Along the Pituitary-Testis-Prostate Axis, Serum Total Testosterone Is a Significant Preoperative Variable Independently Contributing to Separating the Prostate Cancer Population into Prostatectomy Gleason Score Groups

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Abstract. *Aim: To investigate, along the pituitary– testis–prostate axis, the potential of preoperative serum TT in contributing to defining separate prostatectomy Gleason score (pGS) groups of the prostate cancer (PC) population. Materials and Methods: The data of 126 patients operated on for PC were retrospectively reviewed. No patient had previously received 5α-reductase inhibitor, luteinizing hormone (LH)-releasing hormone analogs or testosterone replacement treatment. The patient population was grouped according to the prostatectomy Gleason score (pGS) as 6=3+3, 7=3+4, 7=4+3 and 8-10. Twelve variables were simultaneously investigated in each group: age, prolactin (PRL), follicle stimulating hormone (FSH), LH, total testosterone (TT), free testosterone (FT), estradiol (Er), prostate specific antigen (PSA), percentage of prostate biopsy positive cores (P+), biopsy Gleason score (bGS), overall cancer volume estimated as percentage of prostate volume (V+) and prostate weight (Wi). Univariate analysis of variance (ANOVA), multivariate analysis of variance (MANOVA) and multivariate discriminant analysis were the statistical methods used for evaluating the data. Results: There were 38 patients in pGS 6=3+3, 57 in pGS 7=3+4, 15 in pGS 7=4+3 and 16 in pGS 8-10. ANOVA showed that bGS ($p<0.0001$), P+ ($p<0.0001$), V+ ($p<0.0001$), PSA ($p=0.02$), Wi ($p=0.001$) and TT ($p=0.04$) were significantly different in the four pGS groups. MANOVA tests showed that only bGS ($p<0.0001$) and TT ($p=0.005$) were the significant variables that individually and independently contributed a significant amount to separation of the four pGS groups of the PC population. Multivariate discriminant analysis confirmed that TT ($p=0.005$) and bGS ($p<0.0001$) were the only variables that independently and significantly contributed to separating the pGS groups. Conclusion: along the pituitary– testis–prostate axis, serum TT is a significant preoperative variable that independently contributes to separating the prostate cancer population into pGS score groups. Pretreatment baseline serum TT levels should be measured for their inclusion in nomograms and future neural networks to be considered in the patient population diagnosed with PC.*

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The endocrine system involved in prostate cancer (PC) biology includes the hypothalamus, the pituitary gland, the testes and the adrenals. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted from the gonadotrophic cells located in the anterior pituitary; they are also called gonadotropins because they stimulate the gonads. Most gonadotrophs secrete only LH or FSH, but some appear to secrete both hormones. Prolactin (PRL) is a polypeptide hormone which is secreted by the pituitary lactotroph cells. The interstitial cells of Leydig are responsible for the production of 95% of all circulating androgen in the form of testosterone. Approximately 98% of the circulating androgens are bound to plasma proteins, including a specific beta-globulin, testosterone-binding globulin (TeBG). The free testosterone (FT) in the blood is the physiologically important fraction. LH, FSH, PRL, androgens and estrogens are hormones regulating the function of the prostate.

