Hybrid indocyanine green-Tc-99m-nanocolloid for single-photon emission computed tomography and combined radio- and fluorescence-guided sentinel node biopsy in penile cancer: results of 740 inguinal basins assessed at a single institution

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Platinum Priority – Penile Cancer

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Hybrid Indocyanine Green–^{99m}Tc-nanocolloid for Single-photon Emission Computed Tomography and Combined Radio- and Fluorescence-guided Sentinel Node Biopsy in Penile Cancer: Results of 740 Inguinal Basins Assessed at a Single Institution

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Abstract

Background: Sentinel node (SN) biopsy in penile cancer (PeCa) is typically performed using ^{99m}Tc-nanocolloid and blue dye. Recent reports suggested that the hybrid (radioactive and fluorescent) tracer indocyanine green (ICG)-^{99m}Tc-nanocolloid may improve intraoperative optical SN identification.
Objective: The current study aimed to confirm the reliability of ICG-^{99m}Tc-nanocolloid and to assess whether blue dye is still of added value.
Design, setting, and participants: A total of 400 ≥T1G2N0 PeCa patients were staged with SN biopsy at a single European centre. SNs were preoperatively identified with lymphoscintigraphy and single-photon emission computed tomography. Intraoperatively, SNs were detected via gamma tracing, blue staining, and fluorescence imaging.
Outcome measurements and statistical analysis: All patients (n = 400, 740 groins) received ICG-^{99m}Tc-nanocolloid. Intraoperative SN identification rates were retrospectively evaluated. In those patients who received ICG-^{99m}Tc-nanocolloid and blue dye (n = 266, 492 groins), SN visualisation rates were compared using the McNemar test.
Results and limitations: In total, 740 groins were assessed. No tracer-related (allergic) reactions were reported. All preoperatively defined SNs (n = 1163) were localised intraoperatively. Of all excised SNs, 98% were detectable with gamma probe and 96% were visible with fluorescence imaging. In the analysis of the patients who received ICG-^{99m}Tc-nanocolloid and blue dye, fluorescence imaging yielded a 39% higher SN detection rate than blue dye (95% confidence interval 36–43%, $p < 0.001$). Of the SNs that

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were tumour positive, 100% were intraoperatively visualised by fluorescence imaging, whereas merely 84% of the positive nodes stained blue.
Conclusions: This study confirms that ICG-^{99m}Tc-nanocolloid is a reliable SN tracer for PeCa that significantly improves optical SN detection over blue dye.
Patient summary: Hybrid indocyanine green (ICG)-^{99m}Tc-nanocolloid is a safe and reliable sentinel node (SN) tracer, as established in this large series of 400 penile cancer patients (740 groins). It enables accurate pre- and intraoperative SN identification and significantly improves SN detection rate compared with blue dye, without staining the surgical field or the need for an additional injection.

1. Introduction

The extent of nodal dissemination in penile cancer (PeCa) determines survival. Since currently available noninvasive staging techniques still lack sufficient accuracy to reliably detect small lymph node (LN) metastases, surgical staging remains the standard in clinically node-negative (cN0) patients [1]. To this end, (modified or radical) inguinal lymph node dissection (ILND) is still routinely performed alongside primary tumour surgery in many countries. However, even modified ILND can lead to substantial morbidity and may be considered overtreatment in most patients; since only 20–25% of cN0 patients harbour occult nodal metastasis [2–5]. As an alternative staging modality, dynamic sentinel node biopsy (DSNB) has gradually found its way into routine clinical care [5,6]. The current European Association of Urology guidelines recommend DSNB for cN0 patients with a ≥T1G2 tumour [1]. The procedure is routinely performed using a radiocolloid (ie, technetium-99m [^{99m}Tc]-nanocolloid) combined with an optical (blue) dye [1,5,7]. This combination was shown to be superior to using either modality separately [8]. In an attempt to improve the optical detection sensitivity and assure intraoperative alignment with preoperatively defined radioactive nodes, a dual-labelled (hybrid) tracer consisting of the fluorescent dye indocyanine green (ICG) and ^{99m}Tc-nanocolloid was introduced (ICG-^{99m}Tc-nanocolloid) [9–21]. In 2014, our initial experience with ICG-^{99m}Tc-nanocolloid for DSNB in 65 PeCa patients suggested that this approach could improve optical sentinel node (SN) detection compared with blue dye [10]. The primary aim of this study was to substantiate the reliability and added value of ICG-^{99m}Tc-nanocolloid as an SN tracer for PeCa in a large patient series. In addition, we underline the added benefit of single-photon emission computed tomography (SPECT/CT) and studied whether blue dye is still of added value when exploiting the fluorescence properties of ICG-^{99m}Tc-nanocolloid.

