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Platinum Priority – Prostate Cancer

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Topography of Lymph Node Metastases in Prostate Cancer Patients Undergoing Radical Prostatectomy and Extended Lymphadenectomy: Results of a Combined Molecular and Histopathologic Mapping Study

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Abstract

Background: To determine the anatomic extent of pelvic lymph node dissection (PLND) in prostate cancer (PCa) patients at the time of radical prostatectomy (RP), knowledge about the topography of lymph node (LN) metastases is required.
Objective: Because small-volume LN metastases may be missed by standard histopathologic examination, we performed an anatomic mapping study combining molecular and histopathologic LN examination in PCa patients treated with RP and extended PLND (ePLND).
Design, setting, and participants: A total of 52 patients with intermediate- ($n = 15$) and high-risk ($n = 37$) PCa underwent RP and ePLND without neoadjuvant treatment. ePLND included dissection of the obturator fossa and the external, internal, and common iliac vessels.
Outcome measurements and statistical analysis: LNs $\geq$3 mm in diameter were analysed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for prostate-specific antigen (PSA) expression and by standard histopathology. Topography of positive LNs was determined descriptively.
Results and limitations: Of 1469 dissected LNs (median: 27 LNs per patient), 1186 LNs were $\geq$3 mm. Molecular LN analysis was positive in 127 LNs of 27 patients (52%) including 32 LNs of 12 patients (23%) with histopathologic positive LNs. Molecular examination was negative in 3 of 35 histopathologic positive LNs (9%). Combining both molecular and histopathologic findings, positive LNs were located in the standard PLND field defined by obturator fossa and external iliac vessels in 71%, along the internal iliac vessels in 16%, and along the common iliac vessels in 13%. Of LN-positive patients, 63% had LN metastases outside the standard PLND field. The internal iliac field was involved in 48% and the common iliac field in 37% of node-positive patients. Notably, internal and common iliac vessels were the only positive regions in 7% and 11% of node-positive patients, respectively. A limitation is the small number of patients included.
Conclusions: These findings underline the enhanced sensitivity of qRT-PCR in comparison with standard histopathology for detection of small-volume LN metastases in PCa patients. Our results support an ePLND including the common iliac vessels, at least up to the ureteral crossing, to optimise nodal staging and to remove LNs potentially harbouring metastases.

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1. Introduction

In prostate cancer (PCa) patients treated with radical prostatectomy (RP) and pelvic lymph node dissection (PLND), histopathologic evidence of lymph node (LN) metastases is one of the strongest prognostic factors of poor oncologic outcome [1,2]. Defining the extent of PLND is therefore of crucial interest for the optimal staging and removal of LN metastases.

Anatomic definitions of PLND distinguish a limited PLND (lPLND) from an extended PLND (ePLND). LPLND is defined by a minimal variant restricted to the obturator fossa or a standard variant that also includes the external iliac vessels. EPLND comprises dissection of the obturator fossa and the external, internal and, according to some authors, the common iliac vessels [1–8]. Only an ePLND considers prostatic lymphatic draining sites [8,9], and more LN metastases are removed by an ePLND compared with an lPLND [4,6,10], potentially resulting in a prognostic benefit [1,6,11,12]. Therefore, current guidelines of the European Association of Urology (EAU) recommend an ePLND in PCa patients at the time of RP if a PLND is indicated [13]. Nevertheless, there is no consensus as to what extent an ePLND should be performed. Opinions differ whether the common iliac vessels should be included, and published evidence on their nodal involvement is limited [3–9,12,13].

All clinical LN mapping studies in PCa were based on standard histopathologic LN examination [3–5]. However, the presence of small-volume LN metastases is underestimated by this method [14–16]. Molecular techniques such as polymerase chain reaction (PCR)-based methods have shown higher sensitivities for nodal evaluation in solid tumours including PCa [16–21], and positive molecular results in histopathologic-negative LNs have been prospectively identified as an independent prognostic marker of biochemical recurrence following treatment for localised PCa [14,15].

As a consequence of the demand for a detailed topography of LN metastases, we conducted a LN metastases mapping study combining molecular and histopathologic LN examination in intermediate- and high-risk PCa patients undergoing RP and ePLND including the common iliac vessels.

