INNOVATIVE MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

TOWARDS PERSONALIZED TREATMENT

MARJOLEIN ANKERSMIT

COLOFON

INNOVATIVE MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

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'Success consists of going from failure to failure without loss of enthusiasm'

Sir Winston Churchill

Voor mijn vader

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PART II Molecular image-guided surgery during laparoscopic cholecystectomy

INTRODUCTION AND PERSPECTIVES OF THE THESIS

GENERAL INTRODUCTION

Image-guided surgery refers to any procedure in which a surgeon uses preoperative or intraoperative images in order to directly or indirectly guide resection. Image-guided surgery helps surgeons to perform a safer and less invasive procedure, while obtaining more effective removal of affected tissue. Preoperative and intraoperative imaging modalities have gained enormous interest over the last decades, along with general improvements in healthcare that have resulted in new diagnosis and treatment options, healthy aging and longer survival of patients with previously deadly diseases.

In this thesis we will focus on perioperative imaging during abdominal surgery. Excluding bariatric surgery, more than 55,000 abdominal surgical procedures are performed in the Netherlands annually (source Centraal Bureau voor Statistiek; www.statline.cbs.nl). More than half of these procedures consist of malignant colonic resections or laparoscopic cholecystectomy for symptomatic cholecystolithiasis or gallstone disease. In both surgical procedures perioperative imaging can decrease surgical harm, while improving diagnosis and therapy outcomes. In colon cancer, preoperative imaging may lead to more accurate assessment of tumor spread, while intraoperative imaging may improve complete tumor resection. During laparoscopic cholecystectomy, improvement of optical intraoperative imaging could help to avoid bile duct injury, which would otherwise require re-intervention, a prolonged hospital stay and incure an increased risk of permanent physical damage.

MOLECULAR IMAGING

Next to traditional imaging techniques such as X-ray, ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI), molecular imaging based on exogenously administrated tracers is now available. Single photon emission computed tomography (SPECT) imaging and positron emission tomography (PET) are both highly sophisticated imaging techniques, visualizing metabolically active tissue by measuring the concentration of administrated radiopharmaceuticals. Both imaging modalities can be combined with a CT scan, thus integrating metabolic information with detailed anatomical information. In addition, SPECT and PET allow three-dimensional imaging, which facilitates the dynamic imaging of deeply lying intra-abdominal organs or of organs above or below other organs with significant amounts of activity. This characteristic contrasts with classic scintigraphy, which yields planar data and can only be used to create a two dimensional image. The ability to combine functional

and anatomical data has contributed enormously to the better differentiation of physiological and pathological uptake, more accurate localization of pathology and better characterization of small or equivocal uptake foci.

The most commonly used radionuclide in SPECT is the gamma-photon ^{99m}Tc-nanocoll which has a half-life ($t_{1/2}$) of 6 hrs and can be coupled to various compounds. The most commonly used tracer in PET imaging is ^{18}F-fluorodeoxyglucose (^{18}FDG), which is a marker for tissue uptake of glucose. The great advantage of PET over SPECT is the superior sensitivity and spatial resolution. Additionally, dynamic 3D imaging provides detailed anatomical information on the location of radiopharmaceutical and tracer distribution. These favorable characteristics have expanded the application of PET from imaging of tumor metabolism to selective tumor targets. Nowadays, targeted therapies based on the conjugation and radiolabelling of monoclonal antibodies with PET isotopes are being investigated. These therapies can be aimed exclusively at tumor cells while sparing healthy cells. At the same time new PET-radiocolloids, especially [^{89}Zr]Zr-nanocoll are under development. The prolonged [^{89}Zr]Zr-nanocoll half-life ($t_{1/2}$) of 78.4 hrs improves the quantitative investigation of tracer distribution and therefore allows for a more detailed analysis of metastatic spread of a primary tumor, especially regarding drainage of tumor cells toward lymph nodes [1, 2].

A limitation of radiocolloids is their colorless appearance, which means that they cannot be visualized intraoperatively. Intraoperative guidance towards highly radioactive structures can be achieved by imaging with a portable gamma camera but these cannot differentiate radioactivity between structures [3]. Optical tracers are clearly needed to facilitate intraoperative image-guided surgery. The most frequently used optical tracers are blue dyes (e.g. methylene blue, isosulfan blue or patent blue). Blue dyes are safe when administered locally or in low doses. However, they can induce severe adverse effects such as arrhythmias, coronary vasoconstriction, and hemolytic anemia in patients with renal insufficiency or after intravenous administration of higher doses [4]. Visualization of blue dye through tissue with the naked eye is limited and therefore restricts applications to the identification of superficial structures.

More recently, intraoperative imaging using the near-infrared (NIR) light spectrum has been introduced, using light at wavelengths invisible to the naked eye (between 700-900 nanometers). Advantages of NIR light include high tissue penetration of up to 1 cm, and low autofluorescence. As a result, optimal signal-to-background ratios can be achieved, improving both contrast and the identification of different tissue types [5]. NIR imaging requires a NIR fluorescent agent or so-called fluorophore, combined with an imaging system that is able to both excite and detect

the fluorescent signal. The fluorescent signal can be visualized immediately and most imaging systems are able to combine fluorescence signals with conventional color videos, allowing direct anatomical orientation.

Two fluorophores are currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical applications; methylene blue and Indocyanine Green (ICG). Both are nonspecific contrast agents but with different characteristics. Indocyanine Green has an excitation peak around 800 nm, while methylene blue has an excitation peak of approximately 700 nm. ICG shows better tissue penetration depth and less autofluorescence. Another advantage is the better safety profile of ICG, whether injected locally or systematically. Adverse events have been reported in fewer than 1 in 40,000 patients and most relate to hypersensitivity reactions [6].

Methylene blue and ICG can be used for different surgical applications. Methylene blue is mainly cleared renally and is therefore very useful for ureter mapping to prevent iatrogenic injuries. Indocyanine Green is cleared exclusively by the liver and is excreted into the bile after intravenous administration. It may help reduce bile duct injuries by imaging the biliary tree during laparoscopic cholecystectomy. Additionally, ICG has favorable characteristics that may improve imaging of tumor spread by visualization of lymph node drainage patterns, identification of malignant tissue and finally, to determine adequate resection margins. A third promising fluorophore is IRDye800CW (LI-COR Bioscience, Lincoln, NE), which has an additional NHS ester and can be easily coupled to an antibody without changing its fluorescent properties. Referred to as tumor-targeted imaging, this approach allows for very specific pre- and intraoperative detection of tumor tissue. IRDye800CW is currently available for conjugation to targeted biomolecules for investigational use in clinical trials. However, it has not been studied for diagnostic or therapeutic applications in humans, and has not been FDA approved for this use. Use of IRDye800CW therefore remains limited in current routine treatment of surgical patients.

IMAGE-GUIDED SURGERY IN COLON CANCER

Colorectal cancer is currently the second most frequently diagnosed cancer in the Western world, and is the third most common malignancy, after prostate and lung cancer, in men in the Netherlands. In women, only breast cancer is diagnosed more frequently. As a result of the introduction of a nationwide screening program, increased life expectancy and an aging population, the number of colorectal cancer patients has increased dramatically, from 7100 in

1990 to 14000 in 2017 (source :www.cijfersoverkanker.nl). About two thirds of these patients are diagnosed with colon cancer.

Colon cancer is classified according to stage at diagnosis, as defined by invasion depth of the tumor (T-stage; T1-T4), lymph node and distant metastases (TNM classification) (Figure 1). Early detection is crucial to maximize the chance of cure. Currently, five-year survival ranges from 97% to as low as 8%, depending on disease stage at time of diagnosis [7]. Of newly diagnosed colon cancer cases, early stage T1 and T2 tumors (stage I disease) account for 24% and 34.5%, respectively (source Dutch ColoRectal Audit DCRA-DICA 2017). By this stage a tumor has grown through the muscularis mucosa into the submucosa (T1), and may also have penetrated into the muscularis propria (T2). The general incidence of tumor spread to nearby lymph nodes (N-stage) or distant sites (M-stage) is low in these T1 and T2 tumors. More advanced tumors grow in to the outermost layers of the colon (T3) or though the visceral peritoneum (T4), increasing the risk of metastatic spread dramatically .

Independent of T-stage, accurate pathological examination of resected lymph nodes is a prerequisite, since lymph node metastasis (LNM) serves as the strongest prognostic factor and most important criterion for adjuvant chemotherapy [8]. In the absence of LNM (stage I-II), the 5-year survival of exceeds 95% for stage I and approximately 80% for stage II disease [7, 9-11]. The incidence of LNM in T1/T2 tumors is relatively low, at 8-20%. The proportion of these early staged tumors is expected to increase to approximately 50% due to the introduction of nationwide screening programs [12]. In patients with T1/T2 tumors a complete resection of the primary tumor can be easily achieved with a small resection of the affected colon or endoscopic guided resection. This is an attractive treatment option that avoids exposure to unnecessary surgery-related morbidity and mortality, which are currently as high as 13.5% and 1-5%, respectively [13, 14]. Moreover, previous studies indicate that endoscopic treatment of T1 tumors is safe in patients with low-risk tumors [15]. However, endoscopic resection is insufficient in the majority of T1 tumors categorized as high-risk (71-81%) [16]. In these patients an endoscopic resection is insufficient since LNM are present in 8% of [17]. For T2 tumors the risk of lymph nodes is even higher up to 20%. Currently, it is not possible to distinguish patients with or without lymph node metastases prior to surgery. Therefore, large segmental resection, with en-block excision of all lymph nodes, is unavoidable in the majority of patients with T1 tumors and in all patients with T2 colon cancer [7, 18].

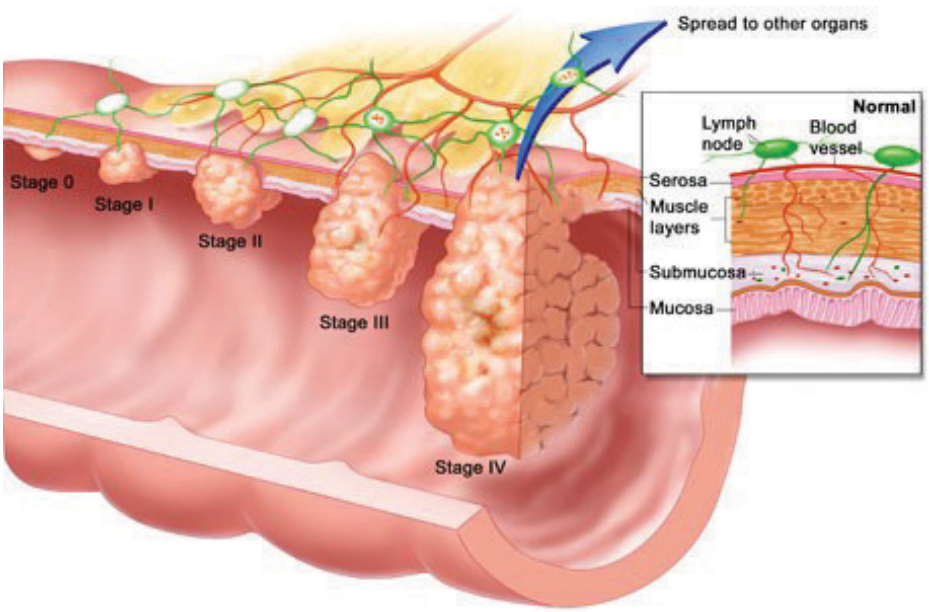

Figure 1. Different stages of colorectal cancer according to the TNM classification system (Picture adapted from; http://www.cancercontrol.net)

On the other hand, up to 20-30% of patients without lymph node metastases show disease recurrence and eventually die within five years of initial treatment, despite complete surgical resection [19]. This high recurrence rate in lymph node-negative patients is probably the result of understaging due to overlooked occult tumor cells (micrometastases or isolated tumor cells) during routine histopathological examination [20]. To improve lymph node staging while decreasing the extent of surgery in node-negative patients, detection of the sentinel lymph node (SLN) could offer a solution.

The sentinel lymph node procedure

Some history

Lymph node involvement as a consequence of the metastatic spread of cancer was already described by Hippocrates, who mentioned a disease similar to currently known lymph node metastases [21]. The investigation of the lymphatic system has a long and fascinating history, with important contributions from several medical scientists. Although some medical pioneers had already described the lymphatic system, probably accidentally, it is generally accepted that it was first properly recognized by the Italian professor Gasparo Asselius (1581-1626) in 1622, who was then Professor of Anatomy at Pavia University in Italy [22]. He noticed 'white vessels' in the

mesentery during dissection of a living dog just after eating. He also observed leakage of white fluid from the vessels after cutting through them and therefore described them as `lacteals`. The anatomy and physiological role of the lymphatic structures was a mystery but began to receive attention from many investigators following Asselius's description. In 1651 the Dutch professor Johannes van Horne (1621-1670) described a main lymphatic collecting vessel which is currently known as the thoracic duct. It was van Horne's student, Frederik Ruysch (1638-1731) (Figure 2), who first demonstrated the drainage of fluids from the organs towards the lymphatic system and finally into the venous blood stream. Concurrently, the Danish physician, mathematician and theologian Thomas Bartholin (1616-1680) studied the lymphatic system of two executed criminals. He confirmed that the lymphatic system was a circulatory system distinct from blood circulation and proposed that lymph originated from blood by filtration. He named the lymph vessels `vasa lymphatica`, a term derived from the Latin word `lympha` which means clear spring water. Anthony Nuck (1650-1692), a professor of anatomy in Leiden, the Netherlands, visualized lymphatics by injection of a mixture of mercury, tin and lead [23]. As a result, the function of the lymph nodes as filters of the lymphatic system was slowly uncovered. The German pathologist, Rudolf Virchow (1821-1902), can be considered the founding father of our modern understanding of the lymphatic system and lymph nodes. He suggested that lymph nodes function as filters in the lymphatic system and also proposed that lymphatic fluid from any given area in the body drains through the lymphatics to specific lymph nodes and subsequently to other lymph nodes. These hypotheses were supported by his own observations during the autopsy of a sailor, in whom carbon pigment from a tattoo on the arm had migrated to a single axillar lymph node.

By the end of the 19th century European surgeons were starting to discuss local treatment of cancer, supplemented with regional lymph node therapy to improve curative efficacy. Supported by the work of Virchow, the prominent British surgeon Herbert Snow (1847-1930) published an article in 1882 in which he advocated elective lymph node dissection in patients with melanoma. Simultaneously, the American surgeon William S. Halsted (1852-1922) developed a mastectomy procedure with en-block axillary resection in breast cancer. From then on, excision of the primary tumor combined with regional node surgery became the standard of care in the surgical treatment for a wide variety of malignancies.

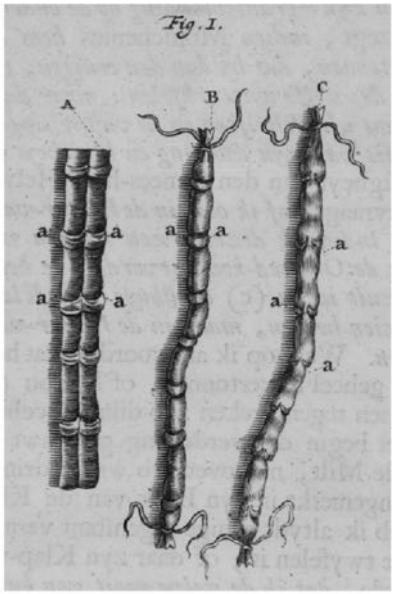

Figure 2 (left). Portrait of Frederik Ruysch (1638-1731)
Figure 3 (right). Anatomical demonstration of ligated and air-filled lymph vessels by Frederik Ruysch (**A**) Divided lymph vessel, (**B**) viewed from the side, (**C**) and from the front. The valves are marked with an (*a*). (Both images adapted from; Arlebout YF (1744) Alle de ontleed-, genees-, en heelkundige werken van Frederik Ruysch. Janssoons van Waesberge, Amsterdam)

In 1960, Gould et al. described the metastatic drainage patterns of parotid tumors towards a lymph node located at the junction of the anterior and posterior facial and referred to this node as the 'sentinel node' [24]. In the same year, the American surgeon Ramon Cabanas studied lymph node metastases in penile carcinoma and noticed that a specific lymph node near the pubic tubercle often harbored tumor cells [25]. Similarly to Gould, he called this node the `sentinel node.` From then on, the imaging of lymphatic drainage patterns using blue dyes and radiocolloid began to be widely investigated. In that period, lymphatic drainage was proposed as a static process occurring in an orderly fashion towards the same fixed location of the sentinel node. Interindividual variability in lymphatic drainage patterns was not taken into account and techniques for SLN identification proved to be unreproducible. As recently as 1992, the American oncologist Donald M. Morton (1934-2014) and pathologist Alastair Cochran finally demonstrated the dynamic patterns of lymphatic drainage, which were found to be variable between patients. This discovery by Morton and Cochran has led to our current concept of the Sentinel lymph node, in which migration of metastatic cells from the primary tumor to a lymph node or nodes occurs in an orderly spread via lymph fluid to the first node or nodes in their paths. This first node or nodes are the so-called Sentinel Lymph Nodes (SLNs) (Figure 4). In 1982 Cochran described an additional advantage of SLN identification, demonstrating that the

presence of small metastases in these nodes is an essential element in the accurate staging of melanoma [26]. Currently, the primary aim of the SLN procedure is to improve the staging of disease by determination of metastases after detailed histopathological assessment of this specific node. In breast cancer and melanoma, the SLN procedure shows high accuracy in the prediction of metastatic spread and is used routinely. However, the SLN procedure appears more challenging in colon cancer and is still under debate.

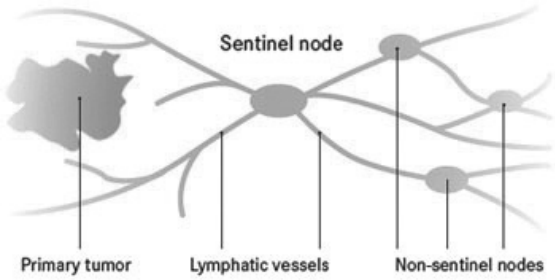

Figure 4. The sentinel lymph node concept [27]

Challenges of sentinel lymph node imaging in colon cancer

The first step of the SLN procedure is identification of the node, followed by excision and extensive histopathological examination consisting of conventional hematoxylin and eosin staining and additional serial-sectioning combined with immunohistochemistry. In melanoma and breast cancer, SLN biopsies are routinely performed using a combination of preoperative colloid planar or SPECT lymphoscintigraphy, intraoperative guidance by gamma-probe and optical guidance of blue-stained nodes after preoperative injection with blue dye. Preoperative (SPECT) lymphoscintigraphy informs the surgeon about the number and localization of radioactive nodes. Intraoperative localization of SLNs is guided by gamma counting of the gamma probe and real-time visualization of blue-stained nodes. In breast cancer and melanoma, detection rates greater than 95% are reported. False negative rates are low, at between 4.6-16% and an overall diagnostic accuracy of 93-97.6% is reported for both malignancies [28,29]. Results for colon cancer vary widely between studies. Additionally, diverse and non-standardized methods mean that results and their interpretation are both of questionable reliability [30]. In general, while high detection rates of over 95% are reported [30,31], reported false negative rates are also high at up to 30% [31], resulting in a lower diagnostic accuracy (88.2%) [31] and pooled sensitivity (76%) [30]. Several patient, tumor and procedure-related factors are offered as possible causes for the currently disappointing performance of the SLN procedure in colon cancer [32].

As in breast cancer and melanoma, it has been proposed that SLN identification in colon cancer is most effective in early disease stages. Higher staged disease, with more advanced transmural tumors and increased risk of lymph node metastases, could theoretically destroy efferent lymphatic pathways. Secondly, large longitudinal tumors can involve adjacent lymphatic patterns, which may increase false negative rates. The number of patients with early staged tumors (T1/T2) is low in the majority of published studies. This is unsurprising, since colon cancer is often asymptomatic until more advanced tumor stages (T3/T4) are reached. With the introduction of nationwide screening programs, the number of early staged tumor is expected to increase. This shift in tumor-stage presentation will facilitate the validation of the SLN technique in colon cancer. Another worrying feature of current SLN performance is the inclusion and combined presentation of results for colon and rectal cancer. The SLN procedure in rectal cancer is questionable, as neoadjuvant chemoradiation therapy may change lymphatic drainage patterns and influence performance of the SLN procedure [33-36].

Other difficulties regarding colon SLN identification are the unpredictable number and location of the SLNs. In contrast to breast cancer and melanoma, it seems that more than one node is frequently assigned as the SLN and they appear to be located near the primary tumor. Since injection of tracer is administrated peritumorally, uptake of tracer in a SLN close to the primary tumor may be hidden by the highly radioactive injection site. This phenomenon is known as the `shine-through effect`. Such SLNs may not be visualized at preoperative imaging. Currently, the gamma-photon ^{99m}Tc –nanocoll is used for SLN identification, a radiocolloid that only allows imaging using planar lymphoscintigraphy or SPECT. The limited resolution of gamma cameras may also hamper precise localization of the SLN. Furthermore, intraoperative detection of the SLNs is difficult since handheld gamma probes cannot differentiate radioactivity arising from the SLN versus the injection site.

Sentinel lymph nodes of the colon are also more often smaller (< 1 cm) and located beneath a thick layer of (fat) tissue. As the penetration depth and particle size of blue dyes are both limited, intraoperative detection of SLNs using blue dye is suboptimal since SLNs are located in an often fatty mesocolon. Secondly, small particle size results in fast migration of blue dye from the SLNs to second echelon nodes, which increases the false negative rate. New technologies that improve the preoperative and intraoperative identification of true SLNs are under investigation. PET/CT lymphoscintigraphy is one such new imaging technique. In oral cancer patients, PET/CT lymphoscintigraphy showed superior results regarding visualization of SLNs near the primary tumor [37]. Additionally, NIR fluorescence imaging using ICG has shown favorable results for SLN identification in several types of cancer [38]. The combination of these new techniques may have a significant impact in the development and implementation of the SLN procedure in colon cancer.

IMAGE-GUIDED SURGERY DURING LAPAROSCOPIC CHOLECYSTECTOMY

Laparoscopic cholecystectomy is the treatment of choice in patients with symptomatic gallstone disease. Around 23,000 laparoscopic cholecystectomies are performed in the Netherlands annually (source Centraal Bureau voor Statistiek; www.statline.cbs.nl). The complication rate after laparoscopic cholecystectomy is 2-12%, with a mortality rate of 0.2% [39]. General complications include wound infection, intra-abdominal abscess formation and postoperative bleeding from the cystic artery.

The most feared complication is bile duct injury (BDI), which has an incidence of 0.5-1.0% [40]. Bile duct injury leads to bile leakage that in turn may cause sepsis, multiple organ failure and even death. It can also lead to obstruction, causing `obstructive jaundice,` potentially leading to a need for liver transplantation [41]. Bile duct injuries play a significant role in morbidity and mortality rates, lower quality of life and extra costs [42]. The main cause of BDI is misidentification of anatomy, which can occur when a surgeon mistakes the common bile duct or an aberrant right hepatic duct for the cystic duct [43]. To ensure that the cystic duct has been identified correctly, it is important to use Strasberg's Critical View of Safety (CVS) [44] (Figure 5). At CVS, the cystic duct and cystic artery are clearly identified and can be clipped and divided safely. Mobilization of the infundibulum is essential to reach CVS. In this procedure a window is created between the cystic duct and cystic artery, and between the cystic artery and liver bed (Calot's triangle).

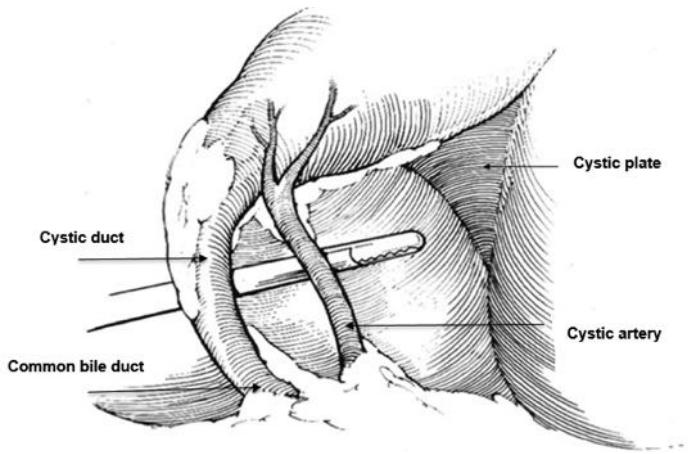

Figure 5. The critical view of safety (Picture adapted from: from Strasberg SM et al. Rationale and use of the critical view of safety in laparoscopic cholecystectomy. J Am Coll Surg. 2010, source 42)

In some cases mobilization of the infundibulum is difficult or cannot be reached due to retraction of the gallbladder against the liver, resulting in an increased risk of bile duct injury. Several factors such as male gender, co-morbidity, complexity, urgency of surgery and conversion are associated with an increased risk of BDI during laparoscopic cholecystectomy [45]. Local risk factors include acute cholecystitis, aberrant anatomy, severe fibrosis after previous inflammation, and bleeding that disturbs the intraoperative view during the procedure [46]. In the Netherlands, conversion to an open procedure is recommended when CVS cannot be reached. However, conversion to an open procedure requires experience, and since laparoscopic cholecystectomy is now the standard surgical approach few surgeons are familiar with open cholecystectomy [47].

Intraoperative cholangiography (IOC) was introduced to improve intraoperative visualization of relevant anatomical procedures. A small catheter is placed into the cystic duct during surgery and after injection of a small amount of contrast fluid an X-ray is taken. Lower rates of BDI are reported with routine use of IOC but selective use has been discouraged [48, 49]. In several countries, including the Netherlands, IOC is not routinely used during laparoscopic cholecystectomy due to the drawbacks of radiation exposure, need for additional equipment and additional costs. An additional serious drawback of IOC is the incision that has to be made in the cystic duct. This can be easily confused with the common bile duct, resulting in bile duct injury. Interpretation of IOC is also difficult when it is not frequently used and it should therefore only be performed by experienced professionals [43].

The use of NIR fluorescence imaging during laparoscopic cholecystectomy, with ICG as contrast agent, is a relatively new technique. After intravenous injection ICG is rapidly cleared by the liver and almost completely excreted in the bile [50]. When NIR fluorescence imaging is used, the outflow of ICG from the gallbladder through the cystic duct can be visualized and may prevent misidentification of the biliary structures [51]. In addition, it could improve procedural efficiency and shorten operation time due to early intraoperative anatomy navigation.

OUTLINE OF THIS THESIS

This thesis consists of two parts. In **the first part** we outline the current performance of the SLN procedure in colon cancer and describe the limitations and difficulties of the procedure. To improve the preoperative and intraoperative guidance towards the SLN, we investigated the use of NIR fluorescence imaging and PET/CT lymphoscintigraphy as SLN mapping techniques. In **the second part**, NIR fluorescence imaging is investigated as an intraoperative imaging technique for visualization of biliary structures during laparoscopic cholecystectomy in patients with mild to severe cholecystolithiasis.

PART I MOLECULAR IMAGE-GUIDED SURGERY IN COLON CANCER

In **Chapter 2,** a general overview is given of the SLN procedure in colon cancer combined with an introduction of NIR fluorescence imaging as a technique for SLN biopsy. Results of the SLN procedure according to current literature in terms of sensitivity, negative predictive value, detection rate and upstaging are evaluated in **Chapter 3**. These results are additionally stratified for several tumor and procedure-related factors to assess their influence on current SLN performance.

In **Chapter 4** and **Chapter 5** we evaluate several variants of the SLN procedure using NIR fluorescence imaging. Additionally, based on current literature we outline the performance and pitfalls of NIR fluorescent SLN mapping in colon cancer.

To improve the SLN procedure in colon cancer, we evaluate the identification and visualization of SLNs using preoperative PET/CT lymphoscintigraphy and intraoperative NIR fluorescence imaging in **Chapter 6**. The combination of these highly sophisticated imaging techniques, further combined with restricted selection criteria, provided essential information regarding number and location of the SLNs. The results of this study could be fundamental to the development of a standardized and accurate SLN procedure in colon cancer.

PART II MOLECULAR IMAGE-GUIDED SURGERY DURING LAPAROSCOPIC CHOLECYSTECTOMY

The use of NIR fluorescence imaging during laparoscopic cholecystectomy has the potential to become a standard part of the surgical procedure and might offer an alternative to the intraoperative cholangiogram.

In **Chapter 7** we investigated the additional value of intraoperative NIR fluorescence imaging of the cystic duct and common bile duct during elective laparoscopic cholecystectomy in patients with uncomplicated gallstone disease. Since early visualization of the biliary anatomy may increase the safety of laparoscopic cholecystectomy in patients with an increased risk of BDI, we investigated this population in **Chapter 8.** An overview of the current performance of imaging of the bile duct with NIR fluorescence imaging is presented as a systematic review in **Chapter 9.** In this chapter we evaluated the NIR fluorescence imaging technique with regard to dosage, timing of ICG administration and patient pathology. Additionally, we performed a meta-analysis of visualization of the cystic duct, common bile duct and common hepatic duct between NIR fluorescence imaging and intraoperative cholangiography.

REFERENCES

1. Pelosi E, Messa C, Sironi S, Picchio M, Landoni C, Bettinardi V, et al. Value of integrated PET/CT for lesion localisation in cancer patients: a comparative study. Eur J Nucl Med Mol Imaging. 2004;31(7):932-9.
2. Rahmim A, Zaidi H. PET versus SPECT: strengths, limitations and challenges. Nucl Med Commun. 2008;29(3):193-207.
3. Vermeeren L, Valdes Olmos RA, Klop WM, Balm AJ, van den Brekel MW. A portable gamma-camera for intraoperative detection of sentinel nodes in the head and neck region. J Nucl Med. 2010;51(5):700-3.
4. Ginimuge PR, Jyothi SD. Methylene blue: revisited. J Anaesthesiol Clin Pharmacol. 2010;26(4):517-20.
5. Frangioni JV. In vivo near-infrared fluorescence imaging. Curr Opin Chem Biol. 2003;7(5):626-34.
6. Benya R, Quintana J, Brundage B. Adverse reactions to indocyanine green: a case report and a review of the literature. Cathet Cardiovasc Diagn. 1989;17(4):231-3.
7. Gunderson LL, Jessup JM, Sargent DJ, Greene FL, Stewart AK. Revised TN categorization for colon cancer based on national survival outcomes data. J Clin Oncol. 2010;28(2):264-71.
8. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004;350(23):2343-51.
9. Manfredi S, Bouvier AM, Lepage C, Hatem C, Dancourt V, Faivre J. Incidence and patterns of recurrence after resection for cure of colonic cancer in a well defined population. Br J Surg. 2006;93(9):1115-22.
10. Brouwer NPM, Bos A, Lemmens V, Tanis PJ, Hugen N, Nagtegaal ID, et al. An overview of 25 years of incidence, treatment and outcome of colorectal cancer patients. Int J Cancer. 2018;143(11):2758-66.
11. Elferink MA, de Jong KP, Klaase JM, Siemerink EJ, de Wilt JH. Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis. 2015;30(2):205-12.
12. Amri R, Bordeianou LG, Sylla P, Berger DL. Impact of screening colonoscopy on outcomes in colon cancer surgery. JAMA Surg. 2013;148(8):747-54.
13. Cahill RA, Bembenek A, Sirop S, Waterhouse DF, Schneider W, Leroy J, et al. Sentinel node biopsy for the individualization of surgical strategy for cure of early-stage colon cancer. Ann Surg Oncol. 2009;16(8):2170-80.
14. Liang J, Fazio V, Lavery I, Remzi F, Hull T, Strong S, et al. Primacy of surgery for colorectal cancer. Br J Surg. 2015;102(7):847-52.
15. Choi PW, Yu CS, Jang SJ, Jung SH, Kim HC, Kim JC. Risk factors for lymph node metastasis in submucosal invasive colorectal cancer. World J Surg. 2008;32(9):2089-94.
16. Bosch SL, Teerenstra S, de Wilt JH, Cunningham C, Nagtegaal ID. Predicting lymph node metastasis in pT1 colorectal cancer: a systematic review of risk factors providing rationale for therapy decisions. Endoscopy. 2013;45(10):827-34.
17. Backes Y, de Vos Tot Nederveen Cappel WH, van Bergeijk J, Ter Borg F, Schwartz MP, Spanier BWM, et al. Risk for Incomplete Resection after Macroscopic Radical Endoscopic Resection of T1 Colorectal Cancer: A Multicenter Cohort Study. Am J Gastroenterol. 2017;112(5):785-96.
18. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471-4.
19. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet. 2005;365(9454):153-65.
20. Sirop S, Kanaan M, Korant A, Wiese D, Eilender D, Nagpal S, et al. Detection and prognostic impact of micrometastasis in colorectal cancer. J Surg Oncol. 2011;103(6):534-7.

21. Karpozilos A, Pavlidis N. The treatment of cancer in Greek antiquity. Eur J Cancer. 2004;40(14):2033-40.

22. Natale G, Bocci G, Ribatti D. Scholars and scientists in the history of the lymphatic system. J Anat. 2017;231(3):417-29.

23. Nieweg OE, Uren RF, Thompson JF. The history of sentinel lymph node biopsy. Cancer J. 2015;21(1):3-6.

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

25. Cabanas RM. An approach for the treatment of penile carcinoma. Cancer. 1977;39(2):456-66.

26. Cochran AJ, Wen DR, Herschman HR, Gaynor RB. Detection of S-100 protein as an aid to the identification of melanocytic tumors. Int J Cancer. 1982;30(3):295-7.

27. Beri A, Janetschek G. Technology insight: radioguided sentinel lymph node dissection in the staging of prostate cancer. Nat Clin Pract Urol. 2006;3(11):602-10.

28. Moncayo VM, Alazraki AL, Alazraki NP, Aarsvold JN. Sentinel Lymph Node Biopsy Procedures. Semin Nucl Med. 2017;47(6):595-617.

29. Wong SL, Hurley P, Lyman GH. Sentinel Lymph Node Biopsy for Melanoma: American Society of Clinical Oncology and Society of Surgical Oncology Joint Clinical Practice Guideline. J Oncol Pract. 2012;8(4):e65-e6.

30. van der Pas MH, Meijer S, Hoekstra OS, Riphagen, II, de Vet HC, Knol DL, et al. Sentinel-lymph-node procedure in colon and rectal cancer: a systematic review and meta-analysis. Lancet Oncol. 2011;12(6):540-50.

31. van der Zaag ES, Bouma WH, Tanis PJ, Ubbink DT, Bemelman WA, Buskens CJ. Systematic review of sentinel lymph node mapping procedure in colorectal cancer. Ann Surg Oncol. 2012;19(11):3449-59.

32. Cahill RA. What's wrong with sentinel node mapping in colon cancer? World J Gastroenterol. 2007;13(47):6291-4.

33. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.

34. Li M, Li JY, Zhao AL, Gu J. Colorectal cancer or colon and rectal cancer? Clinicopathological comparison between colonic and rectal carcinomas. Oncology. 2007;73(1-2):52-7.

35. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012;23(10):2479-516.

36. Stojadinovic A, Nissan A, Protic M, Adair CF, Prus D, Usaj S, et al. Prospective randomized study comparing sentinel lymph node evaluation with standard pathologic evaluation for the staging of colon carcinoma: results from the United States Military Cancer Institute Clinical Trials Group Study GI-01. Ann Surg. 2007;245(6):846-57.

37. Heuveling DA, van Schie A, Vugts DJ, Hendrikse NH, Yaqub M, Hoekstra OS, et al. Pilot study on the feasibility of PET/CT lymphoscintigraphy with 89Zr-nanocolloidal albumin for sentinel node identification in oral cancer patients. J Nucl Med. 2013;54(4):585-9.

38. 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.

39. Deziel DJ. Complications of cholecystectomy. Incidence, clinical manifestations, and diagnosis. Surg Clin North Am. 1994;74(4):809-23.

40. de Reuver PR, Rauws EA, Bruno MJ, Lameris JS, Busch OR, van Gulik TM, et al. Survival in bile duct injury patients after laparoscopic cholecystectomy: a multidisciplinary approach of gastroenterologists, radiologists, and surgeons. Surgery. 2007;142(1):1-9.

41. Booij KA, de Reuver PR, Yap K, van Dieren S, van Delden OM, Rauws EA, et al. Morbidity and mortality after minor bile duct injury following laparoscopic cholecystectomy. Endoscopy. 2015;47(1):40-6.

42. Landman MP, Feurer ID, Moore DE, Zaydfudim V, Pinson CW. The long-term effect of bile duct injuries on health-related quality of life: a meta-analysis. HPB (Oxford). 2013;15(4):252-9.

43. Way LW, Stewart L, Gantert W, Liu K, Lee CM, Whang K, et al. Causes and prevention of laparoscopic bile duct injuries: analysis of 252 cases from a human factors and cognitive psychology perspective. Ann Surg. 2003;237(4):460-9.

44. Strasberg SM, Hertl M, Soper NJ. An analysis of the problem of biliary injury during laparoscopic cholecystectomy. J Am Coll Surg. 1995;180(1):101-25.

45. Keus F, de Jong JA, Gooszen HG, van Laarhoven CJ. Laparoscopic versus open cholecystectomy for patients with symptomatic cholecystolithiasis. Cochrane Database Syst Rev. 2006(4):CD006231.

46. Georgiades CP, Mavromatis TN, Kourlaba GC, Kapiris SA, Bairamides EG, Spyrou AM, et al. Is inflammation a significant predictor of bile duct injury during laparoscopic cholecystectomy? Surg Endosc. 2008;22(9):1959-64.

47. Booij KA, de Reuver PR, van Delden OM, Gouma DJ. [Conversion has to be learned: bile duct injury following conversion to open cholecystectomy]. Ned Tijdschr Geneeskd. 2009;153:A296.

48. Buddingh KT, Weersma RK, Savenije RA, van Dam GM, Nieuwenhuijs VB. Lower rate of major bile duct injury and increased intraoperative management of common bile duct stones after implementation of routine intraoperative cholangiography. J Am Coll Surg. 2011;213(2):267-74.

