Is there a relationship between testosterone and androgen receptor with prostatectomy outcomes?

Bárbara Vieira Lima Aguiar Melão, MD,[1] Sabrina Thalita dos Reis Faria, MD,[2] Kátia Ramos Moreira Leite, MD,[2] Ruan César Aparecido Pimenta, MD,[2] Miguel Srougi, MD,[1] Alberto Azoubel Antunes, MD[1]

[1]Division of Urology, University of São Paulo Medical School, São Paulo, Brazil

[2]Medical Investigation Laboratory (LIM 55), Urology Department, University of São Paulo Medical School, São Paulo, Brazil

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Introduction: *Prostate cancer has a variable natural history and, despite the existence of biochemical recurrence (BCR) predictors, they are still limited in predicting outcomes. The role of testosterone in advanced prostate cancer is well known, however its role in localized prostate cancer is still uncertain. In the present study, we evaluated the relationship of testosterone levels and androgen receptor (AR) expression with oncological and functional outcomes, in patients undergoing radical retropubic prostatectomy (RRP).*

Materials and methods: *Through a retrospective study, patients who underwent RRP, who had at least two preoperative total testosterone dosages, were analyzed and compared according to testosterone levels, oncological and functional outcomes. After analyzing data, tissue samples were selected in a biorepository to carry out the AR and the AR-V7 expression.*

Results: *After applying exclusion criteria, 212 patients were included in the analysis. Thirty-two patients (15.1%) had low testosterone levels and, in this group, a lower rates of erectile function recovery were observed at 24 months (53.1% vs. 71.7%; p = 0.037), a higher rate of BCR (21.9% vs. 9.4%; p = 0.041) and higher International Society of Urological Pathology (ISUP) grade in biopsy products. The AR expression was higher in patients with low testosterone, but there was no difference in relapse rates.*

Conclusions: *Lower levels of testosterone were related to lower rates of erectile function recovery at the end of 24 months after RRP, in addition to conferring higher rates of BCR and higher ISUP grades in biopsy. Furthermore, patients with total testosterone < 300 ng/dL had higher expression of AR, but no difference in BCR rates.*

Key Words: prostate cancer, testosterone, radical prostatectomy, functional outcomes, oncological outcomes, androgen receptor

Introduction

Prostate cancer is the second most common tumor in men, corresponding to about 15% of diagnosed cancers and its incidence has increased over the years.[1] Radical retropubic prostatectomy (RRP) is the most common treatment in localized and locally advanced prostate cancer and aims to eradicate the cancer, preserving, whenever possible, the patient's erectile function and continence.[2] It presents satisfactory long term oncological outcomes, with cancer-specific survival rates that vary across studies, from 80% to 99%.[3,4] Functional outcome data, however, show great variability between series, with erectile function recovery rates ranging from 44% to 73%[5] and post-

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Address correspondence to Dr. Bárbara Vieira Lima Aguiar Melão, Division of Urology, USP Medical School, Av. Dr. Enéas Carvalho de Aguiar, 255, Cerqueira César, São Paulo, SP 05403-000 Brazil

prostatectomy incontinence (PPI) rates from 4% to 31%.[6] This can be explained by different definitions and methodologies of postoperative evaluation, in addition to the characteristics of the patients, the surgeon's experience and the surgical technique used.[5]

The relationship between testosterone and prostate cancer has always been a subject of great interest, since androgens are necessary for prostatic differentiation and throughout life, they continue to participate in homeostasis and prostatic growth through the androgen receptor (AR),[7] which has been implicated in almost all forms of prostate cancer.[8]

The role of testosterone in prostate cancer was first demonstrated in 1941 by Huggins et al, who established the role of castration in prostate cancer regression, and since then, androgen deprivation therapy (ADT) has become the mainstay for the treatment of advanced prostate cancer.[9] Contrary to Huggins' studies, in 2006, Morgentaler proposed the saturation theory, according to which there is a limit to the maximum androgenic stimulus for prostatic growth. When this limit is reached, there would be little or no additional prostatic stimulation with the progressive increase in testosterone, most likely due to AR saturation.[10]

Studies have failed to show a correlation between testosterone levels and the risk of developing prostate cancer,[11-13] but endogenous testosterone has already been associated with oncological outcomes after prostate cancer treatment, with results that are still controversial.[14-21]

Circulating androgen levels play a role in regulating the AR expression,[22] which is generally overexpressed in prostate cancer, with great heterogeneity[23] and with lower immunoactivity in benign tissues.[24] An increase in AR expression has already been described for castration resistant prostate cancer (CRPC),[25] however the prognostic role of AR in prostate cancer is still controversial.[26-28] More than 20 AR variants have already been described, with AR-V7 being the most studied and significant variant, as it produces active protein even in the absence of androgens, which confers resistance to new generation anti-androgens, leading to androgen-independent tumor growth.[29] Studies have already demonstrated a higher prevalence of AR-V7 in CRPC than in hormone sensitive prostate cancer (HSPC),[30] however in the primary prostate cancer setting after surgical treatment, AR-V7 proved to be a predictor of biochemical recurrence (BCR).[31]

Materials and methods

This is a retrospective study, which was approved by the Research Ethics Committee of the University of São Paulo Medical School, under protocol number CAAE: 34544620.6.0000.0068.

First, a retrospective analysis of the medical records of 666 patients diagnosed with prostate cancer, who underwent RRP from January 2014 to December 2017, in a single center and performed by a single surgeon, for whom at least two blood levels of total testosterone were determined.

