



## CORRELATION BETWEEN SERUM TESTOSTERONE, BIOCHEMICAL AND CLINICAL MARKERS IN PROSTATE CANCER PATIENTS

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### ABSTRACT

**Purpose:** Androgens seems to play an important role in prostate cancer (PCa) pathogenesis. Some studies reveal that possible testosterone deficiency could be involved in PCa development and progress. Others indicate that men with high testosterone levels have increased risk in developing more aggressive PCa and poor response to therapy. The objective of the present study is to establish a possible correlation between serum total testosterone levels (TT), prostate specific antigen (PSA) and clinical markers evaluating the aggressiveness of the tumor process in PCa patients.

**Material/Methods:** A total of eighty males, aged from 52 to 84 years (mean age  $66.28 \pm 6.015$  years), with histologically confirmed PCa, entered the study. Diagnosis of PCa was established by systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least). Detected tumors were graded using the Gleason grading system. Serum TT and PSA were analyzed immunochemically. Pearson correlation statistics with a level of significance set at  $p < 0.05$  was used for data analysis.

**Results:** Strong significant positive correlation (Pearson  $r=0.90$ ,  $p<0.01$ ) between PSA and testosterone levels was found in lowest risk group. For the highest risk group there was a negative association between the same parameters (Pearson  $r=-0.38$ ,  $p<0.05$ ). A relatively weak positive relationship (Pearson  $r = 0.29$ ) was detected for patients with less aggressive tumors (Gleason score  $<7$ ) and a negative trend (Pearson  $r = -0.27$ ) in patients with highly aggressive prostate cancer (Gleason  $>7$ ).

**Conclusions:** Our results confirm the hypothesis that low testosterone levels are related to poor prognosis and increased severity of PCa.

**Key words:** Testosterone, Prostate cancer, PSA, Gleason

### INTRODUCTION

Androgens are male hormones playing a critical role in male sexual development and prostate gland physiology. Their synthesis and secretion takes place mostly in male gonads (90%) and to a less extent in adrenal cortex gland (10%). Testosterone, one of the principal androgens in humans, is produced by testicular Leydig cells and is responsible for the development of the epididymis, seminal vesicles in the

fetus, spermatogenesis, and secondary sex characteristics during puberty [1]. Highest levels of testosterone are found at the end of the puberty and their production in men steadily declines with age [2].

In prostate gland, testosterone is responsible for the proliferation of epithelial prostate cells and for the development of the prostate gland, especially in puberty [3]. Inside the prostate cells, testosterone forms dihydrotestosterone (DHT) by the enzyme 5- $\alpha$ -reductase. DHT binds to the androgen receptor (AR) and the complex DHT-AR binds to the androgens response elements on DNA changing gene expression on different genes [4].

There are evidences supporting the role of testosterone in prostate cancer (PCa) pathogenesis and progression. It is not clear whether high or low levels of testosterone are responsible for PCa. In 1941 Huggins and Hodges proposed that high testosterone levels results in PCa progression [5]. After androgen deprivation in animal models a tumor regression was demonstrated [6]. Human studies revealed a relationship between elevated testosterone and PCa development. A meta-analysis of three prospective nested case-control studies demonstrated that patients in the highest quartile of serum testosterone were more likely to develop PCa [7]. In clinical settings, the androgen deprivation therapy is one of the recommended approaches in treatment advanced PCa [8]. Nowadays, according to the International guidelines, adequate data are not available to determine whether there is additional risk of prostate cancer from testosterone replacement [9].

Other studies have reported no association or the opposite - e.g. high PCa risk in patients with low serum testosterone [10]. A study of Korean men undergoing prostate biopsy for suspected PCa indicated that low testosterone was associated with higher PCa risk [11, 12].

There is evidence supporting the link between serum testosterone and PCa severity, evaluated by the Gleason score. Several studies indicated significantly lower serum testosterone in patients with Gleason score 8 or greater [13, 14]. Xylinas et al found that men with TT below 10.4nmol/L were more likely to have high risk PCa with Gleason score  $>7$  and stage pT3-4 [15]. Albisinni S. et al 2012 demonstrated significant correlation between high free testosterone and high-grade PCa, suggesting that the elevated free T/TT ratio is indicative for high-grade PCa [16].

