-specific antigen, a prostate cancer biomarker, several kallikrein proteins, including KLK6 and KLK10, represent promising serum-based markers for diagnosis and prognosis of ovarian cancer (4). Likewise, KLK3, KLK5, and KLK14 have been proposed as serum markers for diagnosis and prognosis of breast carcinomas (4).

Little is known about the role of kallikreins in lung cancer. Thus far, most of the information related to kallikrein expression in the lung was obtained by microarray analysis and real-time reverse transcription-PCR. For instance, microarray profiling of human lung adenocarcinoma showed that *KLK11* is uniquely overexpressed in a subgroup (cluster C2) of neuroendocrine tumors with less favorable outcome (5). Another microarray-based study indicated that *KLK5* and *KLK10* are overexpressed in the squamous cell lung carcinoma subtype (6). In addition, using quantitative real-time reverse transcription-PCR, we previously reported a significant correlation between *KLK5* overexpression and the SCC histotype, as well as a decrease of *KLK7* expression in lung adenocarcinoma, compared with adjacent nonmalignant lung tissues (7). Similarly, we identified only one transcript of *KLK10* in lung tissues using reverse transcription-PCR, whereas *KLK11* expressed at least four alternative transcripts. Subsequently, using multivariate analysis, we found a correlation between *KLK10* overexpression in tumor and the SCC histotype. A similar expression pattern was also observed for these two genes in lung tissue, suggesting coregulation of *KLK10* and *KLK11* expression in this organ (8). Furthermore, expression of KLK5, KLK6, KLK7, KLK10, KLK11, KLK12, KLK13, and KLK14 in the epithelium of the upper and lower respiratory tract (nose, paranasal sinuses, larynx, trachea, bronchial tree) and in their submucosal glands has been reported immunohistochemically (9). In contrast, no staining was detected in the alveolar epithelium of the lung parenchyma. Interestingly, kallikreins were expressed in varying degree only in non-small cell carcinoma and not in neuroendocrine small-cell carcinoma (9).

As there is a critical need to discover novel biomarkers for lung cancer, the aim of this study was to evaluate whether human tissue kallikreins, alone or in combination, can be potential diagnostic biomarkers in serum from patients with NSCLC.

## Materials and Methods

**Clinical samples.** One hundred and one subjects, including 51 cases diagnosed with NSCLC and 50 normal healthy donors were enrolled in this study. Samples were collected at the University of California at Los Angeles Medical Center between October 2004 and March 2006, in accordance with the University of California at Los Angeles Institutional Review Board and patient written informed consent. Peripheral blood was collected from patients at least 4 weeks after receiving prior therapy for patients with advanced disease. In patients who had previously undergone surgical resection, blood was collected after recurrence at least 1 year following surgery. Plasma was collected in EDTA-containing

vacutainer tubes. Samples were centrifuged at 3,000 rpm for 15 min within 1 h of collection, separated, and stored in aliquots at -80°C. Staging was determined by the American Joint Committee on Cancer Guidelines. Distributions of patients by demographic and clinical characteristics are presented in Table 1.

**ELISA for kallikrein measurement in serum.** ELISA-type immunofluorometric procedures developed in-house were used to measure kallikrein levels in serum. Assays used in the present study were of the "sandwich" type, with one antibody used for capture and another for detection. Three types of configurations of ELISA were used in this study, using either monoclonal-monoclonal (KLK5, KLK6, KLK7, KLK8, KLK10, and KLK13), monoclonal-polyclonal (KLK4, KLK11, KLK12, and KLK14), or polyclonal-polyclonal (KLK1) combinations. Detailed information on these methods is provided in Supplementary Table S1. All ELISAs were tested negative for cross-reactivity against other kallikreins. Assay precision within the dynamic range was <10%. These assays were standardized with recombinant proteins produced in yeast or mammalian expression systems. More details about the kallikrein ELISA have recently been published (10).

