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(Citation)
Prostate,80(4):345-351

(Issue Date)
2020-03-01

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90006740

Diagnostic and therapeutic value of pelvic lymph node dissection in the fossa of Marcille in patients with clinically localized high-risk prostate cancer: histopathological and molecular analyses

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Running head: Value of dissection of the fossa of Marcille

Abstract

Background: The optimal extent of lymph node dissection in radical prostatectomy has not been determined. Lymph nodes in the fossa of Marcille, which is an important pelvic lymphatic pathway and candidate for additional dissection, have not been evaluated at the molecular level. Here, we assessed by molecular analysis the presence of occult positive lymph nodes in the fossa of Marcille in patients with clinically localized high-risk prostate cancer.

Methods: Fifty-two patients with clinically localized high-risk prostate cancer underwent pelvic lymph node dissection accompanied by robot-assisted radical prostatectomy. All nodal packets were dissected separately and grouped into right and left obturator, external and internal iliac regions (including common iliac region to ureter crossing), and fossa of Marcille. All lymph nodes were bisected and evaluated by histopathological or molecular analysis using quantitative reverse transcription polymerase chain reaction. The number of positive lymph nodes in the fossa of Marcille and the difference in detection rate were investigated using histopathological and molecular analyses. Perioperative complication rate and predictive factors for biochemical recurrence were evaluated.

Results: In molecular analysis, there were seven positive lymph nodes in the fossa of Marcille in three patients, which were coexistent with positive nodes in other regions.

The detection rate of positive lymph nodes was significantly higher using molecular than histopathological analysis ($p<0.01$). Perioperative complication rate within 90 days after the operation was 25.0% and no Clavien–Dindo grade ≥3 complication was confirmed. Detection of metastasis by histopathological and molecular analysis was a significant factor related to biochemical recurrence in the Cox proportional hazards regression model.

Conclusions: No case of positive lymph nodes in the fossa of Marcille that had skipped over other regions was confirmed. Additional lymph node dissection of fossa of Marcille did not lead to complete resection of molecularly positive lymph nodes.

Key words: reverse transcription polymerase chain reaction; prostatectomy; lymph node dissection

INTRODUCTION

Recently, concurrent extended lymph node dissection has become essential for accurate diagnosis of high-risk clinically localized prostate cancer treated by radical prostatectomy. The optimal extent of lymph node dissection has not been determined, although most templates include at least the external iliac, obturator, and internal iliac areas.[1]

The fossa of Marcille is situated lateral to the common iliac vessels, medial to the psoas major, overlying the proximal obturator nerve, and anterior to the sciatic nerve, and is an important pelvic lymphatic pathway.[2,3] Some authors have evaluated the diagnostic effectiveness of lymph node dissection from the fossa of Marcille.[4,5] However, those authors did not investigate small-volume metastases, which can be missed in histopathological analysis because of sampling error or being overlooked in microscopic examination.[6]

Previous studies, including our own, have demonstrated detection of metastasis by molecular analysis of pathologically negative lymph nodes sampled during radical prostatectomy.[7-9] However, the fossa of Marcille has not been investigated with molecular analysis in patients with prostate cancer. The aim of the present study was to assess by molecular analysis the presence of occult positive lymph nodes in the fossa of Marcille.

MATERIALS AND METHODS

This study was approved by the Institutional Ethics Committee of Kobe University (KUH-170143). We prospectively enrolled 52 patients with clinically localized high-risk prostate cancer [Gleason score at biopsy ≥8 and/or prostate-specific antigen (PSA) ≥20 ng/ml and/or clinical T stage ≥3a]. The patients were scheduled to undergo robot-assisted radical prostatectomy and extended lymph node dissection between January 2017 and March 2019. No eligible patients were treated with neoadjuvant and adjuvant therapy. We excluded patients with double malignancies, or severe cardiopulmonary, hepatic or renal disorder.

