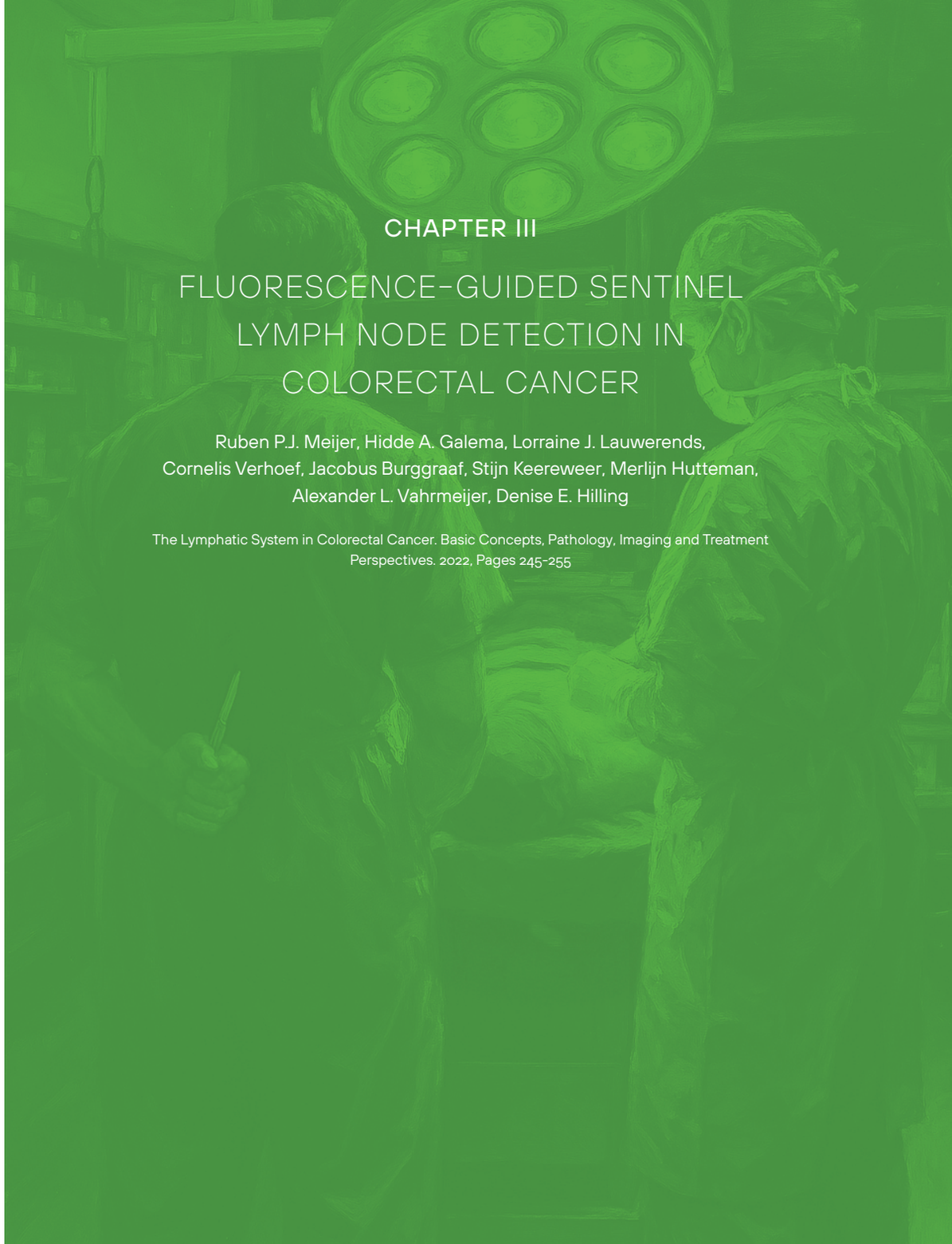
Near-infrared fluorescence imaging in colorectal cancer and its metastases

Meijer, R.P.J.



CHAPTER III

FLUORESCENCE-GUIDED SENTINEL LYMPH NODE DETECTION IN COLORECTAL CANCER

Ruben P.J. Meijer, Hidde A. Galema, Lorraine J. Lauwerends, Cornelis Verhoef, Jacobus Burggraaf, Stijn Keereweer, Merlijn Hutteman, Alexander L. Vahrmeijer, Denise E. Hilling

The Lymphatic System in Colorectal Cancer. Basic Concepts, Pathology, Imaging and Treatment Perspectives. 2022, Pages 245-255

Abstract

Sentinel lymph node (SLN) mapping can be a valuable addition to the treatment of colorectal cancer patients. Nevertheless, conventional lymph node mapping methods using blue dye are limited due to inadequate depth penetration, and the use of a radiocolloid tracer has its logistic hurdles. With near-infrared (NIR) fluorescence imaging, the SLN can be accurately identified in most patients resulting in more accurate lymph node staging. Current technical challenges and the low negative predictive value of the SLN withhold surgeons from its use in daily practice.