49. Flum DR, Dellinger EP, Cheadle A, Chan L, Koepsell T. Intraoperative cholangiography and risk of common bile duct injury during cholecystectomy. JAMA. 2003;289(13):1639-44.

50. Mishra A, Behera RK, Behera PK, Mishra BK, Behera GB. Cyanines during the 1990s: A Review. Chem Rev. 2000;100(6):1973-2012.

51. Verbeek FP, van der Vorst JR, Schaafsma BE, Hutteman M, Bonsing BA, van Leeuwen FW, et al. Image-guided hepatopancreatobiliary surgery using near-infrared fluorescent light. J Hepatobiliary Pancreat Sci. 2012;19(6):626-37.

PART I

MOLECULAR IMAGE-GUIDED SURGERY IN COLON CANCER

NEAR-INFRARED FLUORESCENCE LYMPHATIC LAPAROSCOPY OF THE COLON AND MESOCOLON

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ABSTRACT

During surgery, a surgeon relies on the vision of his eyes and the touch of his hands. While laparoscopic surgery for colon cancer has proven to be safe and effective, it still remains a technically difficult procedure. Although it is associated with reduced haptic feedback, by enforcing the power of visual guidance, the loss of this feedback can be (partly) compensated for. Here we describe how the use of near-infrared dyes and fluorescence laparoscopy could help improve tumour staging and therefore lead to better selection of patients for postoperative adjuvant chemotherapy. More controversially, and analogous to melanoma and breast cancer surgery with sentinel node biopsy, we speculate that local resection with SLN harvesting in early colon cancer might change the therapeutic and surgical strategy in colon cancer.

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths. Standard therapy is an adequate en-bloc resection. Prognosis is, however, associated with the radicality of the first surgery, achieved either by a conventional open or laparoscopic approach, as this is how the stage of disease is determined. The 5-year survival is 90% for patients with stage I disease and 75% for those with stage II disease. Up to 30% of patients with stage I or II disease will develop loco-regional recurrence or distant metastases and will eventually die from CRC [1]. Under-staging during the initial procedure clearly may contribute to this mortality.

Occult tumour cells and micrometastasis in lymph nodes are easily missed by routine histopathological examination [2]. Serial sectioning and additional immunohistochemistry or reverse transcriptase (RT)-PCR could diagnose lymphatic spread more accurately. Ideally, all regional lymph nodes should be examined with these techniques but this would be too expensive and time consuming and therefore not feasible in everyday practice. Focused examination of selective lymph nodes could therefore be a solution. Additionally, in 2013 a national colon cancer screening programme will commence in the Netherlands. It is expected that more early-stage, node-negative colon carcinoma will be diagnosed. Large en-bloc resections for these small lesions could be considered as being unnecessarily traumatic. Nowadays, however, local surgical treatment such as submocosal resection of colonic neoplasia is less applied because of the uncertainty about undetected lymph node metastases. More elegant would be a local resection in combination with a selective lymphadenectomy, analogous to breast cancer surgery. Both these strategies clearly require a means of identifying the most relevant lymph nodes with regard to metastatic significance.

Sentinel lymph node (SLN) concept

The SLN is the first lymph node in the orderly progression of drainage from the primary tumour and has the highest risk of harbouring metastases. The sentinel node concept has been proven to be valid in many solid tumours. Since Morton et al. [3] showed the validity of the concept in melanomas, followed by the subsequent identification of the SLN by lymphatic mapping in breast cancer by Krag et al. [4] and Giuliano et al. [5], the use of sentinel lymph node mapping (SLNM) in the therapeutic strategy has altered profoundly the treatment of these cancers. The conventional pathological work-up of lymph nodes is H&E staining [6,7]. As mentioned earlier, 30% of patients develop recurrences or distant metastases, while lymph node metastases are supposed to be negative by H&E staining. The SLNM technique allows the pathologist to perform detailed analysis on the 1–4 sentinel nodes to detect micrometastatic disease and

improve nodal staging. Several studies indicate that micrometastases are correlated with a significantly poorer prognosis [8–10]. Consequently, this will lead to better selection of patients, who are likely to benefit from adjuvant chemotherapy.

Screening programmes and increasing awareness of the symptoms of colorectal cancer will lead to increased numbers of detection of early-stage colon carcinoma without nodal involvement. Early detection may allow for limited lymph node harvesting. Extensive lymph node resection will lead to potential over-treatment in nodenegative patients but, on the other hand, it is essential to avoid possible under-treatment of patients with falsenegative nodes in cases of limited (sentinel) lymph node harvesting. In these early patients (Tis-T1-T2), therefore, a reliable SLNM technique is especially essential in the case of limited resection.

Sentinel lymph node mapping techniques

A substantial number of studies concerning the feasibility of SLNM in colon carcinoma have been published. The technique for sentinel lymph node identification varies from blue dye (in vivo or ex vivo) or radioactive dye to a combination of radioactive and blue dye. In 2005 Saha et al. [11] published a multicentre trial including 500 patients. SLNM was performed by injecting 0.5–3 ml 1% blue dye circumferentially around the tumour in the subserosal layers. SLNM was successful in 98% of the patients, with an accuracy, sensitivity and negative predictive value of 96%, 90% and 93%, respectively. Des Guetz et al. [12] published a meta-analysis of 33 studies, resulting in a sensitivity of 70% and specificity of 81%. Both studies concluded that lymphatic mapping appears to be readily applicable and it is a useful technique to avoid under-staging. They also noted a wide variation of results shown in the literature. The authors also recommend that for future studies it will be necessary to stratify patients according to their T stage. The SLN procedure is likely to be less reliable in advanced tumour stages with the current techniques.

Recently, van der Pas et al. [13] published a meta-analysis to assess the diagnostic performance of the SLN procedure in terms of sensitivity and detection. To perform detailed analysis, the authors requested individual patient data to stratify for colon and rectal cancer and T stages. The meta-analysis of van der Pas et al. shows a low sensitivity for SLN detection, regardless of T stage, localization or pathological technique used in the overall analysis. In selected high-quality studies a high sensitivity and specificity were reached, similar to those achieved in patients with breast cancer. Therefore the authors suggest that for every patient diagnosed with colon cancer without clinical evidence of lymph node involvement or metastatic disease, an SLN procedure must be considered because of the great benefit of improved staging attributable to the SLN concept.

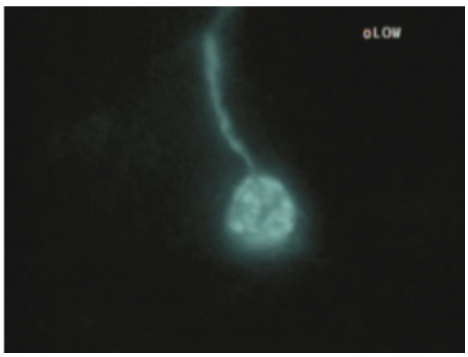
Poor visualization of the tracers used until now is one of the limitations. Blue dye is difficult to detect in mesenteric adipose tissue. Also, blue dye can readily diffuse through the SLN and pass to multiple nodes. Using a combination of blue dye and radioactive tracers could overcome this problem. The use of gamma tracers for SLN in patients with colorectal cancer was first reported by Kitagawa et al. [14]. Since then more data have been published that suggest an improvement of identification, accuracy rate and number of found SLNs by using a combination of blue dye and radioactive tracer [15,16], even though the overall results did not show additional value of the use of radioactive tracers. Using only radioactive tracer presents the problem of signal interference of the injection site and an SLN situated near the tumour. Besides that, the limited ability to visualize the lymphatic vessels and lymph nodes in real time makes radioactive tracers alone less attractive. Near infrared dyes may have the characteristics to solve these problems.

Sentinel lymph node mapping using near-infrared dyes

Near-infrared dyes have a high peak spectral absorption. Wavelengths in the near-infrared (NIR) range (700– 900 nm) penetrate deeply into living tissue compared with visible light and blue dye. Therefore SLNM can also be accurately assessed in mesenteric adipose tissue. Today, multiple near-infrared experimental dyes are available. The choice of a suitable dye for in vivo imaging requires consideration of several issues. Most importantly, the dye should exhibit strong excitation and fluorescence emission in the NIR spectral range, a higher molar extinction coefficient and quantum yield. The dyes should have little or no photobleaching and should cause no phototoxic effects. Functional groups should permit easy conjugates to proteins, antibodies, peptides and other biopolymers, to form readily fluorescent conjugates. Today, however, only Indocyanine Green (ICG) is clinically approved for use. ICG is rapidly cleared from the blood. ICG is based on its fluorescence at approximately 835 nm with a particle size of 7.3 nm. In 2006 Nagata et al. [17] demonstrated successful laparoscopic sentinel node mapping using infrared ray laparoscopy (IRL) with ICG staining. It was shown that IRL was five times superior in localizing the SLN compared with conventional laparoscopy.

We have used a newly developed laparoscopic near-infrared fluorescence imaging system (Olympus Corp., Tokyo, Japan) optimized for in vivo SLN detection with ICG (Pulsion Medical Systems AG, Munchen, Germany). For injection we used a solution of 25 mg ICG diluted in 9 ml 0.9% NaCl and 1 ml Albumin (HSA) (Cealb; Sanquin, Amsterdam, the Netherlands) to prevent the easy passage through the lymph channels and nodes. After a successful feasibility study in a goat model [18] (Figures 1 and 2), we performed the procedure in 20 colorectal cancer patients. Depending on tumour size, 1–4 subserosal, peritumoural injections with 1 ml ICG solution were administered. During the study we improved the injection technique. In the first eight cases, a

spinal needle was used and in subsequent cases a flexible endoscope catheter was used. This catheter gives less spillage of the ICG in the abdominal cavity. After injection, visualization of lymph flow and SLN was performed by the near-infrared laparoscope. The fluorescent dots are identified as SLNs and laparosopically harvested. These nodes were analysed by a pathologist using multisectioning and immunohistochemistry. In nine of the first 10 cases we found an SLN, which were all negative for micrometastases. In four of these cases a positive nonsentinel lymph node was found while the SLN was negative. All of these cases were in the first five patients, where we used a spinal needle for ICG injection, which gave a lot of spillage. In three out of four cases these patients also had large tumours (diameter > 6 cm).



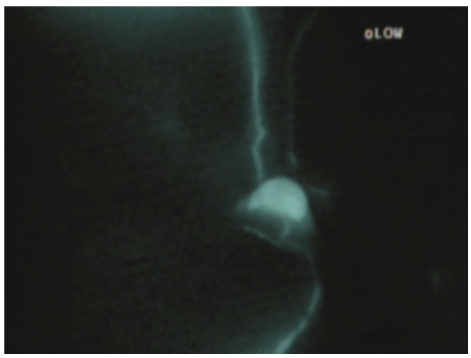


Figure 1 (left) . Fluorescent (sentinel) lymph node seen 3 min after ICG / albumin injection in an animal model.
Figure 2 (right). Fluorescent (sentinel) lymph node with afferent and efferent lymph vessels in an animal model.

DISCUSSION

Strengthened by our personal experience in SLNM in colon cancer patients, we believe SLNM could fulfil an important role in future colon cancer treatment. However, before clinical implementation the SLN procedure needs to be improved. The fluorescent technique seems to be very promising and may overcome many limitations of current techniques. But as we experienced in our study, there are several areas that have to be improved. First, we observed the problem of dye leakage during the injection of ICG. Our final injection technique was performed by a flexible endoscope in the subserosal layer around the tumour. Correct positioning of the needle-tip was found to be very difficult. Spillage of ICG results in a fluorescent abdominal cavity and makes it impossible to detect SLNs. Colonoscopic pre,-or perioperative injection in the submucosal layer could be a solution.

As with blue dye, ICG also has disadvantages. Although the penetration depth in fatty tissue is better than blue dye it is still limited (1.5 cm). We think the combination of radioactive tracers and a new, more fluorescent dye could solve this problem. These new dyes should have better fluorescent characteristics compared with ICG. LI-COR IRDye800 CW seems to be one of the most promising new dyes. The LI-COR IRDye800 CW has favorable characteristics compared with the current available dyes. The fluorescence of this dye is supposed to be many times stronger than that of ICG, making it more visible in fatty tissue. It also has a high labelling density, which makes IRDye800CW easy to conjugate with radioactive tracers or monoclonal antibodies.

CONCLUSION

Accurate identification of SLN may prove an effective means to ensure appropriate surgical staging of patients and help in the selection of patients for adjuvant therapies. Additionally, screening programs and increasing awareness of the symptoms of colon cancer will lead to increased numbers of early colorectal cancer. SLNM may therefore also alter the way we treat these tumours (Tis-T1-T2), similar to breast cancer surgery, especially if new technologies prove compelling. New strategies might make it possible to treat early colon cancer by local resection therapy using only a minimally invasive surgical sentinel node procedure.

REFERENCES

1. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev 2008; Jul 16: CD005390.
2. Hermanek P, Hutter RV, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. Cancer 1999; 86: 2668–73.
3. Morton DL, Wen DR, Wong JH et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992; 127: 392–9.
4. KragDN,WeaverDL,AlexJC,FairbankJT.Surgical resection and radiolocalization of the sentinel lymph node in breast cancer using a gamma probe. Surg Oncol 1993; 2: 335–9.
5. Giuliano AE, Kirgan DM, Guenther JM, Morton DL. Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994; 220: 391–8.
6. Bilchik AJ, Trocha SD. Lymphatic mapping and sentinel node analysis to optimize laparoscopic resection and stagin of colorectal cancer: an update. Cancer Control 2003; 10: 219–23.
7. Saha S, Dan AG, Bilchik AJ et al. Historical review of lymphatic mapping in gastrointestinal malignancies. Ann Surg Oncol 2004; 11(3 Suppl): 245S–9S.
8. Bilchik AJ, Hoon DS, Saha S et al. Prognostic impact of micrometastases in colon cancer: interim results of a prospective multicenter trial. Ann Surg 2007; 246: 568–75.
9. Iddings D, Ahmad A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. Ann Surg Oncol 2006; 13: 1386–92.
10. Liefers GJ, Cleton-Jansen AM, van de Velde CJ et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med 1998; 339: 223–8.
11. Saha S, Sehgal R, Patel M 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: 305–10.
12. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, de MP,Morere JF et al. Is sentinel lymph node mapping in colorectal cancer a future prognostic factor? A meta-analysis. World J Surg 2007; Jun; 31: 1304–12.
13. van der Pas MH, Meijer S, Hoekstra OS et al. Sentinel lymph-node procedure in colon and rectal cancer: a systematic review and meta-analysis. Lancet Oncol 2011; 12: 540–50.
14. Kitagawa Y, Fujii H, Mukai M et al. The role of the sentinel lymph node in gastrointestinal cancer. Surg Clin North Am 2000; 80: 1799–809.
15. SahaS, DanAG, Berman Bet al.Lymphazurin1%versus99mTc sulfur colloid for lymphatic mapping in colorectal tumors: a comparative analysis. Ann Surg Oncol 2004; 11: 21–6.
16. Trocha SD, Nora DT, Saha SS, Morton DL, Wiese D,
17. Bilchik AJ. Combination probe and dye-directed lymphatic mapping detects micrometastases in early colorectal cancer. J Gastrointest Surg 2003; 7: 340–5.
18. Nagata K, Endo S, Hidaka E, Tanaka J, Kudo SE, ShiokawaA. Laparoscopic sentinel node mapping for colorectal cancer using infrared ray laparoscopy. Anticancer Res 2006; 26: 2307–11.
19. van der Pas MH, van Dongen GA, Cailler F, Pelegrin A,Meijerink WJ. Sentinel node procedure of the sigmoid using indocyanine green: feasibility study in a goat model. Surg Endosc 2010; 24: 2182–7.

2

SENTINEL LYMPH NODE PROCEDURE IN COLON CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS OF INDIVIDUAL PATIENT DATA

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SUMMARY

Background The Sentinel Lymph Node (SLN) procedure has not been validated for nodal staging in colon cancer. We aimed to investigate its diagnostic performance and its effect-modifiers.

Methods We performed a systematic review and meta-analysis, searching Embase/Pubmed from database inception until March 25th, 2019 (PROSPERO CRD42016037697). Studies including ≥20 patients, showing positivity rates of SLN-procedures were eligible. We identified concept validation studies characterized by advanced histopathology of SLNs and regional lymph nodes (rLNs). We requested Individual Patient Data (IPD) to stratify for patient, tumour and procedure-related factors. Diagnostic outcome measurements were sensitivity, negative predictive value and detection rate. We also investigated the impact of procedure and tumour-related factors, and estimated the rate of upstaging and prevalence of disease in comparison with standard pathological work-up. Diagnostic outcomes were pooled using a univariate logistic random effects model, and heterogeneity was determined by I^2 and Chi-square tests. Prevalence of disease was calculated as secondary outcome.

Findings We identified 47 studies with 3245 successful SLN-procedures in 3536 patients. Six high quality concept validation studies showed a sensitivity of 0.56 (95%CI:0.53-0.58) with low heterogeneity (Chi^2=2.29 ; df=5 (p=0.808); I^2=0.0%), negative predictive value of 0.69 (95%CI:0.64-0.73) with low heterogeneity, Chi^2=10.36; df=5 (p=0.066); I^2=51.7%) and detection rate of 0.85 (95%CI:0.79-0.89). Diagnostic outcomes were higher for the remaining 41 studies; Diagnostic outcomes were higher for the remaining 41 studies; 0.74 (95%CI:0.69-0.80) with substantial heterogeneity (Chi^2=119.39; df=40 (p<0.0001); I^2=66.5%), negative predictive value of 0.83 (95%CI:0.80-0.86) with substantial heterogeneity (Chi^2=83.18, df=40 (p<0.0001); I^2=51.9%) and detection rate of 0.94 (95%CI:0.91-0.95). Prevalence of disease after conventional H&E-staining was 0.34 (95%CI:0.31-0.37), and 0.48 (95%CI:0.42-0.54) after advanced histopathology of all lymph nodes.

Interpretation The SLN-procedure in colon cancer is currently insufficient due to anatomical and technical difficulties, the wide variation of SLN mapping methods, patient selection and histopathology methods. Future studies should focus on low invasive tumours and real-time imaging of lymph flow towards the SLN. Upon standardization, the SLN-procedure may help to clarify the prognostic relevance of isolated tumour cells and micrometastases.

INTRODUCTION

Colorectal cancer (CRC) is the second most common cancer worldwide [1], and most originate from the colon [2]. Survival rates strongly relate to the invasion of the tumour (T-stage) and the presence of lymph node metastases (LNM). In patients without LNM (stage I-II), 5-years survival exceeds 95% for stage I and approximately 80% for stage II disease [3-6]. Locoregional or systemic disease recurrence in stage I-II disease might results from under-staging at primary presentation due to missed LNM, especially occult tumour cells at routine histopathology [7-9]. On the other hand, with the start of nationwide CRC screening programs the incidence of stage I disease will be diagnosed more frequently (ca. 50%) [10, 11]. In patients with low-risk T1 tumours, endoscopic excision is safe and appropriate. Unfortunately, 71-81 % of T1 tumours are high-risk [12]. Similar to T2-tumours, segmental resection with en-bloc removal of all adjacent lymph nodes is required to determine their nodal status. Since LNM prevalence is low in T1 (6-12%) and T2 (20%) [13, 14], most patients are unnecessarily exposed to surgical morbidity (30%) and mortality (1-5%).

Conventional histopathological lymph node assessment consists of analysis of single 4 µm thick haematoxylin & eosin-stained (H&E-stained) sections per 5mm. Advanced analysis involves serial-sectioning and staining with H&E and immunohistochemistry (IHC). This detects occult disease in up to 33% of patients classified as pN0 with conventional workup [7, 15, 16]. However, these techniques are too expensive and time-consuming for clinical practice (often >20 excised nodes per specimen). To improve lymph node staging while decreasing the extent of surgery in node-negative patients, and to restrain pathological workload, analysis of the sentinel lymph node (SLN) might be helpful. It is hypothesized that SLNs represent the nodal tumour status: tumour-negative SLN implies no LNM. However, validation requires that SLNs as well as regional lymph nodes (rLNs) are analysed with advanced histopathology [17].

In a previous review [18], there was limited data comparing the yield of the SLN-procedure versus an appropriate reference test (advanced histopathology of all excised lymph nodes). In the present study we identified `high quality concept validation studies` (HQ-studies) characterized by assessment of SLNs as well as of rLNs with advanced histopathology. We compared the diagnostic performance of these HQ-studies to all other published studies on SLN in colon cancer. We also investigated the impact of procedure and tumour-related factors by collecting individual patient data (IPD). To estimate the potential clinical relevance of the SLN-procedure, we calculated the rate of upstaging and prevalence of disease versus standard pathological work-up.

METHODS

Search strategy and selection criteria

This review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[19], and registered in the PROSPERO international prospective register of systematic reviews (PROSPERO database CRD42016037697). A comprehensive search was performed in PubMed and Embase from inception to March 25th, 2019 in collaboration with a medical librarian (LS). Search terms included controlled terms (MesH-PubMed, Emtree-Embase) and free text terms, and comprised (including synonyms and closely related words) as index terms or free-text words: 'colorectal cancer' and 'sentinel lymph node' (Supplementary information-SI 1). Duplicate articles were excluded. Systematic and narrative reviews were checked for additional references. Eligibility criteria were: 1) studies designed to assess effectiveness of identification and diagnostic performance of SLN-procedures in colon cancer; 2) ≥20 patients; 3) a clear description of histopathological techniques and specimen handling; 4) results presented for colon or rectal cancer separately. To avoid overlap in duplicate publications, the article with the largest sample size was included. Language restriction was English, German or Dutch.

To validate the SLN-concept, we identified a subgroup of HQ-studies based on the following criteria: [1] ≥20 SLN-procedures annually in each centre; [2] exclusion of patients with clinical signs of nodal involvement or other metastases; [3] advanced histopathology (serial sectioning with H&E-staining and IHC) to SLNs as well as to rLNs; [4] classification of metastases found with serial sectioning and IHC [20]; [5] metastases defined as isolated tumour cells <0.2mm, micrometastases 0.2-2.0mm, metastases >2.0mm; [6] an average lymph node yield of 10 nodes.

Data extraction

MA and SV selected studies independently. Titles and abstracts were screened for full-text eligibility. Full-texts were selected using eligibility criteria (disagreement was resolved in consensus-meetings).

MA and SV independently extracted data from full-text articles using standardized forms, comprising gender, age, number of patients with colon cancer, T-stage, in- or ex-vivo SLN harvesting, site of injection, used tracer(s), definition of SLN, total number of SLN-procedures, total number of SLNs harvested, total number of rLNs, number of failures to identify SLN, and histopathological technique(s) for SLN and other nodes. We contacted all corresponding authors, and if unsuccessful the first or last author, to provide IPD for colon separated from rectal cancer patients separately, and for subgroup-analyses. We requested tumour location,

T-stage, tumour size, number of SLNs, number of SLNs positive with H&E staining and number of SLNs positive at advanced histopathology; total number of rLNs; number of rLNs positive with H&E staining and with advanced histopathology, respectively.

REFERENCE STANDARD AND TEST RESULTS

The quantitative results were used to build contingency tables comprising true positives (TP), true negatives (TN), false negatives (FN) and upstaging (SI 2a & 2b).

We classified SLN-procedures as successful if at least one SLN could be identified. Procedures were considered as failures if no SLNs were found or when the SLNs were the only lymph nodes harvested precluding comparison of pathological findings to regional lymph nodes.
A true positive (TP) SLN was defined as a histopathological tumour-positive SLN with or without advanced histopathology, independent of rLN-status (false positivity rate was zero since false positive histopathology is impossible). In the HQ-studies, SLNs and rLNs were both examined with conventional and advanced histopathology. In these studies, SLNs were classified as TN if no metastases were found after conventional and advanced histopathology in SLNs and regional lymph nodes. We classified the SLN as FN if SLNs contained no metastases after conventional and advanced histopathology in combination with tumour positive rLNs after conventional or advanced histopathology.

In the overall and IPD databases, advanced histopathology was only performed to SLNs. In this context, we classified SLNs as true negatives (TN) if SLNs contained no metastases after conventional and advanced histopathology in combination with tumour-negative rLNs as defined with standard histopathology, and a tumour-negative SLNs in combination with tumour-positive rLNs as false negative (FN).

Sensitivity, negative predictive value (NPV) and detection rate were recalculated from original published results, IPD and HQ-studies. Sensitivity of the SLN-procedure was defined as the number of true positives in patients with positive histopathological findings. The NPV was defined as the number of patients in whom a negative SLN correctly predicted the lymph node status of the total lymph-node yield. Detection rate was the proportion of successful SLN-procedures divided by all executed SLN-procedures.

In the overall database and IPD, patients were considered 'upstaged' in case of positive SLNs at advanced histopathology without tumour-positive rLNs with conventional histopathology. In HQ-studies, patients were considered as 'upstaged' if LNM were detected in SLNs or rLNs with advanced histopathology in absence of LNM after conventional histopathology. Additionally, we used HQ-studies to calculate the LNM-prevalence (percentage of patients with positive tumour findings after conventional H&E-staining and after advanced histopathology of the SLNs and rLNs together).

Risk of bias

To assess risk of bias we used the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [21], classifying this risk into four key domains: patient selection, index test, reference standard, flow and timing. In each domain low, high or unclear risk of bias can be scored. Using QUADAS-2, applicability of study results for our research question was estimated. Risk of bias and applicability were independently assessed by MA and SV. Discrepancies in interpretation were resolved by discussion (SI 3).

Statistical analysis

Specificity and positive predictive value of SLN-procedures was 100% by definition (false positives were impossible). Sensitivity, NPV and detection rate were pooled using univariate logistic random effects models including confidence intervals. Heterogeneity of results was determined by I^2 and Chi-square tests from random effects models. Statistical pooling was conducted with 'metan' and 'xtmelogit' procedures in STATA v13.0. To assess the influence of tumour- and procedure-related factors on sensitivity, NPV, detection rate, upstaging and prevalence logistic Generalized Estimated Equations (GEE) with independent or exchangeable working correlation matrices were conducted. Subgroup-analysis regarding diagnostic parameters were performed for gender, tumour location, -stage, -size, number of SLNs and total harvested lymph nodes (LN yield), injection site, in-vivo/ex-vivo approach and used tracer(s). For categorical variables marginal group estimates of all outcomes were used to compare between groups. Data were analysed with SPSS v22. P-values of 0.05 or less were used as level of statistical significance.

Role of the funding source

There was no funding source.

RESULTS

We identified 47 eligible studies [22-68] (Table 1; selection process: Figure 1, reasons for exclusion: SI 4). The 47 studies comprised 3243 successful SLN-procedures in 3536 patients (91.7%). Mean age was 68.1 years (SD 4.0). Early staged tumours (T1, T2) prevailed in 10.7% (n=305) and 18.6% (n=532), respectively. Most presented with T3 disease 63.3% (n=1811); 7.4% (n=213) had T4-tumours. SLN definitions varied: only first stained (only blue, blue and radioactive, fluorescent or fluorescent and blue) to all stained or radioactive lymph nodes.

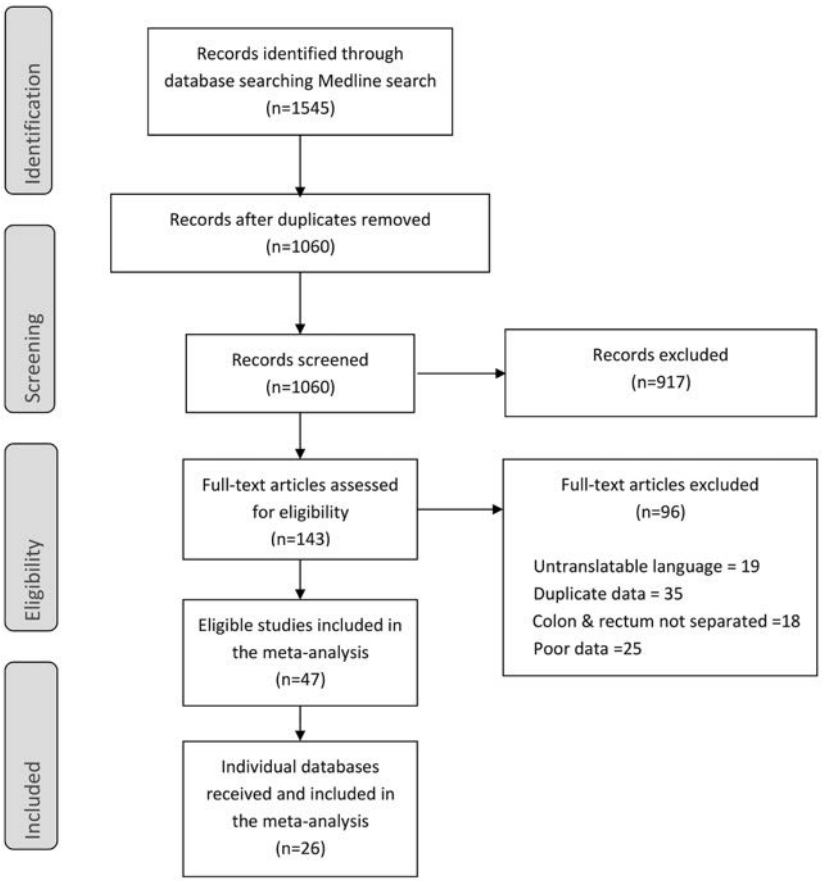

Figure 1. Prisma flowchart

Table 1. Main characteristics of all 47 studies included in the meta-analysis

	Year of publication	Colon tumours	Men	Women	Successful procedures	T1/T2	T3/T4	Definition of the sentinel node	In vivo or ex vivo approach	Tracer	Injection site	Histopathological technique
Merrie[22]	2001	25	NR	NR	23	5	20	High-activity nodes or first blue nodes	In vivo	Patent blue and ^{99m}Tc	Subserosal	H&E, RT-PCR of all lymph nodes
Terwisscha[23]	2006	56	23	33	49	11	45	First blue stained nodes	In vivo	Patent blue and ^{99m}Tc	Subserosal	H&E, IHC serial sectioning of all lymph nodes
Faerden[24]	2008	199	81	118	172	51	148	Four blue-stained nodes nearest the tumour	In vivo	Patent blue	Subserosal	HE, IHC 200 um intervals of all lymph nodes
Markl[25]	2010	28	17	11	21	4	21	All blue stained lymph nodes	Ex Vivo	Indian ink	Both	H&E, IHC and RT-PCR all lymph nodes
van der Zaag[26]	2012	249	113	136	199	61	186	First 1-4 blue stained lymph nodes	Ex vivo	Patent blue	Subserosal	H&E, IHC 500 uM intervals of SLN only (n=65) or all lymph nodes (n=134)
Viehl[27]	2003 and 2012	174	95	79	155	38	136	First blue stained nodes within 10 minutes after injection	In vivo	Isosulfan blue	Subserosal	H&E, IHC serial sectioning of all lymph nodes
Cserni[28]	1999	22	9	13	20	2	20	All blue lymph nodes	Ex vivo	Patent blue	Subserosal	H&E only
Bertoglio[29]	2004	20	NR	NR	19	NM	NM	1-3 lymph nodes close to the tumour site, within 5-10 min after injection	In vivo	Patent blue	Subserosal	H&E, 200 um intervals of SLN only
Nagata[30]	2006	44	NR	NR	44	31	13	Green enhanced lymph nodes within 5 minutes after injection	In vivo	Indocyanine Green	Subserosal	H&E only
Thomas[31]	2006	77	36	41	60	21	40	First lymph node to show isosulfan blue uptake	In vivo	Isosulfan blue	Subserosal	H&E, IHC serial sectioning of SLN only
Bianchi[32]	2007	22	15	9	22	3	11	First blue stained lymph nodes	Ex vivo	Patent blue	Subserosal	H&E IHC 50 um intervals of SLN only

Table 1 (continued). Main characteristics of all 47 studies included in the meta-analysis

	Year of publication	Colon tumours	Men	Women	Successful procedures	T1/T2	T3/T4	Definition of the sentinel node	In vivo or ex vivo approach	Tracer	Injection site	Histopathological technique
Kelder[33]	2007	37	20	17	36	12	25	Blue-stained lymph node	In vivo	Patent blue	Subserosal	H&E, IHC 150 um intervals of SLN only
Kusano[34]	2007	25	NR	NR	21	25	0	SLN appeared as round spots of clear fluorescence	In vivo	Indocyanine Green	Subserosal	H&E only
Liberale[35]	2007	69	37	32	67	24	45	Blue and fluorescent lymph nodes	Ex vivo	Patent blue V and Indocyanine green	Subserosal	H&E, IHC 150 um intervals of SLN only
Lim[36]	2007	119	57	63	118	40	78	Initial blue lymph node	In Vivo	Isosulfan blue and ^{99m}Tc	Subserosal	H&E, 2-3 mm thick only on SLN
Matter[37]	2007	40	20	19	36	6	33	Blue-stained nodes after 5-10 minutes	In vivo	Patent blye	Subserosal	H&E, IHC 200 um intervals of all lymph nodes
Tiffet[38]	2007	48	19	29	45	9	39	First blue stained nodes or radioactive	In vivo	Patent blue and ^{99m}Tc	Subserosal	H&E, IHC serial sectioning of SLN only
Yagci[39]	2007	20	13	7	20	1	19	First blue stained nodes within 5-10 minutes after injection	Ex vivo	Patent blue	Submucosal	H&E, IHC 300 um intervals of all lymph nodes
Quadros[40]	2008	22	7	15	20	2	20	All radioactive or blue nodes	In vivo	Patent blue and ^{99m}Tc	Subserosal	H&E, IHC 40 um intervals of SLN only
Saha[41]	2013	308	150	158	306	98	210	First 1-4 blue stained nodes within 10-15 minutes after injection	Ex vivo	Isosulfan blue	Subserosal	H&E, IHC 20-30 um of SLN only
Braat[42]	2014	55	29	26	51	11	44	First 1-4 blue nodes after injection	Both	Patent blue	Both	H&E, IHC 500 um intervals of SLN only
Murawa[43]	2015	131	66	65	131	40	91	First blue stained node after 5-10 minutes	In vivo	Patent blue	Subserosal	H&E, IHC of SLN only

Table 1 (continued). Main characteristics of all 47 studies included in the meta-analysis

	Year of publication	Colon tumours	Men	Women	Successful procedures	T1/T2	T3/T4	Definition of the sentinel node	In vivo or ex vivo approach	Tracer	Injection site	Histopathological technique
Retter[44]	2011	31	NR	NR	28	6	25	First blue stained nodes within 10 minutes after injection	In vivo	Patent blye V dye	Subserosal	H&E, IHC serial sectioning 10um of SLN only
Nordgard[45]	2012	212	NR	NR	204	47	165	Blue stained lymph nodes and nodes with connecting blue vessels	Ex vivo	Patent blue	Submucosal	H&E, IHC and RT-PCR of SLN only
Ramos[46]	2013	125	80	45	121	41	84	1-4 first blue stained lymph nodes	Ex vivo	Methylene blue	Subserosal	H&E, IHC of six 4 uM sections SLN only
Andersen[47]	2017	29	18	11	22	8	21	All fluorescent nodes OR blue nodes 5 minutes after injection	Both	Methylene blue and Indocyanine green	Subserosal	H&E + multislicing of all lymph nodes, IHC of SLNs and some non-SLNs
Bendavid[48]	2002	20	NR	NR	17	NR	NR	Blue stained lymph nodes	In vivo	Isosulfan blue	Subserosal	H&E, IHC 200 um intervals of SLN only
Paramo[49]	2002	55	28	27	45	14	41	First lymph nodes highlighted with blue	In vivo	Isosulfan blue	Subserosal	H&E, IHC 20 um intervals of SLN only
Dahl[50]	2004	30	17	13	30	2	28	Blue-stained lymph nodes within 10-15 minutes	In vivo	Patent blue and ^{99m}Tc	Subserosal	H&E only at multiple levels of all lymph nodes
Read[51]	2005	38	NR	NR	30	25	13	Blue stained nodes within five minutes after injection	In vivo	Isosulfan blue	Subserosal	Single-sectioning with H&E
Redston[52]	2006	72	NR	NR	66	NR	NR	Lymph node with blue dye uptake within the first 10 minutes	In vivo	Isosulfan blue	Subserosal	H&E, IHC 75 um intervals of all LNs

Table 1 (continued). Main characteristics of all 47 studies included in the meta-analysis

	Year of publication	Colon tumours	Men	Women	Successful procedures	T1/T2	T3/T4	Definition of the sentinel node	In vivo or ex vivo approach	Tracer	Injection site	Histopathological technique
Tuech[53]	2006	34	NR	NR	32	5	27	First blue-stained nodes	Both	Patent blue	Both	H&E, IHC serial sectioning 20 um of SLN only
Bembenek[54]	2007	315	186	129	268	NR	NR	Blue-stained lymph nodes appeared within first 10 minutes	In vivo	Patent blue	Subserosal	H&E, IHC 250 um intervals of SLN only
Covarelli[55]	2007	20	12	8	19	NR	NR	Lymph node present in the maximum verified radiation	In vivo	Patent blue and ^{99m}Tc	Siubserosal	H&E, IHC 200 um intervals of SLN only
Stojadinovic[56]	2007	93	43	50	82	31	62	First blue staining nodes within 5-10 minutes	Ex vivo	Isosulfan blue	Subserosal	H&E, IHC 40 um intervals of SLN only
Dragan[57]	2009	60	NR	NR	58	NR	NR	Blue-stained lymph node within 10 min after injection	Ex vivo	Isosulfan blue	Subserosal	H&E, IHC of SLN only
Albayrak[58]	2011	38	20	18	36	22	16	The first 1-4 blue-nodes after injection	In vivo	Isosulfan blue	Subserosal	Multisectiong and H&E staining
Vilcea[59]	2011	22	NR	NR	19	NR	NR	First blue stained nodes	In vivo	Methylene blue	Subserosal	H&E, micro-sectioning at 0.4 um
Hirche[60]	2012	26	NR	NR	25	11	15	First fluorescent identified lymph nodes	In vivo	Indocyanine Green	Subserosal	H&E, IHC 250 um intervals of SLN only
Oh[62]	2014	34	23	11	34	6	28	All blue lymph nodes	Both	Methylene blue	Subserosal	H&E and serial sectioning 0.5 mm intervals
Pallares[63]	2014	101	56	45	92	31	70	1-4 first blue stained	Ex vivo	Methylene blue	Subserosal	H&E, IHC multislicing 5 us of SLN only
Estrada[64]	2016	78	42	36	78	30	48	1-3 blue stained nodes nearest the tumour	Ex vivo	Methylene blue	Submucosal	H&E, IHC 3-250 um intervals of SLN only

Table 1 (continued). Main characteristics of all 47 studies included in the meta-analysis

	Year of publication	Colon tumours	Men	Women	Successful procedures	T1/T2	T3/T4	Definition of the sentinel node	In vivo or ex vivo approach	Tracer	Injection site	Histopathological technique
Liberale[65]	2016	20	9	11	19	6	14	Blue and fluorescent lymph nodes	Ex vivo	Patent blue V and Indocyanine green	Subserosal	H&E, IHC 150 um intervals of SLN only
Mogoanta[66]	2016	21	NR	NR	18	NR	NR	First blue stained nodes within 10 minutes	Ex vivo	Methylene blue	Subserosal	H&E, IHC serial sectioning 3-4 um thickness of all LNs
Currie[67]	2017	30	NR	NR	27	14	16	All fluorescent nodes	In vivo	Indocyanine green	Submucosal	H&E, IHC 250 um intervals of SLN only
Weixler[68]	2017	220	126	94	219	57	163	First blue nodes within 5-10 minutes after injection OR Any fluorescent hotspot	Both	Isosulfan bye + carbon dye or IRDye800CW	Subserosal	H&E, IHC serial sectioning of all lymph nodes

* The first six studies represent the HQ-studies, followed by all IPD studies and other articles.

