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ORIGINAL ARTICLE

Thymidine Kinase-1 as Additional Diagnostic Marker of Prostate Cancer

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SUMMARY

Background: **Thymidine kinase-1 (TK-1) is associated with proliferation and malignancy and has been extensively studied as a diagnostic biomarker for a variety of tumors, but there are limited data for prostate cancer.**
Methods: **TK-1 concentrations in serum were measured in 59 patients with prostate cancer (mean age 68 years) and in the control group of 28 healthy men (mean age 63 years) using commercially available enzymatic immunoassay (LSBio, Inc., Seattle, WA, USA). The patients were divided with respect to the severity of the disease into two groups according to the European Association of Urology (EAU) guidelines (Stage 1, 2 - less severe tumors, stage 3 - severe tumors).**
Results: **Serum thymidine kinase-1 concentrations were significantly elevated in the group of the patients with prostate cancer compared to the healthy individuals (0.204 pmol/L vs. 0.072 pmol/L, with $p < 0.0001$). Diagnostic efficiency of serum TK-1 concentrations was 0.792 with the specificity of 53.6% and sensitivity of 94.9%. Patients with less severe tumors (Stage 1, 2) and severe tumors (Stage 3) had significantly increased levels of TK-1 as well ($p < 0.0001$). Combination of TK-1 and PSA investigation in patients with PCa improve the diagnostic validity of TK-1 (AUC = 0.87).**
Conclusions: **Concentrations of thymidine kinase 1 are increased in all patients with prostate cancer and even more in patients with severe prostate cancer. Thymidine kinase 1 appears to be a promising additional diagnostic marker promising in patients with prostate cancer.**
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INTRODUCTION

Prostate cancer (PCa) is one the most frequent malignancy of men and the third most common cause of death in Czech Republic men [1]. Currently, prostate-specific antigen (PSA) is the most widely used conventional serum marker including serum free PSA and fPSA/tPSA ratio. PSA velocity, PSA density, [-2] proPSA, and prostate health index (PHI) improve specificity, but values of these biomarkers might be affected by many processes [1,2]. Despite all of these markers, 100% conclusively precise diagnostic test for PCa has not yet been introduced. Thymidine kinase is an en-

zyme, a phosphotransferase (a kinase): 2'-deoxythymidine kinase, ATP-thymidine 5'-phosphotransferase. It is present in two forms in mammalian cells, TK-1 and TK-2. Two human TK genes encode two isoenzymes. The first form, TK-1, is located on chromosome 17q-25.3. TK-1 is present in the cytoplasm of cells and TK-1 is dependent on cell cycle. The second form is mitochondrial enzyme TK-2. This form is located on chromosome 16q21 and is cell cycle-independent [3,4]. TK-1 has been extensively studied as a diagnostic biomarker for a variety of cancer types, because TK-1 is the biomarker of proliferation and is involved in the pathway of DNA precursor synthesis [5-7]. TK-1 has been expressed in proliferating and malignant cells [6,8]. Elevated TK-1 activities or concentrations have been found in many tumors including gastric, ovary, cervical, esophageal, lung, prostate, and breast cancers [9].

The aim of our study was to investigate serum levels of TK-1 as a potential diagnostic biomarker in patients with prostate cancer.

MATERIALS AND METHODS

The serum samples of patients with prostate cancer were obtained in the morning before prostatectomy. The cancer diagnosis was performed by histological examination of tumor specimens obtained by prostate resection. The samples of patients and healthy individuals were aliquoted and frozen immediately and kept at -70°C until TK-1 was analyzed. Serum concentrations of TK-1 were measured in 59 patients (mean age 68, range 45 - 82 years) with prostate cancer, and the control group consisting of 28 healthy men (mean age 63 years, range 54 - 78 years) with non-malignant etiology of the disease including benign prostate hyperplasia. All subjects were informed about the project and signed an informed consent. The characteristics of the patients and healthy subjects are summarized in Table 1. Serum TK-1 levels were assayed using a commercially available immunoassay ELISA kit (LSBio, Inc., Seattle, WA, USA). The analytical parameters of the kit were: detection limit 0.063 pmol/L and working range 0.063 - 4.0 pmol/L (intra-assay CV = 5.3%/inter-assay CV = 8.6%). Serum PSA levels were assayed using the electrochemiluminescence sandwich immunoassay on the Cobas e6000 analyzer (Hitachi, High Technology Corp., Tokyo, Japan). The patients were previously clinically investigated and classified according the TNM classification [3]. For further investigation, the patients were divided into two groups according to the severity of the disease. The first group consisted of patients with less severe tumors (Stage 1 and 2), and the second group consisted of patients with severe tumors (Stage 3).