Concept of sentinel lymph node mapping

Adequate lymph node staging is important in colorectal cancer (CRC) treatment, as lymph node metastases are an important determinant for patient prognosis and an indication for adjuvant systemic treatment. The sentinel lymph node (SLN) is defined as the first group of lymph node(s) draining a tumour. The identification, removal, and analysis of these SLNs (SLN mapping) can therefore be of added value for the staging of CRC patients, and subsequent treatment. SLN mapping was first described in 1960 for parotid cancer and is nowadays standard of care in breast cancer and melanoma patients.[1-3]

The SLN concept could also be of added value in CRC patients. Patients with stage I and II diseases (with no lymph node involvement) still develop distant metastases in up to 30% of cases.[4] This could be the result of, among others, understaging of these patients due to missed lymph nodes with occult tumour cells and micrometastases during routine histopathological examination, or inadequate lymph node harvesting at the time of primary treatment. Routine histopathological examination currently exists of reviewing a single paraffin-embedded slide per lymph node, with the chance of missing tumour cells away from the slide's cutting edge. Extensive histopathological analysis of all lymph nodes using serial sectioning or reverse transcriptase polymerase chain reaction could result in more accurate lymph node staging. However, both methods are expensive and time consuming.[5-7] As the SLN procedure identifies the lymph node(s) with the highest chance of containing metastases, more extensive histopathological analysis of only these lymph nodes is feasible. Furthermore, tumour-negative SLNs create an opportunity for local or endoscopic resection of CRC, especially in early-stage tumours.[8]

Conventional methods for SLN mapping include the use of either blue dye, a radiocolloid tracer, or the combination of both.[2,3] The use of blue dye for SLN mapping in CRC is limited due to inadequate depth penetration and the utilization of radiocolloid tracers has some logistic hurdles.[9] A nuclear medicine physician and an endoscopist are required for tracer injection. Moreover, the gamma probe used for localization does not enable real-time visualization. These shortcomings have increased the interest in novel techniques, such as near-infrared (NIR) fluorescence imaging.

Fluorescence-guided surgery

NIR fluorescence imaging provides the surgeon with real-time information of the surgical field and can aid in differentiation between malignant and healthy tissue during surgery.[10] NIR light (700-900 nm) is not visible to the human eye and has relatively deep (up to 10 mm) tissue penetration. Human tissue itself has low autofluorescence in the NIR light spectrum, resulting in a high signal-to-background ratio.[11] NIR fluorescence-guided surgery requires two components: an NIR camera system and a fluorescent agent (figure 1). These NIR camera systems are composed of an NIR excitation light source, collection optics (including optical filtration), and a camera that can detect emitted NIR light. These systems emit photons with a specific wavelength, which are absorbed by the fluorescent agent. Electrons within these agent's molecules transit to an excited state and fall back to their ground state (figure 2). This will release the stored energy in an emitted photon with a longer wavelength than the exciting light of the NIR camera system, the so-called Stokes shift. This emitted photon is subsequently captured by the camera system. The camera output is usually displayed on a monitor including a merged image of the fluorescence signal and the white light image. Both the camera systems and fluorescent agents have shown great improvements in the last decades and have resulted in the clinical use of NIR fluorescence for different purposes during surgery (e.g., bile duct detection, tissue perfusion).[12-14]

Several dedicated NIR fluorescence imaging systems are clinically used for open, laparoscopic, and robotic surgery. On the other hand, only two fluorescent agents, indocyanine green (ICG) and methylene blue, have been approved for clinical use by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). Both are nontargeted fluorescent agents, meaning these agents do not bind to a specific target. ICG, first described in 1957 for the determination of cardiac output, is the most used agent for fluorescence-guided SLN mapping in various cancer types and can also be used for the visualization of vital structures (e.g., bile ducts), liver tumours, and assessment of tissue perfusion.[14-15]

FIGURE 1 Fluorescence-guided surgery.
NIR fluorescent agents are administered intravenously or locally. NIR fluorescence is visualized using a specialized imaging system for intraoperative imaging. The imaging system uses dedicated NIR excitation light to excite the fluorophore. Collection optics, emission filters, and an image sensor capable of detecting NIR fluorescence emission light. The NIR fluorescence signal is displayed on a monitor in the surgical theater. A simultaneous white light image, which can be merged with the NIR fluorescence image, is desirable.

