

**ORIGINAL RESEARCH**

# Baseline total testosterone is associated with high-grade prostate cancer and predicts disease progression: results and clinical implications in 500 patients treated with robotic surgery

Antonio Benito Porcaro<sup>1,†</sup>, Maria Angela Cerruto<sup>1,\*†</sup>, Andrea Franceschini<sup>1</sup>, Filippo Caudana<sup>1</sup>, Alberto Baielli<sup>1</sup>, Francesco Artoni<sup>1</sup>, Francesca Montanaro<sup>1</sup>, Sonia Costantino<sup>1</sup>, Riccardo Rizzetto<sup>1</sup>, Alessandro Veccia<sup>1</sup>, Matteo Brunelli<sup>2</sup>, Riccardo Giuseppe Bertolo<sup>1</sup>, Alessandro Antonelli<sup>1</sup>

<sup>1</sup>Department of Urology, University of Verona, Azienda Ospedaliera Universitaria Integrata, 37126 Verona, Italy

<sup>2</sup>Department of Pathology, University of Verona, Azienda Ospedaliera Universitaria Integrata, 37126 Verona, Italy

**\*Correspondence**

[mariaangela.cerruto@univr.it](mailto:mariaangela.cerruto@univr.it)  
(Maria Angela Cerruto)

† These authors contributed equally.

**Abstract**

**Background:** To assess baseline total testosterone (TT) as a predictor of both adverse pathology and/or disease progression in an aging population diagnosed with clinical prostate cancer (PCa) treated with robotic surgery. **Methods:** From November 2014 to December 2021, 500 Caucasian patients underwent preoperative TT testing. TT was categorized as subnormal (up to 12 nmol/L) versus normal (above 12 nmol/L). Adverse pathology was defined as adverse tumour grade (International Society of Urological Pathology (ISUP) grade 4/5) or tumour stage (extracapsular extension, seminal vesicle invasion) or pelvic lymph node invasion. Associations between the risk of adverse surgical pathology and disease progression were evaluated using appropriate statistical methods. **Results:** Median (Interquartile Range (IQR)) baseline TT was 14.0 nmol/L (10.6–15.5 nmol/L) with levels being subnormal in 165 (33.0%) and normal in 335 (67%) patients who were more likely to have adverse tumour grades (Odds Ratio (OR) = 2.246, 95% Confidence Interval (CI) 1.123–4.490,  $p = 0.022$ ) as well as to progress (Hazard Ratio (HR) = 1.757, 95% CI 1.199–2.577,  $p = 0.004$ ) after a mean follow-up of 71.5 months. The prognostic impact of baseline TT remained unchanged after adjusting for standard clinicopathological factors. **Conclusions:** Baseline TT was associated with high-grade tumour features and independently predicted disease progression. These findings suggest that baseline TT may help refine risk stratification in patients with prostate cancer, although further validation is needed to clarify its role in prostate cancer biology.

**Keywords**

Clinical prostate cancer; Baseline total testosterone; Aging male; Robot-assisted radical prostatectomy; Prostate cancer progression

# La testosterona total basal se asocia con el cáncer de próstata de alto grado y predice la progresión de la enfermedad: resultados e implicaciones clínicas en 500 pacientes tratados con cirugía robótica

## Resumen

**Antecedentes:** Evaluar la testosterona total basal (TT) como predictor tanto de patología adversa como de progresión de la enfermedad en una población de varones de edad avanzada diagnosticados de cáncer de próstata clínicamente localizado (CP) y tratada con cirugía robótica. **Métodos:** Entre noviembre de 2014 y diciembre de 2021, se evaluó la TT preoperatoria en 500 pacientes caucásicos, clasificándola como subnormal ( $\leq 12$  nmol/L) o normal ( $> 12$  nmol/L). La patología adversa se definió como un grado tumoral adverso (International Society of Urological Pathology (ISUP) 4/5), estadio tumoral adverso (extensión extracapsular o invasión de vesículas seminales) o invasión de ganglios linfáticos pélvicos. Las asociaciones con el riesgo de patología quirúrgica adversa y progresión de la enfermedad se evaluaron mediante métodos estadísticos. **Resultados:** La TT basal mediana (Intervalo intercuartílico (RIC)) fue de 14.0 nmol/L (10.6–15.5 nmol/L), con niveles subnormales en 165 pacientes (33.0%) y normales en 335 (67%). Los pacientes con TT subnormal presentaron una mayor probabilidad de presentar grados tumorales adversos ((OR) odds ratio = 2.246; Intervalo de confianza (IC) 95% 1.123–4.490;  $p = 0.022$ ), así como una mayor probabilidad de progresión de la enfermedad ((HR) Hazard Ratio = 1.757; IC 95% 1.199–2.577;  $p = 0.004$ ) tras un seguimiento medio de 71.5 meses. El impacto pronóstico permaneció sin cambios después del ajuste por factores estándar. **Conclusiones:** La TT basal se asoció con características tumorales de alto grado y predijo de forma independiente la progresión de la enfermedad. Estos hallazgos sugieren que la TT basal podría ayudar a refinar la estratificación del riesgo en pacientes con cáncer de próstata, aunque se necesitan estudios adicionales para aclarar su papel en la biología del cáncer de próstata.