2. Patients and methods

2.1. Data source and patient selection

The current retrospective study relied on an on-going prospective database that collected data on 404 consecutive PeCa patients scheduled for DSNB between January 2011 and December 2018 at a single high-volume European centre (Netherlands Cancer Institute—Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands). Patients were eligible if they had ≥T1G2 penile squamous cell carcinoma with at least one cN0 groin, defined by palpation and inguinal ultrasound with fine-needle aspiration cytology (FNAC) in case of a suspect node [5]. DSNB was performed only in the cN0 groins. ICG-^{99m}Tc-nanocolloid was used as an SN tracer in all patients. Initially, patients also received an additional injection with blue dye (as was conventional). With increasing clinical experience with ICG-^{99m}Tc-nanocolloid, blue dye was gradually phased out. Patients were excluded if it was not known/reported whether blue dye was administered (n = 4). The final population consisted of 400 assessable patients (740 inguinal basins/groins; Fig. 1). The study protocol for the introduction of ICG-^{99m}Tc-nanocolloid was approved by the medical ethics committee (N09DRF, NL 26699.031.09) [10]. Thereafter, a waiver from the institutional review board was received for the data collection/analysis.

2.2. Dynamic sentinel node procedure (DSNB)

DSNB was performed in the same session as the primary tumour surgery (n = 319), or within 6 wk after (diagnostic) resection of the penile tumour (secondary/delayed DSNB, n = 81). DSNB was performed as previously described [10,12,13,19]. A detailed overview with an accompanying video is provided in the Supplementary material. In short, ICG-^{99m}Tc-nanocolloid (GE Healthcare BV, Leiderdorp, The Netherlands) was injected on the same day (n = 351; 88%, 1-d protocol) or the day before surgery (n = 49; 12%, 2-d protocol). The standard dose was 90 MBq. Lymphoscintigraphy was performed directly (0–10 min), at 15 min, and 2 h after the injection, followed by SPECT/CT (Symbia T; Siemens, Erlangen, Germany). When patent blue dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was administered, this was done shortly before surgery. Intraoperatively, SNs were pursued using a gamma probe (Neoprobe; Johnson & Johnson Medical, Hamburg, Germany), mobile gamma camera (Sentinella; OncoVision, Valencia, Spain), followed by SN visualisation using a fluorescence camera (FIS-00, PhotoDynamic Eye; Hamamatsu Photonics, Hamamatsu, Japan). An overview of all equipment is depicted in Supplementary Figure 1 and the accompanying video. In patients who received blue dye, it was also noted whether SNs coloured blue. There was no interference of blue dye and ICG imaging. After SN excision, the mobile gamma camera was used to check for residual SNs (comparison with reference image at the start of surgery). If remaining activity (>10% of the activity of the removed SN) was observed at the same site of a previously excised SN, it was considered an additional SN and also excised. Before closing the wound, the surgical area was palpated to search for clinically suspicious LNs.

All harvested SNs were histopathologically analysed as previously described [10]. In case of one or more tumour-positive SNs, the patient was scheduled to undergo a completion ILND of the affected side.

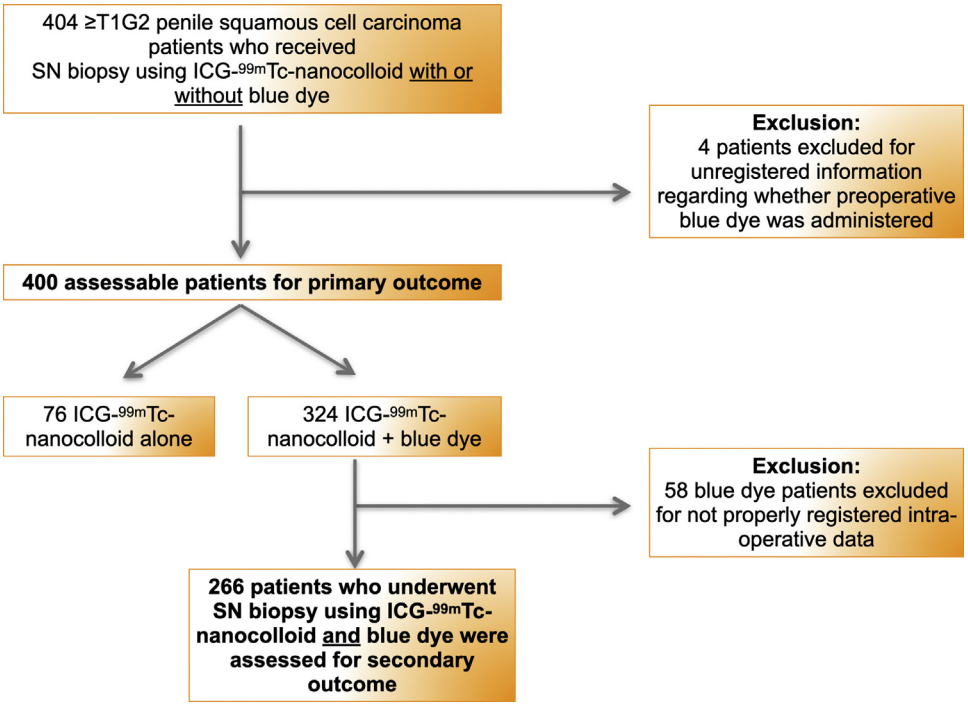

Fig. 1 – Flowchart presenting the study cohort and excluded patients. ICG = indocyanine green; SN = sentinel node.gr1

2.3. Follow-up

Follow-up included physical examination and inguinal ultrasound every 3 mo during the first 2 yr after DSNB and every 6 mo thereafter [1].

