ANTICANCER RESEARCH
International Journal of Cancer Research and Treatment
ISSN: 0250-7005

Serum Thymidine Kinase 1 – Potential Prostate Cancer Biomarker: A Clinical Study

JAKUB REZAC[1], LENKA HANOUSKOVA[2], STEPAN VESELY[3], KAREL KOTASKA[2], ALZBETA KANTOROVA[1], ANNA LINHARTOVA[1], VOJTECH FIALA[1], VIKTOR SOUKUP[1] and OTAKAR CAPOUN[1]

[1]Department of Urology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic;
[2]Department of Medical Chemistry and Clinical Biochemistry, Motol University Hospital, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic;
[3]Department of Urology, Motol University Hospital, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Reprinted from
ANTICANCER RESEARCH *43*: 1675-1680 (2023)

ANTICANCER RESEARCH

International Journal of Cancer Research and Treatment

HighWire Press® STANFORD UNIVERSITY

ISSN (print): 0250-7005
ISSN (online): 1791-7530

Editorial Board

Editorial Office: International Institute of Anticancer Research, 1st km Kapandritiou-Kalamou Rd., Kapandriti, P.O. Box 22, Attiki 19014, Greece. Tel / Fax: +30-22950-53389.

U.S. Branch: Anticancer Research USA, Inc., 111 Bay Avenue, Highlands, NJ 07732, USA.

E-mails: Editorial Office: journals@iiar-anticancer.org
Managing Editor: editor@iiar-anticancer.org

ANTICANCER RESEARCH supports: (a) the establishment and the activities of the INTERNATIONAL INSTITUTE OF ANTICANCER RESEARCH (IIAR; Kapandriti, Attiki, Greece); and (b) the organization of the International Conferences of Anticancer Research. The IIAR is a member of UICC. For more information about ANTICANCER RESEARCH, IIAR and the Conferences, please visit the IIAR website: www.iiar-anticancer.org

Publication Data: ANTICANCER RESEARCH (AR) is published bimonthly from January 1981 to December 2008 and monthly from January 2009. Each annual volume comprises 12 issues. Annual Author and Subject Indices are included in the last issue of each volume. ANTICANCER RESEARCH Vol. 24 (2004) and onwards appears online with Stanford University HighWire Press from April 2009. All published articles are deposited in PubMed Central.

Annual Subscription Rates 2023 per volume: Institutional subscription US$ 1,898.00 (online) or US$ 2,277.00 (print & online). Personal subscription US$ 897.00 (online) or US$ 1,277.00 (print & online). Prices include rapid delivery and insurance. The complete previous volumes of Anticancer Research (Vol. 1-42, 1981-2022) are available at 50% discount on the above rates.

Subscription Orders: Orders can be placed at agencies, bookstores, or directly with the Publisher. (e-mail: subscriptions@iiar-anticancer.org)

Advertising: All correspondence and rate requests should be addressed to the Editorial Office.

Book Reviews: Recently published books and journals should be sent to the Editorial Office. Reviews will be published within 2-4 months.

Articles in ANTICANCER RESEARCH are regularly indexed in all bibliographic services, including Current Contents Life Sciences and Medical Sciences, Science Citation Index Expanded, Index Medicus, Biological Abstracts, PubMed, PubMed Central, Chemical Abstracts, BIOSIS, Previews, Essential Science Indicators, Excerpta Medica, University of Sheffield Biomedical Information Service, Current Clinical Cancer, AIDS Abstracts, Elsevier Bibliographic Database, EMBASE, Compendex, GEOBASE, EMBiology, Elsevier BIOBASE, FLUIDEX, World Textiles, Scopus, Progress in Palliative Care, Cambridge Scientific Abstracts, Cancergram (International Cancer Research Data Bank), MEDLINE, Reference Update - RIS Inc., PASCAL-CNRS, Inpharma-Reactions (Datastar, BRS), CABS, Immunology Abstracts, Telegen Abstracts, Genetics Abstracts, Nutrition Research Newsletter, Dairy Science Abstracts, Current Titles in Dentistry, Inpharma Weekly, BioBase, MedBase, CAB Abstracts/Global Health Databases, Investigational Drugs Database, VINITI Abstracts Journal, Leeds Medical Information, PubsHub, Sociedad Iberoamericana de Información Científica (SIIC) Data Bases.

D.T.P. BY IIAR
PRINTED BY ENTYPO, ATHENS, GREECE. PRINTED ON ACID-FREE PAPER

ANTICANCER RESEARCH *43*: 1675-1680 (2023)
doi:10.21873/anticanres.16319

Serum Thymidine Kinase 1 – Potential Prostate Cancer Biomarker: A Clinical Study

JAKUB REZAC[1], LENKA HANOUSKOVA[2], STEPAN VESELY[3], KAREL KOTASKA[2], ALZBETA KANTOROVA[1], ANNA LINHARTOVA[1], VOJTECH FIALA[1], VIKTOR SOUKUP[1] and OTAKAR CAPOUN[1]

[1]Department of Urology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic;
[2]Department of Medical Chemistry and Clinical Biochemistry, Motol University Hospital, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic;
[3]Department of Urology, Motol University Hospital, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Abstract. *Background/Aim: Serum thymidine kinase 1 (STK1) is a proliferation biomarker that has been used as a diagnostic marker of several malignant diseases. However, there are limited data for prostate cancer (PCa). Patients and Methods: In this study, we retrospectively analysed serum samples from 169 patients with biopsy confirmed PCa, who had been indicated for radical prostatectomy (RP) between 2013-2016. The results were compared with those in serum samples from 39 healthy men. We used commercially available enzymatic immunoassay to determine the levels of STK1. The patients were divided into groups according to the Gleason score (GS) and risk factors for adjuvant radiotherapy (aRT), which were defined as GS 8-10, pT3, and a positive surgical margin. Results: The median serum level of STK1 in PCa patients was 0.289 pmol/l. In the control group, the median value was 0.0116 pmol/l ($p<0.001$). By comparing the patients with GS≤6 vs. 7 vs. ≥8 ($p=0.01$), we found statistically significant differences. In the correlation of STK1 values with risk factors, we found statistically significant differences both in comparison of 0 vs. 1 vs. 2 vs. 3 risk factors ($p=0.021$), as well as ≤1 vs. 2≥ risk factors ($p=0.009$). Conclusion: The levels of STK1 are significantly higher in patients with PCa than those in healthy controls. Furthermore, STK1 values correlate with GS and predefined risk factors for aRT. Therefore, STK1 can be considered as a potential tumour marker of PCa diagnosis and risk stratification.*

Correspondence to: Otakar Capoun, MD, Ph.D., General Faculty Hospital, Ke Karlovu 6, 128 08, Prague, Czech Republic. Tel: +420 242967847, email: otakar.capoun@vfn.cz

Key Words: Prostate cancer, thymidine kinase 1, STK1, serum biomarker.

Prostate cancer (PCa) is the second most common male malignancy, accounting for 14% of all diagnosed cancer cases with the incidence steadily growing (1). Currently, it is the third most common cause of cancer mortality among men, accounting for just over 10% of all cancer-related deaths (2). The prostate-specific antigen (PSA) is currently the most frequently used tumour marker in the early detection of PCa, despite its low specificity and low negative predictive value (3). False positives trigger unnecessary biopsies with a certain rate of complications. Furthermore, PCa represents a wide spectrum of diagnoses, ranging from clinically indolent to aggressive, high-grade cancers. PSA-based screening leads to overdiagnosis and overtreatment (4), escalating the overall cost of treatment. New markers could lead to better differentiation of significant cancer as well as improved monitoring of the disease. Therefore, research for novel cost-effective serum and urine diagnostic biomarkers with higher accuracy is needed.

Although almost one hundred potentially useful urine and serum markers for PCa have been reported, none of these have replaced PSA on its own or in a combination with other tumour markers (3, 5, 6). One of the main cancer characteristics is uncontrolled cell proliferation. Proliferative activity of cancer cells correlates with the aggressiveness of the disease. Predictive markers capable of measuring tumour-cell proliferation are clinically valuable because they may improve chances of early detection of tumour-related diseases, as well as its monitoring during therapy (7). Serum thymidine kinase 1 (STK1) is a proliferation biomarker that has already been used as a diagnostic marker for several malignant diseases (8).

STK1 is a cellular enzyme involved in the salvage pathway of DNA precursor synthesis. It catalyses the conversion of thymidine to deoxythymidine monophosphate, which is further phosphorylated to deoxythymidine di- and triphosphates prior incorporation into DNA (9).