## Palabras Clave

Cáncer de próstata clínico; Testosterona total basal; Envejecimiento masculino; Prostatectomía radical asistida por robot; Progresión del cáncer de próstata

## 1. Introduction

Clinical prostate cancer has become a major public health issue owing to its increasing prevalence in the aging male population. Men at risk may benefit from prostate-specific antigen (PSA) testing, which has been shown to reduce cancer-specific mortality. Therefore, identifying factors associated with unfavorable disease is essential to reduce both overtreatment and undertreatment, which may adversely affect patients' quality of life and long-term outcomes [1–4]. Patients with clinically localized PCa are stratified into prognostic risk groups to guide appropriate treatment decisions. With the main focus of reducing overtreatment issues which have forced the European Association of Urology (EAU) and the National Comprehensive Cancer Network (NCCN) to continuously update guidelines on disease's managements options that may vary from active surveillance (AS) or watchful waiting (WW) to active treatments, including robot-assisted radical prostatectomy (RARP), which may be combined with extended pelvic lymph node invasion (ePLND), or radiation therapy (RT) [1–4]. To date, one of the main challenges in the management of clinical PCa is the identification of additional prognostic factors that may improve patient stratification and facilitate the selection of appropriate treatments. Although multiparametric magnetic resonance imaging (mpMRI) and molecular biology have improved disease characterization, their routine implementation remains limited; the former lacks sufficient reproducibility across multicentre studies, whereas the latter is not widely available in routine clinical practice [1–5]. Prostate biology is closely linked to androgen status, and testosterone levels naturally decline with advancing age. Accordingly, the

International Society of Andrology (ISA) has classified total testosterone (TT) levels as low ( $< 8$  nmol/L), intermediate (8–12 nmol/L) and normal ( $> 12$  nmol/L), with the former group requiring treatment and the latter requiring only monitoring. The role of baseline TT in PCa biology remains controversial, with previous studies reporting conflicting findings; likewise, studies evaluating the association between baseline TT and adverse pathological features and/or disease progression are lacking [6–9]. Therefore, we aimed to test the hypothesis that baseline TT levels are associated with adverse pathological features and may independently predict disease progression in patients undergoing robotic surgery for clinically localized PCa.

## 2. Materials and methods

### 2.1 Study population, data collection and evaluation of parameters

From November 2014 to December 2021, 500 consecutive patients with clinically localized prostate cancer (PCa) were prospectively evaluated at our Institution. All patients underwent robot-assisted radical prostatectomy (RARP), with extended pelvic lymph node dissection (ePLND) performed when indicated according to international guidelines. Surgical specimens were assessed by a dedicated pathologist.

Preoperative serum total testosterone (TT) levels were measured in all patients between 8:00 and 8:30 AM using a radioimmunoassay and expressed in nmol/L. Serum sex hormone-binding globulin (SHBG) was not assessed; therefore, free testosterone was not calculated. Direct measurement of free testosterone using currently available

assays is considered unreliable.

Additional clinical and pathological variables included age, body mass index (BMI), prostate volume (PV), prostate weight (PW), prostate-specific antigen (PSA), percentage of biopsy-positive cores (BPC), biopsy and pathological tumor grade according to the International Society of Urological Pathology (ISUP) classification, tumor stage according to the Tumor, Node, and Metastasis (TNM) system, and European Association of Urology (EAU) risk classification. Postoperative follow-up was conducted according to current guideline recommendations.

Inclusion criteria were as follows:

- Consecutive Caucasian patients diagnosed with clinical prostate cancer;

- Availability of preoperative total testosterone assessment;
- Centralized pathological evaluation of surgical specimens.

Exclusion criteria were as follows:

- Any previous treatment for prostate cancer, including androgen deprivation therapy;
- Incomplete clinical, pathological, or follow-up data.

## 2.2 Evaluation of endpoints and statistical methods

The study aimed to evaluate whether preoperative TT was associated with adverse pathological findings and disease progression after surgery. Accordingly, TT was analyzed both as a continuous variable and as a categorical variable, the latter being dichotomized into subnormal (up to 12 nmol/L) versus normal (above 12 nmol/L) levels according to ISA recommendations [7]. Accordingly, adverse pathology was defined as the presence of high-grade disease (ISUP grade groups 4/5) and/or extracapsular extension (ECE) and/or seminal vesicle invasion (SVI) and/or pelvic lymph node invasion (PLNI). Disease progression was defined as the occurrence of biochemical recurrence or persistence, and/or local recurrence, and/or distant metastases. Continuous variables were summarized as medians with interquartile ranges (IQR), while categorical factors were reported as frequencies (percentages). Associations between baseline TT and adverse clinical and pathological features were assessed using a logistic regression model (univariate analysis), while associations with disease progression were assessed using Cox's proportional hazards model (univariate and multivariate analysis). In order to assess the impact of preoperative TT as both a continuous and categorized variable, we considered three multivariable models involving clinical and pathological variables as well as EAU prognostic risk classes accordingly, the first one was adjusted for age, BMI, PV, PSA, biopsy ISUP grade groups, clinical tumor stage (cT) and clinical nodal status (cN); the second one for pathology ISUP grade groups, pathological tumor stage (pT), surgical margins and pathological nodal status (pN); and finally the third one for EAU prognostic risk classes, which included not only clinical parameters for defining them, but also age, BMI and PV, as well. Patients were censored at the time of disease progression or last follow-up. Likewise, not adjusted Kaplan-Meier estimator time risk curves of normal versus subnormal basal TT levels were displayed and tested for significance (log-rank test). The time interval from surgery

to event occurrence (disease progression) or to last follow-up (censoring status) was measured in months. Adjuvant or salvage treatments were administered after disease progression. The software used to run the analysis was IBM-SPSS version 26 (IBM Corp., Armonk, NY, USA). All tests were two-sided, and  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1 Baseline TT levels associated with the risk of high-grade disease

