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ORIGINAL ARTICLE

Radioisotopic detection of sentinel lymph nodes in clinically localized high-risk prostate cancer

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Summary

Purpose: *To explore the efficacy of a radioisotopic (RI) method in detecting sentinel lymph nodes (SLNs), known as sites of harboring metastases, in localized high-risk prostate cancer (HRPC).*

Methods: *The RI method was applied to 26 males with clinically localized HRPC, subjected to radical prostatectomy in 2006-2008. All had poor pathological characteristics: initial PSA > 15 ng/ml, Gleason score > 7, clinically suspected extracapsular extension, seminal vesicle invasion, and/or positive pelvic lymph nodes (LNs). The radiopharmaceutical (Tc-99m) was injected preoperatively at 4 zones of the periphery of the prostate. Tc-99m-nanocolloid particles were ≤80 nm in size, with total activity of 3 mCi (111 MBq), diluted in 2 mL. One hour after Tc-99m administration, a planar scintigraphy was performed on a gamma camera in anterior, posterior and lateral projections. A high resolution collimator was used, gathering impulses up to 300 000 per frame. The precise location of the SLNs was determined intraoperatively by a gamma probe. The LNs removed by extended pelvic lymphadenectomy were arranged on an anatomical template, examined ex vivo by the gamma probe and scanned again. The LNs were cleaned from the adjacent fatty tissue, fixed in neutral formalin, and then processed separately for histological and immunohistochemical examination.*

Results: *The number of surgically removed LNs ranged from 9 to 38 (mean 13), and the SLNs from 1 to 7 (mean 3). The SLNs were visualized on lymphoscintigraphy as strictly defined, round zones of high activity and were easily recognized intraoperatively by the gamma probe. The scintigraphic images of the scanned anatomical templates correlated well with those prior to surgery. Histology confirmed LN metastases in 11 cases. 94% of the metastatic LNs were SLNs, accurately detected by the RI method. Only 2 metastatic LNs showed no activity prior to, and during the operation. Most of the metastatic LNs (62%) were SLNs, located out of the obturator fossa.*

Conclusion: *The radioisotopic detection of the SLNs in HRPC is an objective and sensitive method that aids the surgeon to take a proper decision regarding the scope of the pelvic LN dissection in each particular case.*

Key words: gamma probe, lymph node dissection, lymphoscintigraphy, prostate cancer, sentinel lymph node

Introduction

Prostate cancer initially disseminates to the pelvic LNs. Early identification of LN metastases (LNM) has an enormous impact on proper staging, disease prognosis, and on the choice of optimal treatment strategy.

The conventional imaging methods (computed tomography/CT, magnetic resonance imaging/MRI, even positron emission tomography/PET) lack the required level of sensitivity and specificity needed to accurately detect LNM. Therefore, the most reliable method still remains the pelvic lymph node dissection (PLND), followed by histological and/or immunohistochemical examination of the removed LNs.

The appropriate dissection template, however, has not yet been determined. Current evidence suggests that LNM are often found outside of the standard area of dissection. Therefore, many authors currently advo-

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cate extended pelvic lymph node dissection (ePLND), though at the price of increased morbidity and operative time. In view of the increasing popularity of the contemporary minimally invasive surgical methods, it becomes evident that a method combining the accuracy of LNM detection with minimal morbidity during surgery is sorely needed.

Recently, the SLN concept has been applied in various malignancies with the intent to limit the boundaries and minimize the morbidity of PLND. The term "sentinel" was first mentioned by Gould in 1960 [1], and widely popularized by Cabanas at the end of the 1970s [2]. The SLN is the first LN staying on the pathway of the lymphatic drainage from the tumor, serving like a filter for the metastatic tumor cells. Hence, the sentinel concept postulates that a negative sentinel node automatically excludes further lymphatic metastatic spread. The lack of reproducibility in the location of the Cabanas SLN in penile carcinoma led to a temporary failure of the SLN dissection until 1993 when Alex and Kreg first used a RI method and a gamma probe for intraoperative SLN identification in malignant melanoma [3]. After the pioneering work of Wawroschek et al. in 1999 [4], the method was successfully applied in prostate cancer.