Table I. *The characteristics of the patients and healthy subjects.*

Variable	Patients=169	Controls=39	*p*-Value
STK1, pmol/l; mean (SD; min - max)	0.289 (0.289; 0.062-1.78)	0.012 (0.0742; 0.0625-0.364)	<0.001
STK1 density, pmol/l/ml; mean (SD; min - max)	0.825 (0.902; 0.066-6.538)	X	X
iPSA, ng; mean (SD; min - max)	9.522 (7.929; 1.770-68.75)	X	X
iPSA density, ng/ml; mean (SD; min - max)	0.258 (0.208; 0.039-1.803)	X	X
Age, years; mean (SD; min - max)	64.928 (6.363; 40.9-79.1)	65.923 (5.441; 55-80)	0.475
Gleason score; n (%)			
6	68 (40)	X	X
7	79 (47)	X	X
8 and more	22 (13)	X	X
Stage; n (%)			
Localised (pT2)	110 (65)	X	X
Locally advanced	59 (35)	X	X
Surgical margin; n (%)			
Positive	47 (28)	X	X
Negative	122 (72)	X	X

STK1: Serum thymidine kinase 1; iPSA: prostate-specific antigen at the time of diagnosis; SD: standard deviation.

The activity of STK1 is cell-cycle dependent and shows a different pattern in normal proliferating cells compared with tumour cells. In normal cells, STK1 activity reaches its peak at late G1 phase/early S phase (10-20-fold increase) and is dramatically reduced to undetectable levels by the end of M phase (10, 11). However, STK1 activity may remain elevated in G2 and M phases of the cell cycle in malignant cells (12), most likely due to disordered regulation of transcription and degradation. High levels of STK1 have been observed in proliferating and malignant cells (13, 14).

In our pilot study, we measured increased concentrations of STK1 in patients with PCa (15). Our aim was to confirm these results in a larger study. We believe that STK1 may prove to be a cost-efficient and minimally invasive diagnostic and monitoring tool for PCa.

Patients and Methods

Patient selection. We performed a retrospective analysis of prospectively collected serum samples from 169 patients with PCa scheduled for radical prostatectomy (RP) between 2013-2016. The results were compared with those in the serum from 39 healthy male volunteers, with an average age of 61 years, without relevant urological or oncological medical history, with negative urine bacterial culture and urine cytology, PSA level under 2 μg/l, and negative digital rectal examination. The Institutional Ethics Committee approved the study. All participants gave their written informed consent.

Sample and data collection. Blood was collected from all patients before RP after overnight fasting via puncture of the cubital vein. The blood was then centrifuged for 10 min at 3,000 rpm (1,450 g) and the serum was aliquoted, immediately frozen, and kept at –70˚C until STK1 was analysed. Radical prostatectomy specimens were examined by a specialized pathologist. Tumours were classified according to the tumour, node and metastasis (TNM) classification (16) and graded according to the Gleason score (GS) (17). Healthy volunteers underwent the same blood sampling procedure.

Concentration measurements. For both groups, we measured the STK1 marker level in the serum with the use of enzyme-linked immunosorbent assay (ELISA), utilizing a commercially available immunoassay technique ELISA kit (LSBio, Inc, Seattle, WA, USA).

The analytical parameters of the kit were as follows: detection limit 0.063 pmol/l and working range 0.063-4.0 pmol/l [intra-assay coefficient of variation (CV)=5.3%/inter-assay CV=8.6%]. Serum PSA levels were measured using the electrochemiluminescence sandwich immunoassay on the Cobas e6000 analyser (Hitachi, High Technology Corp., Tokyo, Japan).

For statistical analysis, the patients were divided into groups in accordance with GS and risk factors for adjuvant radiotherapy (aRT), which were defined as GS 8-10, pT3 or a positive surgical margin.

Statistical analysis. Statistical data analysis was performed using SAS software (SAS Institute Inc., Cary, NC, USA). Basic statistical data such as mean, standard deviation, variance, median, interquartile range, minimum and maximum were calculated for the measured parameters. For categorical variables, their absolute and relative frequencies were examined. Nonparametric tests (Wilcoxon's two-sample test and its generalised variant, the so-called Kruskal–Wallis test) were used to compare the distributions of the examined parameters between the tested groups. We tested the age agreement between the examined groups using Two One Sample Test. The relationships between the parameters were investigated using the Spearman correlation coefficient and were expressed graphically using linear regression. Statistical significance was determined at alpha=5%.

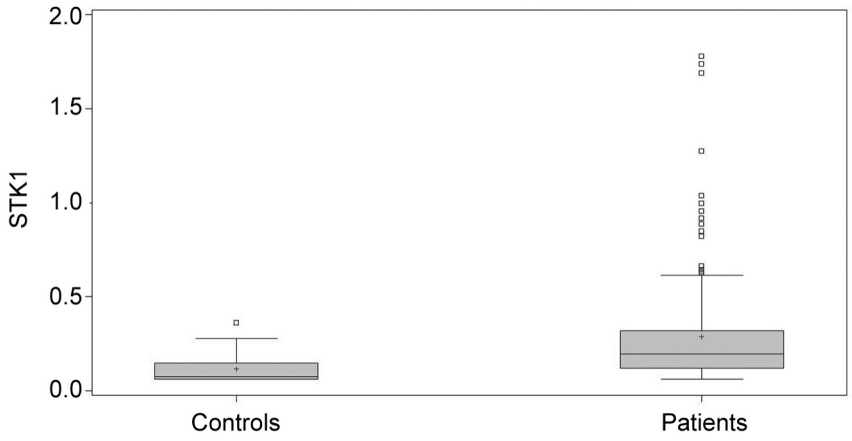

Figure 1. *Levels of serum thymidine kinase 1 (STK1) (pmol/l) in patients with confirmed prostate cancer and healthy controls.*

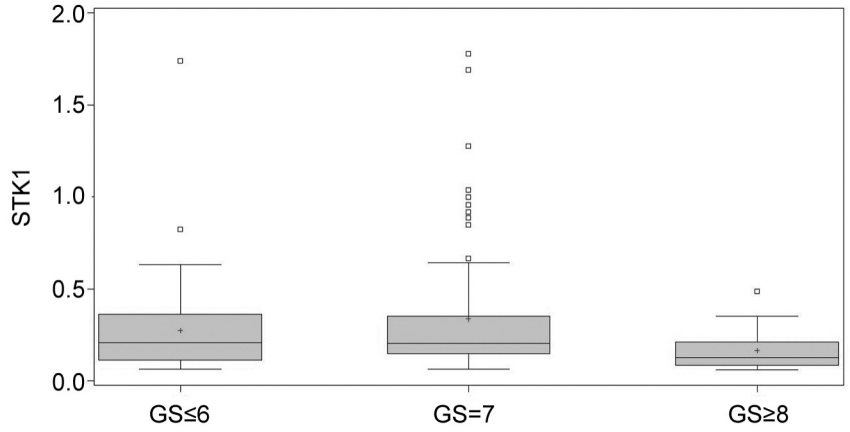

Figure 2. *Correlation between Gleason score and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

Results

The study included 169 patients with PCa aged between 40 and 79 years and 39 healthy subjects aged between 53 and 78 years. The characteristics of the patients and healthy subjects are summarized in Table I.

The serum levels of STK1 in PCa patients were significantly increased as compared to those in the control group of healthy subjects. The median STK1 level detected in PCa patients was 0.289 pmol/l [standard deviation (SD)=0.289; min-max 0.062-1.78], whereas in the control group was 0.012 pmol/l (SD=0.0742; min-max 0.063-0.364) (p<0.001) (Figure 1, Table I).

When comparing patients with GS≤6 *vs.* 7 *vs.* ≥8 (p=0.01) (Figure 2, Table II), we found a statistically significant difference, but not when comparing patients with GS≤6 *vs.* ≥7 (p=1.000). Staging did not show any significant difference neither in the comparison of pT2 *vs.* pT3a *vs.* pT3b (p=0.989), nor in the comparison of pT2 *vs.* pT3a+pT3b (p=1.0) (Table II).

We found a statistically significant correlation of STK1 serum level with risk factors for aRT (GS 8-10, pT3, positive surgical margin), p=0.021 (Figure 3). The lowest levels of STK1 were found in patients with all three risk factors. The difference between patients with no risk factors and to those with at least one risk factor was not significant (p=0.579). Statistically significantly lower levels of STK1 were found when comparing patients with 0 or 1 and patients with 2 or more risk factors (p=0.009) (Table III, Figure 4).

Correlations between STK1 and PSA density, PSA at the time of diagnosis, prostate health index (PHI), and prostate size measured by TRUS were not statistically significantly different.