For SLN concept validation we identified six HQ-studies among the 47 included studies, comprising 669 patients with 563 successful procedures. Of 106 failed procedures, no SLNs were identified in 92 patients and no lymph nodes besides SLNs were found in surgical specimens in fourteen. In two studies the SLN-procedure was performed ex-vivo [25, 26]; both used only blue dye injected in subserosal layers (n=215). Submucosal blue dye injection was additionally performed in sixteen patients [25]. In-vivo subserosal injections were used in the remaining four studies (n=454) [22-24, 27], using blue dye [24, 27] or dye combined with 99mTc-nanocoll [22, 23] as mapping agents. Fifty-three (8%) patients had T1, 79 T2 (12%), 486 T3 (73%) and 51 (8%) had T4 tumours, with mean tumor sizes of 4.7cm (SD 2.0). The majority were located in the sigmoid, none were in the left flexure.

Pooled sensitivity and NPV for all 47 studies are shown in Figure 2 and Figure 3. HQ-studies showed a sensitivity of 0.56 (95%CI:0.53-0.58) with low heterogeneity (Chi^2=2.29; df=5 (p=0.808); I^2=0%, Figure 4), corresponding to a NPV of 0.69 (95%CI:0.64-0.73; with low heterogeneity, Chi^2=10.36; df=5 (p=0.066); I^2=51.7%, Figure 5). Detection rate among HQ-studies was 0.85 (95%CI:0.79-0.89).

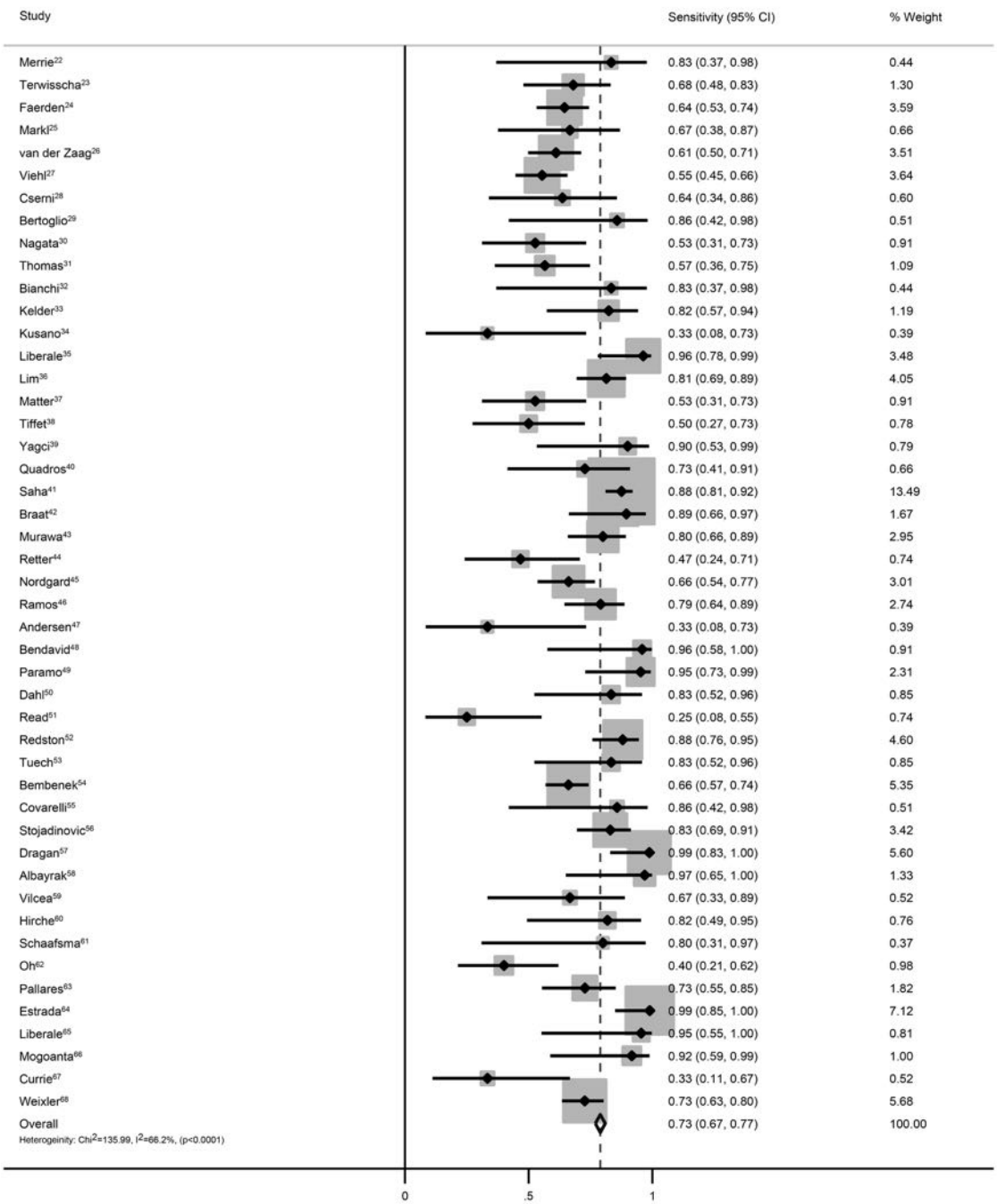

Figure 2. Weighted sensitivity for each individual study*

* Sensitivity was calulcated after advanced examination of only the SLNs
** The first six studies are referred as high quality concept validation studies in the manuscript

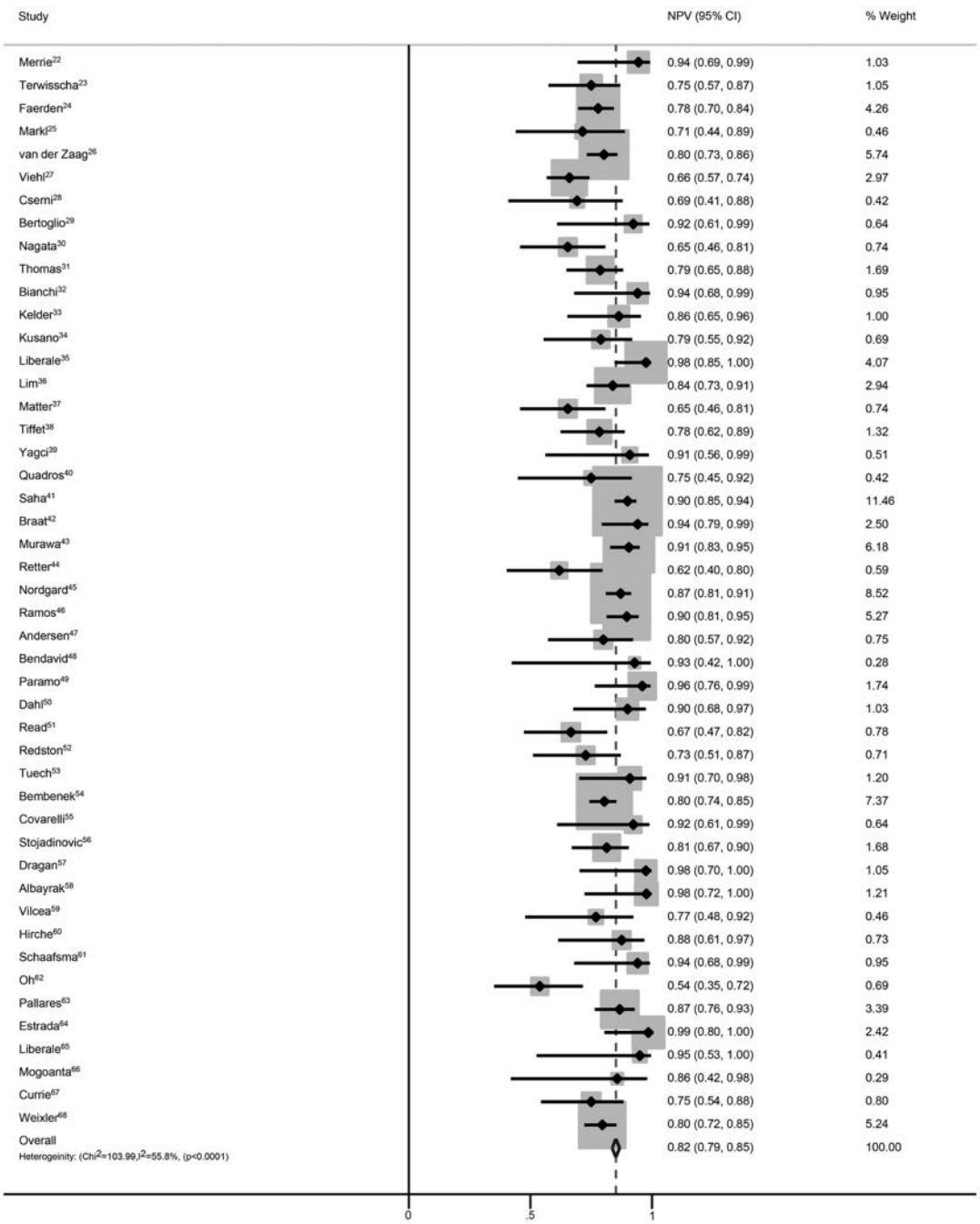

Figure 3. Weighted negative predictive value for each individual study*

* Negative predictive value was calculated after advanced examination of only the SLNs
** The first six studies are referred as high quality concept validation studies in the manuscript

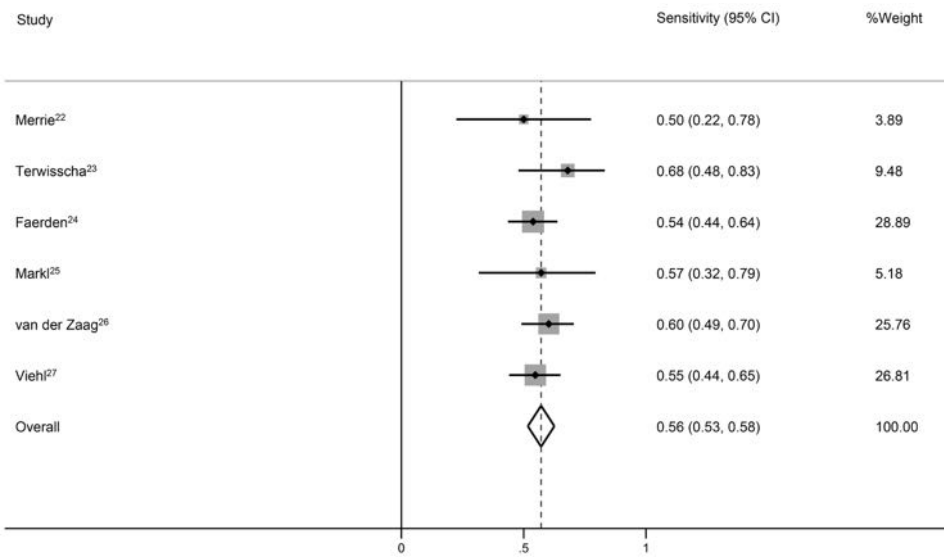

Figure 4. Weighted sensitivity of the high quality concept validation studies*

* Sensitivity after advanced examination of SLNs and regional lymph nodes

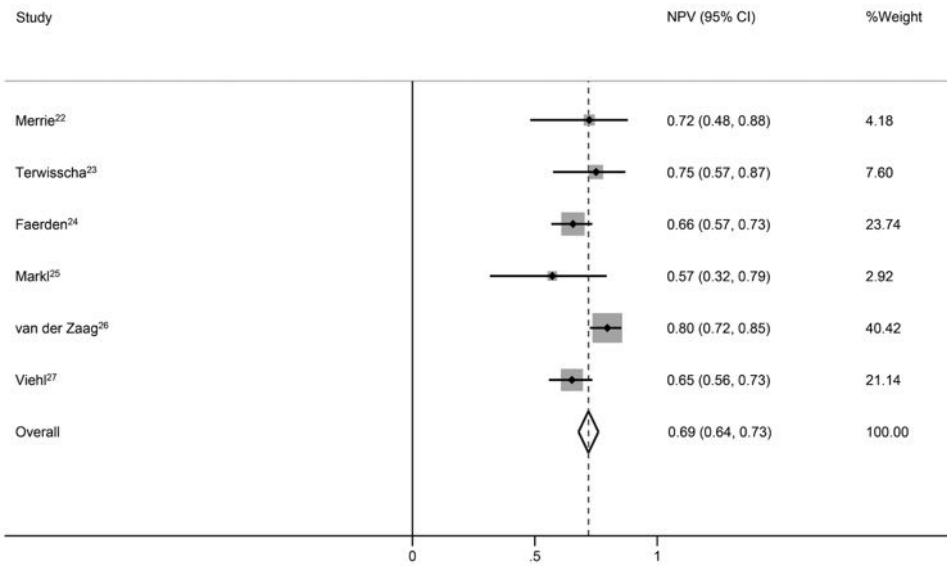

Figure 5. Weighted negative predictive value of high quality concept validation studies*

* Negative predictive value after advanced examination of SLNs and regional lymph nodes

Diagnostic performance was higher in the remaining 41 studies: pooled sensitivity was 0.74 (95%CI:0.69-0.80) with substantial heterogeneity (Chi^2=119.39; df=40 (p<0.0001); I^2=66.5%), and

pooled NPV of 0.83 (95%CI:0.80-0.86), again with substantial heterogeneity (Chi^2=83.18, df=40 (p<0.0001); I^2=51.9%). Pooled detection rate was 0.94 (95%CI:0.91-0.95).

Sources of heterogeneity (focusing on procedure and tumour-related factors) were investigated using IPD from 26 studies including the six HQ-studies [23,26-28,30,35,37-39,41-45,47,48,52,53,56,57,60-62,64,67,68], comprising 2182 patients presenting 2006 successful SLN-procedures (92%). Some did not provide data on tumour stage [29], tumour location [30] upstaging [45], and injection site and in- or ex-vivo approach [42]. Mean tumour size was 4.8 cm (SD 2.3) in 1212 patients out of 13 studies [26-28, 33-35, 37, 38, 40-43, 45].

Subanalyses of diagnostic parameters were performed for HQ-studies (SI 5) and IPD (SI 6). In HQ-studies ex-vivo had a higher sensitivity than in-vivo (ex-vivo; 0.61 [95%CI:0.60-0.610] vs. in-vivo; 0.54 [95%CI:0.54-0.54], p<0.0001; similar trend in IPD: ex-vivo: 0.75 [95%CI:0.66-0.82] vs. in-vivo: 0.66 [95%CI:0.59-0.73], p=0.051). However, detection rates were higher with the in-vivo approach (HQ: in-vivo; 0.87 [95%CI:0.85-0.89] vs. ex-vivo; 0.77 [95%CI:0.76-0.77], p<0.0001). These results were not endorsed by IPD. Results for blue dye, whether or not combined with 99mTc-nanocoll, showed significantly better sensitivity than fluorescent tracers in the IPD (blue dye; 0.71 [95%CI:0.64-0.77], blue dye + 99mTc-nanocoll; 0.74 [95%CI:0.67-0.81] vs. fluorophore; 0.38 [95%CI:0.32-0.34], blue dye + fluorophore; 0.33 [95%CI:0.33-0.33], p<0.0001). Subanalyses for imaging tracers could not be performed in the HQ-studies due to the small number of patients. Numbers of SLNs identified with blue dye and blue dye combined with 99mTc-nanocoll were similar (blue dye: mean 2.6, SD 2.4 vs blue dye combined with 99mTc-nanocoll: mean 3.2, SD 2.7, p=0.068). Site of injection did not affect diagnostic performance.

Stratification for tumour-related factors showed that diagnostic parameters were independent of tumour location and size. In HQ-studies, sensitivity was lower with T1 tumours than in T3 and T4 tumours, but not in IPD (T1; 0.30 [95%CI:0.16-0.51] vs.T3; 0.56 [95%CI:0.51-0.61], p=0.006 vs.T4; 0.61 [95%CI:0.51-0.69], p=0.009). Sensitivity was positively associated with the number of harvested SLNs (>2 SLNs: 0.66 [95%CI:0.59-0.72] vs 2 SLNs; 0.54 [95%CI:0.44-0.63], 1 SLN: 0.43 [95%CI:0.34-0.52]; p=0.001). We observed no relation between number of rLNs and diagnostic parameters.

Prevalence of LNM was 0.34 (95%CI:0.31-0.37) after conventional H&E-staining (SLNs and rLNs), and after advanced histopathology 0.48 (95%CI:0.42-0.54), corresponding to 18% upstaging (Table 2). Prevalence with advanced histopathology of SLNs only was 0.41 (95%CI:0.37-0.45), vs. 0.34 (95%CI:0.31-0.37) with conventional pathology, corresponding with an upstaging of 10% converted from node-negativity to node-positivity.

Table 2. Prevalence of disease shown as mean (95%CI)

H&E staining of SLNs and regional LNs	H&E staining of SLNs and regional LNs + advanced pathology of only SLNs	H&E staining + advanced pathology of both SLNs and regional LNs	Prevalence of LNM after H&E-staining of only the SLN	Prevalence of LNM after H&E-staining and advanced pathology of only SLN
0.34 (0.31-0.37)	0.41 (0.37-0.45)	0.48 (0.42-0.54)	0.22 (0.04-0.26)	0.29 (0.28-0.29)
0.10 (0.05-0.17)	0.15 (0.10-0.21)	0.30 (0.18-0.47)	0.05 (0.02-0.09)	0.12 (0.06-0.22)
0.19 (0.15-0.24)	0.25 (0.19-0.32)	0.38 (0.29-0.48)	0.11 (0.08-0.15)	0.20 (0.25-0.35)
0.41 (0.36-0.45)	0.48 (0.43-0.52)	0.52 (0.44-0.60)	0.28 (0.23-0.34)	0.29 (0.27-0.32)
0.52 (0.46-0.59)	0.59 (0.52-0.66)	0.66 (0.51-0,78)	0.36 (0.30-0.42)	0.41 (0.33-0.49)

DISCUSSION

In this meta-analysis we found that sensitivity of SLN procedures in colon cancer is low, regardless of tumour and procedure-related factors. The difference in sensitivity between HQ-studies and other studies underlines the necessity of similar and appropriate reference tests to both SLNs and regional lymph nodes. We cannot decide whether this low accuracy results from technical difficulties, anatomical complexities (perhaps specific to colon cancer) or from a conceptual problem.

Firstly, colon SLNs are often small (<1 cm) and located beneath a thick layer of fat impeding visibility with optical techniques, explaining low sensitivities with these methods. Secondly, number and location of colon SLNs are unpredictable since lymphatic drainage patterns are unknown. Hence, SLN definitions are inconsistent resulting in harvesting of the first to all tracer accumulating nodes. It was suggested that at least four SLNs are needed for accurate SLN-procedures, based on the assumption that colon cancer has by definition more SLNs than breast cancer and melanoma which typically have a single SLN [69]. This suggests that lymphatic flow in colon cancer is more divergent. Our results do not contradict the hypothesis of better accuracy when harvesting more lymph nodes (>2 SLNs), but this must be interpreted with caution since more harvested SLNs will increase sensitivity anyway. Subanalysis of procedure-related factors showed better results for sensitivity after an ex-vivo procedure while in-vivo harvesting detects twice as many nodes (ex-vivo; 1.6 [SD 2.1] vs in-vivo; 3.2 [SD 2.4], p<0.0001). In addition, no other tumour or procedure-related factors (tracer or injection site) showed better SLN accuracy with more SLNs harvested. The better sensitivity of the ex-vivo approach may result from better real-time visualization of lymph flow dynamics and a more specific mesocolon dissection to detect SLNs [70]. On the other hand, node positive (S)LNs can be missed with the ex vivo approach, for example when they are located outside the resection area.

Real-time visualization of lymph flow dynamics to establish lymphatic drainage patterns might improve SLN-mapping. Therefore, some studies combined preoperative lymphoscintigraphy with intraoperative gamma-probe and blue dye, as in breast cancer and melanoma [22, 23, 36, 38, 40, 50, 55]. This allows for preoperative identification of the number and location of the SLNs, and real-time identification of SLNs versus surrounding fat [55, 71]. However, results were similar for perioperative gamma imaging added to blue dye vs. blue dye only. This may reflect the limited resolution of lymphoscintigraphy impeding proper preoperative SLN identification due to "shine-through" from the tracer depot. Intraoperative identification is difficult because blue dye cannot be seen through fatty tissue, and its relatively small particle size causes rapid passage

through lymphatic channels, limiting its usefulness in detecting first draining nodes [69, 72]. Near-infrared fluorescent tracers have better characteristics for intraoperative detection than blue dye (e.g. larger particle size, real-time and improved optical guidance) [73-76]. In addition, PET/CT (e.g. with 89Zr-nanocoll) may provide an alternative pre-operative localisation tool with much better resolution than SPECT [77]. We hypothesize that the most optimal SLN mapping protocol is combined injection of an optical tracer with a radiocolloid.

Whereas our results suggest that ex-vivo SLN identification is preferable, a disadvantage is potential disruption of natural lymphatic pathways and non-identification of (S)LNs when located outside the resection area [41]. We suggest to use in-vivo injections followed by in-vivo and additionally ex-vivo SLN-identification in fresh resection-specimens.

The potential benefits of SLN-procedures in colon cancer are improved staging, patient management and survival. Serial-sectioning increases detection of (macro)metastases and especially micrometastases and isolated tumour cells. Indeed, LNM-prevalence in HQ-studies was 48% versus 34% after conventional H&E-staining. These missed LNM at initial surgery might help to explain recurrences in patients after apparently curative surgery. However, the prognostic and predictive relevance of occult tumour cells is still unclear. If only micrometastases, rather than isolated tumour cells, are associated with lower 5-years survival, the benefit of the SLN-procedure will be limited since the former are found less often than the latter [7, 78-81]; Unfortunately, we could not distinguish these two categories since most studies did not differentiate these subtypes. Future studies should investigate the prognostic relevance of isolated tumour cells and micrometastases, and establish if adjuvant chemotherapy improves survival in these patients. When the clinical relevance of occult tumour cells is known and a reliable SLN-procedure has been developed, studies investigating SLN-picking, combined with local excision of the primary tumour if the SLN is negative, can be designed. Such management might lower surgery-related morbidity and mortality in early staged tumours [82, 83].

The strength of this meta-analysis is that we implemented a rigorous reference test for the HQ-studies, in which SLNs and regional lymph nodes were both examined with conventional and advanced histopathology. For as far as we know this is has not been performed previously. This revealed on the one hand the overestimated diagnostic performance in studies without it, and the true prevalence of LNM on the other. Another strength of the study is the wealth of individual patient data (ca. two-third of the included population) allowing for additional analyses of tumour- and procedure-related factors. Although we applied statistical methods suited for small sample sizes, subgroup-analyses (especially tumour stage) should be interpreted

with caution. Heterogeneity for primary outcome parameters was low in the HQ-studies, but the tremendous methodological diversity across the other studies may have induces bias. Unfortunately, we did not have IPD of four large studies [54, 63, 64, 68] and of four others [48, 57, 58, 65] of which the last four reported sensitivities up to 100% (852 SLN-procedures, i.e. 27% of our study-population). Although no study met the eligibility criteria for HQ-studies, results might have affected diagnostic outcomes especially regarding subgroup-analysis. Inability to separate colon and rectal cancers, precluded analysis of 18 databases (n=677) (see SI 4).

In conclusion, the SLN procedure has the potential to improve staging while diminishing the surgical trauma in patients with early-staged colon cancer. The SLN performance is currently insufficient due to anatomical and technical difficulties combined with the wide variation of used SLN mapping methods, patient selection and histopathological analysis of lymph nodes. Therefore a standardized SLN-procedure must be developed, focussing on low invasive tumours and real-time imaging of lymph flow towards the SLN. Upon standardization, the SLN-procedure may help to clarify the prognostic relevance of isolated tumour cells and micrometastases. Finally, the SLN-procedure in colon cancer seems more challenging than in other cancers, and considerable expertise will be required before large patient-related studies can be undertaken.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62(1):10-29.
3. Gunderson LL, Jessup JM, Sargent DJ, Greene FL, Stewart AK. Revised TN categorization for colon cancer based on national survival outcomes data. J Clin Oncol. 2010;28(2):264-71.
4. Manfredi S, Bouvier AM, Lepage C, Hatem C, Dancourt V, Faivre J. Incidence and patterns of recurrence after resection for cure of colonic cancer in a well defined population. Br J Surg. 2006;93(9):1115-22.
5. Brouwer NPM, Bos A, Lemmens V, Tanis PJ, Hugen N, Nagtegaal ID, et al. An overview of 25 years of incidence, treatment and outcome of colorectal cancer patients. Int J Cancer. 2018;143(11):2758-66.
6. Elferink MA, de Jong KP, Klaase JM, Siemerink EJ, de Wilt JH. Metachronous metastases from colorectal cancer: a population-based study in North-East Netherlands. Int J Colorectal Dis. 2015;30(2):205-12.
7. Sloothaak DAM, van der Linden RLA, van de Velde CJH, Bemelman WA, Lips DJ, van der Linden JC, et al. Prognostic implications of occult nodal tumour cells in stage I and II colon cancer: The correlation between micrometastasis and disease recurrence. Eur J Surg Oncol. 2017;43(8):1456-62.
8. Weixler B, Warschkow R, Guller U, Zettl A, von Holzen U, Schmied BM, et al. Isolated tumor cells in stage I & II colon cancer patients are associated with significantly worse disease-free and overall survival. BMC Cancer. 2016;16:106.
9. Faerden AE, Sjo OH, Bukholm IR, Andersen SN, Svindland A, Nesbakken A, et al. Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer. Dis Colon Rectum. 2011;54(2):200-6.
10. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-91.
11. Schoen RE, Pinsky PF, Weissfeld JL, Yokochi LA, Church T, Laiyemo AO, et al. Colorectal-cancer incidence and mortality with screening flexible sigmoidoscopy. N Engl J Med. 2012;366(25):2345-57.
12. Bosch SL, Teerenstra S, de Wilt JH, Cunningham C, Nagtegaal ID. Predicting lymph node metastasis in pT1 colorectal cancer: a systematic review of risk factors providing rationale for therapy decisions. Endoscopy. 2013;45(10):827-34.
13. Liang J, Fazio V, Lavery I, Remzi F, Hull T, Strong S, et al. Primacy of surgery for colorectal cancer. Br J Surg. 2015;102(7):847-52.
14. Bokey EL, Chapuis PH, Fung C, Hughes WJ, Koorey SG, Brewer D, et al. Postoperative morbidity and mortality following resection of the colon and rectum for cancer. Dis Colon Rectum. 1995;38(5):480-6; discussion 6-7.
15. van der Zaag ES, Kooij N, van de Vijver MJ, Bemelman WA, Peters HM, Buskens CJ. Diagnosing occult tumour cells and their predictive value in sentinel nodes of histologically negative patients with colorectal cancer. Eur J Surg Oncol. 2010;36(4):350-7.
16. Verrill C, Carr NJ, Wilkinson-Smith E, Seel EH. Histopathological assessment of lymph nodes in colorectal carcinoma: does triple levelling detect significantly more metastases? J Clin Pathol. 2004;57(11):1165-7.
17. Nordgard O, Smaaland R. SLN mapping in colorectal cancer. Lancet Oncol. 2011;12(11):990; author reply -1.

18. van der Pas MH, Meijer S, Hoekstra OS, Riphagen, II, de Vet HC, Knol DL, et al. Sentinel-lymph-node procedure in colon and rectal cancer: a systematic review and meta-analysis. Lancet Oncol. 2011;12(6):540-50.

19. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol. 2009;62(10):1006-12.

20. Hermanek P, Hutter RV, Sobin LH, Wittekind C. International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. Cancer. 1999;86(12):2668-73.

21. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529-36.

22. Merrie AE, van Rij AM, Phillips LV, Rossaak JI, Yun K, McCall JL. Diagnostic use of the sentinel node in colon cancer. Dis Colon Rectum. 2001;44(3):410-7.

23. Terwisscha Van Scheltinga SE, Den Boer FC, Pijpers R, Meyer GA, Engel AF, Silvis R, et al. Sentinel node staging in colon carcinoma: value of sentinel lymph node biopsy with radiocolloid and blue staining. Scand J Gastroenterol Suppl. 2006(243):153-7.

24. Faerden AE, Sjo OH, Andersen SN, Hauglann B, Nazir N, Gravdehaug B, et al. Sentinel node mapping does not improve staging of lymph node metastasis in colonic cancer. Dis Colon Rectum. 2008;51(6):891-6.

25. Markl B, Arnholdt HM, Jahnig H, Spatz H, Anthuber M, Oruzio DV, et al. A new concept for the role of ex vivo sentinel lymph nodes in node-negative colorectal cancer. Ann Surg Oncol. 2010;17(10):2647-55.

26. van der Zaag ES, Bouma WH, Peters HM, Bemelman WA, Buskens CJ. Implications of sentinel lymph node mapping on nodal staging and prognosis in colorectal cancer. Colorectal Dis. 2012;14(6):684-90.

27. Viehl CT, Guller U, Cecini R, Langer I, Ochsner A, Terracciano L, et al. Sentinel lymph node procedure leads to upstaging of patients with resectable colon cancer: results of the Swiss prospective, multicenter study sentinel lymph node procedure in colon cancer. Ann Surg Oncol. 2012;19(6):1959-65.

28. Cserni G, Vajda K, Tarjan M, Bori R, Svebis M, Baltas B. Nodal staging of colorectal carcinomas from quantitative and qualitative aspects. Can lymphatic mapping help staging? Pathol Oncol Res. 1999;5(4):291-6.

29. Bertoglio S, Sandrucci S, Percivale P, Goss M, Gipponi M, Moresco L, et al. Prognostic value of sentinel lymph node biopsy in the pathologic staging of colorectal cancer patients. J Surg Oncol. 2004;85(3):166-70.

30. 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.

31. Thomas KA, Lechner J, Shen P, Waters GS, Geisinger KR, Levine EA. Use of sentinel node mapping for cancer of the colon: 'to map or not to map". Am Surg. 2006;72(7):606-11; discussion 11-2.

32. Bianchi PP, Ceriani C, Rottoli M, Torzilli G, Roncalli M, Spinelli A, et al. Laparoscopic lymphatic mapping and sentinel lymph node detection in colon cancer: technical aspects and preliminary results. Surg Endosc. 2007;21(9):1567-71.

33. Kelder W, Braat AE, Karrenbeld A, Grond JAK, De Vries JE, Oosterhuis JWA, et al. The sentinel node procedure in colon carcinoma: a multi-centre study in The Netherlands. Int J Colorectal Dis. 2007;22(12):1509.

34. 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.

35. Liberale G, Lasser P, Sabourin JC, Malka D, Duvillard P, Elias D, et al. Sentinel lymph nodes of colorectal carcinoma: reappraisal of 123 cases. Gastroenterol Clin Biol. 2007;31(3):281-5.

36. Lim SJ, Feig BW, Wang H, Hunt KK, Rodriguez-Bigas MA, Skibber JM, et al. Sentinel lymph node evaluation does not improve staging accuracy in colon cancer. Ann Surg Oncol. 2008;15(1):46-51.

37. Matter M, Winckler M, Aellen S, Bouzourene H. Detection of metastatic disease with sentinel lymph node dissection in colorectal carcinoma patients. Eur J Surg Oncol. 2007;33(10):1183-90.

38. Tiffet O, Kaczmarek D, Chambonniere ML, Guillan T, Baccot S, Prevot N, et al. Combining radioisotopic and blue-dye technique does not improve the false-negative rate in sentinel lymph node mapping for colorectal cancer. Dis Colon Rectum. 2007;50(7):962-70.

39. Yagci G, Unlu A, Kurt B, Can MF, Kaymakcioglu N, Cetiner S, et al. Detection of micrometastases and skip metastases with ex vivo sentinel node mapping in carcinoma of the colon and rectum. Int J Colorectal Dis. 2007;22(2):167-73.

40. Quadros CA, Lopes A, Araujo I, Fregnani JH, Fahel F. Upstaging benefits and accuracy of sentinel lymph node mapping in colorectal adenocarcinoma nodal staging. J Surg Oncol. 2008;98(5):324-30.

41. Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, et al. Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. Am J Surg. 2013;205(3):302-5; discussion 5-6.

42. Braat AE, Pol RA, Oosterhuis JW, de Vries JE, Mesker WE, Tollenaar RA. Excellent prognosis of node negative patients after sentinel node procedure in colon carcinoma: a 5-year follow-up study. Eur J Surg Oncol. 2014;40(6):747-55.

43. Murawa D, Nowaczyk P, Spychala A, Hunerbein M, Michal M. The Influence of Intraoperative Factors and Histopathological Staging on the Performance of Sentinel Node Biopsy in Colon Cancer. Acta Chir Belg. 2015;115(3):184-90.

44. Retter SM, Herrmann G, Schiedeck TH. Clinical value of sentinel node mapping in carcinoma of the colon. Colorectal Dis. 2011;13(8):855-9.

45. Nordgard O, Oltedal S, Aasprong OG, Soreide JA, Soreide K, Tjensvoll K, et al. Prognostic relevance of occult metastases detected by cytokeratin 20 and mucin 2 mRNA levels in sentinel lymph nodes from colon cancer patients. Ann Surg Oncol. 2012;19(12):3719-26.

46. Jose Domingo Sardon Ramos JEA, Eugenia Campo Cimarras, Baltasar Cermeno Toral, Jose Antonio Romeo Ramirez, Jaione Saenz de Ugarte Sobron, Begona Atares Pueyo, Virginia Moreno Nieto, Maria Cuadra Cestafe, Erika Miranda Serrano. Sentinel lymph node biopsy technique in colon cancer. Experience in 125 cases. Cirugia espanola 2013(91):366-71.

47. Andersen HS, Bennedsen ALB, Burgdorf SK, Eriksen JR, Eiholm S, Toxvaerd 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.

48. Bendavid Y, Latulippe JF, Younan RJ, Leclerc YE, Dube S, Heyen F, et al. Phase I study on sentinel lymph node mapping in colon cancer: a preliminary report. J Surg Oncol. 2002;79(2):81-4; discussion 5.

49. Paramo JC, Summerall J, Poppiti R, Mesko TW. Validation of sentinel node mapping in patients with colon cancer. Ann Surg Oncol. 2002;9(6):550-4.

50. Dahl K, Westlin J, Kraaz W, Winqvist O, Bergkvist L, Thorn M. Identification of sentinel nodes in patients with colon cancer. Eur J Surg Oncol. 2005;31(4):381-5.

51. Read TE, Fleshman JW, Caushaj PF. Sentinel lymph node mapping for adenocarcinoma of the colon does not improve staging accuracy. Dis Colon Rectum. 2005;48(1):80-5.

52. Redston M, Compton CC, Miedema BW, Niedzwiecki D, Dowell JM, Jewell SD, et al. Analysis of micrometastatic disease in sentinel lymph nodes from resectable colon cancer: results of Cancer and Leukemia Group B Trial 80001. J Clin Oncol. 2006;24(6):878-83.

53. Tuech JJ, Pessaux P, Di Fiore F, Nitu V, Lefebure B, Colson A, et al. Sentinel node mapping in colon carcinoma: in-vivo versus ex-vivo approach. Eur J Surg Oncol. 2006;32(2):158-61.

54. 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.

55. Covarelli P, Cristofani R, Boselli C, Servoli A, Burattini MF, Badolato M, et al. Preliminary study on radioguided sentinel node identification in colon cancer. Am Surg. 2007;73(3):222-6.

56. Stojadinovic A, Nissan A, Protic M, Adair CF, Prus D, Usaj S, et al. Prospective randomized study comparing sentinel lymph node evaluation with standard pathologic evaluation for the staging of colon carcinoma: results from the United States Military Cancer Institute Clinical Trials Group Study GI-01. Ann Surg. 2007;245(6):846-57.

57. Dragan R, Nebojsa M, Dejan S, Ivan P, Dragos S, Damir J, et al. Clinical application of sentinel lymph node biopsy for staging, treatment and prognosis of colon and gastric cancer. Hepatogastroenterology. 2009;56(96):1606-11.

