Abstract. Aim: To evaluate prolactin (PRL) physiopathology along the pituitary–testis–prostate axis at the time of initial diagnosis of prostate cancer in relation to the available clinical variables and to the subsequent cluster selection of the patient population. Patients and Methods: The study involved 100 individuals diagnosed with prostate cancer. Age, percentage of positive cores at transrectal ultrasound scan biopsy (P+), biopsy Gleason score (BGS), PRL, luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (TT), free testosterone (FT) and prostate-specific antigen (PSA) were the continuous clinical variables. All patients had histologically proven carcinoma of the prostate and had not previously received hormonal manipulations. Correlation and multiple linear regression analysis of the variables along the pituitary–testis–prostate cancer axis was performed. The prostate cancer population was clustered according to the PRL/P+ ratio into group A (4.20≤PRL/P+≤20), B (20<PRL/P+≤44) and C (44<PRL/P+≤157.83). Correlation, multiple linear regression and analysis of variance of the clustered population along the pituitary-testis-prostate cancer axis was computed. Results: At diagnosis of prostate cancer, pituitary hormones were significantly correlated to FT (PRL, FSH, LH) and to PSA (FSH, LH); also close to significance was the correlation of both PRL and LH to P+ (p=0.07). On multiple linear regression analysis only, PRL was independently predicted by P+ (p=0.02), while PSA independently predicted both FSH (p=0.03) and LH (p=0.01). PRL was significantly correlated to P+ in the prostate cancer population clustered according to the PRL/P+ ratio into groups A, B and C which significantly differed for PRL (p=0.002), P+ (p<0.0001) and BGS (p=0.0001). Conclusion: In the prostate cancer population at diagnosis and along the pituitary–testis–prostate axis, PRL is significantly correlated to P+; moreover, PRL is also independently predicted by P+. Because of the high correlation between PRL and P+, the prostate cancer population at diagnosis might be clustered according to the PRL/P+ ratio into groups A, B and C, which, as a theory, might express prognostic potential for clinical applications. However, confirmatory studies are needed.

The endocrine system involved in prostate cancer biology includes the hypothalamus, the pituitary gland, the testes and the adrenals. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted from the gonadotroph 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 the hormones which regulate the function of the prostate.

Etiological and stimulatory factors of prostate cancer are still not completely understood. The main evidence from the reported literature shows that prostate cancer is androgen dependent (1), increases the levels of prostate-specific antigen (PSA) (2), is related to PSA growth rate for its extension and prognosis (3, 4), and pretreatment total testosterone (TT) and FT serum levels may be abnormal (5-12). Human benign prostatic hyperplasia and prostate cancer tissues have been found to express LH and FSH receptors (13-17). 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 (18). Locally produced PRL has been documented in prostate tumors and it shows tumor growth potency, acting via autocrine/paracrine mechanisms; a novel class of compounds with therapeutic potential to target PRL receptors (PRLR) signaling, namely competitive PRLR antagonists, have also been developed (19, 20).

Prostate cancer is an interesting tumor for clinical endocrine investigation. Unfortunately, at the moment we ignore prostate cancer physiopathology along its natural history (21). The pituitary axis in prostate cancer has long being investigated and it has been suggested that the tumor may produce a substance that alters the normal function of the pituitary–testicular axis which results in abnormal LH and FSH serum levels (5, 9-12, 22-29). It has been suggested that the impact of prostate cancer on the hypothalamic–pituitary axis may be more profound in high-grade prostate cancer (27), but this hypothesis has not been confirmed (30).

This study was aimed at evaluating PRL physiopathology in the pituitary–testis–prostate axis at the time of initial diagnosis of prostate cancer in relation to the available clinical variables and to the subsequent cluster selection of the patient population.

