available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Prostate Cancer

Computerized Quantification and Planimetry of Prostatic Capsular Nerves in Relation to Adjacent Prostate Cancer Foci

Johannes Bründl[a,*], *Sebastian Schneider*[a], *Florian Weber*[b], *Florian Zeman*[c], *Wolf F. Wieland*[a], *Roman Ganzer*[a]

[a] *Department of Urology, University of Regensburg, Caritas St. Josef Medical Center, Regensburg, Germany;* [b] *Institute of Pathology, University of Regensburg, Regensburg, Germany;* [c] *Center for Clinical Studies, University of Regensburg, Regensburg, Germany*

Article info

Article history:
Accepted April 26, 2013
Published online ahead of print on May 7, 2013

Keywords:
Prostate nerve distribution
Prostate cancer
Prostate capsule
Perineural invasion
Capsular nerve growth

Abstract

Background: Perineural invasion is discussed as a significant route of extraprostatic extension in prostate cancer (PCa). Recent in vitro studies suggested a complex mechanism of neuroepithelial interaction.
Objective: The present study was intended to investigate whether the concept of neuroepithelial interaction can be supported by a quantitative analysis and planimetry of capsular nerves in relation to adjacent PCa foci.
Design, setting, and participants: Whole-mount sections of the prostate were created from patients undergoing non–nerve-sparing laparoscopic radical prostatectomy. For each prostate, adjacent sections were created and stained both to identify capsular nerves (S100) and to localize cancer foci (hematoxylin and eosin).
Outcome measurements and statistical analysis: Computerized quantification and planimetry of capsular nerves (ImageJ software) were performed after applying a digital grid to define 12 capsular sectors. For statistical analyses, mixed linear models were calculated using the SAS 9.3 software package.
Results and limitations: Specimens of 33 prostates were investigated. A total of 1957 capsular nerves and a total capsular nerve surface area of 26.44 mm^2 were measured. The major proportion was found in the dorsolateral (DL) region ($p < 0.001$). Adjacent tumor was associated with a statistically significant higher capsular nerve count compared with the capsules of tumor-free sectors ($p < 0.005$). Similar results were shown for capsular nerve surface area ($p < 0.006$). Subsequent post hoc analyses at the sector level revealed that the effect of tumor on capsular nerve count or nerve surface area is most pronounced in the DL region.
Conclusions: The presence of PCa foci resulted in a significantly increased capsular nerve count and capsular nerve surface area compared with tumor-free sectors. The present study supports former in vitro findings suggesting that the presence of PCa lesions may lead to complex neuroepithelial interactions resulting in PCa-induced nerve growth.

* Corresponding author. Department of Urology, University of Regensburg, Caritas St. Josef Medical Center, Landshuter Straße 65, D-93053 Regensburg, Germany. Tel. +49 941 782 3531; Fax: +49 941 782 3545.
E-mail address: johannes.bruendl@ukr.de (J. Bründl).

0302-2838/$ – see back matter
http://dx.doi.org/10.1016/j.eururo.2013.04.043

1. Introduction

Therapeutic regimens for prostate cancer (PCa) differ according to tumor stage [1]. Various studies have focused on the mechanisms of metastatic progression. In addition to the well-accepted routes of hematogenous and lymphatic tumor spread, perineural invasion (PNI) has been discussed over the last few decades as the most significant route of extraprostatic spread in PCa [2]. Furthermore, the detection of PNI in prostate needle biopsy specimens has been identified as a predictor for extraprostatic extension [3] and advanced disease [4]. Although PNI is frequently found in PCa ($\leq$75%), little is known about its exact mechanism. Over a long period, PNI was thought to be a low-resistance pathway [4,5]. Recent cell culture studies, however, suggested a more complex mechanism of neuroepithelial interaction [6]. Nevertheless, there are no data on whether PCa induces capsular nerve growth in vivo. The present study was intended to investigate whether the concept of a neuroepithelial interaction can be supported by a quantitative analysis of capsular nerves in relation to adjacent PCa foci. We investigated this aspect by means of computerized quantification and planimetry of capsular nerves in whole-mount sections.