**Statistical methods.** The relationships between biomarkers and tumor and patient characteristics were examined with the nonparametric Kruskal-Wallis test. Spearman's rank correlation coefficient was used to assess the correlations among biomarkers. Logistic regression was done to calculate the odds ratio (OR) to define the relation between biomarkers and cancer status. ORs were calculated on log-transformed biomarkers and were represented with their 95% confidence interval (95% CI) and two-sided *P* values.

To evaluate the markers for disease screening and diagnosis, the classification accuracy needs to be assessed. Here, we used receiver operating characteristic (ROC) curve, the most commonly used summary of classification accuracy. The ROC curve is a plot of the true positive fraction versus the false positive fraction for the set of rules that classify a subject as "test-positive," that is, if the marker value exceeds a threshold value, where threshold varies over all possible

**Table 1.** Clinical and pathologic characteristics of NSCLC patients

	Controls	Cases
Smoking status		
Yes	15	34
No*	31	17
x <sup>†</sup>	4	
Gender		
Female	20	22
Male	30	29
Age		
Mean (y)	37	63
SD	11	12
Stage		
III		12
IV		39
Histology		
ADC		25
SCC		6
BAC		1
LCC		1
Unspecified NSCLC		18

NOTE: All patients had unresectable stage III or IV NSCLC at the time of sample collection. Surgical procedures occurred >1 y before specimen collection.

Abbreviations: ADC, adenocarcinoma; BAC, bronchioloalveolar carcinoma; LCC, large-cell carcinoma.

\* <100 cigarettes/lifetime.

<sup>†</sup> x, unknown.

**Table 2.** Distribution of markers in serum of controls (0) and patients with NSCLC (1)

Marker	Control/case	n*	Mean (SE) <sup>†</sup>	Median (1st, 3rd quartile)	P <sup>‡</sup>	AUC (95% CI)
KLK1	0	46	1.43 (1.72)	0.68 (0.43, 1.40)	0.126	0.59 (0.48-0.71)
	1	51	2.08 (2.78)	0.83 (0.58, 2.25)		
KLK4	0	46	0.07 (0.20)	0.00 (0.00, 0.00)	0.135	0.57 (0.48-0.65)
	1	51	0.10 (0.24)	0.00 (0.00, 0.14)		
KLK5	0	50	0.46 (0.29)	0.51 (0.20, 0.69)	0.006	0.66 (0.55-0.77)
	1	50	0.48 (0.94)	0.26 (0.12, 0.44)		
KLK6	0	50	3.15 (0.76)	3.01 (2.60, 3.63)	0.837	0.61 (0.39-0.63)
	1	50	3.22 (1.11)	3.03 (2.34, 3.98)		
KLK7	0	50	5.21 (1.74)	5.09 (3.79, 6.17)	0.017	0.64 (0.53-0.75)
	1	50	10.34 (28.61)	4.02 (3.21, 5.13)		
KLK8	0	50	0.47 (0.22)	0.45 (0.32, 0.60)	0.004	0.67 (0.57-0.78)
	1	50	0.62 (1.82)	0.30 (0.17, 0.46)		
KLK10	0	50	0.46 (0.34)	0.41 (0.14, 0.74)	0.020	0.64 (0.53-0.75)
	1	50	0.82 (3.60)	0.17 (0.08, 0.46)		
KLK11	0	50	0.92 (0.43)	0.91 (0.67, 1.13)	0.001	0.70 (0.59-0.80)
	1	50	2.05 (3.75)	1.23 (0.88, 1.65)		
KLK12	0	44	111.67 (74.85)	106.70 (46.89, 169.78)	0.014	0.65 (0.54-0.76)
	1	51	83.15 (96.21)	59.58 (4.44, 117.66)		
KLK13	0	46	0.02 (0.07)	0.00 (0.00, 0.00)	0.007	0.61 (0.54-0.69)
	1	51	0.48 (2.40)	0.00 (0.00, 0.13)		
KLK14	0	46	0.38 (0.22)	0.32 (0.22, 0.51)	0.014	0.65 (0.54-0.76)
	1	51	0.58 (0.91)	0.42 (0.33, 0.58)		

\*Number of patients.  
<sup>†</sup>All values are in µg/L.  
<sup>‡</sup>Wilcoxon rank sum test.

values. It quantifies how well a marker discriminates between diseased and nondiseased individuals.