2.4. Outcomes and statistical analysis

The primary endpoints were the safety (defined as any side effect/event after tracer injection or during surgery) and reliability (SN identification and false-negative [FN] rate) of ICG-^{99m}Tc-nanocolloid as a tracer for DSNB. The secondary endpoints were to evaluate the added benefit of SPECT/CT over conventional lymphoscintigraphy for preoperative SN identification and to determine whether blue dye is still of added value when using ICG-^{99m}Tc-nanocolloid.

Medians and interquartile ranges (IQRs), as well as frequencies and proportions were reported for continuous and categorical variables, respectively. In the entire cohort ($n = 400$), total preoperative SNs visualised by early and late lymphoscintigraphy, and SPECT/CT were assessed. Intraoperatively identified SNs were scored for the modality that enabled their detection. The FN rate of DSNB was calculated according to previously published definitions: FN/(true positive + FN) × 100 or 1–sensitivity × 100 [22]. FN cases were defined as patients with LN recurrence during follow-up after a negative SN procedure at the time of primary surgery in the absence of a local penile recurrence or residual disease. All other negative cases were regarded as true negative procedures.

To evaluate the added value of blue dye, a second analysis on those patients who received ICG-^{99m}Tc-nanocolloid and blue dye (with complete intraoperative documentation on blue vs fluorescent status, $n = 266$; Fig. 1). Herein, the added value of blue dye with regard to fluorescence-based SN visualisation rates was analysed using the McNemar test; 95% confidence intervals (CIs) for the difference in paired proportion were calculated using the Bonett and Price method [23]. Histopathological findings were compared between ICG-^{99m}Tc-nanocolloid and blue dye. Analyses were performed using R software version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), and all tests were two sided with significance level set at $p < 0.05$.

3. Results

3.1. Reliability of DSNB using ICG-^{99m}Tc-nanocolloid

Of the 400 included patients, 11% ($n = 44$) had unilateral cN1 groins. These patients underwent DSNB in the clinically unaffected groin only. Included are 34 patients (8.5%) who underwent repeat DSNB for a recurrent tumour, although preoperative lymphoscintigraphy and SPECT/CT visualised at least one SN in all patients (in 24 patients, this was after tracer reinjection). Nevertheless, there were still nonvisualisations after tracer reinjection in 16 groins. This resulted in a total of 740 assessed groins (Table 1).

A median dose of 89 MBq of ICG-^{99m}Tc-nanocolloid was injected preoperatively (Table 2). No allergic reactions or side effects were observed after tracer injection. A total of 1163 SNs were identified by preoperative imaging (median: three SNs per patient, IQR 2–4). Of those, 43% ($n = 499$), 92% ($n = 1073$), and 99.6% ($n = 1159$) were identified by early lymphoscintigraphy, late lymphoscintigraphy, and SPECT/CT, respectively. SPECT/CT revealed 86 SNs in 65 patients that were not seen on the lymphoscintigrams (7.4% [95% CI 5.9–8.9%]; Fig. 2)

All preoperatively defined SNs were localised intraoperatively. A total of 1324 SN specimens were excised (median: three SNs per patient, IQR 2–4). Of these, 1296 (98%) were detected by gamma probe and 1265 (96%) were

Table 1 – Descriptive characteristics of the entire cohort of 400 patients with ≥T1G2 penile squamous cell carcinoma with at least one cN0 groin, scheduled for DSNB[a]

Variables	Overall (%)
Age at surgery (yr), median (IQR)	69 (60–76)
BMI, median (IQR)	27 (25–30)
Clinical T stage	
T1	145 (36)
T2	198 (50)
T3	47 (12)
Unknown	10 (2.5)
Clinical N stage	
N0	356 (89)
N1	44 (11)
Repeat SN biopsy	34 (8.5)
Delayed/secondary SN biopsy	81 (20)
Total included groins for SN biopsy	
Overall	740
Unilateral	60 (15)
Bilateral	340 (85)
Tracer	
ICG-^{99m}Tc-nanocolloid	76 (19)
ICG-^{99m}Tc-nanocolloid + blue dye	324 (81)

BMI = body mass index; DSNB = dynamic sentinel node biopsy; ICG = indocyanine green; IQR = interquartile range; SN = sentinel node.
[a] All patients received ICG-^{99m}Tc-nanocolloid.

Table 2 – Pre-, intra-, and postoperative findings in all 400 patients (740 groins)

	Overall
Preoperative variables	
Dose of ICG-^{99m}Tc-nanocolloid administered (MBq), median (IQR)	89 (80–123)
Reinjection of ICG-^{99m}Tc-nanocolloid, *n* (%)	24 (6)
Allergic reactions/side effects after injection of:	
ICG-^{99m}Tc-nanocolloid, *n*	0
Blue dye, no.	0
SNs identified at preoperative imaging, *n* (median, IQR)	1163 (3, 2–4)
SNs identified at preoperative lymphoscintigraphy, *n* (median, IQR)	
Early	499 (1, 0–2)
Late	1073 (2, 2–3)
SNs identified at preoperative SPECT/CT, *n* (median, IQR)	1159 (3, 2–4)
Intraoperative variables	
Time from ICG-^{99m}Tc-nanocolloid injection to surgery (h), median (IQR)	14 (5–19)
Preoperative identified SNs localised intraoperatively, %	100
Total number of excised SNs, *n* (median, IQR)	1324 (3, 2–4)
Intraoperative SNs detected by gamma probe, *n* (%)	1296 (98)
Intraoperative SNs visualised with fluorescence camera, *n* (%)	1265 (96)
Postoperative variables	
Total number of SNs identified at pathology, *n* (median, IQR)	1441 (3, 2–4)
Positive SNs, *n* (patients, groins)	95 (70, 84)
Positive SNs detected by gamma probe, *n* (%)	95 (100)
Positive SNs visualised by fluorescence camera, *n* (%)	95 (100)
False-negative SN biopsy per patient, *n* (rate)	8 (10)
False-negative SN biopsy per groin, *n* (rate)	8 (8.7)
Follow-up (mo), median (IQR)	36 (15–62)