Patients and Methods

The study involved 100 individuals diagnosed with prostate cancer. The descriptive statistics of the patient population with treatments performed at the time of this communication are shown in Table I. All the patients had histologically proven carcinoma of the prostate and had not previously received 5α-reductase inhibitors, LH-releasing hormone analogs or testosterone replacement treatment. The 14-core transrectal ultrasound scan (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) was used to grade the tumors. Patients were classified according to primary tumor stage, lymph node and metastatic status, using the categories recommended by the 1997 International Union Against Cancer TNM classification system (31). The total patient population under the testosterone study, still open and progressing, is over 235 individuals, but this communication does not include those patients who were not simultaneously assessed for pituitary hormones. After informed signed consent, simultaneous pretreatment serum samples were obtained from a cubital vein, at least one month after TRUS biopsy, between 8:00–8:30 a.m. for measuring serum PRL, FSH, LH, TT, FT and PSA levels. The samples were analyzed at the same laboratory of our hospital. PRL (range: 3.06-20.04 μg/l), FSH (range: 1.0-14 IU/l), LH (range: 2.0 – 10 IU/l), TT (range: 9-29 nmol/l) and PSA (normal range: 2-4 μg/l) were measured by immunochemiluminescent test performed by ADVIA Centaur XP Immunoassay System (Siemens Company). Free testosterone (FT) levels were measured by immunonephelometric test performed byADVIA Centaur XP Immunoassay System (Siemens Company). Free testosterone (FT) normal range: 31-163 pmol/l) was measured by immunoradiometric test (DSL, USA).

### Table I. Summary and descriptive statistics of the patient population (n=100).

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Mean</th>
<th>Med</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.69</td>
<td>66.68</td>
<td>6.73</td>
<td>50.51</td>
<td>80.41</td>
</tr>
<tr>
<td>PRL (3.06-20.04 μg/l)</td>
<td>9.42</td>
<td>7.95</td>
<td>7.40</td>
<td>3.02</td>
<td>47.69</td>
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<tr>
<td>FSH (1.0-14 IU/l)</td>
<td>9.31</td>
<td>6.30</td>
<td>10.93</td>
<td>1.30</td>
<td>54.80</td>
</tr>
<tr>
<td>LH (2.10-10 IU/l)</td>
<td>6.51</td>
<td>4.20</td>
<td>7.97</td>
<td>1.10</td>
<td>48.00</td>
</tr>
<tr>
<td>TT (9-29 nmol/l)</td>
<td>16.32</td>
<td>15.15</td>
<td>6.67</td>
<td>6.50</td>
<td>40.70</td>
</tr>
<tr>
<td>FT (31-163 pmol/l)</td>
<td>33.01</td>
<td>31.60</td>
<td>10.51</td>
<td>14.10</td>
<td>71.90</td>
</tr>
<tr>
<td>PSA T (2-4 μg/l)</td>
<td>8.58</td>
<td>5.72</td>
<td>7.85</td>
<td>1.31</td>
<td>44.60</td>
</tr>
<tr>
<td>P+</td>
<td>0.35</td>
<td>0.31</td>
<td>0.22</td>
<td>0.06</td>
<td>1.00</td>
</tr>
<tr>
<td>BGS</td>
<td>6.53</td>
<td>6.00</td>
<td>0.80</td>
<td>5.00</td>
<td>9.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical stage n</th>
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<tbody>
<tr>
<td>cT1c</td>
</tr>
<tr>
<td>cT2</td>
</tr>
<tr>
<td>cT3</td>
</tr>
<tr>
<td>cN0</td>
</tr>
<tr>
<td>cM0</td>
</tr>
<tr>
<td>Primary treatment n</td>
</tr>
<tr>
<td>Radical prostatectomy 86</td>
</tr>
<tr>
<td>Radiation 10</td>
</tr>
<tr>
<td>Androgen blockade 3</td>
</tr>
<tr>
<td>Active surveillance 1</td>
</tr>
</tbody>
</table>