To explore the potential that a marker panel can lead to improved performance, we did the ROC analysis first on individual markers and then in combination. We considered an algorithm that renders a single composite score using the linear predictor, fitted from a binary regression model. This algorithm has been justified to be optimal under the linearity assumption (11). A stepwise regression procedure was used to select markers in the panel, sometimes along with clinical variables.

Because an independent validation series was not available for this study, the predictive accuracy of composite scores was evaluated based on resampling of the original data. Specifically, we randomly split the data into a learning set and a test set. The learning set included two thirds of the observations, and the test set one third of the observations. Using the learning set, we first did model selection from which the final selected model gave rise to the linear combination rule. We then calculated two ROC curves for the linear score, one using data from the learning set and the other from the training set. The vertical differences between the two ROC curves gave the overestimation of the sensitivities at given specificities. The whole procedure was repeated 200 times and these differences were averaged to yield an estimate of the expected overestimation. We present both the original ROC curves and the ROC curves that are corrected for overestimation.

All analyses were done using SAS 9.1 (SAS Institute, Inc.) and Splus 7.0 (Insightful Corp.).

## Results

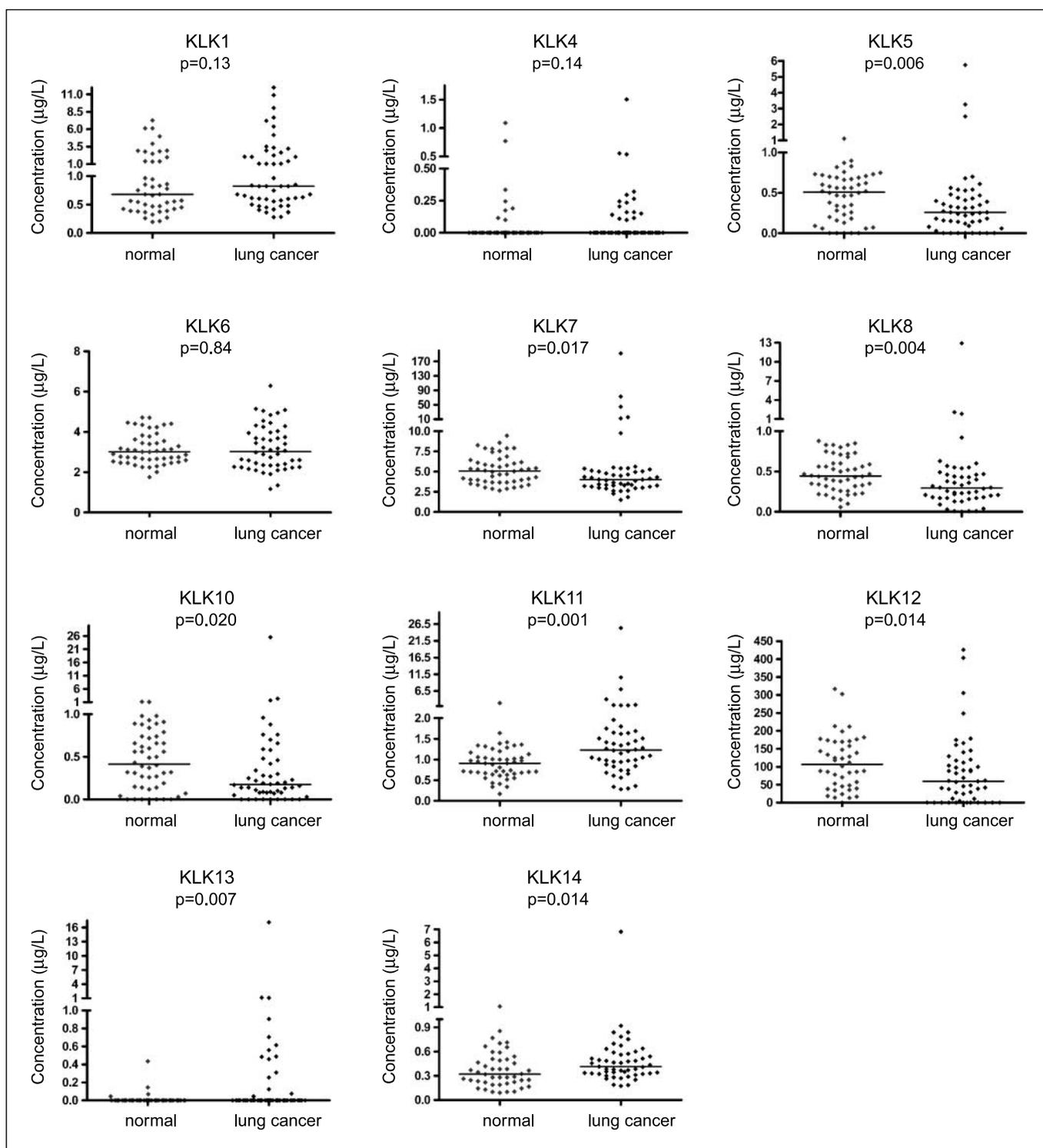
**Distribution of biomarkers.** To determine the clinical utility of the kallikrein family as potential biomarkers in NSCLC, the expression profile of several members of the family was examined by ELISA assays previously developed and validated. The differential expression of KLK1, KLK4-8, and KLK10-14 (after logarithmic transformation of values) was determined by

