Lower Serum Total Testosterone is Associated with Lymph Node Metastases in a Radical Prostatectomy Cohort Study

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Abstract. *Background: Data on testosterone levels of patients with prostate cancer of different grade and stage are inconsistent. We retrospectively investigated serum total testosterone of a radical prostatectomy cohort to further shed light on this problem. Patients and Methods: The preoperative level of serum total testosterone of 217 patients (mean age: 65±5.8 years) undergoing radical prostatectomy between 1989 and 2002 was analyzed for possible associations with Gleason score (≤6 vs. <7 vs. 8-10) and tumor stage (pT2 vs. pT3 vs. N+) with adjustment for age, diabetes and obesity. Patients exhibiting prostate-specific antigen (PSA) levels of >10 ng/ml and biopsy Gleason scores of ≥7 were submitted to standard lymphadenectomy. Results: The multivariate model revealed a significant effect of body mass index (BMI) (p=0.0003) and diabetes (p=0.002) on testosterone levels. Significantly lower testosterone levels were recorded in patients with nodal metastases (p<0.0001) compared to patients with non metastatic disease. No significant associations between testosterone, Gleason score and stage were found in patients with non- metastatic disease. Conclusion: Testosterone levels prior to radical prostatectomy were lower in patients with nodal involvement.*

Measurements of serum testosterone in addition to prostate-specific antigen (PSA) were recently recommended to predict aggressive prostate cancer (1-3). This was based on showing lower testosterone levels in patients with poorly differentiated and advanced disease (4-10). The subject, however, is still controversial (11-14) and further investigation seems reasonable. This study was performed to assess significant determinants of lower testosterone in a cohort of patients who had undergone radical prostatectomy. Data were available from a single institutional, community-based opportunistic screening program which included preoperative measurement of serum total testosterone.

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Key Words: Prostate cancer, serum total testosterone, pelvic nodal involvement.

Patients and Methods

Study population. Medical records were reviewed of 217 non consecutive patients who had undergone retropubic radical prostatectomy between 1989 and 2002 and provided results of preoperative assessments of serum total testosterone. Individuals exhibiting PSA levels of >10 ng/ml and a biopsy Gleason score ≥7 were submitted to standard dissection of obturator and external iliac nodes. Exclusion criteria were neo-adjuvant hormonal therapy and any kind of medication known to influence testosterone levels.

Laboratory testing. Blood samples were drawn between 8.00 and 12.00. Total serum PSA was measured with the LIA-mat Test (Byk-Sangtec Diagnostics, Dietzenbach, Germany) before 1997 and with the AXSYM-Assay (Abbot-Laboratories, Abbott Park, IL, USA) thereafter. Serum total testosterone was analyzed in pooled aliquots of two samples taken 15-20 minutes apart from an antecubital vein after informed consent. The ELIAgen Testosterone kit (Adaltis, Bologna, Italy) was used until September 25th, 2000. After that the laboratory used Testosterone ELISA (DRG, Hamburg, Germany). A method comparison study yielded the Passing-Bablok regression line (15): Adaltis=1.403×DRG+0.344, r=0.922 (N=40). This equation was applied for a method conversion in order to approach the Adaltis testosterone results from measurements made with the DRG kit.

Work up of surgical specimens. Gleason scores were routinely assessed on whole radical prostatectomy specimens and were reclassified on archival material by a single pathologist. Cancer was staged according to the TNM criteria. Between 5 and 15 lymph

Table I. *Frequency of tumor stage, Gleason scores, diabetes and body mass index (BMI).*

Variable	Category	N (%)
Tumor stage	≤pT2	155 (71.4)
	pT3/No	43 (19.8)
	pT3/N+	19 (8.8)
Gleason score	Non metastatic cancer	
	≤6	108 (49.8)
	7	70 (32.3)
	8-10	20 (9.2)
	Lymph node metastases	
	≤6	0 (0,0)
	7 (pattern 3/4)	2 (10.5)
	7 (pattern 4/3)	6 (31.7)
	8-10	11 (57.8)
Diabetes	No	204 (94.0)
	Yes	13 (6.0)
BMI	<30	193 (88.9)
	≥30	24 (11.1)

nodes were removed by standard pelvic node dissection and paraffin sections of nodes were scanned for malignancy.

