

1 **A specific mapping study using fluorescence sentinel lymph node detection in patients with**
2 **intermediate- and high-risk prostate cancer undergoing extended pelvic lymph node**
3 **dissection**

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19 **Word count:** abstract 199, main text 1035

20 **Key words:** Indocyanine Green, Lymphadenectomy, Prostate cancer, Sentinel Lymph Node,

21 Mapping

22 **Abstract**

23 Sentinel lymph node (SLN) detection techniques have the potential to change the standard of
24 surgical care of patients with prostate cancer. We performed a lymphatic mapping study and
25 determined the value of fluorescence SLN detection with indocyanine green (ICG) for the
26 detection of lymph node metastases in intermediate- and high-risk patients undergoing radical
27 prostatectomy and extended pelvic lymph node dissection. A total of 42 patients received
28 systematic or specific ICG injections into the prostate base, the mid-portion, the apex, the left
29 lobe or the right lobe. We found that: 1. external and internal iliac regions encompass the
30 majority of SLNs; 2. common iliac regions contain up to 22% of all SLNs; 3. a prostatic lobe can
31 drain into the contralateral group of pelvic lymph nodes; 4. the fossa of Marcille also receives
32 significant drainage. Among the 12 patients who received systematic ICG injections, 5 (42%) had
33 a total of 29 lymph node metastases. Of these, 16 nodes were ICG-positive, yielding 55%
34 sensitivity. The complex drainage pattern of the prostate and the low sensitivity of ICG for the
35 detection of lymph node metastases reported in our study highlight the difficulties related to the
36 implementation of SNL techniques in prostate cancer.

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38 **Patient summary:** There is controversy over how extensive lymph node dissection should be
39 during prostatectomy. We investigated the lymphatic drainage of the prostate and whether
40 sentinel node fluorescence techniques would be useful to detect node metastases. We found that
41 the drainage pattern is complex and that the sentinel node technique is not able to replace
42 extended pelvic lymph node dissection.

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44 **Main text**

45 The role of pelvic lymph nodes dissection (PLND) during radical prostatectomy (RP)
46 remains a matter of continuous debate (1). Sentinel lymph node (SLN) detection has been
47 advanced as a potential alternative to PLND. In prostate cancer, the technique was first described
48 using ^{99m}technetium bound to a colloid (2). However, radio-guided SLN detection has not come
49 into widespread use. The use of the fluorescent dye indocyanine green (ICG) may open the door
50 to a broader acceptance of SLN techniques in prostate cancer surgery (3). Against this
51 background, we provide a comprehensive description of lymphatic landing sites per anatomical
52 region of the prostate using this SLN technique. We also evaluate the sensitivity of ICG-based
53 fluorescence SLN detection to detect lymph node metastases in intermediate- and high-risk
54 patients.

55 Detailed information on patient selection and detection technique is found in
56 Supplementary Patients and Methods. From November 2012 through September 2015, 42
57 patients presenting with clinically localized intermediate- or high-risk prostate cancer and
58 scheduled for RP gave written informed consent to participate in our prospective study. ICG
59 (Pulsion Medical Systems, Feldkirchen, Germany) was injected transrectally shortly prior to
60 laparotomy. The first 12 patients enrolled received sextant injections (six injections into base,
61 mid-portion, and apex of each prostatic lobe peripheral zone). The next 30 patients received
62 injections into one of these sites: 1. prostate base bilaterally; 2. mid-portion bilaterally; 3. apex
63 bilaterally; 4. left lobe (base, mid-portion, apex); or 5. right lobe (base, mid-portion, apex). A
64 near-infrared-sensitive probe (Fluobeam®, Fluoptics, Grenoble, France) was used to collect
65 fluorescence generated in the tissue under real-time image guidance. Independent of the findings
66 of fluorescence SLN detection, an extended PLND was subsequently performed. An ex-vivo

67 fluorescence examination of all dissected lymph nodes was then carried out. Lymphatic landing
68 sites per anatomical region of the prostate were depicted graphically. Diagnostic statistics
69 assessed the value of ICG in detecting lymph node metastases.