PC is an interesting tumor for clinical endocrine investigation. Unfortunately, at the moment PC physiopathology along its natural history is not considered (1). Etiological and stimulatory factors of PC are still not completely understood. The main evidence from the reported literature shows that PC is androgen dependent (2) and increases the level of prostate-specific antigen (PSA) (3). The pituitary axis in PC has long been investigated and it has been suggested that this tumor type may produce a substance that alters the normal function of the pituitary–testicular axis which results in abnormal serum LH and FSH levels (4-7); however, these findings have not been confirmed (8). Human benign prostatic hyperplasia and PC tissues have been found to express LH and FSH receptors (9-13). These findings suggest that gonadotrophins may promote cancer either indirectly by stimulating testicular production of hormones, or directly through their receptors located in the prostate gland (14). Locally produced PRL has been documented in prostate tumors and exhibits tumor growth potency, acting *via* autocrine/paracrine mechanisms; moreover, a novel class of compounds with therapeutic potential to target PRL receptors signaling, namely competitive PRL receptor antagonists, have also been developed (15, 16). Since the pioneering work of Huggins and Hodges (1), androgens have been universally considered as being pivotal in the regulation of normal prostatic function and malignant prostate growth (4-7, 17-20). However, considerable experimental evidence has accumulated to support an equally important role for estrogens in the development and/or progression of human PC. Estrogens induce systemic effects by acting through the pituitary gland to indirectly lower androgen levels, as well as their effects by directly targeting prostate tissue by specific estrogen receptors (ER). Estrogens and their receptors are implicated in PC development and progression. There is a significant potential for the use of ER-α antagonists and -β agonists to prevent PC and delay disease progression (21). Locally produced or metabolically transformed estrogens may differently affect proliferation activity of PC cells. Estrogens may either stimulate or reduce PC cell growth, also depending on the receptor status. In particular, an imbalance of ER-α antagonists and ER-β expression may be critical to determine the ultimate effects of estrogen on PC cell growth (22).

It has been shown that the natural history of PC is closely related to the prostatectomy Gleason score (pGS) which is the factor that best predicts biochemical recurrence, development of metastases and PC-specific mortality (23-27). The association of PC tumor grade with pretreatment serum levels of hormones along the pituitary– testis– prostate axis is a subject that has long been investigated, but the conclusions are controversial and the topic remains unsettled (6, 7, 17-20, 28-37). The objective of the present study was to investigate the potential for preoperative hormones of the pituitary–testis–prostate axis in contributing to separating PC population into pGS groups.

Materials and Methods

The present analysis was part of a study carried out from 2007 to 2011 aimed at evaluating a potential link between PC and the hypothalamus– pituitary– testis– prostate axis. The data of 126 patients operated on for PC were retrospectively reviewed. Standard retropubic radical prostatectomy (RRP) was the surgical procedure performed, with or without local lymph node dissection (LND). No patient had previously received 5α-reductase inhibitor, LH-releasing hormone analogs or testosterone replacement treatment. The 14-core trans rectal ultrasound and (TRUS)- guided prostate biopsy technique was routinely used and additional cores were taken when a lesion on either TRUS or digital rectal examination was evident. The biopsy Gleason score (bGS) of positive cores was assessed by a pathologist and percentage of positive cores (P+) was computed.

After informed signed consent, pretreatment simultaneous serum samples were taken for measuring serum estradiol (Er), PRL, FSH, LH, TT, FT and PSA levels. The samples were analyzed at the same laboratory of our hospital. PRL (normal range=3.07-20.05 μg/l), FSH (1.0-14 IU/l), LH (2.0–10 IU/l), TT (9-29 nmol/l), Er (normal value <200 pmol/l) and PSA (2-4 ug/l) were measured by immunochemiluminescent test performed by ADVIA Centaur XP Immunoassay System (Siemens Company). FT (normal range=31-163 pmol/l) was measured by immunoradiometric test (DSL, USA). The prostatectomy specimens were fixed *in toto* overnight (10% neutral buffered formhaldeyde), coated with India ink and then weighed (Wi). Tissue sections of 4 μm were prepared in standard fashion and stained with hematoxylin and eosin.

Patients were classified according to primary tumor stage, lymph node and metastatic status, using the TNM categories recommended by the 1997 International Union Against Cancer TNM classification system (38). Invasion of the bladder neck without involvement of the seminal vesicles was staged as pT3a disease. Seminal vesicle invasion was defined as tumor involvement of the muscular wall (pT3b). Surgical margins were stated as free (R–) or involved by cancer (R+). Tumors were graded according to the Gleason grading system and the Gleason score was computed after summing up the two patterns, prevalent and secondary, structuring the tumor. Overall tumor volume was estimated as a percentage of the prostate volume (V+). Biopsy and prostatectomy specimens were assessed by an experienced pathologist.

