ORIGINAL ARTICLE

Prognostic role of perineural invasion in prostate biopsy

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Abstract
Introduction: Despite tumor cell dissemination through the intraprostatic nervous system being considered as a prostate cancer progression mechanism, the significance of perineural invasion in prostate biopsies to predict extraprostatic extension and its use as a potential prognosis factor is controversial.

Materials and methods: Retrospective study carried out at an institution on 208 patients treated with radical prostatectomy (January 2007 – July 2010) in which the presence of perineural invasion and the Gleason score in the preoperative biopsy were determined, as well as the clinical stage and the pre-surgery PSA. We classified the patients in risk groups in accordance with the D’Amico classification. We performed bivariate and multivariate statistical analyses to establish the correlations between the different variables.

Results: We objectified PNI in 18.3% of the prostate biopsies. 71% of the prostatectomy specimens with perineural invasion presented extraprostatic extension in the previous biopsy against 23.1% when this was not found (p < 0.0001) and 47% of the cases showed positive margins with PNI, against 18.3% without perineural invasion (p < 0.0001). In fact, in the multivariate analysis, perineural invasion proved to be an independent risk factor in the presentation of extraprostatic extension and positive margins in the prostatectomy specimen.

Conclusions: The presence of perineural invasion is a useful prognostic factor for predicting extraprostatic extension and the involvement of surgical margin in the radical prostatectomy specimen. We believe that determining it may be a useful tool for improving preoperative diagnosis and planning treatment.

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El papel pronóstico de la invasión perineural en la biopsia prostática

Resumen

Introducción: A pesar de que la diseminación celular tumoral a través del sistema nervioso intraprostático se considera un mecanismo de progresión del cáncer prostático, el significado de la invasión perineural en biopsias de próstata para predecir extensión extraprostática y su utilidad como potencial factor pronóstico es controvertido.

Material y métodos: Estudio retrospectivo llevado a cabo en una institución sobre 208 pacientes tratados con prostatectomía radical (enero 2007-julio 2010) en los que se ha determinado la presencia de invasión perineural y el score de gleason en la biopsia preoperatoria, así como el estadío clínico y el PSA prequirúrgico. Clasificamos los pacientes en grupos de riesgo según la clasificación de D’Amico. Realizamos análisis estadístico bivariante y multivariante para establecer la correlación entre las distintas variables.

Resultados: Se objetivó IPN en el 18,3% de las biopsias prostáticas. Presentaron extensión extraprostática el 71% de los espécimenes de prostatectomía con invasión perineural en la biopsia previa vs. 23,1% cuando no existía este hallazgo ($p < 0,0001$) y márgenes positivos el 47,4% de los casos con IPN, frente a 18,3% sin invasión perineural ($p < 0,0001$). De hecho, en el análisis multivariante la invasión perineural demostró ser un factor de riesgo independiente para presentar extensión extraprostática y márgenes positivos en la pieza de prostatectomía.

Conclusiones: La presencia de invasión perineural es un factor pronóstico útil para la predicción de extensión extraprostática y afectación de márgenes quirúrgicos en la pieza de prostatectomía radical. Consideramos que su determinación puede ser una herramienta útil en la mejora del diagnóstico preoperatorio y en la planificación del tratamiento.

Introduction

Vascular and lymphatic pathways of tumor dissemination are well known, but the perineural one has received less attention.\textsuperscript{1,2} Perineural invasion (PNI) of the nerves is considered a metastatic route in some tumors.\textsuperscript{1} It is considered that when the tumor cells get into the nerve sheaths, they find a facilitator of the migration plan, an area of less resistance to tumor dissemination,\textsuperscript{2} although the multiple layers of collagen and basal membrane of the nerve sheath do not really give low resistance; therefore, it is starting to be believed that the PNI is more an effect of invasion than of simple diffusion.\textsuperscript{1} In addition, several neurotrophins and stromal elements such as fibroblasts probably play an important role in the complex interactions that make the PNI possible.\textsuperscript{1}

There are several comprehensive reviews on PNI and its significance in the prognosis of the patient,\textsuperscript{1,3-8} which feed the key issue of discussion on whether it is worth to have the impact on the therapeutic decision for establishing a risk of tumor dissemination before surgery.\textsuperscript{6,7,9-11} To many, the presence of PNI in the biopsy is an indicator of risk of extraprostatic extension (EPE), of affected margins, and biochemical recurrence.\textsuperscript{5-8,12} For all this, some recommend not following the neurovascular bundles (NVB) in these patients.