ICG = indocyanine green; IQR = interquartile range; SN = sentinel node; SPECT/CT = single-photon emission computed tomography.

visible with fluorescence imaging. Twenty-eight SNs (2.1%) not detected by gamma probe due to radioactive decay were recovered using fluorescence imaging (Fig. 3).

At histopathology, 1441 SNs were identified and 95 were positive (70 patients, 17.5% [70/400]; 84 groins; Table 2). All 95 identified positive SNs were both radioactive and fluorescent (Table 2). Only seven patients experienced an intraoperative complication (1.8%; bleeding requiring ligation) and a total of 86 patients (22%) developed a 30-d postoperative complication, of whom 75 (87%) were minor and required no intervention other than antibiotics.

At a median follow-up of 36 mo (for patients with a true negative procedure; IQR 15–62), eight out of 330 patients with a negative DSNB developed an LN metastasis in the investigated groin and therefore had an FN procedure (eight FN cases and 70 positive patients = FN rate of 10% per patient [95% CI 4.5–19%], and the probability of a negative procedure to become an FN procedure at 2 yr of 2.5%). The LN metastasis was unilateral in all patients; thus, eight out of 740 groins were not staged correctly (eight FN cases and 84 positive groins = FN rate of 8.7% per groin [95% CI 3.8–16%], and the probability of a negative procedure to become an FN procedure at 2 yr of 1.4%). The FN rate was not affected by the repeat DSNB patients. Six of eight patients had recurrence within 12 mo after the SN procedure. All FN recurrences occurred within 24 mo.

3.2. *Blue dye versus ICG-^{99m}Tc-nanocolloid*

An additional analysis was performed in the subcohort that underwent DSNB using ICG-^{99m}Tc-nanocolloid and blue dye. No allergic reactions/side effects were observed after blue dye injection. Of the initial 324 patients, 58 were excluded from the analysis due to incomplete intraoperative reporting of blue status of the SNs (Fig. 1). This resulted in 266 evaluated patients (492 groins; Supplementary Table 1), wherein a total of 754 SNs were identified by preoperative imaging (median: three SNs per patients, IQR 2–4; Table 3). All the preoperatively defined SNs were identified intraoperatively (Table 3 and Supplementary Fig. 1). A total of 900 SNs were excised (median: three SNs per patient, IQR 2–4). Of these, 858 (95%) were visible via fluorescence imaging, while merely 505 (56%) were stained blue (difference 39% [95% CI 43–36%], $p < 0.001$; Fig. 3). In this cohort, SNs not detectable by gamma probe ($n = 20$, 2.2%) were identified and dissected based on fluorescence imaging only ($n = 16$) or blue dye colouration only ($n = 4$; Table 3). The intraoperative fluorescence and blue dye SN detection rate was lower in patients with increased body mass index after correction for clinical T stage and N stage (Supplementary Fig. 2).

Of the 58 positive SNs at histopathology, 100% were radioactive, 100% were fluorescent, and 84% ($n = 49$) were blue at the time of excision (Table 3). None of the SNs identified based on blue dye colouration only (neither radioactive nor fluorescent) were positive at final histopathology. Of those patients in whom blue dye did not identify any SN ($n = 47$), 6% had positive nodes ($n = 3$, detected by both gamma tracing and fluorescence imaging).