2. Patients and methods

The local ethics committee approved the present study, and all patients signed consent approved by a review board before participation. Between March 2010 and June 2011, LN specimens were obtained from 52 consecutive patients without neoadjuvant treatment at our institution who underwent open RP and ePLND for intermediate-risk PCa (Gleason score 7 or prostate-specific antigen [PSA] 10–20 ng/ml or clinical tumour extension cT2b–c) or high-risk PCa (Gleason score 8–10 or PSA >20 ng/ml or clinical tumour extension ≥cT3a).

RP and ePLND were performed by four surgeons (MA, JEG, HK, and TM) according to a predefined template including bilateral dissection of the obturator fossa and the external iliac, internal iliac, and common iliac vessels (Fig. 1). Distally, ePLND was limited by the femoral canal and proximally by the aortic bifurcation.

LNs ≥3 mm were bisected. One half and the lateral edge of the second half were formalin fixed and stained with haematoxylin and eosin for histopathologic examination (pN0 or pN1). The remainder was snap frozen within 30 min after removal and stored at −80 °C for later RNA extraction. LNs >2 cm were bisected, and the resulting pieces were examined like singular LNs <2 cm. LNs <3 mm were only assessed by histopathology because tissue was insufficient for examination by both techniques. The 2010 tumour classification of the Union Internationale Contre le Cancer was applied. Standard histopathology comprised one tissue section per formalin fixed LN piece with a size of up to 5 mm and x tissue sections per LN piece with a size of $x \times 5$ mm.

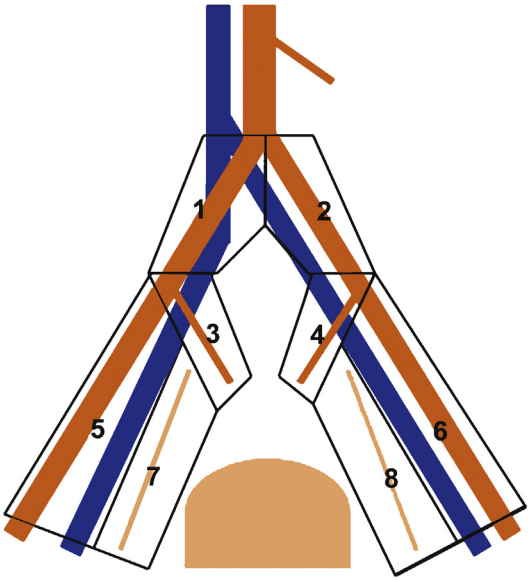

Fig. 1 – Extended pelvic lymph node dissection (PLND) included bilateral dissection of the right and left common iliac vessels (areas 1 and 2), the right and left internal iliac vessels (areas 3 and 4), the right and left external iliac vessels (areas 5 and 6), and the right and left obturator fossa (areas 7 and 8). Areas 5–8 were defined as the standard PLND field.

2.1. Quantitative reverse transcriptase-PCR assay

For RNA extraction, LNs were homogenised using the FastPrep instrument (MP Biomedicals) and mirVana Kit (Ambion). Complementary DNA (cDNA) was generated by using the high-capacity cDNA reverse transcription kit (Invitrogen) according to the manufacturer's description. TaqMan probe and primer sets were used for the detection of PSA and endogenous reference gene expression of hypoxanthine phosphoribosyl-transferase (HPRT) 1 and ubiquitin C (UBC) (Applied Biosystems; Table 1). Endogenous references were selected based on a comparative analysis of six genes as described by Vandesompele et al. [22] using the bioinformatic geNorm analysis software.

All quantitative reverse transcriptase-PCR (qRT-PCR) reactions were performed in a total volume of 10 μl with cDNA equivalent of 50 ng RNA from each LN, TaqMan Gene Expression Master Mix (Life Technologies) as well as TaqMan primer and probe sets according to the manufacturer's description. Each experiment included cDNA from LNCaP cells as a positive control and a nontemplate control.

PSA expression levels were analysed in duplicate, and mean signal over background (Cq) levels were used for further analyses. Results were normalised against the geometric mean of HPRT1 and UBC in relation to a calibrator sample (blood of a healthy volunteer spiked with 10 LNCaP cells per 10^6 peripheral blood mononuclear cells [PBMCs]) with a given relative gene expression (RGE) value of 1.0. Results were depicted using the ΔΔ-Cq method. Sensitivity was determined by the detection of serial dilutions with defined copy numbers ($0–10^3$) of a cloned PSA fragment as well as LNCaP cells ($0–10^3$) in 10^6 PBMC using triplicates.