Statistics. Patients were analyzed for correlations between the independent variables body mass index (BMI), diabetes, Gleason scores and pathological stages, using chi-square tests or Fisher's exact test, where appropriate. The relation of BMI, diabetes, Gleason score and pathological stage to age was analyzed by *t*-tests or a univariate analysis of variance, respectively. Univariate and multivariate regression analyses with forward selection were calculated with testosterone levels as dependent variable and age (years), BMI (≥30 *vs.* <30 kg/m^2), diabetes (yes/no), Gleason scores (≤6 *vs.* 7, 8-10) and pathological stages (organ-confined *vs.* non-organ confined *vs.* nodal involvement) as independent variables. All analyses were carried out with the software package SAS 9.1.3 (SAS Inc., Cary, NC, USA). The significance level was set to $p=0.05$ (two-sided). No adjustment for multiple testing was made.

Results

Patient demographic characteristics. The mean patient age at the time of surgery was 65.0±5.8 years. Severe cardiovascular and pulmonary disease, diabetes with end organ involvement and malignancies other than prostate cancer were absent. The mean PSA level was 11.9±12.6 ng/ml and the mean serum total testosterone level was 488 ng/dl ± 195.2 ng/dl. The frequency of different Gleason scores, tumor stages, diabetes mellitus and BMI ≥ 30 kg/m^2 among 217 patients is summarized in Table I and the Gleason scores at different tumor stages in Table II.

Univariate and multivariate analyses. Significant correlations were found between the independent variables BMI, diabetes (Fisher's exact test: $p=0.008$), stage and Gleason score

Table II. *Stage by Gleason score.*

Stage/Gleason	<6 (%)	7 (%)	8-10 (%)	Total
organ confined	90 (58.1)	54 (34.8)	11 (7.1)	155
pT3/N0	18 (41.9)	16 (37.2)	9 (20.9)	43
pT3/N+	0 (0.0)	8 (3,6)	11(5,06)	19
Total	108	78	31	217

(Fisher's exact test: $p<0.0001$). Univariate analyses of testosterone levels as dependent variable revealed no effect of age but a significant effect of BMI and diabetes (Table III), whereas the effect of age was not significant ($p=0.8$). Patients with a BMI >30 had significantly lower testosterone levels than patients with a BMI ≤30. Testosterone levels were significantly lower in patients with diabetes. The effect of diabetes ($p=0.02$) and BMI ($p=0.0003$) also remained significant in the multivariate analysis. We further analyzed the effect of the Gleason score on testosterone levels by specifying patients with non- metastatic disease and a Gleason score ≤6 as reference group. We found significantly lower testosterone levels in patients with non-metastatic disease and Gleason score 8-10 ($p=0.03$) as well as in those with metastatic disease ($p<0.0001$) compared to the reference. However, in the linear multivariate model with forward selection, only the comparison of patients with metastases with the reference group remained significant. Patients with non-metastatic disease and a Gleason score 7 did not differ significantly in testosterone levels from the reference group ($p=0.2$).

The difference in testosterone levels of patients with non-metastatic disease and tumor stage >pT2 and those with non-metastatic disease with tumor stage pT2 (=reference) was not significant ($p=0.06$) whereas in comparison to the reference group, significantly lower testosterone levels were observed in patients with metastasis ($p<0.0001$).

Discussion

Although recruited between 1989 and 2002, a series of 217 non-consecutive patients were found eligible for analysis. Blood samples were drawn between 8.00 and 12.00 in the morning. A recent report revealed no significant differences in testosterone concentrations in early or late morning serum samples (16). Gleason scores were reassessed for the analysis on archival material. Immunoassays of total testosterone were changed once by the local laboratory but a standard method for conversion of results was applied to provide comparable data.