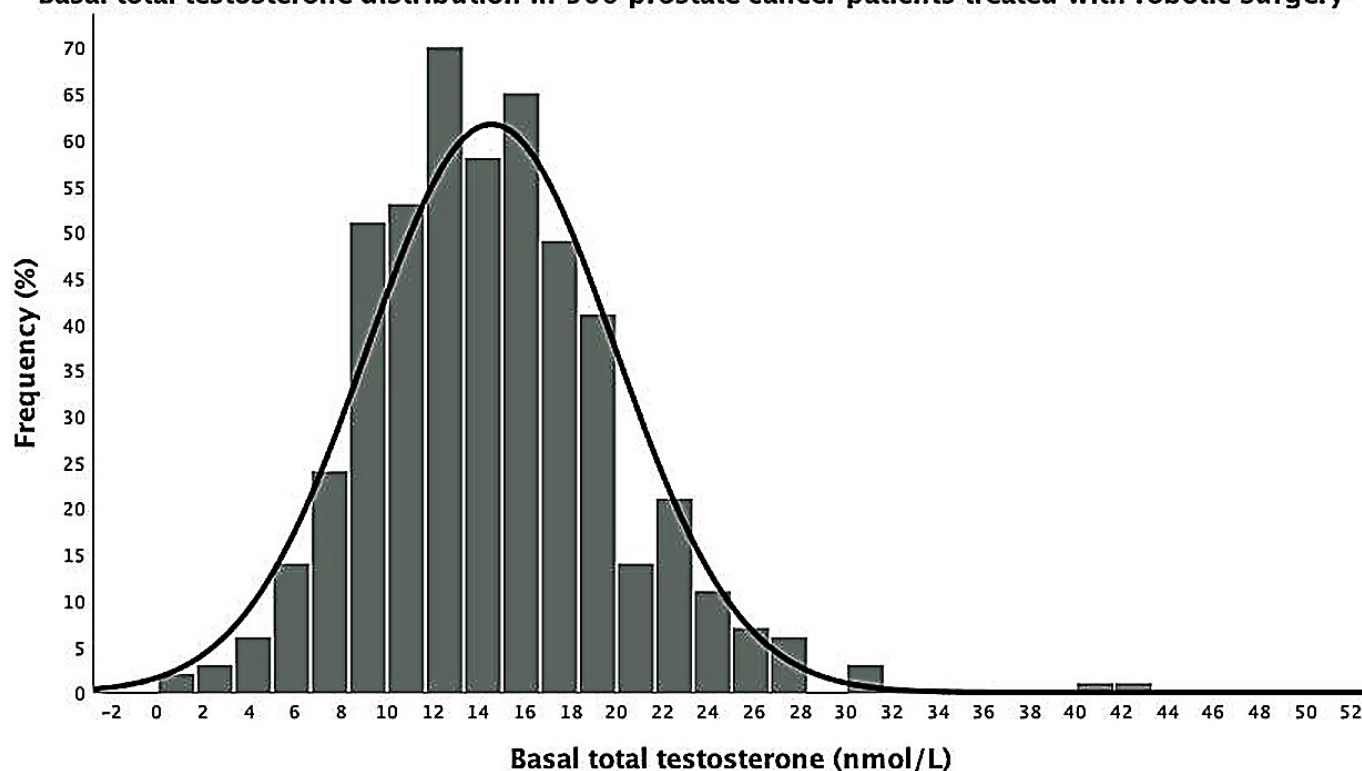
Fig. 1 illustrates the distribution of baseline TT levels. Mean  $\pm$  Standard Deviation (SD) and median (IQR) values were 14.5 nmol/L (5.3 nmol/L) and 14 nmol/L (10.6–15.5 nmol/L), respectively; furthermore, testosterone levels were subnormal in 165 (33.0%) subjects. Demographic features of the Caucasian study population are reported in Table 1, which shows that patients with normal TT levels were more likely to harbor high-grade tumors (ISUP 4/5) on both biopsy and pathology specimens, but less likely to associate with abnormal BMI; conversely, patients with subnormal testosterone levels were less likely to have high-grade disease and more likely to be overweight, as well. EAU prognostic risk classes were as follows: low risk in 129 (25.8%) cases, intermediate risk in 266 (53.2%) subjects, and high-risk in 105 (21%) patients. Extended PLND was performed in 355 patients, with a median (IQR) of 27 (21–33) lymph nodes removed; cancer invasion was identified in 47 (13.2%) subjects. Androgen deprivation following surgery was given in 115 (23%) subjects, and RT was delivered in 106 cases (21.2%), with salvage intent in 44 (8.8%). After a mean (95% CI) follow-up of 71.5 months (59.3–73.6 months), disease progression occurred in 129 patients (25.8%). There were also 13 deaths (2.6%), of which one death (0.2%) was related to PCa.

### 3.2 Baseline TT levels associated with the risk of prostate cancer progression

Clinical and pathological factors associated with PCa progression are summarized in Table 2. Patients presenting with adverse tumor grades and stages were more likely to progress after adjusting for other variables, as well. Likewise, in univariate analysis, baseline TT levels were associated with the risk of PCa progression either as a continuous (HR 1.035, 95% CI 1.002–1.068,  $p = 0.036$ ) or categorical variable (HR 1.757, 95% CI 1.199–2.577,  $p = 0.004$ ). Higher TT levels were associated with an increased risk of disease progression; conversely, patients presenting with subnormal TT levels were less likely to progress, as illustrated in Fig. 2.