Dissected lymph nodes with diameter ≥3 mm were bisected. We sterilized all instruments with RNase Away (Thermo Fisher Scientific, Waltham, MA, USA) and rinsed them with nuclease-free water between the sectioning of each lymph node in order to prevent cross-contamination. One half was frozen immediately and stored at −80°C until assessment with reverse transcription polymerase chain reaction (RT-PCR). The other half was histopathologically examined by one pathologist after it was fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. Lymph nodes <3 mm in diameter were only assessed histopathologically as there was insufficient tissue for both analyses. Primary tumor and lymph nodes were staged according to the 2010 edition of the American Joint Committee on Cancer/Union for

International Cancer Control TNM staging system.[10] Biochemical recurrence was defined as serum PSA level ≥0.2 ng/ml. At the time of biochemical recurrence, the metastatic site was surveyed with fluorodeoxyglucose positron emission tomography (FDG PET)/magnetic resonance imaging (MRI). All treatment physicians were blinded to the results of molecular analysis.

Surgical technique of pelvic lymph node dissection

Robot-assisted radical prostatectomy and pelvic lymph node dissection were performed as described previously.[11] The template for lymph node dissection included the genitofemoral nerve anterolaterally; the branches of the umbilical artery and the bladder wall medially; the ureter crossing of the common iliac cranially; and the deep circumflex vein caudally.

Fibrofatty tissue, including lymph nodes, was sampled according to the standardized anatomical template as follows: bilateral obturator fossa, internal and external iliac region, and fossa of Marcille. For complete dissection, the fossa of Marcille was cleared by medial and lateral traction of fully exposed external iliac vessels (Figure 1). Fatty tissue around the common iliac vessels caudal to the ureter crossing was sampled, along with the internal iliac lymph nodes.

Pelvic lymph node dissection in all eligible patients was performed by two senior

surgeons (NH and MF).

Quantitative RT-PCR

Total RNA was extracted from each lymph node specimen using the acid guanidinium isothiocyanate, phenol chloroform method, and 1 μg total RNA was reverse transcribed to make cDNA using SuperScript® VILO™ Master Mix ezDNase Enzyme (Thermo Fisher Scientific). To analyze expression levels of kallikrein-related peptidase (KLK)3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs, quantitative RT-PCR was performed using the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). Each cDNA was analyzed by quantitative RT-PCR in a 50-μL volume using Taqman™ Fast Advanced Master Mix, and TaqMan Gene Expression Assay (Thermo Fisher Scientific). The primers and TaqMan probe sets for KLK3 (Hs02576345_m1) and GAPDH (Hs99999905_m1) were used for RT-PCR (sequences not disclosed) (Thermo Fisher Scientific). The cycling conditions were 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. All specimens were analyzed in duplicate and the mean values were used for quantification.

To exclude false-positive results, we used 35 lymph nodes from four male patients with bladder cancer as controls: they underwent cystoprostatectomy and prostate cancer

was excluded histopathologically. However, cDNA from all control lymph nodes was not amplified by quantitative RT-PCR using KLK3. Instead, we set the score equivalent to one copy as the lower limit of positive using the calibration curve made with GeneArt Strings DNA fragment including the sequence of KLK3. We defined specimens positive for KLK3 mRNA in real-time RT-PCR as indicating the presence of molecular metastasis.

Endpoints

The primary endpoint was the number of positive lymph nodes in the fossa of Marcille using histopathological and molecular analyses. Secondary endpoints were: perioperative complication rate; the difference in positive lymph node detection rate of whole template between histopathological and molecular analyses; and the relation between biochemical recurrence and presence of positive lymph nodes. The assessment of biochemical recurrence was limited to cases that were observed for at least 6 months after surgery. The perioperative complications within 90 days after the operation were also assessed using the Clavien–Dindo classification.[12] Exploratory endpoints were the prognostic factors for biochemical recurrence during the observation period. The prognostic factors examined were: preoperative PSA level; Gleason score of patients undergoing robot-assisted radical prostatectomy; and the

presence of extraprostatic extension, lymphovascular invasion, positive surgical margin, and metastasis in histopathological and molecular analysis.

Statistical analysis

Differences in positive lymph node detection rate between histopathological and molecular analyses were calculated using Pearson's chi-squared test. Biochemical recurrence-free survival was evaluated by the Kaplan–Meier method, and the difference was determined by log-rank test. The significance of each factor for biochemical recurrence was assessed by the Cox proportional hazards regression model. All statistical analyses were done with JMP version 13.0 (SAS Institute, Cary, NC, USA) and $p<0.05$ was considered to indicate statistical significance.