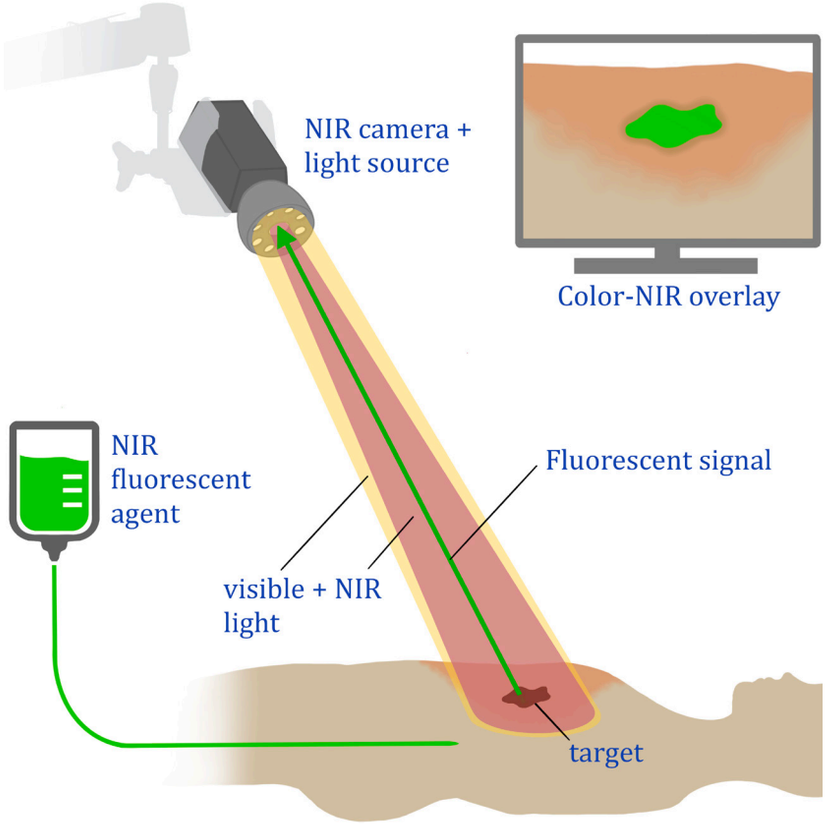
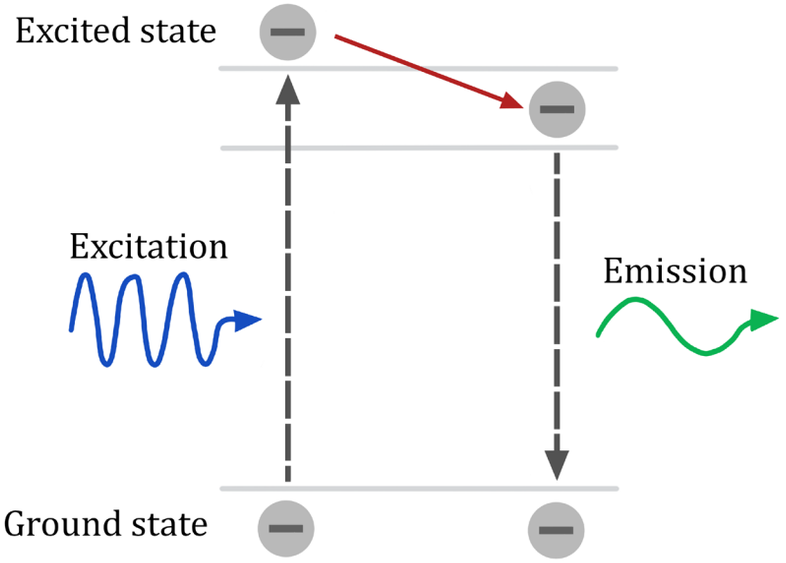


FIGURE 2 Schematic overview of the principle of fluorescence.
A photon in the appropriate wavelength (excitation light) is absorbed by the fluorophore, elevating an electron to an excited state. When the electron transitions back to its ground state, a photon is emitted. This emitted photon is of a longer wavelength and lower energy.



Fluorescence-guided sentinel lymph node detection in colorectal cancer

While peritumoural injection of ICG is the most used technique for NIR fluorescence guided SLN mapping, alternative fluorescent dyes have also been assessed (figure 3). In addition, different injection sites and variable timing of the injection have been investigated.

The injection site of ICG can be either subserosal or submucosal, with a slight preference for the latter.[16-18] The submucosa houses an important part of the intestinal lymphatic system, which might improve the lymphatic uptake of the fluorescent agent from the tumour surrounding tissue.[19] Submucosal injection is performed prior to or during surgery, via endoscopy. Subserosal injection, on the other hand, is performed intraoperatively by the surgeon. In minimally invasive surgery, this requires transcutaneous injection of the fluorescent agent. Correct positioning of the needle and maintaining this position during injection of the fluorescent agent is easier with the submucosal technique and therefore leads to less spillage of ICG.[16-17]