Statistical methods. Summary and descriptive statistics of the PC population was computed. Univariate analysis of variance (ANOVA), multivariate analysis of variance (MANOVA) and multivariate discriminant analysis were the statistical methods used for evaluating the data.

In this study, MANOVA of twelve variables simultaneously analyzing four different pGS categories (pGS: 6=3+3, 7=3+4, 7=4+3, 8-10) was first computed. The groups were simultaneously compared for twelve variables including age, PRL, FSH, LH, TT, FT, Er, PSA, P+, bGS, V+ and Wi. The multivariate between sample sum of squares (SSH) matrix H and the within sample sum of squares (SSE) matrix E were computed; the sum matrix E + H as well as the determinant of |E| and |E + H| were calculated. The mean vectors were compared for significant differences and the likelihood ratio test of H_0: mean (age=PRL=FSH=LH=TT=FT=Er=PSA=P+=bGS=V+=Wi) was given by *Wilks' statistics* (*Wilks' U*). The Wilks' U statistic was computed as the ratio of the determinants of the calculated matrices as follows: Wilks' U=|E|/|E + H|. H_0 was rejected if Wilks' U was equal to or less

than the critical value for α, *p, vH, vE,* where α=0.05, *p*=number of variables (*p*=12), *vH*=degrees of freedom for hypothesis (vH=k-1=3) and vE=degrees of freedom for error (*vE*=N – k=126-4=122). The *F*-test and the *p*-value of the F distribution of the Wilks' statistic were also computed. If the null hypothesis H_0 was rejected, the twelve variables were individually tested in the four groupd of the PC population by ANOVA using the 0.05 level of significance. In order to asses the independent significance of each variable when simultaneously adjusted for the others in separating the pGS groups of the PC population, MANOVA tests on sub-vectors were also computed. The test of one variable simultaneously adjusted for the other eleven was given by the ratio test of the overall Wilks' U including all the twelve variables to the Wilks' U of the other eleven variables (*i.e.* excluding the tested variable). For example, the test for PSA when simultaneously adjusted for the other eleven variables was given as Wilks' U (PSAlage, PRL, FSH, LH, TT, FT, Er, P+, bGS, V+, Wi)=Wilks' U(age, FSH, LH, TT, FT, Er, PSA, P+, bGS, V+, Wi)/Wilks'U (age, PRL, FSH, LH, TT, FT, Er, P+, bGS, V+, Wi). The critical value for Wilks' U (α=0.05, *p*=1, *vH*=3, *vE*=122) was 0.96; the *F*–test and the *p*-value of the *F* distribution were also computed.

The eigenvalues and the eigenvectors of the E^{-1} H calculated matrix were computed for multivariate discriminant analysis. The squared canonical correlation, the pooled covariance matrix (S_{pl}) and the the square roots of the diagonal elements of S_p were obtained. Vectors a_1, a_2 and a_3 were standardized. The significance of the first two vector discriminant functions, of the second and third vector discriminant function were tested by Wilks' U statistics; the *F* approximation and the correlated *p*-values were also computed. Common approaches to assessing the contribution of each variable (in the presence of the other variables) to separating groups were examination of the standardized discriminant function coefficients and, as performed in MANOVA subvector tests, calculation of the Wilks' U statistics and partial *F*-test for each variable in order to assess their ranking order.

Results

Summary and descriptive statistics of the operated PC population (N=126) are reported in Table I. There were 38 patients in pGS group 6=3+3, 57 in group 7=3+4, 15 in group 7=4+3 and 16 in group 8-10.