70 Baseline characteristics of the patients are summarized in Supplementary Table 1. All 42
71 patients had one or more lymph nodes detected by fluorescence. The lymphatic mapping study
72 showed that (Fig. 1 and Supplementary Table 2): 1. the external and internal iliac regions
73 encompass the majority of SLNs; 2. the common iliac regions contain up to 22% of all SLNs; 3. a
74 prostatic lobe can drain into the contralateral group of pelvic lymph nodes; 4. the fossa of
75 Marcille also receives significant drainage; and 5. practically all sites of the prostate can drain to
76 different regions of the pelvis bilaterally.

77 Thus, the drainage pattern did not show that distinct lymphatic pathways exist per
78 prostatic anatomical region. Our results also underscore crossover of lymphatics to the opposite
79 side and that the common iliac regions and the fossa of Marcille should not be overlooked during
80 PLND, as combined they may contain up to a third of all SLNs. The multitude of lymphatic
81 landing sites as well as the individual variability of lymphatic drainage may represent an obstacle
82 to intra-operative SLN detection. In contrast to breast cancer, in prostate cancer lymph node
83 metastases do not follow a pre-defined pathway of metastatic spread and there is no certainty that
84 the histologic status of the SLN reflects the status of the entire pelvic node basin.

85 Among the 12 patients who received systematic ICG injections a median of 15 SLNs per
86 patient were removed (IQR 10-20). Five patients had a total of 29 lymph node metastases (Table
87 1). Of these, 16 were ICG-positive, yielding a sensitivity for the detection of lymph node
88 metastases of 55%. The negative predictive value was 95%, the specificity was 57%, and the
89 predictive positive value 8%. Repeating analyses with patients as unit of analysis showed that

90 four of five patients had ICG-positive lymph node metastases, yielding a sensitivity for the
91 correct staging of patients of 80%.

92 Hruby et al evaluated 38 patients who underwent laparoscopic RP (4). The sensitivity of
93 the SLN technique with ICG to detect metastases was 98% (42 nodes in 15 patients). Yuen et al
94 reported on 66 patients who underwent RP and SNL detection using ICG (5). Only nine
95 metastases in six patients were detected, all of which were ICG-positive. Our median number of
96 nodes removed was 35 vs. 18 in both previous reports. These findings suggest that our template
97 leads to a notably more complete PLND, which would result in more precise sensitivity analyses.
98 In addition, our median number of SLNs removed was 15 (IQR 10-20), which is in the higher
99 range of most previous studies using ICG alone or in combination with a radio-colloid (3–6).
100 Other authors may not have searched as thoroughly for SLNs in surgically less accessible regions
101 such as the proximal part of the common iliac vessels, in the fossa of Marcille and in the
102 presacral regions. In Yuen et al’s study, only 2% and 1% of all SLNs were found in the common
103 iliac and presacral regions, respectively (5).

104 The low sensitivity of ICG to detect metastases in the current study raises the concern of
105 skip metastases. The presence of tumor cells in the lymph nodes may clog the feeding lymphatics
106 and interfere with their ability to take up ICG, as it has been postulated for radio-colloids (7).
107 Interestingly, we could sometimes visualize that lymphatic drainage goes around enlarged nodes.
108 Going forward, emerging molecular imaging modalities using dyes conjugated with tumor-
109 specific peptides may provide added sensitivity (8–10). It has to be noted that it is not possible to
110 discriminate with certainty between primary landing sites and higher levels of drainage.
111 However, the fact that fluorescent lymph nodes appeared 15-30 min after ICG injection and that
112 direct drainage through lymphatic vessels could be seen in some cases suggests that these lymph
113 nodes can be considered SLNs. Our study is limited by its small sample size. Nevertheless, it

114 included a high number of lymph node metastases in the setting of extended PLND, allowing
115 appropriate sensitivity analyses.