Our team was the first in Bulgaria to apply routinely in our practice the RI method of SLN detection in prostate cancer [5]. In our current study we explored the diagnostic possibilities of the RI method, its advantages and drawbacks, and report our initial experience with the first 26 cases.

Methods

The RI method was applied to 26 males, aged between 46 and 77 years (mean 66.3), subjected to radical prostatectomy between January 2006 and June 2008. All patients had histologically confirmed by tru-cut biopsy clinically localized HRPC. The term "clinically localized" PC comprised all potentially curable by radical prostatectomy PC cases. These were, to our view, all non metastatic PC cases (except the regional LNs), i.e. all stages < T4b, according to the TNM classification (2002 edition). The term "high risk" PC included cases with adverse pathological characteristics: initial PSA > 15 ng/ml, biopsy Gleason sum > 7, and clinically suspected extracapsular extension, seminal vesicle invasion, and/or positive pelvic LNs. Fourteen of our patients had already been registered in the Oncologic Dispensary and had received some form of neoadjuvant hormonal therapy prior to surgery.

Patient characteristics are summarized in Table 1.

All patients were informed in detail about the study objectives and the study protocol, and about all potential side effects and complications that might be associated with it. All patients gave their written consent prior to surgery.

Tc-99m-nanocolloid (Nanocoll®; GE Healthcare, Italy) was used as RI tracer to detect SLNs. Its particles were ≤ 80 nm in size, and the total activity of the tracer was 3 mCi (111 MBq), equally diluted in 2 mL. The radiopharmaceutical agent was injected preoperatively, 3-18 hours prior to surgery, at 4 zones of the periphery of the prostate (2 sites per lobe), in order to obtain homogeneous distribution. Two injection techniques were used: 1) under transrectal ultrasound guidance, using a Chiba needle; and 2) under digital rectal control, using the fine Franzen needle. Care was taken to avoid spillage of the RI tracer into the rectum. To reduce possible RI contamination, patients were asked to empty their bladders frequently after the procedure.

Preoperative lymphoscintigraphy

One hour after the radioisotope administration, a planar scintigraphy was performed on a gamma camera using one rotation head DIACAM, Siemens, in 3 projections: anterior, posterior and lateral. A high resolution collimator was used, gathering impulses up to 300 000 per frame. To detect more accurately the SLNs, we placed markers on the left and right spina iliaca anterior and superior for the anterior and the posterior projections, and on the symphysis and the sacrum for the lateral projection.

Intraoperative gamma probe SLN detection

Operation was performed under general anesthesia via a lower abdominal midline incision between the pubic symphysis and the umbilicus. Using an extraperitoneal approach, all major groups of pelvic LNs were consecutively and systematically explored. These groups involved the LNs located bilaterally along the external iliac vessels, in the obturator fossa, along the internal iliac vessels (including the pararectal nodes), along the common iliac vessels up to the aorta bifurcation, and the LNs in the presacral region. The hot nodes in each group were identified by a standard gamma probe (Gamma Finder, SI, Germany), wrapped in a sterile plastic sheath. The gamma probe was kept close to the lymphatic tissue along the main vessels at different angles, to obtain an optimal exposure. Each time at least 3 separate measurements were performed to ensure reproducibility, and the average radioactive count of each node was then recorded. The visual sig-