The exclusion criteria included patients who were unable to neurovascular bundles preservation, patients who were previously incontinent or previously impotent, patients using 5-alpha-reductase inhibitors for less than 6 months before surgery or who had previously undergone testosterone replacement therapy (TRT), patients undergoing neoadjuvant or adjuvant ADT, patients undergoing prior or postoperative pelvic radiotherapy, patients undergoing previous surgery for prostatic hyperplasia, patients with only one dosage of testosterone and those who were not followed up. Ultimately, 212 patients were included.

The preoperative criteria used to define low testosterone levels was a total testosterone level < 300 ng/dL.[32] The pathological staging of prostate cancer followed the classification recommended by the American Joint Committee on Cancer, according to the 8th edition, published in 2017.[33] Risk stratification was based on D'Amico's criteria.[34]

To compare the outcomes, the total testosterone median level was considered and the patients were divided into two groups: group 1 included patients with mean dosages below 300 ng/dL and group 2 included patients with mean total testosterone levels available greater than or equal to 300 ng/dL.

The postoperative criteria for BCR is defined as a prostate-specific antigen (PSA) above 0.2 ng/mL.[35] Continence is defined as the use of < 2 pads/day[36] and potency as the ability to maintain sufficient erections to perform intercourse (with or without the use of phosphodiesterase type 5 inhibitor use), both evaluated in 6, 12 and 24 months after surgery.

After analyzing the clinical data, tissue samples from the patients of interest were selected from a biorepository to carry out the analysis of AR and AR-V7 expression.

TABLE 1. **TaqMan assay tables were used in the study**

Genes	Assay	Company
AR	Hs00171172_m1	Applied Biosystems
AR-V7	Hs04260217_m1	Applied Biosystems
B2M	Hs00187842_m1	Applied Biosystems

Applied Biosystems, CA USA

TABLE 2. **Comparison of oncological outcomes**

		Total testosterone (median)		p value
		< 300 (group 1)*	**≥ 300 (group 2)***	
Diabetes	No	28 (87.5)	161 (89.4)	0.745
	Yes	4 (12.5)	19 (10.6)	
Family history	No	18 (56.3)	105 (58.3)	0.826
	Yes	14 (43.8)	75 (41.7)	
ISUP (biopsy)	1	8 (25)	63 (35)	0.006
	2	8 (25)	78 (43.3)	
	3	9 (28.1)	16 (8.9)	
	4	6 (18.8)	22 (12.2)	
	5	1 (3.1)	1 (0.6)	
ISUP (biopsy)	1 e 2	16 (50)	141 (78.3)	0.001
	3, 4 e 5	16 (50)	39 (21.7)	
Clinical stage	Localized	32 (100)	180 (100)	
	Locally advanced	0	0	
ISUP (specimen)	1	4 (12.5)	45 (25)	0.466
	2	9 (28.1)	56 (31.1)	
	3	11 (34.4)	49 (27.2)	
	4	7 (21.9)	25 (13.9)	
	5	1 (3.1)	5 (2.8)	
ISUP (specimen)	1 e 2	13 (40.6)	101 (56.1)	0.105
	3, 4 e 5	19 (59.4)	79 (43.9)	
Pathological stage	Localized	24 (75)	146 (81.1)	0.424
	Locally advanced	8 (25)	34 (18.9)	
pT3a	Negative	24 (75)	147 (81,7)	0.379
	Positive	8 (25)	33 (18.3)	
pT3b	Negative	32 (100)	178 (98.9)	0.549
	Positive	0	2 (1.1)	
Lymph nodes	Negative	32 (100)	180 (100)	
	Positive	0	0	
Surgical margin	Negative	29 (90.6)	172 (95.2)	0.247
	Positive	3 (9.4)	8 (4.4)	
Risk stratification	Low risk	9 (28.1)	58 (32.2)	0.504
	Intermediate risk	16 (50)	97 (53.9)	
	High risk	7 (21.9)	25 (13.9)	
Recurrence	No	25 (78.1)	163 (90.6)	0.041
	Yes	7 (21.9)	17 (9.4)	
Upgrading	Yes	15 (46.9)	65 (36.1)	0.247
	No	17 (53.1)	115 (63.9)	

*group 1 = patients with testosterone < 300ng/dL; group 2 = patients with testoterone ≥ 300 ng/dL
ISUP = International Society of Urological Pathology

Analysis of AR and AR-V7 gene expression

Cellular RNAs were extracted from samples from clinical specimens using the mirVana kit (Ambion, Austin, TX, USA), according to the manufacturer's instructions. The total RNA was then synthesized using the High-Capacity cDNA Reverse Transcription Kit for complementary DNA synthesis (Applied Biosystems, CA, USA). The target gene sequences were amplified in a 10 µL reaction

mixture containing 5 µL of TaqMan Universal PCR Master Mix and 0.5 µL of TaqMan gene expression, Table 1. All qPCR reactions were performed in duplicate. Data were analyzed using the DataAssist Software (Applied Biosystems, USA) and B2M was used as the endogenous control in the gene expression analysis.

Statistical analysis

Data were presented as median and standard deviation for continuous variables. Comparison between groups was performed using Student's t test and Anova (homogeneous variables) and Mann- Whitney (non-homogeneous variables). Categorical variables were presented as frequencies and percentages and analyzed using the chi-square test. Binary logistic regression was used to calculate risk factors for BCR and postoperative erectile dysfunction. Statistical analysis were performed by SPSS 19.0 software for Windows, and throughout the analysis, p was considered significant when less than or equal to 0.05.

