Positive Association between Preoperative Total Testosterone and Lymph Node Invasion in Intermediate Risk Prostate Cancer

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Key Words
Prostate cancer • Radical prostatectomy • Testosterone • Pelvic lymph node dissection • Lymph node invasion • Prostate specific antigen

Abstract
Introduction: Prostate cancer (PCa) patients who are classified into the intermediate risk category represent a heterogeneous population needing further preoperative risk assessment. Objectives: To evaluate clinical total testosterone (TT) associations with lymph node invasion (LNI) in intermediate risk PCa. Material and Methods: Between November 2014 and July 2016, intermediate risk PCa was assessed in 154 patients who underwent extended pelvic lymph node dissection if the risk of LNI was higher than 5%. Clinical factors associated with the risk LNI were investigated by the multinomial logistic regression model. Results: The risk of LNI was assessed higher than 5% in 40.9% of cases of whom 15.5% had LNI. In the multivariate model, the risk of LNI was independently increased by prostate specific antigen (OR = 1.185; p = 0.021) and TT (OR = 1.004; p = 0.036). As a result, TT was an independent factor that associated with LNI because it increased the risk of LNI by 4% for each increment unit of TT. Conclusion: Preoperative TT independently increased the risk of LNI in the intermediate risk class of PCa patients elected to radical prostatectomy and extended pelvic lymph node dissection. TT might be a useful preoperative factor for stratifying intermediate risk patients because of the positive association of TT with high grade tumors.
Patients are classified into low, intermediate and high cancer class risk categories [6]. In each category, the risk of lymph node invasion (LNI) is assessed by nomograms [2–5, 7], which do not consider the new Gleason grading group system [8, 9]. The risk of extensive LNI associates with the high risk disease [10, 11].

PCa is an endocrine tumor, which depends on androgens [12] and associates with increased levels of prostate-specific antigen (PSA) [13]. Basal levels of total testosterone (TT) have been detected abnormal in the PCa population [14]. The association between TT and PCa is a controversial and unsettled subject, which needs further clinical research [15, 16].

Patients, who are classified into the intermediate risk category, represent a large and heterogeneous population because of the risk of tumor upgrading as well as LNI in the surgical specimen [2–5, 7, 11, 12]. In this category, extended PLND is performed if the risk of LNI is estimated greater than 5% [7]. As a consequence, this protocol generates a sub-population of patients in whom the status of the loco-regional lymph nodes is unknown. In modern cohorts, analyses of clinical factors that associate with the unknown pathological nodal status, which is classified as pNx category, are missing. The aim of this study was to evaluate clinical factors associated with and without LNI compared to cases who did not undergo PLND in the intermediate risk PCa population.

Materials and Methods

The study was retrospective and was approved by the Institutional Review Board. Each patient provided informed-signed consent.

In a period ranging from November 2014 to July 2016, basal levels of TT were measured in 349 patients before RP. The study evaluated 319 cases after excluding 30 patients who were under androgen deprivation therapy. Pretreatment simultaneous serum samples of TT and PSA were obtained from a cubital vein, at least 1 month after biopsies, between 8:00–8:30 a.m. The samples were analyzed at our laboratory. The plasma levels of TT (ng/dl) and PSA (ng/ml) were determined by radioimmunoassay.

Biopsies performed elsewhere were not revised and were accepted by the following criteria: (i) at least 12–14 biopsy cores; (ii) the reported number of positive cores; (iii) measurement of prostate volume (ml). In our institution, the 14-core transperineal guided prostate biopsy technique was used. Prostate volume (ml) was measured by standard methods. In each case, pelvic lymph node staging (cN) was performed by computerized tomography (CT) and/or by multi-parametric magnetic resonance imaging (mpMRI) modalities. When pelvic nodes measured within 1 cm in diameter, clinical staging status was coded cN0. Enlarged pelvic nodes measuring more than 1 cm in diameter were staged as cN1 disease. The metastatic status was investigated by CT, bone scan and occasionally by 11-choline positron emission tomography CT (11C-PET CT).

According to the surgeon’s skills and expertise, surgery was performed by the robot-assisted radical prostatectomy (RARP) procedure in 268 cases (84.0%) or by the retropubic radical prostatectomy (RRP) approach in 51 patients (16.0%). Overall, 152 patients underwent extended PLND, which was performed by RARP in 108 cases (71.1%) and by RRP in 44 patients (28.9%). RARP was delivered by the da Vinci robot system (Intuitive Surgical, Inc., Sunnyvale, CA, USA) and was performed through the transperitoneal approach with antegrade prostatic dissection [17]. RRP was performed according to the technique of Walsh [18].