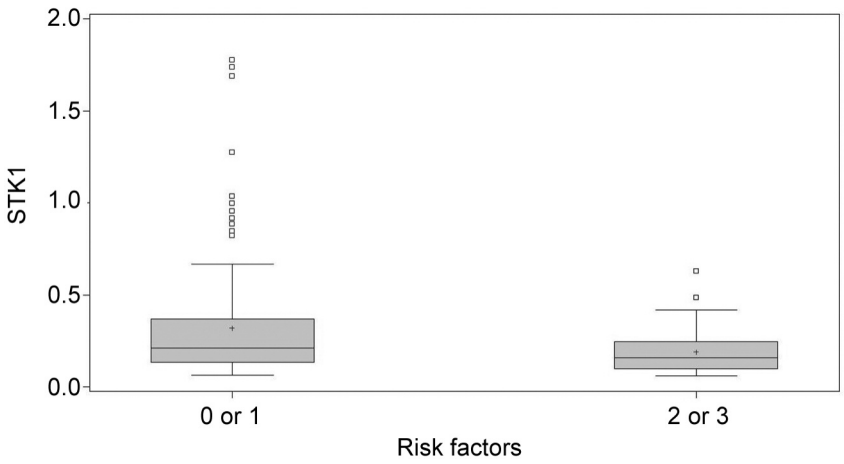

Figure 3. *Correlation between number of risk factors for adjuvant radiotherapy (Gleason score 8-10, pT3, positive surgical margin) and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

Table II. *Correlation between staging and levels of STK1.*

Parameter	STK1, pmol/l; median (SD; min-max)	*p*-Value
GS≤6	0.21 (0.25; 0.063-1.74)	0.098
GS7	0.204 (0.34; 0.062-1.78	
GS≥8	0.127 (0.111; 0.062-0.49)	
GS≤6	0.21 (0.25; 0.063-1.74)	1.0
GS≥7	0.193 (0.31; 0.062-1.78)	
pT2	0.594 (0.862; 0.066-5.235)	0.989
pT3a	0.531 (1.121; 0.134-6.538)	
pT3b	0.715 (0.467; 0.17-1.633)	
pT2	0.594 (0.862; 0.066-5.235)	1
pT3a+pT3b	0.535 (0.985; 0.134-6.538)	

STK1: Serum thymidine kinase 1; SD: standard deviation; GS: Gleason score.

Table III. *Correlation between risk factors for adjuvant radiotherapy (GS 8-10, pT3, positive surgical margin) and levels of STK1.*

Risk factors	STK1, pmol/l; median (SD; min-max)	*p*-Value
0	0.196 (0.342; 0.063-1.78)	0.0211
1	0.265 (0.271; 0.063-1.278)	
2	0.16 (0.124; 0.067-0.634)	
3	0.084 (0.085; 0.062-0.243)	
0	0.196 (0.342; 0.063-1.78)	0.579
1 or 2 or 3	0.191 (0.224; 0.062-1.278)	
0	0.196 (0.342; 0.063-1.78)	0.792
2 or 3	0.195 (0.226; 0.063-1.278)	
0 or 1	0.21 (0.319; 0.063-1.78)	0.009
2 or 3	0.158 (0.122; 0.062-0.634)	

STK1: Serum thymidine kinase 1; GS: Gleason score.

Discussion

We compared serum levels of STK1 in patients with PCa and healthy controls using an enzyme immunoassay method utilising a commercially available kit. We showed that serum levels of STK1 in patients with PCa were significantly higher (median 0.289 pmol/l) than those in the control group (median 0.0116 pmol/l).

While PSA will likely remain the most widely used prostate tumour marker in the near future, the need for other diagnostic methods, either on their own or in combination with other tumour markers, is becoming more and more urgent, in particular because of the sensitivity and specificity of the PSA test. The potential new marker must fulfil several requirements such as cost efficiency, minimal invasiveness and repeatability (18).

The results of our study confirm our hypothesis that higher STK1 level is associated with the diagnosis of PCa. The presented results are in accord with the study of Li *et al.* (19), who demonstrated that STK1 concentration and total PSA were significantly higher in patients with PCa, as compared to patients with benign prostatic hyperplasia (BPH) and healthy individuals [n=123, median 2.5 pmol/l (SD 2.0; min-max 0.2-14.7)]. Furthermore, STK1 concentration was associated with GS, whereas total PSA was not. However, no association was identified between STK1 concentration and total serum PSA. Li *et al.* also indicated the difficulty in differentiating BPH from PCa, *i.e.*, supporting our aim for the identification of markers

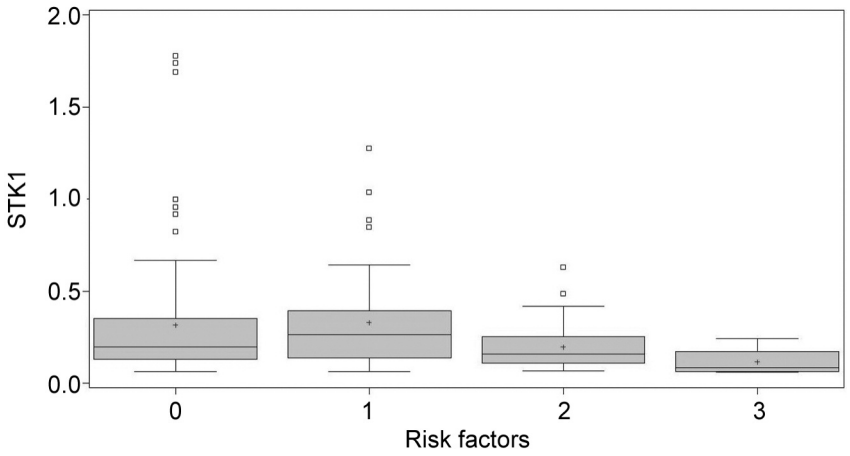

Figure 4. *Correlation between number of risk factors for adjuvant radiotherapy (Gleason score 8-10, pT3, positive surgical margin) and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

to be used in a combination with other molecules. Our results also show the association of STK1 concentrations with the grading of the disease according to GS.

Lundgren *et al.* (20) measured STK1 in 36 patients, who died of PCa [median 0.30 ng/ml (min-max 0.21-0.41)], and in 294 randomly selected healthy men, and showed that high levels of STK1 can predict PCa-related death in 30 years. Another study by Jagarlamudi *et al.* (8) compared STKa (serum thymidine kinase activity) and concentration of STK1 in patients with PCa (n=47) to those of healthy blood donors. The results demonstrated that STKa and STK1 concentration differed significantly between patients with PCa and healthy individuals.

In this study, we measured the levels of STK1 in a higher number of patients with PCa than in previous studies. In addition, unlike others, we added risk factors for aRT after RP (GS 8-10, pT3, positive surgical margin), which may prove as a useful predictive combination of parameters with clinical benefits. Our results confirm that STK1 levels are significantly higher in patients with PCa than in healthy controls. Furthermore, STK1 values correlate with the tumour GS and a number of predefined risk factors for aRT. Therefore, STK1 is a promising tumour marker for PCa. It is important to note that the current study was not a clinical trial following specific criteria, rather, it was based on data collected during routine clinical practice. This may limit the reliability of the conclusions drawn.

In the future, we plan to examine a panel of serum and urine biomarkers which could function as a reliable guidance for PCa diagnosis with higher sensitivity and specificity than PSA. In turn, the number of unnecessary prostate biopsies, which are both invasive and pose significant risks for the patients, will be reduced.

Conflicts of Interest

The Authors have no conflicts of interest to report in relation to this study.

Authors' Contributions

Rezac Jakub drafted the manuscript and prepared the figures. Measurements were performed by Hanouskova Lenka, and Kotaska Karel. Kantorova Alzbeta, Linhartova Anna and Fiala Vojtech edited the manuscript. Supervision was performed by Capoun Otakar, Soukup Viktor, and Vesely Stepan.

Acknowledgements

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organisation 00064203 (University Hospital Motol, Prague, Czech Republic). Supported by the Ministry of Health, Czech Republic – conceptual development of research organisation 00064165, General University Hospital and the First Faculty of Medicine in Prague.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *71(3)*: 209-249, 2021. PMID: 33538338. DOI: 10.3322/caac.21660

2 Health at a Glance 2019: OECD Indicators, 2019. Available at: https://www.oecd-ilibrary.org/sites/ac388762-en/index.html?itemId=/content/component/ac388762-en [Last accessed on August 30, 2022]

3 Filella X and Foj L: Emerging biomarkers in the detection and prognosis of prostate cancer. Clin Chem Lab Med *53(7)*: 963-973, 2015. PMID: 25581761. DOI: 10.1515/cclm-2014-0988

4 Dani H and Loeb S: The role of prostate cancer biomarkers in undiagnosed men. Curr Opin Urol *27(3)*: 210-216, 2017. PMID: 28212119. DOI: 10.1097/MOU.0000000000000384

5 Bratt O and Lilja H: Serum markers in prostate cancer detection. Curr Opin Urol *25(1)*: 59-64, 2015. PMID: 25393274. DOI: 10.1097/MOU.0000000000000128

6 Shariat SF, Semjonow A, Lilja H, Savage C, Vickers AJ and Bjartell A: Tumor markers in prostate cancer I: blood-based markers. Acta Oncol *50(Suppl 1)*: 61-75, 2011. PMID: 21604943. DOI: 10.3109/0284186X.2010.542174

7 He Q, Fornander T, Johansson H, Johansson U, Hu GZ, Rutqvist LE and Skog S: Thymidine kinase 1 in serum predicts increased risk of distant or loco-regional recurrence following surgery in patients with early breast cancer. Anticancer Res *26(6C)*: 4753-4759, 2006. PMID: 17214336.