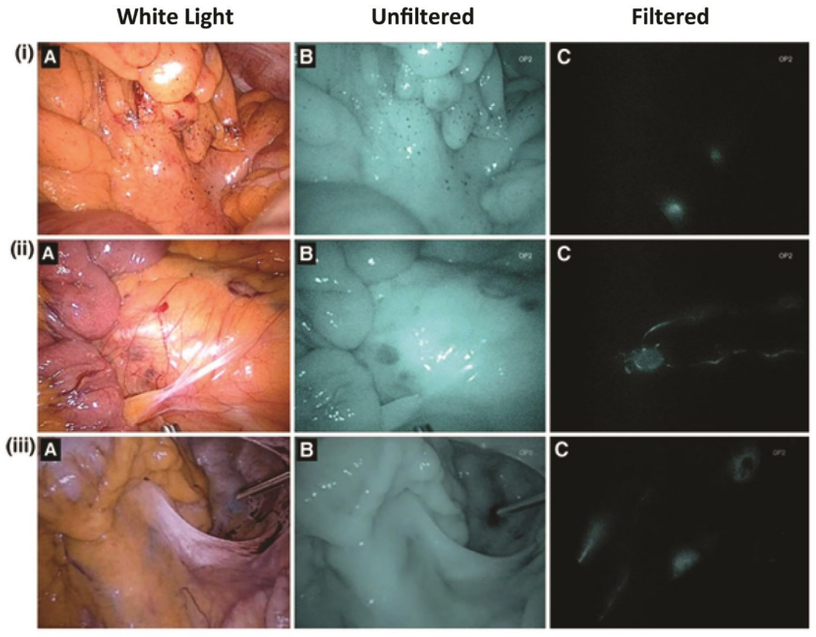
Timing of fluorescent dye administration and assessment has been assessed directly pre- or intraoperatively (both referred to as *in vivo*), and postoperatively (*ex vivo*). *In vivo* administration has some practical drawbacks, particularly for the preferred submucosal injection. For *in vivo* submucosal injection, an endoscopy in the operating room is required directly before or during surgery, which poses a logistical challenge. Furthermore, bowel insufflation might alter the surgical field and therefore hamper the surgical procedure. The alternative, *ex vivo* imaging, is logistically simpler and enables the use of experimental agents. *Ex vivo* imaging also has some disadvantages. Lymphatic flow may be disrupted after resection, and altering the surgical plan (i.e., perform a more limited resection) based on histopathological analysis of the harvested lymph nodes is not possible. Moreover, *ex vivo* fluorescent agent injection and lymph node identification does not facilitate identifying SLNs in patients with aberrant lymph node drainage patterns.[20]

Table 1 summarizes studies that describe fluorescence-guided SLN mapping in CRC patients. A procedure is defined as successful if one or more lymph nodes were identified by fluorescence (the SLN). An upstaged patient is defined as a patient who was staged as No (all lymph nodes being tumour-negative) using conventional histopathology but showed tumour-positive SLNs after additional extensive histopathological assessment of the SLNs. These patients can consequently change from stage I or II CRC to stage III. The percentage of upstaged patients was calculated by dividing the number of upstaged patients by all lymph node-negative patients before extensive histopathological assessment of the SLN.

In all studies, intraoperative identification of the SLNs was performed using ICG and this was successful in most cases. Andersen *et al.* had a remarkably lower success rate of 65.5% in their multicenter trial, with all other studies being single center.[16] This could be explained by a learning curve, which is suggested by Bembenek *et al.* to be more than 22 cases per center, a number none of their centers had reached.[9] The sensitivity of SLN identification with ICG ranged from 0.33 to 1, and the negative predictive value was relatively low, with only three (33%) studies reporting an NPV above 0.9.

FIGURE 3 Intraoperative results of sentinel lymph node mapping using with indocyanine green.

Three patients (I-III) demonstrating NIR fluorescence-guided sentinel lymph node mapping with indocyanine green (A, a white light image; B, an NIR image without filter; and C, a filtered NIR image). (I) Two bright spots in the mesocolon were identified in the filtered view, consistent with sentinel lymph nodes. (II) A bright spot is seen in the filtered view, consistent with a paraaortic sentinel lymph node. (III) A bright spot is seen in the filtered view, alongside the right iliac artery, consistent with a sentinel lymph node. The injection site of the rectal tumour is clearly visible, as well as an efferent channel in the sigmoid mesentery which was found to lead to another sentinel lymph node.



Source: Reproduced by permission from Cahill, R. A., Anderson, M., Wang, L. M., Lindsey, I., Cunningham, C., Mortensen, N. J. (2012) (40). Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. Surgical Endoscopy, 26(1), 197-204.

TABLE 1 Results of clinical trials assessing fluorescence-guided SLN mapping for CRC.