LH, Luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; TT, total testosterone; FT, free testosterone; PSA T, total PSA; P+, biopsy positive cores (%); BGS, biopsy Gleason score; cT, tumor clinical staging; cN, clinical node stage; cM, clinical staging for metastases; med, median; SD, standard deviation; Var, variance.
Results

The results of correlation and multiple linear regression analysis of the test variables along the pituitary–testis–prostate axis are presented in Table II. The close to statistical significance was the correlation of PRL to FT (r = 0.06), P+ (r = 0.07) and age (r = 0.07). FSH was significantly correlated to FT (r = 0.007) and age (r = 0.04). LH was significantly correlated to FT (r = 0.01) and age (r = 0.001). Close to statistical significance was LH correlation to P+ (r = 0.07). On multiple linear regression analysis, PRL was significantly predicted only by P+ (r = 0.02), FSH only by PSA (r = 0.03) as for LH (r = 0.01). The BGS was also significantly correlated to P+ (r < 0.0001), age (r = 0.05) and PSA (r = 0.06); however, on multiple linear regression analysis, BGS was only significantly predicted by P+ (r < 0.0001) (data not reported in Table II). The scatter plot of P+ predicting PRL for the patient population is shown in Figure 1.

Groups A, B and C of the patient population clustered according to the PRL/P+ ratio are shown in Figure 2 and the results of correlation and multiple linear regression analysis of the clusters along the pituitary-testis-prostate cancer axis are reported in Table III, where ANOVA is also shown. PRL was significantly correlated to P+ in cluster A (p = 0.03), B (p < 0.0001) and C (p < 0.0001) and to FT in cluster B (p = 0.03); on multiple linear regression analysis, PRL was significantly predicted by P+ in groups B and C (p < 0.0001) and also by TT in group B (p = 0.06) and C (p = 0.05). FSH was significantly correlated to age (p = 0.03) in group A, to FT (p = 0.05) and P+ (p = 0.06) in group B; on multiple linear regression analysis, it was significantly predicted by TT (p = 0.05), BGS (p = 0.03) and PSA (p = 0.0003) in group A, by TT (p = 0.02) in cluster C. LH was correlated to age (p = 0.01) in group A, to FT (p = 0.05), P+ (p = 0.01) and age (p = 0.008) in group B; on multiple linear regression analysis, LH was significantly predicted by TT (p = 0.04), BGS (p = 0.03), PSA (p = 0.0002) and age (p = 0.05) in cluster A, by age (p = 0.02) in cluster B and by TT (p = 0.03), P+ being of borderline significance (p = 0.08) in group C. The PRL/P+ A, B and C clusters significantly differed for mean values of PRL (p = 0.02), P+ (p < 0.0001) and BGS (p = 0.0001), as shown by ANOVA (see Table III).

The distribution of the BGS scores were different in the the PRL/P+ clusters (A, B, C) and the difference was significant (p = 0.00003), as shown in Table IV. PRL was also significantly correlated to P+ (r = 0.58, p = 0.02) in the BGS cluster C (data not reported in the Table). The patient population with the different BGS groups A, B and C are depicted in Figure 3 which shows evident overlap of the clusters. Scatterplots of the the BGS groups A, B, C subclustered according to the PRL/P+ ratio are shown in

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Table II. Correlation and multiple regression analysis of the pituitary hormones (PRL, FSH, LH) along the testis (TT, FT)–prostate cancer (BG, P+, PSA) system in the patient population (N=100).