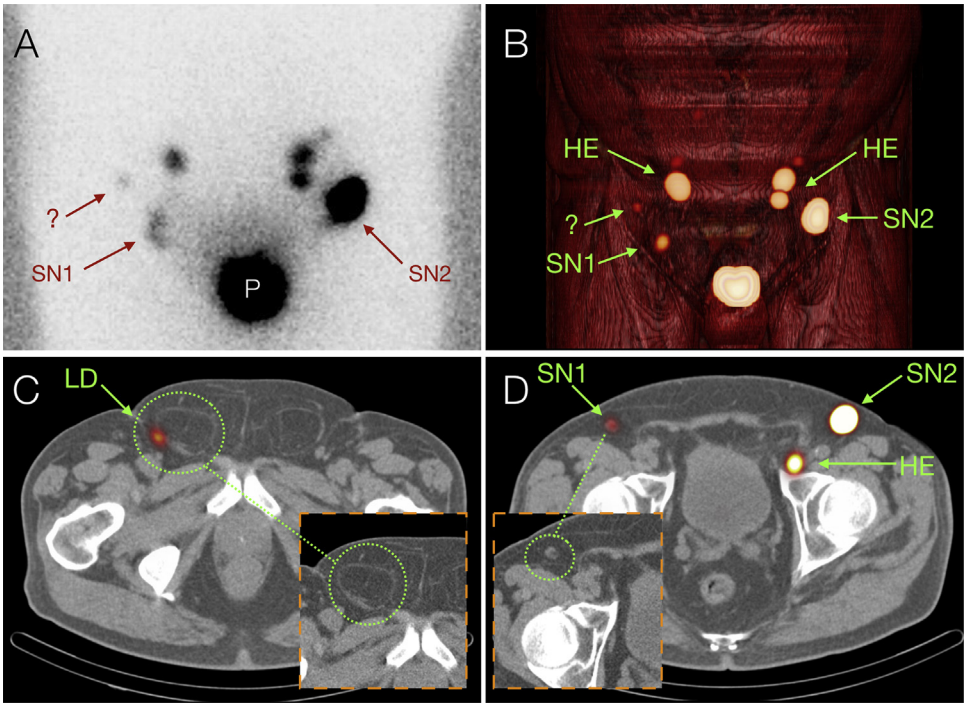

Fig. 2 – Preoperative SN imaging. (A) Early lymphoscintigraphy showing drainage from the penile tumour (P) to bilateral radioactive hot spots. There are two spots on the right side both of which appear early (SN1 and?), both of which could be a potential SN. (B) Coronal SPECT/CT image at 2 h after injection showing the potential SNs and higher echelon nodes (HE). (C) Axial SPECT/CT image where there is no node on the location of the radioactivity, indicating that this corresponds to stasis in a lymphatic duct (LD). This was confirmed at histopathology. (D) The second hotspot observed at lymphoscintigraphy ("?" in A) corresponds to a node on CT and is therefore the true SN on the right side (SN1). This image also illustrates how SPECT/CT helps distinguish an inguinal SN (SN2) from an HE. CT = computed tomography; SN = sentinel node; SPECT/CT = single-photon emission computed tomography.gr2

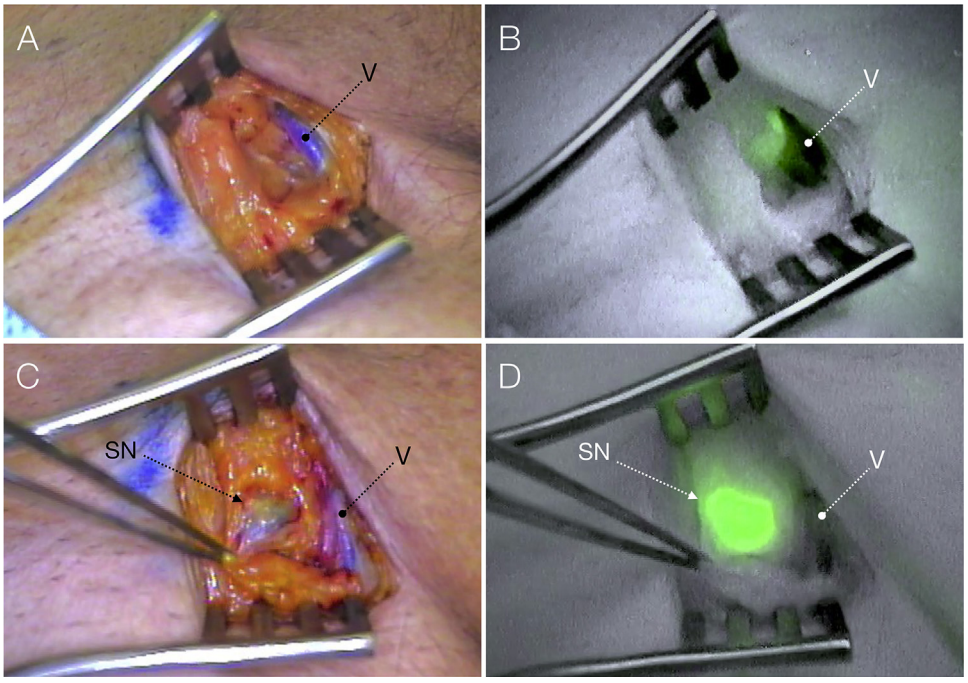

Fig. 3 – Intraoperative SN visualisation using fluorescence imaging. (A) Image showing surgical field with a vein (V), and no LN is visible (yet) by the naked eye. (B) Corresponding fluorescence image showing that the SN can already be visualised below a layer of fat. (C) After further dissection, the SN is localised. The node is also coloured by blue dye, which can be appreciated only when the SN is found (SN). (D) Corresponding fluorescence image clearly showing the fluorescent SN (SN). LN = lymph node; SN = sentinel node.gr3

Table 3 – Pre-, intra-, and postoperative findings in 266 patients who underwent DSNB using ICG-^{99m}Tc-nanocolloid and blue dye