RESULTS

Patient characteristics and perioperative results

Patient characteristics are given in Table 1. The median age of eligible patients was 69 years, median initial PSA was 8.6 ng/ml, and median body mass index was 23.4. Clinical T stage was T2 in 22 patients (42.3%) T3a in 26 (50.0%) and T3b in four (7.7%). Table 2 summarizes the perioperative histopathological outcomes. Thirteen perioperative complications (25.0%) occurred within 90 days after the operation. No severe complications (Clavien–Dindo grade ≥3) were reported. During follow-up, 13 patients (25.0%) experienced biochemical recurrence. Four of these patients had recurrence on the day of surgery because their PSA level never decreased below 0.2 ng/ml. The metastatic site was not found with FDG PET/MRI.

Molecular and histopathological analysis of lymph node status

In quantitative RT-PCR using sequence of GAPDH, cDNA from all specimens were steadily amplified (median and standard deviation of Ct: 24.9 and 0.38, respectively). Based on the analysis using artificial DNA fragments, including KLK3 sequence, we calculated a threshold Ct score of 39.4 for KLK3 expression for determination of the presence of metastasis in molecular analysis.

In total, 1293 lymph nodes were dissected from 52 patients; of which, 1262 lymph

nodes (median 23) had a diameter ≥3 mm and were subjected to histopathological and molecular analyses. Positive KLK3 mRNAs were detected in 96 lymph nodes (7.6%). This detection rate was significantly higher than that of histopathological analysis (0.9%, $p<0.01$). Positive lymph nodes were most frequently detected from obturator fossa (n=46) and the internal iliac region (n=22). There were seven positive lymph nodes from the fossa of Marcille in three patients. Ipsilateral obturator fossa and internal and external iliac lymph nodes of these patients were also positive in molecular analysis. The distribution of positive lymph nodes is shown in Table 3.

Among the 52 patients, seven (13.5%) had positive lymph nodes by histopathological and molecular analyses; nine (17.3%) had histopathologically negative but molecularly positive lymph nodes; and 36 (69.2%) had no positive lymph nodes in either analysis. Molecular analysis detected positive lymph nodes among 16 (30.8%) patients. Notably, no patient was positive by histopathological but negative by molecular analysis.

Association of lymph node status with biochemical recurrence

Biochemical recurrence-free survival was significantly longer in patients without molecular metastasis compared with those with molecular metastasis ($p<0.01$) (Figure 2). Multivariate analysis using a Cox regression hazard model revealed that the presence of histopathological and molecular metastasis was an independent factor

associated with early biochemical recurrence-free survival. (Table 4)

DISCUSSION

We performed pelvic lymph node dissection using a template including the fossa of Marcille and evaluated the presence of metastasis by histopathological and molecular analyses. Histopathological analysis detected no additional positive lymph nodes from the fossa of Marcille, and molecular analysis detected a small number of occult metastases. Furthermore, all patients with molecularly positive lymph nodes in the fossa of Marcille already had multiple lymph node metastasis in other regions.

The fossa of Marcille, landing site of pelvic lymphatic drainage, is considered to be an important site of lymph node metastasis from prostate cancer.[2] A previous mapping study with imaging using indocyanine green has suggested the presence of sentinel lymph nodes in the fossa of Marcille.[3] Because of the importance of the fossa of Marcille, several institutions have added it to their template for pelvic lymph node dissection in radical prostatectomy.[3-5] Previous histopathological analysis has revealed a small number of positive nodes in the fossa of Marcille, although all patients had co-occurrence of lymph node metastasis of other regions.[4,5] However, in histopathological analysis, small-volume metastases can be missed because of sampling error on nodal slicing, or they may simply be missed on microscopic examination.[6,13] Conversely, intraoperative imaging did not identify the true landing site because the positive lymph nodes included many histopathologically cancer-negative nodes. In the present study,

we decided to investigate distribution of positive lymph nodes more accurately by searching for occult metastasis using quantitative RT-PCR. We hypothesized that the fossa of Marcille might have an important role as the first landing site of metastatic prostate cancer if we found that metastasis was only found in the fossa of Marcille in some patients.