MANOVA: Age, PRL, FSH, LH, TT, FT, Er, PRL, PSA, P+, bGS, V+ and Wi summary descriptive statistics of the four pGS groups are given in Table II. The overall Wilks' U statistic was 0.406. In this case, the parameters of the critical Wilks' U distribution were *p*=12, *vH*=3, *vE*=122 and α=0.05. The null hypothesis was rejected because Wilks' U value was less than the critical value (=0.732). The approximate *F*-test value was 3.25 and the *p*-value was highly significant ($p<0.0001$).

Since the null hypothesis regarding the variables for the pGS groups was rejected, we tested the significance of each individual variable by ANOVA using the 0.05 level of significance. As shown in Table II, TT ($p=0.01$), PSA ($p=0.02$), P+ ($p<0.0001$), bGS ($p<0.0001$), V+ ($p<0.0001$) and Wi ($p=0.001$) were significantly different in the four pGS groups. Moreover, in order to assess the independent significance of each variable when simultaneously adjusted for the others in contributing to separate the pGS samples on a multidimensional space, MANOVA tests on sub-vectors were computed. The results showed that on multivariate analysis, only bGS ($p<0.0001$), TT ($p=0.005$) were the significant variables that independently contributed a significant amount to separation of the four pGS groups of the PC population (see Table II). Close to significance was Wi ($p=0.06$).

Multivariate discriminant analysis: The first two eigenvalues and eigenvectors of the $E^{-1}H$ matrix are reported in Table III. The first two eigenvalues accounted for 96% of the total variance, the first for 80% and the second for 16%;

Table I. *Summary and descriptive statistics of the operated prostate cancer population (n = 126).*

Continuous variables

Variable	Mean	Med	SD	Min	Max
Age (years)	65.29	67.00	6.56	45.00	78.00
PRL (3.06-20.04 µg/l)	7.93	7.72	2.93	3.02	17.17
FSH (1.0-14 IU/l)	8.14	6.30	8.75	1.10	54.80
LH (2.10-10 IU/l)	6.00	4.20	6.91	1.10	48.00
TT (nmol/l)	16.31	15.10	6.51	6.50	40.70
FT (pmol/l)	33.79	31.69	11.06	14.10	71.90
Er (<150 pmol/l)	134.28	123.00	52.82	2.48	355.00
PSA T (µg/l)	7.67	5.79	5.61	1.9	38.60
P+ (%)	0.34	0.31	0.20	0.06	1.00
bGS	6.42	6.00	0.75	5.00	9.00
pGS	6.88	7.00	0.77	6.00	10.00
V+ (%)	0.20	0.15	0.16	0.01	1.00
Wi (g)	61.33	50.94	31.12	26.00	207.00

Staging: Clinical and pathology

cT	n (%)	pT	n (%)	R	n (%)	pN	n (%)
1c	70 (0.56)	2a	11 (0.09)	R–	70 (0.56)	pNx	10 (0.81)
2a	36 (0.28)	2b	54 (0.43)	R+	56 (0.44)	pN0	22 (0.17)
2b	18 (0.14)	3a	51 (0.40)			pN+	2 (0.02)
3a	2 (0.02)	3b	10 (0.08)				

Gleason's grading: Biopsy and pathology

bGS	n (%)	pGS	n (%)
≤6	72 (0.57)	6	38 (0.30)
7=3+4	39 (0.31)	7=3+4	57 (0.45)
7=4+3	8 (0.06)	7=4+3	15 (0.12)
8-9	7 (0.06)	8-10	16 (0.13)

TT: Total testosterone; FT: free testosterone; PSA T: total PSA; FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin, Er: estradiol, P+: % biopsy positive cores; bGS: biopsy Gleason score; pGS: pathology Gleason score; V+: cancer as percentage of the prostate volume; Wi: prostate weight; cT: clinical staging; R: surgical margins; cN: clinical node stage; cM: clinical staging for metastases; Med: median; SD: standard deviation; Var: variance.

