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%.

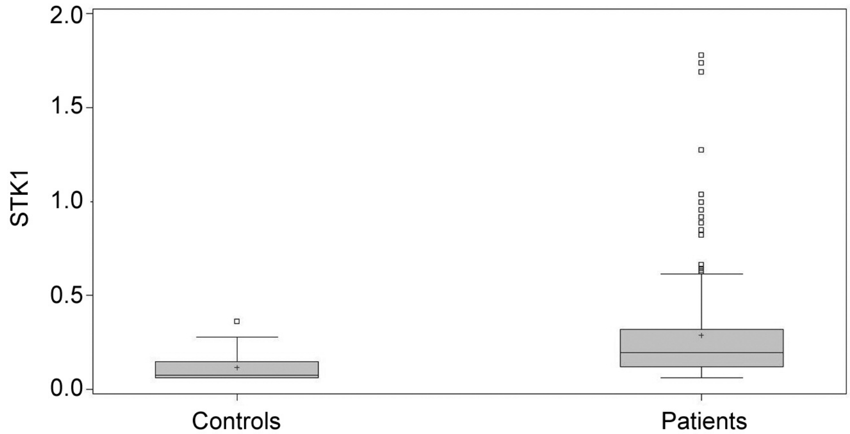

Figure 1. *Levels of serum thymidine kinase 1 (STK1) (pmol/l) in patients with confirmed prostate cancer and healthy controls.*

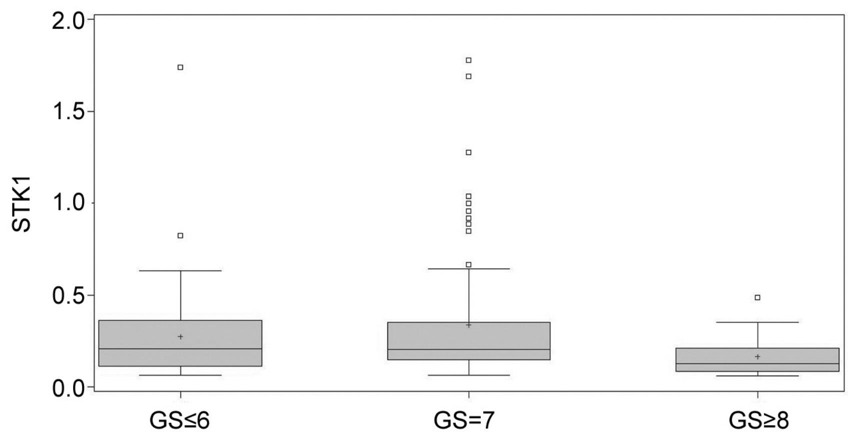

Figure 2. *Correlation between Gleason score and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

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.

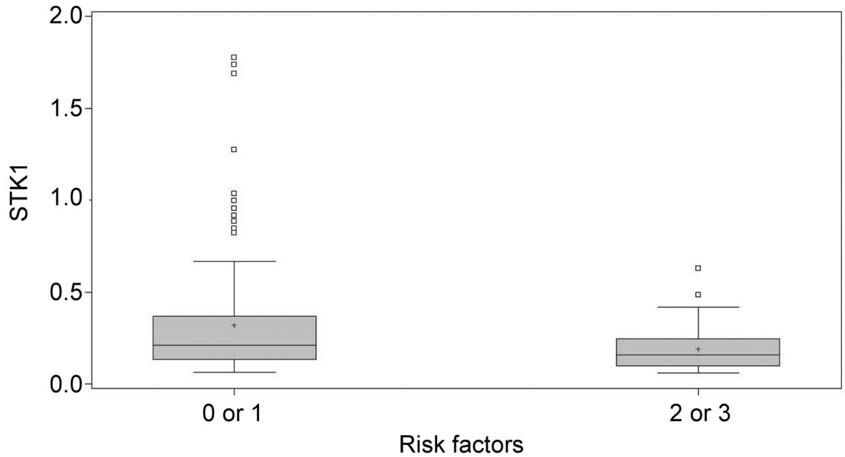

Figure 3. *Correlation between number of risk factors for adjuvant radiotherapy (Gleason score 8-10, pT3, positive surgical margin) and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

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

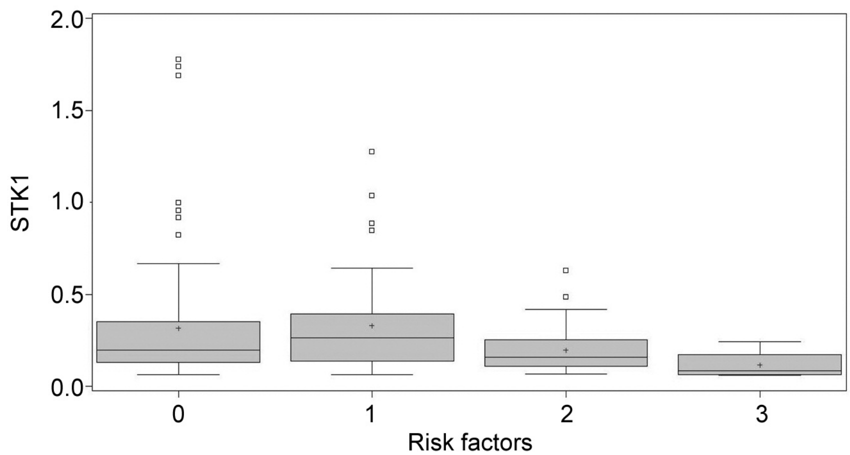

Figure 4. *Correlation between number of risk factors for adjuvant radiotherapy (Gleason score 8-10, pT3, positive surgical margin) and levels of serum thymidine kinase 1 (STK1) (pmol/l).*

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