116 In conclusion, our lymphatic mapping study delineated a complex drainage pattern of the
117 prostate that questions whether targeted lymph node dissection can be implemented in prostate
118 cancer. Together with the low sensitivity for the detection of metastases, these results suggest that
119 for the time being fluorescence SNL detection does not represent an alternative to a meticulously
120 performed PLND in higher-risk patients.

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122 **Conflicts of interest:** the Fluobeam® imaging device was provided at no charge by Fluoptics.
123 However, the authors had complete control of the data and information submitted for publication.

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127 **Figure legend**

128 Fig. 1. Percentages of sentinel nodes detected per drainage region with regard to anatomical sites
129 of the prostate. The ICG injection sites are depicted in the upper right corner. The orange zones
130 represent the external iliac regions, the yellow zones the internal iliac regions, the green zones the
131 common iliac regions, and the regions delineated by dashed lines the fossas of Marcille.

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136 **6. References**

- 137 1. Studer UE. Should pelvic lymph node dissection be performed with radical prostatectomy?
138 Yes. *J Urol.* 2010;183:1285–1287.
- 139 2. Wawroschek F, Wagner T, Hamm M, et al. The influence of serial sections,
140 immunohistochemistry, and extension of pelvic lymph node dissection on the lymph node
141 status in clinically localized prostate cancer. *Eur Urol.* 2003;43:132–136; discussion 137.
- 142 3. Van Der Poel HG, Buckle T, Brouwer OR, Valdés Olmos RA., Van Leeuwen FW.
143 Intraoperative laparoscopic fluorescence guidance to the sentinel lymph node in prostate
144 cancer patients: clinical proof of concept of an integrated functional imaging approach
145 using a multimodal tracer. *Eur Urol.* 2011;60:826–833.
- 146 4. Hruby S, Englberger C, Lusuardi L, et al. Fluorescence guided targeted pelvic lymph node
147 dissection for intermediate and high risk prostate cancer. *J Urol.* 2015;194:357–363.
- 148 5. Yuen K, Miura T, Sakai I, Kiyosue A, Yamashita M. Intraoperative fluorescence imaging
149 for detection of sentinel lymph nodes and lymphatic vessels during open prostatectomy
150 using indocyanine green. *J Urol.* 2015;194:371–377.
- 151 6. Jeschke S, Lusuardi L, Myatt A, Hruby S, Pirich C, Janetschek G. Visualisation of the
152 lymph node pathway in real time by laparoscopic radioisotope- and fluorescence-guided
153 sentinel lymph node dissection in prostate cancer staging. *Urology.* 2012;80:1080–1086.
- 154 7. Weckermann D, Dorn R, Holl G, Wagner T, Harzmann R. Limitations of radioguided
155 surgery in high-risk prostate cancer. *Eur Urol.* 2007;51:1549–1556; discussion 1556-1558.
- 156 8. Nakajima T, Mitsunaga M, Bander NH, Heston WD, Choyke PL, Kobayashi H. Targeted,
157 activatable, in vivo fluorescence imaging of prostate-specific membrane antigen (PSMA)
158 positive tumors using the quenched humanized J591 antibody - indocyanine green (ICG)
159 conjugate. *Bioconjug Chem.* 2011;22:1700–1705.
- 160 9. Ulmert D, Evans MJ, Holland JP, et al. Imaging androgen receptor signaling with a
161 radiotracer targeting free prostate-specific antigen. *Cancer Disc* 2012;2(4):320-7.
- 162 10. Cai QY, Yu P, Besch-Williford C, et al. Near-infrared fluorescence imaging of gastrin
163 releasing peptide receptor targeting in prostate cancer lymph node metastases. *Prostate.*
164 2013;73:842–854.