EVALUATION OF HUMAN KALLIKREINS 5, 6, 7, 8, 10, 11 AS PROGNOSTIC MARKERS OF OVARIAN CANCER PATIENTS

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ABSTRACT

Introduction: Experimental results indicate that the expression and proteolytic activity of human tissue kallikreins (hKs) are differentially regulated in tumours, mainly adenocarcinomas, and are often associated with patient prognosis [1]. Kallikreins 5,6,7,8,10 and 11 have been found at increased levels in patients with ovarian carcinoma [1,2]. Notably, pre-surgical serum hK6 and hK10 levels can increase the diagnostic sensitivity of CA125 in patients with early stage ovarian cancer and are independent markers for poor prognosis [3,4].

Methods: Serum samples from 91 patients with ovarian carcinoma were collected after surgery and prior to each chemotherapy cycle, and were assessed for the levels of the tumour marker CA125 and kallikreins 5, 6, 7, 8, 10 and 11. Histology, stage, chemotherapy treatment and outcome were reported for each patient. 43 patients died within two years of follow-up while 48 patients survived (overall survival = OS); 23 of the surviving patients were classified as free of disease progression (progression-free survival = PFS).

Results: In ROC analyses, CA125, hK6, hK8 and hK11 showed the best sensitivity and specificity to distinguish patients with disease-free survival or over-all survival from patients who died within two years. In the group of patients with less than two-year survival, eight patients had CA125 values below the traditional cut-off of 30U/ml. Both, hK6 and hK8, were above their respective cut-off in all eight patients (determined as 95th percentile of hK6 and hK8 in healthy women, n = 200) indicating that the combination of CA125 with kallikreins can improve prognosis. Univariate logistic regression was utilized to determine the association of analytes as continuous quantitative variables. The results from quartile analysis show significant association with risk for death at 2-years for all markers but hK7.

Conclusions: Univariate assessments of the kallikreins as continuous and categorical variables demonstrate the predictive power of human tissue kallikreins and CA125 as prognostic markers in ovarian cancer.

INTRODUCTION – HUMAN KALLIKREINS

Human Tissue Kallikreins [1]

- **Genes: Chromosome 19q13.4 (300kb)**
- **Proteins: Serine Proteases with Trypsin or Chymotrypsin-like activity**

CA125 in ovarian cancer [5]

- **Low specificity as a diagnostic marker (elevated in other types of cancer or non-cancerous gynecological conditions)**
- **Low sensitivity**
- **Good biomarker for monitoring**

Kallikreins as Cancer Biomarkers

- **PSA/hK2 is utilized to monitor prostate cancer patients**
- **hK6 (Zyme / Protease M / Neurosin), hK10 (NES1), hK11, hK8, hK5 and hK14 may represent novel ovarian cancer biomarkers**
- **hK11 may represent a novel prostate cancer biomarker**
- **hK5 and hK14 may represent novel breast cancer biomarkers**

HYPOTHESIS

We hypothesize that:

Kallikreins 5,6,7,8,10 and 11 are novel prognostic markers of ovarian cancer

OBJECTIVES

- **Quantification of hK 5,6,7,8,10 and 11 in pre-surgical serum of 98 ovarian cancer patients**
- **Statistical analysis: Distribution of variables per characteristics of patient and tumour**
- **Relation of kallikreins to 2-yr Survival**

MATERIALS AND METHODS

Kallikrein protein	Assay configuration	Detection limit (pg/L)	Reference
hK5	Mono-mono	0.05	*
hK6	Mono-mono	0.05	6
hK7	Mono-mono	0.2	7
hK8	Mono-mono	0.05	*
hK10	Mono-mono	0.02	6, 8
hK11	Mono-poly	0.05	9

* Unpublished results

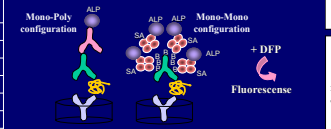


Figure 1: Assay configurations for immunological quantification of hKs

PATIENT INFORMATION

- **Patients n=92, Stage I / II n=19, Stage III/ IV n=73, 57 years median age (22-80 years)**
- **All draws were before first chemotherapy, often month or years after surgery**
- **Therapeutic effect: complete remission (CR) in 28 patients, partial remission (PR) in 20 patients, stable disease (SD) in 8 patients and progressive disease (PD) in 36 patients**
- **44 patient died within 2 years, 48 patients survived (21 patients disease-free)**
- **CA125 values were measured for all patients – for 31 patients the CA125 was below the cut-off point of 30U/ml and remained non-informative in 28 patients over the course of chemotherapy.**
  Note: all 19 patients with stage I/ II had non-informative CA125 values

SURVIVAL ANALYSIS - RESULTS

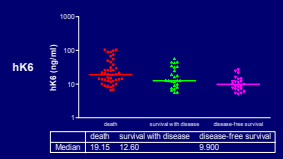
Table 1: Distribution of analytes per quartile and number of patients