In this study, in patients with clinically localized high-risk prostate cancer, molecular analysis revealed seven occult lymph node metastases in the fossa of Marcille that were not detected by histopathological analysis. However, all these positive lymph nodes coexisted with those in several other regions. Our results did not confirm the hypothesis that the fossa of Marcille is one of the first landing sites of metastasis of prostate cancer.

It has been previously documented that removal of a large number of lymph nodes at radical prostatectomy is associated with a linear increase in the probability of detecting lymph node invasion.[14,15] In contrast, pelvic lymph node dissection and its extension are associated with higher complication rate, longer operation time and longer hospital stay in comparison with limited (obturator fossa only) and extended (obturator fossa, and external and internal iliac nodes) lymph node dissection.[16] In particular, the increase in lymphocele is of concern.[17] Therefore, investigation of the optimal extent of lymph node dissection is essential and the morbidity of pelvic lymph node dissection

should always be considered, especially when the newly dissected region is anticipated to add little clinical information.[18] A small amount of cancer tissue might be removed with lymph node dissection of fossa of Marcille. However, it is difficult to remove the root of the metastasis at the same time because positive lymph nodes in the fossa of Marcille indicate already-extensive lymph node metastasis. Our study suggests that additional lymph node dissection of the fossa of Marcille might not provide sufficient benefit to overcome the disadvantage of radical prostatectomy.

Our study confirmed the importance of molecular metastasis in early biochemical recurrence. Previous studies have proved that the presence of molecular metastasis is related to oncological prognosis after radical prostatectomy.[6,8] However, ours is believed to be the first study to assess the predictive power of molecular metastasis with a template including the fossa of Marcille, focusing on early biochemical recurrence. Adjuvant therapy is preferable for patients at high risk of early biochemical recurrence.[19] National Comprehensive Cancer Network (NCCN) guidelines recommend adjuvant androgen deprivation therapy as category 1 therapy for pN1 cases, while extra beam radiotherapy alone is recommended for pN0 cases.[20] Positive nodes in molecular analysis might be an important factor for recommendation of adjuvant androgen deprivation therapy, although metastasis in histopathological and molecular analysis cannot be identified.

Several limitations of this study should be considered. First, the single-arm design and small number of patients limit the conclusions that can be drawn. The small number of eligible patients was caused by exclusion of those with intermediate-risk clinically localized prostate cancer, which often includes many patients with low risk of node-positive cancer. Second, small metastasis in only one side of a bisected lymph node might lead to a false-negative result. Third, some of the recurrent cases may have included local recurrence. It might have been desirable to exclude these patients using 68Ga-PSMA/PET, but this cannot be performed in Japan. However, in this study, 12 cases (80.0%) had minimal positive surgical margin length (<3 mm), which was less likely to have contributed significantly to local recurrence.[21] Finally, the observation period after pelvic lymph node dissection was too short. Further observation and case accumulation are necessary to evaluate the importance of the fossa of Marcille in long-term prognosis.

CONCLUSIONS

Molecular analysis suggests that clinically localized high-risk prostate cancer including metastasis in the fossa of Marcille has a high probability of extensive metastasis. Our study suggests that addition of lymph node dissection in the fossa of Marcille is not sufficient to accomplish complete resection of lymph node metastasis. Molecular metastasis might be a strong predictor of early biochemical recurrence.

CONFLICT OF INTEREST

The authors declare that there were no conflict of interests.

ACKNOWLEDGMENTS

We thank Cathel Kerr, BSc, PhD, and H. Nikki March, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

