CIRCULATING THYMIDINE KINASE 1 AND TUMOR M2 PYRUVATE KINASE ARE POTENTIAL PREDICTORS FOR DISEASE RECURRENCE IN RENAL CELL CARCINOMA AFTER NEPHRECTOMY

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INTRODUCTION

Thymidine Kinase 1 (TK1) is an enzyme involved in DNA synthesis by incorporating thymidine and, therefore, is considered to be an important proliferation marker. The recently developed high-sensitive assay DiviTum® has a much higher analytical sensitivity for TK1 activity than previous assays. This allows detecting even small changes in proliferation activity of malignant tumor disease.

Pyruvate kinase (PK) is a key enzyme in glycolysis. The dimeric form of PK isoenzyme type M2 (TuM2-PK) was shown to be overexpressed in proliferating and tumor cells, including Renal Cell Carcinoma (RCC). Both markers are released into circulation by tumor necrosis and cell turnover.

STUDY AIMS

To characterize the association of circulating TK1 and TuM2-PK levels with the main clinicopathological parameters in RCC. To estimate the predictive value of these two markers for disease recurrence after nephrectomy.

PATIENTS AND METHODS

IRB approved protocol. HMO-0438-08.

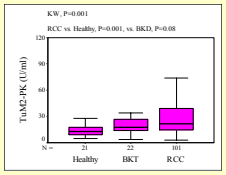
Study design. A. Healthy - 21. B. Benign kidney tumors (BKT) - 22. C. RCC - 101.

Methods. Serum TK1 activity and plasma levels of TuM2-PK were measured preoperatively or before systemic treatment by ELISA kits: DiviTum® (Biovica, Sweden) and ScheBo®Tech (Germany), respectively.

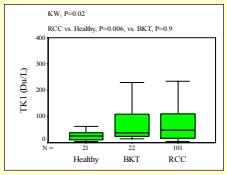
Statistics. Non-parametric tests were used for comparisons between numeric variables (Kruskal-Wallis, Mann-Whitney, Chi-square, Fisher's exact and Spearman's rho). For analyses of the sensitivity-specificity relation of the assays, receiver operating characteristic (ROC) curves were constructed, and the areas under these curves (AUC) were calculated. The models for the probability of disease recurrence as a function of the serum TK1 activity and plasma TuM2-PK levels were constructed using logistic regression analysis. A value of $P < 0.05$ was considered significant.

RESULTS

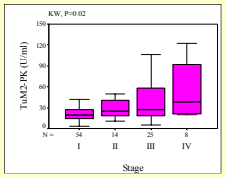
Association of TuM2-PK and TK1 with tumor stage and grade.



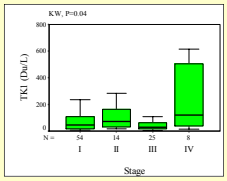
Patients with RCC had significantly higher circulating TuM2-PK levels and TK1 activity compared to healthy individuals but not compared to patients with BKT.

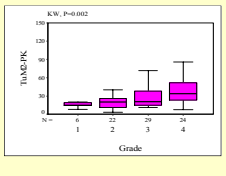


RCC patients (n=101)		
Age, y	Median (Range)	60 (22-80)
Sex	Males / Females	65 / 36
Histology	Clear Cell / Chromophobe / Papillary	79 / 12 / 10
Stage	I / II / III / IV	54 / 14 / 25/ 8
Nuclear grade	I / II / III / IV / not applicable	6 / 22 /29 / 24 / 20
Tumor necrosis	Yes / No	29 / 73
Median follow-up	months (range)	61.3 (8-113)

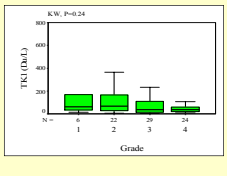


There was a significant association between circulating TuM2-PK and TK1 levels and tumor stage. Patients with stage T3 tumors demonstrated low TK1 activity.



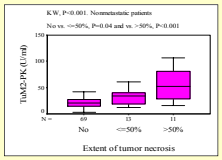


Elevated plasma TuM2-PK levels were associated with high grade tumors. Patients with high grade tumors demonstrated a low serum TK1 activity.

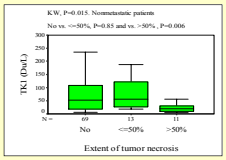


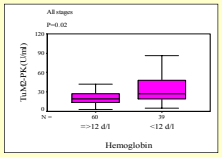
Tumor Necrosis, Anemia, TuM2-PK and TK1.

Association of tumor necrosis with stage and grade in 93 pts with nonmetastatic RCC			
Parameter	No. of pts	No. of pts with necrosis (%) - extensive (%)	P-Value
pT Stage			
1	54	6 (11) - 2 (4)	P<0.001
2	14	4 (29) - 1 (7)	Extensive
3	25	14 (56) - 8 (32)	P=0.001
Grade			
1	6	0 (0) - 0 (0)	P<0.001
2	22	2 (9) - 0 (0)	
3	29	5 (17) - 2 (7)	Extensive
4	24	13 (54) - 8 (33)	P=0.002

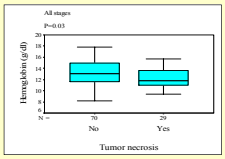


The presence of extensive tumor necrosis (>50%) was associated with high level of plasma TuM2-PK, however, it adversely affected the serum TK1 activity.





TuM2-PK showed a significant increase in patients with anemia. Anemia was found to be more prevalent in patients with necrotic tumors.



TK1 and TuM2-PK in recurrence prediction

Of the 71 patients with clear cell RCC (ccRCC) who underwent nephrectomy, 13 patients (18.3%) developed metastatic disease. There was no recurrence in patients with non-ccRCC. The optimal cut-off values for prediction of disease recurrence, derived from the analysis of the ROC curves, corresponded to 30 U/ml and 170 Du/L, respectively. These cut-off levels yielded the sensitivity 84.6%, 38.5% and specificity 79.3%, 89.7% for TuM2-PK and TK1, respectively. The combination of these two markers resulted in a sensitivity of 100% and specificity of 74.1%. Older age, higher stage at presentation, tumor necrosis, elevated TuM2-PK and TK1 were found more prevalent among patients who developed disease recurrence after nephrectomy. In a multivariate logistic regression model only stage pT3, TuM2-PK and TK1 remained significant.

Multivariate analysis			
Characteristics	No. of pts	Odds Ratio (CI)	P Value
Age (ys)			
≤60	36	1	
>60	35	3.8 (0.4-35.0)	0.24
pT Stage			
1	39	1	
2	10	1.5 (0.7-32.9)	0.82
3	22	49.5 (3.2-566.0)	0.005
Necrosis			
No	52	1	
Yes	19	1.2 (0.1-19.0)	0.91
TuM2-PK (U/ml)			
≤30	48	1	
>30	23	21.2(2.8-160.9)	0.003
TK1(Du/L)			
≤170	60	1	
>170	11	18.2 (1.4-231.2)	0.03

CONCLUSIONS

The presence of tumor necrosis is associated with increased plasma levels of TuM2-PK.

The presence of tumor necrosis has adverse affect on the TK1 activity, explaining the low activity of this enzyme in serum of patients with high stage and grade RCC.

Preoperative TuM2-PK and TK1 predict disease recurrence in ccRCC patients after nephrectomy. The measurements of TuM2-PK and TK1 may offer valuable prognostic information in selecting patients for adjuvant therapy after nephrectomy.