On multivariate analysis, baseline TT remained an independent predictor of disease progression both as a continuous and as a categorized variable. Accordingly, patients presenting with normal TT when compared with those having subnormal levels were more likely to progress after adjusting for clinical (HR 1.573, 95% CI 1.040–2.377,  $p = 0.032$ ) and pathological (HR 1.604, 95% CI 1.081–2.379,  $p = 0.019$ ) variables. Likewise, this trend was also confirmed across EAU prognostic risk classes. Accordingly, EAU intermediate- and high-risk classes, when compared with the low-risk class, were less

**Basal total testosterone distribution in 500 prostate cancer patients treated with robotic surgery**



**FIGURE 1. Basal total testosterone levels.** Distribution of basal total testosterone (TT) levels in 500 patients with clinical prostate cancer treated with robotic surgery. Mean (standard deviation) as well as median (interquartile range) TT levels were 14.5 nmol/L (5.3 nmol/L) and 14.0 nmol/L (10.6–15.5 nmol/L), respectively. Functionally, patients were categorized as subnormal or normal according to basal TT levels up to 12 nmol/L versus above that level, respectively.

**TABLE 1. Clinical and pathological demography of 500 PCa patients stratified by baseline total testosterone (TT) levels before robotic surgery.**

|                           | Population       | Subnormal TT<br>(up to 12 nmol/L)<br>n = 165 (33%) | Normal TT<br>(>12 nmol/L)<br>n = 335 (67%) | OR (95% CI)         | p-value |
|---------------------------|------------------|----------------------------------------------------|--------------------------------------------|---------------------|---------|
| <b>Clinical variables</b> |                  |                                                    |                                            |                     |         |
| Age (yr)                  | 65.0 (60.2–70.0) | 65.0 (61.5–70.0)                                   | 65.0 (60.0–70.0)                           | 0.991 (0.963–1.019) | 0.520   |
| BMI (kg/m <sup>2</sup> )  | 25.6 (23.8–28.1) | 26.4 (24.4–28.7)                                   | 25.3 (23.4–27.7)                           | 0.897 (0.845–0.952) | <0.0001 |
| PV (mL)                   | 40.0 (30.0–52.2) | 43.0 (34.0–56.0)                                   | 40.0 (30.0–52.0)                           | 0.992 (0.982–1.002) | 0.126   |
| PSA (ng/mL)               | 6.8 (5.0–8.9)    | 6.6 (5.0–8.2)                                      | 6.8 (5.1–9.4)                              | 0.996 (0.973–1.021) | 0.770   |
| BPC (%)                   | 31.2 (19.1–50.0) | 28.5 (16.6–50.0)                                   | 33.3 (20.0–50.0)                           | 1.006 (0.997–1.014) | 0.196   |
| cT1c                      | 322 (64.4)       | 112 (67.9)                                         | 210 (62.7)                                 | 1.000               |         |
| cT2/3                     | 178 (35.6)       | 53 (32.1)                                          | 125 (37.3)                                 | 1.258 (0.848–1.867) | 0.255   |
| cN0                       | 474 (94.8)       | 154 (93.3)                                         | 320 (94.5)                                 | 1.000               |         |
| cN1                       | 26 (5.2)         | 11 (6.7)                                           | 15 (4.5)                                   | 0.656 (0.294–1.463) | 0.303   |
| ISUP 1                    | 166 (33.2)       | 61 (37.0)                                          | 105 (31.3)                                 | 1.000               |         |
| ISUP 2/3                  | 269 (53.8)       | 90 (54.5)                                          | 179 (53.4)                                 | 1.155 (0.771–1.732) | 0.484   |
| ISUP 4/5                  | 65 (13.0)        | 14 (8.5)                                           | 51 (15.2)                                  | 2.116 (1.083–4.137) | 0.028   |

TABLE 1. Continued.

|                        | Population       | Subnormal TT<br>(up to 12 nmol/L)<br>n = 165 (33%) | Normal TT<br>(>12 nmol/L)<br>n = 335 (67%) | OR (95% CI)         | p-value |
|------------------------|------------------|----------------------------------------------------|--------------------------------------------|---------------------|---------|
| Pathological variables |                  |                                                    |                                            |                     |         |
| PW (g)                 | 52.0 (41.0–65.0) | 70.0 (55.0–80.0)                                   | 64.0 (50.0–80.2)                           | 0.995 (0.985–1.004) | 0.284   |
| ISUP 1                 | 57 (11.4)        | 23 (13.9)                                          | 34 (10.1)                                  | 1.000               |         |
| ISUP 2/3               | 335 (67.0)       | 117 (70.9)                                         | 218 (65.1)                                 | 1.260 (0.709–2.240) | 0.430   |
| ISUP 4/5               | 108 (21.6)       | 25 (15.2)                                          | 83 (24.8)                                  | 2.246 (1.123–4.490) | 0.022   |
| pT2                    | 400 (80.0)       | 139 (84.4)                                         | 261 (77.9)                                 | 1.000               |         |
| pT3a                   | 46 (9.2)         | 10 (6.1)                                           | 36 (10.7)                                  | 1.917 (0.924–3.979) | 0.081   |
| pT3b                   | 54 (10.8)        | 16 (9.7)                                           | 38 (11.3)                                  | 1.265 (0.681–2.350) | 0.457   |
| R0                     | 369 (73.8)       | 129 (78.2)                                         | 240 (71.6)                                 | 1.000               |         |
| R1                     | 131 (26.2)       | 36 (21.8)                                          | 95 (28.4)                                  | 1.418 (0.914–2.201) | 0.119   |
| pNx/0                  | 453 (90.6)       | 152 (92.1)                                         | 301 (89.9)                                 | 1.000               |         |
| pN1                    | 47 (9.4)         | 13 (7.9)                                           | 34 (10.1)                                  | 1.321 (0.677–2.576) | 0.415   |