Table 1. Patient characteristics and tumor parameters

No.	*Age (years)*	*Initial PSA (ng/ml)*	*Stage*	*Surgical margins*	*Extracapsular invasion*	*Seminal vesicle invasion*	*Gleason score*	*SLN/TLN**	*MLN/SLN***	*LNM Site****
1	63	52	pT2c	–	No	No	8	3/13	1m/3	III (1)
2	67	11	pT3b	–	Yes	Yes	9	5/14	1/5	III (1)
3	64	16	pT2b	–	No	No	7	3/10	0/3	–
4	70	27	pT2b	–	No	No	7	2/9	0/2	–
5	68	45	pT3b	–	Yes	Yes	9	7/11	6/7	I (1); II (2); III (2); IV (1)
6	58	7	pT3a	–	Yes	No	8	3/10	2/3	II (1); IV (1)
7	67	2	pT3b	–	Yes	Yes	8	1/14	0/1	–
8	69	4	pT3b	–	Yes	Yes	8	2/18	0/2	–
9	77	8	pT3b	Apex	Yes	Yes	4	2/14	0/2	–
10	68	47	pT3b	–	Yes	Yes	5	5/13	4/5	II (2); III (2)
11	73	18	pT3b	–	Yes	Yes	9	3/10	0/3	–
12	65	7	pT3b	Bladder	Yes	Yes	7	3/12	0/3	–
13	61	30	pT3b	–	Yes	Yes	7	2/10	0/2	–
14	65	60	pT3b	–	Yes	Yes	8	3/9	0/3	–
15	61	17	pT3b	–	Yes	Yes	6	3/14	0/3	–
16	46	79	pT3b	Bladder	Yes	Yes	9	4/10	3/4	II (1); III (2)
17	75	50	pT3b	–	Yes	Yes	5	5/38	0/5	–
18	75	9	pT3b	–	Yes	Yes	7	1/10	0/1	–
19	76	8	pT3b	Lateral	Yes	Yes	7	3/10	2/3	I (1); III (1)
20	59	118	pT3b	Lateral	Yes	Yes	9	5/13	4/5	II (3); III (1)
21	62	36	pT3a	–	Yes	No	8	3/11	0/3	–
22	59	9	pT4	Bladder	Yes	No	8	3/12	2/3	I (1); II (1)
23	69	8	pT3a	–	Yes	No	9	7/23	7/7	I (2); II (2); III (3)
24	64	24	pT3b	–	Yes	Yes	8	3/20	0/3	–
25	74	32	pT2b	–	No	No	8	2/11	2m/2	II (1); III (1)
26	70	26	pT3b	Bladder	Yes	Yes	9	1/10	0/1	–

*Sentinel lymph nodes (SLN)/ Total number of harvested lymph nodes, ** Metastatic lymph nodes / SLN: sentinel lymph nodes (micrometastases are marked with "m"), *** I: External iliac; II: Obturator; III: Internal iliac; IV: Common iliac. Numbers in parentheses show the total number of metastatic lymph nodes found per site

nal was supplemented by an acoustic signal at different levels, which facilitated the detection of the hot nodes. To reduce superfluous radioactivity and interference of the signal from the injection site, the prostate was protected by a lead shield, wrapped in a sterile plastic bag. The radioactivity level measured above the aorta bifurcation was used as a background count level. LNs showing radioactivity twice or more than the radioactivity of the background level, were defined as "hot" nodes. Hot nodes that expressed an extremely high radioactive signal were resected first, to not interfere with the rest with lower count rates.

Postoperative gamma probe SLN detection and lymphoscintigraphy

The intraoperative gamma probe SLN detection was followed by extended PLND, removing all the lymphatic tissue ("hot" LNs and "cold" LNs) within the above mentioned anatomical boundaries. The surgically removed LNs were cleaned from the adjacent adipose and connective tissue, and were arranged on an anatomical template, which was examined by the gamma probe on the back table *ex vivo* again. To identify any preoperatively missed SLN, the same template was also scanned on the gamma camera and the scintigraphic images were compared with those prior to operation.

All LNs removed were finally fixed in neutral formalin, and then processed separately (by main groups and side of location) for routine histological (hematoxylin & eosin /H&E) and immunohistochemical (prostate specific antigen /PSA & cytokeratin /CK) examination.

Following ePLND, we performed a standard retropubical radical prostatectomy, according to the previously described surgical technique [6]. Whenever technically feasible and oncologically justified, unilateral or bilateral preservation of the neurovascular bundles was implemented.