Results

Altogether 212 patients were included in the study. The median age of patients was 60 (± 9.12) years, 89.15% did not have diabetes or family history of prostate cancer (58.01%). The median total testosterone was 446.41 (± 161.3) ng/dL, ranging from 102 to 1445 ng/dL, and 32 of them (15.1%) lower than 300 ng/dL.

The median preoperative PSA was 5.27 (± 3.23) ng/dL, most patients had ISUP 2 and 3 in the biopsy products, corresponding to 33.5% and 40.56%, respectively; and testosterone correlated with higher ISUP grade in biopsy ($p < 0.006$), as shown in Table 2.

In the pathological analysis of the surgical specimen, which was performed by a single pathologist, the median prostatic volume was 41.47 (±19.71) g, extra-prostatic extension was present in 18.87% patients and seminal invasion in only 2 patients (0.94%). The positive surgical margin rate was 5.19% and BCR was observed in 11.32% of the patients, with testosterone being positively related to recurrence ($p = 0.041$). The median time for recurrence was 34 (± 13.68) months and the median follow up duration was 40 (± 14.08) months. Despite higher rates of extra-prostatic extension tumors, higher ISUP grade in the surgical specimen and higher rates of positive surgical margin in the group of patients with low testosterone levels, including with a higher upgrading, it was not statistically significant, Table 2.

As for the functional outcomes, 90.57% of the patients were continent at the 6-month postoperative evaluation, only 6 (2.83%) were incontinent 24 months after surgery. Erectile function was recovery in 34.9% of the study population at 6 months, gradually increasing at 12 months (41.5%) and 24 months (68.87%). Furthermore, recovery of erectile function was greater in group 2, with a statistically significant difference only in the 24 months` evaluation ($p = 0.037$), Table 3.

TABLE 3. **Comparison of functional outcomes**

		Total testosterone (median)		p value
		< 300 (group 1)*	**≥ 300 (group 2)***	
Urinary incontinence				
6 months	Continent	28 (87.5)	164 (91.1)	0.520
	Incontinent	4 (12.5)	16 (8.9)	
12 months	Continent	29 (90.6)	173 (96.1)	0.177
	Incontinent	3 (9.4)	7 (3.9)	
24 months	Continent	30 (93.8)	176 (97.8)	0.206
	Incontinent	2 (6.3)	4 (2.2)	
Erectile function				
6 months	Present	8 (25)	66 (36.7)	0.202
	Absent	24 (75)	114 (63.3)	
12 months	Present	15 (46.9)	109 (60.6)	0.148
	Absent	17 (53.1)	71 (39.4)	
24 months	Present	17 (53.1)	129 (71.7)	0.037
	Absent	15 (46.9)	51 (28.3)	
Use of PDE5I	No	4 (12.5)	19 (10.9)	0.794
	Yes	28 (87.5)	155 (89.1)	

*group 1 = patients with testosterone < 300 ng/dL; group 2 = patients with testosterone ≥ 300 ng/dL

TABLE 4. **Logistic regression for risk factors for biochemical recurrence**

Variables	Biochemical recurrence		
	OR	95%CI	p value
TT (normal vs. low)	0.489	0.159-1.503	0.212
Age (continuous)	1.027	0.969-1.088	0.370
PSA (continuous)	3.056	0.754-12.381	0.118
Biopsy ISUP (2/3 vs. 4/5)	1.943	0.839-4.499	0.121
Specimen ISUP (2/3 vs. 4/5)	4.168	1.585-10.959	0.004
Pathological stage (T2 vs. T3)	1.517	0.684-3.365	0.306
Surgical margin (positive vs. negative)	11.521	2.563-51.778	0.001

OR = odds ratio; CI = confidence interval; TT = total testosterone; PSA = prostate-specific antigen; ISUP = International Society of Urological Pathology

To assess independent risk factors for BCR, logistic regression analysis was performed, which showed that patients with a positive surgical margin (p = 0.001) and with high-risk ISUP grade in the surgical specimen (p = 0.004) were independently associated with BCR. However, total testosterone was not independently associated with relapse, Table 4. Likewise, logistic regression also showed independently correlation of age (p < 0.001) with erectile function recovery, but not of testosterone with erectile function (p = 0.082), Table 5.

TABLE 5. **Logistic regression for risk factors for the recovery of the erectile function**

Variables	Erectile function		
	OR	95%CI	p value
TT (normal vs. low)	2.085	0.911-4.77	0.082
Age (continuous)	0.917	0.881-0.954	< 0.001

OR = odds ratio; CI = confidence interval; TT = total testosterone

TABLE 6. **AR and AR-V7 expression**

	Expression	
	AR	AR-V7
Group 1*		
With recurrence	4 (100%)	2 (50%)
Without recurrence	6 (100%)	6 (100%)
Group 2*		
With recurrence	7 (100%)	4 (57,1%)
Without recurrence	5 (100%)	4 (80%)

*group 1 = patients with testosterone < 300 ng/dL
group 2 = patients with testosterone ≥ 300 ng/dL

For the AR gene expression analysis, 10 patients from group 1 were selected, 4 patients who relapsed and 6 patients who did not relapsed. And from group 2, 7 patients who recurred and 5 patients without recurrence were selected. All selected patients expressed AR and the prevalence of AR-V7 expression was according to Table 6

Subsequently, a comparison was made between groups 1 and 2, showing a statistically significant higher AR expression among patients in group 1, Figure 1A. Afterwards, the patients were compared in terms of recurrence, and no difference was observed in gene expression between those who relapsed and those who did not, Figure 1B. Afterwards, patients in group 1 were compared for recurrence, with no difference in AR expression being observed, Figure 1C. The same comparative analysis was also performed in group 2, with no statistically significant difference in AR expression between patients who relapsed or not, Figure 1D.

