# Advancing surgical guidance: from (hybrid) molecule to man and beyond

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# CHAPTER 8

## OPTIMISATION OF FLUORESCENCE GUIDANCE DURING ROBOT-ASSISTED LAPAROSCOPIC SENTINEL NODE BIOPSY FOR PROSTATE CANCER

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## ABSTRACT

**BACKGROUND** The hybrid tracer was introduced to complement intraoperative radiotracing towards the sentinel nodes (SNs) with fluorescence guidance.

**OBJECTIVE** Improve in vivo fluorescence-based SN identification for prostate cancer by optimizing hybrid tracer preparation, injection technique, and fluorescence imaging hardware.

**DESIGN, SETTING, AND PARTICIPANTS** Forty patients with a Briganti nomogram-based risk >5% of lymph node metastases were included. After intraprostatic tracer injection, SN mapping was performed (lymphoscintigraphy and single photon emission computed tomography with computed tomography (SPECT/CT)).

In groups 1 and 2, SNs were pursued intraoperatively using a laparoscopic gamma probe followed by fluorescence imaging. In group 3, SNs were initially located via fluorescence imaging. Compared with group 1, in groups 2 and 3, a new tracer formulation was introduced that had a reduced total injected volume (2.0 mL vs. 3.2 mL but increased particle concentration. For groups 1 and 2, the Tricam SLII with D-Light C laparoscopic fluorescence imaging system was used. In group 3, the laparoscopic fluorescence imaging system was upgraded to an Image 1 HUB HD with D-Light P system.

**INTERVENTION** Hybrid tracer-based SN biopsy, extended pelvic lymph node dissection, and robot-assisted radical prostatectomy.

**OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS** Number and location of the preoperatively identified SNs, in vivo fluorescence-based SN identification rate, tumor status of SNs and lymph nodes, postoperative complications, and biochemical recurrence (BCR).

**RESULTS AND LIMITATIONS** Mean fluorescence-based SN identification improved from 63.7% (group 1) to 85.2% and 93.5% for groups 2 and 3, respectively (p=0.012). No differences in postoperative complications were found. BCR occurred in three pN0 patients.

**CONCLUSIONS** Stepwise optimization of the hybrid tracer formulation and the laparoscopic fluorescence imaging system led to a significant improvement in fluorescence-assisted SN identification. Preoperative SPECT/CT remained essential for guiding intraoperative SN localization.

## INTRODUCTION

Sentinel node (SN) biopsy using a radioactive tracer was introduced for prostate cancer to minimize the extent of the pelvic lymph node dissection (PLND) while retaining diagnostic accuracy [1]. The concept behind SN biopsy is to identify the lymph nodes that are most likely to contain metastatic cells in case migration from the primary prostate tumor has occurred, the so-called SNs. Visualization of this direct drainage pathway transcends the anatomic location of the SN. Therefore, this technique also enables the identification of potential tumor-bearing lymph nodes outside the extended PLND (ePLND) template [2,3] that would otherwise have been missed. When performing SN biopsy in combination with an ePLND, improved lymphatic staging can be achieved; pathologists can evaluate the SNs more extensively, decreasing the possibility of sampling errors, which can result in improved diagnostic accuracy [4,5].

Since its introduction, the procedure has been subject to various refinements. In the past 15 years, the surgical technique has shifted from a mainly open procedure to a laparoscopic and later a robot-assisted procedure. For preoperative SN mapping, following the injection of a radioactive tracer, lymphoscintigrams are taken. The introduction of single photon emission computed tomography with computed tomography (SPECT/CT) resulted in improved anatomic SN localization, allowing better planning of the operation and reducing operative time [6].

To date, intraoperative SN identification is based primarily on the use of a (laparoscopic) gamma probe (radioguided approach). The recent introduction of fluorescence imaging during surgery was shown to aid the surgeon in optical, fluorescence-based, visualization of the SNs [7,8]. Yet, the limited penetration depth of the near-infrared fluorescent dye indocyanine green (ICG; <1.0 cm) prohibits preoperative SN mapping, meaning that during surgery meticulous scanning of, and beyond, the entire ePLND template is required [2,6]. This exploration is extensive and time-consuming and may potentially miss SNs. Hence, the use of ICG is often combined with radiocolloid-based preoperative SN mapping methods [8]. To facilitate the integrated use of preoperative imaging with fluorescence guidance, we introduced the hybrid tracer ICG-$^{99m}$Tc-nanocolloid [6]. Being both radioactive and fluorescent, a single ICG-$^{99m}$Tc-nanocolloid administration allows for preoperative SN mapping as well as intraoperative fluorescence guidance to these exact hot spots. In our previous studies, the hybrid nature of this tracer was shown to complement the radio-guided approach and outperformed blue dye [9,10].

Following our initial feasibility study in prostate cancer [11], 40 additional prostate cancer patients were included. In these patients, we systematically evaluated whether optimization of the tracer formulation and fluorescence imaging hardware improvements could help increase in vivo fluorescence-based SN identification during robot-assisted laparoscopic procedures.