8 Jagarlamudi KK, Hansson LO and Eriksson S: Breast and prostate cancer patients differ significantly in their serum Thymidine kinase 1 (TK1) specific activities compared with those hematological malignancies and blood donors: implications of using serum TK1 as a biomarker. BMC Cancer *15*: 66, 2015. PMID: 25881026. DOI: 10.1186/s12885-015-1073-8

9 Eriksson S, Munch-Petersen B, Johansson K and Eklund H: Structure and function of cellular deoxyribonucleoside kinases. Cell Mol Life Sci *59(8)*: 1327-1346, 2002. PMID: 12363036. DOI: 10.1007/s00018-002-8511-x

10 Jagarlamudi KK and Shaw M: Thymidine kinase 1 as a tumor biomarker: technical advances offer new potential to an old biomarker. Biomark Med *12(9)*: 1035-1048, 2018. PMID: 30039979. DOI: 10.2217/bmm-2018-0157

11 Sherley JL and Kelly TJ: Regulation of human thymidine kinase during the cell cycle. J Biol Chem *263(17)*: 8350-8358, 1988. PMID: 3372530.

12 Chang ZF and Huang DY: The regulation of thymidine kinase in HL-60 human promyeloleukemia cells. J Biol Chem *268(2)*: 1266-1271, 1993. PMID: 8419329.

13 He Q, Skog S and Tribukait B: Cell cycle related studies on thymidine kinase and its isoenzymes in Ehrlich ascites tumours. Cell Prolif *24(1)*: 3-14, 1991. PMID: 2009315. DOI: 10.1111/j.1365-2184.1991.tb01506.x

14 Gasparri F, Wang N, Skog S, Galvani A and Eriksson S: Thymidine kinase 1 expression defines an activated G1 state of the cell cycle as revealed with site-specific antibodies and ArrayScan assays. Eur J Cell Biol *88(12)*: 779-785, 2009. PMID: 19726104. DOI: 10.1016/j.ejcb.2009.06.005

15 Hanousková L, Řezáč J, Veselý Š, Průša R and Kotaška K: Thymidine kinase-1 as additional diagnostic marker of prostate cancer. Clin Lab *66(6)*, 2020. PMID: 32538039. DOI: 10.7754/Clin.Lab.2019.191026

16 The European Association of Urology (EAU) prostate cancer guidelines, 2022. Available at: https://uroweb.org/guidelines/prostate-cancer [Last accessed on August 30, 2022]

17 Gleason DF: Classification of prostatic carcinomas. Cancer Chemother Rep *50(3)*: 125-128, 1966. PMID: 5948714.

18 Biomarkers in cancer: an introductory guide for advocates. Available at: https://cancer.wisc.edu/research/wp-content/uploads/2019/05/Biomarkers-in-Cancer.pdf [Last accessed on December 15, 2022]

19 Li S, Zhou J, Wang Y, Zhang K, Yang J, Zhang X, Wang C, Ma H, Zhou J, He E and Skog S: Serum thymidine kinase 1 is associated with Gleason score of patients with prostate carcinoma. Oncol Lett *16(5)*: 6171-6180, 2018. PMID: 30333882. DOI: 10.3892/ol.2018.9345

20 Lundgren PO, Tribukait B, Kjellman A, Norming U, Jagarlmudi K and Gustafsson O: Serum thymidine kinase 1 concentration as a predictive biomarker in prostate cancer. Prostate *82(8)*: 911-916, 2022. PMID: 35294068. DOI: 10.1002/pros.24335

Received January 14, 2023
Revised January 28, 2023
Accepted January 31, 2023

Instructions for Authors 2023

General Policy. ANTICANCER RESEARCH (AR) will accept original high quality works and reviews on all aspects of experimental and clinical cancer research. The Editorial Policy suggests that priority will be given to papers advancing the understanding of cancer causation, and to papers applying the results of basic research to cancer diagnosis, prognosis, and therapy. Each article should include a concrete conclusion constituting a "new piece of knowledge" backed up by scientific evidence. AR will also accept the following for publication: (a) Abstracts and Proceedings of scientific meetings on cancer, following consideration and approval by the Editorial Board; (b) Announcements of meetings related to cancer research; (c) Short reviews (of approximately 120 words) and announcements of newly received books and journals related to cancer, and (d) Announcements of awards and prizes.

AR provides for the prompt print and online publication of accepted articles, generally within 1-2 months from final acceptance. Manuscripts will be accepted on the understanding that they report original unpublished works in the field of cancer research that are not under consideration for publication by another journal, and that they will not be published again in the same form. All authors should sign a submission letter confirming the approval of their article contents. All material submitted to AR will be subject to peer-review, when appropriate, by two members of the Editorial Board and by one suitable outside referee. All manuscripts submitted to AR are urgently treated with absolute confidence, with access restricted to the Managing Editor, the journal's secretary, the reviewers and the printers.The Editors reserve the right to improve manuscripts on grammar and style.

The Editors and Publishers of AR accept no responsibility for the contents and opinions expressed by the contributors. Authors should warrant due diligence in the creation and issuance of their work.

AR is a hybrid open-access journal (a subscription journal in which some of the articles are open access). All articles that are published as open access are with gold OA, which means that the final published version is permanently and freely available to anyone. Our open access articles are distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) 4.0 international license (https://creativecommons.org/licenses/by-nc-nd/4.0).

NIH Open Access Policy. The journal acknowledges that authors of NIH-funded research retain the right to provide a copy of the published manuscript to the NIH four months after publication in ANTICANCER RESEARCH, for public archiving in PubMed Central.

UKRI Open Access Policy. UKRI-funded Authors retain the right to distribute the final published version of their accepted article, such as via an institutional and/or subject repository (e.g. EuropePMC), under a Creative Commons Attribution 4.0 International (CC BY 4.0) licence (https://creativecommons.org/licenses/by/4.0/).

Copyright. Once a manuscript has been published in ANTICANCER RESEARCH, which is a copyrighted publication, the legal ownership of all published parts of the paper has been transferred from the Author(s) to the journal. Material published in the journal may not be reproduced or published elsewhere without the written consent of the Managing Editor or Publisher.

Format. Two types of papers may be submitted: (i) Full papers containing completed original work (without supplementary data), and (ii) review articles concerning fields of recognisable progress. Papers should contain all essential data in order to make the presentation clear. Reasonable economy should be exercised with respect to the number of tables and illustrations used. Papers should be written in clear, concise English. Spelling should follow that given in the "Shorter Oxford English Dictionary".

Manuscripts. Submitted manuscripts exceeding 4 printed pages will be subject to excess page charges. The 4 printed pages correspond approximately to twelve (12) document pages (~250 words per double-spaced typed page in Arial 12), including abstract, text, tables, figures, and references. Excess pages are charged US$ 230.00 each. All manuscripts should be divided into the following sections: (a) *First page* including the title of the presented work [not exceeding fifteen (15) words], full names and full postal addresses of all Authors, name of the Author to whom proofs are to be sent, key words, an abbreviated running title, an indication "review", "clinical", "epidemiological", or "experimental" study, and the date of submission. (Note: The order of the Authors is not necessarily indicative of their contribution to the work. Authors may note their individual contribution(s) in the appropriate section(s) of the presented work or before the Acknowledgements); (b) *Abstract* not exceeding 250 words, organized according to the following headings: Background/Aim – Materials and Methods/Patients and Methods – Results – Conclusion; (c) *Introduction;* (d) *Materials and Methods/Patients and Methods;* (e) *Results;* (f) *Discussion;* (g) *Conflicts of Interest;* (h) *Authors' Contributions;* (i) *Acknowledgements;* (j) *References.* All pages must be numbered consecutively. Footnotes should be avoided. Review articles may follow a different style according to the subject matter and the Author's opinion. Review articles should not exceed 35 pages (approximately 250 words per double-spaced typed page) including all tables, figures, and references.

Figures (graphs and photographs). All figures should appear at the end of the submitted document file. Once a manuscript is accepted all figures should be submitted separately in either jpg, tiff or pdf format and at a minimum resolution of 300 dpi. Graphs must be submitted as pictures made from drawings and must not require any artwork, typesetting, or size modifications. Figures should be prepared at a width of 8 or 17cm with eligible symbols, lettering and numbers. The number of each figure must be indicated. Pages that include color figures are subject to color charges (US$350.00 per page).

Tables. All tables should appear at the end of the submitted document file. Each table may have 2-10 vertical columns. Once a manuscript is accepted, each table should be submitted separately, typed double-spaced. Tables should be numbered with Roman numerals and should include a short title.