The AR-V7 expression was also higher in group 1, Figure 2A, however, did not have statistically significant differences in expression when patients were compared in terms of BCR in group 1, Figure 2B or in group 2, Figure 2C. Statistical analysis was not possible in group 1 because only 2 patients expressed AR-V7.

Discussion

The main goal of surgical treatment in localized prostate cancer is the complete removal of the tumor, preserving, whenever possible, patient's continence and erectile function. The natural history of patients with prostate cancer is very variable, and the known predictive factors do not always predict disease

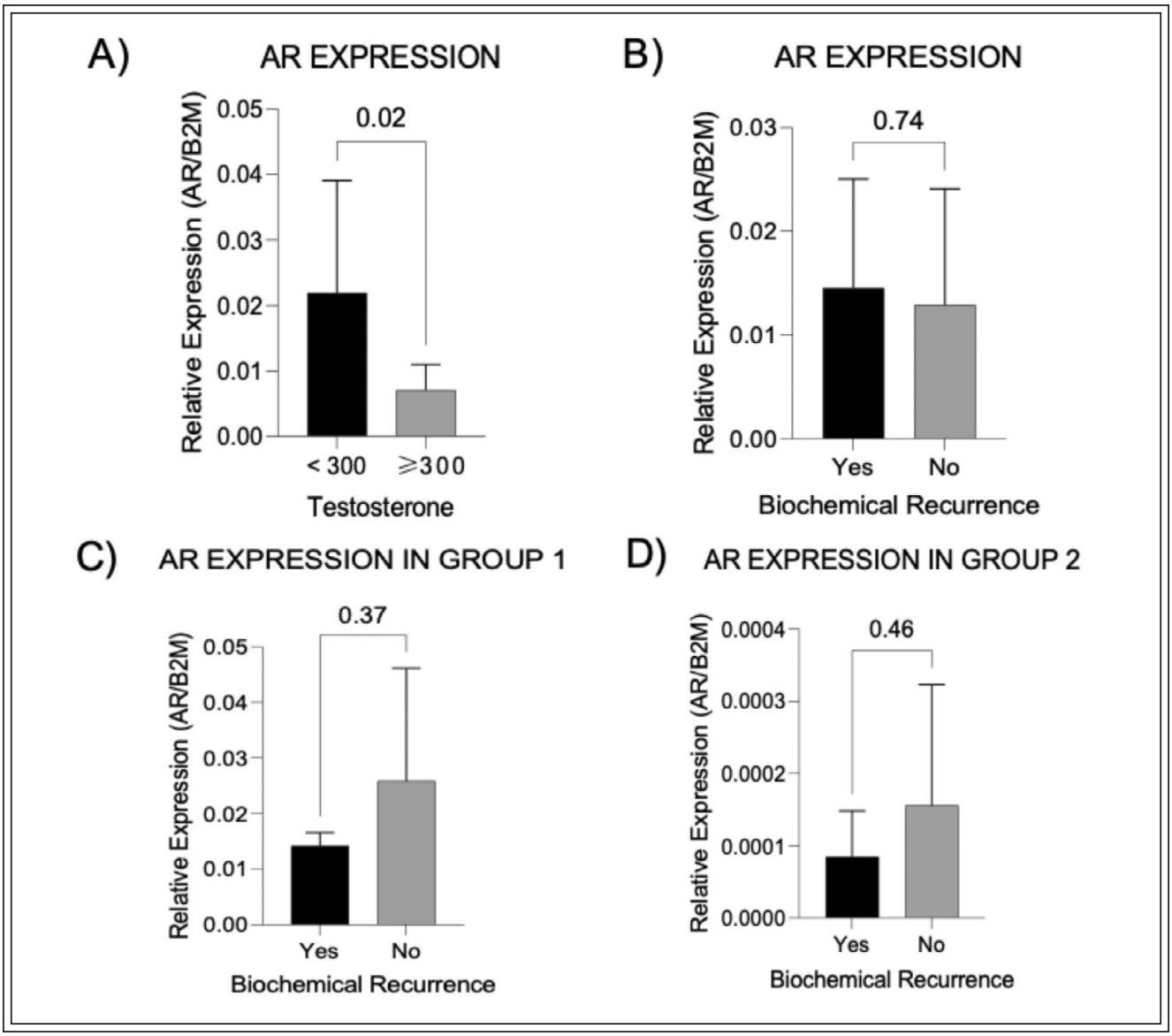

Figure 1. Comparative analysis of androgen receptor (AR) expression.

outcomes, as highlighted for Kehinde et al, and are therefore limited in their prognostic capacity.[37] Testosterone has a well-established role in advanced prostate cancer setting, but it still has a controversial role in localized setting, not being recognized as a predictive or prognostic factor.