	Overall
Preoperative variables	
SNs identified at preoperative imaging, *n* (median, IQR)	754 (3, 2–4)
SNs identified at preoperative lymphoscintigraphy, *n* (median, IQR)	
Early	345 (1, 0–2)
Late	693 (2, 2–3)
SNs identified at preoperative SPECT/CT, *n* (median, IQR)	750 (3, 2–4)
Intraoperative variables	
Preoperatively identified SNs localised intraoperatively (gamma probe, fluorescence camera, blue), %	100
Total number of excised SNs, *n* (median, IQR)	900 (3, 2–4)
Intraoperative SNs detected by gamma probe, *n* (%)	880 (98)
Intraoperative SNs visualised with fluorescence camera, *n* (%)	858 (95)
Intraoperative SNs that stained blue, *n* (%)	505 (56)
Intraoperative SNs visualised with fluorescence camera only (neither radioactive nor blue), *n* (%; patients)	16 (1.8; 7)
Intraoperative SNs identified with blue colouration only (neither radioactive nor fluorescent), *n* (%; patients)	4 (0.44; 3)
Postoperative variables	
Total number of SNs identified at pathology, *n* (median, IQR)	966 (3, 2–4)
Positive SNs, *n* (patients, groins)	58 (44, 51)
Positive SNs detected by gamma probe, *n* (%)	58 (100)
Positive SNs visualised by fluorescence camera, *n* (%)	58 (100)
Positive SNs visualised by blue dye, *n* (%)	49 (84)
False-negative SN biopsy per patient, *n* (rate)	5 (10)
False-negative SN biopsy per groin, *n* (rate)	5 (8.9)
Follow-up (mo), median (IQR)	42 (18–65)

DSNB = dynamic sentinel node biopsy; ICG = indocyanine green; IQR = interquartile range; SN = sentinel node; SPECT/CT = single-photon emission computed tomography.

4. Discussion

In the current study, we present the largest cohort of PeCa patients who underwent DSNB using SPECT/CT and ICG-^{99m}Tc-nanocolloid. We confirmed that ICG-^{99m}Tc-nanocolloid is safe, and allows for accurate pre- and intraoperative SN localisation, thereby vastly improving optical SN detection compared with blue dye.

SPECT/CT revealed additional SNs in 16% of patients and improves the visualisation rate to 99.6%. This finding is in line with previous studies on PeCa [24,25] and might be explained by the increased sensitivity and resolution of SPECT itself, together with the improved anatomical localisation of LNs offered by the three-dimensional information provided (Fig. 2) [26,27]. These strengths of SPECT/CT encourage its inclusion in urological guidelines as a preoperative imaging modality for DSNB procedures [1].

The fact that no allergic reactions or side effects were observed in relation to the injection of ICG-^{99m}Tc-nanocolloid in any of the 400 patients affirms that the tracer is safe. The slightly higher SN detection rate with the gamma probe than with fluorescence imaging can mainly be explained by the superior tissue penetration of radioactive gamma rays in relation to the fluorescent signal. Indeed, body mass index negatively impacted intraoperative fluorescence-based SN identification, but not the radioguidance, which is in line with previous findings [19]. Conversely, fluorescence imaging does not suffer from decay. This is exemplified by the observation that nearly 2% of the SNs missed by gamma probe were visualised by fluorescence imaging. Further, the operating surgeons appreciated the fluorescence guidance as a means to optically confirm intraoperative SN localisation, sooner than blue dye can be detected (Fig. 3). Together, these findings underscore how the integrated use of fluorescence imaging and radioguidance, when using ICG-^{99m}Tc-nanocolloid, complements to the otherwise standard radioguided procedure [13]. This said, the radioactive signature of ^{99m}Tc still remains indispensable [21].

The reliability of ICG-^{99m}Tc-nanocolloid in assessing LN status in cN0 PeCa patients is affirmed by the finding that merely eight of 740 groins were not staged correctly with DSNB using ICG-^{99m}Tc-nanocolloid (accuracy = 99%; eight FN cases and 84 positive groins = FN rate of 8.7% per groin, and the probability of a negative procedure to become an FN procedure at 2 yr of 1.4%). The FN rate was not affected by T stage or patients who underwent a second/repeat DSNB, and is in line with previous reports using the standard approach with ^{99m}Tc-nanocolloid with blue dye (FN rates ranging from 5% to 22%) [28,29].

The addition of new technologies such as SPECT/CT and fluorescence imaging can help the surgeon to localise the target lesion, but the question remains whether it is logical to expect this to directly translate into a further decrease of the FN rate. First, since DSNB is a combined effort of multiple departments (nuclear medicine, urology, and pathology), either stage of the procedure can potentially cause an FN case, and not all of them can be influenced by improving pre- or intraoperative SN visualisation. Second, as the overall FN rate is highly dependent on the total number of positive events (which tends to be low due to rarity of the disease), the calculation is heavily influenced by each FN case and by improved patient selection (ie, by ultrasound and FNAC), which is supported by the wide 95% CI. Third, biological variability in sequential dissemination, lymph flow variation, and in transit tumour cells are also theoretical reasons for FN procedures [30]. As chemical tracers are not tumour cells and the biology of metastasis is not fully understood yet, it is highly likely that the DSNB procedure will never become 100% accurate.