Results

Preoperative patient characteristics are shown in Table 1. Mean patient age was 66.3 years (range 46-

77); mean PSA level at presentation (prior to hormonal therapy) was 28.8 ng/ml (range 2.0-118.0). Pathological stage was pT2 in 4 cases; pT3 in 21 cases; and pT4 in 1 case. Seminal vesicle involvement was found in 18/26 (69.2%) cases. The pathological Gleason score was < 7 in 4 cases, 7 in 6 cases, and >7 in 16 cases. Positive surgical margins were found in 7/26 (26.9%) cases. They were in the apical region of the prostate in 1 case, along the lateral side in 2 cases and at the prostatovesical segment in 4 cases (Table 1).

The surgically removed LNs varied in number from 9 to 38 (mean 13 per case), and the identified SLNs from 1 to 7 (mean 3 per case). A total of 349 LNs, among which 84 SLNs, were harvested and examined.

All patients tolerated well the intraprostatic injection of the Tc-99m-nanocolloid. There were no subjective symptoms, side effects or complications that might be associated with the administration of the RI tracer.

SLNs were detected in all studied patients. The SLNs were visualized on preoperative lymphoscintigraphy as strictly defined, round zones of high activity. In some cases the lymphatic pathways were visualized as well.

The hot nodes were easily recognized intraoperatively by the gamma probe, which detected the radioactive signal they generated. The radioactivity level varied between the individual patients and strongly depended on the time period between the intraprostatic injection of RI tracer and the SLN detection during surgery. The highest LN radioactivity was recorded soon after the Tc-99m-nanocolloid administration and decreased with time. Although it was not fixed as a rule and varied widely on an individual basis, the highest level of radioactivity was measured among the internal iliac (including the pararectal) LNs. The LN radioactivity measured *in vivo* was less than that measured *ex vivo*.

In most cases the scintigraphic images of the postoperatively scanned anatomical templates correlated well with those prior to surgery, although the visualized hot nodes could not be accurately addressed as belonging to a certain group of LNs (Figure 1 a, b). With the postoperative lymphoscintigraphy, used as a validation count mark for all (100%) SLNs detected, the preoperative lymphoscintigraphy detected 73/84 SLNs (87%); the intraoperative *in vivo* gamma probe - 77/84 SLNs (91.7%); and the postoperative *ex vivo* gamma probe - 82/84 SLNs (97.6%).

Hot LNs were located in all main regions of the male pelvis (Figure 2 a, b), including the presacral region below the aorta bifurcation.

The results of the histological study confirmed

A

Right

Left

B

Right

Left

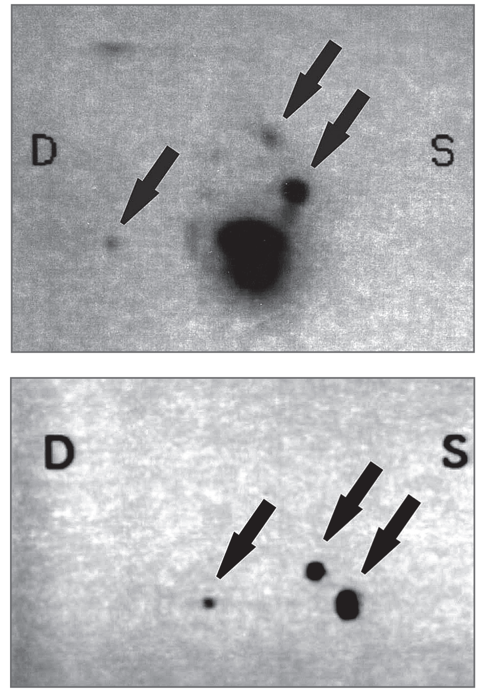

Figure 1. Comparison between A: preoperative and **B:** postoperative lymphoscintigraphy.