In the present study, low testosterone levels were statistically associated with higher rates of BCR, although not independently in the multivariate analysis. Massengill et al evaluated 879 patients' records treated with radical prostatectomy and found low pretreatment testosterone levels as an independent predictor of extraprostatic disease, but not with BCR.[21] Some other studies also associated low levels of testosterone with worse oncological outcomes[16-19] and higher rates of BCR;[38] however, the data are still conflicting and the use of testosterone as a preoperative predictive factor is still controversial. In patients eligible for active surveillance (AS), Ferro et al demonstrated that low testosterone levels were associated with disease progression, suggesting that levels of this androgen should be evaluated in patients who are candidates for AS.[39]

The mechanism for testosterone effect is not completely understood. One hypothesis for worse pathological outcomes would be the fact that lower androgen levels could generate an active

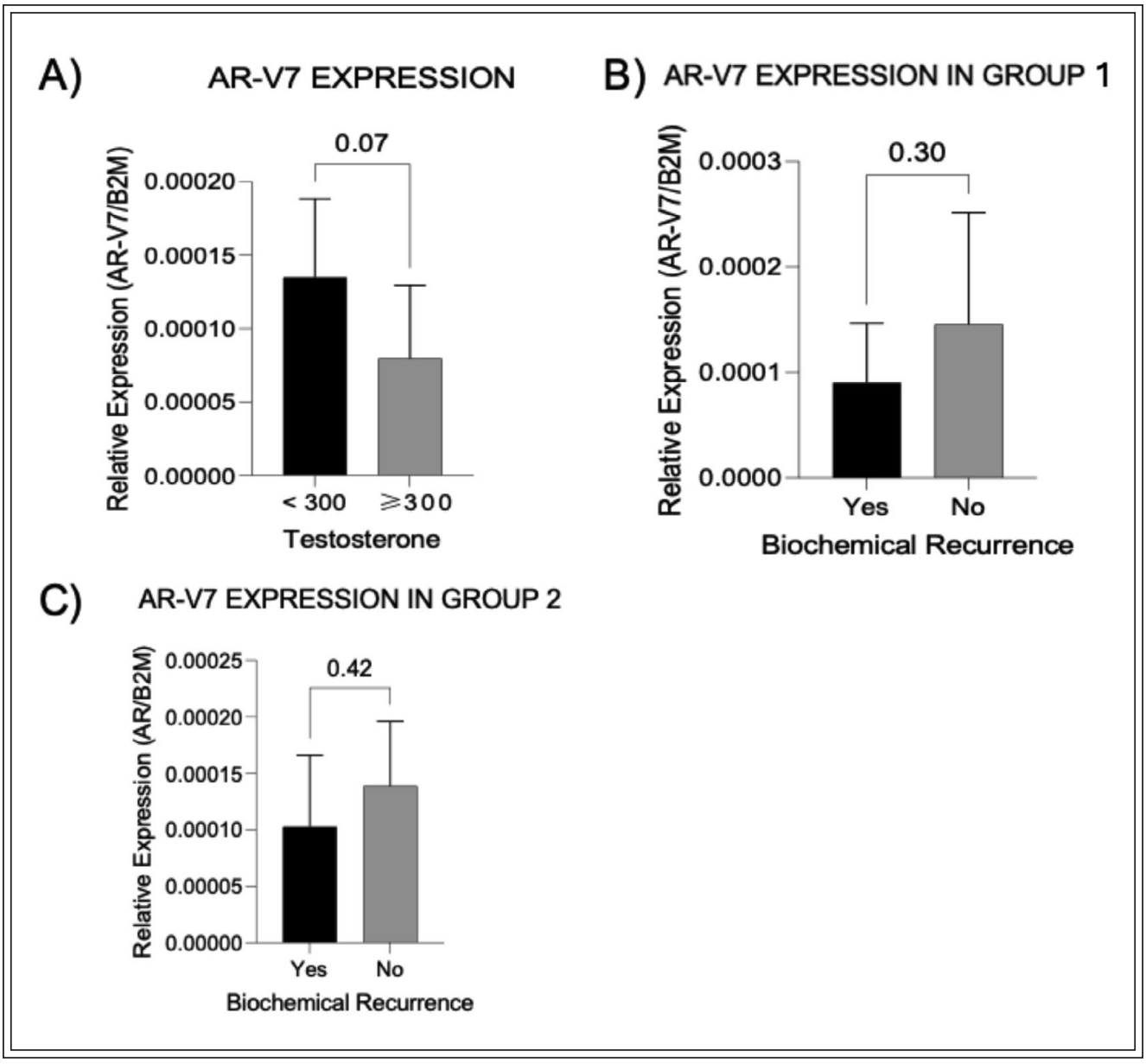

Figure 2. Comparative analysis of AR-V7 expression.

environment of androgen deficiency, which could work as a promoter for the development of more aggressive prostate cancer. Furthermore, high-grade tumors seem to exert negative feedback, decreasing the secretion of the hypothalamic-pituitary axis.[40] This theory comes from the analysis by Miller et al who reported a significant increase in gonadotropin and testosterone levels after radical prostatectomy in patients with prostate cancer.[41]

Furthermore, in our study, we observed lower rates of erectile function recovery in patients with low testosterone levels in the 24-month postoperative evaluation, although not independently associated contrary to age, independently associated with poorer functional recovery, therefore have already be shown to be valuable in patient counseling.[42] Although testosterone is involved in the male sexual response, the relationship between testosterone and erectile dysfunction is not linear,[43,44] being more significant in individuals with low testosterone levels,[45] but even in this scenario, the androgen level that would be responsible for causing erectile dysfunction is very controversial in the literature and is influenced by other factors, mainly age and comorbidities, which

end up attenuating this relationship.[46] Furthermore, testosterone has an established role in regulating penile functioning, with studies in animal models demonstrating upregulation of the production of nitric oxide (NO) synthetase, which produces NO, a key mediator of erectile function,[47] which would also help to understand the best erectile response in patients with normal testosterone. With the results found in our study, it would be advisable for patients to be informed preoperatively about the lower possibility of recovery of erectile function after surgery for prostate cancer when serum testosterone levels are reduced.