The discrepancy in the literature concerning the link between testosterone levels and PCa development and aggressiveness motivated us to study the relationship between serum TT levels, PSA and clinical markers evaluating the aggressiveness of the tumor process in PCa patients.

## MATERIALS AND METHODS

### Patients

A total of eighty males, aged from 52 to 84 years (mean age  $66.28 \pm 6.015$  years), with histologically confirmed PCa, entered the study. Diagnosis of PCa was established by systemic transrectal ultrasound-guided tru-cut prostate biopsies (10 cores at least). The biopsy specimens were processed for standard histological (hematoxylin and eosin staining) and immunohistochemical (PSA, pancytokeratine (CK) and high molecular weight CK /HMW CK/) examination. All malignant tumors were clinically staged using the TNM classification, v. 2009. Detected tumors were graded using the Gleason grading system. Tumors with Gleason score  $\geq 7$  were defined as high-grade tumors, those with Gleason score = 7 intermediate grade and with Gleason score  $< 7$  – as low-grade tumors. Each of these three groups comprised 23, 34, and 22 male patients, respectively.

The patients were divided into three risk groups according to EAU guidelines [17]. The stratification was based on PSA levels, Tumour Node Metastasis (TNM) classification of PCa and Gleason score of biopsy-detected PCa: low risk group (n=7) - patients with PSA  $< 10$  ng/ml, Gleason score  $< 7$  and stage cT1-T2a; intermediate risk group (n=34) - patients with PSA levels between 10 ng/ml and 20 ng/ml or Gleason score = 7 or stage cT2b; high risk group (n=37) – patients with PSA  $> 20$  ng/ml or Gleason score  $> 7$  and stage cT2c or higher – i.e. locally advanced cancer (any PSA, any GS, cT3-4, or cN+).

### Laboratory examinations

**PSA determination:** PSA serum levels were measured by standard chemiluminescent enzyme immunometric assay, using the IMMULITE 2000 automated system.

**Total testosterone determination:** TT serum levels were measured by standard chemiluminescent enzyme immunometric assay, using the IMMULITE 2000 automated system.

**Other covariates:** Detailed information, regarding patient height, weight, age, family history of PCa, and history of benign prostatic hyperplasia (BPH) was collected by interview at the time of hospitalization.

**Statistics:** Categorical variables were presented as frequencies (%). GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA) was used for statistical analysis of continued variables. Means  $\pm$  SD were determined. Student t-test for comparison of means of different parameters was used. The level of significance was set at  $p < 0.05$ . A non-parametric Pearson correlation analysis was performed to evaluate the associations between serum total testosterone levels and other tested parameters.

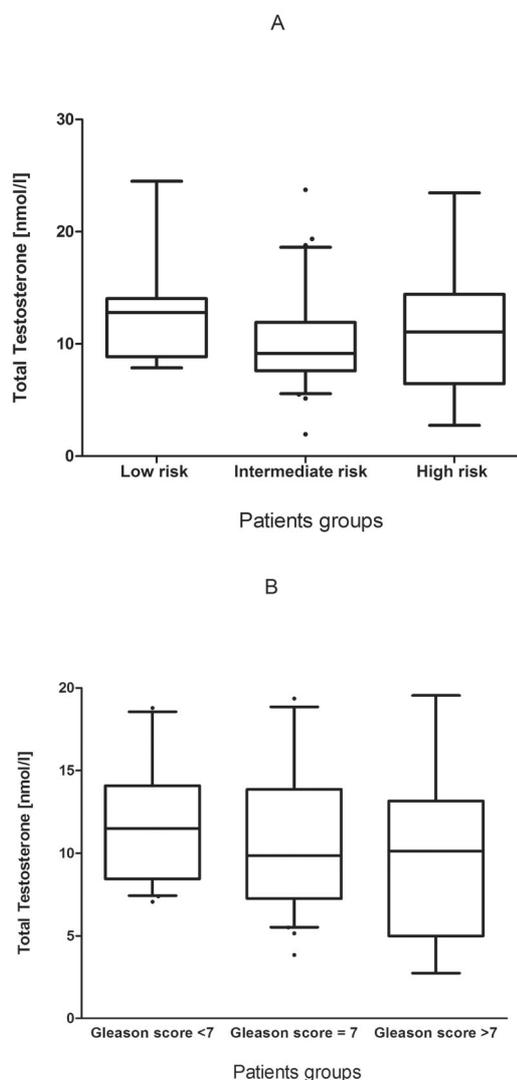
The study was approved by the local Ethics Committee, following the guidelines of the Declaration of Helsinki.