	Agent	Patients	Diagnosis	Procedure	Injection site	Number SLNs	Success rate	Sensitivity	NPV	Upstaged patients* (%)
INTRAOPERATIVE										
Andersen (16)	ICG:HA	29	CC	L	SS	1 (0-3)*	65.50%	0.33	0.76	1 (12%)
Ankersmit (17)	ICG:HA	29	CC	L	14 SS; 15 SM	2 (0-6)*	89.70%	0.44	0.8	3 (13%)
Cahill (39)	ICG	18	CRC	L	SM	3.6 (1-5)**	100%	1	1	0 (0%)
Carrara (40)	ICG	95	CRC	L	SS	1.5 (1-5)**	96.8	0.73	0.96	1 (1%)
Currie (41)	ICG	30	CC	L	SM	3 (1-4)***	90%	0.33	0.75	1 (5%)
Hirche (42)	ICG	26	CC	O	SS	1.7 (0-5)**	96%	0.82	0.87	3 (21%)
Kusano (43)	ICG	26	CRC	O	SS	2.6 (± 2.4)****	88.50%	0.5	0.81	NR
Nagata (44)	ICG	48	CRC	L	SS	3.5 (± 1.7) ****	97.90%	0.44	0.89	NR
Noura (45)	ICG	25	RC	O	SM	2.1 (± 0.8)****	92%	1	1	NR
Watanabe (46)	ICG	31	CC	L	SS	10.4 (± 4.73)****	100%	0.67	0.93	NR
POSTOPERATIVE										
Hutteman (18)	HSA800	24	CRC	NA	SM	3 (1-5)*	100%	0.89	0.94	NR
Liberale (47)	ICG	20	CC	NA	SS	1 (0-4)*	95%	0.57	0.81	3 (23%)
Schaafsma (48)	HSA800	22	CC	NA	SM	3.5 (± 1.9)****	95%	0.8	0.94	NR
Weixler (49)	HSA800	50	CC	NA	SS	4.4 (± 2.2)****	98%	0.64	0.74	5 (17%)

The number of detected SLNs are presented as: *median with range, **mean with range, ***median with interquartile range, ****mean with standard deviation. The sensitivity is calculated by dividing the number of procedures with a tumour-positive SLN (true positives) by the sum of true positive and false negative procedures. The negative predictive value is determined by dividing the amount of true negative procedures by the sum of true negative and false negative procedures. Upstaged patients are defined as patients with no tumour involvement on conventional histopathology of all lymph nodes, but a tumour-positive SLN at advanced histopathology. The percentage of upstaged patients is calculated as: upstaged patients/upstaged patients + true negatives. Abbreviations: CC = Colon cancer; CRC = colorectal cancer; HA = human albumin; ICG = indocyanine green; L = laparoscopic; NA = not applicable; NPV = negative predictive value; NR = not reported; O = open; RC = rectal cancer; SLN = sentinel lymph nodes; SM = submucosal; SS = subserosal.

HSA800 (IRDye 800CW conjugated to human serum albumin) is another fluorescent agent used for *ex vivo* SLN mapping in CRC patients, which has not been approved by the FDA or EMA yet. Preclinical studies have shown an advantage of HSA800 over ICG regarding lymphatic entry, flow, fluorescence yield, and reproducibility. This is most likely a result of its bigger hydrodynamic diameter, resulting in improved retention in the SLN.[21] Clinical *ex vivo* studies with HSA800 have shown comparable results to *in vivo* assessment with ICG with a wide range in sensitivity (0.64-0.89) and negative predictive value (0.74-0.94).

Future perspectives

Fluorescence-guided SLN mapping has the potential to improve adequate staging in CRC patients. Despite its advantages and several published clinical studies, it is not used in common day practice. This might be the result of technical and logistic hurdles. Moreover, it is unknown if the upstaging of patients with micrometastatic lymph nodes and subsequent adjuvant treatment will lead to improved patient outcomes.

The number of early-stage CRC patients is expected to increase in the coming years, due to the introduction of nationwide screening programs.[22,23] With this increasing number of early-stage CRC patients, the number of lymph node-negative patients is also expected to rise, since 90% of the T1 tumours are No.[24,25] Especially in these patients SLN mapping might be valuable. Because of the low incidence of lymph node metastases in these patients, a reliable SLN procedure showing a tumour-negative SLN enables the possibility for local excision without an extensive lymphadenectomy, thereby potentially lowering perioperative morbidity.[8]

The relatively low negative predictive value of the SLNs (the probability that in case of a tumour-negative SLN, all other regional lymph nodes are tumour negative) is an important reason that this procedure is not yet implemented in daily practice. It withholds surgeons from performing a local excision and omitting an oncological resection based on a tumour-negative SLN. The low NPV is mainly a consequence of a high false negative rate (tumour-negative SLNs in the presence of a tumour-positive regional lymph node). One explanation for this high false negative rate is the occurrence of the so-called skip metastases, which are reported in 10-22% of the cases.[26,27] Tumour size could be another reason for this high false negative rate. T3-T4 tumours showed false negative results in 23% of the cases compared to 2% of the T1-T2 tumours.[28] It is suggested that these more invasive tumours (T3-T4) alter the lymphatic flow, resulting in skip metastases.

Based on the promising preliminary results, the interest in neoadjuvant treatment for colon cancer has increased in recent years.[29,30] This novel treatment strategy could influence the success rate of SLN mapping, as research in other tumour types suggest altered lymphatic flow after neoadjuvant treatment.[31] As a result, it could be preferable to perform SLN mapping prior to neoadjuvant therapy.