58. Albayrak Y, Oren D, Gundogdu C, Kurt A. Intraoperative sentinel lymph node mapping in patients with colon cancer: study of 38 cases. Turk J Gastroenterol. 2011;22(3):286-92.

59. Vilcea ID, Vasile I, Mirea CS, Mesina C, Enache SD, Tenovici M, et al. Sentinel lymph node study in colorectal cancer using serial sectioning and Hematoxylin-Eosin staining: importance and limitations. Rom J Morphol Embryol. 2011;52(1 Suppl):379-83.

60. 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.

61. 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.

62. Oh SY, Kim DY, Kim YB, Suh KW. Clinical application of sentinel lymph node mapping in colon cancer: in vivo vs. ex vivo techniques. Ann Surg Treat Res. 2014;87(3):118-22.

63. Pallares-Segura JL, Balague-Pons C, Dominguez-Agustin N, Martinez C, Hernandez P, Bollo J, et al. The role of sentinel lymph node in colon cancer evolution. Cir Esp. 2014;92(10):670-5.

64. Estrada O, Pulido L, Admella C, Hidalgo LA, Clave P, Sunol X. Sentinel lymph node biopsy as a prognostic factor in non-metastatic colon cancer: a prospective study. Clin Transl Oncol. 2017;19(4):432-9.

65. 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.

66. Mogoanta SS, Calota F, Vasile I, Crafciuc AV, Gherghinescu MC, Sapalidis K, et al. Histological and immunohistochemical study on sentinel lymph node in colorectal cancer - values and limitations. Rom J Morphol Embryol. 2016;57(1):65-74.

67. 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.

68. 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.

69. Viehl CT, Guller U, Langer I, Laffer U, Oertli D, Zuber M. Factors influencing the success of in vivo sentinel lymph node procedure in colon cancer patients: Swiss prospective, multicenter study sentinel lymph node procedure in colon cancer. World J Surg. 2013;37(4):873-7.

70. Park JS, Chang IT, Park SJ, Kim BG, Choi YS, Cha SJ, et al. Comparison of ex vivo and in vivo injection of blue dye in sentinel lymph node mapping for colorectal cancer. World J Surg. 2009;33(3):539-46.

71. Sandrucci S, Mussa B, Goss M, Mistrangelo M, Satolli MA, Sapino A, et al. Lymphoscintigraphic localization of sentinel node in early colorectal cancer: results of a monocentric study. J Surg Oncol. 2007;96(6):464-9.

72. Heller S, Zanzonico P. Nuclear probes and intraoperative gamma cameras. Semin Nucl Med. 2011;41(3):166-81.

73. Hojo T, Nagao T, Kikuyama M, Akashi S, Kinoshita T. Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer. Breast. 2010;19(3):210-3.

74. Cahill RA. What's wrong with sentinel node mapping in colon cancer? World J Gastroenterol. 2007;13(47):6291-4.

75. Takahashi N, Nimura H, Fujita T, Yamashita S, Mitsumori N, Yanaga K. Quantitative assessment of visual estimation of the infrared indocyanine green imaging of lymph nodes retrieved at sentinel node navigation surgery for gastric cancer. BMC Surg. 2016;16(1):35.

76. Rocha A, Dominguez AM, Lecuru F, Bourdel N. Indocyanine green and infrared fluorescence in detection of sentinel lymph nodes in endometrial and cervical cancer staging - a systematic review. Eur J Obstet Gynecol Reprod Biol. 2016;206:213-9.

77. Ankersmit M, Hoekstra OS, van Lingen A, Bloemena E, Jacobs M, Vugts DJ, et al. Perioperative PET/CT lymphoscintigraphy and fluorescent real-time imaging for sentinel lymph node mapping in early staged colon cancer. Eur J Nucl Med Mol Imaging. 2019;46(7):1495-505.

78. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ, van der Zaag ES, Tanis PJ, Bemelman WA, et al. The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. Eur J Surg Oncol. 2014;40(3):263-9.

79. Iddings D, Ahmad A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. Ann Surg Oncol. 2006;13(11):1386-92.

80. Nicastri DG, Doucette JT, Godfrey TE, Hughes SJ. Is occult lymph node disease in colorectal cancer patients clinically significant? A review of the relevant literature. J Mol Diagn. 2007;9(5):563-71.

81. Saha S, Elgamal M, Cherry M, Buttar R, Pentapati S, Mukkamala S, et al. Challenging the conventional treatment of colon cancer by sentinel lymph node mapping and its role of detecting micrometastases for adjuvant chemotherapy. Clin Exp Metastasis. 2018;35(5-6):463-9.

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

83. Cahill RA, Bembenek A, Sirop S, Waterhouse DF, Schneider W, Leroy J, et al. Sentinel node biopsy for the individualization of surgical strategy for cure of early-stage colon cancer. Ann Surg Oncol. 2009;16(8):2170-80.

SUPPLEMENTARY INFORMATION S1

PubMed Search History March 25, 2019 (Read from bottom-up)

Search	PubMed Query	Items found
#3	#1 AND #2	560
#2	"Sentinel Lymph Node Biopsy"[Mesh] OR (sentinel[tiab] AND ("Lymph Nodes"[Mesh] OR lymph[tiab] OR lymphatic[tiab] OR node[tiab] OR nodes[tiab] OR nodal[tiab]))	15679
#1	"Colorectal Neoplasms"[Mesh] OR colorectal cancer*[tiab] OR colorectal carcinoma*[tiab] OR colorectal tumor*[tiab] OR colorectal tumour*[tiab] OR colorectal neoplas*[tiab] OR colonic cancer*[tiab] OR colonic carcinoma*[tiab] OR colonic tumor*[tiab] OR colonic tumour*[tiab] OR colonic neoplas*[tiab] OR colon cancer*[tiab] OR colon carcinoma*[tiab] OR colon tumor*[tiab] OR colon tumour*[tiab] OR colon neoplas*[tiab] OR rectum cancer*[tiab] OR rectum carcinoma*[tiab] OR rectum tumor*[tiab] OR rectum tumour*[tiab] OR rectum neoplas*[tiab] OR rectal cancer*[tiab] OR rectal carcinoma*[tiab] OR rectal tumor*[tiab] OR rectal tumour*[tiab] OR rectal neoplas*[tiab] OR anus cancer*[tiab] OR anus carcinoma*[tiab] OR anus tumor*[tiab] OR anus tumour*[tiab] OR anus neoplas*[tiab] OR anal cancer*[tiab] OR anal carcinoma*[tiab] OR anal tumor*[tiab] OR anal tumour*[tiab] OR anal neoplas*[tiab]	238682

Embase.com Search History March 25, 2019 (Read from bottom-up)

Search	Embase.com Query	Items found
#3	#1 AND #2	985
#2	'Sentinel Lymph Node'/exp OR 'Sentinel Lymph Node Biopsy'/exp OR (sentinel:ti,ab,kw AND ('Lymph Node'/exp OR 'Lymph Node Biopsy'/exp OR lymph:ti,ab,kw OR lymphatic:ti,ab,kw OR node:ti,ab,kw OR nodes:ti,ab,kw OR nodal:ti,ab,kw))	27177
#1	'Large Intestine Cancer'/exp OR 'Intestine Cancer'/de OR 'rectum tumor'/exp OR (colorectal NEAR/3 cancer*):ti,ab,kw OR (colorectal NEAR/3 carcinoma*):ti,ab,kw OR (colorectal NEAR/3 tumor*):ti,ab,kw OR (colorectal NEAR/3 tumour*):ti,ab,kw OR (colorectal NEAR/3 neoplas*):ti,ab,kw OR (colon* NEAR/3 cancer*):ti,ab,kw OR (colon* NEAR/3 carcinoma*):ti,ab,kw OR (colon* NEAR/3 tumor*):ti,ab,kw OR (colon* NEAR/3 tumour*):ti,ab,kw OR (colon* NEAR/3 neoplas*):ti,ab,kw OR (rectum NEAR/3 cancer*):ti,ab,kw OR (rectum NEAR/3 carcinoma*):ti,ab,kw OR (rectum NEAR/3 tumor*):ti,ab,kw OR (rectum NEAR/3 tumour*):ti,ab,kw OR (rectum NEAR/3 neoplas*):ti,ab,kw OR (rectal NEAR/3 cancer*):ti,ab,kw OR (rectal NEAR/3 carcinoma*):ti,ab,kw OR (rectal NEAR/3 tumor*):ti,ab,kw OR (rectal NEAR/3 tumour*):ti,ab,kw OR (rectal NEAR/3 neoplas*):ti,ab,kw OR (anus NEAR/3 cancer*):ti,ab,kw OR (anus NEAR/3 carcinoma*):ti,ab,kw OR (anus NEAR/3 tumor*):ti,ab,kw OR (anus NEAR/3 tumour*):ti,ab,kw OR (anus NEAR/3 neoplas*):ti,ab,kw OR (anal NEAR/3 cancer*):ti,ab,kw OR (anal NEAR/3 carcinoma*):ti,ab,kw OR (anal NEAR/3 tumor*):ti,ab,kw OR (anal NEAR/3 tumour*):ti,ab,kw OR (anal NEAR/3 neoplas*):ti,ab,kw	377247

SUPPLEMENTARY INFORMATION SI 2A

3 X 2 contingency table when only SLNs are examined with advances histopathology

	LN +	LN-
SLN +	A (True Positive)	B (True positive)
SLN-	C (False negative)	D (True negative)
SLN ultra-staging +	E (True positive)	F (True positive/upstaging)

SLN +	The sentinel lymph node(s) positive confirmed with conventional single section H&E staining
SLN -	The sentinel lymph node(s) negative for metastases after conventional single section H&E staining and advanced histopathological examination
SLN ultra-staging +	The sentinel lymph node(s) positive after advanced histopathological examination but negative with conventional single section H&E staining
LN +	Lymph nodes positive with single section H&E staining
LN-	Lymph nodes negative for metastases after conventional single section H&E staining and advanced histopathological examination

Sensitivity = (A+B+E+F)/ ((A+B+E+F)+C)
Negative predictive value = D /(D+C)
Upstaging = F / (F+D)

SUPPLEMENTARY INFORMATION SI 2B

3x3 contingency table when all lymph nodes are examined by advanced histopathology

	LN +	LN-	LN ultra-staging +
SLN +	A (True Positive)	B (True positive)	G (True positive)
SLN-	C (False negative)	D (True negative)	H (False negative/ upstaging)
SLN ultra-staging +	E (True positive)	F (True positive/ upstaging)	I (True positive/ upstaging)

SLN +	The sentinel lymph node(s) positive confirmed with conventional single section H&E staining
SLN -	The sentinel lymph node(s) negative for metastases after conventional single section H&E staining and advanced histopathological examination
SLN ultra-staging +	The sentinel lymph node(s) positive after advanced histopathological examination but negative with conventional single section H&E staining
LN +	Lymph nodes positive with single section H&E staining
LN-	Lymph nodes negative for metastases after conventional single section H&E staining and advanced histopathological examination
LN ultra-staging +	Lymph nodes positive after advanced histopathological examination but negative with conventional single section H&E staining

Sensitivity = (A+B+E+F+G+I)/ ((A+B+E+F+G+I)+ C+H)
Negative predictive value = D /(D+C+H)
Upstaging = (F+H+I)/ ((F+H+I) +D)

Prevalence after only H&E-staining of SLNs and regional lymph nodes = (A+B+C+E+G) / (A+B+C+D+E+F+G+H+I)
Prevalence after advanced examination of *only* SLNs = (A+B+C+E+F+G+I) / (A+B+C+D+E+F+G+H+I)
Prevalence after advanced examination of the *total* LN yield = (A+B+C+E+F+G+H+I)/ (A+B+C+D+E+F+G+H+I)

Supplementary Information 3. QUADAS-2

	Risk of Bias				Applicability concerns		
Study	**Patient selection**	**Index test**	**Reference standard**	**Flow and Timing**	**Patient selection**	**Index test**	**Reference standard**
Merrie[22]	?	☺	☺	☺	☺	☺	☺
Terwisscha[23]	☺	☺	☺	☺	☺	☺	☺
Faerden[24]	☺	☺	☺	☺	☺	☺	☺
Markl[25]	☹	☺	☺	☺	☺	☺	☺
Van der Zaag[26]	☺	☺	☺	☺	☺	☺	☺
Viehl[27]	☺	☺	☺	☺	☺	☺	☺
Cserni[28]	?	☺	☹	☺	☺	☺	☹
Bertoglio[29]	☺	☺	☹	☺	☺	☺	☹
Nagata[30]	?	☺	☹	☺	☺	☺	☹
Thomas[31]	☺	☺	☺	☺	☺	☺	☹
Bianchi[32]	☺	☺	☺	☺	☺	☺	☹
Kelder[33]	☺	☺	☺	☺	☺	☺	☹
Kusano[34]	?	☺	☹	?	☺	☺	☹
Liberale[35]	☺	☺	☺	☺	☺	☺	☹
Lim[36]	☺	☺	☺	☺	☺	☺	☹
Matter[37]	☺	☺	☺	☺	☺	☺	☹
Tiffet[38]	☹	☺	☺	☺	☺	☺	☹
Yagci[39]	☹	☺	☺	☺	☺	☺	☹
Quadros[40]	☺	☺	☺	☺	☺	☺	☹
Saha[41]	☺	☺	☺	☺	☺	☺	☹
Braat[42]	☺	☺	☺	☺	☺	☺	☹
Murawa[43]	☺	☺	☺	☺	☺	☺	☹
Retter[44]	☹	☺	☺	☺	☺	☺	☹
Nordgard[45]	☺	☺	☺	☺	☺	☺	☹
Ramos[46]	☹	☺	☺	☺	☺	☺	☹
Andersen[47]	☹	☺	☺	☺	☺	☺	☹
Bendavid[48]	?	☺	☺	☺	☺	☺	☹
Paramo[49]	☺	☺	☺	☺	☺	☺	☹
Dahl[50]	☺	☺	☺	☺	☺	☺	☹
Read[51]	?	☺	☹	☺	☺	☺	☹
Redston[52]	☹	☺	☺	☺	☹	☺	☹
Tuech[53]	☺	☺	☺	☺	☺	☺	☹

Supplementary Information 3 (continued). QUADAS-2

	Risk of Bias				Applicability concerns		
Study	**Patient selection**	**Index test**	**Reference standard**	**Flow and Timing**	**Patient selection**	**Index test**	**Reference standard**
Bembenek[54]	☺	☺	☺	☺	☺	☺	☹
Covarelli[55]	?	☺	☺	☺	☺	☺	☹
Vilcea[59]	☺	☺	☹	☺	☺	☺	☹
Hirche[60]	☺	☺	☺	☺	☺	☺	☹
Schaafsma[61]	☺	☺	☹	☺	☺	☺	☹
Oh[62]	☺	☺	☹	☺	☺	☺	☹
Pallares[63]	?	☺	☺	☺	☺	☺	☹
Estrada[64]	☺	☺	☺	☺	☺	☺	☹
Liberale[65]	☺	☺	☺	☺	☺	☺	☹
Mogoanta[66]	☺	☺	☺	☺	☺	☺	☹
Currie[67]	☺	☺	☺	☺	☺	☺	☹
Weixler[68]	☺	☺	☺	☺	☺	☺	☹

☺ Low risk ☹ High Risk ? Unclear Risk

Explanation QUADAS-2

Using QUADAS-2, risk of bias can be classified into four key domains: patient selection; index test; reference standard, flow and timing. In each domain low, high or unclear risk of bias can be scored. High risk of bias is scored if one of the questions on the form is answered with 'yes'; unclear risk of bias is scored if one of the questions is answered with 'unclear' and low risk of bias is scored if all questions in the domain are scored 'no'. Beside risk of bias assessment, the applicability of the domain on the author's research question can be assessed as high or low. If in,- or exclusion criteria were not described we assessed risk of bias as unclear. Risk of bias and applicability for the reference standard were considered as high when no histopathological techniques additional to conventional H&E-staining were applied to all lymph nodes. Risk of bias for flow and timing was considered as unclear when not all patients were mentioned in the results.

Results QUADAS-2

The majority of the studies scored low risk of bias for patient selection. Those studies with high risk of bias did not exclude patients suspicious for distant or lymphatic metastases after preoperative conventional work-up [25,38,39,44,46,47,52]. Risk of bias and concerns for applicability regarding the index test were all estimated as 'low risk' since all SLNs and regional lymph

nodes were undertaken by conventional H&E-staining. Fourty-one studies showed high risk of bias due to the reference standard because no histopathological techniques additional to conventional H&E-staining were applied to all lymph nodes [28-68]. Risk of bias for flow and timing was considered as unclear in one study since not all patients were mentioned in the results [34]. High concerns for applicability was found in one study in which the authors included patients with invasive colorectal carcinoma and did not report in,- or exclusion criteria [52].

Supplemental data 4. Studies excluded after full-text review

First author, year	Title	Additonal information
UNTRANSLATABLE LANGUAGE		
Abdurakhmonov, 2007	Intraoperative staging of colorectal cancer	Russian
Basilo, 2006	Sentinel lymph node detection in colorectal cancer: importance, techniques and results	Portugese
Coccetta, 2010	The sentinel lymph node mapping in colon cancer	Italian
Chen, 2006	Ex vitro sentinel lymph node mapping in colorectal carcinoma	Chinese
Duben, 2010	Lymphatic mapping and biopsy of sentinel lymph node using combined methodology of in vivo application of patent blue and radionuclide and ex vivo detection of metastatic affection of lymph nodes in colorectal carcinoma	Czech
Fabrega, 2001	The sentinel node: first cases in Panama	Spanish
Forte, 2006	The clinical significance of lymph node micrometastases and of concept of sentinel lymph node	Italian
Kolev, 2006	Sentinel lymph node mapping in patients with colorectal cancer	Bulgarian
Pasquini, 2005	Feasibility and reliability of the study of sentinel lymph nodes with immunohistochemical technique in colon carcinoma	Italian
Pozza, 2002	Impact of sentinel lymph node in the staging of colorectal carcinoma	Italian
Ramos, 2012	The sentinel lymph node technique in colon cancer	Spanish
Sandrucci, 2005	Lymposcintigraphic localization of sentinel lymph nodes in colorectal carcinoma in early stage: results of a single center study and proposal of a multicenter protocol	Italian and duplicate with Sandrucci, 2007; Lymphoscintigraphic localization of sentinel node in early colorectal cancer: results of a monocentric study *(not included)*
Sefr, 2003	Sentinel node biopsy in colorectal carcinoma- pilot study	Czech
Sefr, 2006	Lymphatic mapping and sentinel node biopsy in colorectal cancer	Czech

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Toma, 2002	Detection of sentinel lymphatic region with activated carbon particles in lymph node dissection for colorectal cancer	Japanese
Vajda, 2002	Sentinel lymph node mapping in colorectal cancer	Hungarian
Wang, 2005	Mapping the sentinel lymph node ex vivo and finding the micrometastasis by CK-immunostaining in carcinoma of the colon and rectum	Chinese
Zhang, 2007	Sentinel lymph node mapping in colorectal cancer: study of 45 cases	Chinese
DUPLICATE REPORTS OR DATA		
Bembenek, 2005	Detection of lymph node micrometastases and isolated tumor cells in sentinel and nonsentinel lymph nodes of colon cancer patients	Duplicate with Bembenek, 2007; Sentinel lymph node biopsy in colon cancer; a prospective multicenter trial *(Included)*
Bembenek, 2005	Optiemierung des staging beim kolonkarzinom durch sentinel-lymphknoten-biopsie	Duplicate with Bembenek, 2007; Sentinel lymph node biopsy in colon cancer; a prospective multicenter trial *(Included)*
Bertagnolli, 2004	Sentinel node staging of resectable colon cancer: results of a multicenter study	Duplicate with Redston, 2006: Analysis of micrometastatic disease in sentinel lymph nodes from resectable colon cancer: results of cancer and Leukemia Groep B trial 80001
Bilchik, 2007	Prognostic impact of micrometastases in colon cancer: interim results of a prospective multicenter trial	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Bilchik , 2001	Molecular staging of early colon cancer on the basis of sentinel node analysis: a multicenter phase II trial	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Braat, 2004	Successful sentinel node identification in colon carcinoma using Patent blue V	Duplicate with Braat, 2014; Excellent prognosis of node negative patients after sentinel node procedure in colon carcinoma: a 5-year follow-up studie *(Included)*

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Cahill, 2009	Sentinel node biopsy for the individualization of surgical strategy for cure of early-stage colon cancer	Duplicate with Bembenek, 2007; Sentinel lymph node biopsy in colon cancer; a prospective multicenter trial *(Included)* And Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Dan, 2004	1% lymphazurin vs 10% fluorescein for sentinel node mapping in colorectal tumors	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Evangelista, 2002	Sentinel lymph node mapping in colorectal cancer: a feasibility study	Duplicate with Bertoglio, 2004: Prognostic value of sentinel lymph node biopsy in the pathological staging of colorectal cancer *(Not included)*
Feig, 2001	A caution regarding lymphatic mapping in patients with colon cancer	Duplicate with Lim, 2008: Sentinel lymph node evaluation does not improve staging accuracy in colon cancer *(Included)*
Iddings, 2007	Association of angiogenesis markers with lymph node metastasis in early colorectal cancer	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Kelder, 2006	RT-PCR and immuohistochemical evaluation of sentinel lymph nodes after in vivo mapping with Patent Blue V in colon cancer patients	Duplicate with Kelder, 2007: The sentinel node procedure in colon carcinoma: a multi-center study in the Netherlands.
Lasser, 2003	Is sentinel lymph node mapping relevant for colon cancer? A feasibility study	Article in French Duplicate with Liberale, 2007: Sentinel lymph nodes of colorectal carcinoma: reappraisal of 123 cases *(Included)*

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Murawa, 2011	One hundred consecutive cases of sentinel lymph node mapping in colon cancer- the results of prospective, single-centre feasibility study with implementation of immunohistochemical staining	Duplicate with Murawa, 2015; The influence of intraoperative factors and histopathological staging on the performance of sentinel node biopsy in colon cancer *(Included)*
Nordgard, 2009	Quantitative RT-PCR detection of tumor cells in sentinel lymph nodes isolated from colon cancer patients with an ex vivo approach	Duplicate with Nordgard, 2012: Prognostic relevance of occult metastases detected by cytokeratin 20 and mucin 2 mRNA levels in sentinel lymph nodes from colon cancer patients *(Included)*
Nowaczyk, 2012	Analysis of sentinel lymph node biopsy results in colon cancer in regard of anthropometric features of the population and body composition assessment formulas	Duplicate with Murawa, 2011; One hundred consecutive cases of sentinel lymph node mapping in colon cancer- the results of prospective, single-centre feasibility study with implementation of immunohistochemical staining *(Included)*
Oltedal, 2010	Detection of occult metastases in sentinel lymph nodes from colon cancer patients by k-ras mutations peptide nucleic acid clamp PCR	Duplicate with Nordgard, 2012: Prognostic relevance of occult metastases detected by cytokeratin 20 and mucin 2 mRNA levels in sentinel lymph nodes from colon cancer patients *(Included)*
Patten, 2004	A prospective evaluation of radiocolloid and immunohistochemical staining in colon carcinoma lymphatic mapping	Duplicate with Lim, 2008: Sentinel lymph node evaluation does not improve staging accuracy in colon cancer *(Included)*
Ramos, 2012	The sentinel lymph node technique in colon cancer	Duplicate with Ramos, 2013; Sentinel lymph node biopsy technique in 125 cases *(included)*
Saha, 2004	Lymphazurin 1% versus 99mTc sulfur colloid for lymphatic mapping in colorectal tumors: a comparative analysis	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Saha, 2006	A multicenter trial of sentinel lymph node mapping in colorectal cancer: prognostic implications for nodal staging and recurrence	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Trocha, 2003	Molecular staging of early colon cancer on the basis of sentinel node analysis: a multicenter phase II trial	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Tsoulias, 2000	Lymphatic mapping and focused analysis of sentinel lymph nodes upstage gastrointestinal neoplasms.	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Viehl, 2013	Factors influencing the success of in vivo sentinel lymph node procedure in colon cancer patients: Swiss, prospective multicenter study	Duplicate with Viehl, 2012: Sentinel lymph node procedures leads to upstaging of patients with resectable colon cancer: Results of the swiss prospective multicenter study sentinel lymph node procedure in colon cancer *(included)*
Van der Zaag, 2009	Improving staging accuracy in colon and rectal cancer by sentinel lymph node mapping: a comparative study	Duplicate with van der Zaag, 2012: Implications of sentinel lymph node mapping in nodal staging and prognosis in colorectal cancer *(included)*
Van der Zaag, 2010	Diagnosing occult tumour cells and their predictive value in sentinel nodes od histologically negative patients with colorectal cancer	Duplicate with van der Zaag, 2012: Implications of sentinel lymph node mapping in nodal staging and prognosis in colorectal cancer *(included)*
Van der Zaag, 2011	Decreased incidence of isolated tumor cells in lymph nodes after laparoscopic resection of colon cancer	Duplicate with van der Zaag, 2012: Implications of sentinel lymph node mapping in nodal staging and prognosis in colorectal cancer *(included)*
Van der Zaag, 2011	Categorization of occult tumour cells in lymph node in patients with colon cancer not reliable enough	Duplicate with van der Zaag, 2012: Implications of sentinel lymph node mapping in nodal staging and prognosis in colorectal cancer *(included)*
Waters, 2000	Sentinel lymph node mapping for carcinoma of the colon; a pilot study	Duplicate with Thomas, 2006: Use of sentinel node mapping for cancer of the colon: ' to map or not to map'
Weixler, 2017	Comparative analysis of tumor cell dissemination on the sentinel lymph nodes and the bone marrow in patients with nonmetastasized colon cancer: A prospective multicenter study	Duplicate with Weixler 2017: 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 *(Included)*
Wiese, 2010	Ultrastaging of sentinel lymph nodes (SLNs) cs. Non-SLNs in colorectal cancer – do we need both?	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Wiese, 2009	A prospective study of false-positive diagnosis of micrometastatic cells in the sentinel lymph nodes in colorectal cancer	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Wood, 2001	Focused examination of sentinel lymph nodes upstaged early colorectal carcinoma	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
Wood, 2002	One hundred consecutive cases of sentinel lymph node mapping in early colorectal carcinoma: detection of missed micrometastases	Duplicate with Saha 2013: Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation *(included)*
RESULT COLON AND RECTAL CANCER NOT SEPARATED		
Bell, 2005	Ex vivo sentinel lymph node mapping in colorectal cancer	
Bertoglio, 2004	Prognostic value of sentinel lymph node biopsy in the pathological staging of colorectal cancer	
Codignola, 2005	Is there any role for sentinel node mapping in colorectal cancer staging? Personal experience and review of the literature	
Codignola, 2005	Is there any role for sentinel node mapping in colorectal cancer staging? Personal experience and review of the literature	
Demirbas, 2005	Should sentinel lymph node mapping be performed for colorectal cancer?	
Esser, 2001	The role of sentinel lymph node mapping in staging of colon and rectal cancer	
Fitzgerald, 2002	Ex vivo sentinel lymph node biopsy in colorectal ancer: a feasibility study	Also < 20 colon carcinoma patients included
Ivanov, 2009	Intraoperative sentinel lymph node mapping in patients with colorectal cancer	

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Joosten, 1999	Intraoperative lymphatic mapping and the sentinel node concept in colorectal carcinoma	
Kitagawa, 2002	Sentinel node mapping for colorectal cancer with radioactive tracer	Also < 20 colon carcinoma patients included
Murawa, 2007	Evaluation of the sentinel lymph node biopsy in colorectal carcinoma including the results of immunohistochemical examinations	
Park, 2009	Comparison of ex vivo and in vivo injection of blue dye in sentinel lymph node mapping for colorectal cancer	
Sanducci, 2007	Lymphoscintigraphic localization of sentinel node in early colorectal cancer: results of a monocentric study	
Smith, 2005	Ex vivo sentinel lymph node mapping in colon cancer: improving the accuracy of pathological staging	
Sommariva, 2010	Factors affecting false negative rates on ex vivo sentinel lymph node mapping in colorectal cancer	
Stojanosi, 2017	Sentinel lymph node detection in colorectal cancer- first experience	
Van Schaik, 2007	Ex vivo sentinel lymph node 'mapping' in colorectal cancer	
Wong, 2004	Validation of ex vivo lymphatic mapping in hematoxylin-eosin node negative carcinoma of the colon and rectum	
POOR DATA		
Broderick-Villa 2004	Ex vivo lymphatic mapping: a technique to improve pathological staging in colorectal cancer	Inclusion of less than 20 colon carcinoma patients
Ceranic, 2010	Validation and feasibility of ex vivo sentinel lymph node 'mapping' by methylene blue in colorectal cancer	Inclusion of less than 20 colon carcinoma patients
Cai, 2012	Colorectal cancer lymph node staining by activated carbon nanoparticles suspension in vivo or methylene blue in vitro	No reporting of positivity rate

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Chand, 2018	Feasibility of fluorescence lymph node imaging in colon cancer: FLICC	Inclusion of less than 20 colon carcinoma patients
Fricker, 2006	Sentinel node mapping for staging of colorectal cancer	Original paper not found
Gurzu, 2011	Practical value of the complex analysis of sentinel lymph nodes in colorectal carcinomas	Inclusion of less than 20 colon carcinoma patients
Hutteman, 2011	Clinical translation of ex vivo sentinel lymph node mapping using preoperative radioisotope injection in cases of extraperitoneal rectal cancer	Inclusion of less than 20 colon carcinoma patients
Larussen, 2014	Blue dye injection does not induce dissemination of epithelial cells during SLN procedure in colon cancer	Outcome measurements not extractable from original data.
Li, 2010	Applied study of sentinel lymph node biopsy in colorectal cancer	Outcome measurements not extractable from original data.
Lind, 2017	Prognostic relevance of an epigenetic biomarker panel in sentinel lymph nodes from colon cancer patients	Outcome measurements not extractable from original data
Marhic, 2017	Molecular analysis of sentinel lymph node in colon carcinomas by one-step nucleic amplification (OSNA) reduces time to adjuvant chemotherapy interval	No description of sentinel lymph node detection and results.
Murawa, 2007	Evaluation of the sentinel node biopsy in colorectal carcinoma including the results of immunohistochemical examinations	Inclusion of less than 20 colon carcinoma patients
Nissan, 2012	United states military cancer institute clinical trials group (USMCI G1-01) randomized controlled trial comparing targeted nodal assessment and ultrastaging with standard pathological evaluation for colon cancer	No reporting of positivity rate
Oltedal, 2018	The prognostic relevance of Sentinel Lymph Node Metastases Assessed by PHGR1 mRNA Quantification in Stage I to III Colon Cancer	No description of sentinel lymph node detection and results.

Supplemental data 4 (continued). Studies excluded after full-text review

First author, year	Title	Additonal information
Rivet, 2011	Ex vivo sentinel lymph node mapping in laparoscopic resection of colon cancer	Only percentages are given in original data
Roseano, 2003	Sentinel lymph node mapping in the management of colorectal cancer	Inclusion of less than 20 colon carcinoma patients
Soni, 2011	Comparison of nodal positivity between SLNM vs conventional surgery in colon cancer patients with < 12 and > 12 lymph nodes harvested	No reporting of positivity rate
Visscher, 2013	Quantitative analysis of superparamagnetic contrast agent in sentinel lymph nodes using ex vivo vibrating sample magnetrometry	Experimental mapping technique
Vogelaar, 2014	The diagnostic value of one-step nucleic acid amplification (OSNA) for sentinel lymph nodes in colon cancer patients	Outcome measurements not extractable from original data.
Wakeman, 2011	Lymph node yield following injection of patent blue V dye into colorectal cancer	No reporting of positivity rate
Wang, 2012	Ex vivo localization and immunohistochemical detection of sentinel lymph node micrometastasis in patients with colorectal cancer can upgrade tumor staging	Outcome measurements not extractable from original data.
Weixler, 2015	Intranodal mapping using carbon dye results in more accurate lymph node staging in colon cancer patients	No reporting of positivity rate
Yan, 2014	A multi-center study of using carbon nanoparticles to track lymph node metastases in T1-2 colorectal cancer	No reporting of positivity rate
Zielinski, 2011	Sentinel lymph node in colorectal cancer 5-years follow-up	Outcome measurements not extractable from original report.

Supplementary Information 5. Subanalysis of the high quality concept validation studies*

		Patients (n)	Successful procedures (n)	Sensitivity* % (95% CI)	Detection rate % (95% CI)
Overall		669	563	0.56 (0.53-0.58)	0.85 (0.79-0.89)
Patient and tumour related factors					
Gender					
	Male	299	242	0.53 (0.45-0.61)	0.81 (0.74-0.86)
	Female	345	298	0.58 (0.51-0.65)	0.87 (0.80-0.91)
Tumour location					
	Caecum	181	155	0.58 (0.50-0.65)	0.87 (0.75-0.93)
	Ascendens	139	117	0.55 (0.43-0.66)	0.84 (0.78-0.88)
	Right flexure	15	14	0.45 (0.18-0.75)	0.92 (0.83-0.97)
	Transversum	48	38	0.42 (0.29-0.57)	0.80 (0.75-0.84)
	Left flexure	0	0	N/A	N/A
	Descendens	45	38	0.64 (0.51-0.75)	0.85 (0.71-0.93)
	Sigmoid	234	195	0.56 (0.53-0.59)	0.84 (0.81-0.86)
Tumour stage					
	T1	53	46	0.30 (0.16-0.51)	0.85 (0.76-0.91)
	T2	79	64	0.53 (0.41-0.65)	0.79 (0.69-0.86)
	T3	486	408	0.56 (0.51-0.61)	0.85 (0.80-0.89)
	T4	51	45	0.61 (0.51-0.69)[a]	0.89 (0.71-0.96)[d]
Tumour size					
	< 5 cm	254	215	0.54 (0.52-0.57)	0.84 (0.81-0.87)
	> 5 cm	132	105	0.58 (0.47-0.69)	0.80 (0.62-0.90)
Number of SLNs					
	SLN =1	149	148	0.43 (0.34-0.52)	-
	SLN = 2	117	116	0.54 (0.44-0.63)	-
	SLN > 2	310	298	0.66 (0.59-0.72)[b]	-
Total LN yield					
	< 10	165	144	0.56 (0.53-0.59)	0.85 (0.79-0.90)
	> 10	489	418	0.55 (0.53-0.57)	0.86 (0.79-0.91)

Supplementary Information 5 (continued). Subanalysis of the high quality concept validation studies*

		Patients (n)	Successful procedures (n)	Sensitivity* % (95% CI)	Detection rate % (95% CI)
Procedure related factors					
Injection site					
	Subserosal	653	550	0.56 (0.53-0.58)	0.85 (0.79-0.89)
	Submucosal	16	13	0.57 (0.57-0.57)	0.83 (0.82-0.84)
In or ex vivo approach					
	In vivo	454	398	0.54 (0.54-0.54)	0.87 (0.85-0.89)
	Ex vivo	215	165	0.61 (0.60-0.61)[c]	0.77 (0.76-0.77)[e]
Imaging tracer					
	Blue dye	558	491	0.55 (0.53-0.56)	0.83 (0.77-0.88)
	Blue dye +^{99m}Tc	81	72	0.64 (0.54-0.72)	0.89 (0.86-0.92)
	Fluorophore	0	-	N/A	N/A
	Blue dye + fluorophore	0	-	N/A	N/A

* Sensitivity after advanced examination of SLNs and regional lymph nodes

[a] T1 vs T3 p=0.006, T1 vs T4 p=0.009
[b] 1 SLN vs 2 SLNs p=0.001; 1 SLN vs >2 SLNs p < 0.0001, 2 SLNs vs > 2 SLNs p<0.0001
[c] In vivo vs ex vivo p< 0.0001
[d] T2 versus T4 p=0.023
[e] in vivo versus ex vivo p< 0.000

Supplementary Information 6. Subanalysis of Individual patient data*

		Patients (n)	Successful procedures (n)	Sensitivity* % (95% CI)	Detection rate % (95% CI)
Overall		2182	2006	0.69 (0.63-0.75)	0.92 (0.89-0.94)
Patient and tumour related factors					
Gender					
	Male	828	747	0.72 (0.63 -0.79)	0.91 (0.86-0.94)
	Female	848	781	0.71 (0.64-0.78)	0.93 (0.89-0.96)
Tumour location					
	Cecum	426	394	0.75 (0.64-0.83)	0.94 (0.89-0.96)
	Ascendens	528	490	0.71 (0.63-0.78)	0.92 (0.88-0.95)
	Right flexure	61	58	0.80 (0.58-0.92)	0.93 (0.84-0.97)
	Transversum	176	156	0.65 (0.52-0.76)	0.87 (0.82-0.91)
	Left flexure	38	35	0.59 (0.33-0.81)	0.89 (0.77-0.95)
	Descendens	167	154	0.57 (0.43-0.71)	0.92 (0.86-0.96)
	Sigmoid	728	663	0.70 (0.64-0.76)	0.91 (0.88-0.94)
Tumour stage					
	T1	274	249	0.65 (0.47-0.79)	0.89 (0.84-0.93)
	T2	367	337	0.71 (0.61-0.79)	0.93 (0.87-0.96)
	T3	1350	1245	0.69 (0.62-0.75)	0.93 (0.89-0.95)
	T4	170	156	0.69 (0.60-0.76)	0.92 (0.86-0.96)
Tumour size					
	< 5 cm	784	726	0.69 (0.57-0.79)	0.93 (0.89-0.95)
	> 5 cm	428	387	0.69 (0.59-0.78)	0.91 (0.84-0.95)
Number of SLNs					
	SLN =1	450	446	0.61 (0.49-0.71)	-
	SLN = 2	466	463	0.66 (0.56-0.74)	-
	SLN > 2	1117	1097	0.74 (0.69-0.79)[c]	-
Total LN yield					
	< 10	598	560	0.68 (0.61-0.73)	0.92 (0.88-0.95)
	> 10	1439	1328	0.72 (0.63-0.79)	0.93 (0.89-0.95)

Supplementary Information 6 (continued). Subanalysis of Individual patient data*

		Patients (n)	Successful procedures (n)	Sensitivity* % (95% CI)	Detection rate % (95% CI)
Procedure related factors					
Injection site					
	Subserosal	1879	1719	0.68 (0.61-0.74)	0.91 (0.88-0.94)
	Submucosal	248	236	0.73 (0.60-0.83)	0.94 (0.86-0.98)
In or ex vivo approach					
	In vivo	1097	1719	0.66 (0.59-0.73)	0.93 (0.89-0.95)
	Ex vivo	1001	925	0.75 (0.66-0.82)	0.91 (0.83-0.95)
Imaging tracer					
	Blue dye	1712	1563	0.71 (0.64-0.77)[a]	0.92 (0.89-0.95)[d]
	Blue dye + ^{99m}Tc	365	350	0.74 (0.67-0.81)[b]	0.93 (0.88-0.96)
	Fluorophore	69	65	0.38 (0.32 -0.45)	0.93 (0.71-0.98)
	Blue dye + fluorophore	29	22	0.33 (0.33-0.33)	0.76 (0.76-0.76)

*Sensitivity calculated after advanced examination of only SLNs

[a] Blue dye vs fluorophore and blue dye + fluorophore p< 0.0001 (both)
[b] Blue dye + ^{99m}Tc vs fluorophore and blue dye + fluorophore p< 0.0001 (both)
[c] 1 SLN vs >2 SLNs p=0.016 and 2 SLNs vs >2 SLNs p=0.020
[d] Blue dye + fluorophore vs blue dye, blue dye + ^{99m}Tc and fluorophore p< 0.0001

LAPAROSCOPIC SENTINEL LYMPH NODE IDENTIFICATION IN PATIENTS WITH COLON CARCINOMA USING A NEAR-INFRARED DYE: DESCRIPTION OF A NEW TECHNIQUE AND FEASIBILITY STUDY

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ABSTRACT

Background After promising results were obtained from studies in large animals, a technique using indocyanine green (ICG) is being introduced for sentinel lymph node (SLN) biopsy in colon cancer patients.