OR, odds ratio; CI, confidence interval; continuous variables are reported as medians (IQR, interquartile ranges) and categorical factors as frequencies (percentages); ISUP, International Society of Urologic Pathology tumour grade group formulation; see also materials and methods for abbreviations; TT, total testosterone; BMI, body mass index; PV, prostate volume; PSA, prostate-specific antigen; BPC, biopsy-positive cores; cT, clinical tumor; cN, clinical node; PW, prostate weight; pT, pathological tumor; R, residual tumor classification; pN, pathological node.

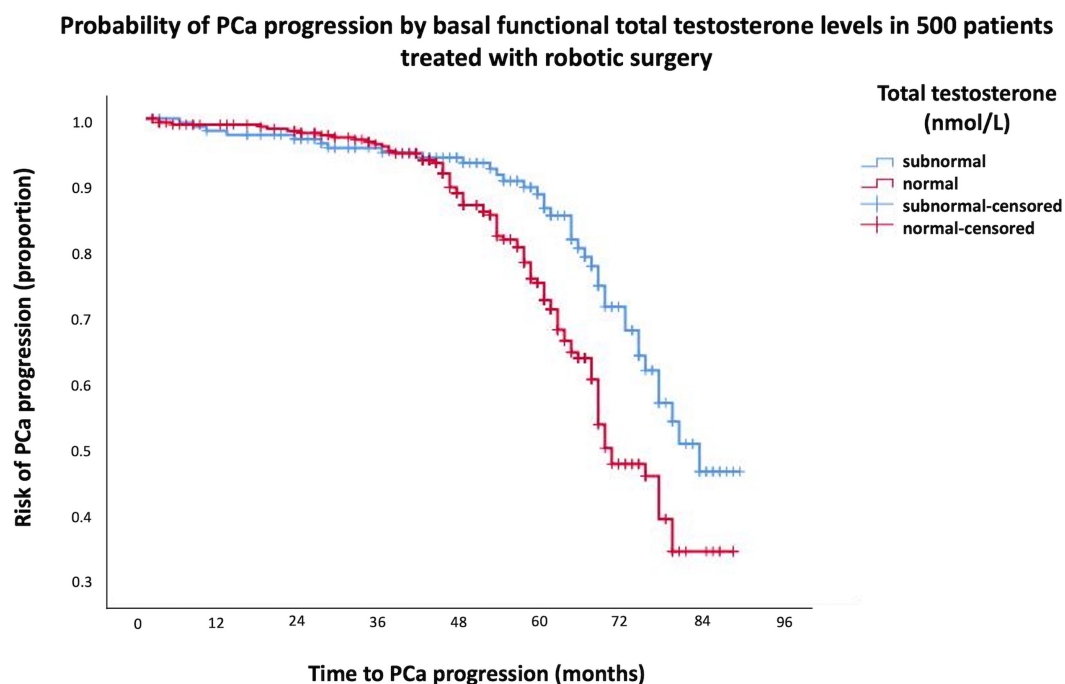
TABLE 2. Risk of prostate cancer progression by standard clinical and pathological factors after robotic surgery in 500 patients with clinical localized disease.

|                                 | No PCa progression | PCa progression  | Univariate analysis |         | Multivariate analysis |         |
|---------------------------------|--------------------|------------------|---------------------|---------|-----------------------|---------|
| Statistics                      | 371 (74.2)         | 129 (25.8)       | HR (95% CI)         | p-value | HR (95% CI)           | p-value |
| Age (yr)                        | 65.0 (61.0–70.0)   | 65.0 (60.0–70.0) | 1.007 (0.980–0.615) | 0.615   | 0.995 (0.966–1.024)   | 0.732   |
| BMI (kg/m <sup>2</sup> )        | 25.6 (23.7–28.1)   | 25.4 (23.7–28.5) | 0.984 (0.933–1.037) | 0.546   | 0.968 (0.920–1.018)   | 0.207   |
| PV (mL)                         | 41.3 (31.5–53.0)   | 39.0 (30.6–50.5) | 0.998 (0.988–1.009) | 0.705   | 0.998 (0.987–1.009)   | 0.703   |
| PSA (ng/mL)                     | 6.5 (4.9–8.5)      | 7.4 (5.4–11.5)   | 1.045 (1.030–1.060) | <0.0001 | 1.040 (1.021–1.058)   | <0.0001 |
| BPC (%)                         | 28.5 (16.6–50.0)   | 42.8 (25.0–64.2) | 1.017 (1.010–1.024) | <0.0001 | 1.010 (1.002–1.017)   | 0.011   |
| TT<br>(continuous,<br>nmol/L)   | -                  | -                | 1.035 (1.002–1.068) | 0.036   | -                     | -       |
| TT<br>(normal vs.<br>subnormal) | -                  | -                | 1.757 (1.199–2.577) | 0.004   | -                     | -       |
| ISUP 1 (%)                      | 139 (37.5)         | 27 (20.9)        | 1.000               |         | 1.000                 |         |
| ISUP 2/3 (%)                    | 201 (54.2)         | 68 (52.7)        | 2.148 (1.373–3.361) | 0.001   | 1.887 (1.192–2.985)   | 0.007   |
| ISUP 4/5 (%)                    | 31 (8.4)           | 34 (26.4)        | 5.010 (3.007–8.348) | <0.0001 | 3.464 (2.006–5.984)   | <0.0001 |
| cT1c (%)                        | 242 (65.2)         | 80 (62.0)        | 1.000               |         | 1.000                 |         |
| cT 2/3 (%)                      | 129 (34.8)         | 49 (38.0)        | 2.385 (1.647–3.453) | <0.0001 | 2.019 (1.348–3.023)   | 0.001   |