<table>
<thead>
<tr>
<th>Pituitary</th>
<th>Analysis</th>
<th>Testis</th>
<th>Prostate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>STAT</td>
<td>TT</td>
</tr>
<tr>
<td>PRL</td>
<td>COR</td>
<td>r</td>
<td>-0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>MLR</td>
<td>coef</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.96</td>
</tr>
<tr>
<td>FSH</td>
<td>COR</td>
<td>r</td>
<td>-0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>MLR</td>
<td>coef</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.34</td>
</tr>
<tr>
<td>LH</td>
<td>COR</td>
<td>r</td>
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<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>MLR</td>
<td>coef</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-Value</td>
<td>0.48</td>
</tr>
</tbody>
</table>

STAT, Statistics; r, Pearson's correlation coefficient; COR, correlation analysis; MLR, multiple linear regression analysis; coef, regression coefficients.

computed. Correlation and multiple linear regression analysis of the clustered population along the pituitary–testis–prostate cancer axis was performed. Analysis of variance (ANOVA) of the variables between the groups was computed. The patient population was also clustered according to the BGS in group A (BGS ≤6), B (BGS = 3+4) and C (BGS ≥4+3). A contingency table relating the BGS groups (A, B, C) to PRL/P+ clusters (A, B, C) was constructed and the Chi-squared test was performed in order to detect statistical significance. PRL versus P+ scatter plots were computed for the patient population clustered in BGS groups (A, B, C) and for each BGS group subclustered according to PRL/P+ ratio into A, B, C.
Figure 4 which shows that the BGS groups (A, B, C) are not homogenous because they include significantly different PRL/P+ subclusters (see Figure 4).

Discussion

PRL correlation in the prostate cancer population is unclear, controversial and has not been fully investigated. It has been shown that PRL did not correlate any prostate cancer variable (6, 9); however, it has also been demonstrated that PRL was inversely associated with PSA in univariate analysis and that correlation disappeared on multivariate analysis (32). The prognostic value of PRL in the prostate cancer population is also unclear; indeed, it has been shown that serum PRL levels were significantly lower in metastatic prostate cancer with a good response to hormonal treatment (12), but it has also been demonstrated that PRL did not relate to survival (11). To the best of our knowledge, this is the first clinical study showing a significant correlation of serum PRL to P+; PRL was also independently predicted by P+ $p=0.02$ on multiple linear regression analysis (see Table II). The pituitary–testis–prostate cancer axis was simultaneously investigated in clinically localized prostate cancer of the present study, which showed that pituitary hormones were significantly correlated to the testis and to prostate cancer; moreover, they were significantly predicted by prostate cancer clinical variables (P+, PSA) on multiple linear regression analysis (see Table II) showing a direct functional interacting relationship of PRL on prostate cancer biology (P+ and PSA). These findings suggest that there is also an evident association between pituitary hormones and prostate cancer phenotype. We also showed that P+ significantly predicted the BGS on multiple linear regression analysis and this finding suggests a potential prognostic role of PRL in the natural history of prostate cancer (33-36). The results of the present paper confirm the findings of our previous investigations in the prostate cancer population; we have shown that LH and FSH were highly correlated to FT and independently predicted by PSA; moreover, the high correlation of BGS and P+ has also been confirmed (37, 38). Interestingly, the present findings have confirmed that FT and TT might have key roles in prostate cancer biology along the pituitary–testis–prostate axis, expressing complicated feedback systems which, in part, might be explained by both linear and non-linear mathematical laws (39-42). The significant correlation and prediction of PRL by P+ allowed us to cluster the patient population into groups A, B and C according to the PRL/P+ ratio (see Figures 1 and 2). PRL serum levels in PRL/P+ clusters were all significantly correlated to P+ (see Table III) and significantly differed for
PRL, P+ and BGS (see Tables III and IV), suggesting that they might have a potential prognostic role in prostate cancer (33-36). Interestingly, PRL/P+ cluster A showed features indicating a high-risk group in which both FSH and LH were significantly and independently predicted by TT, FT and PSA along the pituitary–testis–prostate cancer axis (see Table III). PRL/P+ clusters B and C were also significantly correlated and predicted along the pituitary–testis–prostate system by TT and P+ (see Table III). Moreover, PRL/P+ cluster B showed features indicating a model where all pituitary hormones were simultaneously correlated along the endocrine system (see Table III). The PRL/P+ ratio selected significant potential prognostic clusters in which the risk of progression might be assessed as high (group A), intermediate (group B) and low (group C); the PRL/P+ clusters might also express potential as clinical models for further investigations.