# METHODS

## PATIENTS

Between December 2010 and July 2013, 40 patients with localized prostate cancer and a Briganti nomogram-estimated risk >5% of lymph node metastases were included after informed consent was obtained. Patients were scheduled for robot-assisted radical prostatectomy (RARP) and SN biopsy followed by an ePLND. The first nine patients were included under registration of the feasibility study (N09IGF), and the patient population was completed through off-label use of the hybrid tracer. Three groups were formed for statistical analysis. In group 1 (n=11; December 2010-April 2011), the previously described hybrid tracer preparation [11] and the Tricam SLII with D-Light C laparoscopic fluorescence imaging system (KARL STORZ Endoskope GmbH & Co. KG, Tuttlingen, Germany) was used. In group 2 (n=13; April 2011-November 2012), the particle concentration was increased, and the injected volume decreased. In group 3 (n=16; December 2012-July 2013), the tracer formulation was identical to that used in group 2, but an upgraded laparoscopic fluorescence imaging system (Image 1 HUB HD with D-Light P system (KARL STORZ Endoskope GmbH & Co. KG)) was introduced.

## TRACER PREPARATION

Two different tracer formulations were used. In group 1 we prepared the hybrid tracer as previously described (0.4 mL in the syringe; referred to as the previously described tracer formulation) [11]. In groups 2 and 3 we used the new tracer formulation.

The new tracer formulation was prepared as follows: $^{99m}$Tc-nanocolloid was made by adding 2.0 mL pertechnetate (approximately 300 MBq) to a vial of nanocolloid (GE Healthcare, Eindhoven, The Netherlands). ICG-$^{99m}$Tc-nanocolloid was then formed by adding 0.05 mL (0.25 mg) of ICG solution (5.0 mg/mL; Pulsion Medical, Feldkirchen, Germany) to the vial. After in situ formation of ICG-$^{99m}$Tc-nanocolloid, the tracer was subtracted from the vial and diluted with saline to a total volume of 2.0 mL in the syringe. Procedures were performed in accordance with the Dutch guidelines for good manufacturing practice and with approval of the local pharmacist.

## TRACER INJECTION

The hybrid tracer was injected transrectally into the peripheral zone of each quadrant of the prostate under ultrasound guidance [11]. In group 1, four deposits of 0.1 mL ICG-$^{99m}$Tc-nanocolloid were given. After each injection, the needle was flushed with 0.7 mL saline (total injected volume: 3.2 mL). In groups 2 and 3, patients received four deposits of 0.5 mL ICG-$^{99m}$Tc-nanocolloid (total injected volume: 2.0 mL).

## PREOPERATIVE SENTINEL NODE MAPPING

Static planar lymphoscintigraphy was performed 15 min and 2 h after injection, followed by a SPECT and low-dose CT scan (Symbia T; Siemens Healthcare, Erlangen, Germany).

SPECT and low-dose CT images were fused, and a three-dimensional (3D) SPECT/CT-based volume-rendering reconstruction was created using OsiriX medical imaging software (Pixmeo, Geneva, Switzerland). Images were analyzed by an experienced nuclear medicine physician according to previously described criteria [12].

### SURGICAL PROCEDURE

Operations were performed by HGvdP using the da Vinci S Surgical system (Intuitive Surgical Inc., Sunnyvale, CA, USA). Patients first underwent SN biopsy, followed by ePLND and RARP.

In the case of a one-sided SN non-visualization following preoperative imaging, an ePLND was performed on that side. The ePLND comprised all lymph nodes in the internal, obturator, and external regions proximal of the ureter vessel crossing and distally from the pubic bone. SNs outside the ePLND template were defined as described by Meinhardt et al. [13]. Preoperatively acquired SPECT/CT images and the 3D volume-rendered image were used as a virtual roadmap for the localization of the individual SNs. Intraoperatively, in groups 1 and 2, SNs were initially pursued using an laparoscopic gamma probe (Europrobe 2; Eurorad S.A., Eckbolsheim, France) followed by confirmatory fluorescence imaging. In group 3, SNs were initially localized via fluorescence imaging followed by ex vivo confirmation via gamma tracing. Real-time fluorescence images were introduced into the da Vinci S system via the TilePro function [11].

In this study, fluorescence imaging was performed using two generations of laparoscopic fluorescence imaging systems: the Tricam SLII with D-Light C system (groups 1 and 2) and the Image 1 HUB HD with D-Light P system (group 3) (both KARL STORZ Endoskope GmbH & Co. KG).

### PATHOLOGIC EXAMINATION

Lymph nodes and SNs were formalin fixed, cut at 2 mm, and paraffin embedded. Lymph nodes sections were stained with haematoxylin and eosin. SNs were cut at three levels (150 µm intervals), and sections were haematoxylin and eosin stained. In addition, on the second level, an immunohistochemical stain was performed using a CAM5.2 antibody (catalogue number 345779; Becton Dickinson Biosciences, San Jose, CA, USA). Prostatectomy specimens were formalin fixed, paraffin embedded, and classified according to the 2009 TNM classification.

### FOLLOW-UP

Postoperative complications (within 90 days after surgery) were scored using the Clavien-Dindo score [14]. Patients were evaluated for biochemical recurrence (BCR; prostate-specific antigen >0.1 ng/mL) during follow-up.

### STATISTICAL ANALYSIS

For continuous variables, the mean or median and interquartile range (IQR; 25-75%) is

given. For discrete variables, frequencies and percentages are reported. Study endpoints were as follows: 1) intraoperative fluorescence-based SN identification rate; defined for each patient as ((number of SNs intraoperatively visualized via fluorescence imaging)/(total number of SNs seen on preoperative imaging)) x 100%; 2) postoperative complications, and 3) BCR.