TABLE 2. Continued.

|            | No PCa progression | PCa progression  | Univariate analysis      |                 | Multivariate analysis |                 |
|------------|--------------------|------------------|--------------------------|-----------------|-----------------------|-----------------|
| Statistics | 371 (74.2)         | 129 (25.8)       | HR (95% CI)              | <i>p</i> -value | HR (95% CI)           | <i>p</i> -value |
| cN0 (%)    | 356 (96.0)         | 80 (62.0)        | 1.000                    |                 | 1.000                 |                 |
| cN1 (%)    | 15 (4.0)           | 49 (38.0)        | 1.839 (0.991–3.412)      | 0.053           | 1.052 (0.553–2.001)   | 0.878           |
| PW (g)     | 52.0 (42.0–67.0)   | 50.0 (40.0–62.0) | 0.997 (0.998–1.007)      | 0.611           | 0.999 (0.989–1.009)   | 0.845           |
| ISUP 1     | 54 (14.6)          | 3 (2.3)          | 1.000                    |                 | 1.000                 |                 |
| ISUP 2/3   | 272 (73.3)         | 63 (48.8)        | 4.856<br>(1.522–15.494)  | 0.008           | 4.322 (1.347–13.863)  | 0.014           |
| ISUP 4/5   | 45 (12.1)          | 63 (48.8)        | 20.318<br>(6.341–65.098) | <0.0001         | 10.938 (3.255–36.753) | <0.0001         |
| pT2        | 324 (87.3)         | 76 (58.9)        | 1.000                    |                 | 1.000                 |                 |
| pT3a       | 29 (7.8)           | 17 (13.2)        | 2.599 (1.530–4.415)      | <0.0001         | 1.821 (1.044–3.177)   | 0.035           |
| pT3b       | 18 (4.9)           | 36 (27.9)        | 4.526 (3.028–6.767)      | <0.0001         | 1.751 (1.086–2.822)   | 0.022           |
| R0         | 289 (77.9)         | 80 (62.0)        | 1.000                    |                 | 1.000                 |                 |
| R1         | 82 (22.1)          | 49 (38.0)        | 1.774 (1.239–2.540)      | 0.002           | 0.960 (0.648–1.422)   | 0.838           |
| pNx/0      | 364 (98.1)         | 89 (69.0)        | 1.000                    |                 | 1.000                 |                 |
| pN1        | 7 (1.9)            | 40 (31.0)        | 4.751 (3.260–6.924)      | <0.0001         | 1.992 (1.246–3.186)   | 0.004           |

Continuous variables are reported as medians (IQR, interquartile ranges) and categorical factors as frequency (percentage); ISUP, International Society of Urologic Pathology tumour grade group formulation; HR, hazard ratio; CI, confidence interval; PCa, prostate cancer; BMI, body mass index; PV, prostate volume; PSA, prostate-specific antigen; BPC, biopsy-positive cores; TT, total testosterone; cT, clinical tumor; cN, clinical node; PW, prostate weight; pT, pathological tumor; R, residual tumor classification; pN, pathological node.



**FIGURE 2. Kaplan-Meier survival risk curves of PCa.** Kaplan-Meier survival risk curves of prostate cancer (PCa) progression according to baseline total testosterone (TT) levels, which were categorized as subnormal (up to 12 nmol/L) versus normal (above 12 nmol/L) in 500 patients with clinical PCa treated with robotic surgery. Accordingly, mean (95% confidence interval) survival time of disease progression was longer for patients presenting with subnormal TT levels (75.1 months; 95% CI: 71.8–78.4 months) than those with normal testosterone levels (68.9 months; 95% CI: 66.3–73.6 months), and this difference was significant (Mantel-Cox log-rank test:  $p = 0.003$ ).

likely to progress for subnormal testosterone levels, as detailed in Table 3.

#### 4. Discussion

The relationship between testosterone and prostate biology has been extensively investigated over the last decades [1–3, 6–10]. Several studies explored associations between baseline testosterone and prostate cancer (PCa) pathology and progression [8–10]. Nevertheless, available evidence remains inconsistent, and robust prospective studies are still needed [8–10]. Previous reports have shown positive, inverse, or null associations between testosterone levels and PCa outcomes [8–10]. Baseline testosterone remains a relevant variable in aging men at increasing risk of PCa [1–3].

Although our results derive from a large Caucasian population, their generalizability should be interpreted with caution. Epidemiological factors associated with PCa include age, ethnicity, BMI, metabolic status and testosterone levels. Similarly, variations in testosterone levels related to these factors in both the general and cancer populations will present a challenge to urologists in the near future, given the ageing, multiethnic population that needs to be educated about health and cancer risk factors [1, 2, 6].