In 2009, Liebig et al\textsuperscript{1} published a comprehensive review on PNI reflecting that research on the molecular mechanisms involved is scarce and its prevalence is not established. A better understanding of the PNI could lead to improving therapeutic strategies, and to that effect, understanding the structure of the nerve sheath is vital.\textsuperscript{1}

Materials and methods

We assessed the presence of PNI in prostate nerves in the prostate biopsies performed in 226 patients undergoing radical prostatectomy at our center from January 2007 to July 2010, discarding 18 biopsies performed in other hospitals, so the study focuses on 208 patients. In the results of margins and EPE, a case has been withdrawn for being T0. Gleason, PNI, vascular invasion, the presence of fatty infiltration, the proportion of tissue affected by carcinoma and the presence of high-grade PNI are evaluated histologically, although not all data have been included in the study results. Prostate biopsies were performed with an 18G needle guided by ultrasound. Generally, 5 cylinders were taken from each lobe, and in some patients with 1–3 previous negative biopsies, saturation biopsies were performed (20 samples). Biopsies included a maximum of 3 cylinders per block, serially sections them. We documented the number of cylinders of each sample and its size. Three patients were diagnosed by transurethral resection of the prostate. In doubtful cases or with a small amount of atypical inconclusive glands, 34BE12 cytokeratin immunostaining and/or P-63 was performed to assess prostate basal cells. We also performed immunostaining with PSA to distinguish between high-grade prostate cancer and urothelial carcinoma or other type, where considered necessary.

The prostatectomy specimens were fixed in formalin, painting their surface for evaluation of the margins. The apex, bladder neck and seminal vesicles (SV) were included in separate blocks with a sagittal cut each, including both hemisections. The prostate was cut into 2–3 mm slices perpendicular to the longitudinal axis. Each of the sections was...
Prognostic role of perineural invasion in prostate biopsy

Figure 1  Prostatic cylinder with sources for adenocarcinoma and several images of perineural invasion.

divided into halves or quarters for inclusion into the paraffin block. The prostate was included in its entirety with detailed guidance on each cut reflected in an illustrated scheme.

We define PNI as the presence of adenocarcinoma within the perineurium, within the nerve, surrounding the nerve, or invading ganglionic tissue. This finding is feasible in prostate cylinder biopsy (Fig. 1). In doubtful cases, immunostaining with S-100 allows to bring out the nerve bundles, and mixed cytokeratin immunostaining (CKAE1/AE3) stains tumor cell nests, which sometimes makes easier the visibility of intra- and perineural invasion of the adenocarcinoma (Fig. 2).

We analyzed other factors such as age, previous PSA, clinical stage, and Gleason, as well as final tumor stage and presence of margins in the prostatectomy specimen. Biochemical relapse has not been assessed for having a short follow-up. We divided patients into low risk (PSA ≤ 10, Gleason ≤ 6 and T1-2a), high risk (PSA > 20 or Gleason 8–10, or T2c or higher) and the rest in intermediate risk (D’Amico classification). We have used the definition of overstaging (Gleason score increase, appearance of tertiary degree or change to a higher order of primary and secondary Gleason) and understaging (Gleason score decrease or changes to a lower order of primary and secondary Gleason) when comparing the histological data of the prostate biopsy with surgical specimen data.

The database and statistical analysis were performed using SPSS v12.0 for Windows. First, a univariate analysis was performed using measures of centralization (mean and median) and dispersion (standard deviation and range) for quantitative variables and proportions or percentages for qualitative ones. After that, a bivariate analysis was performed using chi-square test or Fisher’s exact test to compare proportions and Student’s t-test for the comparison of means. Finally, for those affected margins and EPE as dependent variables, multivariate analysis was performed by means of logistic regression modeling, including the variables that showed statistically significant (p < 0.25) in bivariate analysis and those that we considered potential

Figure 2  (A) IHC with S-100 bringing out nerve bundle with perineural invasion by adenocarcinoma (the arrow points to the source for the adenocarcinoma), (B) intra- and perineural invasion demonstrated with CK AE1/AE3 that stains the tumor cells (the arrow points to the nerve bundle).
confounding factors (PSA, Gleason, stage and PNI). The association was considered statistically significant if \( p < 0.05 \), excluding the model variable when it did not reach that significance and when its presence did not change the effect of other variables in a relevant way, thus, the confounding slant is reduced. No interactions between variables were studied due to the limited sample size. Also, the simultaneous inclusion of variables that showed clear collinearity was avoided. The goodness of model fit was assessed with the corresponding table of classification.

Results

The mean age in the series was 63.7 years, the pre-biopsy PSA 6.8 (1.1–29.7), and the Gleason sum 6.6. In biopsies, we had unilateral disease in 68.7% of the cases, but in the prostatectomy, only 26.4% were unilateral. IPN was detected in the biopsy in 38 cases (18.3%) and in the prostatectomy in 151 (72.6%). For this work, the PNI of the specimen was not analyzed, which will be evaluated in the future taking the clinical evolution and tumor relapse into account.