A meta-analysis by Ankersmit *et al.* showed a pooled upstaging (no tumour involvement on conventional histopathology, but a tumour-positive SLN at advanced histopathology) in 15% of the patients.[17] This means that roughly one out of seven patients is wrongly classified as No without the use of fluorescence imaging and extensive histopathological assessment of the SLN. These patients would not have been upstaged to stage III and wrongfully been withheld adjuvant therapy, which theoretically leads to worse survival.

As emphasized, the use of fluorescence-guided SLN mapping with ICG increases the detection rate of SLNs in CRC patients and can result in upstaging in a substantial number of patients. Nevertheless, this concept still requires postoperative histopathological analysis. Direct intraoperative feedback regarding the malignancy status of any lymph node could be provided with the use of tumour-targeted fluorescence-guided surgery. Tumour-targeted agents consist of a fluorophore conjugated to a targeting component and therefore possess strong binding affinity for a specific cancer-associated molecular target or biomarker.[32] Unfortunately, tumour-targeted tracers tend to show a relatively high false positive rate (fluorescent lymph node without tumour localization) of 7%-33% for lymph node imaging, due to aspecific tracer localization.[33-35] On the other hand, it is still debated whether a small tumour load (micrometastases and lymph nodes with isolated tumour cells) accumulate enough volume of the tracer to produce a sufficiently enhanced fluorescent signal. Nevertheless, tumour-targeted agents do not only allow for the identification of lymph node metastases but also other metastases, the primary tumour and tumour-positive resection margins.[36] Several tumour-targeted agents are currently studied in phase II and III trials (SGM-101 in Locally Advanced and Recurrent Rectal Cancer.[37,38]

Conclusions

Fluorescence-guided SLN mapping in CRC can be a valuable addition to detect micrometastases and occult metastases in locoregional lymph nodes. It can result in upstaging in a significant part of the patients, whom otherwise would not have received adjuvant therapy. The low negative predictive value appears to be an important reason for the delayed introduction to current standard of care. Tumour-targeted fluorescent agents might overcome these shortcomings in the future.

REFERENCES

1 Gould EA, Winship T, Philbin PH, Kerr HH. Observations on a "sentinel node" in cancer of the parotid. Cancer. 1960;13:77-8.

2 He PS, Li F, Li GH, Guo C, Chen TJ. The combination of blue dye and radioisotope versus radioisotope alone during sentinel lymph node biopsy for breast cancer: a systematic review. BMC Cancer. 2016;16:107.

3 Valsecchi ME, Silbermins D, de Rosa N, Wong SL, Lyman GH. Lymphatic mapping and sentinel lymph node biopsy in patients with melanoma: a meta-analysis. J Clin Oncol. 2011;29(11):1479-87.

4 Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev. 2008;2008(3):CD005390.

5 Doekhie FS, Mesker WE, Kuppen PJ, van Leeuwen GA, Morreau H, de Bock GH, *et al.* Detailed examination of lymph nodes improves prognostication in colorectal cancer. Int J Cancer. 2010;126(11):2644-52.

6 Liefers GJ, Cleton-Jansen AM, van de Velde CJ, Hermans J, van Krieken JH, Cornelisse CJ, *et al.* Micrometastases and survival in stage II colorectal cancer. N Engl J Med. 1998;339(4):223-8.

7 Yamamoto H, Murata K, Fukunaga M, Ohnishi T, Noura S, Miyake Y, *et al.* Micrometastasis Volume in Lymph Nodes Determines Disease Recurrence Rate of Stage II Colorectal Cancer: A Prospective Multicenter Trial. Clin Cancer Res. 2016;22(13):3201-8.

8 Cahill RA, Leroy J, Marescaux J. Localized resection for colon cancer. Surg Oncol. 2009;18(4):334-42.

9 Bembenek AE, Rosenberg R, Wagler E, Gretschel S, Sendler A, Siewert JR, *et al.* Sentinel lymph node biopsy in colon cancer: a prospective multicenter trial. Ann Surg. 2007;245(6):858-63.

10 Vahrmeijer AL, Hutteman M, van der Vorst JR, van de Velde CJ, Frangioni JV. Image-guided cancer surgery using near-infrared fluorescence. Nat Rev Clin Oncol. 2013;10(9):507-18.

11 Frangioni JV. New technologies for human cancer imaging. J Clin Oncol. 2008;26(24):4012-21.

12 Griffiths M, Chae MP, Rozen WM. Indocyanine green-based fluorescent angiography in breast reconstruction. Gland Surg. 2016;5(2):133-49.

13 van den Hoven P, Ooms S, van Manen L, van der Bogt KEA, van Schaik J, Hamming JF, *et al.* A systematic review of the use of near-infrared fluorescence imaging in patients with peripheral artery disease. J Vasc Surg. 2019;70(1):286-97 e1.