Table II. *Summary statistics, (ANOVA)and (MANOVA) of the prostate cancer population (N=126) grouped according to the prostatectomy Gleason score.*

pGS groups	Statistic	Age (years)	PRL (μg/l)	FSH (IU/l)	LH (IU/l)	TT (nmol/l)	FT (pmol/l)	Er (pmol/l)	PSA (μg/l)	P+ (%)	bGS bGS	V+ (%)	Wi (g)
6=3+3	Mean	64.60	7.78	6.66	4.79	15.78	34.88	138.43	7.91	0.23	5.78	0.13	76.75
(n=38)	Median	66.50	8.18	6.70	4.35	14.3	33.90	121.31	6.07	0.17	6.00	0.08	69.00
	SD	6.69	2.36	3.04	2.27	5.75	10.93	60.16	6.75	0.15	0.47	0.17	40.72
	Min	51.00	3.02	1.10	1.10	7.00	16.60	31.00	2.77	0.06	5.00	0.01	26.00
	Max	75.00	14.02	13.52	10.55	33.6	65.40	355.00	38.60	0.69	7.00	1.00	207.0
7=3+4	Mean	65.01	8.06	9:05	6.46	16.73	32.71	129.10	6.25	0.34	6.49	0.18	56.0
(n=57)	Median	67.00	7.64	5.70	4.19	15.70	31.10	123.17	5.44	0.33	6.00	0.20	46.60
	SD	6.82	3.02	10.72	8.04	7.27	10.76	47.18	3.24	0.19	0.50	0.10	28.60
	Min	45.0	3.30	1.40	1.31	6.50	14.80	2.48	1.90	0.06	7.00	0.01	26.70
	Max	78.0	16.79	51.6	45.8	40.70	67.50	240.00	17.20	0.83	7.00	0.70	159.00
7=4+3	Mean	66.80	7.57	6.98	5.14	12.88	31.60	143.58	10.25	0.45	6.86	0.32	50.92
(n=15)	Median	66.00	6.70	6.60	4.20	12.90	30.00	128.10	7.74	0.42	7.00	0.30	48.41
	SD	6.20	3.13	4.37	2.81	2.63	9.85	52.88	6.78	0.24	0.63	0.18	13.84
	Min	5.10	4.06	1.40	2.10	8.20	15.50	87.00	3.39	0.15	6.00	0.08	31.20
	Max	76.00	17.17	15.50	10.44	16.00	58.40	264.00	29.40	1.00	8.00	0.70	80.00
8-10	Mean	66.50	8.16	9.50	8.05	19.32	37.12	134.12	9.74	0.46	7.31	0.33	50.39
(n=16)	Median	67.50	7.17	6.75	4.35	19.05	35.95	130.5	6.99	0.50	7.00	0.30	50.00
	SD	5.76	3.77	12.47	11.29	6.75	13.34	56.28	6.98	0.18	0.87	0.20	14.02
	Min	54.00	4.62	2.40	2.40	9.20	14.10	25.00	3.60	0.08	6.00	0.08	36.20
	Max	73.00	15.75	54.80	48.00	31.30	71.90	217.00	22.20	0.79	9.00	0.75	93.80
ANOVA	Stat	Age	PRL	FSH	LH	TT	FT	Er	PSA	P+	bGS	V+	Wi
	F-Test	0.61	0.17	0.60	1.01	2.8	0.98	0.40	3.17	7.96	31.00	9.38	5.23
	p-value	0.60	0.91	0.50	0.38	0.04	0.40	0.74	0.02	<0.0001	<0.0001	<0.0001	0.001
MANOVA	Stat	Age	PRL	FSH	LH	TT	FT	Er	PSA	P+	bGS	V+	Wi
	Wilks' U	0.97	0.99	0.99	0.99	0.89	0.98	0.96	0.97	0.99	0.69	0.97	0.96
	F-Test	0.95	0.11	0.11	0.11	4.48	0.52	1.50	0.83	0.11	16.23	0.87	2.48
	p-value	0.41	0.95	0.95	0.95	0.005	0.66	0.21	0.47	0.95	<0.0001	0.45	0.06