	Minimum -25th percentile	25th - Median percentile	Median - 75th percentile	75th - Maximum percentile
CA125	5 -15	16-109	110-511	512-8050
hK5	0.01 – 0.1	0.11 – 0.16	0.17-0.36	0.37-4.2
hK6	5 - 9	9.1 – 13.8	14 – 25.5	26.1 - 200
hK7	0.6 – 2.3	2.4 - 3.2	3.3 – 4.4	4.5 - 23.3
hK8	2.8 – 6.9	7 – 11.1	11.2 - 15	16 -44
hK10	0.4 – 1.7	1.8 – 2.9	3 – 5.4	5.7 - 90
hK11	0.2 – 0.59	0.6 – 0.9	0.9 – 1.4	1.4 – 7.2

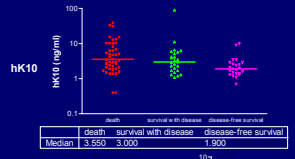
Figure 2: CA125 level in first sample is lower in patients with positive outcome

	death	survival w ith disease	disease-free survival
Median	345.0	161.5	12.00

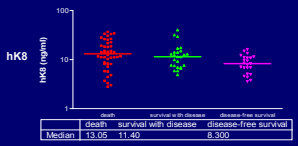
Figure 3: hk6, 8 and 10 level in first sample is lower in patients with positive outcome (hK5, 7 and 11 were not informative: hK5 included for comparison purposes)



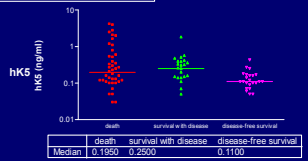
	death	survival with disease	disease-free survival
Median	19.15	12.60	9.900



	death	survival with disease	disease-free survival
Median	3.550	3.000	1.900

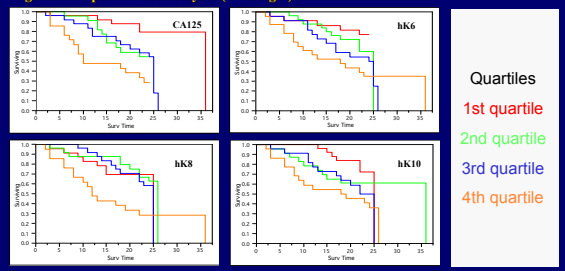


	death	survival with disease	disease-free survival
Median	13.05	11.40	8.300



	death	survival with disease	disease-free survival
Median	0.1950	0.2500	0.1100

Figure 4: Kaplan-Meier analyses (All Stages)

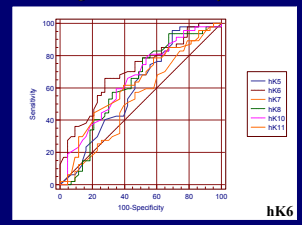


Summary of Kaplan Meyer Analysis

Analyte	Log-rank p-value (All Stages)	Log-rank p-value (Stage III)
CA125	0.0010	0.0666
hK5	0.1077	0.3160
hK6	0.0364	0.0269
hK7	0.6113	0.7242
hK8	0.0222	0.0381
hK10	0.0481	0.1396
hK11	0.0673	0.3074

SURVIVAL ANALYSIS – RESULTS (cont.)

Figure 6: First draw - 2-year survival versus death



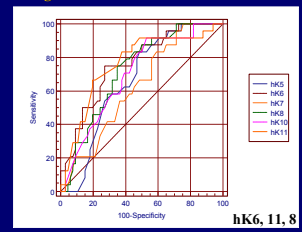
CA125 = AUC 0.74

POSITIVE GROUP
Sample size = 47
NEGATIVE GROUP
Sample size = 43

Areas Under ROC Curves

hk5 = 0.605
hK6 = 0.707
hK7 = 0.531
hK8 = 0.628
hK10 = 0.653
hK11 = 0.627

Figure 7: First draw - disease-free survival versus death and survival with disease



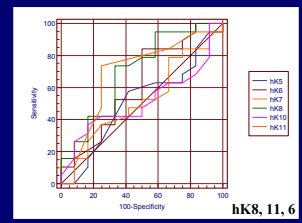
CA125 = AUC 0.86

POSITIVE GROUP
Sample size = 24
NEGATIVE GROUP
Sample size = 66

Areas Under ROC Curves

hk5 = 0.664
hK6 = 0.761
hK7 = 0.628
hK8 = 0.723
hK10 = 0.698
hK11 = 0.760

Figure 8: First draw - disease-free survival versus death and survival with disease in patients with CA125 below 30U/ml



POSITIVE GROUP
Sample size = 19
NEGATIVE GROUP
Sample size = 12

Areas Under ROC Curves

hk5 = 0.511
hK6 = 0.616
hK7 = 0.533
hK8 = 0.700
hK10 = 0.515
hK11 = 0.686

CONCLUSIONS

- **Kallikreins displayed significant associations with the outcomes evaluated when investigated in univariate assessments**
- **Across the outcome measures and the univariate and multivariate regressions performed, the most consistent prognostic markers were CA125, hK6, and hK8**

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