14 van Manen L, Handgraaf HJM, Diana M, Dijkstra J, Ishizawa T, Vahrmeijer AL, *et al.* A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. J Surg Oncol. 2018;118(2):283-300.

15 Fox IJ, Brooker LG, Heseltine DW, Essex HE, Wood EH. A tricarbocyanine dye for continuous recording of dilution curves in whole blood independent of variations in blood oxygen saturation. Proc Staff Meet Mayo Clin. 1957;32(18):478-84.

16 Andersen HS, Bennedsen ALB, Burgdorf SK, Eriksen JR, Eiholm S, Toxværd A, *et al. In vivo* and *ex vivo* sentinel node mapping does not identify the same lymph nodes in colon cancer. Int J Colorectal Dis. 2017;32(7):983-90.

17 Ankersmit M, Bonjer HJ, Hannink G, Schoonmade LJ, van der Pas M, Meijerink W. Near-infrared fluorescence imaging for sentinel lymph node identification in colon cancer: a prospective single-center study and systematic review with meta-analysis. Tech Coloproctol. 2019;23(12):1113-26.

18 Hutteman M, Choi HS, Mieog JS, van der Vorst JR, Ashitate Y, Kuppen PJ, *et al.* Clinical translation of *ex vivo* sentinel lymph node mapping for colorectal cancer using invisible near-infrared fluorescence light. Ann Surg Oncol. 2011;18(4):1006-14.

19 Miller MJ, McDole JR, Newberry RD. Microanatomy of the intestinal lymphatic system. Ann N Y Acad Sci. 2010;1207 Suppl 1(Suppl 1):E21-8.

20 Tuech JJ, Pessaux P, Regenet N, Bergamaschi R, Colson A. Sentinel lymph node mapping in colon cancer. Surg Endosc. 2004;18(12):1721-9.

21 Ohnishi S, Lomnes SJ, Laurence RG, Gogbashian A, Mariani G, Frangioni JV. Organic alternatives to quantum dots for intraoperative near-infrared fluorescent sentinel lymph node mapping. Mol Imaging. 2005;4(3):172-81.

22 Cardoso R, Guo F, Heisser T, Hackl M, Ihle P, De Schutter H, *et al.* Colorectal cancer incidence, mortality, and stage distribution in European countries in the colorectal cancer screening era: an international population-based study. Lancet Oncol. 2021;22(7):1002-13.

23 Navarro M, Nicolas A, Ferrandez A, Lanas A. Colorectal cancer population screening programs worldwide in 2016: An update. World J Gastroenterol. 2017;23(20):3632-42.

24 Fields AC, Lu P, Hu F, Hirji S, Irani J, Bleday R, *et al.* Lymph Node Positivity in T1/T2 Rectal Cancer: a Word of Caution in an Era of Increased Incidence and Changing Biology for Rectal Cancer. J Gastrointest Surg. 2021;25(4):1029-35.

25 Ricciardi R, Madoff RD, Rothenberger DA, Baxter NN. Population-based analyses of lymph node metastases in colorectal cancer. Clin Gastroenterol Hepatol. 2006;4(12):1522-7.

26 Bao F, Zhao LY, Balde AI, Liu H, Yan J, Li TT, *et al.* Prognostic impact of lymph node skip metastasis in Stage III colorectal cancer. Colorectal Dis. 2016;18(9):O322-9.