Overall MANOVA: Wilks' U=0.406< Wilks' U critical 0.732 (F-test=3.25, p-value <0.0001). MANOVA tests on subvectors. TT: Total testosterone; FT: free testosterone; PSA T: total PSA; FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin, Er: estradiol, P+: % biopsy positive cores; bGS: biopsy Gleason score; pGS: pathology Gleason score; V+: cancer as percentage of the prostate volume; Wi: prostate weight.

moreover, the third was not assessed because it accounted for only 4% of the total variance in the population. The squared canonical correlation between each of the two discriminant functions and the grouping variables were r_1^2=0.50 and r_2^2=0.16. The Wilks'U statistic was significant for the first (a_1: p<0.0001) and second (a_2: p=0.0007) eigenvector (ai). Thus the mean vectors lay largely in the two-dimensional space and two discriminant functions (z_1, z_2) sufficed to describe most of the separation among the four prostatectomy GS groups. The standardized discriminant coefficients of the first two eigenvectors (a^*_1 and a^*_2) are also reported in Table II. As shown, bGS (0.45), TT (0.19) and, to a lesser extent, Wi, contributed most to separating the groups in that order for the first eigenvector (a_1); moreover, TT (0.26), more than bGS (0.08), contributed most in separating the groups in that order for the second eigenvector (a_2). On multivariate discriminant analysis, as already assessed by MANOVA tests on sub-vectors, TT and bGS were the variables that independently and significantly contributed to separating the pGS groups; moreover, the variables ranked as: bGS (p<0.0001) and TT (p=0.005). The summary statistics of the first (z1) and second (z2) discriminate function are also reported in Table II.

Figure 1 shows a scatter plot of the first two discrimnant functions (z_1, z_2) for the data of the different pGS groups of the population (N=126) summarized in Table II. The first and, to a lesser extent, second discriminant functions (z_1, z_2)

Table III. *Multivariate discriminant analysis of the prostate cancer population (N=126).*

ei	eigenvalue	pv	ai	Age	PRL	FSH	LH	TT	FT	Er	PSA	P+	bGS	V+	Wi	Wilks' U	*F*-Test	*p*-Value
e1	1.02	0.80	a1	–0.01	–0.01	0.01	–0.02	–0.03	0.005	0.002	–0.007	–0.43	–0.80	–0.30	0.005	3.97	3.97	<0.0001
e2	0.20	0.96	a2	–0.01	0.01	0.03	–0.02	0.04	0.0004	–0.002	–0.013	–0.85	0.15	–0.48	–0.006	0.67	2.38	0.0007
Standardized coefficients			a*i															
			a*1	0.07	0.03	0.13	0.16	0.19	0.06	0.12	0.04	0.07	0.45	0.05	0.16			
			a*2	0.10	0.03	0.32	0.17	0.26	0.005	0.12	0.07	0.14	0.08	0.06	0.01			
Partial F of each variable			Stat															
			F	0.95	0.11	0.11	0.11	4.48	0.52	1.50	0.83	0.11	16.23	0.87	2.48			
			p	0.41	0.95	0.95	0.95	0.005	0.66	0.21	0.47	0.95	<0.0001	0.45	0.06			

		z1					z2				
		Overall	pGS				Overall	pGS			
Statistics of	Statistic		3+3	3+4	4+3	8-10		3+3	3+4	4+3	8-10
z1 and z1	Mean	–6.45	–5.77	–6.52	–6.89	–7.44	0.05	–0.05	0.16	–0.19	0.17
	Median	–6.37	–5.84	–6.59	–6.89	–7.27	0.05	–0.03	0.16	–0.10	0.11
	SD	0.79	0.56	0.53	0.58	0.79	0.38	0.35	0.35	0.37	0.39
	Min	–9.23	–7.31	–7.49	–7.86	–9.23	–0.94	–0.86	–0.81	–0.94	–0.71
	Max	–4.54	–4.54	–5.43	–5.97	–6.03	0.96	0.54	0.96	0.25	0.73