If we analyze the series according to the D’Amico risk classification, 81 patients were low risk (38.9%), 122 intermediate (58.7%) and 5 high risk (2.4%). The mean PSA in the cases with PNI (8.3 \( \pm \) 5) was higher than in those without PNI (6.4 \( \pm \) 2.7) \( p = 0.000 \). In fact, patients with PNI suffered more frequent PSA > 10 (26.3%) than those without PNI (10%) \( p = 0.007 \). Clinical stage was 169 (81.2%) T1c, 3 (1.4%) T1b, 32 (15.4%), T2a, 2 (1%) T2b and 2 (1%) T3a. In prostatectomy, 66 patients were pT3 (31.7%), and the overall positive margin rate 23.7%. The percentage of pT3 according to the clinical classification was 0% for T1b, 28% for T1c, 46.9% in T2a and 100% of T2b–T3a.

Table 1 shows the PNI percentage for risk of D’Amico, grouped Gleason (6 vs. 7–8), clinical stage (T1 vs. T2–3) and grouped PSA (10 or < vs. >10), the difference being significant in all of them. In Gleason group 6, 2.2% had PNI compared with 30.5% Gleason \( \geq 7 \) \( p = 0.000 \) group. Of the stages T1, 12.9% had PNI compared with the 44.4% of the T2–3 \( p = 0.000 \). And of the PSA \( \leq 10 \), 15.5% had PNI compared with 37% of those with PSA > 10 \( p = 0.007 \). This table also provides the bivariate study of the EPE and the margins with relation to PNI, Gleason, clinical stage, and PSA. The margins were only significant with relation to PNI (47.4% in the group with PNI and 18.3% without PNI, \( p = 0.000 \)). The EPE was associated with PNI, they were pT3 71% (27/38) with, and 23.1% (39/169) without PNI \( p = 0.000 \), with grouped Gleason (21.3% in Gleason 6 vs. 39.8% in Gleason 7–8) \( p = 0.005 \) and with clinical stage (27.5% in T1 vs. 52.8% in T2–3) \( p = 0.003 \), but not with grouped PSA \( p = 0.10 \).

Table 2 shows the PNI multivariate analysis, PSA, Gleason, and clinical stage as a prognosis of affected margins or
EPE. The only significant margin was PNI and in EPE all of them, but at removing the PNI, none was significant. The only significant one in our study in terms of EPE and margins was the PNI. There are under- and overstaging degrees in all patients and groups of low and intermediate risk. In the low-risk one, there has been an increased Gleason in 38.3% of the cases (31/81), and in the intermediate-risk group 11.5% (14/122). Globally, we have an overstaging degree of 21.7% and an understaging of 18.4% (Table 3).

### Table 3  Graded under- and overstaging (changes in biopsy and specimen Gleason) in the whole series, and in intermediate- and low-risk groups.

<table>
<thead>
<tr>
<th>Biopsy Gleason</th>
<th>Same number</th>
<th>Decrease (understaging)</th>
<th>Increase (overstaging)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The whole series</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>54 (60.7%)</td>
<td>35 (39.3%)</td>
</tr>
<tr>
<td>7</td>
<td>115</td>
<td>70 (60.9%)</td>
<td>10 (8.7%)</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>3 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>208</td>
<td>124 (59.9%)</td>
<td>45 (21.7%)</td>
</tr>
<tr>
<td><strong>Intermediate risk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>7</td>
<td>113</td>
<td>69 (61.1%)</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>73 (59.8%)</td>
<td>14 (11.5%)</td>
</tr>
<tr>
<td><strong>Low risk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>81</td>
<td>50 (61.7%)</td>
<td>31 (38.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>395</td>
<td>261 (66.1%)</td>
<td>136 (33.9%)</td>
</tr>
</tbody>
</table>

Discussion

The nerve sheath has three layers: epineurium, perineurium and endoneurium.1 In works on PNI, tumor cells are described within each of them, but it remains to be established which the main location of the PNI is and what histopathological signs are needed to define it. The definition of Batsakis is accepted: tumor cells in, around or through the nerves.

Villers et al. showed two areas where the nerves of the NVB pass through the prostatic capsule, an upper neural pedicle at the base and a lower one at the apex, which are the areas with the greatest capsular penetration.2 Likewise, the prostatic apex is the area most likely to present affected margins, and where the distance between the capsule and the NVB is shorter; perhaps that is one of the reasons why the apex is an extension risk zone, even in small tumors.2

In addition to assessing the PNI, it is possible to measure the number and percentage of affected nerves,3 tumor length, number of affected cylinders, percentage of each cylinder affected, maximum percentage of cylinder affected, and total tumor percentage.13 Prediction classifications of EPE, SV, and lymph node involvement, such as Partin,14,15 or risk of biochemical relapse ones of D’Amico based on Gleason, PSA and clinical category,16–18 although there are many other models.19 However, a full consensus on how to predict the behavior of cancer has not been reached, and presumably important parameters have remained out of these attempts to stratify the disease prior to surgery. The PNI stands out among them.