Kruskal-Wallis testing. As shown in Table 2, the distributions of biomarkers between cases and controls were significantly different at the 0.05 level for KLK5, KLK7-8, and KLK10-14. More precisely, KLK5, KLK7, KLK8, KLK10, and KLK12 exhibited a reduced expression level, whereas KLK11, KLK13, and KLK14 were overexpressed in lung cancer patients, compared with samples taken from healthy individuals (Fig. 1). Among different members of the kallikrein family, KLK13 was of particular interest as it was detected in almost 26% of cancers, as opposed to only 4% of normal individuals.

With the exception of KLK5, the distribution of markers did not seem to differ by smoking status and gender among cases and controls (Supplementary Table S2). Serum levels of KLK5 were lower in samples taken from females ( $P = 0.02$ ) and/or smokers ( $P = 0.03$ ). Similarly, distributions of markers were not significantly different between tumor stages (Supplementary Table S2). However, in the case of KLK11, of 37 patients with stage IV NSCLC, 21 samples (almost 60%) exhibited expression level higher than median, compared with only 25% in stage III (III-A and III-B) samples.

To assess correlations among markers of the kallikrein family, Spearman's rank correlation coefficients were calculated (Supplementary Table S3). Several kallikreins were statistically correlated with each other. For instance, KLK7 seemed to be moderately correlated to KLK8, KLK10, KLK12, and KLK13 (Spearman's rank correlations ranged from 0.25 to 0.47,  $P < 0.05$ ). By contrast, KLK1 and KLK4 expressions were not statistically correlated with any of the other members of the family.

**Association of biomarkers with lung cancer risk.** Logistic regression models were used to examine a possible association between kallikrein biomarkers and lung cancer risk (Table 3). Based on univariate analysis, higher values of KLK11 (OR, 2.28;



**Fig. 1.** Scatter plots of individual kallikrein levels ( $\mu\text{g/L}$ ) in the sera of healthy volunteers (*normal*) and patients with NSCLC (*lung cancer*). Horizontal lines, median values. *P* values were determined by the Wilcoxon rank sum test.

*P* = 0.004), KLK13 (OR, 2.03; *P* = 0.009), and KLK14 (OR, 2.00; *P* = 0.007) were associated with higher risk of NSCLC. Among kallikreins down-regulated in serum of patients with lung cancer, lower values of KLK8 (OR, 0.53; *P* = 0.024) and KLK12 (OR, 0.44; *P* = 0.006) were also correlated with higher risk of lung cancer. As expected, smoking history, but not

gender, was found to be strongly associated with lung cancer risk (OR, 4.13; *P* = 0.001).