TT: Total testosterone; FT: free testosterone; PSA T: total PSA; FSH: follicle-stimulating hormone, LH: luteinizing hormone, PRL: prolactin, Er: estradiol, P+: % biopsy positive cores; bGS: biopsy Gleason score; pGS: pathology Gleason score; V+: cancer as percentage of the prostate volume; Wi: prostate weight: ei: Eigenvalue, pr of var: pv: proportion of variance, ai: eigenvector; e1, e2, e3: first, second and third eigenvalue; a1, a2, a3: first, second and third eigenvector; a*1, a*2, a*3: first, second and third standardized eigenvector; z1 : first discrimninat function, z2: second discriminant function.

effectively separated the pGS groups. As evident from the bi-plot, pGS group 7=3+4 was closer to the pGS 6=3+3, while the pGS 7=4+3 was closer to the pGS 8-10 cluster (see Figure 1).

Discussion

In the present study, multivariate data on samples from different pGS populations were collected. Although in some cases it makes sense to isolate each variable and study it separately, in the main it does not. In most instances, the variables are related in such a way that when analyzed in isolation they may often fail to reveal the full structure of the data. With the great majority of multivariate data sets, all the variables need to be examined simultaneously in order to uncover the patterns and the key features of the data. The two multivariate techniques used in the present analysis were primarily inferential. Their aim was to display or extract any signal in the data in the presence of noise, and to discover what the data has to tell us. The prime interest was in assessing whether the populations involved had different mean vectors on the measurements taken. For this, MANOVA showed that in a multidimensional set of variables of PC patients at diagnosis, only bGS and TT were the significant and independent variables, proving that the pGS populations involved had significant different mean vectors on the measurements taken. In our opinion, the significant variables on ANOVA (PSA, P+, V+, Wi) depended on pGS and TT in a way that needs further investigation; moreover, the non-significant variables (PRL, FSH, LH, FT, Er) might depend on pGS and TT by the way of complicated feed-back systems that need to explored.

A further question that was of interest for the grouped multivariate data was whether or not it was possible to use the measurements made to construct a classification rule derived from the original observations, the training set, that might allow new individuals having the same set of measurements, but no group label, to be allocated to a group in such a way that misclassifications are minimized. The relevant technique used was the discriminant function analysis which showed that the multivariate data could be displayed in a two-dimensional space by the first two eigenvectors since these eigenvalues together accounted for 96% of the variance (see Table III and Figure 1). The bGS contributed most in separating the groups for the first eigenvector (standardized eigenvector coefficient 0.45) and

TT for the second eigenvector (standardized eigenvector coefficient 0.26). Interestingly, looking at Figure 1, it seems that the population might be clustered mainly in two groups since the pattern 7=3+4 overlapped into 6=3+3 while the pattern 7=4+3 blended into and pGS 8-10 group (see Figure 1); however, the hypothetical group including pGS (6 and 3+4) and pGS (4+3 and 8 to 10) might be subclustered by the second discriminant function where the mean values of TT are higher in the more aggressive tumors of each group (pGS 3+4 *versus* 6, and pGS 8-10 *versus* 4+3, see Table II and III). As a theory, discriminant function analysis might assess different tumor phenotypes that the actual grading systems are unable to distinguish.