Statistical analysis

The differences between the subgroups were tested for the statistical significance by the nonparametric Mann-Whitney test. A value of $p < 0.005$ was considered statistically significant. Receiving operating curve (ROC) analysis was used to examine the diagnostic efficiency. Analysis of variance was used to evaluate the correlation of TK-1 levels with age and PSA levels. The statistical software MedCalc version: 18.02.01 (Ostende, Belgium) was used for the statistical analysis.

RESULTS

The serum levels of thymidine kinase-1 in patients with prostate cancer were significantly increased compared with the control group of healthy men (median = 0.204 pmol/L vs. median = 0.072 pmol/L, $p < 0.0001$, Mann-Whitney test, Figure 1). Diagnostic efficiency of serum TK-1 expressed as AUC calculated from the ROC analysis was 0.792 (specificity = 53.6% and sensitivity = 94.9%) (Figure 2). The serum levels of TK-1 in patients with pT1, 2 and pT3 stages were significantly increased compared with the control group ($p < 0.0001$ and $p = 0.0026$, respectively, one-way analysis of variance) (Figure 3). The AUC for the combination of TK-1 and PSA is higher than those for the individual TK-1 (AUC = 0.87 vs. AUC = 0.79, $p = 0.19$) (Figure 4).

DISCUSSION

The results of the pilot study confirmed the relevant role of TK-1 in the prostate cancer diagnosis.

We proved that the serum levels of TK-1 were significantly increased in the patients with prostate cancer compared with the healthy individuals. These results correlate with results of other studies [6,8]. These findings correlated with the results previously published in study of Jagarlamundi et al. [6] with AUC = 0.88, sensitivity = 0.64, and specificity = 0.96. The presented study showed very good diagnostic efficiency of serum TK-1 values (AUC = 0.79, with specificity 54% and sensitivity 95%). The differences in AUC found in our study and in study of Jagarlamundi et al. are not significant ($p = 0.14$).

These results show that investigation of serum TK-1 levels indicate that the determination of TK-1 concentration in serum might be a useful test even for the screening of individuals for prostate cancer risk. Recent publications mentioned the investigation of catalytic activity of TK-1 with respect to the tumor proliferation and progression; nevertheless, the diagnostic power of the TK-1 concentration seems to be higher than for the TK-1 catalytic activity. TK-1 concentrations show greater sensitivity for the solid tumors and show more consistent TK-1 values in different disease types [7]. We found significant differences between serum TK-1 concentrations in control group of healthy individuals and patients according to the pT1 and 2, and pT3 clinical stages ($p < 0.0001$ and $p = 0.0026$, respectively). These results correlate with the fact, that TK-1 expres-

Table 1. Characteristics of patients and healthy subjects.

	Control group (n = 28)	Patients (n = 59)	p-value
Mean age (range)	64 (55 - 78)	68 (45 - 82)	-
S-TK-1 (pmol/L)	0.072 (0.063 - 0.364)	0.204 (0.063 - 1.40)	< 0.0001
PSA (µg/L)	1.25 (0.29 - 2.34)	7.32 (3.02 - 19.27)	< 0.0001

Age expressed as mean (min - max); concentrations S-Thymidine kinase-1 and PSA are expressed as median (min - max), p-value is calculated with the Mann-Whitney test.

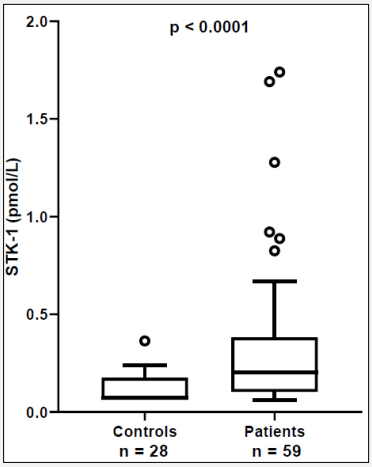

Figure 1. Serum TK-1 levels in healthy individuals and patients with prostate cancer (pmol/L).

Results are expressed as box-and-whisker plots with medians (IQR, 25th - 75th percentiles).

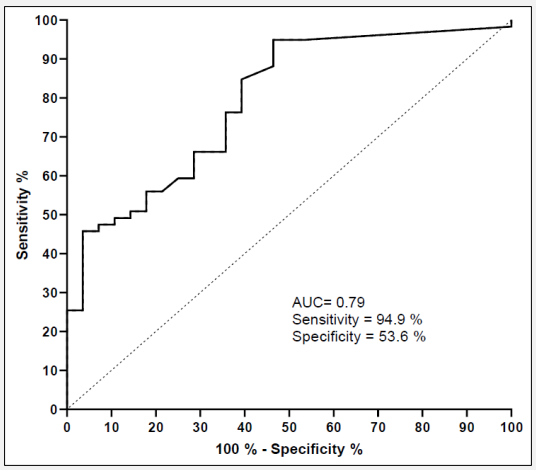

Figure 2. Diagnostic efficiency of TK-1.