A one-way analysis of variance was performed for evaluation of the number of postoperative complications in the three groups. We used the nonparametric Kruskal-Wallis test for evaluation of between-group differences in intraoperative fluorescence-based SN identification rate and the number of harvested SNs and lymph nodes. For BCR-free survival, we performed a log-rank test comparing groups 1 and 2 with group 3. A chi-square test was performed to evaluate whether there was a difference in pN1 patients among the three groups. Statistical analysis was performed using SPSS version 20 (IBM Corp., Armonk, NY, USA).

In general, viewing our 40 patients as a random sample of the entire population, our null hypothesis is that the unknown distributions of these rates in the population are the same across the three groups. A p-value <0.05 was considered significant.

# RESULTS

## PREOPERATIVE IMAGING

Patient characteristics are shown in Table 1. At least one SN was preoperatively identified in 38 of the 40 patients. Bilateral non-visualization occurred in two patients (5.0%) and unilateral non-visualization in five patients (12.5%). Lymphoscintigraphy and SPECT/CT imaging identified a total of 119 SNs (median: 3, IQR 0-2). Results per subgroup are specified in supplementary information Table SI1.

Changing the hybrid tracer formulation did not yield a significant difference in the number of preoperatively visualized SNs (Table 2). However, with the new tracer formulation flushing was no longer necessary between placement of the difference tracer deposits, thereby reducing injection time and increasing the ease of the procedure.

## INTRAOPERATIVE SENTINEL NODE IDENTIFICATION

Six of the preoperatively identified SNs could not be resected because of the risk of injury or mechanical limitations of the robot (location: pararectal region inside the mesorectal fascia (n=3), presacral region (n=2), and right iliac region (n=1) Table 3).

In seven patients, 14 additional SNs were removed during surgery based on their fluorescent and radioactive appearance in the same region as the SNs detected with preoperative imaging. In retrospect, in six of these seven patients, lumph node clusters could be visualised on CT (Figure 1). Overall, 127 SNs (median: 3 per patient; IQR: 2-4; Table 2) were identified during surgery. In 16 patients (40.0%), an SN was located outside the ePLND template (Figures 2 and 3; Table 3).

**Table 1.** Patient characteristics

| | ***Total*** | ***Group 1*** | ***Group 2*** | ***Group 3*** |
|---|---|---|---|---|
| *No. patients* | 40 | 11 | 13 | 16 |
| *Age, median (IQR)* | 64 (60-68) | 62 (59-69) | 64 (61-67) | 65 (60-70) |
| *Preoperative PSA-level (ng/mL), median (IQR)* | 8.5 (6.4-13.9) | 12.0 (8.1-17.2) | 9.0 (6.9-15.5) | 6.8 (5.2-9.0) |
| *Clinical T-stage* | | | | |
| - 1c (%) | 7 | 2 | 3 | 2 |
| - 2a (%) | 3 | 1 | 1 | 1 |
| - 2b (%) | 10 | 4 | 3 | 4 |
| - 2c (%) | 9 | 1 | 3 | 4 |
| - 3a (%) | 9 | 3 | 2 | 4 |
| - 3b (%) | 2 | 0 | 1 | 1 |
| *Biopsy Gleason sum score* | | | | |
| - 6 (%) | 4 | 4 | 0 | 0 |
| - 7 (%) | 30 | 6 | 8 | 16 |
| - 8 (%) | 5 | 1 | 4 | 0 |
| - 9 (%) | 1 | 0 | 1 | 0 |
| - 10 (%) | 0 | 0 | 0 | 0 |
| *Pathologic T-stage* | | | | |
| - 2a (%) | 0 | 0 | 0 | 0 |
| - 2b (%) | 6 | 2 | 1 | 3 |
| - 2c (%) | 16 | 3 | 5 | 8 |
| - 3a (%) | 16 | 5 | 6 | 5 |
| - 3b (%) | 2 | 1 | 1 | 0 |
| *Pathologic Gleason sum score* | | | | |
| - 6 (%) | 4 | 0 | 2 | 2 |
| - 7 (%) | 28 | 9 | 6 | 14 |
| - 8 (%) | 3 | 1 | 2 | 0 |
| - 9 (%) | 4 | 0 | 3 | 0 |
| - 10 (%) | 1 | 1 | 0 | 0 |

IQR = interquartile range; no. = number; PSA = prostate specific antigen.

**Table 2.** Intraoperative sentinel node identification and ex vivo measurements

| | ***Total (n=38[@])*** | ***Group 1*** | ***Group 2*** | ***Group 3*** | ***p-value*** |
|---|---|---|---|---|---|
| *Number of intraoperatively detected SNs, per patient, median (IQR)* | 3 (2-4) | 2 (2-3) | 4 (2.5-4) | 4 (2-4) | 0.2[a] |
| *In vivo SN identification* | | | | | |
| - Fluorescence-based SN identification rate# in vivo, per patient,mean % (mean % corrected for malfunctioning equipment) | 72.9% (84.0%*) | 50.9% (63.7%*) | 63.8% (85.2%*) | 93.5% (93.5%*) | 0.005a (0.012a) |
| - Radioactivity-based SN detection in vivo, per patient, mean % | 100% | 100% | 100% | NA | - |
| *Ex vivo SN measurements* | | | | | |
| - Fluorescence-based SN detection ex-vivo, % | 96.9% | 92.6% | 97.7% | 100.0% | - |
| - Radioactivity-based SN detection ex vivo, % | 100.0% | 100.0% | 100.0% | 100.0% | - |
| *Time per combined SN, ePLND and prostatectomy procedure (h), median (IQR)* | 2:07 (2:00-2:12) | 2:01 (1:50-2:01) | 2:04 (2:00-2:14) | 2:06 (2:02-2:14) | 0.2[b] |

[@] Two patients were excluded due to non-visualization on preoperative images (one patient in group 1, and one patient in group 2).