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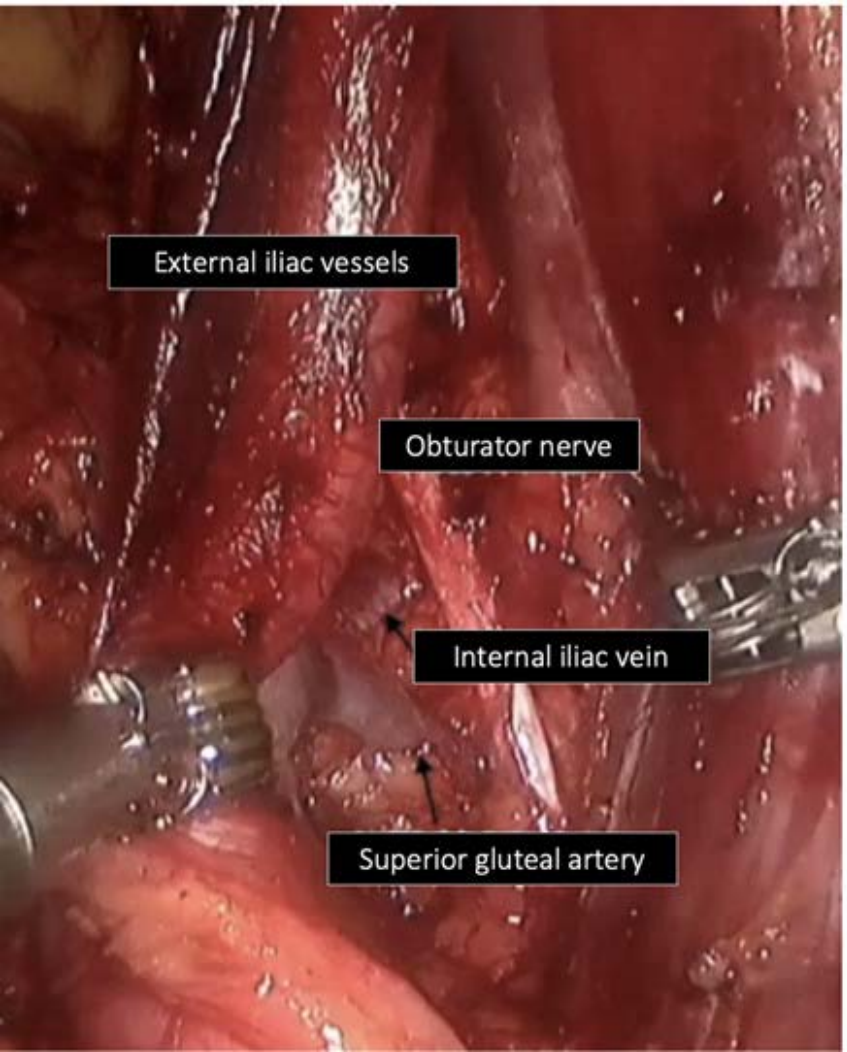
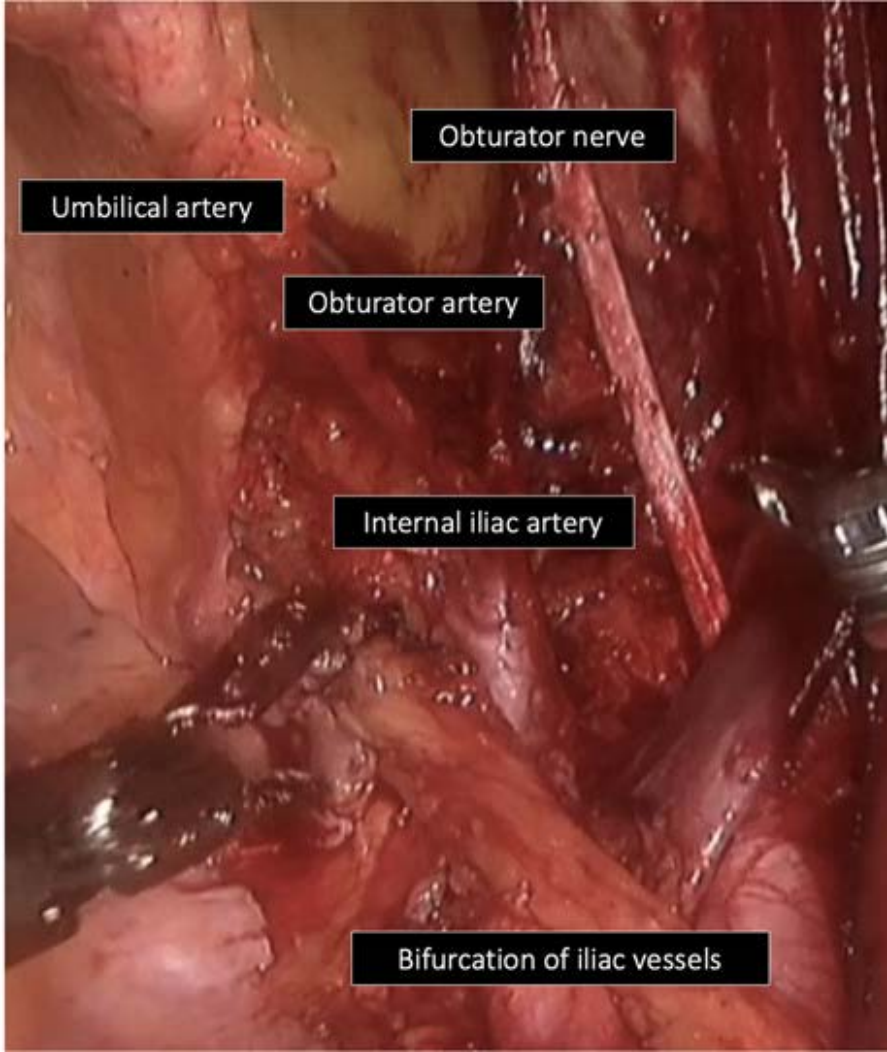
Figure legends