References. Authors must assume responsibility for the accuracy of the references used. Citations for the reference sections of submitted works should follow the form below and must be numbered consecutively. In the text, references should be cited by number in parenthesis. Examples: 1 Kenyon J, Liu W and Dalgleish A: Report of objective clinical responses of cancer patients to pharmaceutical-grade synthetic cannabidiol. Anticancer Res *38(10):* 5831-5835, 2018. PMID: 30275207. DOI: 10.21873/anticanres.12924 (PMIDs and DOIs only if applicable). 2 McGuire WL and Chamnes GC: Studies on the oestrogen receptor in breast cancer. In: Receptors for Reproductive Hormones. O' Malley BW, Chamnes GC (eds.). New York, Plenum Publ Corp., pp 113-136, 1973. 3 Global Health Estimates 2015: Disease Burden by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva, World Health Organisation, 2016. Available at: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index2.html [Last accessed on April 3, 2018]. (The web address should link directly to the cited information and not to a generic webpage).

Nomenclature and Abbreviations. Nomenclature should follow that given in "Chemical Abstracts", "Index Medicus", "Merck Index", "IUPAC -IUB", "Bergey's Manual of Determinative Bacteriology", The CBE Manual for Authors, Editors and Publishers (6th edition, 1994), and MIAME Standard for Microarray Data. Human gene symbols may be obtained from the HUGO Gene Nomenclature Committee (HGNC) (http://www.gene.ucl.ac.uk/). Approved mouse nomenclature may be obtained from http://www.informatics.jax.org/. Standard abbreviations are preferable. If a new abbreviation is used, it must be defined on first usage.

Clinical Trials. Authors of manuscripts describing clinical trials should provide the appropriate clinical trial number in the correct format in the text.

For International Standard Randomised Controlled Trials (ISRCTN) Registry (a not-for-profit organization whose registry is administered by Current Controlled Trials Ltd.) the unique number must be provided in this format: ISRCTNXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by "ISRCTN"). Please note that there is no space between the prefix "ISRCTN" and the number. Example: ISRCTN47956475.

For Clinicaltrials.gov registered trials, the unique number must be provided in this format: NCTXXXXXXXX (where XXXXXXXX represents the unique number, always prefixed by 'NCT'). Please note that there is no space between the prefix 'NCT' and the number. Example: NCT00001789.

Ethical Policies and Standards. ANTICANCER RESEARCH agrees with and follows the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors in 1978 and updated in October 2001 (www.icmje.org). Microarray data analysis should comply with the "Minimum Information About Microarray Experiments (MIAME) standard". Specific guidelines are provided at the "Microarray Gene Expression Data Society" (MGED) website. Presentation of genome sequences should follow the guidelines of the NHGRI Policy on Release of Human Genomic Sequence Data. Research involving human beings must adhere to the principles of the Declaration of Helsinki and Title 45, U.S. Code of Federal Regulations, Part 46, Protection of Human Subjects, effective December 13, 2001. Research involving animals must adhere to the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society. The use of animals in biomedical research should be under the careful supervision of a person adequately trained in this field and the animals must be treated humanely at all times. Research involving the use of human foetuses, foetal tissue, embryos and embryonic cells should adhere to the U.S. Public Law 103-41, effective December 13, 2001.

Submission of Manuscripts. Please follow the Instructions for Authors regarding the format of your manuscript and references. Manuscripts must be submitted only through our online submission system at: http://www.iiar-submissions.com/login.html. In case a submission is incomplete, the corresponding Author will be notified accordingly. Questions regarding difficulties in using the online submission system should be addressed to: email: journals@iiar-anticancer.org

Galley Proofs. Unless otherwise indicated, galley proofs will be sent to the corresponding Author of the submission. Corrections of galley proofs should be limited to typographical errors. Reprints, PDF files, and/or Open Access may be ordered after the acceptance of the paper. Authors of online open access articles are entitled to a complimentary online subscription to Anticancer Research for the current year and all previous digital content since 2004 (upon request to the Subscriptions Office). Galley proofs should be returned corrected to the Editorial Office by email (iiar@iiar-anticancer.org) within 24 hours.

Erratum. An erratum is published to correct any error(s) that might have been introduced to the article by the publisher during the publication process. Authors who may notice an error in the final published article should contact directly the Editorial Office.

Corrigendum. A corrigendum is published to change a part or to correct any error(s) that might have been introduced by the author(s). Authors should contact our Editorial Office, and our Editor will decide on the appropriate course of action.

Specific information and additional instructions for Authors

1. Anticancer Research (AR) closely follows the new developments in all fields of experimental and clinical cancer research by (a) inviting reviews on topics of immediate importance and substantial progress in the last three years, and (b) providing the highest priority for rapid publication to manuscripts presenting original results judged to be of exceptional value. Theoretical papers will only be considered and accepted if they bear a significant impact or formulate existing knowledge for the benefit of research progress.

2. Anticancer Research will consider the publication of conference proceedings and/or abstracts provided that the material submitted fulfils the quality requirements and instructions of the journal, following the regular review process by two suitable referees.

3. An acknowledgement of receipt, including the article number, title and date of receipt is sent to the corresponding author of each manuscript upon receipt. If this receipt is not received within 5 days from submission, the author should contact the Editorial Office to ensure that the manuscript (or the receipt) was not lost in the mail or during electronic submission.

4. Each manuscript submitted to AR is sent for peer-review (single-blind) in confidence to two-three suitable referees with the request to return the manuscript with their comments to the Editorial Office within 12 days from receipt. If reviewers need a longer time or wish to send the manuscript to another expert, the manuscript may be returned to the Editorial Office with a delay. All manuscripts submitted to AR, are treated in confidence, without access to any person other than the Managing Editor, the journal's secretary, the reviewers and the printers.

5. All accepted manuscripts are carefully corrected in style and language, if necessary, to make presentation clear. (There is no fee for this service). Every effort is made (a) to maintain the personal style of the author's writing and (b) to avoid change of meaning. Authors will be requested to examine carefully manuscripts which have undergone language correction at the pre-proof or proof stage.

6. Authors should pay attention to the following points when writing an article for AR:
   - The Instructions to Authors must be followed in every detail.
   - The presentation of the experimental methods should be clear and complete in every detail facilitating reproducibility by other scientists. • The presentation of results should be simple and straightforward in style. Results and Discussion should not be combined into one section. • Results given in figures should not be repeated in tables.
   - Photographs should be clear with high contrast, presenting the actual observation described in the legend and in the text. Each legend should provide a complete description, being self-explanatory, including technique of preparation, information about the specimen and magnification.
   - Statistical analysis should be elaborated wherever it is necessary. Simplification of presentation by giving only numerical or % values should be avoided.
   - Fidelity of the techniques and reproducibility of the results, should be points of particular importance in the discussion section. Authors are advised to check the correctness of their methods and results carefully before writing an article. Probable or dubious explanations should be avoided.
   - Authors should not cite results submitted for publication in the reference section. Such results may be described briefly in the text with a note in parenthesis (submitted for publication by… authors, year).
   - References. Each article should address, list and discuss the entire spectrum of current publications relevant to its field.
   - By following these instructions, Authors will facilitate a more rapid review and processing of their manuscripts and will provide the readers with concise and useful papers.

7. Following review and acceptance, a manuscript is examined in language and style, and galley proofs are rapidly prepared. Second proofs are not sent unless required.

8. Authors should correct their galley proofs very carefully and preferably twice. An additional correction by a colleague always proves to be useful. Particular attention should be paid to chemical formulas, mathematical equations, symbols, medical nomenclature etc. Any system of correction marks can be used in a clear manner, preferably in red. Additions or clarifications are allowed provided that they improve the presentation but do not bring new results (no fee).

9. All Authors will be asked to supply author contribution and conflict of interest forms.

10. Articles submitted to AR may be rejected without review if:
    - they do not fall within the journal's policy.
    - they do not follow the instructions for authors.
    - language is unclear.
    - results are not sufficient to support a final conclusion.
    - results are not objectively based on valid experiments.
    - they repeat results already published by the same or other authors before the submission to AR.
    - plagiarism is detected by plagiarism screening services.

    (Rejection rate (2022): 71%).

11. Authors who wish to prepare a review should contact the Managing Editor of the journal in order to get confirmation of interest in the particular topic of the review. The expression of interest by the Managing Editor does not necessarily imply acceptance of the review by the journal.

12. Authors may inquire information about the status of their manuscript(s) by calling the Editorial Office at +30-22950-53389, Monday to Friday 9.00-16.00 (Athens time), or by sending an e-mail to journals@iiar-anticancer.org

13. Authors who wish to organize and edit a special issue on a particular topic should contact the Managing Editor.

14. Authors, Editors and Publishers of books are welcome to submit their books for immediate review in AR. There is no fee for this service.