Subjects and Methods Colon cancer patients without clinical signs of metastatic disease, presenting at the VU University Medical Center (Amsterdam, The Netherlands) or Kennemer Gasthuis (Haarlem, The Netherlands), were asked to participate in the study. During laparoscopy, a subserosal injection of 2.5 mg of ICG diluted in 1 mL of 0.9% NaCl plus 2% human albumin was performed using a percutaneously inserted long rigid or flexible needle. After injection, a near-infrared laparoscope (Olympus Corp., Tokyo, Japan) was used for lymph flow and SLN visualization. The SLNs were laparoscopically harvested and analyzed by a senior pathologist using multisectioning and immunohistochemistry.

Results Fourteen patients were included (six women, eight men), with a median age of 75.5 (interquartile range [IQR], 67.8–81.0) years and a median body mass index of 25.1 (IQR, 22.7–26.0) kg/m2. Median tumor diameter was 4.5 (IQR, 3.4–7.0) cm. At least one SLN was identified in all patients, with a median number of 2.0 (IQR, 2.0–3.3) SLNs. The median time between injection and identification of the SLN was 15.0 (IQR, 13.3–29.3) minutes. Positioning of the needle tip into the subserosal layer was found to be more effective using the flexible needle. When this flexible needle was used, less spill of dye was observed. All SLNs were negative. We observed four false-negative nodes, all after using a rigid needle. None of the patients showed an adverse reaction to the ICG injection.

Conclusions Preliminary results of laparoscopic sentinel node identification using a near-infrared dye show this procedure is safe and feasible. It was possible to detect lymph nodes in all patients. Large tumor size, drainage to adjacent lymphatic vessels, and the use of a rigid needle might contribute to false-negative nodes.

INTRODUCTION

The concept of sentinel lymph node (SLN) biopsy has been validated in patients with melanoma, breast and vulvar cancer [1]. Cabanas was the first to describe this concept for penile carcinoma. The lymph flow dynamics were used to identify the first lymph node upon which the tumor drains. When tumor spread occurs, this SLN is most likely the first to be infested by tumor cells. This concept has been used for optimizing surgical treatment for breast cancer and melanoma patients [2,3]. The primary purpose of SLN mapping in colon cancer is to upstage tumors whose metastases would remain undetected by conventional pathological examination and to identify aberrant lymphatic drainage.

The gold standard for colon cancer treatment today is a been validated in patients with melanoma, breast, and segmental resection including resection of all regional lymph nodes. For tumor staging, lymph nodes are harvested manually by the pathologist and are assessed using single-section examination followed by hematoxylin and eosin (H&E) staining. The outcome of this histopathological examination is the most important indication for adjuvant chemotherapy. Following this current strategy for colon cancer management, up to 30% of Stage I and II patients (by definition, node negative) will eventually develop metastases and die from colon cancer [4]. This might be caused by a suboptimal pathological examination of the lymph nodes. Using the conventional single-section H&E staining, only a limited part of the lymph node is investigated, and therefore (micro)metastases can be easily missed. Identification of the SLN, subsequent serial section, and the use of immunohistochemistry, as used in other forms of cancer, might be of value in upstaging nodenegative colon cancer patients. Until now, tracers currently used for SLN identification in colon cancer patients have shown limitations such as poor tissue penetration of blue dye in the case of a fatty mesentery and signal interference if the SLN is located near the tumor when using radioactive tracers [5,6]. Here we describe a new minimally invasive technique for SLN mapping in patients with colon carcinoma.

SUBJECTS AND METHODS

The study protocol was approved by the ethical committee of the VU University Medical Center, Amsterdam, The Netherlands, and by the local ethical committee of the Kennemer Gasthuis, Haarlem, The Netherlands (registration number NL2461302908). Patients at least 18 years old, who gave oral and written informed consent, were included in this validation study. Right after the SLN procedure, each patient received standard surgery. Exclusion criteria were pre- and

perioperatively gross lymph node involvement, distant metastases, advanced disease with invasion of adjacent structures, prior colorectal surgery, metastatic or T4 disease discovered during intraoperative staging, contraindications to laparoscopy, rectal cancer, and allergy to iodine. Main study end points were identification rate of SLNs, number of false-negative SLNs, number of patients who are upstaged by ultrastaging techniques, number and status of aberrant lymph nodes, and accuracy and conformity of the SLN status.

Surgical procedure

Patients meeting the inclusion criteria were prepped, draped, and placed under general anesthesia. Laparoscopic access was obtained in the traditional fashion, and abdominal exploration was performed to rule out intra-abdominal metastases. Injection of the dye was performed by the same surgeon (W.J.H.J.M.) in all cases. In the first 7 patients a rigid spinal needle was used for injection. During the final 7 patients two different kinds of flexible endoscopic needles were used for transcutanous injection (sclerosering and Wang needle). During two of the seven procedures a sclerosering needle (FH Medical BV, Veenendaal, The Netherlands) with a tube length of 230 cm, tube diameter of 2.35 mm, and needle projection of 5 mm was used. In 5 patients a Wang transbronchial cytology needle (ConMed Corp., Utica, NY) with a tube length of 130 cm, tube diameter of 1.9 mm, and needle projection of 13 mm was used. The short tube length and small diameter of the Wang transbronchial cytology needle, allowed for a more controlled way of injecting tracer material. Alternatively, using a longer flexible needle makes it easier to place the needle tip in the subserosal layer of the colon and keep it positioned during tracer administration. The use of indocyanine green (ICG)/albumin solution was based on previous results obtained from animal studies [6]. First, 1.0 mL of saline was injected into the colon wall to ensure correct placement of the needle (confirmed by a raising bleb), followed by the injection of ICG (ICG-Pulsion; Pulsion Medical Systems AG, Mu¨nchen, Germany) solution. The ICG solution consisted of 25 mg of ICG diluted in 1.0 mL of human albumin (20%) and 9 mL of NaCl (0.9%). One to three peritumoral injections were performed depending on tumor size. The maximum injected volume contained 1.0 mL of ICG solution (< 2.5 mg). For lymph vessel and SLN identification, a newly developed, CE-approved, near-infrared (NIR) laparoscope (Olympus Corp., Tokyo, Japan) was used. The system consisted of a Visera laparoscopic system equipped with a xenon light source for excitation of ICG and filter sets for optimal wavelength selection. The optic device used has adjusted coated lenses to allow for optimal infrared transparency. A more detailed description of this laparoscope was previously published [6]. The first one to four lymph nodes, accumulating tracer material and presenting as bright fluorescent dots using the NIR modes, were defined as SLN(s). After

identification, the SLN or SLNs were being harvested laparoscopically. After SLN harvesting, conventional laparoscopic medial to lateral oncologic resection was conducted.

Pathological assessment

A senior pathologist manually searched the fresh resection specimen for lymph nodes. Lymph nodes found were bisected along the longest axis and embedded in paraffin. All lymph nodes were stained with H&E. If the SLNs were tumor negative after routine H&E staining, the SLNs were sectioned (3–4 lm thick) at 150-lm intervals and examined at three levels with H&E as well as using immunohistochemistry with the epithelial marker CAM 5.2. Metastases between 0.2 mm and 2.0 mm were referred to as micrometastases. Metastases smaller than 0.2 mm were described as isolated tumor cells.

RESULTS

Fourteen patients were included: six women and eight men. The median age of the patients was 75.5 (interquartile range [IQR], 67.8–81.0) years. Patients had a median body mass index of 25.1 (IQR, 22.7–26.0) kg/m2. The tumors were located in the sigmoid (n= 6), colon transversum (n= 1), cecum (n= 5), and colon ascendens (n= 2). The median diameter of the tumors was 4.5 (IQR, 3.4–7.0) cm (Table 1).

Table 1. Patient characteristics

Patient	Sex	Age (years)	BMI (kg/m^2)	Tumor site	Tumor diameter (cm)	T-stage
1	Female	81	25.9	Colon ascendens	7.0	T3
2	Male	80	25.9	Cecum	7.0	T3
3	Male	74	24.5	Colon transversum	2.5	T2
4	Male	84	22.9	Sigmoid	10.0	T3
5	Male	82	22.2	Sigmoid	4.5	T3
6	Female	54	31.2	Cecum	4.0	T2
7	Male	81	30.1	Cecum	3.0	T2
8	Female	77	26.4	Sigmoid	9.5	T3
9	Male	81	25.8	Sigmoid	4.5	T3
10	Female	68	21.0	Sigmoid	3.5	T3
11	Male	74	25.1	Colon ascendens	0.8	T3
12	Female	46	22.0	Cecum	6.0	T3
13	Female	74	23.8	Cecum	4.5	T2
14	Male	67	25.1	Sigmoid	3.5	T3

During all procedures the flow of dye in the lymphatic vessels could be followed in real time by the bright fluorescent signal (Figure 1). Proper excitation of the dye occurred almost immediately after ICG injection (approximately 1 minute). The absence of autofluorescence of intra-abdominal structures and a high signal-to-noise ratio made lymph vessel and lymph node identification possible (Figure 2). During all procedures at least one to four lymph nodes could be identified and harvested. The (sentinel) lymph nodes were being dissected using the white-light modus of the NIR system. Guided by the greenish color of the ICG itself and by switching back to the NIR modes, complete removal of the (sentinel) node could be optimized. There was no need for conversion in any of the procedures.

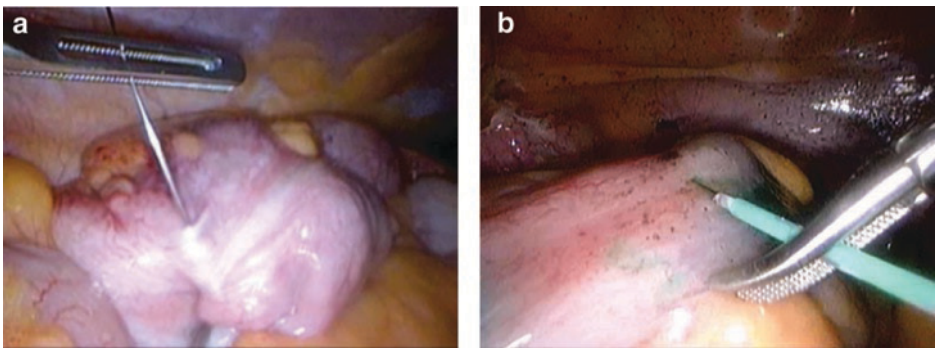


Figure 1. Positioning of the needle near the tumor with (a) a rigid needle and (b) a flexible needle**.**

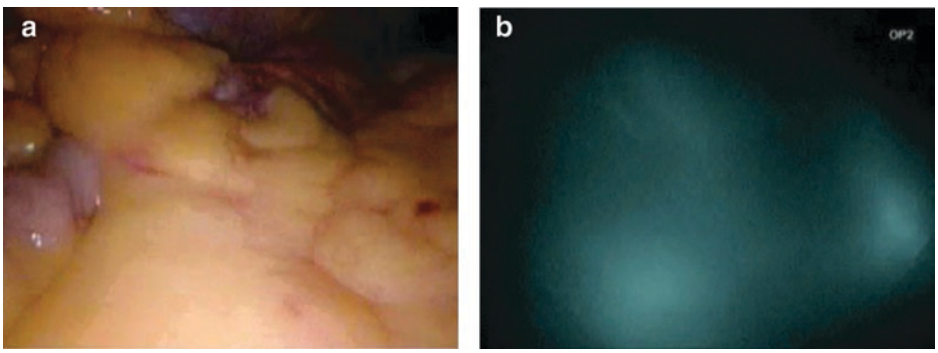


Figure 2. Observation of mesentery with two lymph nodes (a) with normal light and (b) using near-infrared.

During five procedures more than one injection was used because of the size of the tumor (Table 2). The median time between dye injection and SLN identification was 15 (IQR, 13.3–29.3) minutes. Laparoscopic harvesting of (sentinel) lymph nodes was successful in all 14 patients

(100%). The median peroperative number of SLNs found was 2.0 (IQR, 2.0–3.3), and the median number of non-SLNs found by the pathologist in the resected specimen was 10.5 (IQR, 4.8–22.3). In 10 patients no metastases were found during evaluation of the SLN and non-SLNs. In 4 patients a positive non-SLN was found, whereas the presumed SLN was negative (false negative SLN). No adverse events or complications occurred as a result of laparoscopic SLN identification using ICG.

Table 2. Procedure characteristics

Patient	Needle used	Number of injections	Time between injection and SLN identification (minutes)
1	Rigid	81	25.9
2	Rigid	80	25.9
3	Rigid	74	24.5
4	Rigid	84	22.9
5	Rigid	82	22.2
6	Rigid	54	31.2
7	Rigid	81	30.1
8	Sclerosering needle	77	26.4
9	Sclerosering needle	81	25.8
10	Wang needle	68	21.0
11	Wang needle	74	25.1
12	Wang needle	46	22.0
13	Wang needle	74	23.8
14	Wang needle	67	25.1

[a] Each injection consisted with 1 mL of indocyanine green/human albumin/NaCl solution

The usage of a rigid spinal needle made the procedure technical more difficult. In particular, correct positioning of the needle tip into the subserosal layer, for example, depended much on the right angle between colon wall and needle. More spillage of dye occurred when using the rigid needle in contrast to the flexible (Wang) needle. Intra-abdominal spillage of dye made the SLN identification difficult in the first 5 cases, because of the fluorescent abdominal cavity. Time between injection and lymph node identification took longer when spillage occurred. During the final 7 cases a flexible endoscopic needle was used. Positioning of the needle tip into the colon wall was found to be much easier, especially when using the Wang transbronchial cytology needle. No spillage was reported during the final seven procedures. We also observed that NIR light is able to penetrate relatively deeply through living tissue. During our study lymph nodes located 1.5 cm in the fatty tissue could be identified.

DISCUSSION

This study was conducted to describe and assess the feasibility of a new technique using NIR dyes for SLN detection and harvesting in colon cancer patients.

The major issue in our current study is occurrence of the false-negative (sentinel) nodes in 4 patients. We believe the following problems contribute to these findings:

1. Correct placement of the needle close to the tumor is essential for adequate lymph node mapping. However, if the peritumoral injection of the NIR dye is not close enough to the tumor or in the case of big tumors, this can lead to drainage into adjacent lymph vessels instead of the vessels draining on the SLN. As a result SLNs will be missed.

2. During the first seven procedures a rigid spinal needle was used for dye injection. Correct positioning of the needle tip was found to be difficult and occasionally lead to spillage of dye. During the final seven procedures the rigid needle was replaced by a flexible needle. Using a flexible needle, positioning of the needle tip into the colon wall was found to be easier. Intra-abdominal space is limited, and maneuvering of a flexible needle was shown to be more manageable, making finding a correct position easier.

3. Also, the SLN mapping in large-sized tumors (> 7 cm in diameter) seems to show less favorable results regarding sensitivity. Obliteration of the lymph vessels by ingrowth of tumor tissue or obstruction by gross metastases in the lymph node, resulting in an alternative route of lymph flow, could be an explanation.

During animal experiments we observed the problem of dye leakage [6]. Spilled ICG will stick to intra-abdominal tissues, resulting in a fluorescent abdominal cavity. Rinsing the abdominal cavity with saline after spillage at the injection site does solve this problem only slightly. We addressed this problem by a pre-injection of saline to assure a correct localization of the needle. Correct placement of the needle gives bulging of the colon wall.

Other injection strategies could be interesting for future studies. Perioperative colonoscopic injection, for example, could be an alternative [7]. When spillage of dye occurs, the tracer material will leak into the lumen of the colon, making spillage less of a problem. During animal experiments this approach showed favorable results (unpublished data). Different injection

routes like intratumoral or submucosal injection by per- or preoperative colonoscopy is a subject of our future studies.

SLN mapping using patent blue and radioisotope tracers, analogous to breast cancer, is noted to have limitations when used in colon cancer patients [8,9]. Some authors have concluded that the SLN concept is not suitable for colon cancer patients because high false-negative ratios were seen [8,9]. However, results from a meta-analysis addressing sensitivity of the SLN procedure in colorectal cancer patients showed favorable results when selecting for high-quality studies. Sensitivities as high as 90% with a detection rate of 96% could be achieved [10]. In addition, recently published studies showed high sensitivity rates when the procedures were accurately performed [11,12]. The type of dye chosen might also have confounded the results of some studies. Ink, for example, show poor tissue penetration, especially in patients with extensive intra-abdominal fat. When radioactive tracers are used, the signal from the injection site might interfere when the SLN is localized nearby the tumor. NIR dyes with their unique characteristics could help to overcome these problems. We clearly show here that lymph nodes in obese patients can be adequately identified using ICG up to 1.5 cm in fatty mesentery.

During our procedures we diluted ICG with saline and human albumin. This was based on previous animal experiments [6]. During these experiments we were not able to detect SLNs when using plain ICG. We could identify lymph vessels, but it seemed that ICG washed through the lymph nodes and did not accumulate in the nodes. After human albumin was added, SLNs could be identified. In addition, human albumin seems to increase the fluorescent capacities of ICG. Other groups reported good kinetics using only ICG [13,14]. The need for human albumin and its concentration for ICG dilution also deserve further analysis. The period of time between dye injection and SLN identification varied from 2.5 to 40 minutes. Tracer material used was equal during all cases. Intra-abdominal lymph node drainage patterns are not consistent, and SLNs can be located at different locations. Exploring the abdomen in search for the SLN can be time consuming, especially when spillage of dye occurred, coloring more intra-abdominal anatomic structures fluorescent. Lymph vessels can be identified within seconds after dye injection when they are located at the surface. Depending on the depth of localization, a solid amount of dye entrapped in the lymph node will be needed to ensure enough tissue penetration for identification. In the patient in whom the sentinel node was identified in 2.5 minutes, the lymph node was located near the tumor and at the surface of the mesentery.

ICG was used because this dye is clinical grade. Several companies worldwide are busy developing new NIR dyes for clinical purposes. Toxicological studies are needed, but they

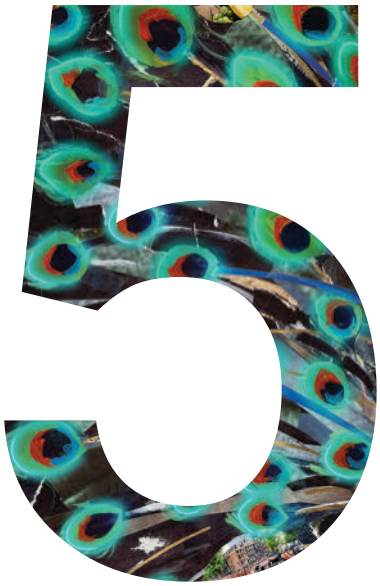
are time consuming, and the first clinical-grade dyes are still being awaited. These new dyes are reputed to have better fluorescent characteristics compared with ICG. Nevertheless, we demonstrated that ICG was capable of SLN detection by using the NIR modes of an NIR laparoscope.

CONCLUSIONS

Laparoscopic (sentinel) lymph node mapping using ICG/albumin/NaCl solution is feasible in patients with colon cancer. However, this novel technique needs more refinement. In particular, the site of injection (subserosal or intramucosal injection and intra- or peritumoral injection) is a point of discussion. More studies concerning the concentration and amount of dye to be injected, the additional value of human albumin, method of injection, and validation of the SLN technique using ICG are warranted.

REFERENCES

1. Cabanas RM. An approach for the treatment of penile carcinoma. Cancer 1977;39:456–466.
2. Thompson JF. The Sydney Melanoma Unit experience of sentinel lymphadenectomy for melanoma. Ann Surg Oncol 2001;8:44S–47S.
3. Veronesi U, Paganelli G, Viale G, Luini A, Zurrida S,Galimberti V, Intra M, Veronesi P, Robertson C, Maisonneuve P, Renne G, De Cicco C, De Lucia F, Gennari R. A randomized comparison of sentinel-node biopsy with routine axillary dissection in breast cancer. N Engl J Med 2003;349:546–553.
4. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapyfor completely resected stage II colon cancer. Cochrane Database Syst Rev 2008;(3):CD005390.
5. Meijerink WJ, van der Pas MH, van der Peet DL, Cuesta MA,Meijer S. New horizons in colorectal cancer surgery. Surg Endosc 2009;23:1–3.
6. van der Pas MH, van Dongen GA, Cailler F, Pelegrin A,Meijerink WJ. Sentinel node procedure of the sigmoid using indocyanine green: Feasibility study in a goat model. Surg Endosc 2010;24:2182–2187.
7. Cahill RA, Anderson M, Wang LM, Lindsey I, CunninghamC, 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;26:197–204.
8. Faerden AE, Sjo O, Andersen SN, Hauglann B, Nazir N,Gravedaug B, Moberg I, Svinland A, Nesbakken A, Bakka A. Sentinel node mapping does not improve staging of lymph node metastasis in colonic cancer. Dis Colon Rectum 2008; 51:891–896.
9. Thomas KA, Lechner J, Shen P, Waters GS, Geisinger KR,Levine EA. Use of sentinel node mapping for cancer of the colon: 'To map or not to map.' Am Surg 2006;72:606–611.
10. van der Pas MH, Meijer S, Hoekstra OS, Riphagen II, de Vet HC, Knol DL, van Grieken NC, Meijerink WJ. Sentinel lymph-node procedure in colon and rectal cancer: A systematic review and meta-analysis. Lancet Oncol 2011;12: 540–550.
11. Saha S, Seghal R, Patel M, Doan K, Dan A, Bilchik A, BeutlerT, Wiese D, Bassily N, Yee C. A multicenter trial of sentinel lymph node mapping in colorectal cancer: Prognostic implications for nodal staging and recurrence. Am J Surg 2006; 191:305–310.
12. Terwisscha Van Scheltinga SE, Den Boer FC, Pijpers R,Meyer GA, Engel AF, Silvis R, Meijer S, van der Sijp JR. Sentinel node staging in colon carcinoma: Value of sentinel lymph node biopsy with radiocolloid and blue staining. Scand J Gastroenterol Suppl 2006;(243):153–157.
13. 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;25:103–108.
14. Nagata K, Endo S, Hidaka E, Tanaka J, Kudo SE, ShiokawaA. Laparoscopic sentinel node mapping for colorectal cancer using infrared ray laparoscopy. Anticancer Res 2006;26: 2307–2311.



NEAR-INFRARED FLUORESCENCE IMAGING FOR SENTINEL LYMPH NODE IDENTIFICATION IN COLON CANCER: A PROSPECTIVE SINGLE-CENTER STUDY AND SYSTEMATIC REVIEW WITH META-ANALYSIS

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Tech Coloproctol. 2019;23:1113-1126

ABSTRACT

Background Near-infrared (NIR) fluorescence imaging has the potential to overcome the current drawbacks of sentinel lymph node mapping (SLNM) in colon cancer. Our aim was to provide an overview of current SLNM performance and of factors influencing successful sentinel lymph node (SLN) identification using NIR fluorescence imaging in colon cancer.

Methods A systematic review and meta-analysis was conducted to identify currently used methods and results. Additionally, we performed a single-center study using indocyanine green (ICG) as SLNM dye in colon cancer patients scheduled for a laparoscopic colectomy. SLNs were analyzed with conventional hematoxylin-and-eosin staining and additionally with serial sectioning and immunohistochemistry (extended histopathological assessment). A true positive procedure was defined as a tumor-positive SLN either by conventional hematoxylin-and-eosin staining or by extended histopathological assessment, independently of regional lymph node status. SLN procedures were determined to be true negatives if SLNs and regional lymph nodes revealed no metastases after conventional and advanced histopathology. SLN procedures yielding tumor-negative SLNs in combination with tumor-positive regional lymph nodes were classified as false negatives. Sensitivity, negative predictive value and detection rate were calculated.

Results This systematic review and meta-analysis included 8 studies describing 227 SLN procedures. A pooled sensitivity of 0.63 (95%CI 0.51-0.74), negative predictive value 0.81 (95%CI 0.73-0.86) and detection rate of 0.94 (95%CI 0.85-0.97) were found. Upstaging as a result of extended histopathological assessment was 0.15 (95%CI 0.07-0.25). In our single-center study we included 30 patients. Five false negative SLNs were identified, resulting in a sensitivity of 44% and negative predictive value of 80%, with a detection rate of 89.7%. Eight patients had lymph node metastases, in 3 cases detected after extended pathological assessment, resulting in an upstaging of 13% (3 of 23 patients with negative nodes by conventional hematoxylin and eosin staining).

Conclusions Several anatomical and technical difficulties make SLNM with NIR fluorescence imaging in colon cancer particularly challenging when compared to other types of cancer. As a consequence, reports of SLNM accuracy vary widely. Future studies should try to standardize the SLNM procedure and focus on early stage colon tumors, validation of tracer composition, injection mode and improvement of real-time optical guidance.

INTRODUCTION

Colon cancer is one of the most common malignancies in the Western world and the number of early stage tumors (T1 and T2) identified is expected to increase as a result of the introduction of nationwide screening programs [1]. Lymph node metastases are the strongest predictive factor for patient survival [2]. The low risk of lymph node metastasis in these early stage tumors makes local excision of the primary tumor an attractive treatment option [3]. However, current treatment by segmental resection with en-bloc resection of lymph nodes is unavoidable as long as uncertainties about undetected lymph node metastasis remain. Despite complete segmental resection, up to 20-30% patients with early stage disease will develop distant metastasis and eventually die from colon cancer [4]. This high recurrence rate in node-negative colon tumors could be the result of understaging due to missed occult tumor cells (e.g. isolated tumor cells or micrometastases) during routine histopathological examination or inadequate lymph node harvesting [5-8]. Detailed examination of all lymph nodes using serial sectioning and immunohistochemistry (IHC) is desirable. However, these techniques are time-consuming and expensive and are therefore not appropriate for daily practice. On the other hand, the majority of colon cancer patients without lymph node metastasis are exposed to unnecessary surgery-related morbidity and mortality, currently of 13.5% and 2.0%, respectively [9].

The concept of sentinel lymph node mapping (SLNM) in colon cancer as a staging technique has been described frequently, with variable results [10]. The limited penetration depth and fast migration of current blue dyes, resulting in high false negative rates, is frequently mentioned as a serious drawback of SLNM. Near-infrared (NIR) fluorescence imaging for SLNM has several properties that are advantageous for the SLN procedure in colon cancer and has already shown promising results in several types of cancer [11-13]. The relatively high penetration depth and real-time optical guidance are important benefits in SLN identification, since lymph node drainage patterns of colon cancer are unknown and SLNs are generally in an unfavorable location beneath a fatty mesocolon.

Since this technique has increased interest in SLNM in colon cancer, it is important to obtain a broader understanding of the technique and of the factors that influence the success of NIR- fluorescence imaging for SLN identification. Therefore, a systematic review and meta-analysis was conducted to provide an overview of the current performance of SLNM with NIR fluorescence imaging in colon cancer, and of the factors influencing its sensitivity, detection rate, negative predictive value and upstaging rates. These outcomes are compared with results

and experience of a prospective single-center study performed in our hospital which aimed to determine SLNM accuracy using NIR fluorescence imaging in 30 colon cancer patients.

MATERIALS AND METHODS

Systematic literature review and meta-analysis

The protocol for the review was registered in the international prospective register of systematic reviews (PROSPERO, registration number CRD42018110076). A systematic literature search was performed in PubMed MEDLINE and Embase from inception through October 2nd 2018, in collaboration with a medical librarian (LS). The literature search was in compliance with the screening guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [14]. Two authors (MA and WJHJM) independently conducted the literature search. In case of disagreement regarding inclusion or exclusion, a paper was discussed to establish consensus. The following medical subject headings (MeSH) were used as index terms; 'colorectal neoplasms', 'Sentinel Lymph Node Biopsy', 'Indocyanine Green' and 'Fluorescent dyes'. The full PubMed search strategy is detailed in Supplementary Information 1. Systematic reviews and narrative review articles identified during the search were checked for additional references. Articles were included if they fulfilled the following criteria: 1) description of the use of NIR imaging for SLNM in colon cancer; 2) prospective design to assess the effectiveness of identification and diagnostic performance of the SLN procedure in colon cancer; 3) separate results for colon and rectal cancer. Studies that performed SLNM only in rectal carcinoma were excluded. To avoid overlapping patient data in duplicate publications, the article with the largest sample size was included. Studies published in English, German or Dutch were included. Articles were systematically screened by title, followed by abstract screening and finally full-text screening.

Variables collected consisted of gender, age, body mass index (BMI), number of patients with colon cancer, T-stage of disease, tumor size, in vivo or ex vivo injection, tracer injection site, tracer composition, tracer concentration, injected dose, investigator's definition of SLN, total number of SLNs, total number of regional lymph nodes, number of SLN procedures, number of failed SLN procedures, histopathological technique(s) used, imaging system, and any adverse effects of SLNM. The quantitative results were used to build 3x2 contingency tables to estimate numbers of true positives (TP), true negatives (TN) and false negatives (FN) (Supplementary Information 2, Figure 1). A true positive procedure was defined as a tumor-positive SLN with or without advanced histopathology analysis, independently of regional lymph node status. In this

context a false positive rate was zero by definition since false positive histopathological findings are not possible. SLN procedures were determined to be true negatives if SLNs and regional lymph nodes revealed no metastases after conventional and advanced histopathology. SLN procedures yielding tumor-negative SLNs in combination with tumor-positive regional lymph nodes were classified as false negatives.

As false positives were not possible, the specificity and positive predictive value of the SLN procedure was 100% by definition. The diagnostic parameters *sensitivity, negative predictive value, detection rate,* and *upstaging* were recalculated from the included data.
Sensitivity of the SLN-procedure was defined as the number of true positives in patients with positive histopathological findings (TP/TP+FN). The negative predictive value was defined as the number of patients in whom a negative SLN correctly predicted the lymph node status of the total lymph node yield (TN/TN+FN). *Detection rate* was the proportion of successful SLN-procedures divide by all executed SLN procedures. Patients were considered as `*upstaged*` in case of positive SLNs at advanced histopathology without tumour-positive regional lymph nodes with conventional histopathology. The percentage of upstaged patients was calculated by dividing the number of upstaged patient with the number of TN patients after conventional histopathology (upstaged patients /upstaged patients + TN).

Quality assessment of the studies was based on the revised tool for the quality assessment of diagnostic accuracy studies (QUADAS-2) [15]. Risk of bias was independently assessed by two reviewers (MA and WJHJM). Discrepancies in interpretation were resolved by discussion (Table 1).

Table 1 . Results of quality assessment of the studies included according to QUADAS-2

	Risk of Bias				Applicability concerns		
Study	**Patient selection**	**Index test**	**Reference standard**	**Flow and Timing**	**Patient selection**	**Index test**	**Reference standard**
Andersen[22]	☹	☺	☺	☺	☹	☺	☺
Currie[21]	☺	☺	☺	☺	☺	☺	☺
Hirche[23]	☺	☺	☺	☺	☺	☺	☺
Hutteman[26]	☹	?	☹	?	☹	☺	☹
Liberale[24]	☺	☺	☺	☺	☺	☺	☺
Schaafsma[25]	☹	☺	☹	☺	☹	☺	☹
Watanabe[27]	?	☺	☹	☺	?	?	☹
Weixler[20]	☹	☺	☺	☺	☹	☺	☺

☺ Low risk ☹ High Risk ? Unclear Risk

We used the data from the 3x2 tables to calculate sensitivity, negative predictive value, detection rate and upstaging. Individual results were presented graphically by plotting these values with their 95% confidence intervals in forest plots. An bivariate random-effects approach for the meta-analysis of sensitivity [16] and negative predictive value was used [17]. We investigated heterogeneity visually by examining the forest plots and statistically by including covariates in the bivariate models through conducting subgroup analysis. The following sources of heterogeneity were assessed 1) Used tracer (indocyanine green, IRDye800CW whether or not combined with blue dye); 2) Number of injections (2-4 injections vs. random number of injections); 3) Injection technique (in vivo or ex vivo); 4) Injection site (subserosal vs. submucosal); 5) Time between injection and SLN mapping (directly after injection, 3-10 minutes after injection and > 15 minutes after injection). We incorporated these factors as covariates in the bivariate models to examine the effect of potential sources of bias and variation across subgroups of studies.

Pooled estimates of detection rates and upstaging with their corresponding 95% confidence intervals were calculated using Freeman-Tukey double arcsine transformation within a random effects model framework. Heterogeneity of combined study results was assessed by I^2, and its connected Chi-square test for heterogeneity, and the corresponding 95% confidence intervals were calculated. Statistical analysis were performed using R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria) with packages "mada" and "meta."

Prospective single-center study

We performed a prospective single-center study which was approved by the Medical Ethics Committee of the UMC-Amsterdam, Vrije Universiteit Amsterdam, The Netherlands. The study was registered in the Clinical trials database (NCT02122523). Included patients were at least 18-years-old with proven colon cancer and scheduled for laparoscopic colectomy. All patients provided oral and written informed consent. Exclusion criteria included pre- and peroperative gross lymph node invasion, distant metastases, prior colorectal surgery, advanced disease with invasion of adjacent structures, metastatic or T4 disease on preoperative imaging or discovered during intraoperative staging, rectal cancer, contraindications for laparoscopy and allergy to iodine. The first 14 patients received a transcutaneously performed subserosal injection of ICG and the subsequent 15 patients had submucosal tracer injection by colonoscopy.

Injection of ICG was performed in vivo in all patients after general anesthesia. The injection solution consisted of 25 mg ICG diluted in 1.0 ml human albumin (20%) and 9.0 ml NaCl (0.9) [18]. Injection of dye followed prior injection of 1.0 ml NaCl (0.9%) to ensure correct needle placement in the submucosal layer in both injection techniques. A subserosal injection consisted of 1-3

peritumoral injections with a sclerosing needle. For submucosal injection, a colonoscopy was executed directly after general anesthesia and placement of a laparoscopic port. ICG was administrated by 1-4 submucosal injections at the base of the tumor using a V960 injection needle (Prince Medical, Gutenberg, France). In both groups the surgical procedure started with exposure of the peritoneal cavity and operative field, followed by mobilization of the colon medial to lateral. The mesocolon was inspected with conventional imaging and additionally with a NIR 30° laparoscope (Olympus, Tokyo, Japan). All fluorescent lymph nodes identified with the NIR camera were considered SLN(s), intraoperatively harvested and presented to the pathologist separately from the rest of the specimen. All lymph nodes were bisected along the longest axis, paraffin embedded and stained with H&E. Serial sectioning (3-4 µm thick) at 150 µm intervals was followed by H&E staining. Immunohistochemistry for the epithelial carcinoembryonic-antigen (CEA) (Clone 1117; DAKO Netherlands M7072), CAM 5.2 (3455799; BD Biosciences Netherlands) and CK19 (M0888, clone RCK 108; DAKO The Netherlands) followed when no metastases were found after routine H&E staining. Metastases were classified according to the TNM 5 guidelines. Metastases identified by serial sectioning and IHC were classified as reported by Hermanek and colleagues [19]. Sensitivity, detection rate, negative predictive value and upstaging were calculated by comparing the results of SLNM and pathological examination for all lymph nodes and expressed as a median (range).

RESULTS

Systematic review and meta-analysis

We identified 8 eligible studies published between January 2006 and August 24th 2018 presenting 227 SLN procedures [20-27]. Since no additional articles were found by cross-checking references, these 8 studies were analyzed and critically appraised. The selection process is shown in Figure 1. We excluded the study of van der Pas et al. [28] because these results were reported in our single-center study. An overview of included articles is given in Table 2 and Table 3. None of the studies reported adverse events.

BMI was reported in 7 studies and varied widely from 19 to 40 kg/m^2 [25,24,26,23,21,22,27]. None of the studies mentioned BMI as a potential factor of influence on SLN performance. Four studies described tumor size, which varied between 9-100 mm [21,26,25,24]. All studies disclosed tumor stage. Early staged T1 and T2 tumors were found in 41 (18%) and 57 (25%) patients, respectively. T3- tumors were diagnosed in 113 (50%) reported patients and T4- tumors in 16 patients (7%).