Using multivariate analysis, KLK11 (OR, 2.55; *P* = 0.004), KLK12 (OR, 0.47; *P* = 0.021), KLK13 (OR, 2.61; *P* = 0.005), and KLK14 (OR, 2.62; *P* = 0.004) were confirmed to be significant predictors of lung cancer risk, when logistic

regression models were adjusted for clinical variables, smoking status, and gender (Table 3).

**Diagnostic accuracy of biomarkers.** The clinical usefulness of kallikreins in distinguishing NSCLC from noncancer was confirmed using ROC curve analysis (Fig. 2). When different candidates were considered individually, most of them, for example, KLK5, KLK7-10, or KLK12-14, had a moderate discriminatory capacity for classifying patients into cancer and noncancer groups, with an area under the curve (AUC) ranging from 0.61 to 0.70 (Table 2; Fig. 2). KLK11 showed the highest AUC (0.70; 95% CI, 0.58-0.82) in differentiating between cases and controls. We further investigated if panels of markers could improve the diagnostic accuracy. We used a stepwise model selection procedure that took all the baseline markers into consideration. Using the linear predictors from the final model, we found that combining a few of these markers improved the classification capacity. Indeed, a panel of markers, including KLK4, KLK8, KLK10, KLK11, KLK12, KLK13, and KLK14, yielded an AUC of 0.90 (95%CI, 0.87-0.97; Fig. 3). After correcting for potential overfitting, the adjusted AUC was still at 0.80. After taking into account the clinical variables, including gender and smoking, along with KLK8, KLK11, KLK12, KLK13, and KLK14 markers, the AUC of the ROC curve was 0.92 (95% CI, 0.85-0.98; Fig. 3), with a corrected AUC of 0.82. Smoking and gender alone had an AUC of 0.67. These data suggest that a panel of tissue kallikrein biomarkers, along with clinical variables, may represent a powerful diagnostic modality for lung cancer.

## Discussion

In this study, we compared serum levels of 11 kallikreins from 50 healthy individuals and 51 patients with NSCLC to evaluate the clinical utility of this gene family as diagnostic markers for lung cancer. Using ELISA immunoassays developed in-house, we observed alterations in expression between cases and controls for several kallikreins. Based on the median

values, KLK11, KLK13, and KLK14 were found to be significantly overexpressed in cases compared with controls, whereas KLK5, KLK7, KLK8, KLK10, and KLK12 seemed to be down-regulated. The profile of kallikrein down-regulation in serum from patients with NSCLC is similar to the one observed in breast (12-14), prostate (15, 16), and testicular tumors (17, 18), where kallikrein transcripts and proteins are generally down-regulated. Furthermore, we previously reported a decrease of *KLK10* mRNA in cancerous lung tissues and *KLK7* transcripts in lung adenocarcinoma in comparison with their adjacent tissue counterparts (7, 8). By contrast, an overexpression of *KLK11*, *KLK13*, and *KLK14*, as well as nine other kallikreins (*KLK2-8*, *KLK10*, and *KLK15*), has been shown in ovarian carcinoma tissues, cell lines, and/or serum, at the mRNA and/or protein levels (19-21). More recently, Borgono et al. (22) showed that serum KLK14 levels were significantly elevated in prostate cancer patients compared with healthy males. Surprisingly, we observed that serum KLK11 concentration is up-regulated in NSCLC patients compared with the control group, although its mRNA level was found to be lower in cancerous lung tissues compared with noncancerous lung tissues (8, 23). This discrepancy between low mRNA levels and elevation of serum protein in lung cancer may be attributed to angiogenesis and/or destruction of lung architecture during progression, thereby facilitating the leakage of these secreted proteins to the general circulation.

In a previous study, we found that *KLK11* is represented by at least four alternative transcripts in lung tissues. Among them, one was identical to the brain type *KLK11* mRNA (24) in noncancerous and cancerous lung tissues. On the other hand, a longer coding splice variant, containing an additional exon in the 5' untranslated region, was only detected in cancerous lung tissues (8). Given that the majority of the putative proteins encoded by splice variants are predicted to be secreted (25, 26), the above-mentioned cancer-specific *KLK11* variant may be useful as a biomarker.