A large study in Caucasian patients treated surgically showed no association between testosterone and recurrence [11]. However, it reported an association with unfavorable tumour grade [11]. A study in Asian patients with localized PCa showed that preoperative testosterone predicted biochemical recurrence [12]. In that study, testosterone did not predict unfavorable pathology [12]. Another Asian cohort showed that both low and high testosterone predicted poor prognosis [13]. A Caucasian cohort showed that low testosterone predicted unfavorable pathology and outcomes [14]. Baseline testosterone should be evaluated alongside established prognostic systems such as EAU or NCCN risk classifications [1–3, 15, 16]. Its prognostic value should also be independent of high-grade disease, defined as ISUP 4/5 [15, 16]. In the present study, baseline testosterone was associated with adverse tumor grade at biopsy and at final pathological examination. Adverse tumor grade was

defined as ISUP 4/5 [15, 16]. Moreover, baseline testosterone independently predicted disease progression after adjustment for established prognostic factors. This association remained significant after adjustment for standard prognostic factors [1–3]. In our study, we focused on disease progression as a single composite endpoint, including biochemical recurrence, local recurrence and metastatic progression; although this single outcome may appear to differ in their specific biological and clinical significance, it seems more likely to be correlated with the natural history of prostate cancer; accordingly, biochemical persistence/recurrence may precede or accompany locoregional recurrence and/or systemic recurrence, which are more likely to be detected using Positron Emission Tomography-Prostate-Specific Membrane Antigen (PET-PSMA) or more advanced molecular imaging in the near future [1, 2, 15, 16].

Our results showed a positive association between basal testosterone and adverse tumour grade (ISUP 4/5 in the surgical specimen) and disease progression after robotic surgery in a large PCa population, including all EAU prognostic risk classes. Accordingly, higher baseline testosterone levels were associated with increased risk of disease progression, consistent with pathological findings and disease prognosis. Similarly, a study of 338 patients with low-risk prostate cancer and features indicating AS showed an inverse association between basal testosterone levels and adverse pathological features in the surgical specimen, including tumor upstaging, upgrading, unfavorable disease, positive surgical margins, and predominant Gleason 4 [17]. Accordingly, lower testosterone levels predicted adverse pathological features. However, this study only included Caucasian patients and did not control for this factor [17]. Furthermore, it did not include all EAU prognostic risk classes and did not evaluate disease progression [17]. On the other hand, a study of basal testosterone showed no association with the risk of PCa at biopsy or unfavorable tumor grade in the placebo arm of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial, which included 3255 patients, of whom 819 were diagnosed with prostate cancer. However, the study had limitations, including a mixed ethnic population, the inclusion of only low-to-intermediate

**TABLE 3. Multivariate analysis showing the impact of baseline TT levels on the risk of prostate cancer progression after robotic surgery in 500 patients presenting with clinically localized disease.**

| Groups of Variables             | Baseline TT (nmol/L) |         | TT normal vs. subnormal (nmol/L) |         |
|---------------------------------|----------------------|---------|----------------------------------|---------|
|                                 | HR (95% CI)          | p-value | HR (95% CI)                      | p-value |
| Risk after adjusting for:       |                      |         |                                  |         |
| (a) clinical factors            | 1.039 (1.004–1.076)  | 0.028   | 1.573 (1.040–2.377)              | 0.032   |
| (b) pathology factors           | 1.038 (1.006–1.071)  | 0.019   | 1.604 (1.081–2.379)              | 0.019   |
| (c) EAU prognostic risk classes |                      |         |                                  |         |
| EAU intermediate vs. low        | 2.675 (1.545–4.631)  | <0.0001 | 2.585 (1.493–4.474)              | 0.001   |
| EAU high vs. low                | 5.985 (3.394–10.554) | <0.0001 | 5.581 (3.159–9.861)              | <0.0001 |

*In order to assess the impact of preoperative TT as both a continuous and categorized variable, we considered three multivariable models involving clinical and pathological variables as well as EAU prognostic risk classes, adjusting them for the aforementioned variables. HR, hazard ratio; CI, confidence interval; TT, total testosterone; EAU, European Association of Urology; baseline TT categorized as subnormal if up to 12 nmol/L and normal if >12 nmol/L.*

risk categories, and the failure to evaluate patient follow-up after diagnosis with PCa [18].

Interestingly, a collaborative analysis of 20 prospective trials involving 6933 patients revealed that men with low free testosterone levels were less likely to develop prostate cancer overall. However, there was no follow-up to corroborate this finding, which was consistent with our results [19].

Testosterone levels also showed stratification ability within each EAU risk group [1, 15, 16]. Patients with subnormal testosterone levels represented 33% of the cohort. These patients were less likely to present with adverse tumor grades. They were also less likely to experience disease progression. Patients with higher testosterone levels showed increased risk of adverse pathology. They also had a higher risk of disease progression. Testosterone demonstrated independent prognostic value across each EAU risk category [1–3, 15, 16]. These findings support the hypothesis that long-term androgen exposure may influence prostate cancer biology and clinical behavior [6, 20–22]. Baseline testosterone may represent an additional prognostic biomarker in the natural history of PCa. These results may have implications for clinical practice and disease biology. Identifying additional prognostic factors is crucial in patients with localized PCa [1–3]. These factors should be easily applicable in routine clinical practice [1–3]. They should also provide independent information beyond standard risk classifications [1–3]. Our results support a role for baseline testosterone in this context. Higher TT levels were associated with high-grade tumor features, particularly ISUP grade groups 4/5 [15, 16]. They also predicted disease progression. Prostate cancer remains a hormone-dependent malignancy [6, 20]. Increasing testosterone levels were associated with worse outcomes in our cohort. Conversely, subnormal testosterone levels were associated with more favorable pathology [6, 18]. These patients also had a lower risk of progression. Subnormal testosterone was present in one-third of patients. This variable may help refine risk stratification within EAU categories [1, 15, 16]. Future studies should incorporate testosterone into predictive models. These models may include nomograms or artificial intelligence tools. Such approaches could improve stratification of PCa biology. PSA-based screening continues to increase PCa diagnosis worldwide [3]. Our findings also support biological hypotheses linking testosterone and prostate cancer [6, 20–22]. Prostate growth disorders are influenced by age, ethnicity, metabolic status, BMI, and testosterone levels [6, 21, 22]. These variables may affect the prostate microenvironment and cellular interactions [17–19]. In our study, subnormal testosterone appeared protective. It was associated with lower rates of aggressive disease. Prostate cancer is a well-established endocrine-dependent tumor [6, 17]. Androgen deprivation therapy is widely used in clinical practice [6, 20]. This treatment aims to reduce baseline TT. In advanced disease, treatments target intracellular androgen signaling [20]. This occurs even in castrate systemic conditions [20]. Our results are consistent with hormone-sensitive and castration-resistant disease stages [20]. Baseline testosterone may influence tumor microenvironment dynamics [20–22]. It may affect tumour growth and differentiation patterns [20–22]. Lower testosterone levels may protect against poorly differentiated tumors. These tumors are often