In the present study, no difference was observed in continence recovery related to testosterone levels. Similarly, Paula Domino et al found no evidence of an association between hypogonadism and delay on urinary continence recovery among 1209 patients treated by radical prostatectomy.[48] This correlation, to date, has been little studied and some authors suggested periurethral changes in hypogonadal men,[49] while others demonstrated a correlation between urinary function and sexual function only in eugonadal patients. The bladder expresses PDE5 in smooth muscle cells and in the endothelium of veins and its androgen-dependent PDE5 activity could explain this finding.[50]

As testosterone is the main androgen in the body and its receptor is the AR, we analyzed the expression of AR and one of its most studied variants, AR-V7, in a subgroup of selected patients. Our study demonstrated greater AR expression in patients with low testosterone levels, which probably occurs to maintain an adequate androgen response,[22] with studies even demonstrating sensitization of prostate cancer cells to lower levels of androgens.[51] However, in the comparative analysis between patients with testosterone < 300 ng/dL, there was no difference in AR or AR-V7 expression between patients who relapsed or not. That is, although low testosterone was associated with relapse in our population and with increased AR expression, this higher expression was not observed in patients who relapsed. This may be explained by the fact that, separately, the expression of AR and AR-V7 is not a predictive factor for recurrence.

In the CRPC setting, AR and its variants are associated with ADT resistance and a greater chance of disease progression.[52] In the localized prostate cancer setting, the relationship between AR expression and prognosis after surgical treatment of prostate cancer is still controversial, with some studies relating increased expression with worse prognosis[27,28,53] and others not demonstrating prognostic value in AR expression.[24,54] The choice of tissue studied may influence the results, since tissue from prostate biopsy, used in many studies, may not adequately represent the pathological profile of the tumor. In our study, a biorepository was used with a tissue sample from surgical specimen, selected to better represent the tumor in each patient. Schatzl et al evaluated the association of testosterone with androgen receptor expression in patients with newly diagnosed prostate cancer and correlated low testosterone levels with an increase in androgen receptor density, as we also identified in our study, however Schatzl used tissue samples from biopsy.[55]

The active variants of AR, with special importance to AR-V7, had their emergence associated with the progression of prostate cancer to the CRPC setting.[56] However, new studies have demonstrated its presence in prostate cancer tissue never submitted to ADT and in BPH tissues,[57] which was corroborated by our study. AR-V7, considered a prognostic factor for first-line hormone therapy and prostatectomy, correlated to lower progression free survival (PFS)[52] and overall survival (OS)[30] in advanced prostate cancer, has already been associated with higher rates of BCR in a cohort of patients with localized prostate cancer with a high risk of recurrence.[31,58] However, our study failed to demonstrate this association, perhaps due to the small number of patients in the analysis and the fact that not only high-risk patients were being analyzed. A meta-analysis, which gathered the main studies on the subject up to December 2019, demonstrated that the prevalence of AR-V7 is significantly higher in CRPC than in HSCP, probably being an adaptive response to AR-target therapies, and that it could be a useful biomarker in these patients.[30]

The strengths of this study lie in the fact that all patients analyzed underwent RRP by a single surgeon at a large volume tertiary center, which reduces the variability in functional and pathological outcomes due to the surgical technique or surgeon's experience. All surgical specimens were evaluated by the same pathologist, specialized in genitourinary tumors, minimizing inter-observer interpretation differences. In addition, pre and postoperative assessments of all patients were performed by the same experienced team. All patients had a confirmatory total testosterone measurement and patient inclusion criteria were strict. In addition, the tissues samples used for molecular analysis came from surgical specimens, examined and selected to better represent the tumor.

However, the study has limitations that must be considered. First, the study is retrospective. Testosterone measurements were not performed in the same laboratory or using the same methodology. Functional assessments were not carried out using

validated questionnaires, which could cause inter-observer variability, even within the same team. Erectile function is known to be influenced by factors that have not been evaluated in this population, such as obesity, high blood pressure, and smoking.

The study highlights the importance and impact of testosterone on the outcomes after definitive surgery for prostate cancer, but prospective studies with a larger population are needed to confirm these findings and to support testosterone measurement as a predictive and, perhaps, prognostic factor in the localized prostate cancer setting.