## RESULTS

### Total testosterone levels in PCa patients

Highest TT was indicated for the low risk group ( $13.43 \pm 5.42$  nmol/L). The intermediate and high-risk patients groups revealed TT below the cut-off value of 11.3 nmol/L. Surprisingly, we found lowest TT for the intermediate risk group ( $10.19 \pm 5.03$  nmol/L) (Fig. 1A). No statistically significant differences in serum TT levels were indicated between these risk groups for biochemical recurrence of localized and locally advanced PCa. We also compared the TT according to Gleason score. Highest TT levels ( $12.06 \pm 4.39$  nmol/L), closer to the lower reference limit were indicated for the patients with Gleason score  $< 7$  (Fig. 1B). The group with highest Gleason score  $> 7$  demonstrated lowest TT ( $9.65 \pm 4.55$  nmol/L). A borderline statistical significance ( $p = 0.078$ ) was found for these two groups. There was no statistical significance in TT when the high- and the low-Gleason score groups were compared to the patients with an intermediate Gleason score = 7 ( $p > 0.05$ ).

**Fig. 1.** Total testosterone levels in PCa patients



(A) stratification by the risk for biochemical recurrence of localized and locally advanced prostate cancer;

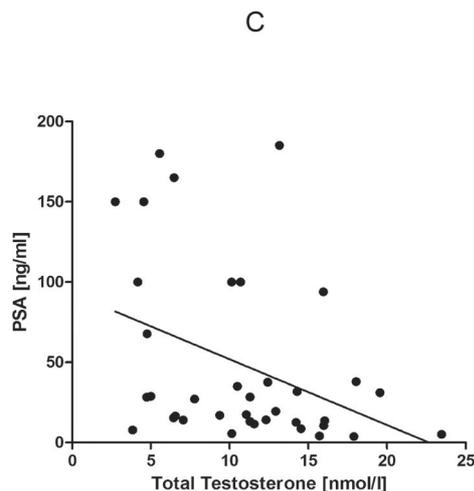
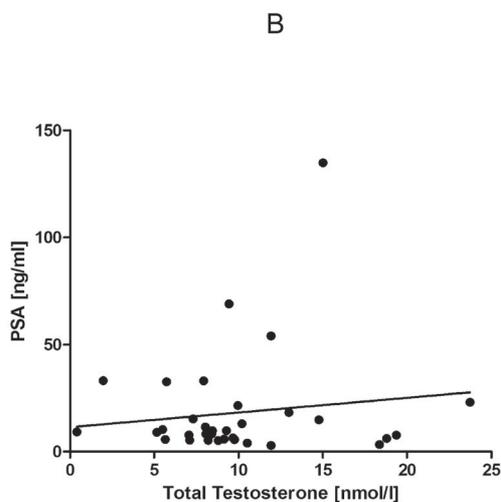
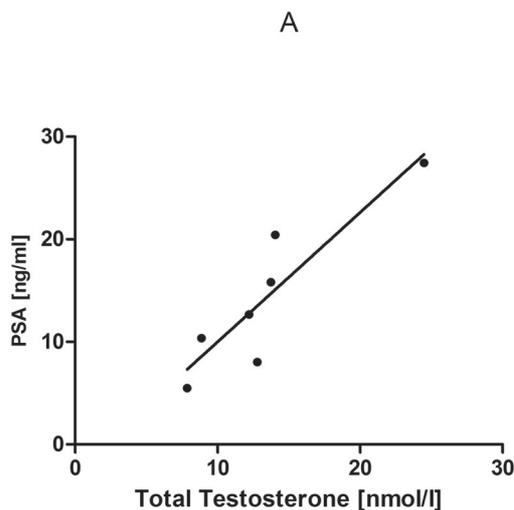
p.0.05 for all risk groups

(B) stratification by the Gleason score;  $p = 0.078$  for the group with Gleason score  $<7$  vs the group with Gleason score  $>7$ ;  $p > 0.05$  for the groups with Gleason score  $<7$ , Gleason score  $>7$  vs the group with Gleason score = 7.