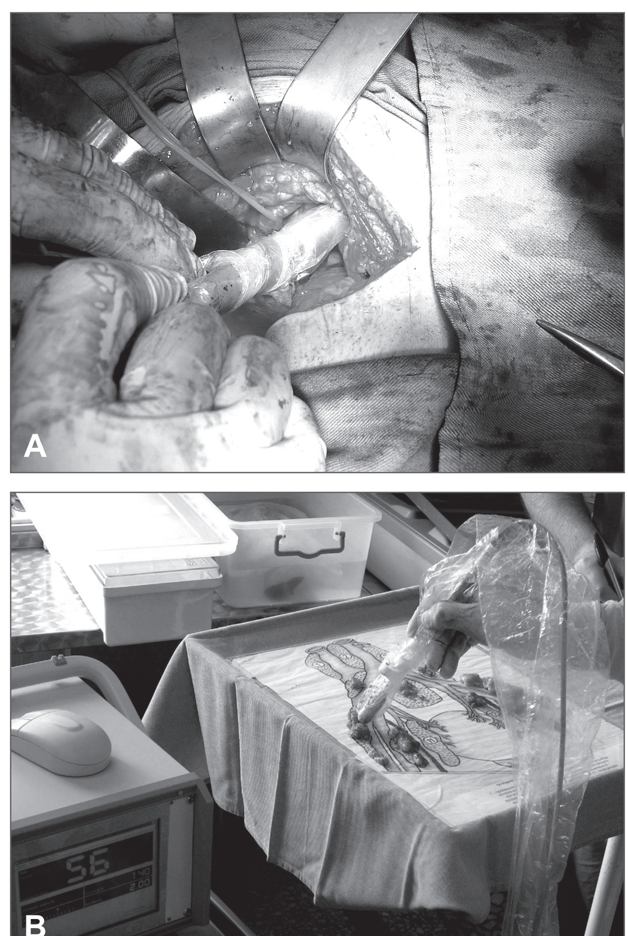

Figure 2. Identification of sentinel (“hot”) lymph nodes: **A:** *in vivo*; **B:** *ex vivo*.

LN metastases in 11 cases (42.3%). A total of 34 metastatic LNs were found, of which 32/34 (94.1%) were SLNs, accurately detected by the RI method. Only 2 metastatic LNs showed no activity prior to, and during the operation. In the first case this result looked quite surprising, because the affected LN was macroscopically gross and firm, apparently abnormal and involved by the tumor (Figure 3 a, b). Interestingly, in both of these cases neoadjuvant hormonal therapy had been administered prior to surgery.

Most of the metastatic LNs (62%) were SLNs, located exclusively out of the obturator fossa: external iliac 5/34 (14.7%); internal iliac 14/34 (41.2%) and common iliac 2/34 (5.9%). The obturator LNs were 13/34 (32.4%; Table 2). Although a few hot LNs within the presacral LN group were clearly identified, both by the preoperative lymphoscintigraphy and by the intraoperative application of the gamma probe device, the consecutive pathomorphological examination did not reveal any metastases in this region. The highest percentage (41.2%) of LNM was found among the LNs along the internal iliac vessels (Figure 4), which corresponded with the observation that the highest radioactivity counts had been found in this region as well. Most LNM were macrometastases, involving large areas (up to 90%) of the LN tissue, but there were 3 cases with micrometastases as well. LNM were multiple in 9 cases and solitary in 2.

Table 2. Distribution of metastases in pelvic lymph nodes

Main groups of pelvic lymph nodes	*Distribution of lymph node metastases n (%)*
Obturator (right / left)	13/34 (32.4)
External iliac (right / left)	5/34 (14.7)
Internal iliac (right / left)	14/34 (41.2)
Common iliac (right / left)	2/34 (5.9)
Presacral lymph nodes	0/34 (0)