**Table 3.** Logistic regression with disease status as outcome

	n	Univariate		Multivariate	
		OR (95% CI)	P	OR (95% CI)	P*
Clinical variables					
Smoking					
Nonsmoker	48	1	0.001	1	0.001
Smoker	49	4.13 (1.77-9.65)		4.14 (1.77-9.68)	
Gender					
Female	42	1	0.749	1	0.879
Male	59	0.88 (0.4-1.94)		1.07 (0.45-2.52)	
Marker					
KLK1	97	1.34 (0.89-2.03)	0.165	1.49 (0.94-2.38)	0.090
KLK4	97	1.36 (0.89-2.07)	0.154	1.23 (0.78-1.93)	0.369
KLK5	100	0.75 (0.49-1.13)	0.166	0.84 (0.53-1.32)	0.451
KLK6	100	0.97 (0.65-1.43)	0.867	1.06 (0.69-1.62)	0.791
KLK7	100	0.96 (0.65-1.42)	0.835	1.11 (0.72-1.7)	0.633
KLK8	100	0.53 (0.3-0.92)	0.024	0.58 (0.32-1.04)	0.067
KLK10	100	0.77 (0.52-1.16)	0.213	0.84 (0.54-1.29)	0.417
KLK11	100	2.28 (1.29-4.01)	0.004	2.55 (1.36-4.78)	0.004
KLK12	95	0.44 (0.24-0.79)	0.006	0.47 (0.25-0.89)	0.021
KLK13	97	2.03 (1.19-3.46)	0.009	2.61 (1.34-5.08)	0.005
KLK14	97	2.00 (1.21-3.32)	0.007	2.62 (1.37-5.01)	0.004

\*Adjusted for clinical variables smoking and gender.