\# Intraoperative fluorescence-based SN identification rate is defined as: defined for each patient as: ((number of SNs intraoperatively visualized via fluorescence imaging) / (total number of SNs seen on preoperative imaging)) x 100%.

* Intraoperative fluorescence-based SN identification rate after correction for non-visualization due to malfunctioning equipment.

[a] = Kruskal-Wallis test; [b] = ANOVA-test; n = number; NA = not applicable; IQR = interquartile range; SN = sentinel node; n = number of patients.

**Table 3.** Number and location of the intraoperatively identified sentinel nodes

| | *Total* | *Group 1* | *Group 2* | *Group 3* |
|---|---|---|---|---|
| *SNs removed from ePLND template, no. (% total)* | | | | |
| - Left obturator region | 19 (15.0%) | 4 (3.1%) | 4 (3.1%) | 11 (8.7%) |
| - Right obturator region | 28 (22.0%) | 7 (5.5%) | 10 (7.9%) | 11 (8.7%) |
| - Left external region | 18 (14.2%) | 6 (4.7%) | 8 (6.3%) | 4 (3.1%) |
| - Right external region | 16 (12.6%) | 5 (3.9%) | 5 (3.9%) | 6 (4.7%) |
| - Left internal region | 9 (7.1%) | 0 | 5 (3.9%) | 4 (3.1%) |
| - Right internal region | 7 (5.5%) | 0 | 2 (1.6%) | 5 (3.9%) |
| - Left common Iliac trunk | 10 (7.9%) | 3 (2.4%) | 4 (3.1%) | 3 (2.4%) |
| - Right common Iliac trunk | 2 (1.6%) | 0 | 0 | 2 (1.6%) |
| Subtotal | 109 (85.8%) | 25 (19.7%) | 38 (29.9%) | 46 (36.2%) |
| *SN removed outside ePLND template, no. (% total)* | | | | |
| - Pararectal (mesorectal fascia) region | 5 (3.9%) | 1 (0.8%) | 0 | 4 (3.1%) |
| - Presacral region | 5 (3.9%) | 1 (0.8%) | 2 (1.6%) | 2 (1.6%) |
| - Paravesical region | 4 (3.1%) | 0 | 0 | 4 (3.1%) |
| - Right umbilical ligament | 1 (0.8%) | 0 | 1 (0.8%) | 0 |
| - Left umbilical ligament | 1 (0.8%) | 0 | 1 (0.8%) | 0 |
| - Para-aortal region | 2 (1.6%) | 0 | 2 (1.6%) | 0 |
| Subtotal | 18 (14.2%) | 2 (1.6%) | 6 (4.7%) | 10 (7.9%) |
| Total | 127 (100.0%) | 27 (21.3%) | 44 (34.6%) | 56 (44.1%) |
| *Not removed SN, no.* | | | | |
| - Pararectal region | 3 | 1 | 1 | 1 |
| - Presacral region | 2 | 0 | 1 | 1 |
| - Right iliac region | 1 | 1 | 0 | 0 |
| Total | 6 | 2 | 2 | 2 |

SN = sentinel node; ePLND = extended pelvic lymph node dissection; no. = number.

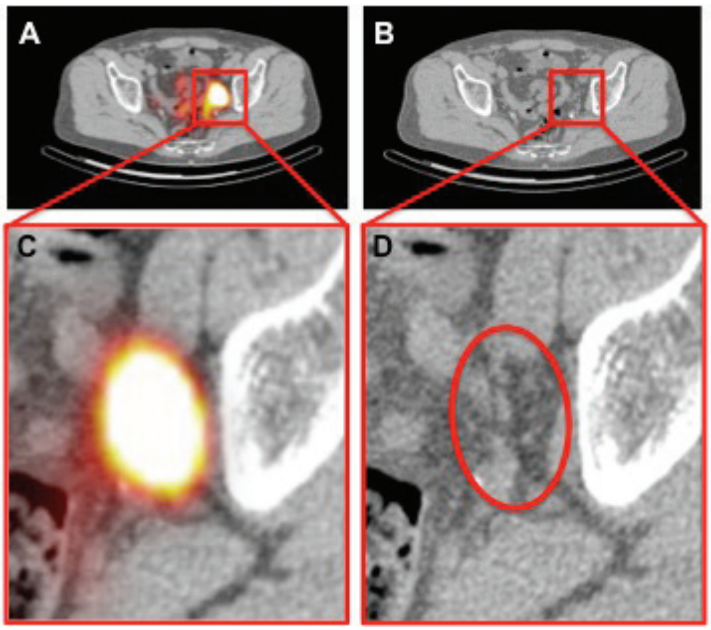


**Figure 1.** Clustered lymph nodes as seen on SPECT/CT imaging. A) Fused SPECT/CT image showing the location of a single radioactive hotspot in the left obturator region; B) Corresponding CT image; C) Zoom showing the radioactive hotspot; D) Multiple lymph nodes were visualized on the CT image in the area of the radioactive hotspot. SPECT/CT = single photon emission computed tomography combined with computed tomography; CT = computed tomography.