27 Saha S, Sehgal R, Patel M, Doan K, Dan A, Bilchik A, *et al.* A multicenter trial of sentinel lymph

node mapping in colorectal cancer: prognostic implications for nodal staging and recurrence. Am J Surg. 2006;191(3):305-10.
28 Burghgraef TA, Zweep AL, Sikkenk DJ, van der Pas M, Verheijen PM, Consten ECJ. *In vivo* sentinel lymph node identification using fluorescent tracer imaging in colon cancer: A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2021;158:103149.
29 Karoui M, Rullier A, Piessen G, Legoux JL, Barbier E, De Chaisemartin C, *et al.* Perioperative FOLFOX 4 Versus FOLFOX 4 Plus Cetuximab Versus Immediate Surgery for High-Risk Stage II and III Colon Cancers: A Phase II Multicenter Randomized Controlled Trial (PRODIGE 22). Ann Surg. 2020;271(4):637-45.
30 Seymour MT, Morton, D. FOxTROT: an international randomised controlled trial in 1052 patients (pts) evaluating neoadjuvant chemotherapy (NAC) for colon cancer. Journal of Clinical Oncology. 2019;37(15).
31 Kuehn T, Bauerfeind I, Fehm T, Fleige B, Hausschild M, Helms G, *et al.* Sentinel-lymph-node biopsy in patients with breast cancer before and after neoadjuvant chemotherapy (SENTINA): a prospective, multicentre cohort study. Lancet Oncol. 2013;14(7):609-18.
32 Hernot S, van Manen L, Debie P, Mieog JSD, Vahrmeijer AL. Latest developments in molecular tracers for fluorescence image-guided cancer surgery. Lancet Oncol. 2019;20(7):e354-e67.
33 de Valk KS, Deken MM, Handgraaf HJM, Bhairosingh SS, Bijlstra OD, van Esdonk MJ, *et al.* First-in-Human Assessment of cRGD-ZW800-1, a Zwitterionic, Integrin-Targeted, Near-Infrared Fluorescent Peptide in Colon Carcinoma. Clin Cancer Res. 2020;26(15):3990-8.
34 Lu G, van den Berg NS, Martin BA, Nishio N, Hart ZP, van Keulen S, *et al.* Tumour-specific fluorescence-guided surgery for pancreatic cancer using panitumumab-IRDye800CW: a phase 1 single-centre, open-label, single-arm, dose-escalation study. Lancet Gastroenterol Hepatol. 2020;5(8):753-64.
35 Rosenthal EL, Moore LS, Tipirneni K, de Boer E, Stevens TM, Hartman YE, *et al.* Sensitivity and Specificity of Cetuximab-IRDye800CW to Identify Regional Metastatic Disease in Head and Neck Cancer. Clin Cancer Res. 2017;23(16):4744-52.
36 de Valk KS, Deken MM, Schaap DP, Meijer RP, Boogerd LS, Hoogstins CE, *et al.* Dose-Finding Study of a CEA-Targeting Agent, SGM-101, for Intraoperative Fluorescence Imaging of Colorectal Cancer. Ann Surg Oncol. 2021;28(3):1832-44.
37 SGM-101 in Locally Advanced and Recurrent Rectal Cancer (SGM-LARRC) [Internet]. clinicaltrials.gov. 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT04642924.
38 Performance of SGM-101 for the Delineation of Primary and Recurrent Tumour and Metastases in Patients Undergoing Surgery for Colorectal Cancer [Internet]. Clinicaltrials.gov. 2018. Available from: https://clinicaltrials.gov/ct2/show/NCT03659448.
39 Cahill RA, Anderson M, Wang LM, Lindsey I, Cunningham C, Mortensen NJ. Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. Surg Endosc. 2012;26(1):197-204.
40 Carrara A, Motter M, Amabile D, Pellecchia L, Moscatelli P, Pertile R, *et al.* Predictive value of the sentinel lymph node procedure in the staging of non-metastatic colorectal cancer. Int J Colorectal Dis. 2020;35(10):1921-8.
41 Currie AC, Brigic A, Thomas-Gibson S, Suzuki N, Moorghen M, Jenkins JT, *et al.* A pilot study to assess near infrared laparoscopy with indocyanine green (ICG) for intraoperative sentinel lymph node mapping in early colon cancer. Eur J Surg Oncol. 2017;43(11):2044-51.
42 Hirche C, Mohr Z, Kneif S, Doniga S, Murawa D, Strik M, *et al.* Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. Int J Colorectal Dis. 2012;27(3):319-24.
43 Kusano M, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M. Sentinel node mapping guided by indocyanine green fluorescence imaging: a new method for sentinel node navigation surgery in gastrointestinal cancer. Dig Surg. 2008;25(2):103-8.
44 Nagata K, Endo S, Hidaka E, Tanaka J, Kudo SE, Shiokawa A. Laparoscopic sentinel node mapping for colorectal cancer using infrared ray laparoscopy. Anticancer Res. 2006;26(3B):2307-11.
45 Noura S, Ohue M, Seki Y, Tanaka K, Motoori M, Kishi K, *et al.* Feasibility of a lateral region sentinel node biopsy of lower rectal cancer guided by indocyanine green using a near-infrared camera system. Ann Surg Oncol. 2010;17(1):144-51.
46 Watanabe J, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K. Evaluation of lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time indocyanine green fluorescence imaging. Int J Colorectal Dis. 2017;32(2):201-7.
47 Liberale G, Vankerckhove S, Galdon MG, Larsimont D, Ahmed B, Bouazza F, *et al.* Sentinel Lymph Node Detection by Blue Dye Versus Indocyanine Green Fluorescence Imaging in Colon Cancer. Anticancer Res. 2016;36(9):4853-8.
48 Schaafsma BE, Verbeek FP, van der Vorst JR, Hutteman M, Kuppen PJ, Frangioni JV, *et al. Ex vivo* sentinel node mapping in colon cancer combining blue dye staining and fluorescence imaging. J Surg Res. 2013;183(1):253-7.
49 Weixler B, Rickenbacher A, Raptis DA, Viehl CT, Guller U, Rueff J, *et al.* Sentinel Lymph Node Mapping with Isosulfan Blue or Indocyanine Green in Colon Cancer Shows Comparable Results and Identifies Patients with Decreased Survival: A Prospective Single-Center Trial. World J Surg. 2017;41(9):2378-86.