**Associations between total testosterone and PSA levels in PCa patients**

A strong positive correlation between PSA and TT (Pearson  $r = 0.90$ ,  $p < 0.01$ ) was demonstrated for the low risk group (Fig. 2A). The same trend, although nonsignificant (Pearson  $r = 0.13$ ,  $p > 0.05$ ), was retained for the intermediate risk group (Fig. 2B). The high-risk group revealed the opposite trend, a significant moderate negative correlation (Pearson  $r = -0.38$ ,  $p < 0.05$ ) between PSA and TT serum levels (Fig. 2C).

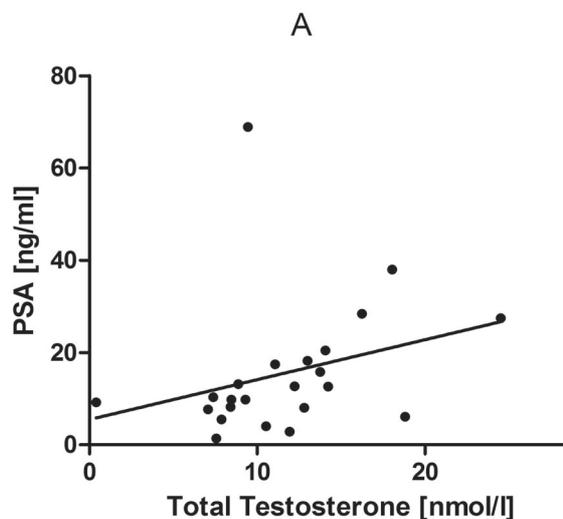
**Fig 2.** Association between total testosterone and PSA levels in PCa patients stratified by the risk for biochemical recurrence of localized and locally advanced prostate cancer

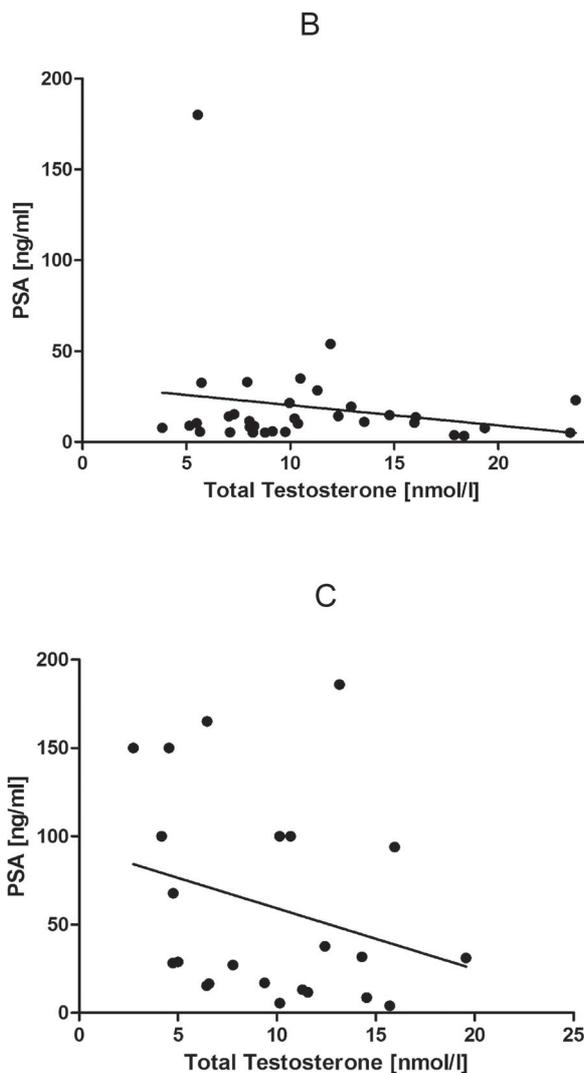


A. low risk group  
 B. intermediate risk group  
 C. high risk group  
 Legend:  
 (A) Pearson  $r = 0.90$ ;  $p < 0.01$   
 (B) Pearson  $r = 0.13$ ;  $p > 0.05$   
 (C) Pearson  $r = -0.38$ ;  $p < 0.05$