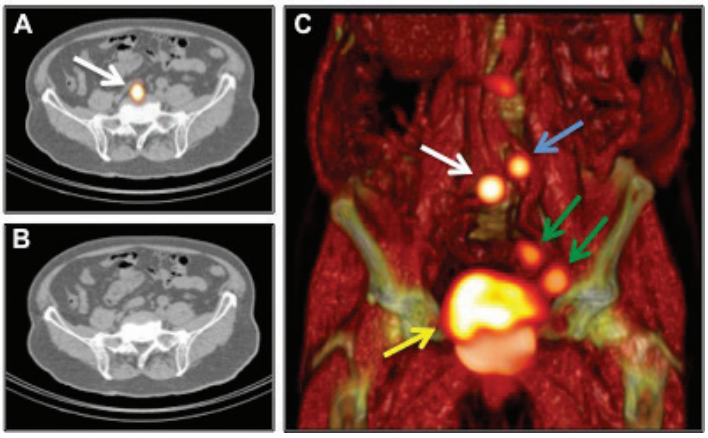


**Figure 2.** Illustration of the localization of sentinel nodes outside the extended pelvic lymph node dissection template. A) Fused SPECT/CT image showing the location of a single radioactive hotspot at the aorto-caval level (white arrow); B) Corresponding CT image; C) three dimensional volume rendering showing the injection site (yellow arrow), the aorto-caval sentinel node (white arrow) as well as two sentinel nodes at the external iliac and obturator region (green arrows). A higher-echelon para-aortic lymph node (blue arrow) was also visualized. SPECT/CT = single photon emission computed tomography combined with computed tomography; CT = computed tomography.

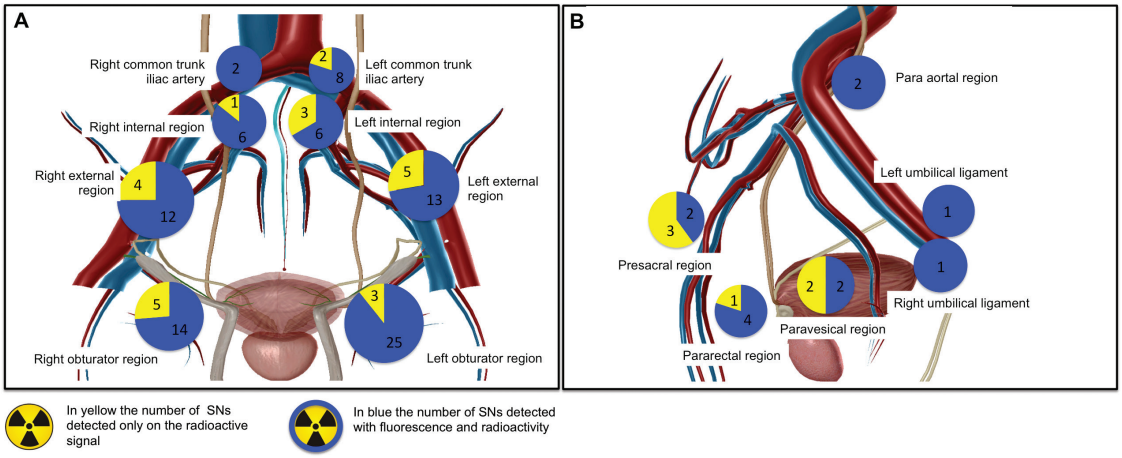


**Figure 3.** Intraoperative sentinel node location. A) Sentinel nodes inside extended pelvic lymph node dissection template; B) Sentinel nodes outside the extended pelvic lymph node dissection template. Intraoperatively, nodes were detected using radioguidance (yellow) or radio- and fluorescence guidance (blue). The schematic images used to illustrate the location of the detected sentinel nodes were generated with Visible Body software (Argosy Publishing Inc, Newton Upper Falls, MA, USA).

The two patients who had non-visualization on preoperative imaging were excluded from the intraoperative SN detection outcome analysis. With every stepwise modification, the intraoperative fluorescence-based SN visualization rate increased (Table 2). The mean optical SN visualization percentage modestly increased from 50.9% (group 1) to 63.8% (group 2) after the tracer formulation was altered. In these two groups, in five patients (group 1: two patients; group 2: three patients), none of the SNs could be intraoperatively visualized via fluorescence imaging for reasons of malfunctioning equipment (damaged light cable). After excluding these patients, the in vivo fluorescence-based SN visualization percentage of groups 1 and 2 was found to be 63.7% and 85.2%, respectively. Following the introduction of the upgraded laparoscopic fluorescence imaging system (group 3), the mean intraoperative visualization percentage went up to 93.5% (p=0.012). Ex vivo measurements in the operating room revealed a fluorescent signal in 123 of the 127 excised SNs (96.9%), while all excised SNs were radioactive (100.0%; Table 2).