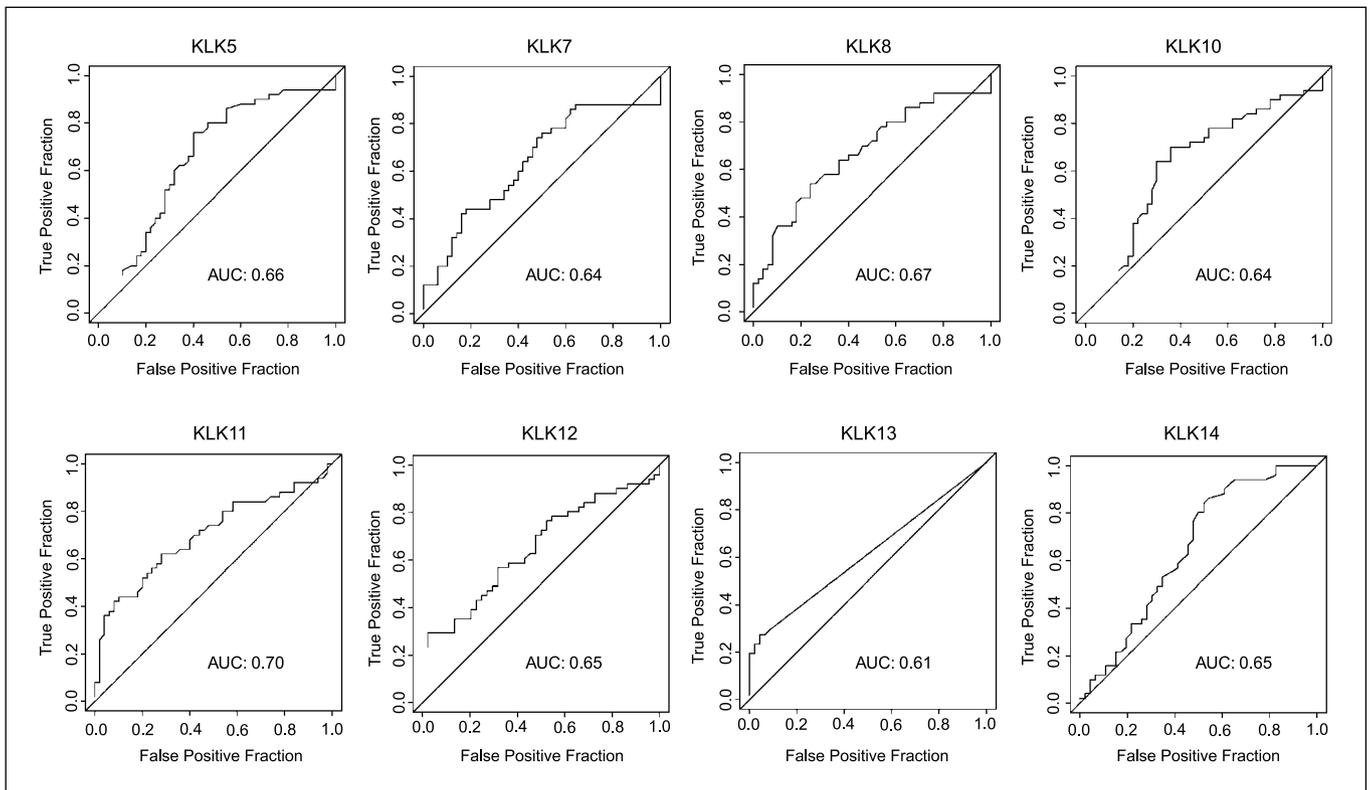


Fig. 2. ROC curve analysis of KLK5, KLK7, KLK8, KLK10, KLK11, KLK12, KLK13, and KLK14 in differentiating NSCLC cases and controls. The AUC is given in each panel.

Of the different kallikreins measured, we found that KLK4 and KLK13 were expressed at very low levels in most of the serum samples of this study. Indeed, KLK4 and KLK13 proteins were above the detection limit (0.1 and 0.05 µg/L, respectively) in only 15% of controls and 30% of cases for KLK4, and in 4% of controls and 26% of cases for KLK13. It is known that serine protease inhibitors, such as α<sub>2</sub>-macroglobulin, can form complexes with certain serum kallikreins, including KLK2, KLK3, KLK4, KLK5, and KLK13 (27–31). Binding of KLK4 and KLK13 with serum proteinase inhibitors may lead to complexes that are not recognized by ELISA, as shown for prostate-specific

antigen (32). Alternatively, these kallikreins may be expressed/secreted at low levels. For instance, *KLK4*/*KLK4* was found to be expressed in ovarian cancer tissues using reverse transcription-PCR, Southern blot, and immunoblotting (33–35). However, a recent study indicated that the concentration of KLK4 was very low in ovarian cancer effusions, suggesting that KLK4 could be expressed in ovarian cancer cells but not secreted to the extracellular medium (36). The most recent data on kallikrein expression in normal lung tissue indicate that KLK7, KLK10, KLK11, and KLK12 are most prominently expressed (10).