We evaluated the relationships between PSA and TT levels for the three Gleason score groups (Fig. 3A, B, C). A positive association (Pearson  $r = 0.29$ ;  $p > 0.05$ ) was demonstrated for the PCa patients with a Gleason score  $<7$  (Fig. 3A). For patients with Gleason score = 7 a weak but negative trend (Pearson  $r = -0.19$ ;  $p > 0.05$ ) was indicated between PSA and TT (Fig. 3B). For the group with most aggressive PCa (Gleason score  $>7$ ) the same negative trend was retained however it becomes stronger (Pearson  $r = -0.27$ ;  $p > 0.05$ ) (Fig. 3C).

**Fig. 3.** Association between total testosterone and PSA levels in PCa patients stratified by the Gleason score





- (A) Gleason score <7
- (B) Gleason score = 7
- (C) Gleason score >7

Legend:

- (A) Pearson  $r = 0.29$  ;  $p > 0.05$
- (B) Pearson  $r = -0.19$ ;  $p > 0.05$
- (C) Pearson  $r = -0.27$ ;  $p > 0.05$

## DISCUSSION

We found highest testosterone levels for the low risk group and for the patients with less aggressive PCa evaluated by the Gleason score. Lowest TT levels were indicated for the group with highest Gleason score >7. Although the difference in TT between groups with Gleason score below 7 and above 7 is not significant ( $p > 0.05$ ), it confirms the notion that lower TT is associated with increased risk, higher Gleason scores, and overall tumor burden [18, 19]. It was established that high ratio of PSA to testosterone was associated with increased PCa risk in men with moderate elevation of PSA (3–10 ng/ml) and in testosterone defi-

cient men with PSA values <4.0 ng/ml [20]. Based on these results it was suggested that reduced testosterone increases the risk of PCa for any PSA value [21]. It is well documented in the literature that there is a U-shaped relationship between serum sex hormones and PCa aggressiveness, with the lowest and highest levels associated with high-risk PCa [22]. According to Khera et al, a more than 50% increased risk of high Gleason score was established in men with testosterone levels below 10nmol/l compared to men with normal testosterone [23].

Our study revealed strong positive correlation between testosterone and PSA levels in patients with low risk of recurrence. The same association was established for the patients with less aggressive PCa (Gleason score < 7). It is well documented that androgens are important for prostate growth and development and that testosterone induce prostate growth increasing also PSA [24]. After binding to a specific androgen receptor (AR) the complex TT-AR translocates into the nucleus and binds to specific AR-response elements in the promoters of androgen-regulated genes in DNA altering their transcription. The normal expression of these genes in a healthy prostate is responsible for balanced production of PSA and other prostate-specific proteins [25].

In the high-risk group and in cases with advanced PCa and high Gleason score we established a negative correlation between serum PSA and TT. If PSA is an indirect measure for PCa severity and aggressiveness, this means that low levels of testosterone are associated with higher aggressive of PCa. There are multiple studies reporting a negative association between TT and high-grade PCa [26, 27]. Recently the saturation hypothesis explains the ‘paradoxal’ findings that low TT is related to high-grade PCa tumors [21, 28]. According to the saturation model high TT concentrations stimulate prostate growth and function, reflected by PSA concentrations, to a limit e.g. saturation point of about 8 nmol/L TT. Higher TT concentrations above the saturation point result in minimal or absent changes in PSA [19, 21, 28]. A possible explanation of the saturation hypothesis is that at low TT concentrations the AR is very sensitive to induce prostate gland growth. At high TT concentrations, the AR becomes saturated and no able to stimulate AR-dependent genes for prostate growth, e.g. the effects of testosterone on prostate gland are reduced.

## CONCLUSION

Our results confirm the hypothesis that low testosterone levels are related to poor prognosis and increased severity of PCa. More studies need to be performed in this area to clarify how exactly testosterone is involved in PCa pathogenesis.

## ACKNOWLEDGMENTS

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