(This text is a combination of advice and suggestions contributed by Editors, Authors, Readers and the Managing Editor of AR).

Clin. Lab. 2020;66:1045-1049

ORIGINAL ARTICLE

Thymidine Kinase-1 as Additional Diagnostic Marker of Prostate Cancer

Lenka Hanousková [1], Jakub Řezáč [2], Štěpán Veselý [2], Richard Průša [1], Karel Kotaška [1]

[1] *Department of Medical Chemistry and Clinical Biochemistry, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic*
[2] *Department of Urology, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic*

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 immuno-assay (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.**
(Clin. Lab. 2020;66:1045-1049. DOI: 10.7754/Clin.Lab.2019.191026)

Correspondence:
Karel Kotaška
Department of Medical Chemistry and
Clinical Biochemistry
2nd Faculty of Medicine
Charles University
University Hospital Motol
V Úvalu 84
150 06 Prague 5
Czech Republic
Email: kotaska@email.cz

Manuscript accepted November 27, 2019

KEY WORDS

prostate cancer, thymidine kinase-1

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] pro-PSA, 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 biomark-er 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 con-trol 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.

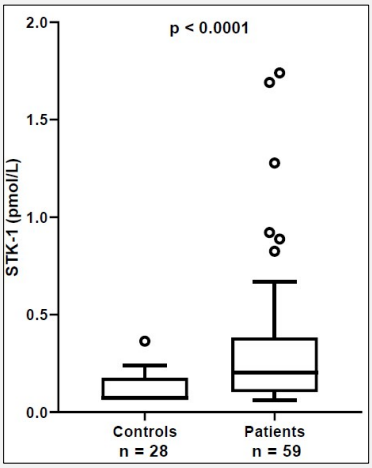

Figure 1. Serum TK-1 levels in healthy individuals and patients with prostate cancer (pmol/L).

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

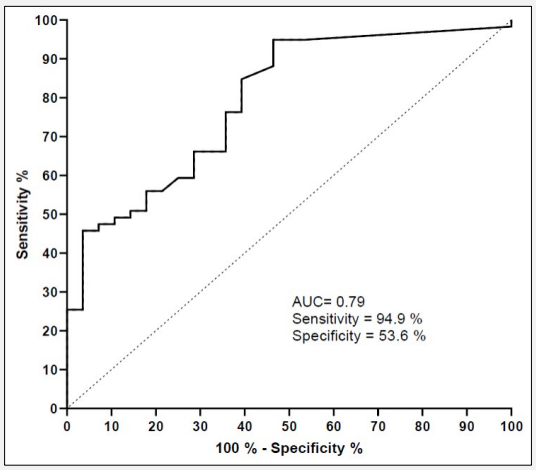

Figure 2. Diagnostic efficiency of TK-1.

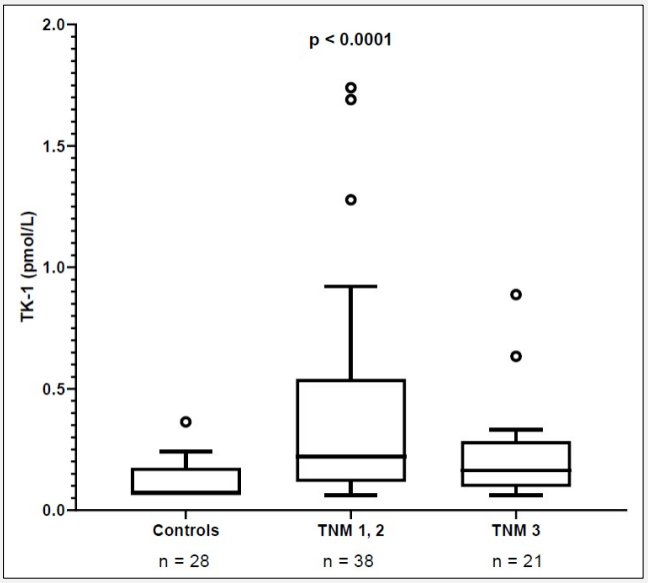

Figure 3. Serum TK-1 levels in patients with less severe and severe prostate cancer tumors.

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

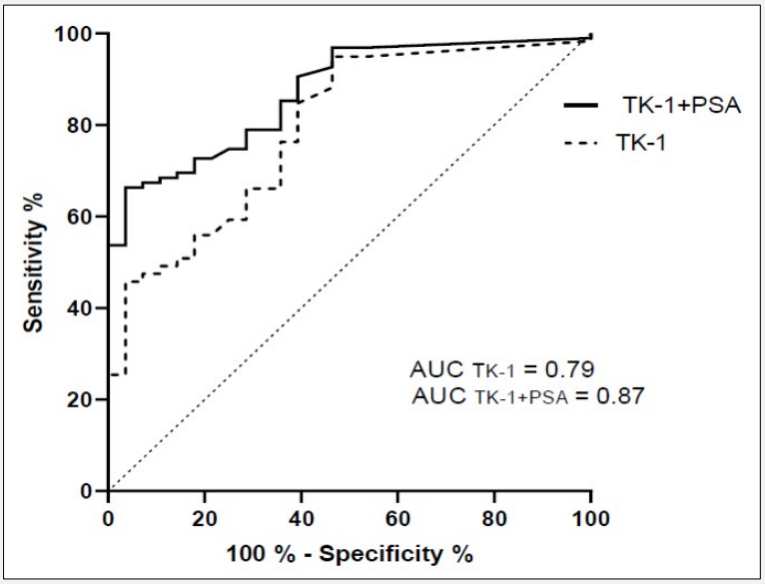

Figure 4. The diagnostic performance of TK-1 and PSA in patients with CaP.

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.

Acknowledgment:
Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic).

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

References:

1. Geryk E, Dítě P, Kozel J, et al. Other primary neoplasm in patients with prostate cancer in comparison of its incidence, mortality and prevalence. Onkologie 2010;4(2):89-94. https://www.onkologiecs.cz/pdfs/xon/2010/02/08.pdf

2. Ayyildiz SN, Ayyildiz A. PSA, PSA derivatives, proPSA and prostate health index in the diagnosis of prostate cancer. Turk J Urol 2014;40(2):82-8 (PMID: 26328156).

3. Heidenreich A, Aus G, Bolla M, et al. EAU guidelines on prostate cancer. Eur Urol 2008;53(1):68-80 (PMID: 17920184).

4. Aufderklamm S, Todenhofer T, Gakis G, et al. Thymidine kinase and cancer monitoring. Cancer Lett 2012;316(1):6-10 (PMID: 22 068047).

5. Karlstrom AR, Neumuller M, Gronowitz JS, Kallander CF: Molecular forms in human serum of enzymes synthesizing DNA precursors and DNA. Mol Cell Biochem 1990;92(1):23-35 (PMID: 2155379).

6. Jagarlamudi KK, Hansson LO, Eriksson S. Breast and prostate cancer patients differ significantly in their serum Thymidine kinase 1 (TK1) specific activities compared with those hematological malignancies and blood donors: implications of using serum TK1 as a biomarker. BMC Cancer 2015;15:66 (PMID: 258810 26).

7. Jagarlamudi KK, Shaw M. Thymidine kinase 1 as a tumor biomarker: technical advances offer new potential to an old biomarker. Biomark Med 2018, 12(9):1035-48 (PMID: 30039979).

8. Li S, Zhou J, Wang Y, Zhang K, et al. Serum thymidine kinase 1 is associated with Gleason score of patients with prostate carcino-ma. Oncol Lett 2018;16(5):6171-80 (PMID: 30333882).

9. Xiang Y, Zeng H, Liu X, et al. Thymidine kinase 1 as a diagnos-tic tumor marker is of moderate value in cancer patients: A meta-analysis. Biomed Rep 2013;1(4):629-37 (PMID: 24648999).