There are disparate works on PNI in relation to EPE and margins. The PNI of the biopsy was assessed as a prognosis for therapeutic decision, with significant results in several studies.5–8,10,12,20 Other works do not consider that PNI is an important prognostic factor.13,21 On the other hand, some works detect a relationship between the PNI and the positive margins5,12,14; suggesting not to preserve the NVB in cases of PNI, with the intention of reducing the margins.10 Not all studies support the relationship between the PNI and the affected margins.5,13,20 In the literature, there is a variety of data, definitions, and variable criteria in the systematic search of the PNI, which prevent a proper comparison.4

In addition, many of the prognostic signs of the disease are interrelated. Therefore, neuroanatomical and molecular studies are needed to better establish mechanisms of tumor growth.22

Although in 1994, the College of American Pathologists recommended the assessment of the PNI in biopsies, later Bostwick et al. considered the PNI as a factor not studied enough to demonstrate prognostic value, but rather PSA, stage, Gleason and margins.23 We believe there is gained experience, and our own casuistry so endorses it, in order to consider the PNI as a significant prognostic factor of the EPE and margins.

In the literature, there is PNI in 7–48% of prostatic biopsies5–10,12,20,21 and in 31.9–79% of prostatectomies.12,14,24–27 The problem with the PNI of the prostatectomy specimen is different, being able to think whether a prognostic factor of biochemical relapse and survival should be considered. Staging errors do not fully explain these differences. In fact, there is more overstaging related to PSA, with lower prostate volume and greater tumor volume, but not with PNI.28

Most authors consider that the PNI is a risk factor for EPE,5,11,30 but not a contraindication for surgery, and they continue considering this option as the most appropriate, although without respecting the NVB.11 In our experience, the PNI behaves as a prognostic factor of affected margins and EPE. Ficarra et al.29 and Bastacky et al.30 consider the PNI a marker of margins and EPE, respectively. It seems and assumed also that the PNI in low-risk patients increases the biochemical relapse at 5 years, its independent prognostic role not being clear in the intermediate or high ones.10 In our study, we did not assess the impact of the PNI on the biochemical relapse, given our short follow-up. Ng et al. believe that the PNI in the prostatectomy specimen is significantly associated with greater stage, larger tumor.
volume, lower prostate weight, higher Gleason score, more EPE, greater involvement of SV and margins, but not PSA, lymph node involvement, presence of PNI or biochemical relapse.\(^5\) Quinn et al. see that the PNI of the biopsy correlates with the stage, the biopsy Gleason and specimen, the number of affected cylinders, the EPE, the affected margins, but not with the PSA pre-treatment, or with the lymph node involvement.\(^12\) For them, in the multivariate study, the PNI only relates to biochemical relapse in patients with PSA > 10,\(^12\) suggesting that the PNI can be important in the biopsy of high-risk patients with PSA > 10.

Cannon et al. think that the NVB can be preserved even if there is PNI;\(^3\) and Holmes et al.\(^11\) observe that most of the times the EPE occurs outside of the NVB, whether they have PNI or not. Nonetheless, they do not propose the removal of the NVB in cases of PNI, although knowledge of the risk of EPE can help in the therapeutic decision, mainly in whether to respect the NVB or not, without being a surgical contraindication.\(^11\) Sometimes the NVB has been recommended to be resected on the side affected by the PNI.\(^10\) Other authors consider that the PNI suggests sacrificing the NVB.\(^30\) In this sense, D’Amico et al. observe that when resecting the NVB on the affected side, the margins are reduced, thus, considering resecting the NVB in the low-risk cases with PNI.\(^10\)

The identification of the PNI should possibly modify the therapeutic decisions, but estimating the risk only for this finding is very difficult because the published evidence is often methodologically inadequate or comparable.\(^4\) If the PNI is a prognostic factor in a group of patients at increased risk of biochemical relapse, it may be helpful in identifying patients who require a more aggressive treatment.\(^9\) Similarly, while it is not clarified whether the PNI is an absolute contraindication for NVB preservation, other markers should be sought to help at this point.

The low-risk patients with PNI might benefit from a treatment more targeted for intermediate-risk ones, and high-risk patients should be informed of the high possibility of requiring postoperative radiotherapy.\(^7\)

In summary, the PNI is a prognostic factor of risk of EPE and affected margins. More comprehensive and prospective studies should be performed to assess the actual role of this finding in the prognosis of patients with prostate cancer and in therapeutic decision making.

Conflict of interest

The authors declare that they have no conflict of interest.

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