

Evaluation of TKTL1 as a biomarker in serum of prostate cancer patients

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Introduction Monocyte associated transketolase-like 1 (TKTL1) as a cancer biomarker has become popular with alternative practitioners, but plays no role in conventional medicine. This investigation evaluates the potential of serum TKTL1 as a biomarker for prostate cancer.

Material and methods Patients (n = 66) undergoing curative radical prostatectomy (RPE) for biopsy-proven PCa were included in the study. Controls (n = 10) were healthy, age-matched, male volunteers. 10 ml of peripheral blood was drawn from patients several days before surgery and from controls. Serum TKTL1 was measured using the ELISA method.

Results The median age at tumor diagnosis was 66 years and median serum PSA was 8.0 ng/ml. Nearly 96% of PCas submitted to surgery were clinically significant. Compared to healthy controls, serum TKTL1 was significantly lower in PCa patients (p = 0.0001, effect size indicator $r = Z/\sqrt{n} = 0.4179$). No correlation was apparent between serum TKTL1 and serum PSA, Gleason sum, tumor stage or further clinical and pathologic parameters.

Conclusions Reduced serum TKTL1 in PCa patients stands in opposition to TKTL1 epitope detection in monocytes (EDIM) based studies, whereby increased TKTL1 in monocytes of tumor patients has been reported. Since serum TKTL1 does not correlate with clinical parameters in the current investigation, further research is needed to clarify whether serum TKTL1 has potential as a biomarker for PCa.

Key Words: complementary medicine ↔ prostate cancer ↔ diagnosis ↔ biomarker ↔ TKTL1

INTRODUCTION

In Europe, prostate cancer (PCa) is the most common malignancy in males and the third most common cause of cancer mortality. Currently, more extensive and earlier diagnosis has led to a decrease in PCa related mortality [1, 2]. Despite an extended search for a more specific biomarker than prostate specific antigen (PSA), PSA still remains the only widely employed diagnostic and follow-up marker for PCa [3]. The predictive capability of the 4 ng/ml

PSA threshold as a biopsy indicator is deficient since 20–40% of PCa cases are thereby missed [4]. Thus, other accurate diagnostic PCa biomarkers, especially for aggressive and potentially life-threatening PCa, are needed.

Since increased aerobic glycolysis or the 'Warburg effect' has been identified as common to neoplastic cells [5], this mechanism promises potential for diagnostic and therapeutic targets. The enzyme transketolase-like 1 (TKTL1), therefore, comes into investigational focus since it is a crucial enzyme for sugar

fermentation, linking glucose and fat metabolism without pyruvate dehydrogenase [6, 7]. When over-expressed, TKTL1 activates the pentose phosphate pathway (PPP), accelerating tumor cell growth and supporting tumor survival and systemic dissemination [8]. Thus, it has been speculated that TKTL1 could become a cancer biomarker. Accordingly, an assay was developed to evaluate TKTL1 based on the fluorometric epitope detection of specific antibodies in CD14/CD16 positive monocytes, following tumor cell phagocytosis and digestion (EDIM-test) [9]. Still, the role of TKTL1 as a cancer biomarker is controversial [10, 11] and the EDIM-test has not been approved for routine clinical application. Nevertheless, though TKTL1 does not play a role in conventional medicine, it has gained high popularity in alternative/complementary medicine, not only as a diagnostic but also as a prediction marker to assess the risk of metastatic progression.

The goal of the current investigation was to compare the TKTL1 serum level in patients with a clinically localized PCa to that in healthy controls. Furthermore, the investigation was directed towards establishing whether the TKTL1 serum level correlates with clinical and histologic parameters of the tumor, thus facilitating identification of patients harboring life-threatening disease requiring definitive treatment. For this purpose, the serum concentration of TKTL1 in PCa patients and healthy controls was analyzed by means of an enzyme-linked immunosorbent assay (ELISA), which is a highly standardized detection system [12], and correlated to clinical and histologic parameters.