10. Hanouskova L, Řezáč J, Veselý Š,et al. Diagnostic benefits of mindin as a prostate cancer biomarker. Journal of Medical Biochemistry 2019 (Ahead of Print). https://content.sciendo.com/view/journals/jomb/ahead-of-print/article-10.2478-jomb-2019-0008.xml

sciendo

J Med Biochem 2019; 38 DOI: 10.2478/jomb-2019-0008

UDK 577.1 : 61 ISSN 1452-8258

J Med Biochem 38: 1–4, 2019

Short Communication
Kratko saop{tenje

DIAGNOSTIC BENEFITS OF MINDIN AS A PROSTATE CANCER BIOMARKER
DIJAGNOSTI^KE PREDNOSTI MINDINA KAO BIOMARKERA RAKA PROSTATE

Lenka Hanousková[1], Jakub Řezá~[2], [těpán Veselý[2], Richard Prů{a[1], Karel Kota{ka[1]

[1]Department of Medical Chemistry and Clinical Biochemistry, Charles University, Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic
[2]Department of Urology, Charles University Second Faculty of Medicine and University Hospital Motol, Prague, Czech Republic

Summary

Background: It has been shown that decreased expression and activity of extracellular matrix protein mindin correlate with various types of cancers including breast, colon and lung cancers. The aim of the presented study was to investigate the serum mindin levels in prostate cancer.
Methods: Mindin concentrations in serum were measured in 56 patients with prostate cancer (mean age 68 years) and in control group of 29 healthy men (mean age 64 years) using commercially available enzymatic immunoassay (Cusabio, WuHan, China). The patients were divided with respect to the severity of the disease into two groups according to the EAU guidelines (stage 1, 2 – less severe tumours, stage 3, 4 – severe tumours).
Results: Serum mindin concentrations were significantly elevated in the group of healthy individuals unlike in the patients with prostate cancer (2.12 ng/mL vs 0.78 ng/mL, with P=0.0007, AUC=0.705). Patients with less severe tumours (stage 1, 2) and severe tumours (stage 3, 4) had significantly decreased levels of S-mindin as well (P=0.0037), although the difference in serum mindin concentrations between the patients with less severe and severe tumours was not significant.
Conclusions: Concentrations of mindin were decreased in patients with prostate cancer and reduced in patients with less severe prostate cancer as well. Mindin appears to be a promising diagnostic marker useful in the diagnosis of prostate cancer.

Keywords: mindin, prostate cancer, biomarker

Kratak sadr`aj

Uvod: Pokazalo se da je smanjena ekspresija i aktivnost ekstracelularnog proteinskog matriksa mindina korelira sa razli~itim tipovima raka, uklju~uju}i rak dojke, debelog creva i plu}a. Cilj prikazane studije je bio da se ispita serumski nivo mindina kod karcinoma prostate.
Metode: Koncentracije mindina u serumu su merene kod 56 bolesnika sa karcinomom prostate (srednja starost 68 godina) i u kontrolnoj grupi od 29 zdravih mu{karaca (srednja starost 64 godine) kori{}enjem komercijalno dostupnog enzimskog testa (Cusabio, VuHan, Kina). Pacijenti su prema te`ini bolesti podeljeni u dve grupe prema EAU smernicama (faza 1, 2 – manje te{ki tumori, faza 3, 4 – te{ki tumori).
Rezultati: Koncentracije serumskog mindina bile su zna~ajno povi{ene u grupi zdravih pojedinaca, za razliku od pacijenata sa rakom prostate (2,12 ng/mL u odnosu na 0,78 ng/mL, sa P=0,0007, AUC=0,705). Pacijenti sa manje ozbiljnim tumorima (faza 1, 2) i te{kim tumorima (faza 3, 4) imali su i zna~ajno sni`ene nivoe S-mindina (P=0,0037), iako razlika u koncentracijama mindina u serumu izme u pacijenata sa manje te{kim i ozbiljnim tumori nije bila zna~ajna.
Zaklju~ak: Koncentracije mindina su smanjene kod pacijenata sa rakom prostate, a smanjene su i kod pacijenata sa manje te{kim oblikom raka prostate. Izgleda da je mindin obe}avaju}i dijagnosti~ki marker i da je koristan u dijagnostici raka prostate.

Klju~ne re~i: mindin, rak prostate, biomarker

Address for correspondence:
Karel Kota{ka
Department of Medical Chemistry and Clinical Biochemistry
2nd Faculty of Medicine, Charles University
University Hospital Motol
V Uvalu 84
150 06 Prague 5

List of abbreviations: AUC, Area under the curve; CV, Coefficient of variation; EAU, European Association of Urology; ELISA, Enzyme-linked immunosorbent assay; IQR, interquartile range; Pca, prostate carcinoma; PHI, prostate health index; PSA, Prostate-specific antigen; PSADT, Prostate-specific antigen doubling time; ROC, Receiver operating characteristics.

Introduction

Prostate cancer (PCa) is one the most frequently diagnosed tumours of men and the third most common cause of cancer death of Czech men (1). Currently, the only biomarker in wide clinical use for the diagnosis and prognosis of prostate cancer is the prostate-specific antigen (PSA). Other markers useful for the diagnostics of prostate cancer are free PSA and Free/Total PSA ratio, complexed PSA, pro PSA - 2proPSA and Prostate Health Index (PHI). Total PSA, % fPSA, f/tPSA, and other PSA derivatives, PSAD, PSA velocity, PSADT, age-specific PSA do not decrease the number of unnecessary biopsies performed for diagnostic purposes. Despite all of these markers, a 100% conclusively precise diagnostic test for PCa has not been introduced yet (2).

PSA can be detected in the serum of a blood sample and is considered to be currently the most useful tumour marker (3). PSA can be used for prostate cancer screening and monitoring of the response to the treatment. PSA seems to be useful for the detection of prostate cancer of the men whose total PSA concentration in the 4–10 mg/L range (4). Investigation of the serum PSA levels does not have a direct correlation with increasing grade and stage of prostate cancer (5). 20 % of patients have PSA levels less than 4 mg/L, 25 % of the patients have PSA levels in the interval of 4–10 mg/L. Moreover, some aggressive forms of prostate cancer can be PSA negative (6). Due to the limitations of PSA as a biomarker, there is still a need for new biomarkers that can be used as prognostic indicators of prostate cancer for effective differentiation between indolent and aggressive disease (7).

Mindin, also called spondin 2, is an extracellular matrix protein which is encoded by the SPO2 gene located in the chromosome 4p16.3. Spondin 2 belongs to the F-spondin family of secreted extracellular matrix proteins. The members of F-spondin family have three domains: FS1 (for F-spondin), FS, and thrombospondin type 1 repeats. Mindin exerts a broad spectrum of effects on the innate immune system and its role in cancer is currently investigated (8). Recently, mindin is mentioned as a candidate biomarker for prostate cancer diagnosis (9).

The study aimed to investigate serum levels of mindin as a potential diagnostic biomarker in patients with prostate cancer.

Materials and Methods

Serum samples of patients with prostate cancer were obtained in the morning before prostatectomy. The cancer diagnosis was performed by histological examination of tumour specimens obtained by prostate resection. The samples of patients and healthy individuals were frozen immediately, aliquoted and kept at -70 °C until mindin was analyzed. Serum concentrations of mindin were measured in 56 patients (mean age 68 years, range 45–82 years) with prostate cancer, and in the control group consisting of 29 healthy men (mean age 64 years, range 55–78 years). Informed consent was obtained for all of the individuals included in the study. The characteristics of the patients and healthy subjects are summarized in *Table I*.

Serum mindin levels were assayed using commercially available immunoassay technique ELISA kit (Cusabio, WuHan, China). The analytic characteristics of the diagnostic kit were as follows: detection limit 0.78 ng/mL and working range 3.12–200 ng/mL, with a mean coefficient of variation (CV)=11%. The patients were previously clinically investigated and classified according to the European Association of Urology (EAU) guidelines (10). 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 tumours (stages 1 and 2), the second group consisted of patients with severe tumours (stages 3 and 4).

Results

Differences between subgroups were tested for statistical significance by the nonparametric Mann-Whitney test. The value of $P<0.005$ was considered statistically significant. Receiving operation analysis (ROC) was used to investigate the diagnostic efficiency. The analysis of variance was used to evaluate the relationship of mindin levels with the age and PSA levels. Statistical software MedCalc version: 18.02.01 (Ostende, Belgium) was used for statistical analysis.

Serum levels of mindin in patients with prostate cancer were significantly decreased in the control group expressed as medians (2.12 ng/mL vs 0.78 ng/ml, P=0.0007, Mann-Whitney test, *Figure 1*). Diagnostic efficiency of serum mindin expressed as AUC calculated from the ROC analysis was 0.705 (specificity=73 % and sensitivity=64 %) (*Figure 2*). Serum levels of mindin in patients with less severe tumours (stages 1 and 2) and severe tumours (stages 3 and 4) were significantly decreased compared with the control group as well. (P=0.0037, One-way analysis of variance, *Figure 3*).

Discussion

The results of the pilot study confirm the relevant role of mindin in the prostate cancer diagnosis. We proved that the serum levels of mindin were significantly decreased in patients with prostate cancer compared with healthy individuals. These results differ from the results of other studies showing elevated mindin levels in patients with prostate cancer

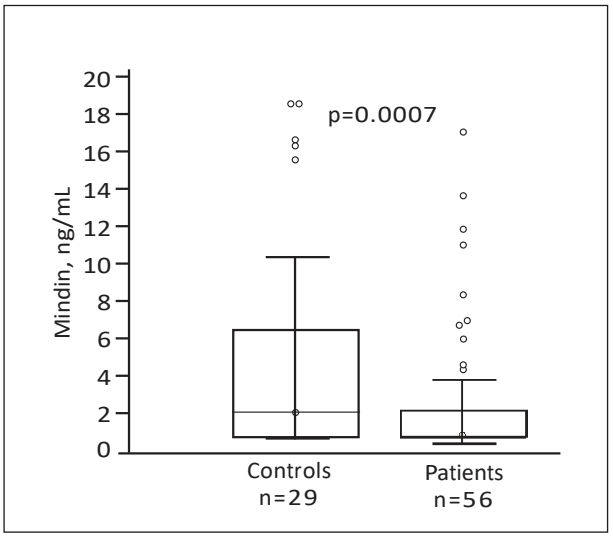

Figure 1 Serum mindin levels in healthy individuals and patients with prostate cancer.
Results are expressed as Box-and-whisker plots with medians (IQR 25. – 75. percentile).