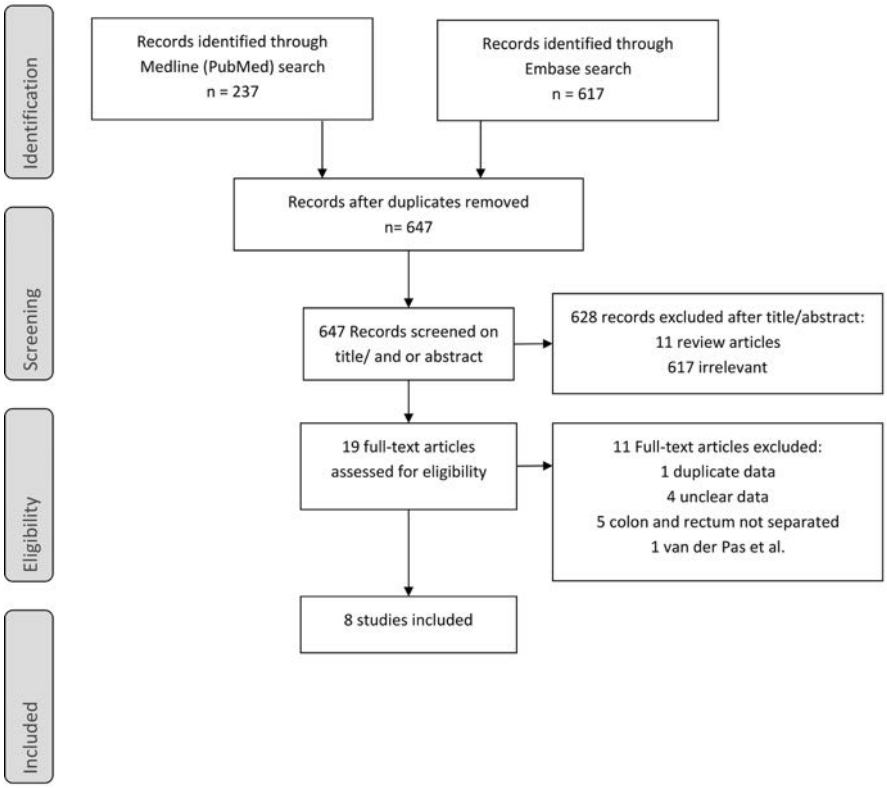


Figure 1. PRISMA 2009 Flow Diagram

As fluorescent mapping agent, ICG was used in 5 studies sourced from different companies [22,21,27,24,23]. ICG was dissolved in distilled water in 3 studies [22,24,27] and humanized-serum albumin (HSA) was added in 1 study [22]. Three studies used IRDy800CW conjugated to HSA and dissolved in PBS [26,25,20] (Li-Cor, Lincoln, NE,USA). In 4 studies injection of a fluorescent tracer was combined with administration of blue dye [22,24,25,20]. The concentration of the fluorescent dyes varied between 0.5-5.0 mg/ml. In all studies injection occurred around the tumor. The number of injections varied between 2-4 injections proximal and distal to the tumor [22,27,23] or circumferentially [21,24], up to a random number of injections depending on tumor size [26,25,20]. A lower volume of tracer was injected when the number of injections was determined by tumor size compared to those using 2-4 standard injections. Administration of tracer occurred in vivo [22,21,23,27] or ex vivo [26,24,25,20]. Procedures using IRDye800CW as a tracer were all performed ex vivo, since the dye was not Food and Drug Administration -approved during the performance of the studies. Both techniques allow subserosal or submucosal injection. The submucosal injection technique was used in 3 studies [21,26,25] and subserosal in 5 studies [22,23,27,20,24]. SLNs were identified directly after dye injection [22,24], after 3-10 minutes [21,23,26,25] or more than 15 minutes

after injection [20,27]. As shown in table 2, the highest number of SLNs were identified when SLNM was performed more than 15 minutes after injection.

In 5 studies SLNs were serial sectioned and stained with IHC when no (sentinel) lymph node metastases were found after conventional H&E staining [22,21,23,24,20]. Intervals used in the serial sectioning of 3-5 um sections varied between studies and ranged from 50 to 250 um. A cytokeratin antibody was used for IHC in all 5 studies, as cytokeratin is highly expressed in metastases of colorectal origin.

An overall pooled sensitivity of 0.63 (95% CI 0.51-0.74) and negative predictive value of 0.81 (95% CI 0.73-0.86) were estimated (Figure 2 and Figure 3). The pooled detection rate was estimated to be 0.96 (95% CI 0.88-1.0) (Figure 4). Upstaging could be calculated in 5 studies [22,21,23,24,20] and a pooled upstaging of 0.15 (95% CI 0.07-0.25) was estimated (Figure 5). Thus, 15% of patients with negative SLNs assessed with conventional histopathology, showed occult tumor cells (isolated tumor cells or micrometastases) after examination with advanced histopathology. None of the subgroup analyses showed statistically significant differences between subgroups in terms of sensitivity, negative predictive value or detection rate (Supplementary Information, Table 2).

Table 2. Characteristics of included studies

Study	Patients	Successful procedures	Male:Female	Age (Years)	BMI (kg/m²)	Tumor diameter (mm)	T1	T2	T3	T4	Tumor location	Number LNs	Number SLNs
Andersen[22]	29	19	18:11	69 (36-79)	26 (19-31)	na	1	7	19	2	Cecum to sigmoid	24 (9-24)	1 (0-3)
Currie[21]	30	27	na	69 (61-73)	26.2 (19-31)	37 (29-49)	6	8	14	2	Cecum to sigmoid	34 (27-39)	3 (1-4)
Hirche[23]	26	25	na	67 (47-87)	28.4	na	6	5	14	1	Cecum to sigmoid	32.9 (10-143)	1.7 (0-5)
Hutteman[26]	19	19	na	64 ± 16.6	27.5 ± 6.17	42 ± 13	1	4	11	3	Cecum to sigmoid	16.2 ± 5.34	3.2 ± 1.01
Liberale[24]	20	20	9:11	70 (43-87)	26.3 (18-36)	39 (0-100)	5	1	10	4	Cecum to sigmoid	22.4 (5-41)	1.5 (0-4)
Schaafsma[25]	22	21	12:10	69 (41-88)	25 (20-40)	37 (9-90)	2	7	10	3	Cecum to sigmoid	20.5 ± 8.1	3.5 ± 1.9
Watanabe[27]	31	31	22:9	67.5 ± 12.2	23.6 ± 3.24	na	13	18	0	0	Splenic flexure	17.5 ± 7.6	10.4 ± 4.73
Weixler[20]	50	49	31:19	68 ± 11.2	na	na	7	7	35	1	Cecum to sigmoid	23.4 ± 9.5	4.4 ± 2.2

Table 3. Technical characteristics of SLN mapping of included studies

Study	Infrared system	Fluorescent tracer	Tracer composition	Concentration	Injected dose	Injection technique	Site of injection	Time of SLN mapping	Histopathological examination for SLNs
Andersen[22]	SPIES (Karl Storz, Holte, Denmark)	ICG; ICG Pulsion, Pulsion Medical Systems, Munich, Germany	ICG: HSA diluted in H2O and blue dye	ICG 25 mg H2O 9 ml HSA 1 ml	2 x 0.5 ml proximal an distal from the tumor	In vivo	Subserosal	Intraoperative directly and 20 min after injection	Five series of 50 um interval, 3 um thickness/each; 1st section H&E and 3th section with IHC for cytokeratin A
Currie[21]	Laparoscopic NIR-imaging system (Olympus Corporation, Tokyo, Japan)	ICG; ICG Pulsion, Pulsion Medical Systems, Munich, Germany	na	5 mg/ml	4 x 1 ml circumferentially	In vivo	Submucosal	Intraoperative 7 (IQR 6 -8) min after injection	Standard H&E-staining. If negative for metastases, serial sectioning in slices of 4 um at 250 um intervals, staining of each level with H&E and IHC for pan-cytokeratin antibody
Hirche[23]	IC-View (ICG Pulsion, Pulsion Medical Systems, Munich, Germany)	ICG stock solution	na	5 mg/ml	2.0 (range 1-4) ml around the tumor	In vivo	Subserosal	Intraoperative 3-10 min after injection and ex vivo	H&E-staining at 250 um. If negative for metastases, re-examination by serial sectioning at 5 um and H&E-staining and IHC for cytokeratin antibody for each section.
Hutteman[26]	Mini-FLARE	IRDye800CW; Li-Cor, Licoln, NE	IRDye800CW : HSA diluted in PBS	3 : 1	1 ml circumferentially	Ex vivo	Submucosal	Ex vivo 5 min after injection and tracer massage	H&E-staining at 4 um sections
Liberale[24]	Photodynamic Eye PDE (Hamamatsu, Japam)	ICG (Pulsion, Paris, France)	ICG diluted in H2O and blue dye	0.5 mg/ ml	4 x 0.5 ml circumferentially	Ex vivo	Subserosal	Ex vivo directly after injection and during pathological examination	Standard H&E staining. If negative for metastases, serial sectioning using three slices at 150 um interval stained with H&E, if still negative IHC for anti-pan- cytokeratin

Table 3 (continued). Technical characteristics of SLN mapping of included studies

Study	Infrared system	Fluorescent tracer	Tracer composition	Concentration	Injected dose	Injection technique	Site of injection	Time of SLN mapping	Histopathological examination for SLNs
Schaafsma[25]	Mini-FLARE	IRDye800CW; Li-Cor, Lincoln, NE	IRDye800CW : HSA diluted in PBS and blue dye	1.5 : 1	1 ml circumferentially	Ex vivo	Submucosal	Ex vivo 5 min after injection and tracer massage	H&E-staining at 4 um sections
Watanabe[27]	In vivo: D-Light P System (Karl Storz, Tuttlingen, Germany) Ex vivo: HyperEye Medical System, Mizuho corporation, Tokyo, Japan	Diagnogreen; Daiichi Pharmaceuticals, Tokyo, Japan	ICG diluted in H2O	2.5 mg/ml	2 x 1 ml proximal and distal from the tumor	In vivo	Subserosal	Intraoperative 30 min after injection and ex vivo	H&E staining
Weixler[20]	Mini-FLARE	IRDye800CW; Li-Cor, Lincoln, NE	IRDye800CW : HSA diluted in PBS and blue dye	3 : 1	0.4 ± 0.2 ml Number of injections depends on tumor size	Ex vivo	Subserosal	15 min after injection	Serial sectioning at 3 levels, H&E at the 1st section of each level. If negative for metastases then IHC for cytokeratin 19 for a second section.

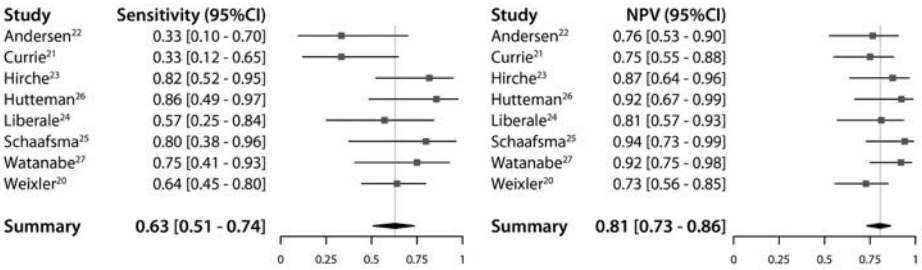


Figure 2 (left). Pooled sensitivity
Figure 3 (right). Pooled negative predictive value

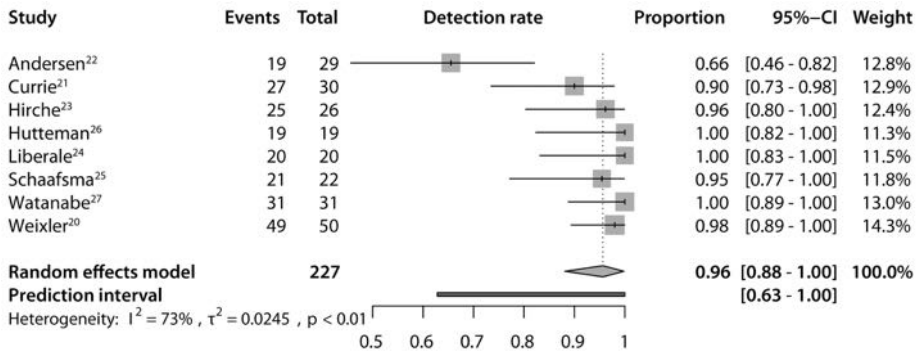


Figure 4. Pooled detection rate

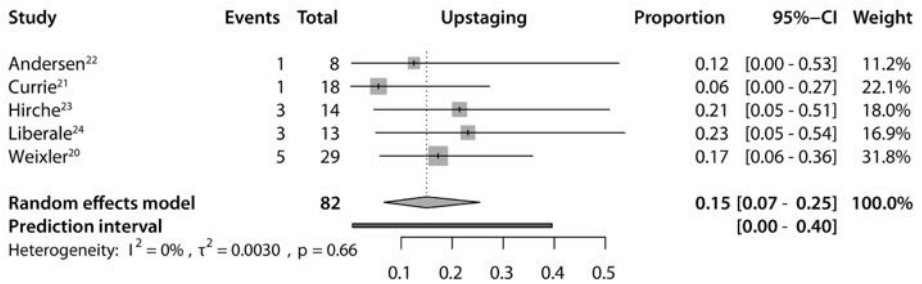


Figure 5. Pooled upstaging

Prospective single-center study

A total of 30 patients were included. One patient was excluded due to intraoperatively detected gross lymph node metastasis. Patient characteristics and results for each patient are shown in Table 4. No adverse events related to ICG were noticed. Subserosal dye injection occurred in 14 patients and submucosal injection in 15 patients. In 2 patients (patient 22 and 28) no fluorescent SLNs were intraoperatively identified resulting in an overall detection rate of 89.7%,

with a median of 2 (range 0-6) SLNs and 13 (range 1-34) regional lymph nodes identified. Of the 29 patients, nine patients showed lymph node metastases. Five of these patients showed metastases in the regional lymph nodes but not in the SLN (patient 1, 2, 4, 10, 17. These SLNs are considered as false negatives. In one patient, lymph node metastases were found in both the SLNs and regional lymph nodes (patient 27). These SLNs are classified as true positives. In three procedures, SLNs showed metastases in *only* the SLNs which were found at advanced histopathology (patient 15, 16 and 18). In two of these patients, SLNs contained isolated tumor cells found with additional IHC (patient 16 and 18) and in one patient SLN metastases (> 2 mm) were detected after serial sectioning and H&E staining (patient 15). These three patients were converted from node negatives to node positives and therefore considered as ' upstaged' and also true positives. As a result, the sensitivity of the procedure was 44% (4/9), negative predictive value 80% (20/25) and upstaging 13% (3/23).

Results for sensitivities after subserosal injection and submucosal injection were 0 versus 80%, respectively. Negative predictive values were 71% after subserosal injection and 91% using the submucosal injection technique.

Table 4. Patient characteristics and results of each patients

Patient	Diagnosis	Tumour location	BMI (kg/m²)	Tumour size (cm)	Injection side	Number of injections	SLN found	Malignant SLN	Non-SLNs	Malignant regional lymph nodes	SLN-status
1	T3N1M0	Ascendens	25.9	7.0	SS	1	3	0	34	1	FN
2	T3N2M0	Cecum	25.9	7.0	SS	1	1	0	22	8	FN
3	T2N0M0	Transversum	24.5	2.5	SS	1	2	0	1	0	TN
4	T3N1M0	Sigmoid	22.9	10	SS	2	2	0	6	1	FN
5	T3N0M0	Sigmoid	22.2	4.5	SS	1	4	0	5	0	TN
6	T2N0M0	Cecum	31.2	4.0	SS	1	2	0	9	0	TN
7	T2N0M0	Cecum	30.1	3.0	SS	1	2	0	25	0	TN
8	T3N0M0	Sigmoid	26.4	9.5	SS	2	1	0	4	0	TN
9	T3N0M0	Sigmoid	25.8	4.5	SS	1	3	0	10	0	TN
10	T3N1M0	Sigmoid	21.0	3.5	SS	3	2	0	4	2	FN
11	T3N0M0	Ascendens	25.1	0.8	SS	1	1	0	27	0	TN
12	T1N0M0	Cecum	22.0	6.0	SS	1	6	0	32	0	TN
13	T2N0M0	Cecum	23.8	4.5	SS	3	2	0	14	0	TN
14	T3N0M0	Sigmoid	25.1	3.5	SS	3	6	0	14	0	TN
15	T3N1M0	Sigmoid	25.6	4.5	SM	4	4	1	12	0	TP/Upstaging
16	T3N0M0	Transversum	23.3	5.5	SM	2	2	2	21	0	TP/Upstaging
17	T3N1M0	Sigmoid	26.1	6.5	SM	4	4	0	17	3	FN
18	T2N0M0	Sigmoid	26.3	4.0	SM	1	4	1	11	0	TP/Upstaging
19	T1N0M0	Transversum	29.3	0.9	SM	1	0	0	30	0	TN
20	T3N0M0	Sigmoid	29.0	5.0	SM	4	3	0	15	0	TN
21	T2N0M0	Sigmoid	22.9	0.8	SM	1	4	0	8	0	TN
22	T3N0M0	Sigmoid	29.0	5.0	SM	3	0	0	12	0	TN

Table 4 (continued). Patient characteristics and results of each patients

Patient	Diagnosis	Tumour location	BMI (kg/m^2)	Tumour size (cm)	Injection side	Number of injections	SLN found	Malignant SLN	Non-SLNs	Malignant regional lymph nodes	SLN-status
23	T1N0M0	Cecum	35.2	4.5	SM	1	2	0	13	0	TN
24	T1N0M0	Sigmoid	27.1	2.0	SM	1	2	0	13	0	TN
25	T1N0M0	Cecum	24.4	2.2	SM	1	2	0	14	0	TN
26	T1N0M0	Sigmoid	29.3	2.0	SM	1	2	0	7	0	TN
27	T4N1M0	Transversum	43.1	7.0	SM	4	4	2	29	1	TP
28	T1N0M0	Transversum	33.0	2.3	SM	1	0	0	5	0	TN
29	T1N0M0	Sigmoid	27.9	3.3	SM	3	3	0	9	0	TN

DISCUSSION

The concept of SLNM to improve lymph node staging in colon cancer has been investigated extensively [10]. Due to the wide variation in reported outcomes and frequent lower accuracy of results compared to breast cancer and melanoma, overall treatment decision making based on SLN assessment is still not safe. The location of colonic SLNs in the fatty mesocolon and limited penetration depth of blue dye through fatty adipose tissue are frequently mentioned as serious obstacles to accurate SLNM. NIR imaging using a fluorophore as mapping agent has been proposed as a more effective technique for SLNM in colonic malignancies due to its high tissue penetration of up to 1 cm [29].

In this meta-analysis, we included 8 studies that described the use of a fluorophore as a mapping agent for SLNM in colon cancer. We found wide variation between studies regarding the NIR fluorescent SLNM techniques employed, resulting in large differences in reported outcomes. Subgroup analyses were therefore performed to identify the technically-related factors influencing SLNM accuracy. These technical factors included the tracer used, injection site, in vivo or ex vivo SLNM performance, number of injections and timing between tracer administration and SLN identification. Additionally tracer composition, dosage and concentrations all varied between studies. Pooled detection rates and negative predictive values both showed acceptable results, at 0.96 (95%CI 0.88-1.0) and 0.81 (95%CI 0.73-0.86), respectively. However, an overall low sensitivity of 0.63 (95%CI 0.51-0.74), accompanied by wide variation (33%-85%), was reported among included studies. Although subgroup analysis showed comparable results for SLN accuracy, we believe that several technical factors contributed to the poorer results for SLNM using NIR fluorescence imaging in colon cancer compared to those reported in other types of cancer [30].

The first technical factor which should be validated is site of injection for tracer administration. Although meta-analysis results showed no significant differences after subserosal or submucosal injection of dye, notably outcomes in terms of sensitivity and negative predictive value were found after subserosal injection in our single-center study. According to current literature, this can be explained by the greater accuracy of injection near the tumor after submucosal injection, which also probably improves uptake by all tumor-draining lymphatic vessels [26,31-33]. Another possible explanation is difficulty regarding correct needle positioning and maintaining position during tracer administration. We have personally experienced this problem, especially when using the subserosal injection technique. To improve the accuracy of needle positioning, an injection of 1.0 ml saline in the colon wall was administrated before

ICG. The raised `bleb` was helpful in confirming correct positioning of the needle into the colon wall. However, a unfavourable side effect of the injection of saline prior to ICG, is the increase of hydrostatic pressure at the injection site. This resulted in dislocation of the needle in several patients and extravasation of dye into the peritoneum, which then made SLN identification impossible in these patients due the high intrinsic background fluorescence in the abdominal cavity. We experienced no spillage of dye using the submucosal injection technique. Only 1 of the reviewed studies described spillage of dye, which in that case occurred after injection into the subserosal layer, but the authors did not mention this as a cause of false negative SLN procedures [22]. Independently of the injection technique used, correct needle placement and careful administration of tracer without spillage of dye is crucial to a successful SLN procedure. There appears to be a steep learning curve for accurate tracer administration and we therefore recommend that this procedure should only be performed by an experience surgeon or gastroenterologist.

Tracer administration and SLN identification can be performed in vivo or ex vivo. Advantages of the ex vivo SLN procedure include avoidance of patient exposure to dye, interruption of the surgical procedure, and the relative simplicity of the technique. In addition, ex vivo SLNM is thought to allow a more aggressive dissection of the mesocolon, resulting in improvement of SLN identification [34]. However, ex vivo SLN identification after extraction of the specimen disrupts natural lymphatic pathways. Moreover, an ex vivo approach may exclude SLNs located outside the resection area, whereas an in vivo SLN procedure might have identified aberrant lymph node drainage patterns and thus changed the mesocolonic resection margins [35]. In our study we did not modify our resection margins, but several reviewed studies identified metastatic SLNs which would not have been included in the standard resection specimen [22,36,27]. Furthermore, an in vivo approach can potentially facilitate SLN picking, combined with local excision of the primary tumor when no metastases are found in the SLNs. This treatment approach would dramatically change therapy options for patients with early staged tumors, and could potentially decrease surgery-related morbidity rates while improving patient survival [3]. It must be emphasized that node picking interrupts the standard oncological approach. Secondly, a higher number of lymph nodes retrieved is associated with prolonged disease free survival due to better lymph node staging [37]. These facts underline the need for a highly sensitive SLNM technique before minimization of surgery can be justified in cases where no metastases are found in the SLNs [38].

Optimal timing of tracer administration is another technical factor which needs to be improved. Despite the helpful larger diameter of ICG or possibility of using HSA, the tracer still shows fast

migration to higher echelon lymph nodes, resulting in a greater number of fluorescent regional lymph nodes. Designating a large number of fluorescent lymph nodes as SLNs is undesirable since advanced histopathological examination must be applied to all these nodes, which is expensive, time-consuming and probably not cost-effective. Another contributing factor is time of harvesting between injection and SLN detection. As shown by the results in table 2 of the reviewed studies, numbers of assigned SLNs were highest when SLNM was performed more than 15 minutes after injection [27,20]. A high number of SLNs should be avoided, as non-SLNs could be mistaken for SLNs and unnecessary costs will be incurred. Therefore surgeons should attempt to follow lymph flow drainage patterns directly after injection which would overcome prolonged time intervals between tracer administration and SLNM.

A wide variety of dye compositions, concentrations and dosages were employed in the reviewed studies. It is important to note that ICG and IRDye800CW should be seen as distinct tracers, as they have their own specific chemical and physical properties. An ICG dose of around 500 ug has been suggested as optimal for SLNM but this has never been clearly investigated in colon cancer [39,12,40,41]. Humanized-serum-albumin is frequently added to ICG and IRDye800CW, which may improve metabolic activity and result in a brighter fluorescent signal. In addition, HSA should increase the hydrodynamic diameter, leading to better retention of dye in the SLN. However, results for fluorescent tracer combined with HSA in SLNM are inconclusive [42,18,43]. More research to optimize concentration, dosage and composition is necessary to improve the fluorescent signal and to allow differences in signal intensity between the SLN and surrounding tissue to be distinguished.

To improve the NIR fluorescence imaging SLN procedure in colon cancer, patient and tumor-related characteristics should be investigated in addition to technical factors. Currie et al. [21] reported a sensitivity of 33% and argued that this was caused by inclusion of large tumors (>35 mm) and high number of T3 and T4 tumors, which are associated with more advanced tumor stages. Weixler et al. [20] and Liberale et al. [24] agreed that the inclusion of T3-T4 tumors is a potential cause of high false negative rates, but not tumor size itself. Tumor stage and size are both frequently mentioned as causes of high false negative rates in current literature [44]. It is well-established that higher stage disease with more advanced transmural tumors increase the risk of lymph node metastasis. Secondly, large longitudinal and transserosal tumors could theoretically destroy efferent lymphatic pathways and may involve adjacent lymphatic drainage patterns, which can increase false negative rates. It must be emphasized that these factors have never been verified as confounders in large studies [45,10]. However, since more advanced tumor stages already meet criteria for adjuvant chemotherapy, and treatment of these tumors

will not be altered by a SLN procedure, we suggest that future studies should try to include only early stage tumors.

High BMI and additional mesocolonic adiposity are associated with a decreased sensitivity for SLNM [46,45]. Although none of the included studies confirmed this association, we experienced technical difficulties during SLNM in patients with a fatty mesocolon. First, it must be emphasized that effective penetration of fluorescence is still limited in fatty mesocolon. Additionally, more fatty tissue requires more dissection, leading to additional risk of disrupting the lymphatic vessels, with leakage of dye into the abdominal cavity as a consequence. As a result SLN identification becomes more challenging, since adhesion of dye to fat compromises the NIR view and fluorescing fat could be mistaken for an SLN [21].

Finally, the pathological assessment of SLNs has a major influence on the accuracy of the procedure. Only advanced histopathological techniques are able to detect occult tumor cells (e.g. isolated tumor cells and micrometastases), although the prognostic significance of these occult tumor cells is still unclear. Nevertheless, it has been suggested that micrometastases are associated with a significant reduction in 5-year survival [5,8]. Therefore, studies of the SLN procedure should use serial sectioning and IHC for histopathological examination to all SLNs when no lymph node metastasis are found after conventional H&E staining [47]. Limitations of the results presented here include the marked variability in the methods used and the small number of patients included in each study, including our own single-center study.

CONCLUSIONS

Evidence regarding SLNM with NIR fluorescence imaging in colon cancer is still limited. Better standardization of the technique will be necessary in future trials. These studies should concentrate on early stage tumors and focus on tracer composition, injection mode and the improvement of real-time optical guidance to the SLN. Moreover, due to several anatomical and technical difficulties, the SLN procedure in colon cancer seems to be more challenging compared to other types of cancer, and considerable expertise will be required before large patient-related studies can be undertaken to validate SLNM as part of the standard surgical treatment in colon cancer.

REFERENCES

1. de Wijkerslooth TR, de Haan MC, Stoop EM, Deutekom M, Fockens P, Bossuyt PM, Thomeer M, van Ballegooijen M, Essink-Bot ML, van Leerdam ME, Kuipers EJ, Dekker E, Stoker J (2010) Study protocol: population screening for colorectal cancer by colonoscopy or CT colonography: a randomized controlled trial. BMC Gastroenterol 10:47. doi:10.1186/1471-230X-10-47
2. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zaninelli M, Clingan P, Bridgewater J, Tabah-Fisch I, de Gramont A, Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer I (2004) Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 350 (23):2343-2351. doi:10.1056/NEJMoa032709
3. Cahill RA, Leroy J, Marescaux J (2009) Localized resection for colon cancer. Surg Oncol 18 (4):334-342. doi:10.1016/j.suronc.2008.08.004
4. Figueredo A, Coombes ME, Mukherjee S (2008) Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev (3):CD005390. doi:10.1002/14651858.CD005390.pub2
5. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ, van der Zaag ES, Tanis PJ, Bemelman WA, Buskens CJ (2014) The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. Eur J Surg Oncol 40 (3):263-269. doi:10.1016/j.ejso.2013.12.002
6. Wasif N, Faries MB, Saha S, Turner RR, Wiese D, McCarter MD, Shen P, Stojadinovic A, Bilchik AJ (2010) Predictors of occult nodal metastasis in colon cancer: results from a prospective multicenter trial. Surgery 147 (3):352-357. doi:10.1016/j.surg.2009.10.008
7. Markl B, Herbst C, Cacchi C, Schaller T, Krammer I, Schenkirsch G, Probst A, Spatz H (2013) Prognostic significance of histologically detected lymph node micrometastases of sizes between 0.2 and 2 mm in colorectal cancer. Int J Colorectal Dis 28 (7):977-983. doi:10.1007/s00384-012-1636-y
8. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, Hermans J, van Krieken JH, Cornelisse CJ, Tollenaar RA (1998) Micrometastases and survival in stage II colorectal cancer. N Engl J Med 339 (4):223-228. doi:10.1056/NEJM199807233390403
9. Liang J, Fazio V, Lavery I, Remzi F, Hull T, Strong S, Church J (2015) Primacy of surgery for colorectal cancer. Br J Surg 102 (7):847-852. doi:10.1002/bjs.9805
10. van der Pas MH, Meijer S, Hoekstra OS, Riphagen, II, de Vet HC, Knol DL, van Grieken NC, Meijerink WJ (2011) Sentinel-lymph-node procedure in colon and rectal cancer: a systematic review and meta-analysis. Lancet Oncol 12 (6):540-550. doi:10.1016/S1470-2045(11)70075-4
11. Kusano M, Tajima Y, Yamazaki K, Kato M, Watanabe M, Miwa M (2008) Sentinel node mapping guided by indocyanine green fluorescence imaging: a new method for sentinel node navigation surgery in gastrointestinal cancer. Dig Surg 25 (2):103-108. doi:10.1159/000121905
12. Mieog JS, Troyan SL, Hutteman M, Donohoe KJ, van der Vorst JR, Stockdale A, Liefers GJ, Choi HS, Gibbs-Strauss SL, Putter H, Gioux S, Kuppen PJ, Ashitate Y, Lowik CW, Smit VT, Oketokoun R, Ngo LH, van de Velde CJ, Frangioni JV, Vahrmeijer AL (2011) Toward optimization of imaging system and lymphatic tracer for near-infrared fluorescent sentinel lymph node mapping in breast cancer. Ann Surg Oncol 18 (9):2483-2491. doi:10.1245/s10434-011-1566-x
13. Rocha A, Dominguez AM, Lecuru F, Bourdel N (2016) Indocyanine green and infrared fluorescence in detection of sentinel lymph nodes in endometrial and cervical cancer staging - a systematic review. Eur J Obstet Gynecol Reprod Biol 206:213-219. doi:10.1016/j.ejogrb.2016.09.027
14. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 62 (10):1006-1012. doi:10.1016/j.jclinepi.2009.06.005

15. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM, Group Q- (2011) QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155 (8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009

16. Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH (2005) Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 58 (10):982-990. doi:10.1016/j.jclinepi.2005.02.022

17. Leeflang MM, Deeks JJ, Rutjes AW, Reitsma JB, Bossuyt PM (2012) Bivariate meta-analysis of predictive values of diagnostic tests can be an alternative to bivariate meta-analysis of sensitivity and specificity. J Clin Epidemiol 65 (10):1088-1097. doi:10.1016/j.jclinepi.2012.03.006

18. van der Pas MH, van Dongen GA, Cailler F, Pelegrin A, Meijerink WJ (2010) Sentinel node procedure of the sigmoid using indocyanine green: feasibility study in a goat model. Surg Endosc 24 (9):2182-2187. doi:10.1007/s00464-010-0923-3

19. Hermanek P, Hutter RV, Sobin LH, Wittekind C (1999) International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. Cancer 86 (12):2668-2673

20. Weixler B, Rickenbacher A, Raptis DA, Viehl CT, Guller U, Rueff J, Zettl A, Zuber M (2017) 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 41 (9):2378-2386. doi:10.1007/s00268-017-4051-2

21. Currie AC, Brigic A, Thomas-Gibson S, Suzuki N, Moorghen M, Jenkins JT, Faiz OD, Kennedy RH (2017) 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 43 (11):2044-2051. doi:10.1016/j.ejso.2017.05.026

22. Andersen HS, Bennedsen ALB, Burgdorf SK, Eriksen JR, Eiholm S, Toxvaerd A, Riis LB, Rosenberg J, Gogenur I (2017) In vivo and ex vivo sentinel node mapping does not identify the same lymph nodes in colon cancer. Int J Colorectal Dis 32 (7):983-990. doi:10.1007/s00384-017-2777-9

23. Hirche C, Mohr Z, Kneif S, Doniga S, Murawa D, Strik M, Hunerbein M (2012) Ultrastaging of colon cancer by sentinel node biopsy using fluorescence navigation with indocyanine green. Int J Colorectal Dis 27 (3):319-324. doi:10.1007/s00384-011-1306-5

24. Liberale G, Vankerckhove S, Galdon MG, Larsimont D, Ahmed B, Bouazza F, Moreau M, El Nakadi I, Donckier V, Bourgeois P, Institute DGftCAoFIatJB (2016) Sentinel Lymph Node Detection by Blue Dye Versus Indocyanine Green Fluorescence Imaging in Colon Cancer. Anticancer Res 36 (9):4853-4858. doi:10.21873/anticanres.11048

25. Schaafsma BE, Verbeek FP, van der Vorst JR, Hutteman M, Kuppen PJ, Frangioni JV, van de Velde CJ, Vahrmeijer AL (2013) Ex vivo sentinel node mapping in colon cancer combining blue dye staining and fluorescence imaging. J Surg Res 183 (1):253-257. doi:10.1016/j.jss.2013.01.003

26. Hutteman M, Choi HS, Mieog JS, van der Vorst JR, Ashitate Y, Kuppen PJ, van Groningen MC, Lowik CW, Smit VT, van de Velde CJ, Frangioni JV, Vahrmeijer AL (2011) Clinical translation of ex vivo sentinel lymph node mapping for colorectal cancer using invisible near-infrared fluorescence light. Ann Surg Oncol 18 (4):1006-1014. doi:10.1245/s10434-010-1426-0

27. Watanabe J, Ota M, Suwa Y, Ishibe A, Masui H, Nagahori K (2017) Evaluation of lymph flow patterns in splenic flexural colon cancers using laparoscopic real-time indocyanine green fluorescence imaging. Int J Colorectal Dis 32 (2):201-207. doi:10.1007/s00384-016-2669-4

28. van der Pas MH, Ankersmit M, Stockmann HB, Silvis R, van Grieken NC, Bril H, Meijerink WJ (2013) Laparoscopic sentinel lymph node identification in patients with colon carcinoma using a near-infrared dye: description of a new technique and feasibility study. J Laparoendosc Adv Surg Tech A 23 (4):367-371. doi:10.1089/lap.2012.0407

29. Nagata K, Endo S, Hidaka E, Tanaka J, Kudo SE, Shiokawa A (2006) Laparoscopic sentinel node mapping for colorectal cancer using infrared ray laparoscopy. Anticancer Res 26 (3B):2307-2311

30. Zhang X, Li Y, Zhou Y, Mao F, Lin Y, Guan J, Sun Q (2016) Diagnostic Performance of Indocyanine Green-Guided Sentinel Lymph Node Biopsy in Breast Cancer: A Meta-Analysis. PLoS One 11 (6):e0155597. doi:10.1371/journal.pone.0155597

31. Cahill RA, Anderson M, Wang LM, Lindsey I, Cunningham C, Mortensen NJ (2012) Near-infrared (NIR) laparoscopy for intraoperative lymphatic road-mapping and sentinel node identification during definitive surgical resection of early-stage colorectal neoplasia. Surg Endosc 26 (1):197-204. doi:10.1007/s00464-011-1854-3

32. Miller MJ, McDole JR, Newberry RD (2010) Microanatomy of the intestinal lymphatic system. Ann N Y Acad Sci 1207 Suppl 1:E21-28. doi:10.1111/j.1749-6632.2010.05708.x

33. Pedrazzani C, Lauka L, Sforza S, Ruzzenente A, Nifosi F, Delaini G, Guglielmi A (2015) Management of nodal disease from colon cancer in the laparoscopic era. Int J Colorectal Dis 30 (3):303-314. doi:10.1007/s00384-014-2075-8

34. Park JS, Chang IT, Park SJ, Kim BG, Choi YS, Cha SJ, Park ES, Kwon GY (2009) Comparison of ex vivo and in vivo injection of blue dye in sentinel lymph node mapping for colorectal cancer. World J Surg 33 (3):539-546. doi:10.1007/s00268-008-9872-6

35. Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, Ganatra B, Kaushal S, Desai D, Mannam S (2013) Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. Am J Surg 205 (3):302-305; discussion 305-306. doi:10.1016/j.amjsurg.2012.10.029

36. Chand M, Keller DS, Joshi HM, Devoto L, Rodriguez-Justo M, Cohen R (2018) Feasibility of fluorescence lymph node imaging in colon cancer: FLICC. Tech Coloproctol 22 (4):271-277. doi:10.1007/s10151-018-1773-6

37. Backes Y, Elias SG, Bhoelan BS, Groen JN, van Bergeijk J, Seerden TCJ, Pullens HJM, Spanier BWM, Geesing JMJ, Kessels K, Kerkhof M, Siersema PD, de Vos Tot Nederveen Cappel WH, van Lelyveld N, Wolfhagen FHJ, Ter Borg F, Offerhaus GJA, Lacle MM, Moons LMG, Dutch TCRCWG (2017) The prognostic value of lymph node yield in the earliest stage of colorectal cancer: a multicenter cohort study. BMC Med 15 (1):129. doi:10.1186/s12916-017-0892-7

38. Soni M, Wiese D, Korant A, Sirop S, Chakravarty B, Gayar A, Bilchik A, Beutler T, Ratz D, Saha S (2011) Comparison of nodal positivity between SLNM vs conventional surgery in colon cancer patients with <12 and >/=12 lymph nodes harvested. Am J Surg 202 (2):207-213. doi:10.1016/j.amjsurg.2010.06.028