## PATHOLOGIC EXAMINATION

Overall, histopathological analysis of the excised tissues yielded 467 lymph nodes: 160 nodes in the SN specimens and 307 additional nodes resected from the subsequent ePLND template. In eight patients, a total of 32 tumor-positive nodes were found: 16 SNs and 16 lymph nodes (Table 4; supplementary information Table SI2). In three patients, the SN was

the only tumor-positive node. In three other patients, next to a tumor-positive SN, a tumor-positive lymph nodes was also found. Strikingly, in one of these three patients, next to two tumor-positive SNs, we found 12 tumor-positive lymph nodes (supporting information Table SI2). In the last two positive patients, SNs were tumor free, but a tumor-positive lymph nodes was found. In one of these two patients, the positive lymph nodes was found in the ePLND tissue (false-negative SN biopsy procedure). In the other patient, a small positive lymph nodes (3 mm) was found in the prostatectomy specimen. This particular lymph nodes was not seen on preoperative images.

On a per-patient basis, the sensitivity of the SN biopsy procedure was 75.0% (six out of eight pN1 patients correctly staged with SN biopsy), with a negative predictive value of 94.1%. On a per-tumor-positive node basis, this sensitivity is 50.0% (16 tumor-positive SNs on a total of 32 positive nodes; Table 4).

**Table 4.** Pathological node evaluation

| | ***Total*** | ***Group 1*** | ***Group 2*** | ***Group 3*** | ***p-value*** |
|---|---|---|---|---|---|
| *No. patients pN1* | 8 | 2 | 3 | 3 | 0.9[b] |
| *SN evaluation* | | | | | |
| - No. harvested SNs / patient, median (IQR) | 4 (2.3-5.0) | 3 (2.0-3.0) | 4 (2.5-5.5) | 4 (3.0-5.8) | 0.028[a] |
| - Total no. SNs | 160 | 29 | 57 | 74 | |
| - Total no. tumor-positive SNs | 16 | 1 | 5 | 9 | |
| *LN evaluation* | | | | | |
| - No. harvested LNs from ePLND/ patient, median (range) | 8 (4.5-11.0) | 4 (4.0-10.0) | 6 (4.5-12.0) | 9 (7.3-11.0) | 0.2[a] |
| - Total no. LNs | 307 | 65 | 95 | 147 | |
| - Total no. tumor-positive LN | 16 | 1 | 13 | 2 | |
| *SN + LN evaluation* | | | | | |
| - Total no. removed nodes per patient (SN + ePLND), median (IQR) | 12 (9.0-14.8) | 9 (6.0-11.0) | 11 (8.0-15.5) | 12 (11.0-16.0) | 0.026[a] |
| - Total no. harvested SNs + LNs | 467 | 94 | 152 | 221 | |

[a] = Kruskal-Wallis test; [b] = Chi-square test.
no. = number of patients; IQR = interquartile range; No. = number; SN = sentinel node; LN = lymph node; ePLND = extended pelvic lymph node dissection; pN1 = positive for regional lymph node metastases.

## FOLLOW-UP

No significant differences in postoperative complications were found among the three groups (p=0.9; Table 5). Although follow-up was relatively short, in patients without nodal metastases (pN0), the Kaplan-Meier curve showed an improvement in BCR-free survival in group 3 (n=0; total follow-up 25 months) versus men in groups 1 and 2 (n=3; total follow-up 38 months; p=0.2; Figure 4).

**Table 5.** Patient follow-up

| | ***Total*** | ***Group 1*** | ***Group 2*** | ***Group 3*** |
|---|---|---|---|---|
| *Follow-up months, median (range)* | 10.5 (3.0-35.0) | 22 (5.0-35.0) | 12 (8.0-22.0) | 8 (3.0-12.0) |
| *Complications* | | | | |
| - Clavien-Dindo | | | | |
| - Lymphocele (Clavien-Dindo IIIa) | 2 | 0 | 2 | 0 |
| - Urinary tract infection (Clavien-Dindo II) | 2 | 0 | 1 | 1 |
| - Postoperative bowel obstruction (Clavien-Dindo II) | 1 | 1 | 0 | 0 |
| - Micturition obstruction (Sachse Ureterotomy) (Clavien -Dindo IIIb) | 1 | 1 | 0 | 0 |
| - Postoperative wound infection (Clavien-Dindo II) | 1 | 0 | 0 | 1 |
| - Hematoma of the ventral abdominal wall (Clavien-Dindo I) | 1 | 0 | 1 | 0 |
| - Epididymitis (Clavien-Dindo II) | 1 | 0 | 0 | 1 |
| - Hydronephrosis (Clavien-Dindo IIIa) | 1 | 1 | 0 | 0 |
| Total | 10 | 3 | 4 | 3 |
| - Erectile dysfunction | 24 | 5 | 6 | 13 |
| - Micturition problems | 11 | 2 | 4 | 5 |

Follow-up in postoperative complications reported within 90 days after the prostatectomy combined with extended pelvic lymph node dissection and sentinel node procedure.