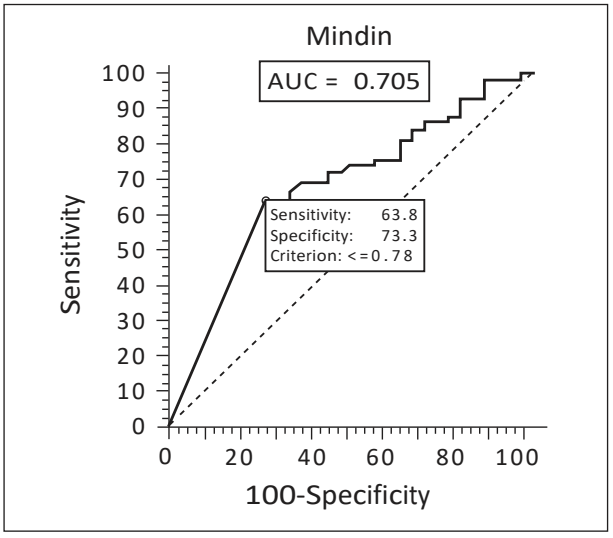

Figure 2 Diagnostic efficiency of mindin.

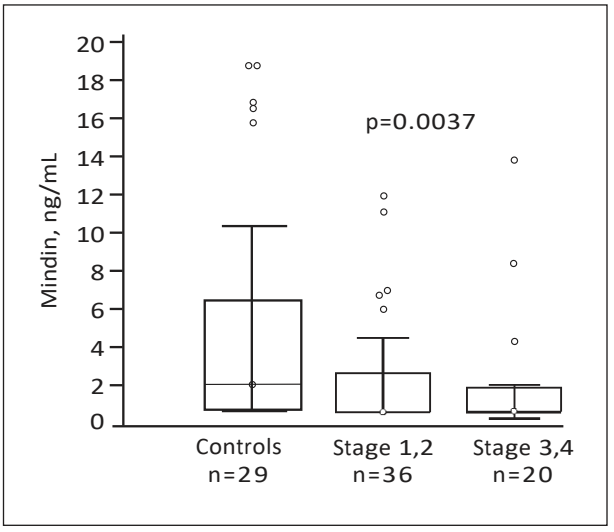

Figure 3 Serum mindin levels in patients with less severe and severe prostate cancer tumours.
Results are expressed as Box-and-whisker plots with medians (IQR 25. – 75. percentile)
Stage 1, 2 – patients with less severe tumours
Stage 3, 4 – patients with severe tumours

(11, 12, 14). Presented results are in concordance with the study of Wang et al. (8) showing significantly decreased serum mindin in tumour lesions of patients compared with adjacent control tissues by colon, lung, gastric, oesophageal, and breast cancer. Wang et al. (8) mentioned an important role of EGR-1, which directly regulates mindin expression at the transcriptional level, and this regulates both mindin mRNA and protein expression in vitro to further define EGR-1 mediated regulation of mindin expression. The relationship between EGR-1 expression and prostate cancer was previously mentioned. Gregg et al. showed that EGR-1 is downregulated in patients with prostate carcinoma according to their clinical considerations (18 of 20 patients in pT2 and pT3 clinical stages showed normal or downregulated EGR-1 expression) (12). Contrary to the previously mentioned reports of Wang et al. (8) and Gregg et al. (12), studies of other authors showed that in patients with prostate cancer EGR-1 stimulates tumour cell growth and its expression level increases with the degree of malignancy (11, 13). This seems to be specific to the prostate tumour cells because, in mammary and lung tumours as well as most normal tissues, EGR-1 expression is low. This contradictory findings regarding EGR-1 expression confirmed the bivalent role of EGR-1 either as a tumour suppressor or oncogene with respect to EGR-1 regulation and the degree of the malignancy.

Our results show good diagnostic sensitivity of mindin with AUC of 0.705. We prove that serum mindin levels are not dependent on the PSA levels, and the age (P=0.42, analysis of variance). The serum levels of mindin differ in patients with prostate cancer. We found significant differences between serum mindin concentrations related to the staging of cancer. The patients with less severe tumours belonging to stages 1 and 2 had lower serum mindin level than patients with severe tumours stages 3 and 4 as shown in *Figure 3*. These results correlate with the results found in other cancer types presented by Wang et al., (8) who presented decreased levels in patients with less severe tumours belonging to stages 1 and 2. In our study, the difference between patients in stages 1 and 2 and 3 and 4 is not significant.

The presented study is a pilot study, and thus the results need to be confirmed on a large number of samples. Various enzymatic immunoassays used for

mindin evaluation show discrepant results, as indicated in the presented study and the study of Wang et al. (8) with similar results, which are different from the study of Luccarelli et al. (14). This finding seems to indicate that the concentrations of mindin in patients with prostate cancer are assay dependent.

The concentration of mindin is decreased in patients with prostate cancer. Mindin concentration is not related to the age and PSA levels. Mindin appears to be a promising diagnostic marker useful in the diagnosis of prostate cancer.

Acknowledgements. Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic).

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Geryk E, Dítě P, Kozel J, [tampach R, Kubí~ek P, Odehnal J: Other primary neoplasm in patients with prostate cancer in comparison of its incidence, mortality and prevalence. Onkologie 2010, 4(2): 89–94.
2. Ayyildiz SN, Ayyildiz A: PSA, PSA derivatives, proPSA and prostate health index in the diagnosis of prostate cancer. Turk J Urol 2014, 40(2): 82–88.
3. Bickers B, Aukim-Hastie C: New molecular biomarkers for the prognosis and management of prostate cancer—the post PSA era. Anticancer Res 2009, 29(8): 3289–98.
4. Buhmeida A, Pyrhonen S, Laato M, Collan Y: Prognostic factors in prostate cancer. Diagn Pathol 2006, 1:4.
5. Shariat SF, Canto EI, Kattan MW, Slawin KM: Beyond prostate-specific antigen: new serologic biomarkers for improved diagnosis and management of prostate cancer. Rev Urol 2004, 6(2): 58–72.
6. Pryor MB, Schellhammer PF: The pursuit of prostate cancer in patients with a rising prostate-specific antigen and multiple negative transrectal ultrasound-guided prostate biopsies. Clin Prostate Cancer 2002, 1(3): 172–6.
7. Jeli} M, Mandi} A, Kladar N, Sudji J, Bo`in B, Srdjenovi} B. Lipid Peroxidation, Antioxidative Defense and Level of 8-Hydroxy-2-Deoxyguanosine in Cervical Cancer Patients. J Med Biochem 2018; 37: 336–45.
8. Wang LF, Liu YS, Yang B, Li P, Cheng XS, Xiao CX, Liu JJ, Li S, Ren JL, Guleng B: The extracellular matrix protein mindin attenuates colon cancer progression by blocking angiogenesis via Egr-1-mediated regulation. Oncogene 2018, 37(5): 601–15.
9. Qian X, Li C, Pang B, Xue M, Wang J, Zhou J: Spondin-2 (SPON2), a more prostate-cancer-specific diagnostic biomarker. PLoS One 2012, 7(5): e37225.
10. Heidenreich A, Aus G, Bolla M, Joniau S, Matveev VB, Schmid HP, Zattoni F: EAU guidelines on prostate cancer. Eur Urol 2008, 53(1): 68–80.
11. Gitenay D, Baron VT: Is EGR1 a potential target for prostate cancer therapy? Future Oncol 2009, 5(7): 993–1003.
12. Gregg JL, Brown KE, Mintz EM, Piontkivska H, Fraizer GC: Analysis of gene expression in prostate cancer epithelial and interstitial stromal cells using laser capture microdissection. BMC Cancer 2010 10:165.
13. Parra E, Ortega A, Saenz L: Down-regulation of Egr-1 by siRNA inhibits growth of human prostate carcinoma cell line PC-3. Oncol Rep 2009, 22(6): 1513–8.
14. Lucarelli G, Rutigliano M, Bettocchi C, Palazzo S, Vavallo A, Galleggiante V, Trabucco S, Di Clemente D, Selvaggi FP, Battaglia M et al.: Spondin-2, a secreted extracellular matrix protein, is a novel diagnostic biomarker for prostate cancer. J Urol 2013, 190(6): 2271–7.

Received: February 4, 2019

Accepted: February 20, 2019