MATERIAL AND METHODS

Patients (n = 66) undergoing curative radical prostatectomy (RPE) for biopsy-proven PCa in the Department of Urology, Goethe-University, Frankfurt am Main, Germany, were included in the investigation. Controls (n = 10) were healthy, age-matched, male volunteers. Approval to carry out the investigation was granted by the local medical ethics committee. Firstly, 10 ml peripheral blood was drawn from patients several days before surgery and from controls. Blood samples were allowed to coagulate and then centrifuged at 3000 rpm at +4°C for 10 minutes. The serum supernatant was stored at -80°C until further processing. After thawing, the concentration of TKTL1 was determined in PCa patient and control serum using a commercially available ELISA kit (SEH018Hu, Cloud-Clone Corp, Houston, TX, USA; sensitivity: <0.055 ng/ml with no significant cross-reactivity or interference between TKTL1 and analogues). All assays were done in duplicate and

the concentration was calculated from a standard curve using a 4-parameter curve fit (Magellan software, Tecan). Univariate analysis was performed by the Wilcoxon-Man-Whitney-test for comparison between two groups and the Kruskal-Wallis-test with the Iman-Conover-method (Bonferroni-Holm-corrected) for more than two groups. Correlation

Table 1. Clinical and histopathological demographics of 66 PCa patients

Parameter	All evaluable men (n=66) Median (range) or n (%)
Age, years	66 (46–88)
pT-stage	
≤2	47 (71.2)
≥3	19 (28.8)
Extracapsular infiltration	15 (22.7)
Infiltration of the seminal vesicle	4 (6.1)
cT-stage	
<3	63 (95.5)
≥3	3 (4.5)
Serum-PSA, ng/ml	8.0 (1.8–57.0)
PSAD, ng/ml/ml	0.2 (0.1–1.2)
Abnormal DRE	33 (50)
Prostate volume, ml	32 (19–90)
Gleason Sum (Biopsy)	
≤6	29 (43.9)
7	25 (37.9)
≥8	12 (18.2)
Highest Gleason Pattern (Biopsy)	
3	26 (39.4)
4	31 (47.0)
5	5 (7.6)
Gleason Sum (RPE)	
6	10 (15.1)
7	39 (59.1)
≥8	17 (25.8)
Highest Gleason Pattern (RPE)	
3	10 (15.1)
4	43 (65.2)
5	13 (19.7)
Change of Gleason Score	
upgrade	30 (45.5)
downgrade	8 (12.1)
Clinically significant PCa (Epstein)	63 (95.5)
D'Amico Classification	
Low risk	16 (24.2)
Intermediate risk	27 (41.0)
High risk	23 (34.8)
N+	7 (10.6)
R+	14 (21.2)
L+	10 (15.1)
V+	1 (1.5)
Pn+	47 (71.2)

Values expressed as median with range or number (%). RPE – radical prostatectomy; DRE – digital rectal examination, PSAD – PSA density

between two parameters was evaluated through Spearman's coefficient. The statistical program applied was BiASfur Windows (Version 9.11, Dr. rer. nat. Hanns Ackermann, epsilon-publishers, Frankfurt, Germany). The null hypothesis (TKTL1 concentration in serum of PCa patients does not differ from that of healthy volunteers) was rejected if p-values were less than 0.05.

The clinical tumor stage was classified according to the 7th edition of the AJCC and the pathologic tumor stage was determined according to the 6th edition of the TNM classification. Tumors were graded with the Gleason Sum (GS). Clinical and histological characteristics were collected from patient charts. Risk classification was determined according to D'Amico. Imaging was carried out according to the currently valid guidelines of the European Association of Urology. Epstein criteria were used to assess the clinical significance of the tumor.

RESULTS

Clinical and pathologic demographics of 66 patients are shown in Table 1. The median age at tumor diagnosis was 66 years (range 46–88) and the median serum PSA was 8.0 ng/ml (range 1.8–57.0) (median age control group: 60 years (55–72); control PSA: 2.8 ng/ml (2.0–4.0)). Nearly all PCas submitted to surgery were clinically significant. All patients were clinically free of visceral or bone disease. None of the patients had evident clinical signs of infection or acute or chronic inflammation at surgery. Histologically, all tumors were conventional acinar adenocarcinomas. Univariate analysis of the ELISA investigation demonstrated that serum TKTL1 was significantly lower in the serum of PCa patients, compared to healthy controls ($p=0.0001$, effect size indicator $r = Z/\sqrt{n} = 0.4179$, Figure 1). However, correlation between serum TKTL1 and serum PSA ($p = 0.38$), biopsy and prostatectomy Gleason sum ($p = 0.79$ and 0.89 , respectively), prostate volume ($p = 0.23$), PSA density ($p = 0.80$), clinical and pathologic T-stage ($p = 0.66$ and 0.65), highest Gleason pattern in the biopsy and prostatectomy specimen ($p = 0.83$ and 0.74 , respectively), upgrade of Gleason sum from biopsy to prostatectomy ($p = 0.86$), Pn-, L-, V-, N- and R status ($p = 0.32, 0.88, 0.30, 0.90$ and 0.32 , respectively), extracapsular extension, seminal vesicle invasion as well as D'Amico classification ($p = 0.75, 0.89$ and 0.34 , respectively) did not reach statistical significance.