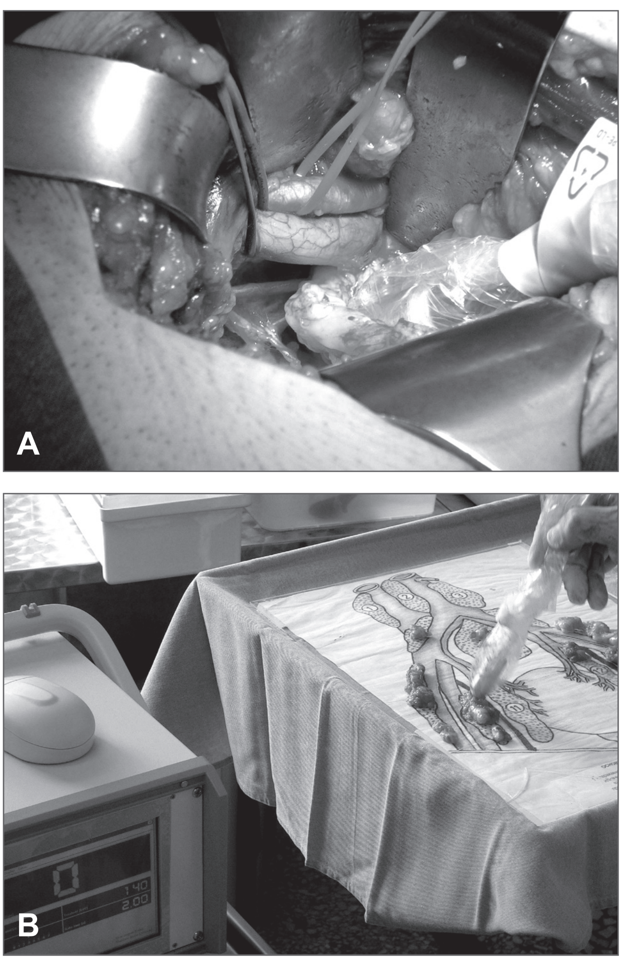

Figure 3. False-negative result - metastatic lymph node in the obturator region, showing complete lack of radioactivity: **A:** *in vivo* detection; **B:** *ex vivo* detection.

Discussion

Studies on the pelvic lymphatic system have pointed out that the internal iliac LNs stay on the main, primary route of lymphatic drainage from the prostate. Therefore, these nodes are considered to be the first echelon, while the common iliac LNs and the lumbar LNs are considered as the second, and the third echelon of nodes, draining the prostate, respectively [7]. Hence, it becomes evident that the minimal or modified PLND, which includes only the nodes located in fossa obturatoria, is insufficient to cover all potential metastatic tumor sites. Even with the commonly accepted standard variant of PLND that additionally includes the nodes

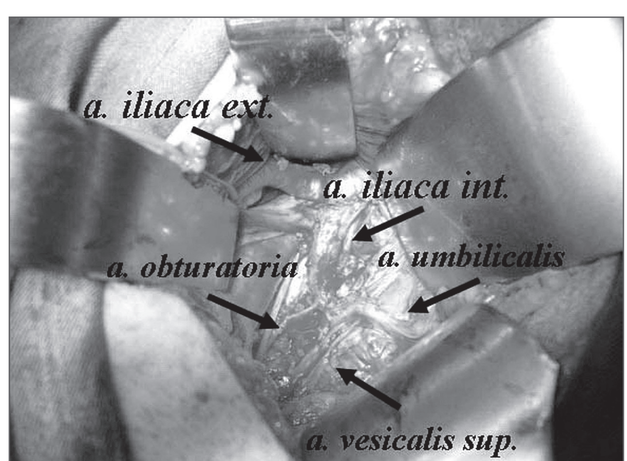

Figure 4. The area along the internal iliac vessels with the highest radioactivity counts, which correlated with a high concentration of metastatic lymph nodes.

around the external iliac vessels, about two thirds of the LNM still remain undetected [8]. There is accumulated evidence in the current literature that proves the significance of the ePLND, removing also the internal iliac and (in some cases) the common iliac LNs. According to current data, ePLND significantly increases the yield of both total LNs and LNM. LNM are detected in about 5-6%, 20-25%, and 30-40% of low-, intermediate- and high-risk PC, respectively [9].