39. Levinson KL, Mahdi H, Escobar PF (2013) Feasibility and optimal dosage of indocyanine green fluorescence for sentinel lymph node detection using robotic single-site instrumentation: preclinical study. J Minim Invasive Gynecol 20 (5):691-696. doi:10.1016/j.jmig.2013.03.013

40. van der Vorst JR, Hutteman M, Gaarenstroom KN, Peters AA, Mieog JS, Schaafsma BE, Kuppen PJ, Frangioni JV, van de Velde CJ, Vahrmeijer AL (2011) Optimization of near-infrared fluorescent sentinel lymph node mapping in cervical cancer patients. Int J Gynecol Cancer 21 (8):1472-1478. doi:10.1097/IGC.0b013e31822b451d

41. van der Vorst JR, Schaafsma BE, Verbeek FP, Swijnenburg RJ, Hutteman M, Liefers GJ, van de Velde CJ, Frangioni JV, Vahrmeijer AL (2013) Dose optimization for near-infrared fluorescence sentinel lymph node mapping in patients with melanoma. Br J Dermatol 168 (1):93-98. doi:10.1111/bjd.12059

42. Hutteman M, Mieog JS, van der Vorst JR, Liefers GJ, Putter H, Lowik CW, Frangioni JV, van de Velde CJ, Vahrmeijer AL (2011) Randomized, double-blind comparison of indocyanine green with or without albumin premixing for near-infrared fluorescence imaging of sentinel lymph nodes in breast cancer patients. Breast Cancer Res Treat 127 (1):163-170. doi:10.1007/s10549-011-1419-0

43. Davies-Venn CA, Angermiller B, Wilganowski N, Ghosh P, Harvey BR, Wu G, Kwon S, Aldrich MB, Sevick-Muraca EM (2012) Albumin-binding domain conjugate for near-infrared fluorescence lymphatic imaging. Mol Imaging Biol 14 (3):301-314. doi:10.1007/s11307-011-0499-x

44. Cahill RA (2007) What's wrong with sentinel node mapping in colon cancer? World J Gastroenterol 13 (47):6291-6294

45. Viehl CT, Guller U, Langer I, Laffer U, Oertli D, Zuber M (2013) Factors influencing the success of in vivo sentinel lymph node procedure in colon cancer patients: Swiss prospective, multicenter study sentinel lymph node procedure in colon cancer. World J Surg 37 (4):873-877. doi:10.1007/s00268-013-1910-3

46. Bembenek AE, Rosenberg R, Wagler E, Gretschel S, Sendler A, Siewert JR, Nahrig J, Witzigmann H, Hauss J, Knorr C, Dimmler A, Grone J, Buhr HJ, Haier J, Herbst H, Tepel J, Siphos B, Kleespies A, Koenigsrainer A, Stoecklein NH, Horstmann O, Grutzmann R, Imdahl A, Svoboda D, Wittekind C, Schneider W, Wernecke KD, Schlag PM (2007) Sentinel lymph node biopsy in colon cancer: a prospective multicenter trial. Ann Surg 245 (6):858-863. doi:10.1097/01.sla.0000250428.46656.7e

47. Faerden AE, Sjo OH, Bukholm IR, Andersen SN, Svindland A, Nesbakken A, Bakka A (2011) Lymph node micrometastases and isolated tumor cells influence survival in stage I and II colon cancer. Dis Colon Rectum 54 (2):200-206. doi:10.1007/DCR.0b013e3181fd4c7c

SUPPLEMENTARY INFORMATION 1

PubMed History 2 Oct 2018

Search	Query	Items found
#4	#1 AND #2 AND #3	237
#3	"Indocyanine Green"[Mesh] OR "Fluorescent Dyes"[Mesh] OR indocyanine green[tiab] OR wofaverdin[tiab] OR vophaverdin[tiab] OR vofaverdin[tiab] OR tricarbocyanin*[tiab] OR ujoviridin[tiab] OR icg [tiab] OR fluorescen*[tiab] OR fluorochrome*[tiab] OR fluorogenic*[tiab] OR cw800*[tiab] OR irdye*[tiab] OR cardio-green[tiab] OR cardiogreen[tiab] OR diagnogreen[tiab] OR "fox green"[tiab] OR ic green[tiab]	463848
#2	"Sentinel Lymph Node Biopsy"[Mesh] OR "Sentinel Lymph Node"[Mesh] OR ((sentinel[tiab] OR lymph[tiab] OR lymphatic[tiab]) AND (node*[tiab] OR nodal[tiab]))	196119
#1	"Colorectal Neoplasms"[Mesh] OR ((colorectal[tiab] OR colon*[tiab] OR rectal[tiab] OR rectum[tiab] OR anus[tiab] OR anal[tiab] OR recti[tiab] OR recto[tiab] OR pararect*[tiab] OR retrorect*[tiab]) AND (neoplasm*[tiab] OR cancer*[tiab] OR tumor[tiab] OR tumour[tiab] OR tumors[tiab] OR tumours[tiab] OR malign*[tiab] OR mass[tiab]))	314134

Embase History 2 Oct 2018

Search	Query	Items found
#4	#1 AND #2 AND #3	617
#3	'indocyanine green'/exp OR 'fluorescent dye'/exp OR 'indocyanine green':ab,ti,kw OR wofaverdin:ab,ti,kw OR vophaverdin:ab,ti,kw OR vofaverdin:ab,ti,kw OR tricarbocyanin*:ab,ti,kw OR ujoviridin:ab,ti,kw OR icg:ab,ti,kw OR fluorescen*:ab,ti,kw OR fluorochrome*:ab,ti,kw OR fluorogenic*:ab,ti,kw OR cw800*:ab,ti,kw OR irdye*:ab,ti,kw OR 'cardio green':ab,ti,kw OR cardiogreen:ab,ti,kw OR diagnogreen:ab,ti,kw OR 'fox green':ab,ti,kw OR 'ic green':ab,ti,kw	623846
#2	'sentinel lymph node biopsy'/exp OR 'sentinel lymph node'/exp OR ((sentinel:ti,ab,kw OR lymph:ti,ab,kw OR lymphatic:ti,ab,kw) AND (node*:ti,ab,kw OR nodal:ti,ab,kw))	279597
#1	'large intestine tumor'/exp OR ((colorectal:ti,ab,kw OR colon*:ti,ab,kw OR rectum:ti,ab,kw OR rectal:ti,ab,kw OR anus:ti,ab,kw OR anal:ti,ab,kw OR recti:ti,ab,kw OR recto:ti,ab,kw OR pararect*:ti,ab,kw OR retrorect*:ti,ab,kw) AND (neoplas*:ti,ab,kw OR cancer*:ti,ab,kw OR tumor*:ti,ab,kw OR tumour*:ti,ab,kw OR malign*:ti,ab,kw OR mass:ti,ab,kw))	491479

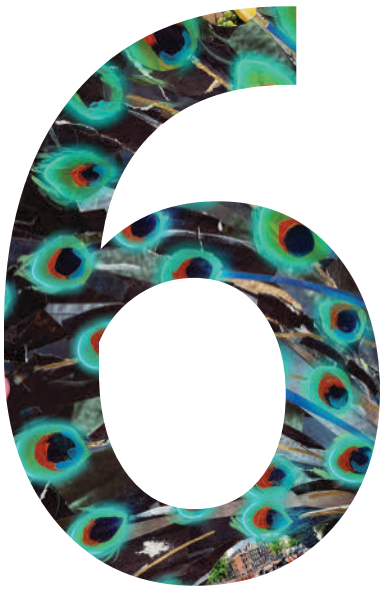
SUPPLEMENTARY INFORMATION 2

Table 1. Contingency table 3 x 2

	LNs with metastases after H&E staging	LN without metastases
SLNs with metastases after H&E staging	True positives (TP)	True positives (TP)
SLNs without metastases after H&E staging	False negatives (FN)	True negatives (TN)
SLNs with metastases after serial sectioning and immunohistochemistry	True positives (TP)	True positives (TP)/ upstaged patients

Table 2. Outcomes of tracer composition and injection technique to primary outcomes

Variable	Studies	Sensitivity 95%CI	Negative predictive value 95%CI	Detection rate 95%CI
Tracer				
Fluorescent tracer alone	4	0.69 (0.41-0.88)	0.85 (0.73-0.83)	0.98 (0.91-1.0)
ICG	5	0.57 (0.36-0.76)	0.81 (0.72-0.88)	0.94 (0.80-1.0)
IRDye800CW	3	0.69 (0.52-0.82)	0.86 (0.63-0.95)	0.98 (0.93-1.0)
Additional blue dye	4	0.60 (0.45-0.74)	0.78 (0.70-0.86)	0.93 (0.76-1.0)
Number of injections				
2-4 injections	5	0.57 (0.56-0.76)	0.81 (0.72-0.88)	0.94 (0.80-1.0)
Random	3	0.69 (0.52-0.82)	0.86 (0.63-0.95)	0.98 (0.93-1.0)
Injection technique				
In vivo	4	0.57 (0.30-0.81)	0.81 (0.71-0.89)	0.91 (0.74-1.0)
Ex vivo	4	0.67 (0.52-0.80)	0.82 (0.68-0.91)	0.99 (0.95-1.0)
Injection site				
Subserosal	5	0.64 (0.51-0.76)	0.80 (0.71-0.87)	0.95 (0.83-1.0)
Submucosal	3	0.66 (0.27-0.91)	0.86 (0.67-0.95)	0.96 (0.87-1.0)
SLN mapping				
Directly after injection	2	0.47 (0.22-0.73)	0.79 (0.62-0.90)	0.88 (0.41-1.0)
3-10 min after injection	4	0.67 (0.54-0.78)	0.84 (0.73-0.91)	0.96 (0.89-1.0)
More than 15 min after injection	2	0.66 (0.49-0.80)	0.83 (0.54-0.95)	0.99 (0.95-1.0)



PERIOPERATIVE PET/CT LYMPHOSCINTIGRAPHY AND FLUORESCENT REAL-TIME IMAGING FOR SENTINEL LYMPH NODE MAPPING IN EARLY STAGED COLON CANCER

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ABSTRACT

Purpose Using current optical imaging techniques and gamma imaging modalities, perioperative sentinel lymph node (SLN) identification in colon cancer can be difficult when the SLN is located near the primary tumour or beneath a thick layer of (fat) tissue. Sentinel lymph node mapping using PET/CT lymphoscintigraphy combined with real-time visualization of the SLN using near-infrared imaging, has shown promising results in several types of cancer and may facilitate the successful identification of the number and location of the SLN in early colon cancer.

Methods Clinical feasibility of PET/CT lymphoscintigraphy using preoperative endoscopically injected [^{89}Zr]Zr-Nanocoll and intraoperative injection of the near-infrared (NIR) tracer Indocyanine Green (ICG) was evaluated in ten early colon cancer patients. Three preoperative PET/CT scans and an additional ***ex vivo*** scan of the specimen were performed after submucosal injection of [^{89}Zr]Zr-Nanocoll. All SLNs and other lymph nodes underwent extensive pathological examination for metastases. A histopathological proven lymph node visible at preoperative PET/CT and identified at PET/CT of the specimen was defined as SLN.

Results A total of 27 SLNs were harvested in seven out of eight patients with successful injection of both tracers. In one patient no SLNs were assigned preoperatively. In two patients injection of [^{89}Zr]Zr-Nanocoll failed due to incorrect needle positioning. Twenty-one (78%) SLNs were found intraoperatively using NIR-imaging. Eleven of the 27 (41%) SLNs were located near the primary tumour (< 2 cm). Those six SLNs not found intraoperatively with NIR-imaging were all located close to the tumour. In all seven patients at least one SLN could be assigned at preoperative imaging 24 hrs after tracer administration. One SLN contained metastases detected by immunohistochemistry. No metastases were found in the non-SLNs.

Conclusions This study shows the potential of preoperative PET/CT lymphoscintigraphy to inform the surgeon about the number and location of SLNs in patients with early colon cancer. The additional use of NIR-imaging allows for intraoperative identification of these SLNs which are invisible with conventional white light imaging. Further research is necessary to improve and simplify the technique. We recommend perioperative SLN identification using a preoperative lymphoscintigraphy scan just before surgery approximately 24 hrs after injection. Additionally an postoperative scan of the specimen combined with intraoperative real-time NIR-imaging should be performed.

INTRODUCTION

Colorectal cancer (CRC) is the second most common malignancy in the Western World and the fourth leading cancer-related cause of death worldwide [1]. Lymph node involvement is still the strongest prognostic factor and serves as the most important selection criterion for adjuvant chemotherapy [2]. The introduction of CRC screening programs will increase the number of early staged CRC (T1-T2 disease) [3, 4]. The low risk of lymph node metastases in these early staged tumours makes local excision of the primary tumour an attractive treatment option [5]. However, uncertainty regarding undetected lymph node metastases makes current treatment of segmental resection with en-bloc resection of lymph nodes unavoidable in early CRC. Despite complete surgical resection, up to 20 to 30% of patients with early CRC show disease recurrence and eventually die within five years of initial treatment [6, 7]. This high recurrence rate in node negative patients is probably the result of understaging due to missed metastases in lymph nodes during routine histopathological examination [8-10]. Conversely, the majority of patients with true negative lymph nodes are exposed to unnecessary surgery-related morbidity and mortality at rates of up to 13.5 and 2.0%, respectively [11]. Sentinel lymph node (SLN) identification could offer a solution by detecting the lymph nodes with the most direct drainage from the primary tumour and therefore with the greatest chance of harbouring metastases.

In melanoma and breast cancer, SLN biopsies are routinely performed using a combination of preoperative colloid SPECT lymphoscintigraphy and perioperative guidance by gamma-probe and preoperatively injected blue dye. The combination of these techniques allows for preoperative identification of the number and location of the SLNs, and real-time identification of the SLNs versus surrounding fat [12-15]. SLN identification in colon cancer seems more challenging than in breast cancer and melanoma. Firstly, SLNs of the colon are more often smaller (< 1 cm),located beneath a thick layer of (fat) tissue and not visible with conventional white light imaging. Secondly, number and location of SLNs of colon carcinoma are less predictable. Additionally, it seems that more than one node can be assigned as SLN frequently and they appear to be often located near the tumour. An additional difficulty of SLN identification in colon cancer is the absence of the intraoperative sense of touch since laparoscopy is the preferred surgical approach for colon resections. These drawbacks underlines the need for a high quality optic tracer. The limited resolution of planar or SPECT scintigraphy may preclude proper preoperative SLN identification due to the shine-through effect from the tracer depot [16, 17]. Intraoperative identification is difficult because blue dye cannot be seen through fatty tissue and its relatively small particle size causes rapid passage through lymphatic channels, limiting its usefulness in detecting the earliest tumour draining lymph nodes [18, 19].

Near-infrared fluorescent tracers, and especially Indocyanine Green (ICG), exhibit more favourable characteristics for intraoperative detection of SLNs compared to blue dye (e.g. larger particle size and real-time, high/resolution optical guidance) [20]. PET scanners have a better spatial resolution than conventional gamma imaging modalities, and allow for dynamic 3D imaging. In oral cancer patients, [^{89}Zr]Zr-Nanocoll PET/CT provided detailed anatomical localization of tracer-foci and potentially improved identification of the SLNs even when they were located near the injection spot [21]. In colorectal cancer preoperative surgical planning using PET/CT combined with real-time NIR-staining of the SLNs using ICG might offer a solution for successful SLN biopsy.

The main purpose of this study is to establish if preoperative [^{89}Zr]Zr-Nanocoll PET/CT imaging is a useful technique to identify the number and location of SLNs in early colon cancer. Concordance and accuracy between number, location and histopathological outcomes of preoperative and postoperative assigned SLNs at PET/CT imaging and intraoperative identified SLNs using real-time fluorescent NIR-imaging are determined. Secondly, we aimed to investigate the pharmacokinetics of [^{89}Zr]Zr-Nanocoll to optimize the logistics of SLN biopsies involving radiolabelled nanocolloid.

MATERIALS AND METHODS

Patients

Patients were eligible if at least 18 years of age and scheduled for a laparoscopic resection of a histopathologically proven colon carcinoma or suspected malignant lesion seen during colonoscopy. Oral and written consent was mandatory for inclusion. Exclusion criteria were reduced physical condition (ASA IV), suspected or proven lymph node involvement or distant metastases seen on routine preoperative imaging (CT-scan), a tumour too large to pass endoscopically, claustrophobia and allergy for iodine. The Medical Ethics Committee of the Amsterdam UMC - Vrije Universiteit Amsterdam and the National Competent Authority approved the study. The study is registered in the Clinical Trial database (NCT02850783).

Study design

The study protocol (Figure 1) consisted of three preoperative PET/CT scans after injection of 0.4 mL median 2.12 (1.69-2.85) MBq [^{89}Zr]Zr-Nanocoll approximately 46 [43-48] hrs before surgery and intraoperative injection of ICG. Detailed information concerning the injection technique can be found in the supplementary material (Supplementary material 1). The PET/CT scan nr. 1 (Ingenuity; Philips Healthcare) consisted of 3-5 dynamic frames of 5 minutes each and started circa one hour after tracer injection. Two static PET/CT images were made one day (scan nr. 2) and two days (scan nr 3) after tracer administration (3-5 frames, 5 minutes each), respectively. Before surgery the results of the PET/CT images were compared with respect to the total number, localization and intensity of possible SLNs by a senior nuclear medicine physician (O.S.H). Results were discussed with the operating surgeon. A SLN was defined when focal tracer accumulation was evident in the mesocolon. At start of surgery after general anaesthesia, a second colonoscopy served to inject the fluorescent tracer Indocyanine Green (ICG) at the base of the tumour using one single injection. During surgery, a NIR laparoscopic device was used (Olympus; Tokyo, Japan). The SLNs were detected by means of fluorescence and the `hot spot` locations preoperatively assigned at PET/CT. Intraoperative fluorescent SLNs were marked with a suture. All patients underwent conventional oncological laparoscopic resection after SLN(s) marking with a suture. A fourth PET/CT scan (scan nr. 4) of the surgical specimen was made directly after surgery to confirm correct identification of preoperatively identified `hot spots` and to facilitate comparison of PET/CT imaging and pathological findings (Figure 2). After identification and additional suturing of radioactive lymph nodes found at PET/CT or with NIR- imaging, the specimen was transferred to the department of Pathology for examination of the specimen including all lymph nodes.

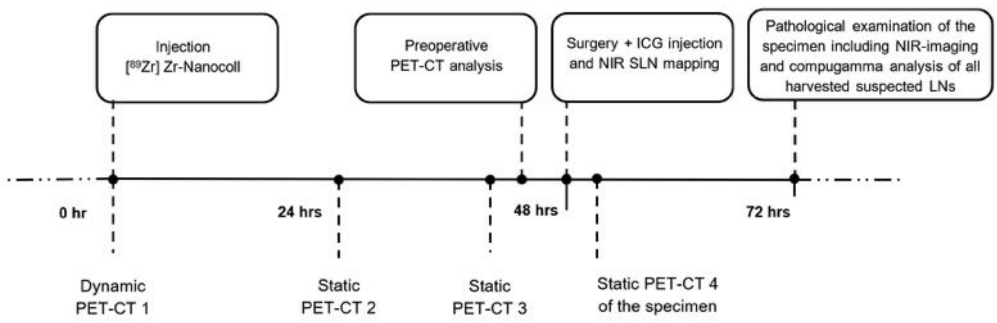

Figure 1. Time schedule of the study protocol

[^{89}Zr]Zr-Nanocoll

[^{89}Zr]Zr-Nanocoll is the PET corollary of ^{99m}Tc-Nanocoll used for SPECT scintigraphy. Heuveling et al. [25] showed that Nanocoll pharmacokinetics are independent of the radiolabel (either ^{89}Zr or ^{99m}Tc) and the 78 hrs physical half-life of ^{89}Zr-Nanocoll allows for flexibility in SLN observation time. [^{89}Zr]Zr-NCS-Bz-DFO-Nanocoll (hereafter called [^{89}Zr]Zr-Nanocoll) was produced according to the previously reported method in Heuveling et al. [25] under good manufacturing practice compliant conditions. [^{89}Zr]Zr-Nanocoll was filter sterilized which resulted in a sterile final product with less than 2.5 endotoxin units/mL. The radiochemical purity was > 99.9%.

Histopathology

Examination of the specimen by the pathologist followed after fixation of the specimen in formalin for at least 24 hrs. After fixation the pathologist harvested the perioperative suture-marked structures assigned as SLNs first and stored them separately. Thereafter the pathologist searched for additional lymph nodes by palpation and slicing of the whole specimen. Round, smooth and rigid structures similar to lymph nodes were harvested and stored separately too. Location of each potential SLN and all non-SLNs were compared with the locations assigned at PET/CT imaging. All lymph nodes were reassessed for fluorescence with the NIR-laparoscope and gamma well counter to reveal radioactivity.

All harvested SLNs and non-SLNs were bisected along the longest axis, paraffin embedded and stained with haematoxylin & eosin (H&E). Individual SLNs were embedded separately. If the lymph nodes were negative after routine H&E staining, all nodes were sectioned (3-4 µm thick) at 150 µm intervals and examined at three levels with H&E-staining and immunohistochemistry with the epithelial marker CEA (Clone 1117; DAKO Netherlands M7072), CAM 5.2 (3455799; BD Biosciences Netherlands) and CK19 (M0888, clone RCK 108; DAKO The Netherlands). Metastases between 0.2 mm and 2.0 mm were classified as micrometastases, and metastases smaller than 0.2 mm as isolated tumour cells according to the TNM 5 classification.

Image analysis

All PET/CT scans including the PET/CT of the specimen, were postoperatively reanalyzed and the results compared with respect to the total number and location of foci by a nuclear medicine physician (O.S.H) who was blinded to surgical findings and pathology results.

The location of all lymph nodes found by the pathologist were compared with pre, -and postoperatively assigned SLNs at PET/CT imaging using Vinci software (Vinci 2.36.0; Max-

Planck-Institut fur Neurologische Forschung, Cologne, Germany). Volumes of interest (VOI) were used to delineate the amount of radioactivity for pharmacokinetics [26].

Definition of sentinel lymph nodes

A histopathological proven lymph node visible at preoperative PET/CT and identified at PET/CT of the specimen was classified as SLN. Lymph nodes only stained by ICG or unstained nodes identified by the pathologist were defined as other lymph nodes.

Statistical analysis

Data were analysed using SPSS software (SPSS version 22.0; SPSS, Inc.; Chicago, IL). Point estimates and distribution were expressed as median and range, respectively. The Mann-Whitney U test was performed to determine the statistical significance of continuous variables. Results were considered as statistically significant at a *P* level of less than 0.05.

RESULTS

We included ten patients with early colon cancer, and patient characteristics are shown in Table 1. None of the patients experienced any adverse reaction following [^{89}Zr]Zr-Nanocoll administration.

Table 1. Basic patient characteristics

Patient	Gender	Age	BMI (kg/m^2)	ASA I-III	Tumour side	Tumour size (cm)	T-stage	N-stage
1	M	74	24.1	II	Sigmoid	0.5	1	N0
2	M	67	23.0	II	Sigmoid	2.8	1	N0
3	M	65	21.9	II	Flexura hepatica	4.8	3	N0
4	M	75	36.1	III	Flexura lienalis	1.0	3	N1
5	F	65	27.4	II	Cecum	3.4	2	N0
6	M	77	23.8	II	Sigmoid	3.0	2	N0
7	M	74	23.3	II	Colon ascendens	3.2	1	N0
8	M	76	26.6	II	Sigmoid	3.9	2	N0
9	M	69	27.8	I	Sigmoid	2.5	2	N0
10	F	63	22.6	II	Flexura lienalis	4.0	2	N0
Total		71.5 (63-77)	24.0 (21.9-36.1)			3.1 (0.5-4.8)		

In one patient (Table 2, patient 4, foci A) a SLN with isolated tumour cells was found. This SLN was detected with both imaging modalities and located > 2 cm from the primary tumour. No metastases were found in other SLNs or lymph nodes in the remaining patients. ICG injection was successful in all patients, but [^{89}Zr]Zr-Nanocoll administration failed in two cases (Tables 1; patients 3 and 4). In one patient tracer had been injected through the subserosal colonic layer in the abdominal cavity (patient 3). In another patient injection failed due to needle luxation outside the tumour during [^{89}Zr]Zr-Nanocoll administration (patient 4). Both resulted in low uptake of radioactivity in the primary tumour and high background radiation due to spill in the abdominal cavity or colonic lumen. Both patients were excluded from further analysis.

At preoperative PET/CT imaging we assigned 24 potential SLN foci (Table 2). One subdiaphragmatic and one preaortic potential lymph nodes (Table 2, patient 1) were not harvested because they were located too far from the resection margins, which would hamper the conventional resection. Another focus was perioperative found but did not contain lymphatic tissue Table 2, patient 1, focus A). In this patient no other SLNs were assigned preoperatively. After exclusion of these three foci, a final number of 21 foci in seven out of eight patients with successful injection of both tracers were identified. Some foci proved to contain more than one SLN, so that these 21 foci compromised 27 true SLNs. All 21 foci were preoperatively assigned by the nuclear medicine consultant just before surgery. All 27 SLNs revealed fluorescence of which 21 (78%) were detected intraoperatively using NIR-imaging. Eleven of the 27 (41%) SLNs were located near the primary tumour (< 2 cm). Those six SLNs not found intraoperatively with NIR-imaging were all located close to the tumour.

An additional 14 lymph nodes were identified at PET/CT imaging of the specimen. All revealed fluorescence of which 10 were identified by intraoperative NIR-imaging. Six out of these 14 lymph nodes were located near the primary tumour; three were only visible at PET/CT of the specimen and another three with both imaging modalities. Another 10 foci were only intraoperatively identified with NIR-imaging. Four of these fluorescent foci contained fat tissue (patient 1; foci B, C, D, E). The remaining foci revealed six lymph nodes which all were located far from the primary tumour.

All specimens were submitted for pathological examination. An additional 172 suspected lymph nodes were harvested from the specimen by the pathologist. Histopathological examination showed 102 true lymph nodes. Fat or blood vessels were found at 70 foci of which fifteen showed fluorescence probably as a result of dye leakage after disruption of lymphatic vessels during specimen extraction.

Of the 102 true lymph nodes, 41 nodes (40%) showed fluorescence with radioactivity counts of 14.3 10^{-3}[0.36-13.3^{-3}] MBq/node and 0.97 [0.028-9.1] %ID/node, which was significantly lower than the SLN radioactivity; 68.3 x 10^{-3}[4.7-215.9^{-3}] MBq/node and 4.7 [0.32-13.2] %ID/node, (p = 0.0001). Mean radioactivity of lymph nodes without uptake of ICG was 1.2 x 10^{-3} (0.33-8.3 x 10^{-3}) MBq and 0.08 (0.03-0.55) %ID/node, which was significantly less than activity in the SLNs and 41 fluorescent lymph nodes, (both p=0.0001). None of the lymph nodes additionally found in the specimen showed metastases.

Table 2. Results of sentinel lymph node identification with PET/CT and near-infrared imaging for each patient

Patient	Foci	LN status after pathology	Assigned as SLN by PET-CT imaging before surgery	Marked intraoperatively using NIR-ICG	Additional ex vivo positive for NIR-ICG	Marked postoperatively at PET/CT of the specimen	Identified at postoperative analysis of the 1st PET/CT	Identified at postoperative analysis of the 2nd PET/CT	Identified at postoperative analysis of the 3th PET/CT
1	A	no LN tissue	Yes	Yes		Yes	-	-	Yes
	B	no LN tissue	-	Yes		-	-	-	-
	C	no LN tissue	-	Yes		-	-	-	-
	D	no LN tissue	-	Yes		-	-	-	-
	E	no LN tissue	-	-	Yes	Yes	-	-	-
	F	LN	-	Yes		Yes	-	-	-
	LN diaphragm	Not harvested	Not harvested				-	Yes	Yes
	LN pre-aortal	Not harvested	Not harvested				-	Yes	Yes
	7 x not found						1x not found	4 x not found	2 x not found
2	A	2 x SLN	Yes	Yes		Yes	Yes	Yes	Yes
	B	SLN	Yes	Yes		Yes	Yes	Yes	Yes
	C	SLN	Yes	Yes		Yes	Yes	Yes	Yes
	D	SLN	Yes	Yes		Yes	Yes	Yes	Yes
	E	2 x SLN	Yes	Yes		Yes	-	Yes	Yes
	F	SLN	Yes	Yes		Yes	-	-	Yes
3	A	SLN	Yes	Yes		Yes	-	Yes	Yes
	B	SLN	Yes	Yes		Yes	-	Yes	Yes
	C	LN	-	Yes		-	-	-	-
	D	LN	-	Yes		-	-	-	-
	1 x not found								1 x not found
4	A	SLN ITC (IHC)	Yes	Yes		Yes	-	Yes	Yes
	B	SLN	Yes	Yes		Yes	-	-	Yes
	C	SLN	Yes	Yes		Yes	-	Yes	-
	D	LN	-	Yes		Yes	-	-	-
	E	LN	-	Yes		-	-	-	-
	F	LN	-	Yes		Yes	-	-	-
	G	LN	-	-	Yes	Yes	-	-	-
	5 x not found		-					1x not found	4x not found
5	A	2 x SLN	Yes	Yes		Yes	Yes	Yes	-
	B	SLN	Yes	-	Yes	Yes	-	-	Yes
	C	LN	-	Yes		Yes	-	-	-
	D	LN	-	-	Yes	Yes	-	-	-
	E	LN	-	Yes		Yes	-	-	-
	F	2 x LN	-	Yes		Yes	-	-	-
	3 x not found		-				1x not found		2 x not found

Table 2 (continued). Results of sentinel lymph node identification with PET/CT and near-infrared imaging for each patient

Patient	Foci	LN status after pathology	Assigned as SLN by PET-CT imaging before surgery	Marked intraoperatively using NIR-ICG	Additional ex vivo positive for NIR-ICG	Marked postoperatively at PET/CT of the specimen	Identified at postoperative analysis of the 1st PET/CT	Identified at postoperative analysis of the 2nd PET/CT	Identified at postoperative analysis of the 3th PET/CT
6	A B C D E F 5 x not found 1 x no LN tissue	SLN SLN LN LN LN LN	Yes Yes - - - - - -	Yes Yes Yes Yes Yes Yes - 1 x no LN tissue		Yes Yes Yes - - - -	Yes Yes - - - - 2 x not found -	Yes - - - - - 1 x not found -	Yes Yes - - - - 2 x not found -
7	A B C 1 x not found	2 x SLN SLN SLN	Yes Yes Yes	Yes Yes -	 Yes	Yes Yes Yes	- Yes - 1 x not found	Yes Yes Yes	Yes Yes Yes
8	A B C D E F G 1 x not found	SLN SLN 3 x SLN LN LN LN LN	Yes Yes Yes - - - - -	Yes - - Yes - Yes -	 Yes Yes Yes Yes	Yes Yes Yes Yes Yes Yes Yes	- Yes - - - - -	Yes Yes Yes - - - -	Yes Yes Yes - - - - 1 x not found
Total	71 foci 21 foci with 27 SLN 19 foci with 20 LN 23 not found 6 no LN tissue 2 not harvested			21 x SLN 16 LN	6 x SLN 4x LN	27 x SLN 14 x LN	11 x SLN	23 x SLN	24 x SLN

LN= Lymph Node; SLN = Sentinel Lymph Node; NIR-ICG = Near infrared-Indocyanine Green

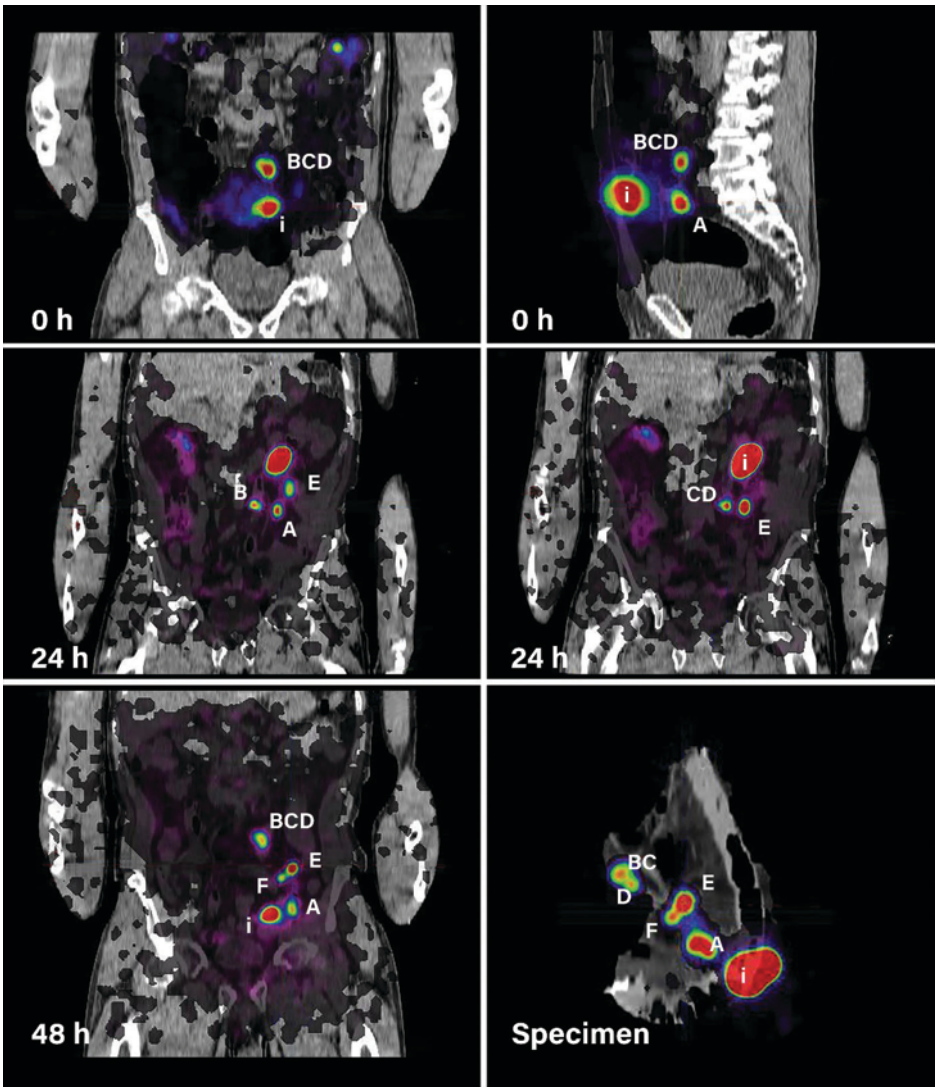

Figure 2. SLN identification at PET/CT images of patient 2.

Injection side is shown as `i` in all images. Foci A, B, C and D showed in total five sentinel lymph nodes and were already apparent at first scan (0 h after injection). Foci E represented two sentinel lymph nodes first seen at second PET/CT scan (24 h after injection) and F was first seen at the third scan after 48 hr. Lymph nodes E and F were probably hidden behind the other `hotter` nodes. All SLNs were intraoperatively identified using near-infrared imaging.

Pharmacokinetics and biodistribution

The pharmacokinetics of [^{89}Zr]Zr-Nanocoll is shown in Figure 3. Pharmacokinetics were calculated using the amount of radioactivity in seven out of the eight SLNs identified at all PET/CT scans. Exclusion of one SLN occurred since no reliable VOI be drawn due to its location near (< 2 cm) the tumour. PET/CT scans were made 0.8 (0.17-1.37) hr, 24.18 (16.07-26.62) hrs, 42.02 (40.08-42.33) hrs and 48.34 (46.2-5.25) hrs after injection, respectively. Not all SLNs were seen at all three preoperative PET/CT scans. Eleven SLNs were visible at the first PET/CT scan. At the second PET/CT scan 23 SLNs were seen. One SLN seen at the first PET/CT was not visible at the second scan and 13 were first identified at the second. Twenty-four SLNs were marked at the third PET/CT just before surgery. Three SLNs seen at the second PET/CT scan were not visible at the third scan. Another three SLNs were first identified at this time-point. (Figure 4).