When evaluating whether the use of blue dye still has complementary value in a DSNB procedure performed with ICG-^{99m}Tc-nanocolloid, we observed that fluorescence guidance yielded a 39% higher SN detection rate than blue dye (95% vs 56%; $p < 0.001$). Furthermore, all SNs ($n = 58$) that were tumour positive at final histopathology could be visualised intraoperatively by fluorescence imaging, whereas 16% ($n = 9$) were missed by blue dye (Table 3). Moreover, 6% of patients with no SNs that coloured blue had positive nodes, and none of the patients with SNs identified based on blue dye colouration only ($n = 3$; four SNs) were positive at final histopathology. All these findings substantiate the results from previous studies with smaller sample sizes and

heterogeneous cohorts that ICG-^{99m}Tc-nanocolloid improves optical SN detection compared with blue dye [10,19]. Hence, we advocate including ICG-^{99m}Tc-nanocolloid as an optional DSNB tracer in a future update of PeCa guidelines. When also considering the drawbacks of blue dye described in the literature such as the need for a second injection, blue staining of the injection site, very limited tissue penetration, and limited effective time window [7,9,10], this study supports omitting blue dye when using ICG-^{99m}Tc-nanocolloid. Regarding costs, there is little difference between blue dye and ICG (estimated additional costs per patient approximately €10, as a vial of ICG can be used for multiple SN procedures). Since most nuclear medicine departments that perform lymphoscintigraphy already have a SPECT/CT scanner, additional costs are mostly limited to the extra scanning time (fees vary across the healthcare systems of different countries). What remains is the one-time investment in a fluorescence camera, which lies within the same price range as the gamma-ray detection probe and are expected to decrease in the near future due to its expanding use and novel (hybrid) camera systems [31]. Based on the above, we have currently adopted ICG-^{99m}Tc-nanocolloid as the new standard SN tracer at our centre (without concomitant injection of blue dye).

Despite its strengths, our analysis is not devoid of limitations. As is the case of virtually all PeCa studies, our report is based on a retrospective analysis with all of its inherent major limitations. The generalisability of our findings may be limited to centres with similar caseload, and our (retrospective) findings still call for multicentre validation with other PeCa centres. Lastly, the observed FN rate is not negligible. This means that there is still room for improvement, and forthcoming efforts should be aimed at finding the optimal balance between maximising diagnostic accuracy and minimising morbidity. Future studies are in preparation to further evolve the procedure from urological, nuclear medicine, and pathological points of view.

5. Conclusions

This study firmly establishes the safety and reliability of hybrid ICG-^{99m}Tc-nanocolloid for SPECT/CT and combined radio- and fluorescence-guided SN identification in a large cohort of PeCa patients. Fluorescence imaging enabled by ICG-^{99m}Tc-nanocolloid vastly improves intraoperative optical SN detection over blue dye, without staining the surgical field or the need for an additional injection.

Author contributions: Oscar R. Brouwer had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.*Study concept and design:* Dell'Oglio, Mazzone, van Leeuwen, Brouwer.
Acquisition of data: de Vries.
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2020.09.007.

References

[1] European Association of Urology. Guidelines on penile cancer. Arnhem, The Netherlands: EAU Guidelines Office; 2020.
[2] Bevan-Thomas R, Slaton JW, Pettaway CA. Contemporary morbidity from lymphadenectomy for penile squamous cell carcinoma: the M. D. Anderson Cancer Center Experience. J Urol 2002;167:1638–42.
[3] Nelson BA, Cookson MS, Smith Jr JA, Chang SS. Complications of inguinal and pelvic lymphadenectomy for squamous cell carcinoma of the penis: a contemporary series. J Urol 2004;172:494–7.
[4] Ficarra V, Zattoni F, Cunico SC, et al. Lymphatic and vascular embolizations are independent predictive variables of inguinal lymph node involvement in patients with squamous cell carcinoma of the penis: Gruppo Uro-Oncologico del Nord Est (Northeast Uro-Oncological Group) Penile Cancer data base data. Cancer 2005;103:2507–16.
[5] Zou ZJ, Liu ZH, Tang LY, et al. Radiocolloid-based dynamic sentinel lymph node biopsy in penile cancer with clinically negative inguinal lymph node: an updated systematic review and meta-analysis. Int Urol Nephrol 2016;48:2001–13.
[6] Horenblas S, Jansen L, Meinhardt W, Hoefnagel CA, de Jong D, Nieweg OE. Detection of occult metastasis in squamous cell carcinoma of the penis using a dynamic sentinel node procedure. J Urol 2000;163:100–4.
[7] Sadeghi R, Gholami H, Zakavi SR, Kakhki VR, Tabasi KT, Horenblas S. Accuracy of sentinel lymph node biopsy for inguinal lymph node staging of penile squamous cell carcinoma: systematic review and meta-analysis of the literature. J Urol 2012;187:25–31.
[8] Pesek S, Ashikaga T, Krag LE, Krag D. The false-negative rate of sentinel node biopsy in patients with breast cancer: a meta-analysis. World J Surg 2012;36:2239–51.
[9] Jung SY, Kim SK, Kim SW, et al. Comparison of sentinel lymph node biopsy guided by the multimodal method of indocyanine green fluorescence, radioisotope, and blue dye versus the radioisotope method in breast cancer: a randomized controlled trial. Ann Surg Oncol 2014;21:1254–9.
[10] Brouwer OR, van den Berg NS, Matheron HM, et al. A hybrid radioactive and fluorescent tracer for sentinel node biopsy in penile carcinoma as a potential replacement for blue dye. Eur Urol 2014;65:600–9.