Studies investigating on the potential relation between TT and tumor grade have shown controversial results, proving that TT might be associated with the Gleason score (20, 31, 39, 40), but might not (17, 30, 36, 37, 41, 42); moreover, when the relation was significant, lower serum levels of TT were found to be related to high-grade tumors. However, the results and conclusions of studies stating that significantly lower levels of TT were detected in patients with high-grade tumors have to be evaluated according to the employed methodology for grouping the patients and to the limitations of the bGS on the pGS. Our study showed that significantly and independently higher mean levels of TT were detected in the pGS 8-10 group (see Table II); however these results are difficult to compare with other studies. For example, Zhang *et al*. (39) showed that on ANOVA, significantly lower levels of TT were detected in patients with high-grade PC tumors; however, if we consider the methodology of the investigation, we see that tumor grades were assessed according to the limitations of the bGS, and high-grade tumors included the 8-9 group and 'moderate cancers' included bGS 5-7. If we consider the data of the present study, we could state that TT serum levels were lower in patients with high-grade tumors including the pGS 8-10 group (19.2 nmol/l) than in the 'moderate group' including together pGS ≤7 cancer (36.80 nmol/l, data not reported). The limits between the bGS and pGS were also evident in the present study; moreover, multivariate discriminant function analysis also outlined the potential misclassifications of the pGS (see Table III and Figure 1). In our opinion, before determining significance of lower serum levels in patients with high-grade tumors, an evident and independent association between serum TT levels and the GS groups has to be assessed. Our study approached the problem by using advanced mathematical and statistical methods in order to show, beyond any doubt, the association between pretreatment serum TT levels and the grade of the tumor; as a result, of the twelve investigated variables in the multidimensional space, only the bGS, as expected, and TT were independent factors contributing to the portioning of the pGS groups (see Table II).

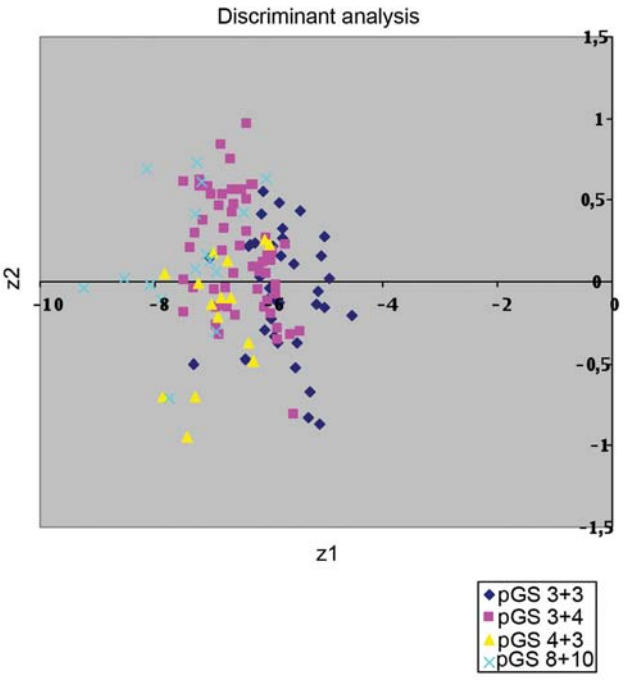

Figure 1. *Scatter plot of the first (z1) and second (z1) discriminant functions of the groups of the patient population (n=126).*

The present study showed the mean TT level to be significantly in the bGS 8-10 group than in bGS 6, 3+4 and 4+3 groups (see Table II). As a theory, the increasing grade of PC tumors represents a progression of events in the cancer population where a spectrum of potential negative changes might occur of the androgen receptors. As a result, progression from hormone-sensitive to castration-resistant disease might occur along the natural history of prostate cancer. We speculate that higher mean serum levels of TT in high-grade cancer might be related to the prevalence of the population resistant to castration where regressive events occurred at the androgen receptor, leading to increased serum levels of TT because of the developed independence from androgens. Once again, our study stresses the potential importance of TT along the natural history of PC, where a clinical continuum occurs from localized disease, to metastasis and castration resistance, finally ending in death from disease.

The present study might have some limitations related to the number of patients included and the absence of comparable studies. However, it shows the importance of TT as a potential prognostic marker along the natural history of PC.

Conclusion

Along the pituitary– testis– prostate axis, the preoperative serum level of TT is a significant variable that independently contributes to separating pGS groups of the patient

population. Pretreatment baseline serum TT levels should be measured and their inclusion in nomograms and neural network programs considered.

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