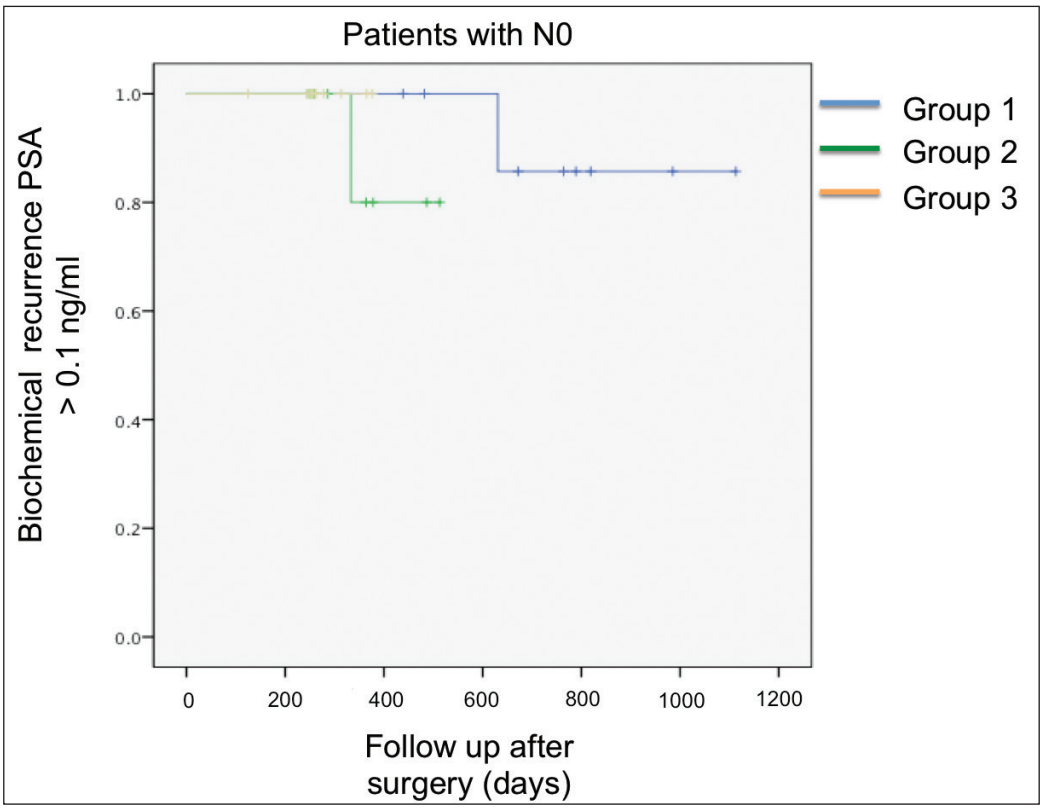


**Figure 4.** Kaplan Meier curve illustrating the biochemical recurrence. The blue line represents group 1 and 2 in which three biochemical recurrences were found. The green line represents group 3.

## DISCUSSION

This study demonstrates that optimization of the hybrid tracer formulation and injection technique, as well as upgrading the laparoscopic fluorescence imaging system, improved in vivo fluorescence-based SN identification during RARP. Without altering the efficacy of preoperative SN mapping, the new tracer formulation increased the injected amount of ICG-$^{99m}$Tc-nanocolloid particles 2.5-fold and reduced the injected volume 1.6-fold. In combination with initial laparoscopic gamma probe exploration, the in vivo fluorescence visualization efficiency increased by 21.5% (group 1 vs. group 2). This increase contradicts our previous findings in breast cancer patients, where a 2-fold increase in the amount of injected particles did not lead to a change in fluorescence visualization efficiency [15]. Feedback from previous studies has taught us that the SN has to be exposed within mms of the surface to allow for in vivo fluorescence-based detection [9,10,16]. Hence, an explanation for this finding may lie in the time taken for the now more routine surgical

exploration; findings in group 1 turned out lower than the reported 85% in our feasibility study, which was based on the same approach.

After upgrading the laparoscopic fluorescence imaging system (group 3), the mean intraoperative fluorescence-based SN visualization percentage increased to 93.5%, transforming the procedure in a potential driver to improve intraoperative localization of tumor-positive SNs, even within the standard ePLND template. This improvement may provide better nodal staging (the "Will Rogers" phenomenon) and help improve the BCR-free survival rate, as was seen in group 3 [17]. The tailored filter settings allowed visualization of the near-infrared fluorescence signal (displayed in blue) as an integral part of the patient anatomy (displayed in "normal" colored view; Figure 5). Despite a slight loss in sensitivity this continuous exploration of the surgical field via fluorescence imaging proved extremely valuable for the localization of the SN(s). In combination wit the 3D information that SPECT/CT provided, this improvement may render initial exploration with the laparoscopic gamma probe in vivo redundant, provided that the fluorescence-based SN identification rate equals that of its radioactive counterpart. This is attractive because fluorescence does not suffer from the shine-through phenomenon from the tracer deposits in the prostate, as is the case for the radioguided approach [18].