associated with a high mutational burden. However, these hypotheses require further investigation [6, 20–22].

Although our results derive from patients with prostate cancer across all EAU risk classes treated with robotic surgery, these findings should be interpreted within the context of a surgical cohort. Their applicability to other treatment settings, including radiation therapy, remains hypothesis-generating and requires validation in dedicated non-surgical cohorts. Therefore, extrapolation beyond the surgical setting should be made with caution. These findings may be of clinical interest, although their impact on treatment decision-making outside the surgical setting requires further validation.

Several limitations of the present study should be acknowledged. First, the retrospective nature of the study may have introduced selection and information biases. Second, testosterone levels were measured only once. Third, biopsy specimens from other centers were not reviewed. Serum sex hormone-binding globulin (SHBG) was not measured. Therefore, calculation of free testosterone was not performed. Direct measurement of free testosterone using current assays is considered unreliable [7–9]. Most studies investigating androgens and prostate cancer use total testosterone. Total testosterone is considered more reliable than free testosterone [7–9]. Fourth, we did not stratify survival analyses according to adjuvant or salvage treatments. These treatments were administered after surgery and disease progression. Our analysis focused on baseline testosterone at diagnosis. Adjusted models confirmed the independent prognostic value of testosterone. However, despite multivariable adjustment, residual confounding cannot be entirely excluded. Larger multicenter studies with longer follow-up may further address this issue. One further potential limitation of our study is that the results relate only to patients who have undergone surgery. Accordingly, they may not be representative of all patients with localized prostate cancer who opt for active surveillance and radiation therapy.

One further limitation of our study is that the results relate only to patients treated surgically. Accordingly, they may not be representative of all patients with localized prostate cancer managed with active surveillance or radiation therapy. In addition, the study was single-center and included only Caucasian patients, which may limit the external validity of the findings. Therefore, extrapolation beyond this surgical cohort should be made with caution and requires confirmation in broader, multi-ethnic, and treatment-specific populations.

Despite these limitations, the study has notable strengths. Baseline TT was measured in the morning under standardized conditions [7]. Testosterone levels fluctuate during the day due to circadian rhythm. A large multi-ethnic study showed that testosterone levels are 20–25% lower in the afternoon in men aged 30–40 years. This difference decreases with age and is about 10% at 70 years [23]. That study recommended measuring testosterone in the morning to ensure reliable assessment [23]. In our study, testosterone was measured in the morning. Therefore, our results are consistent with current recommendations and may be considered reliable. All surgical specimens were evaluated by a dedicated pathologist. This ensured consistency in pathological assessment.

## 5. Conclusions

Baseline TT was associated with adverse pathological characteristics and independently predicted disease progression in patients undergoing robotic surgery for clinically localized PCa [1–3, 15, 16]. Higher TT levels were associated with less favorable pathological findings and a higher risk of progression, whereas subnormal TT levels were associated with more favorable disease features [6, 21, 22]. Baseline TT may provide additional prognostic information and contribute to a more refined risk stratification strategy in the surgical management of PCa [1–3, 15, 16]. However, these findings should be interpreted in light of the retrospective design and require external validation, particularly in non-surgical cohorts.

## AVAILABILITY OF DATA AND MATERIALS

Data are contained within the article.

## AUTHOR CONTRIBUTIONS

ABP and MAC—conceptualized the research. AA, FC and AB—performed the research. FA, FM, SC and RR—performed data curation. AF, AV, MB and RGB—provided help and advice on methodology and manuscript review. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the Declaration of Helsinki. As a retrospective observational study planned before September 2024, the study was granted exempt status from formal IRB approval. Informed consent for data collection within the database and its use in scientific research was obtained from all subjects involved in the study.

## ACKNOWLEDGMENT

Not applicable.

## FUNDING

This research received no external funding.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**How to cite this article:** Antonio Benito Porcaro, Maria Angela Cerruto, Andrea Franceschini, Filippo Caudana, Alberto Baielli, Francesco Artoni, Francesca Montanaro, Sonia Costantino, Riccardo Rizzetto, Alessandro Vecchia, Matteo Brunelli, Riccardo Giuseppe Bertolo, Alessandro Antonelli. Baseline total testosterone is associated with high-grade prostate cancer and predicts disease progression: results and clinical implications in 500 patients treated with robotic surgery. *Revista Internacional de Andrología*. 2026; 24(3): 19-28. doi: 10.22514/j.androl.2026.027.