Conclusion

From the data analyzed, we concluded that lower testosterone serum levels correlate with worse recovery of erectile function in patients after RRP, at the end of 24 months of evaluation, in addition to conferring higher rates of BCR and higher ISUP grade in biopsy products, although it does not seem to influence other post-surgical pathological outcomes. In addition, patients with total testosterone < 300 ng/dL have higher AR expression, which does not seem to directly influence relapse rates, since there was no difference in expression between patients who relapsed and those who did not within the same group. □

References

1. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136(5):E359-E386.
2. Adolfsson J. Watchful waiting and active surveillance: the current position. *BJU Int* 2008;102(1):10-14.
3. Bill-Axelson A, Holmberg L, Garmo H et al. Radical prostatectomy or watchful waiting in prostate cancer - 29-year follow-up. *N Engl J Med* 2018;379(24):2319-2329.
4. Hamdy FC, Donovan JL, Lane JA et al. 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. *N Engl J Med* 2016;375(15):1415-1424.
5. Ayyathurai R, Manoharan M, Nieder AM, Kava B, Soloway MS. Factors affecting erectile function after radical retropubic prostatectomy: results from 1620 consecutive patients. *BJU Int* 2008;101(7):833-836.
6. Ficarra V, Novara G, Rosen RC et al. Systematic review and meta-analysis of studies reporting urinary continence recovery after robot-assisted radical prostatectomy. *Eur Urol* 2012;62(3):405-417.
7. Schaeffer EM, Marchionni L, Huang Z et al. Androgen-induced programs for prostate epithelial growth and invasion arise in embryogenesis and are reactivated in cancer. *Oncogene* 2008;27(57):7180-7191.
8. Lamb AD, Massie CE, Neal DE. The transcriptional programme of the androgen receptor (AR) in prostate cancer. *BJU Int* 2014; 113(3):358-366.
9. Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *CA Cancer J Clin* 1972;22(4):232-240.
10. Morgentaler A, Traish AM. Shifting the paradigm of testosterone and prostate cancer: the saturation model and the limits of androgen-dependent growth. *Eur Urol* 2009;55(2):310-320.
11. Eaton NE, Reeves GK, Appleby PN, Key TJ. Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. *Br J Cancer* 1999;80(7):930-934.
12. Roddam AW, Allen NE, Appleby P, Key TJ, Group EHaPCC. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. *J Natl Cancer Inst* 2008;100(3): 170-183.
13. Boyle P, Koechlin A, Bota M et al. Endogenous and exogenous testosterone and the risk of prostate cancer and increased prostate-specific antigen (PSA) level: a meta-analysis. *BJU Int* 2016;118(5):731-741.
14. Porcaro AB, Petrozziello A, Ghimenton C et al. Associations of pretreatment serum total testosterone measurements with pathology-detected Gleason score cancer. *Urol Int* 2014;93(3): 269-278.
15. Porcaro AB, Tafuri A, Sebben M et al. Positive association between preoperative total testosterone levels and risk of positive surgical margins by prostate cancer: results in 476 consecutive patients treated only by radical prostatectomy. *Urol Int* 2018;101(1):38-46.
16. Teloken C, Da Ros CT, Caraver F, Weber FA, Cavalheiro AP, Graziottin TM. Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. *J Urol* 2005;174(6):2178-2180.
17. Imamoto T, Suzuki H, Fukasawa S et al. Pretreatment serum testosterone level as a predictive factor of pathological stage in localized prostate cancer patients treated with radical prostatectomy. *Eur Urol* 2005;47(3):308-312.
18. Isom-Batz G, Bianco FJ, Kattan MW, Mulhall JP, Lilja H, Eastham JA. Testosterone as a predictor of pathological stage in clinically localized prostate cancer. *J Urol* 2005;173(6):1935-1937.
19. Lane BR, Stephenson AJ, Magi-Galluzzi C, Lakin MM, Klein EA. Low testosterone and risk of biochemical recurrence and poorly differentiated prostate cancer at radical prostatectomy. *Urology* 2008;72(6):1240-1245.
20. Kratzik C, Womastek I, Bieglmayer C et al. Lower serum total testosterone is associated with lymph node metastases in a radical prostatectomy cohort study. *Anticancer Res* 2011;31(10):3615-3618.
21. Massengill JC, Sun L, Moul JW et al. Pretreatment total testosterone level predicts pathological stage in patients with localized prostate cancer treated with radical prostatectomy. *J Urol* 2003;169(5):1670-1675.
22. Burnstein KL. Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. *J Cell Biochem* 2005; 95(4):657-669.
23. Deng Q, Tang DG. Androgen receptor and prostate cancer stem cells: biological mechanisms and clinical implications. *Endocr Relat Cancer* 2015;22(6):T209-T220.
24. Qiu YQ, Leuschner I, Braun PM. Androgen receptor expression in clinically localized prostate cancer: immunohistochemistry study and literature review. *Asian J Androl* 2008;10(6):855-863.
25. Chen CD, Welsbie DS, Tran C et al. Molecular determinants of resistance to antiandrogen therapy. *Nat Med* 2004;10(1):33-39.
26. Minner S, Enodien M, Sirma H et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. *Clin Cancer Res* 2011;17(18):5878-5888.