It has been assessed that core biopsy of the prostate may under- and overgrade the final combined Gleason grade (43); indeed, an exact Gleason score match is present in 41% of cases, while 48% are under- and 17% overgraded (44); the number of positive cores is also a significant predictor of upgrading (45). BGS group A (≤6) accounted for 56% of the patient population (see Table IV) and they had a risk of 52% of being upgraded on the final pathologic Gleason score, as shown by the literature (44). Interestingly, 53% of BGS group A was at risk of upgrading because of being included in PRL/P+ cluster A (n=12) and B (n=18), which represent high-intermediate prognostic groups (see Table IV and Figure 4A). The BGS group B included PRL/P+ clusters A (n=11, 37%), B (n=15, 50%) and C (n=4, 13%); it was evident looking at the scatter plot that the subclusters represented different prognostic groups in which the risk of up- and downgrading were related to PRL/P+ clusters A and C respectively (see Figure 4B). Interestingly, SBG group C (≥4+3) did not include, as expected, PRL/P+ cluster C; the risk of downgrading was also low and might be related to the PRL/P+ cluster B (n=3, 22% of BGS group C), as evident from Figure 4C. Interestingly, PRL was significantly correlated and predicted by P+ (r=0.58, p=0.02) in the BG cluster C (≥4+3); this finding has been confirmed by literature reports showing that PRL protein was correlated to high Gleason scores (46) and was expressed in a large proportion of hormone-refractory clinical human prostate carcinomas and in prostate cancer metastases (47). The prostate cancer population grouped by the BGS was not homogeneous because of the large intergroup overlap, as evident from Figure 3; moreover, when the BG groups were selected according to the PRL/P+ ratio, more homogeneous subclusters were detected (see Figure 4).

The present investigation is limited by the small number of patients, but it is intriguing for its findings and challenging for its potential applications in managing clinical prostate cancer. However, confirmatory studies are needed.

Figure 4. PRL versus P+ scatter plots of the patient population clustered into groups A, B, C according to the PRL/P+ ratio for biopsy Gleason score (BGS) ≤6 (A), BGS=3+4 (B) and BGS≥4+3 (C).
Conclusion

In the prostate cancer population at diagnosis and along the pituitary–testis–prostate axis, PRL is significantly correlated to and predicted by P+. The prostate cancer population at diagnosis might thus be clustered according to the PRL/P+ ratio into groups A, B and C which, as a theory, might express prognostic potential and clinical applications in prostate cancer. However, confirmatory studies are needed.
Table IV. Contingency table relating the biopsy Gleason score (BGS) groups to the PRL/P+ clusters in the patient population (n=100).

<table>
<thead>
<tr>
<th>BGS</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥6</td>
<td>O 12</td>
<td>18</td>
<td>26</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>E 19.04</td>
<td>20.16</td>
<td>16.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-E −7.04</td>
<td>−2.16</td>
<td>9.20</td>
<td></td>
</tr>
<tr>
<td>3+4</td>
<td>O 11</td>
<td>15</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>E 10.20</td>
<td>10.80</td>
<td>9.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-E 0.80</td>
<td>4.20</td>
<td>−5.00</td>
<td></td>
</tr>
<tr>
<td>≥4+3</td>
<td>O 11</td>
<td>3</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>E 4.76</td>
<td>5.04</td>
<td>4.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>O-E 6.24</td>
<td>−2.04</td>
<td>9.20</td>
<td></td>
</tr>
</tbody>
</table>

Statistics

Chi squared test=25.5
p-Value=0.00003
Degrees of freedom=4

O, Observed; E, Expected.

References


30 Fodstad P, Bjoro T, Torlakovic G and Fossa SD: No association of serum gonadal or pituitary hormone with prognostic parameters in stages T1 to T3 pN0M0 prostate cancer. J Urol 168: 1188-1192, 2002.