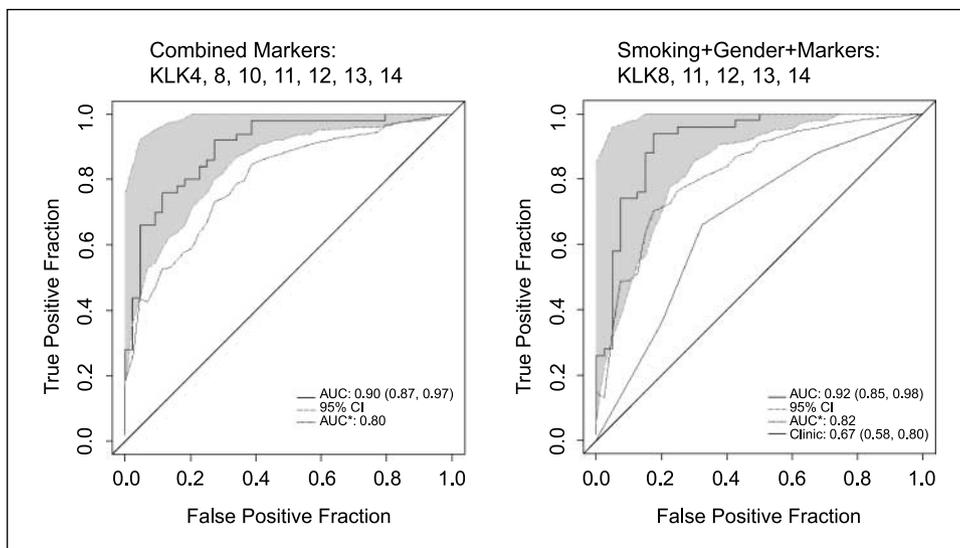


Fig. 3. ROC curve analysis of combined kallikreins in differentiating NSCLC cases and controls. Black and red lines, uncorrected and corrected AUC value for potential overfitting, respectively. Shaded area, 95% CI. Additional clinical variables of smoking status and gender are included in the right panel. The AUC for smoking and gender alone is also shown (AUC 0.67).

Tissue kallikreins are an emerging family on the cancer proteolysis scene. They may exert their function at various stages of cancer initiation and/or progression (4, 37, 38). In our study, we observed that KLK10 was down-regulated in sera of patients with NSCLC. In our study, we also highlighted a significant decrease of KLK8 in sera of patients with NSCLC. More recently, it was reported that KLK8 cleaved fibronectin, thereby suppressing tumor cell invasion and conferred a favorable outcome in early-stage NSCLC (39).

Several other kallikreins, such as KLK11, KLK13, and KLK14, show an increase in serum of patients with NSCLC. These kallikreins may be involved in the promotion of cancer cell growth, angiogenesis, and invasion. For example, several studies reported the cleavage of insulin-like growth factor binding proteins *in vitro* by kallikreins, including insulin-like growth factor binding protein 3 processing by KLK11 in estrogen receptor-positive breast cancer cells (40) and insulin-like growth factor binding proteins 2 and 3 by KLK14 (22). Reciprocally, the bioavailability of insulin-like growth factor binding protein ligands (i.e., insulin-like growth factors) is increased, which in turn stimulates cancer cell growth. Furthermore, via degradation of extracellular matrix components (e.g., collagens I-III, fibronectin) and proteins of basement membranes (e.g., laminin, collagen IV), KLK13 and KLK14 may also contribute to tumor cell invasion and metastasis (22, 29). KLK14 was also found to cleave vitronectin *in vitro*, which in turn may decrease integrin-mediated and urokinase receptor-mediated cell adhesion, facilitating tumor cell detachment (22).

Thus far, kallikreins have mainly been described as individual potential biomarkers. Combination of multiple members and/or other tumor markers could further improve

their utility. For instance, although KLK3/prostate-specific antigen is clinically used as the main prostatic biomarker, serum KLK2 and KLK11 have been shown to function as complementary biomarkers (19, 41–43). Similarly, combined serum KLK6 and KLK10 can increase the diagnostic sensitivity of CA-125 in patients with early stage (I/II) ovarian cancer (4). It has recently been shown that a combination of eight kallikreins (KLK5-8, KLK10, KLK11, KLK13, and KLK14) in effusion samples can achieve areas under the ROC curve of 0.994 and 0.961 in separating ovarian cancer from benign and other cancer groups, respectively (36). In lung cancer, biomarker panels have already been investigated. Molina et al. (44) did a prospective, multicenter study to determine the clinical utility of five well-known tumor markers (carcinoembryonic antigen, CA-125, SCC antigen, CYFRA 21-1, and neuron-specific enolase) in relation to clinical and pathologic variables used in NSCLC. Using a panel of three markers (CYFRA 21-1, CA-125, and carcinoembryonic antigen), they found high sensitivity for NSCLC (87% for locoregional and 93% for advanced disease).

Here, we report for the first time that a panel of kallikreins may be useful for diagnosis of NSCLC. Seven kallikreins (KLK4, KLK8, and KLK10-14) achieved an AUC of 0.90 between lung cancer cases and controls, which was higher than the AUC of any single kallikrein (ranging from 0.57 for KLK4 to 0.70 for KLK11). Because our cohort is relatively small, further studies will be necessary to validate our findings.

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