All PCa patients’ population was classified according to the D’Amico criteria [6] and staged by the 2002 American Joint Committee on Cancer staging system for PCa. Extended PLND was performed in all high risk cases and in intermediate risk patients when the risk of LNI was more than 5% [7]. In low risk patients, extended PLND was performed in a selected cases who showed high risk of tumor upgrading towards high patterns [19].

The dedicated pathologist assessed the surgical specimens, which were processed according to the Stanford protocol [20]. Tumors were classified by the new grading group system [8, 9]. Nodal packets were grouped into left and right nodes, tagged and submitted in separate packages. The removed lymph nodes were assessed for histopathological analysis after hematoxylin and eosin staining. Immunohistochemical staining was performed when appropriate. In each case, the number of removed and metastatic nodes was assessed. Prostate and nodal specimens were then staged according to the 2002 American Joint Committee on Cancer staging system for PCa.

Statistical Methods

All patients with PCa were classified into 3 groups including cases who did not undergo extended PLND because of the low risk of LNI (< 5%) and subjects with or without LNI. Summary statistics was computed. The association of clinical factors among the groups was assessed. Data on continuous variables are reported as medians with their respective ranges and differences among groups analyzed by the Kruskal-Wallis test. Data on categorical variables are presented as percentages, and differences among groups analyzed with Pearson’s chi-squared test or Fisher exact test as appropriate. Clinical factors associated with the risk of LNI was investigated by the multinomial logistic regression model (univariate and multivariate analysis). The software used to run the analysis was IBM-SPSS version 20. All tests were two-sided with p < 0.05 considered to indicate statistical significance.

Results

The whole PCa patients’ cohort included 319 patients who were distributed as follows: (i) low risk, 95 cases (29.8%); intermediate risk, 154 cases (48.3%); (ii) high risk, 70 patients (21.9%). Figure 1 shows each clinical category stratified by the pN status.

In the intermediate risk population (n = 154), RARP was performed in 133 cases (86.4%) and RRP in 21 patients (13.6%) and extended PLND was performed by
RARP in 46 cases and by RRP in 17 patients. In the same group, PLND was performed in 63 cases (40.9%) and metastases were detected in 11 cases (17.5% of the intermediate risk PLND population and 7.1% of the entire intermediate risk population), the remaining 91 patients (59.1%) did not undergo PLND (pNx status) because the risk of LNI was less than 5%.

In the intermediate risk PCa population, the median number of removed nodes was 24. The median number of removed nodes resulted 24 for RARP cases and 20 for RRP patients, but the difference was not significant (p = 0.058). Summary statistics and cross classification of clinical factors with the pathologic lymph node status of the intermediate risk group are reported in table 1. In the same population, the median values of age, PSA and TT were 65 years, 6.8 ng/ml and 371 ng/dl, respectively. Clinical staging of the tumor (cT) was cT1c in 102 cases (66.2%) and cT2b in 52 patients (33.8%).

Among the sub-groups of the intermediate risk PCa, median values of TT were significantly higher in patients with (443.2 ng/dl) or without LNI (421.4 ng/dl) than cases with unknown LNI (354 ng/dl) and the difference was significant (p = 0.023). The proportion of biopsy positive cores were significantly (p < 0.0001) higher in subjects with (41%) or without (40%) LNI than patients with unknown LNI (25%). Patients with LNI had significantly (p = 0.032) higher rates of bGG 3 tumors (36.4%) than subjects without (28.8%) or unknown LNI (23.3%). In the surgical specimen, high grade tumors (grade group 4–5) were significantly higher (p < 0.0001) in patients with LNI (63.6%) than cases without (17.3%) or unknown LNI (9.9%). The distribution of age, body mass index, PSA, prostate volume and cT were not significant.

Although PSA distribution was not significant among the 3 groups, it was included into the multinomial logistic regression models because of its importance in PCa biology [13]. The probability of undergoing extended PLND with absent LNI was independently increased by TT (odds ratio, OR = 1.002; p = 0.048), biopsy positive cores (OR = 278.348; p < 0.0001), bGG 2 (OR = 7.921; p = 0.002) and bGG 3 (OR = 8.826; p = 0.003), as shown in table 2. The risk of LNI was independently increased by PSA (OR = 1.185; p = 0.021) and TT (OR = 1.004; p = 0.036), as shown in table 3. As a result, TT was an in-
dependent factor that associated with LNI because it increased the risk of LNI by 4% for each increment unit of TT. Figure 2 shows the distribution of TT median levels among the groups stratified by pathologic tumor grade groups. As shown, TT median levels were higher in cases with a risk of LNI higher than 5%.