DISCUSSION

TKTL1, as evaluated by the EDIM-test, has been propagated as a reliable cancer biomarker [9], since

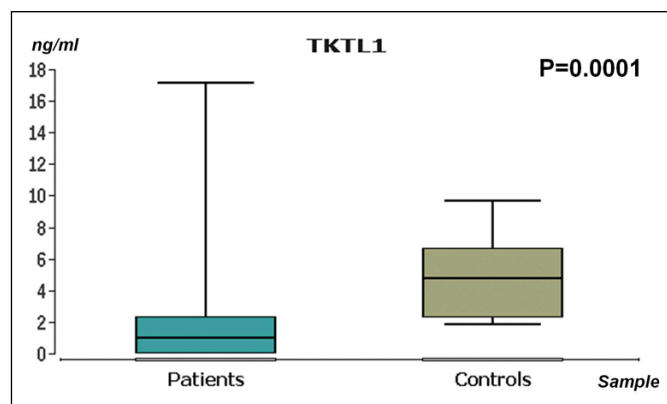


Figure 1. TKTL1 serum concentration (ng/ml) in PCa patients and controls. Box: lower line – quartile Q1 (25% – quantile); middle line – median; upper line – quartile Q3 (75% – quantile); aerials – extreme values.

a positive correlation between TKTL1 expression and tumor progression has been reported [7]. However, investigations negating the reliability of TKTL1 have also been published [11, 13], making a definitive assessment regarding TKTL1 reliability as a biomarker questionable. Indeed, the decreased serum TKTL1 found in PCa patients in the present investigation stands in opposition to the increase in monocyte associated TKTL1 claimed by the proponents of the EDIM-test. However, the different methods and localities, where TKTL1 was measured, in serum and in macrophages, could account for the differing results. Speculatively, assuming the accuracy of the EDIM test together with the theoretical background proposed by Coy and colleagues [6], an inverse correlation between TKTL1 detected in macrophages and extracellular TKTL1 in serum could occur, since tumor cells undergo phagocytosis. TKTL1 could therefore be sequestered in macrophages and the TKTL1 level in serum be reduced. Still, this hypothesis is speculative and requires further evaluation. Although, in the present investigation, serum TKTL1 levels differed in cancer patients and healthy persons, no correlation was apparent between serum TKTL1 and clinical or pathologic parameters. Conflicting reports about TKTL1 expression associated with various cancer entities and clinical outcome have been published by several investigators [10, 13, 14]. TKTL1, determined with the same immunohistochemical method in colorectal cancer tissues, has been positively associated with tumor progression and TKTL1-expression level in one study [15], whereas another study pointed to a significant decrease in TKTL1-expression associated with metastasis [16]. Recently, the DNA demethylating agent 5-aza-2'-deoxycytidine (5-Aza) has been reported

to augment expression of TKTL1 in melanoma cells and was associated with enhanced invasion of the tumor cells [17], whereas others have demonstrated reduced invasion of melanoma cells under 5-Aza treatment [18]. Grimm et al. correlated an increase of EDIM scores with a metabolic shift from aerobic to anaerobic conditions [19], whereas this correlation could not be confirmed by others [20]. Possibly, these inconsistencies have led to the recommendation of combining TKTL1 quantification with a standardized panel of established blood biomarkers [19]. The ambivalence of TKTL1 expression in so many different investigations shows that the role of TKTL1 may be more complex than initially thought. This study was designed as a pilot investigation and thus includes a limited number of patients. No specific imaging or blood tests were performed in controls to exclude incidental cancers. Since patients only underwent a general health check, this could contribute to a potential, though unlikely, bias for the high serum TKTL1 expression found in this cohort. The study was designed to assess the diag-

nostic potential of TKTL1, not its prognostic ability. Since PCa, in most cases, is associated with slow progression, long-term follow-up would be required to follow the course of serum TKTL1 during the course of the disease.

CONCLUSIONS

Serum TKTL1 was decreased in patients with clinically localized PCa, but failed to facilitate identification of patients with aggressive disease, who might particularly benefit from definitive cancer treatment. Based on these results, we cannot currently advise introducing serum TKTL1 into clinical practice. Further long-term studies including larger patient cohorts and simultaneous measurement of serum TKTL1 and macrophage sequestered TKTL1 are warranted to clarify the role of TKTL1 in PCa and resolve its applicability as a PCa biomarker.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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