Figure 1. Our technique of pelvic lymph node dissection. Left: the surface of the external iliac and common iliac vessels up to the ureter crossing was cleared. The branches of the internal iliac artery, such as the umbilical cord artery or superior bladder artery, were cleanly exposed. Right: lymph nodes in the fossa of Marcille were obtained mainly by medial retraction of the external iliac vessels to reveal the space medial to the psoas major. Mobility of the external iliac vessels led to complete dissection of the fossa of Marcille.

Figure 2. Biochemical recurrence-free survival with molecular metastasis or without molecular metastasis, by molecular analysis. Biochemical recurrence-free survival was significantly longer in patients without molecular metastasis compared with those with molecular metastasis ($p<0.01$).


External iliac vessels
Obturator nerve
Internal iliac vein
Superior gluteal artery


Obturator nerve
Umbilical artery
Obturator artery
Internal iliac artery
Bifurcation of iliac vessels

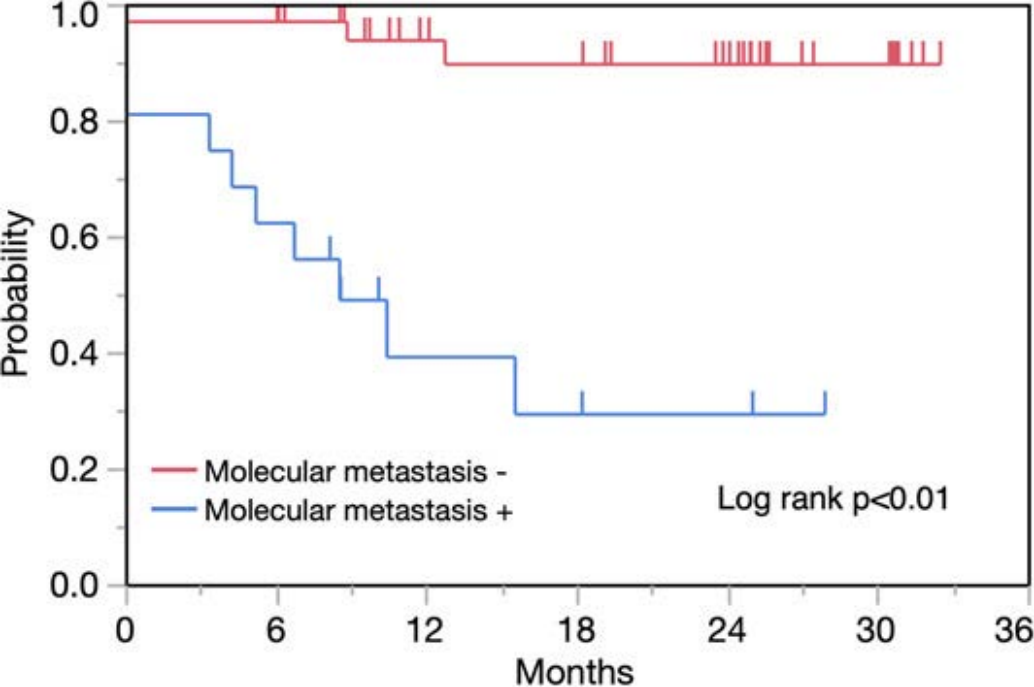

1.0
0.8
0.6
0.4
0.2
0.0
Probability
Molecular metastasis -
Molecular metastasis +
Log rank p<0.01
0
6
12
18
24
30
36
Months