Table 1. Cross classification of clinical factors with lymph node status in intermediate risk prostate cancer (n = 154)

| Clinical factors | Population | LNI                   | p    |
|------------------|------------|-----------------------|------|
|                  |            | Unknown (n = 91; 59.1%)| Absent (n = 52; 33.8%)| Present (n = 11; 7.1%)|      |
| Age, years       | median (range) | 65 (51–76) | 65 (51–76) | 65.5 (52–76) | 65 (55–74) | 0.776 |
| BMI, kg/m²       | median (range) | 26 (19.2–35.5) | 25.7 (20.3–35.5) | 26.2 (19.2–32.9) | 25 (21.7–31.6) | 0.994 |
| PSA, ng/ml       | median (range) | 6.8 (0.7–19.9) | 6.8 (0.7–19.9) | 6.5 (1.5–15.1) | 8.5 (4.9–19.1) | 0.21  |
| TT, ng/dl        | median (range) | 371 (119–1,478.1) | 354 (119–1,060.5) | 421.4 (200–1,478.1) | 443.2 (291.9–1,021.3) | 0.023 |
| PV, ml           | median (range) | 40 (15–106.8) | 39.1 (15–75.7) | 40 (18–106.8) | 51.4 (19.9–84.8) | 0.17  |
| BPC (proportion) | median (range) | 0.29 (0.06–1.00) | 0.25 (0.06–1.00) | 0.40 (0.09–0.35) | 0.41 (0.07–0.58) | <0.0001 |
| cT, n (%)        |             | 102 (66.2) | 64 (70.3) | 30 (57.7) | 8 (72.7) | 0.274 |
| bGG, n (%)       |             | 52 (33.8) | 27 (29.7) | 22 (42.3) | 3 (27.3) | 0.032 |
| Group 1–3        |             | 34 (22.1) | 27 (30.0) | 4 (7.7) | 3 (27.3) | <0.001 |
| Group 4–5        |             | 80 (51.9) | 43 (46.7) | 33 (63.5) | 4 (36.4) |        |
| pGG, n (%)       |             | 129 (83.8) | 82 (90.1) | 43 (82.7) | 4 (36.4) |        |
| Group 1–3        |             | 25 (16.2) | 9 (9.9) | 9 (17.3) | 7 (63.6) |        |
| BMI = Body mass index; PV = prostate volume; BPC = biopsy positive cores; bGG = biopsy Gleason grade group; pGG = pathology Gleason grade group.

Table 2. Clinical factors associated with absent LNI in intermediate risk prostate cancer by the multinomial logistic regression model

| Factors | pN0 vs. pNx (univariate analysis) | pN0 vs. pNx (multivariate analysis) |
|---------|-----------------------------------|------------------------------------|
|         | OR 95% CI | p     | OR 95% CI | p     |
| PSA     | 1.001 | 0.913–1.097 | 0.984 | 1.002 | 1.000–1.005 | 0.048 |
| TT      | 1.003 | 1.001–1.005 | 0.016 | 96.943 | 9.495–989.778 | <0.0001 |
| BPC     | 278.348 | 18.855–4109.216 | <0.0001 | 1.002 | 1.000–1.005 | 0.048 |
| bGG     | 9.921 | 2.179–28.793 | 0.002 | 7.921 | 2.179–28.793 | 0.002 |
| Group 1–3 | 4.821 | 18.855–4109.216 | <0.0001 | 8.826 | 2.150–36.228 | 0.003 |

pN0 = No lymph node invasion; pNx = lymph node invasion unknown; OR = odds ratio; CI = confidence interval; BPC = biopsy positive cores; bGG = biopsy Gleason grading group.
Table 3. Clinical factors associated with LNI in intermediate risk prostate cancer by the multinomial logistic regression model

| Factors | pN1 vs. pNx (univariate analysis) | pN1 vs. pNx (multivariate analysis) |
|---------|----------------------------------|------------------------------------|
|         | OR   | 95% CI    | p   | OR   | 95% CI    | p   |
| PSA     | 1.183 | 1.028–1.361 | 0.019 | 1.185 | 1.025–1.369 | 0.021 |
| TT      | 1.003 | 1.000–1.007 | 0.035 | 1.004 | 1.000–1.007 | 0.036 |
| BPC     | 21.504 | 0.494–935.174 | 0.111 |
| bGG     | 1 (3 + 3) | reference group | | | | |
|         | 2 (3 + 4) | 0.837 | 0.714–4.034 | 0.825 | | |
|         | 3 (4 + 3) | 1.714 | 0.343–8.507 | 0.58 | | |

pN1= Lymph node invasion; pNx = lymph node invasion unknown; OR = odds ratio; CI = confidence interval; BPC = biopsy positive cores; bGG = biopsy Gleason grading group.