2. Materials and methods

2.1. Specimens

All specimens were obtained from patients undergoing non–nerve-sparing endoscopic extraperitoneal radical prostatectomy for biopsy-proven PCa. None of the patients received androgen-deprivation therapy prior to surgery. The surgical technique was standardized, as has been described previously [7]. Prostate specimens were cut into slices of approximately 3-mm thickness to fit whole-mount preparation and fixed in 4% buffered formalin for 36 h before being embedded in paraffin. Specimens were cut with a special microtome (HM 430, Microm International GmbH, Walldorf, Germany) into transverse serial whole-mount sections with a thickness of 10 μm. Histopathologic analysis was performed on transverse whole-mount sections, whereas the most distal part of the apex was processed in the sagittal direction according to the Stanford protocol [8]. For the present study, consecutive whole-mount sections were selected from one area of each specimen containing at least one tumor focus.

2.2. Staining

Each specimen was stained with hematoxylin and eosin (HE) for tumor localization and histopathologic analysis. Adjacent sections were stained for prostatic nerve fibers using a polyclonal antibody against the neural protein S100 (Dako, Hamburg, Germany). The specific immunohistochemical staining of nerves was confirmed by a single pathologist comparing selected pairs of HE and S100 sections. Tumor foci were marked on HE-stained sections by one pathologist, whereas no tumor marking was performed on the corresponding S100 sections.

2.3. Nerve quantification and planimetry

Sections were digitized using a high-resolution flatbed photo scanner device (Perfection V750 Pro, Epson, Meerbusch, Germany) at a resolution of 3200 dpi. For analysis, digital copies of each of the HE and S100 whole-mount sections were centrally covered with a grid dividing each section into 12 sectors numbered clockwise [7,9]. Sectors were then combined into the following regions: 12–1: ventral (V); 2–3 and 10–11: ventrolateral (VL); 4–5 and 8–9: dorsolateral (DL); and 6–7: dorsal (D) (Fig. 1). Image analysis was done with ImageJ software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA), as described previously [7,10]. As the existence of a true capsule of the prostate is a subject of debate, we defined the capsule as the condensed fibromuscular tissue (CFT) at the edge of the prostate (Fig. 1D, 2B, and 2C) [11–13]. No nerves have been analyzed in sectors containing no CFT. In a first step, the prostate capsule of each sector was marked in S100 sections at high resolution by one pathologist. Thereafter, nerve quantification and planimetry of total nerve surface area were performed using color thresholds under visual monitoring without knowledge of tumor location. In a second step, the tumor area within each sector was measured digitally on the corresponding HE-stained sections.

2.4. Statistics

Because of skewed distributions of capsular nerve count and capsular nerve surface area, we used linear mixed models based on ranks for our analyses [14]. At first, we analyzed solely the impact of tumor presence (no/yes), region (sectors 1/12, 2/11, 3/10, 4/9, 5/8, 6/7), and its interaction term on capsular nerve count and capsular nerve surface area. Where significant differences were identified, Bonferonni post hoc tests were performed for pairwise comparisons. In the mixed model, region was used as a repeated effect, and the correlation structure between regions was specified as compound symmetry. In addition, to take account of additional covariates, Gleason score, prostate-specific antigen (PSA) level, pathologic T stage, digital rectal examination (DRE), and prostate volume were added to each model in a subsequent analysis.

To enhance interpretation of the results, we report capsular nerve count and capsular nerve surface area as median, interquartile range (IQR [q1–q3]), and 95% confidence interval. Data of the least squares estimates based on ranks are not shown. A p value <0.05 was considered statistically significant. Statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA) by way of the procedure PROC MIXED.

3. Results

Specimens of 33 patients were available for this study. In all whole-mount sections used for this study, the capsule of the prostate was intact and without artifacts. The capsule had a median diameter of 1.13 mm (IQR: 0.88–1.45). Adjacent PCa foci had no statistical influence on the diameter ($p = 0.34$). A total of 1957 capsular nerves and a total capsular nerve surface area of 26.44 mm^2 were counted. Baseline data are shown in Table 1.

3.1. Capsular nerve distribution

The number of capsular nerves differed significantly between the different regions ($p < 0.001$). Most nerves (75.7%) were located in the DL region. Similar results were obtained with nerve planimetry, with 78.3% of the total capsular nerve surface area located dorsolaterally ($p < 0.001$). All details of nerve quantification and planimetry are shown in Table 2.

3.2. Nerve quantification in proximity to adjacent tumor foci

Sectors with tumor foci were associated with a statistically significant higher capsular nerve count compared with tumor-free sectors (median: 4.0 [IQR: 0.0–12.0] and