It has been clearly documented in the literature that age, acute or chronic illness, diabetes and obesity strongly impact on serum testosterone. Severe comorbidities were absent

Table III. *Uni- and multivariate analysis (testosterone as dependent variable).*

	Univariate analysis		Multivariate analysis	
Variable	Coefficient (95% CI)	*p*-Value	Coefficient (95% CI)	*p*-Value
Age (per 5 years)	3 [25|19]	0.8	8 [12|28]	0.4
BMI (<30/≥30)	173 (97|249)	<0.0001	139 (65|214)	0.0003
Diabetes (no/yes)	165 (62|268)	0.002	113 (15|210)	0.02
Gleason ≤6 (N0)	Reference			
Gleason 7 (N0)	39 (15|92)	0.2	30 (0.22|0.81)	0.3
Gleason 8-10 (N0)	96 (9|183)	0.03	77 (7|162)	0.07
Gleason 8-10 (N+)	216 (129|303)	<0.0001	210 (127|295)	<0.0001
Stage pT2 (N0)	Reference			
Stage pT2 (N0)	58 (3|119)	0.06	40 (187|99)	0.2
Stage >pT2 (N+)	206 (120|291)	<0.0001		

from our patients who were submitted to radical surgery of prostate cancer. Our analysis confirmed previous reports of substantial influence of diabetes and obesity on testosterone levels (17, 18). The prevalence of obesity in our study patients was 11.1% which corresponded to the prevalence of obesity in community dwelling men without prostate cancer (19). A significant correlation between age and testosterone levels was absent; most likely this was due to the restrictive age spectrum. Lower testosterone levels in patients with extra-prostatic extension of prostate cancer than of patients with organ confined disease were reported in the literature (6-8). In our analysis, a significant association of testosterone levels with Gleason scores and pT staging was not revealed in patients without lymph node metastases. However, in patients with metastasis, testosterone levels were significantly lower as compared to those without metastasis with Gleason scores ≤6 and/or tumor stage pT2. The majority of patients (98.5%) who exhibited metastasis in obturator and external iliac nodes presented with Gleason scores of 7 (pattern 4/3) or 8-10, which indicates aggressive disease. Patients with PSA levels of >10 ng/ml and biopsy Gleason scores of ≥7 were submitted to standard pelvic node dissection. Meanwhile poor staging accuracy has been documented by standard pelvic node dissection since a substantial proportion of metastatic nodes may be missed (20). Extended pelvic node dissection was not carried out in our institution before 2002 and the retrospective design of the study precludes an evaluation of testosterone levels of patients undergoing extended pelvic node dissection. Thus, our results are confined to metastasis of obturator and external iliac nodes.

Mechanisms underlying an association between testosterone and prostate cancer grade and stage are still unclear. Theoretically, low testosterone may be responsible for poor tumor differentiation but serum testosterone does not truly reflect the intraprostatic androgenic milieu and the complex biological action of androgens in the prostate. A recent report revealed lower 17-beta-estradiol (E2) in patients with prostate cancer of Gleason pattern of >4+3, while testosterone levels were in the normal range (21). Previous observations of a possible impact of prostate cancer on the hypothalamic pituitary testicular axis (22) were not confirmed in a recent study (23). Lower testosterone was also observed in men harboring non-androgen-related cancer (24) and there is evidence that the prevalence of hypogonadism in male cancer patients exceeds the prevalence of hypogonadism in a non-cancer population (25). Our results confirmed a strong influence of diabetes and obesity on serum total testosterone and furnishes evidence of lower testosterone levels in patients with advanced prostate cancer. A significant association between lower testosterone level and tumor stage was confined to metastatic involvement of obturator and external iliac nodes. The prevalence of lymph node metastases at the time of radical prostatectomy and high Gleason scores (≥4+3) are indicative of aggressive disease and our analysis supports previous reports of an inverse association between Leydig cell function and aggressive prostate cancer (1-4).

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Received June 10, 2011
Revised August 17, 2011
Accepted August 18, 2011