Discussion

The incidence of PCa with secondary nodal involvement ranges between 1.1 and 33.3%, and depends on the median number of removed nodes [2–5, 21–23]. It has been suggested that approximately a number of 20 pelvic nodes is the landmark for a sufficient PLND [24]. In a cohort of patients who were operated by the open approach, the mean number of removed nodes was 28 [25]. In an intermediate-high risk cohort of patients who were operated by RARP, the median number of removed nodes was 20 [26]. In our study, the intermediate cancer class risk subpopulation represented 48.3% of the entire cohort. The risk of LNI was assessed as < 5% in 59.1% of cases who did not undergo extended PLND. Among cases who had extended PLND, LNI was detected in 17.5% of patients who represented 7.1% of the entire subpopulation, as shown in figure 1. The overall median number of removed nodes was 20, which was adequate in both RARP (median = 24) and RRP cases (median = 20).

Clinical factors associated with LNI are well known from the literature and include PSA, clinical stage of the tumor, biopsy Gleason score and proportion of biopsy positive cores [2–5, 7, 11, 21, 27]; however, analysis of factors including TT are missing. In a large multicentre study, predictors of LNI were as follows: (i) none in the D’Amico low risk category; (ii) PSA, biopsy Gleason pattern 4 and proportion of biopsy positive cores in the intermediate risk class; and (iii) proportion of biopsy positive cores in the high risk category [21]. In the intermediate risk category, PLND was performed in 64.9% of cases and analysis of factors were performed in this group including patients with and without LNI [21]. Our study, investigated clinical factors associated with LNI in the intermediate risk category, which was large and heterogeneous. In the intermediate risk category, it has been shown that both TT and PSA increased the risk of LNI when compared to cases who did not undergo lymph node dissection because of the low risk of nodal metastases (< 5%). Indeed, the risk of LNI for 1 unit increase of TT raised by 4% (table 3). This is the first study showing a positive association between preoperative serum levels of TT and risk of LNI in the intermediate risk category, which is heterogeneous and need stratification.
The association between TT and PCa is controversial and the subject is still unsettled [15, 16]. Recently, it has been shown that, in patients with low to intermediate risk disease, basal levels of TT independently predicted tumor upgrading to high risk disease in the surgical specimen [28, 29]. The prediction of tumor upgrading by preoperative TT relates to the association between TT and high grade tumors in the surgical specimen [30]. The present study has shown that the intermediate risk category is heterogeneous because including low, intermediate and high grade tumors. In the surgical specimen, high grade tumors were detected less frequently in patients with unknown LNI (9.9%) than cases without (17.3%) or with (63.6%) LNI. So far, patients who did not undergo ePLND included a subpopulation having less aggressive tumors than cases with or without LNI. The positive association of TT with the risk of LNI are explained by the diagram of figure 2, which shows that preoperative median TT levels increased by increasing levels of LNI. The step forward is to consider TT as a further preoperative factor in evaluating patients who are classified into the intermediate risk category.

There are limits in our study. First, it was a retrospective investigation and biased by this type of studies. Second, the number of patients were limited. Third, we did not evaluate the dimensions of the metastatic nodes. Fourth, prostate biopsies were not all performed in our institution; however, the inclusion criteria of the study avoided potential biases related to this issue. Fifth, the biopsies performed elsewhere were not reviewed by our dedicated pathologist. Sixth, TT was not measured by gas chromatography-mass spectrometry, which is the standard method to measure circulating TT. However, beyond these limits, our study has many strengths and shows new insights into the controversial and heterogeneous intermediate PCa risk category.

**Conclusion**

Preoperative TT independently increased the risk of LNI in the intermediate risk class of PCa patients elected to RP and extended PLND. TT might be a useful preoperative factor for stratifying intermediate risk patients because of the positive association of TT with high grade tumors.

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