[11] van Leeuwen AC, Buckle T, Bendle G, et al. Tracer-cocktail injections for combined pre- and intraoperative multimodal imaging of lymph nodes in a spontaneous mouse prostate tumor model. J Biomed Optics 2011;16:016004.
[12] Buckle T, van Leeuwen AC, Chin PT, et al. A self-assembled multimodal complex for combined pre- and intraoperative imaging of the sentinel lymph node. Nanotechnology 2010;21: 355101.
[13] Brouwer OR, Buckle T, Vermeeren L, et al. Comparing the hybrid fluorescent-radioactive tracer indocyanine green-99mTc-nanocolloid with 99mTc-nanocolloid for sentinel node identification: a validation study using lymphoscintigraphy and SPECT/CT. J Nucl Med 2012;53:1034–40.
[14] van der Poel HG, Buckle T, Brouwer OR, Valdes Olmos RA, van Leeuwen FW. Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate cancer patients: clinical proof of concept of an integrated functional imaging approach using a multimodal tracer. Eur Urol 2011;60:826–33.
[15] van den Berg NS, Brouwer OR, Klop WM, et al. Concomitant radio- and fluorescence-guided sentinel lymph node biopsy in squamous cell carcinoma of the oral cavity using ICG-(99m)Tc-nanocolloid. Eur J Nucl Med Mol Imaging 2012;39:1128–36.
[16] Matheron HM, van den Berg NS, Brouwer OR, et al. Multimodal surgical guidance towards the sentinel node in vulvar cancer. Gynecol Oncol 2013;131:720–5.
[17] van den Berg NS, Brouwer OR, Schaafsma BE, et al. Multimodal surgical guidance during sentinel node biopsy for melanoma: combined gamma tracing and fluorescence imaging of the sentinel node through use of the hybrid tracer indocyanine green-(99m)Tc-nanocolloid. Radiology 2015;275:521–9.
[18] Stoffels I, Leyh J, Poppel T, Schadendorf D, Klode J. Evaluation of a radioactive and fluorescent hybrid tracer for sentinel lymph node biopsy in head and neck malignancies: prospective randomized clinical trial to compare ICG-(99m)Tc-nanocolloid hybrid tracer versus (99m)Tc-nanocolloid. Eur J Nucl Med Mol Imaging 2015; 42:1631–8.
[19] KleinJan GH, van Werkhoven E, van den Berg NS, et al. The best of both worlds: a hybrid approach for optimal pre- and intraoperative identification of sentinel lymph nodes. Eur J Nucl Med Mol Imaging 2018;45:1915–25.
[20] Schaafsma BE, Verbeek FP, Rietbergen DD, et al. Clinical trial of combined radio- and fluorescence-guided sentinel lymph node biopsy in breast cancer. Br J Surg 2013;100:1037–44.
[21] van Leeuwen FW, Hardwick JC, van Erkel AR. Luminescence-based imaging approaches in the field of interventional molecular imaging. Radiology 2015;276:12–29.
[22] Nieweg OE, Estourgie SH. What is a sentinel node and what is a false-negative sentinel node? Ann Surg Oncol 2004;11:169S–73S.
[23] Fagerland MW, Lydersen S, Laake P. Recommended tests and confidence intervals for paired binomial proportions. Stat Med 2014; 33:2850–75.
[24] Saad ZZ, Omorphos S, Michopoulou S, et al. Investigating the role of SPECT/CT in dynamic sentinel lymph node biopsy for penile cancers. Eur J Nucl Med Mol Imaging 2017;44:1176–84.
[25] Naumann CM, Colberg C, Juptner M, et al. Evaluation of the diagnostic value of preoperative sentinel lymph node (SLN) imaging in penile carcinoma patients without palpable inguinal lymph nodes via single photon emission computed tomography/computed tomography (SPECT/CT) as compared to planar scintigraphy. Urol Oncol 2018;36, 92 e17-24.
[26] Jimenez-Heffernan A, Ellmann A, et al. Results of a prospective multicenter international atomic energy agency sentinel node trial on the value of SPECT/CT over planar imaging in various malignancies. J Nucl Med 2015;56:1338–44.
[27] Kraft O, Havel M. Sentinel lymph nodes and planar scintigraphy and SPECT/CT in various types of tumours. Estimation of some factors influencing detection success. Nucl Med Rev Cent East Eur 2013; 16:17–25.
[28] Protzel C, Alcaraz A, Horenblas S, Pizzocaro G, Zlotta A, Hakenberg OW. Lymphadenectomy in the surgical management of penile cancer. Eur Urol 2009;55:1075–88.
[29] Neto AS, Tobias-Machado M, Ficarra V, et al. Dynamic sentinel node biopsy for inguinal lymph node staging in patients with penile cancer: a systematic review and cumulative analysis of the literature. Ann Surg Oncol 2011;18:2026–34.
[30] Nieweg OE. False-negative sentinel node biopsy. Ann Surg Oncol 2009;16:2089–91.
[31] Polom W, Markuszewski M, Rho YS, Matuszewski M. Use of invisible near infrared light fluorescence with indocyanine green and methylene blue in urology. Part 2. Cent Eur J Urol 2014;67:310–3.