The percentage of LNM in our study was much higher than predicted by the Partin tables and the preoperative prognostic nomograms. It might be explained with the high-risk subset of patients treated in our series, but also by the fact that most of these prognostic algorithms and nomograms had been based on historical data, accumulated from the experience with limited PLND. Therefore, they often underestimate the risk of LN involvement, which is actually higher than predicted [10]. The first nomogram, based on the results of ePLND was recently developed [11]. This nomogram could be useful if it is prospectively and internationally validated by other institutions.

Although metastases are found predominantly in the SLNs (94% in our study), MLN are found among the non SLNs ("cold" LN) as well [12]. We had 2 such false-negative cases. This is because the metastatic process occasionally blocks the lymphatic drainage from the prostate. The same phenomenon may also occur when neoadjuvant hormonal therapy had been applied prior to surgery, or when a preceding transurethral resection of the prostate had been performed. These are some of the apparent limitations that lead to a decrease in the sensitivity of the RI method. Other limitations are the cost and the duration of the procedure, which significantly prolongs the time spent for an ePLND. With regard to these limitations, some authors are skeptic about the widespread use of the RI method, especially when HRPC is considered. Others, on the contrary, believe that the best way to remove all positive LNs in HRPC is the combination of extended and sentinel PLND [12].

The preoperative lymphoscintigraphy showed the minimal number of SLNs that had to be detected during surgery. Besides, it allowed us to locate obscured and surgically difficult accessible LNs intraoperatively. It also showed us the side of the potential metastatic tumor spread. The postoperative scanning of the template on the gamma camera, compared with the preoperative scintigraphic images, helped us verify how sensitive the method was. Our results showed that 87% of the SLNs detected by the gamma probe on the back table could be visualized preoperatively. The basic limitation of the method, if used solely for preoperative diagnosis, is the uncertainty with which the visualized hot nodes could be denoted to belong to a certain group of pelvic LNs. This limitation could be overcome if single-photon emission computed tomography (SPECT) fused with computed tomography (SPECT/CT) or magnetic resonance imaging (SPECT/MRI) data is used. However, these methods are expensive and sophisticated. They require longer acquisition time and the expertise of trained nuclear medicine specialists, dedicated to search thoroughly all LNs [8].

To our view, the RI method proved its benefits in detecting the SLNs, considered as the potential metastases' landing sites. It helped us focus exclusively on the LNs along the internal iliac artery and its branches, where the majority of SLNs and MLNs were found.

The limited anatomical space and the abundance of vessels in this region often make PLND hazardous and difficult. However, our study proves strongly the necessity of enlarging the boundaries of PLND in HRPC. Our results showed that 62% of the metastatic LNs were found exclusively outside the obturator fossa. Almost half (47%) of the metastatic LNs were found outside the standard area of dissection. This is a strong argument in favor of ePLND, which should embrace the internal iliac LNs, and probably the neighbouring pararectal, presacral and paravesical SLNs, as well, the precise separation of which is difficult to be done *in vivo*. Although there were SLNs detected among the presacral LNs, located in the area under the promontorium, between the two common and the two internal iliac arteries, we did not find any metastatic LN in this region. We assume that some presacral LNs, especially those that stayed close to the internal iliac artery and the common iliac artery, had probably been placed in the same bottles and were sent to the pathologists together with the internal iliac or common iliac LNs, thus increasing both the yield of LNs and LNM in these two groups. According to Mattei et al. [8], dissection of the presacral LNs is hazardous, as it may compromise the functional results of radical prostatectomy in terms of preservation of patient's continence and erectile function. Therefore, their "super extended" template of PLND is limited to the external (inner half), obturator, internal and the distal common iliac LNs up to the crossing point of the ureter [8]. Whether this is the optimal surgical template of PLND or not, is an issue that still remains to be solved in the future.

Conclusion

The radioisotopic detection of the SLNs in prostate cancer is an objective and sensitive method that aids the surgeon to take a proper decision regarding the scope of the PLND in each particular case. When combined with ePLND, it shows certain advantages, especially in case of the most challenging HRPC.

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