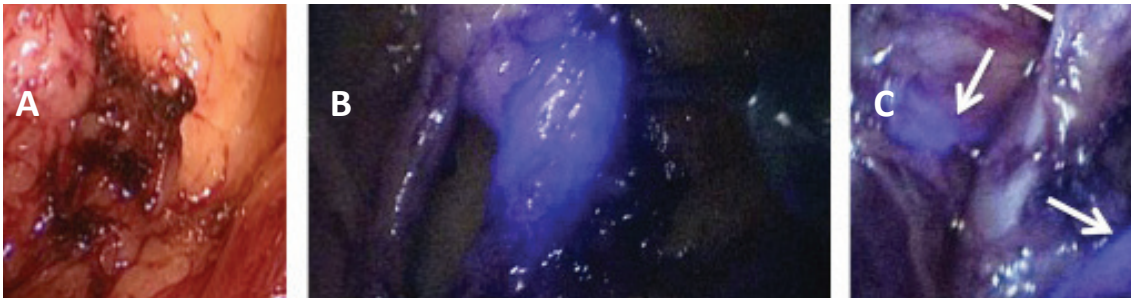


**Figure 5.** Intraoperative sentinel node (and lymphatic duct) identification via fluorescence guidance. A) White light image illustrating the area that harbors the sentinel node; B) Fluorescence guidance clearly shows the contours of the sentinel nodes. The adjusted filter settings of the Image 1 HUB HD + D-light P system (KARL STORZ Endoskope GmbH & Co. KG) allows clear visualization of anatomical detail in the background; C) Lymphatic ducts visualized via fluorescence imaging (white arrows).

Of the 16 tumor-positive SNs that were resected during the operation, one was located outside the ePLND template (6.3%). This finding underlines previous reports stating that metastatic spread may occur beyond the ePLND template [13,19]. In five of the eight patients with positive nodes, we found positive lymph nodes beyond the resected lumph nodes; in total 16 additional tumor-positive lymph nodes were recovered from the ePLND specimens. It must be noted that one patient accounts for 75% of these positive lymph nodes (supporting information Table SI2). Based on our findings, we believe that SN identification via the hybrid approach (including SPECT/CT) combined with ePLND provides the best approach for nodal staging in combination with RARP.

The main limitations of the study are the small patient population, the possibility that SN identification rates may increase over time because of a learning curve, and the relatively low number of overall nodes removed. The cost-effectiveness and the independent use of intraoperative fluorescence guidance remain to be investigated; currently, an international multicenter study is being initiated to address this question. Still an important question remains to be answered: What is the best hybrid tracer injection technique? In our current, ongoing, study (N12IGP) we will evaluate whether the location of hybrid tracer injection (intraprostatic vs. intratumoral) is relevant for the detection and localization of tumor-positive SNs.

## CONCLUSION

Altering the hybrid tracer formulation and injection technique and upgrading the laparoscopic fluorescence imaging system significantly improved in vivo fluorescence-based SN identification. Further improvement of in vivo fluorescence-based SN detection, reaching rates similar to that of the conventional radio-guided approach, may make intraoperative laparoscopic gamma tracing redundant. Still, SPECT/CT remains an essential tool for preoperative SN localization.

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# SUPPORTING INFORMATION

**Table SI1**. Preoperative imaging results

| | *Total* | *Group 1* | *Group 2* | *Group 3* |
|---|---|---|---|---|
| *No. patients* | 40 | 11 | 13 | 16 |
| *Injected dose (MBq), median (IQR)* | 217.8 (205.3-228.7) | 218.2 (205.7-236.7) | 223.8 (207.8-236.7) | 209.6 (203.8-222.8) |
| *Preoperative imaging results, no. per patient* | | | | |
| - SNs on early lymphoscintigrams, median (IQR) | 1 (0-2) | 1 (0-2) | 1 (0-2) | 1 (0-2) |
| - SNs on late lymphoscintigrams, median (IQR) | 2 (1-3) | 2 (2-3) | 2 (1-3) | 2 (2-3) |
| - SNs on SPECT/CT, median (IQR) | 3 (0-6) | 2 (2-3) | 4 (2-4) | 3 (2-4) |
| - Higher-echelon LNs, median (IQR) | 0 (0-1) | 1 (0-2) | 0 (0-1) | 0 (0-.75) |
| *Time from injection to surgery (h), median (IQR)* | 5:44 (4.15-5.07) | 4:34 (4:15-5:05) | 5:05 (4:14-5:35) | 4:42 (4:13-5:04) |

MBq = Mega Bequerel; IQR = interquartile range; SN = sentinel node; LN = lymph node; n = number of patients; no. = number; SPECT/CT = single photon emission computed tomography combined with computed tomography

**Table SI2.** pN1 pathological findings

| *Patient* | *No. tumor-positive SNs* | *Location tumor-positive SNs* | *No. tumor-positive LNs from ePLND* | *Total no. tumor-positive SNs + LNs from ePLND* |
|---|---|---|---|---|
| 1 | 0/1 | - | 1/3 | 1/4 |
| 2 | 1/7 | Right external region | 0/4 | 1/13 |
| 3 | 1/12 | Right external region | 1/7 | 2/19 |
| 4 | 2/2 | Left internal region, Right obturator region | 12/13 | 14/15 |
| 5 | 3/3 | Left external region (2x), Left paravesical region | 0/8 | 3/11 |
| 6 | 0/3 | - | 1/12 | 1/15 |
| 7 | 5/9 | Left internal region (4x), Right obturator region | 0/12 | 5/21 |
| 8 | 4/6 | Left external region, Left obturator region, Right internal region, Right obturator region | 1/13 | 5/19 |
| Total | 16/43 | | 16/72 | 32/115 |

SN = sentinel node; LN = lymph node; ePLND = extended pelvic lymph node dissection.