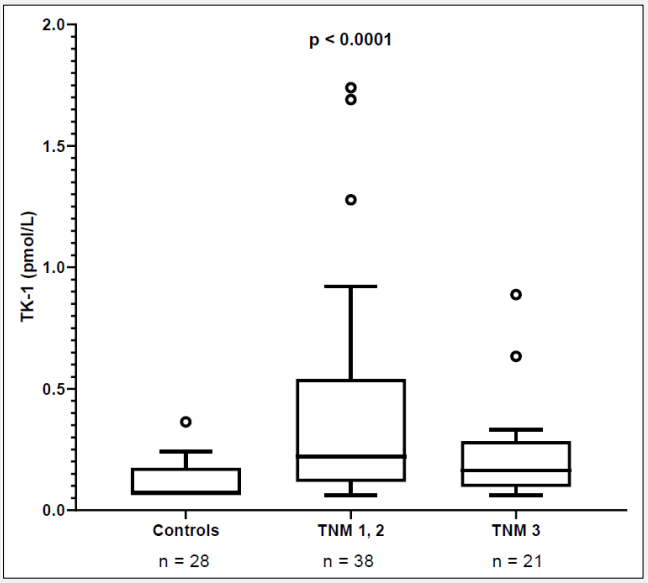

Figure 3. Serum TK-1 levels in patients with less severe and severe prostate cancer tumors.

Results are expressed as box-and-whisker plots with medians (IQR, 25th - 75th percentiles).

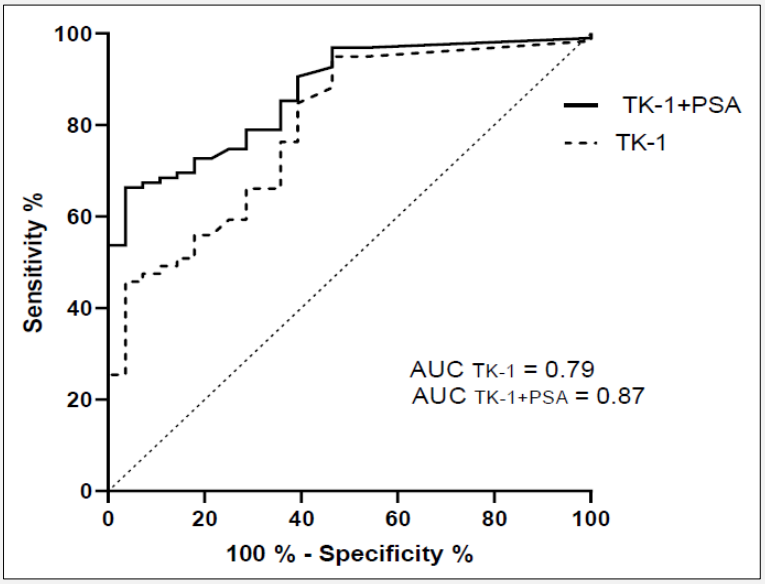

Figure 4. The diagnostic performance of TK-1 and PSA in patients with CaP.

sion is mostly associated with tumor proliferation. Recent publications also show the association of TK-1 concentrations with the grading of the disease according to the Gleason score (GS). We found significant differences between control group of healthy individuals and patients with GS = 5 + 6 and GS = 7 ($p = 0.002$ and $p < 0.0001$, respectively) as well. The results of our study were consistent with those published by Jagarlamudi et al. and Li et al. [6,8], which demonstrated that TK-1 levels correlate with the Gleason score. We also found that serum TK-1 levels are not significantly associated with PSA levels and age ($p = 0.41$, analysis of variance). We also confirmed that the AUC for the combination of TK-1 and PSA is higher than for the individual TK-1 (AUC = 0.87 vs. AUC = 0.79, $p = 0.19$). This finding lead to the opinion, that the combination of S-TK-1 and PSA investigation in patients with PCa improved the diagnostic validity of TK-1.

We also investigated other possible prostate cancer biomarkers (endoglin, TIMP-1, SPINK-1, chromogranin A and annexin A3), but none of them showed significant diagnostic power compared to TK-1 (AUC varied from 0.51 to 0.62). Our previous study confirmed that investigation of mindin levels in serum seems to be relevant for the diagnosis of prostate cancer. The concentrations of mindin in patients with prostate cancer are significantly decreased compared with the control group with AUC = 0.70 and are also correlated with the Gleason score and the staging of the cancer [10].

The presented study is just a pilot study, and our results need to be confirmed with a larger number of samples. It is important to emphasize, that TK-1 concentrations are assay dependent.

CONCLUSION

Concentrations of thymidine kinase-1 in serum are increased in patients with prostate cancer. They are not significantly related to the age and PSA levels. TK-1 appears to be a promising additional diagnostic marker useful in the diagnosis of prostate cancer.

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Declaration of Interest:
The authors state that there are no conflicts of interest regarding the publication of this article.

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