Table 1. Characteristics of eligible patients

	n = 52
Age (median, IQR)	69 (64-74)
Pretreatment PSA, ng/mL (median, IQR)	8.6 (5.6-17.6)
BMI (median, IQR)	23.4 (22.4-25.4)
Clinical T stage	
cT2a	17 (32.7%)
cT2c	5 (9.6%)
cT3a	26 (50.0%)
cT3b	4 (7.7%)
Gleason sum at prostate biopsy	
6	1 (1.9%)
7	17 (32.7%)
≥8	34 (65.4%)

Abbreviations: IQR, interquartile range; PSA, prostate-specific antigen.

Table 2. Perioperative, histopathological, and oncological outcomes

	n = 52
Pathological stage	
pT2	24 (46.2%)
pT3a	17 (32.7%)
pT3b	11 (21.2%)
pN1	7 (13.5%)
Gleason sum of radical prostatectomy	
6	0 (0.0%)
7	28 (53.8%)
≥8	24 (46.2%)
Positive surgical margin	15 (28.8%)
Lymphovascular invasion	13 (25.0%)
Observation period (mo, median, IQR)	19.0 (10.6-25.2)
Biochemical recurrence	13 (25.0%)
Observation period until BCR (mo, median, IQR)	6.7 (3.3-10.4)
Perioperative complication ≤POD 90	

Abbreviation: BCR, biochemical recurrence; IQR, interquartile range.

Table 3. Distribution of dissected lymph nodes

N = 52	Obturator fossa	Internal iliac	External iliac	Marcille	Total
No. of dissected LNs assessed by histopathological and molecular analysis					
Total	493	185	361	223	1262
Median, IQR	9 (6-12)	3 (1-6)	6 (4-8)	4 (2-8)	23 (20-28)
No. of positive LNs in histopathological analysis					
	4 (0.8%)	4 (2.2%)	3 (0.8%)	0 (0%)	11 (0.9%)
No. of positive LNs in molecular analysis					
	46 (9.3%)	22 (11.8%)	21 (5.8%)	7 (3.1%)	96 (7.6%)
Difference of detection rate between two analyses					
P value				<.01	
No. of N + patients in histopathological analysis					
	3 (5.8%)	3 (5.8%)	2 (3.8%)	0 (0%)	7 (13.5%)
No. of N + patients in molecular analysis					
	9 (17.3%)	9 (17.3%)	7 (13.4%)	3 (5.7%)	16 (30.8%)
Difference of detection rate between two analyses					
P value					.03

Abbreviation: IQR, interquartile range; LNs, lymph nodes.

Table 4. Univariate and multivariate Cox regression analysis of factors influencing biochemical recurrence-free survival

	HR	95% CI	*P* value	HR	95% CI	*P* value
+Histopathological analysis						
Preoperative PSA ≥10	1.76	0.58-5.50	.30			
Gleason sum (RARP) ≥8	3.61	1.17-13.44	.02	2.61	0.71-11.36	.15
Extraprostatic extension	5.25	1.41-33.97	.01	1.62	0.17-15.46	.66
Lymphovascular invasion	4.38	1.44-13.72	.01	2.62	0.76-9.42	.12
Positive surgical margin	6.63	2.14-24.54	<.01	1.92	0.45-10.07	.39
Lymph node positive	11.23	3.69-37.65	<.01	6.02	1.63-28.67	.01
+Molecular analysis						
Preoperative PSA ≥10						
Gleason sum (RARP) ≥8				2.00	0.57-8.25	.28
Extraprostatic extension				2.15	0.22-1.74	.28
Lymphovascular invasion				3.11	0.87-11.89	.09
Positive surgical margin				2.66	0.62-13.95	.19
Lymph node positive	10.63	3.19-48.06	<.01	10.21	2.33-64.42	<.01

Abbreviations: CI, confidence interval; HR, hazard ratio; PSA, prostate-specific antigen; RARP, robot-assisted radical prostatectomy.