27. Henshall SM, Quinn DI, Lee CS et al. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. *Cancer Res* 2001;61(2):423-427.
28. Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. *Am J Surg Pathol* 2004;28(7):928-934.
29. Zhu Y, Sharp A, Anderson CM et al. Novel junction-specific and quantifiable in situ detection of AR-V7 and its clinical correlates in metastatic castration-resistant prostate cancer. *Eur Urol* 2018;73(5):727-735.
30. Wang Z, Shen H, Liang Z, Mao Y, Wang C, Xie L. The characteristics of androgen receptor splice variant 7 in the treatment of hormonal sensitive prostate cancer: a systematic review and meta-analysis. *Cancer Cell Int* 2020;20:149.
31. Hu R, Dunn TA, Wei S et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res* 2009;69(1): 16-22.
32. Mulhall JP, Trost LW, Brannigan RE et al. Evaluation and management of testosterone deficiency: AUA guideline. *J Urol* 2018;200(2):423-432.
33. TNM classification of malignant tumours, 8th edition. 2017.
34. D'Amico AV, Whittington R, Malkowicz SB et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 1998;280(11):969-974.
35. Pisansky TM, Thompson IM, Valicenti RK, D'Amico AV, Selvarajah S. Adjuvant and salvage radiotherapy after prostatectomy: ASTRO/AUA guideline amendment 2018-2019. *J Urol* 2019;202(3):533-538.
36. Wei JT, Dunn RL, Marcovich R, Montie JE, Sanda MG. Prospective assessment of patient reported urinary continence after radical prostatectomy. *J Urol* 2000;164(3 Pt 1):744-748.
37. Kehinde EO, Maghrebi MA, Anim JT. The importance of determining the aggressiveness of prostate cancer using serum and tissue molecular markers. *Can J Urol* 2008;15(2):3967-3974.
38. Li T, Sun X, Chen L. Free testosterone value before radical prostatectomy is related to oncologic outcomes and post-operative erectile function. *BMC Cancer* 2019;19(1):87.
39. Ferro M, Lucarelli G, Bruzzese D et al. Low serum total testosterone level as a predictor of upstaging and upgrading in low-risk prostate cancer patients meeting the inclusion criteria for active surveillance. *Oncotarget* 2017;8(11):18424-18434.
40. Gan S, Liu J, Chen Z et al. Low serum total testosterone level as a predictor of upgrading in low-risk prostate cancer patients after radical prostatectomy: a systematic review and meta-analysis. *Investig Clin Urol* 2022;63(4):407-414.
41. Miller LR, Partin AW, Chan DW et al. Influence of radical prostatectomy on serum hormone levels. *J Urol* 1998;160(2): 449-453.
42. Arezki A, Sadri I, Zakaria AS et al. Age-stratified potency outcomes of bilateral nerve sparing robotic-assisted radical prostatectomy. *Can J Urol* 2023;30(1):11424-11431.
43. Kupelian V, Shabsigh R, Travison TG, Page ST, Araujo AB, McKinlay JB. Is there a relationship between sex hormones and erectile dysfunction? Results from the Massachusetts Male Aging Study. *J Urol* 2006;176(6 Pt 1):2584-2588.
44. Buena F, Swerdloff RS, Steiner BS et al. Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. *Fertil Steril* 1993;59(5): 1118-1123.
45. O'Connor DB, Lee DM, Corona G et al. The relationships between sex hormones and sexual function in middle-aged and older European men. *J Clin Endocrinol Metab* 2011;96(10): E1577-E1587.
46. Cunningham GR, Stephens-Shields AJ, Rosen RC et al. Association of sex hormones with sexual function, vitality, and physical function of symptomatic older men with low testosterone levels at baseline in the testosterone trials. *J Clin Endocrinol Metab* 2015;100(3):1146-1155.
47. Park KH, Kim SW, Kim KD, Paick JS. Effects of androgens on the expression of nitric oxide synthase mRNAs in rat corpus cavernosum. *BJU Int* 1999;83(3):327-333.
48. Paula Domino M, Vertosick EA, Vickers AJ, Eastham JA, Sandhu JS. The association between low preoperative serum testosterone and post-radical prostatectomy urinary function. *Urology* 2023;180:190-193.
49. Wolfe AR, Ortiz NM, Baumgarten AS et al. Most men with artificial urinary sphincter cuff erosion have low serum testosterone levels. *Neurourol Urodyn* 2021;40(4):1035-1041.
50. Gacci M, Corona G, Apolone G et al. Influence of serum testosterone on urinary continence and sexual activity in patients undergoing radical prostatectomy for clinically localized prostate cancer. *Prostate Cancer Prostatic Dis* 2010;13(2):168-172.
51. Chen Y, Sawyers CL, Scher HI. Targeting the androgen receptor pathway in prostate cancer. *Curr Opin Pharmacol* 2008;8(4): 440-448.
52. Li H, Zhang Y, Li D et al. Androgen receptor splice variant 7 predicts shorter response in patients with metastatic hormone-sensitive prostate cancer receiving androgen deprivation therapy. *Eur Urol* 2021;79(6):879-886.
53. Inoue T, Segawa T, Shiraishi T et al. Androgen receptor, Ki67, and p53 expression in radical prostatectomy specimens predict treatment failure in Japanese population. *Urology* 2005;66(2): 332-337.
54. Sweat SD, Pacelli A, Bergstralh EJ, Slezak JM, Bostwick DG. Androgen receptor expression in prostatic intraepithelial neoplasia and cancer. *J Urol* 1999;161(4):1229-1232.
55. Schatzl G, Madersbacher S, Haitel A et al. Associations of serum testosterone with microvessel density, androgen receptor density and androgen receptor gene polymorphism in prostate cancer. *J Urol* 2003;169(4):1312-1315.
56. Sharp A, Coleman I, Yuan W et al. Androgen receptor splice variant-7 expression emerges with castration resistance in prostate cancer. *J Clin Invest* 2019;129(1):192-208.
57. Hillebrand AC, Pizzolato LS, Neto BS, Branchini G, Brum IS. Androgen receptor isoforms expression in benign prostatic hyperplasia and primary prostate cancer. *PLoS One* 2018;13(7): e0200613.
58. Chen X, Bernemann C, Tolkach Y et al. Overexpression of nuclear AR-V7 protein in primary prostate cancer is an independent negative prognostic marker in men with high-risk disease receiving adjuvant therapy. *Urol Oncol* 2018;36(4):161. e19-.e30.