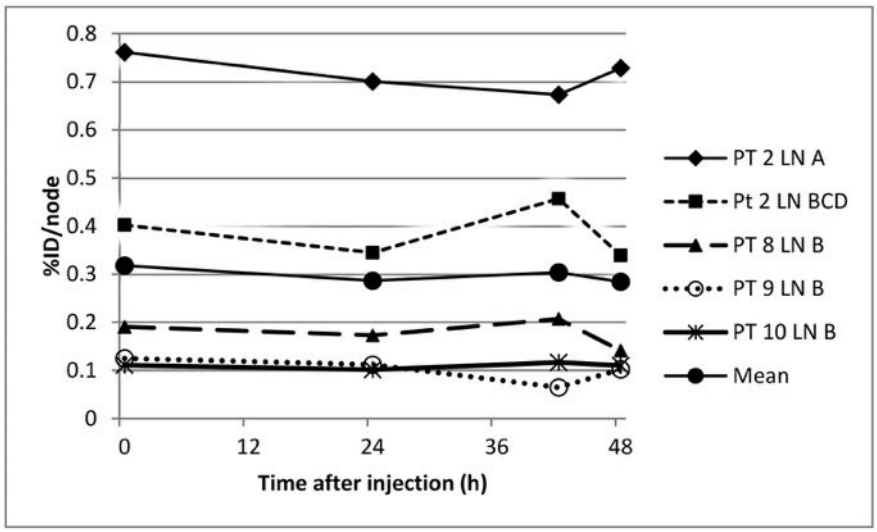

Figure 3. Lymph node kinetics of [^{89}Zr]Zr-Nanocoll

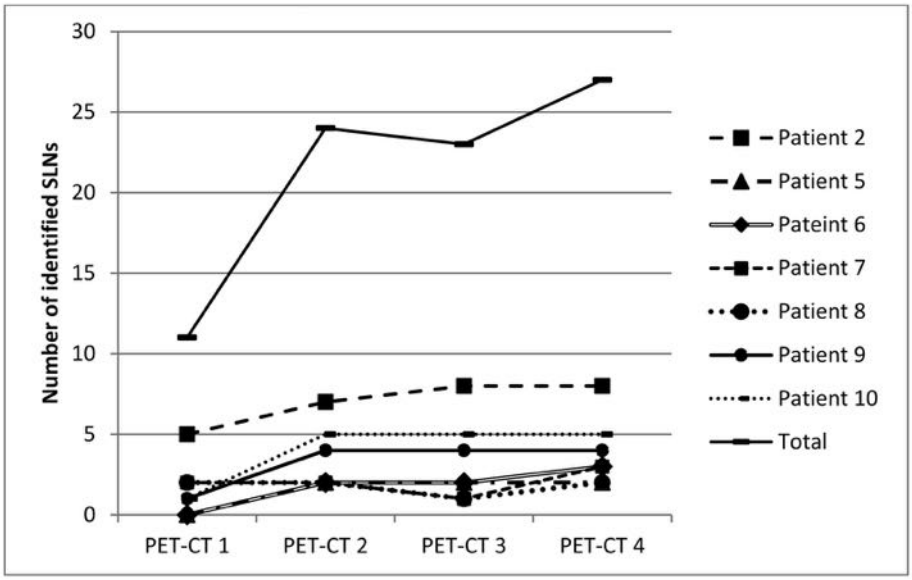

Figure 4. Sentinel lymph node identification by PET/CT imaging over time

DISCUSSION

In the present study we demonstrated the feasibility of PET/CT lymphoscintigraphy combined with optical real-time NIR-imaging using [^{89}Zr]Zr-Nanocoll and ICG to identify the SLNs in early stage colon cancer patients. Perioperative SLN identification succeeded in seven out of eight patients in which a median number of three SLNs were found. All SLNs revealed radioactivity and fluorescence, but six SLNs were not identified with NIR-imaging intraoperatively. These six lymph nodes were all located near (< 2 cm) the primary tumour and were probably covered due to the shine-through effects from the injection depot. When attempting to identify SLNs near the tumour, our results demonstrate that PET/CT imaging is a reliable technique, although PET/CT of the surgical specimen may improve the accuracy. Overall, these findings suggest that PET/CT lymphoscintigraphy combined with NIR-imaging could be a useful method for SLN identification in patients with early staged colon cancer. The preoperative PET/CT images could guide the surgeon to the number and location of SLNs which are currently unknown for colon cancer. Identification of these SLNs with NIR-imaging allows for intraoperative detection of these SLNs which is not possible with conventional white light imaging since SLNs are not visible with the naked eye.

To the best of our knowledge, this is the first study using [^{89}Zr]Zr-Nanocoll radiocolloid and PET/CT with NIR- imaging as SLN mapping technique in colon cancer. The majority of studies in the literature used SPECT/CT alone or in combination with an optical tracer, typically blue dye. The limited resolution of gamma-cameras combined with the restricted visibility of blue dye through skin and fatty tissue and its rapid distribution through lymphatic channels, makes it difficult to detect SLNs in colon cancer. In particular since lymphatic drainage patterns, location and number of SLNs are unknown and unpredictable. The here presented biodistribution data of [^{89}Zr]Zr-Nanocoll over time, combined with the extended pathological examination of the specimen provided essential anatomical information on lymphatic drainage patterns of the primary tumour towards SLNs.

The SLN procedure is based on the concept that tumour metastases occur in an orderly and sequential manner. The SLN(s) is/are the first lymph node(s) that receives lymphatic drainage directly from the primary tumour an therefore has the highest probability of harbouring metastases [27]. Based on this theory, we hypothesized that the lymph nodes with the highest radioactive counts (`hottest` nodes) are most likely the first draining nodes from the primary tumour and therefore can be considered as SLNs. These `hottest` nodes are by definition the nodes visible at PET/CT imaging. Therefore we classified a node as SLN when it was a histopathological proven lymph node visible at preoperative imaging and identified at PET/CT of the specimen. Similarly to breast cancer and melanoma more than one `hot' node were assigned as SLNs in all patients. This phenomenon could be attributed to passing of the tracer through the actual SLN into other nodes or due to divergent drainage patterns from the primary tumour [28-30]. Not all identified SLNs were visible at PET/CT imaging at all time points. Moreover, 14 lymph nodes were first seen at PET/CT of the specimen and therefore not classified as SLN. Both could be the result of physical bowel movements which changes the anatomical position of that part of the colon containing the injection depot and precludes SLN detection, as visualized in Figure 2. However, it can be argued that lymph nodes first seen at postoperative PET/CT should also be considered as SLNs, especially when these lymph nodes are located near the primary tumour. For accurate SLN identification we therefore recommend combined preoperative and postoperative PET/CT. The preoperative imaging could guide the surgeon towards the SLN intraoperatively whereas the postoperative imaging may help the pathologist to identify additional potential SLNs to perform additional serial slicing and immunohistochemistry. Based on our results we recommend one preoperative lymphoscintigraphy scan approximately 24 hrs after tracer injection since the majority of the SLNs were found at the second PET/CT scan (85%) and only three additional SLNs were found at the third PET/CT scan. When the only aim of SLN mapping in colon cancer is improvement of lymph node staging, the here presented

method would not hamper introduction of the technique. For SLN biopsy combined with local excision of the primary tumour, this lack of a highly sensitive intraoperative SLN identification technique is a serious drawback

To improve intraoperative SLN detection, intraoperative detection using a handheld PET-probe would be desirable. Unfortunately development of such PET-probes is expensive and quite challenging due to high-energy photons that need a large collimated and shielded detector [19, 31]. Following lymph flow drainage patterns in real-time using NIR-imaging, would also be an attractive option to facilitate intraoperative detection. However in the present study we could not identify fluorescent lymphatic drainage patterns. This is probably the result of the limited penetration depth of NIR-light hampering identification of fluorescent structures beneath a thick layer of fatty mesocolonic tissue.

Besides the limited tissue penetration of NIR-light we also noticed the differences in lymph node identification between NIR and PET/CT- imaging. Firstly, six SLNs located near the primary tumour were not identified using NIR-imaging. This failure is probably the results of tissue overlying the nodes and background fluorescence from the primary tumour. These results suggest that PET/CT is a more reliable imaging modality for SLN mapping in colon cancer. However we must emphasize that the number of included patients is small and NIR-imaging has shown to be a valuable tool to identify SLNs near the primary tumour in several other types of cancer. Another six intraoperatively identified lymph nodes revealed to be fluorescent only. Although these nodes were not located near the primary tumour nor contained metastases, it is uncertain whether these lymph nodes are true non-SLNs. In the currently used method ICG and [^{89}Zr]Zr-nanocoll were injected separately which could have caused different lymphatic drainage patterns of each tracer. Thereby, a considerably higher number of fluorescent-stained lymph nodes were found compared to SLNs identified at PET/CT imaging. This is the consequence of the small hydrodynamic diameter of ICG resulting in fast migration to higher echelon lymph nodes especially when the time-interval between injection and SLN identification expands.

To overcome these problems we recommend a single simultaneous injection of ICG combined with a radiocolloid. Several studies have shown promising results in multiple types of cancer using the hybrid tracer ICG-^{99m}Tc-Nanocolloid, which allows for preoperative SPECT/CT lymphoscintigraphy combined with intraoperative NIR-imaging and gamma-probe guided SLN detection [32, 33]. An advantage of ICG-^{99m}Tc-Nanocolloid is the high availability in several countries whereas [^{89}Zr]Zr-Nanocoll has not been FDA approved yet. The applicability of this technique in

colon cancer should be reinvestigated using knowledge derived from the here presented results on drainage patterns and tracer characteristics.

There were foci we classified as potential SLN at PET/CT imaging preoperatively which were not found in the specimen (see Table 2). Preoperative SLN identification was difficult in several cases due to background scattering. During reassessment of the PET/CT images we noticed that the majority of these foci were in retrospect suspected to be located into the lumen of the colon, so that some radioactive stool (due to leakage of [^{89}Zr]Zr-Nanocoll from the injection spot) had been considered as SLN.

For quantification of radioactivity uptake, we calculated the uptake of [^{89}Zr]Zr-Nanocoll as a percentage of the injection dose per node using the gamma well counter. Due to logistical restrictions we were not able to weigh nodes and therefore could not express uptake levels as injected dose per gram node (%ID/gram). However, the SLNs contained the highest amount of radioactivity compared to the other nodes, indicating that while additional analyses using %ID/gram might have provided more information concerning biodistribution of [^{89}Zr]Zr-Nanocoll, it would not have changed the results of assigned SLNs.

An important limitation of the present study was the 20% failure rate of [^{89}Zr]Zr-Nanocoll injection. Correct needle placement and careful administration of tracer with limited spillage of dye is crucial for a successful SLN procedure. However, submucosal injection is difficult and even more challenging preoperatively when the patient is awake and exposed to the discomfort of a colonoscopy. For accurate tracer injection and image analysis there appears to be a steep learning curve. We therefore advocate that tracer injection should only be performed by an experienced surgeon or gastroenterologist and image analysis by a senior nuclear medicine consultant.

Other disadvantages of the presented study were the limited number of patients, the extensive research protocol and high costs of PET/CT imaging devices. To better determine clinical implications a simplified protocol study including more patients should be performed.

CONCLUSION

Perioperative PET/CT lymphoscintigraphy using [^{89}Zr]Zr-Nanocoll provides useful anatomical localization information on SLNs in colon cancer, and is able to detect nodes near the primary

tumour. Use in combination with real-time staining of the SLN is crucial to intraoperatively identify the nodes, since lymph nodes are not visible with conventional white light imaging. The limited penetration depth, the low sensitivity in detecting SLNs near the tumour, and its fast biodistribution are serious drawbacks of ICG for the SLN mapping technique in colon cancer. As a consequence, pre- and postoperative PET/CT lymphoscintigraphy is essential to disclose SLNs to the surgeon and pathologist, respectively. Further research should focus on simplification of the here presented technique and evaluation of sensitivity rates before SLN mapping can be integrated in the daily treatment of patients with colon cancer. For tracer administration we recommend a single submucosal injection using a composed tracer consisting of a radiocolloid and optical dye. Perioperative SLN identification should consist of a preoperative lymphoscintigraphy scan just before surgery, approximately 24 hrs after tracer injection. We suggest it should be combined with a postoperative scan of the specimen and intraoperative NIR imaging to identify the preoperatively assigned SLNs.

REFERENCES

1. Torre LA, Siegel RL, Ward EM, Jemal A. Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev. 2016;25:16-27. doi:10.1158/1055-9965.EPI-15-0578.
2. Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004;350:2343-51. doi:10.1056/NEJMoa032709.
3. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17:1471-4. doi:10.1245/s10434-010-0985-4.
4. de Wijkerslooth TR, de Haan MC, Stoop EM, Deutekom M, Fockens P, Bossuyt PM, et al. Study protocol: population screening for colorectal cancer by colonoscopy or CT colonography: a randomized controlled trial. BMC Gastroenterol. 2010;10:47. doi:10.1186/1471-230X-10-47.
5. Cahill RA, Leroy J, Marescaux J. Localized resection for colon cancer. Surg Oncol. 2009;18:334-42. doi:10.1016/j.suronc.2008.08.004.
6. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev. 2008:CD005390. doi:10.1002/14651858.CD005390.pub2.
7. Weitz J, Koch M, Debus J, Hohler T, Galle PR, Buchler MW. Colorectal cancer. Lancet. 2005;365:153-65. doi:10.1016/S0140-6736(05)17706-X.
8. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ, van der Zaag ES, Tanis PJ, Bemelman WA, et al. The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. Eur J Surg Oncol. 2014;40:263-9. doi:10.1016/j.ejso.2013.12.002.
9. Sirop S, Kanaan M, Korant A, Wiese D, Eilender D, Nagpal S, et al. Detection and prognostic impact of micrometastasis in colorectal cancer. J Surg Oncol. 2011;103:534-7. doi:10.1002/jso.21793.
10. Weixler B, Warschkow R, Guller U, Zettl A, von Holzen U, Schmied BM, et al. Isolated tumor cells in stage I & II colon cancer patients are associated with significantly worse disease-free and overall survival. BMC Cancer. 2016;16:106. doi:10.1186/s12885-016-2130-7.
11. Liang J, Fazio V, Lavery I, Remzi F, Hull T, Strong S, et al. Primacy of surgery for colorectal cancer. Br J Surg. 2015;102:847-52. doi:10.1002/bjs.9805.
12. Covarelli P, Cristofani R, Boselli C, Servoli A, Burattini MF, Badolato M, et al. Preliminary study on radioguided sentinel node identification in colon cancer. Am Surg. 2007;73:222-6.
13. Lim SJ, Feig BW, Wang H, Hunt KK, Rodriguez-Bigas MA, Skibber JM, et al. Sentinel lymph node evaluation does not improve staging accuracy in colon cancer. Ann Surg Oncol. 2008;15:46-51. doi:10.1245/s10434-007-9629-8.
14. Quadros CA, Lopes A, Araujo I, Fregnani JH, Fahel F. Upstaging benefits and accuracy of sentinel lymph node mapping in colorectal adenocarcinoma nodal staging. J Surg Oncol. 2008;98:324-30. doi:10.1002/jso.21112.
15. Sandrucci S, Mussa B, Goss M, Mistrangelo M, Satolli MA, Sapino A, et al. Lymphoscintigraphic localization of sentinel node in early colorectal cancer: results of a monocentric study. J Surg Oncol. 2007;96:464-9. doi:10.1002/jso.20848.
16. de Haas RJ, Wicherts DA, Hobbelink MG, van Diest PJ, Vleggaar FP, Borel Rinkes IH, et al. Sentinel lymph node mapping in colon cancer using radiocolloid as a single tracer: a feasibility study. Nucl Med Commun. 2012;33:832-7. doi:10.1097/MNM.0b013e328353bc0c.

17. Tiffet O, Kaczmarek D, Chambonniere ML, Guillan T, Baccot S, Prevot N, et al. Combining radioisotopic and blue-dye technique does not improve the false-negative rate in sentinel lymph node mapping for colorectal cancer. Dis Colon Rectum. 2007;50:962-70. doi:10.1007/s10350-007-0236-3.

18. Viehl CT, Guller U, Langer I, Laffer U, Oertli D, Zuber M. Factors influencing the success of in vivo sentinel lymph node procedure in colon cancer patients: Swiss prospective, multicenter study sentinel lymph node procedure in colon cancer. World J Surg. 2013;37:873-7. doi:10.1007/s00268-013-1910-3.

19. Heller S, Zanzonico P. Nuclear probes and intraoperative gamma cameras. Semin Nucl Med. 2011;41:166-81. doi:10.1053/j.semnuclmed.2010.12.004.

20. Hojo T, Nagao T, Kikuyama M, Akashi S, Kinoshita T. Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer. Breast. 2010;19:210-3. doi:10.1016/j.breast.2010.01.014.

21. Heuveling DA, van Schie A, Vugts DJ, Hendrikse NH, Yaqub M, Hoekstra OS, et al. Pilot study on the feasibility of PET/CT lymphoscintigraphy with 89Zr-nanocolloidal albumin for sentinel node identification in oral cancer patients. J Nucl Med. 2013;54:585-9. doi:10.2967/jnumed.112.115188.

22. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin. 2012;62:10-29. doi:10.3322/caac.20138.

23. Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012;23:2479-516. doi:10.1093/annonc/mds236.

24. Kapteijn BA, Nieweg OE, Muller SH, Liem IH, Hoefnagel CA, Rutgers EJ, et al. Validation of gamma probe detection of the sentinel node in melanoma. J Nucl Med. 1997;38:362-6.

25. Heuveling DA, Visser GW, Baclayon M, Roos WH, Wuite GJ, Hoekstra OS, et al. 89Zr-nanocolloidal albumin-based PET/CT lymphoscintigraphy for sentinel node detection in head and neck cancer: preclinical results. J Nucl Med. 2011;52:1580-4. doi:10.2967/jnumed.111.089557.

26. Frings V, van Velden FH, Velasquez LM, Hayes W, van de Ven PM, Hoekstra OS, et al. Repeatability of metabolically active tumor volume measurements with FDG PET/CT in advanced gastrointestinal malignancies: a multicenter study. Radiology. 2014;273:539-48. doi:10.1148/radiol.14132807.

27. Morton DL, Wen DR, Wong JH, Economou JS, Cagle LA, Storm FK, et al. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg. 1992;127:392-9.

28. Camp ER, Cendan JC, Feezor R, Lind DS, Wilkinson E, Copeland EM. The hottest sentinel lymph node is not always the positive node. Am Surg. 2004;70:475-8; discussion 8.

29. Carlson GW, Murray DR, Thourani V, Hestley A, Cohen C. The definition of the sentinel lymph node in melanoma based on radioactive counts. Ann Surg Oncol. 2002;9:929-33.

30. Lyman GH, Giuliano AE, Somerfield MR, Benson AB, 3rd, Bodurka DC, Burstein HJ, et al. American Society of Clinical Oncology guideline recommendations for sentinel lymph node biopsy in early-stage breast cancer. J Clin Oncol. 2005;23:7703-20. doi:10.1200/JCO.2005.08.001.

31. Heuveling DA, Karagozoglu KH, Van Lingen A, Hoekstra OS, Van Dongen G, De Bree R. Feasibility of intraoperative detection of sentinel lymph nodes with 89-zirconium-labelled nanocolloidal albumin PET-CT and a handheld high-energy gamma probe. EJNMMI Res. 2018;8:15. doi:10.1186/s13550-018-0368-6.

32. Van Den Berg NS, Buckle T, Kleinjan GI, Klop WM, Horenblas S, Van Der Poel HG, et al. Hybrid tracers for sentinel node biopsy. Q J Nucl Med Mol Imaging. 2014;58:193-206.

33. KleinJan GH, van Werkhoven E, van den Berg NS, Karakullukcu MB, Zijlmans H, van der Hage JA, 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. doi:10.1007/s00259-018-4028-x.

34. Tan K.Y. Kawamura M, Sasaki, Tsujinaka, Maeda, Nobuki, Konishi. Distribution of the first metastatic lymph node in colon cancer and its clinical significance. Colorectal Disease. 2009;12:44-7. doi:10.1111/j.1463-1318.2009.01924.x.

35. McMasters KM, Reintgen DS, Ross MI, Wong SL, Gershenwald JE, Krag DN, et al. Sentinel lymph node biopsy for melanoma: how many radioactive nodes should be removed? Ann Surg Oncol. 2001;8:192-7.

36. Martin RC, 2nd, Edwards MJ, Wong SL, Tuttle TM, Carlson DJ, Brown CM, et al. Practical guidelines for optimal gamma probe detection of sentinel lymph nodes in breast cancer: results of a multi-institutional study. For the University of Louisville Breast Cancer Study Group. Surgery. 2000;128:139-44.

SUPPLEMENTARY MATERIAL 1

Detailed description of the injection technique

Colonic lavage occurred the day before the first colonoscopy with 2L Moviprep©.

During first colonoscopy the PET-tracer [^{89}Zr]Zr-Nanocoll was injected using a V960 injection needle with a luminal volume of 1.2 mL (Prince Medical, Gutenberg, France). After endoscopic tumour localization correct submucosal needle placement was confirmed by a raising bleb of NaCl 0.9% directly followed by injection of [^{89}Zr]Zr-Nanocoll and final flushing with 2.0 mL NaCl 0.9% to achieve maximal tracer administration. Afterwards the injection needle was measured for radioactivity to establish the exact amount of injected dose of [^{89}Zr]Zr-Nanocoll.

The second colonoscopy was performed directly after general anaesthesia and laparoscopic port placement. After localization of the tumour, a laparoscopic clamp was placed 5-10 cm proximal to the tumour to prevent distension of the proximal bowels. Injection of ICG/NaCl solution (2.5 mg/mL) was performed by the same senior gastroenterologist, again using a V960 injection needle. ICG/NaCl was administrated by a single shot at the base of the tumour similar to injection of [^{89}Zr]Zr-Nanocoll. After confirmation of correct needle placement in the colonic submucosa using NaCl 0.9%, 0.5 mL (0.2-2.0 ml) ICG/NaCl was administered.

6

PART II

MOLECULAR IMAGE-GUIDED SURGERY DURING LAPAROSCOPIC CHOLECYSTECTOMY

COMPARING NEAR-INFRARED IMAGING WITH INDOCYANINE GREEN TO CONVENTIONAL IMAGING DURING LAPAROSCOPIC CHOLECYSTECTOMY: A PROSPECTIVE CROSSOVER STUDY

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ABSTRACT

Background The aim of this study was to test and validate a novel non-invasive method for intraoperative visualization of extra-hepatic bile ducts during laparoscopic cholecystectomy. Injury to the common bile duct (CBD) is a rare but major complication of laproscopic cholecystectomy. Most injuries occur when anatomy is unclear due to presence of anatomatic variations, acute inflammation, or adhesions.

Patients and methods Thirty patients were included, and each received an intravenous injection of 0.05 mg/kg Indocyanine Green (ICG) (ICG-Pulsion®, PULSION Medical Systems AG, Munich, Germany) prior to the start of surgery. Laparoscopic cholecystectomy was performed according to standard procedures. The CBD and cystic duct (CD) were visualized before and during dissection of the liver hilus using a conventional laparoscopic camera and a recently developed Near Infrared camera (NIR) (Olympus, Tokyo, Japan).

Results Using ICG-NIR, the CBD and CD could be visualized 11 minutes ($p = 0.008$) and 8.6 minutes ($p = 0.001$) earlier than with a conventional camera. Both early (20/30 patients) and late (26/30 patients) identification of the CBD with ICG-NIR was significantly more frequent compared to conventional images (2/30 and 10/30 respectively, $p = <0.001$). One post-operative bilioma required re-admission and endoscopic retrograde cholangiopancreatography (ERCP) with stent placement.

Conclusions Identification of the CBD and CD using a low dose of ICG and the NIR camera was both faster and more frequent compared to conventional laparoscopic images during elective laparoscopic cholecystectomy.

INTRODUCTION

Laparoscopy has been the gold standard for elective cholecystectomies in patients with uncomplicated cholecystolithiasis since the 1990's. The introduction of laparoscopy for elective cholecystectomy has led to an increase in injuries to the extra-hepatic bile ducts[1]. The Critical View of Safety (CVS) was introduced as a safety measure to reduce injuries and has now become an integral part of standard laparoscopic cholecystectomy [2]. However, despite an initial decrease in bile duct injuries with the introduction of CVS, current literature suggests that bile ducts injuries are still in the 0.26-0.6% range [3-8]. Although this complication is rare, a patient with a common bile duct injury (CBD) injury is likely to have re-interventions, prolonged hospital admission, a decreased quality of life and is faced with a 5%-9% mortality risk [9-13]. The 2011 consensus meeting of the European Association for Endoscopic Surgery (EAES), Turin, Italy confirmed that prevention of bile duct injury remains a priority in the improvement of laparoscopic cholecystectomies. Although the CVS is a guideline for safe dissection and is used as a checkpoint before clipping and cutting of the key structures in the liver hilus, it neither clarifies anatomy prior to dissection nor guarantees that injuries will not occur after the completion of dissection.

Intraoperative cholangiogram (IOC) is currently the most commonly used method to provide real-time images of biliary structures during surgery when searching for CBD stones or bile duct injury is suspected during surgery. However, IOC requires x-ray equipment operated by trained personnel and the surgeon must cannulate the bile ducts in order to inject the contrast agent. In addition to patient exposure to radiation, IOC use often prolongs laparoscopic cholecystectomy, and additional costs have been reported [14;15]. The routine or selective use of IOC is still controversial in current literature [4;5;16-23] and it is not routinely used in most centers during elective laparoscopic cholecystectomy. A novel technique using a fluorescent dye and near-infrared (NIR) light can be used with laparoscopy and offers early visualization of structures containing this dye. In this study we used Indocyanine Green (ICG) (ICG-Pulsion®, PULSION Medical Systems AG, Munich, Germany) as fluorescent dye. ICG is a fluorescent contrast agent, which has been successfully used in the assessment of liver function, arterial (micro) circulation, tissue perfusion and ophthalmic imaging [24]. After intravenous injection, ICG becomes protein-bound and emits light with a wavelength of 830 nanometers when assessed with near-infrared light [25;26]. ICG is exclusively excreted into the bile, which would enable fluorescent imaging of the bile ducts during laparoscopic cholecystectomy using a Near-Infrared camera.

This study was designed to test and validate the novel non-invasive technique using ICG and NIR light (ICG-NIR) for early visualization of the extra hepatic bile ducts in order to improve anatomical

identification of the CBD, in addition to the CVS during laparoscopic cholecystectomy. The aim of this study was to investigate the additional value of the ICG-NIR technique during elective laparoscopic cholecystectomy. We hypothesized that the CBD will be visualized earlier during dissection due to the use of ICG-NIR compared with conventional imaging (CI). In contrast, no differences in the frequency of identification of the cystic duct (CD) using ICG-NIR or CI are expected because identification of the CD is an integral part of the CVS.

METHODS

Study design

This study was a prospective single-center, observational, cross-over study to evaluate the feasibility of ICG-NIR during laparoscopic cholecystectomy. The protocol for this study was approved by the institutional ethics committee of the VU University Medical Centre, Amsterdam, The Netherlands.

Patients

Patients were selected for participation from the outpatient clinic of the VU Medical Center, Amsterdam. Inclusion criteria were uncomplicated cholecystolithiasis, age between 18 and 80 years and completion of preoperative workup including abdominal ultrasound and laboratory tests. Exclusion criteria were complicated cholecystolithiasis, (biliary pancreatitis, cholestasis, hospital admission related to cholecystolithasis, acute cholecystitis, endoscopic retrograde cholangiopancreatography/ stent placement, or impaired liver function), ultrasound examination showing dilated intra- or extrahepatic bile ducts, cysts, abscesses or choledocholithiasis, extended comorbidity (ASA > III), iodine allergy, thyroid disease (hyper-/hypothyroidism), and use of listed medication interfering with hepatic ICG uptake (anti-convulsion medication, sodium bisulfite, haloperidol, heroine, pethidine, metamizole, methadone, morphine, nitrofurantoin, opium alkaloids, phenobarbital and phenylbutazon).

NIR laparoscopic camera

The NIR camera used for this study was developed by Olympus (Olympus, Tokyo, Japan). A filter was designed to fit a rigid 0° laparoscope (Olympus, Tokyo, Japan) (Figure 1). The laparoscopic camera image can be switched from laparoscopic CI to NIR imaging using a lever, and the excitation barrier filter only admits light with a wavelength above 800 nanometers. The NIR camera displays all fluorescent structures in green against a black (non-fluorescent) background. ICG has a peak emission of 830 nanometers when assessed with near-infrared light. Following

intravenous injection, ICG becomes bound to plasma proteins and is excreted exclusively in bile by the liver, without passing the enterohepatic circulation. ICG is pharmacologically inactive and is also not metabolized.

Figure 1. Near-infrared filter attached to rigid 0° conventional laparoscope

Procedures

After receiving oral and written information, patients were asked to participate and informed consent was obtained. Patients were scheduled and prepared for elective laparoscopic cholecystectomy according to standard preoperative procedures. In the operating room, patients received a single intravenous bolus injection of 0.05 mg/kg ICG diluted in water immediately after induction of standard general anesthesia. During the cholecystectomy both the prototype NIR camera (Olympus, Tokyo) and a conventional laparoscopic camera (EndoEye, Olympus, Tokyo) were used. Before commencing dissection of the liver hilus was begun, the NIR scope was used to screen for fluorescent bile ducts in and near the liver bed. The NIR scope was used at the start of dissection, early during dissection, late during dissection and at CVS to visualize bile structures.

Bile ducts, visualized by either NIR scope or CI, were identified by the operating surgeon and the investigator. The visualized structures were noted on the scoring form, together with time of observation. The laparoscopic cholecystectomy was performed conform with standard surgical procedures and the CVS. After CVS was obtained and once removal of the gallbladder was completed, the NIR scope was used to screen for bile leakage from either the liver bed or the extra-hepatic bile ducts. All NIR images were recorded on video for later review. Following surgery, patients recovered on the surgical ward and were discharged according to standard postoperative procedures.

Statistical analysis

Data were collected and then analyzed using SPSS (version 15.0, Chicago, IL, USA). A formal power analysis was performed in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA) using

the results of a pilot series of 7 patients showing an significant earlier visualization of the CBD with ICG-NIR compared with CI. A power of 90% required 27 patients, assuming that proportions of discordant pairs were 70% (image on ICG-NIR but not on conventional) and 20% (image on conventional camera but not on ICG-NIR). The total sample size was therefore fixed at 30.The performance of the conventional and ICG-NIR camera was compared using the McNemar test, times to visualization with CI and ICG-NIR were compared using the non-parametric Wilcoxon signed ranks test. Results were considered statistically significant when $p<0.05$. When applicable, standard deviation (±) or percentages (%) are given.

RESULTS

An overview of inclusions and exclusions is shown in Figure 2. Patients' characteristics are shown in Table 1. Average operating time was 71 minutes (± 20.2). No complications occurred due to intravenous injection of ICG. Three complications were noted during laparoscopic cholecystectomy. One patient suffered from bleeding due to a trocar incision after completion of the cholecystectomy. A liver hematoma was noted in another patient due to traction on the falciform ligament. Both complications were controlled laparoscopically with bipolar coagulation without further need for intervention. In a third patient, a laceration of the CD was noted early during dissection of the liver hilus. The laceration was double clipped and both NIR and CI showed no bile leakage after clipping. Postoperatively, the patient was re-admitted with a bilioma. An endoscopic retrograde cholagiopancreatography identified bile leakage from the location of the clips (Strasberg Classification type A bile duct injury) on the remaining CD. A stent was placed. Excluding the readmission, 14/30 (46.7%) patients were operated as outpatients, 12/30 (40%) patients were admitted for one night and 4/30 patients stayed longer than one night.

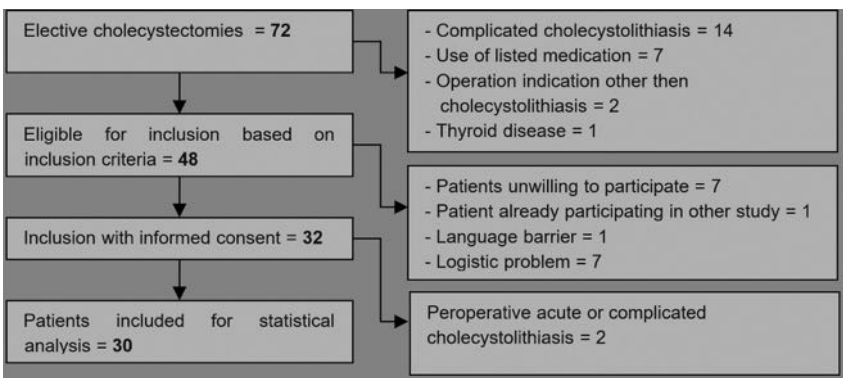

Figure 2. Inclusions and exclusions

Table 1. Patient characteristics BMI: body mass index. ASA: American Society of Anesthesiology

Patients characteristics n = 30	
Male : female ratio	9 : 21
Age in years	49.7 (± 16.7)
BMI	27.5 (± 4.32)
ASA classification I : II : III	13 : 11 : 6
Additional surgical procedures	1 Nissen fundoplication

A single intravenous bolus injection of ICG was administered immediately after induction of general anesthesia, and surgery began 10 minutes (± 8.0) after injection of ICG. Observations after injection of ICG with NIR and CI were performed at start of dissection), early during dissection , late during dissection and at CVS (Figures 3-5) . Results of ICG-NIR and conventional identification of the CBD and CD are shown in Table 2. The average time to positive identification of the CD with ICG-NIR of 37.9 (± 21.4) minutes after injection compared to 46.5 (± 23.2) minutes on CI (Wilcoxon signed ranks test, $p < 0.001$). During dissection of the liver hilus, one biliary and two arterial anatomical variations were encountered. The NIR camera was used to differentiate between biliary and non-biliary (arterial) structures, allowing the biliary nature of the known structures to be confirmed and from there allowing assessment of the non-biliary structures.

An additional cystic artery and extended right hepatic artery with short CD were identified, excluding the presence of an aberrant bile duct. No clear CVS could be obtained with CI in 4 patients due to adhesions and anatomical variations. Both the CD and the CBD were identified using the NIR camera, allowing safe dissection and transection of the CD and cystic artery.

Table 2. Results

		Observation time (minutes)			
Structure visualization modality		**First (26[2-50])**	**Second (38[5-88])**	**Third (43[22-98])**	**Fourth (52[20-125])**
CD					
	ICG	10/30	22/30	24/30	29/30
	Conventional	1/30	7/30	14/30	29/30
	P	0.004	0.000	0.013	1.000
CBD					
	ICG	20/30	24/30	24/30	26/30
	Conventional	2/30	4/30	7/30	10/30
	P	0.000	0.000	0.000	0.000

Observation time is given in minutes (mean[range]).
CBD, common bile duct; CD; ICG, indocyanine green.

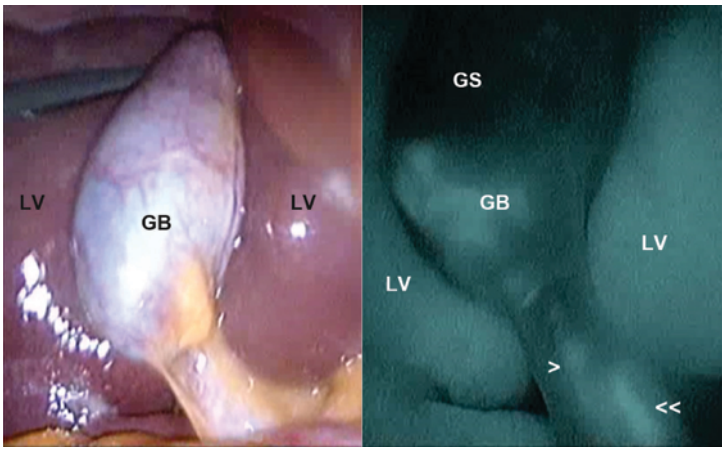

Figure 3. Conventional vs. ICG-NIR observation before start of dissection.
LV: liver; GB: gallbladder; > cystic duct; << common bile duct; GS: black contour of large gallstone

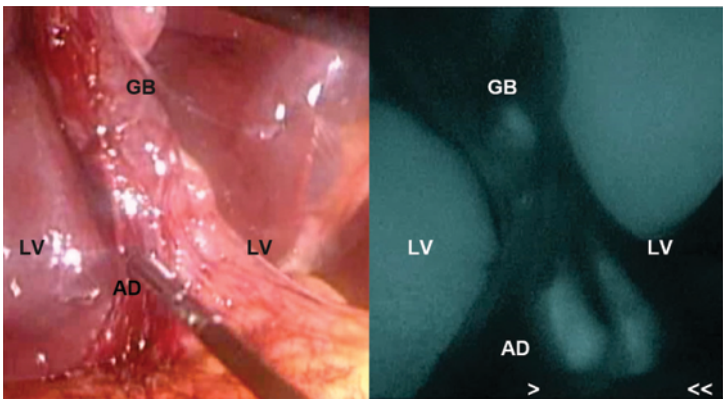

Figure 4. Conventional vs. ICG-NIR observation early during dissection.
LV: liver; GB: gallbladder; AD: adhesion; > cystic duct; << common bile duct

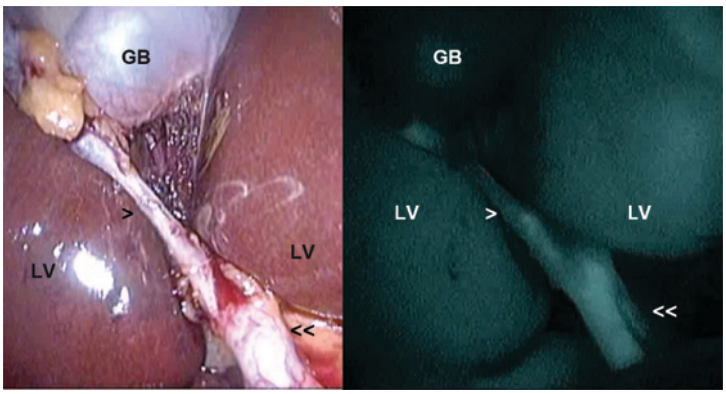

Figure 5. Conventional vs ICG-NIR observation after dissection.
LV: liver; GB: gallbladder; > cystic duct; << common bile duct

DISCUSSION

The results of this pilot study comparing ICG-NIR with CI during laparoscopic cholecystectomy indicate that the NIR camera allows a significantly earlier identification of both the CBD and the CD. In addition, the CBD was identified significantly more frequently with NIR imaging early during dissection and at CVS compared with CI The failure of the NIR-ICG technique to identify the CBD in the remaining patients (10/30) could have been caused by the relatively deep position of the CBD interacting with the limited penetration depth of ± 1.0 cm for ICG, preventing detection of the CBD in such cases. We stress that no additional attempts were made during surgery to identify the CBD when not visible with CI or NIR-ICG because surgical exploration of the hilus to identify the CBD is not standard during laparoscopic cholecystectomy and could increase the risk of bile duct injury. Although identification is not part of the dissection during standard laparoscopic cholecystectomy, we found the identification of the CBD useful for choosing the level of dissection in the hilus at safe distance of the identified CBD, and for limiting dissection of the CD when the CBD was visualized at close proximity.

Although the CD could be identified significantly earlier with the NIR camera, the differences gradually declined during dissection, and at CVS no differences between NIR and CI remained (table 2).This finding supports our hypothesis that no differences between NIR and CI could reasonably be expected at CVS, since positive identification of the CD is mandatory for CVS.

During this study, the use of ICG-NIR was strictly observational. If the CD or CBD wasidentified with NIR but not with CI, dissection was continued with aid of CI and performed following standard laparoscopic dissection.

Doses of ICG described in the literature range from a single bolus injection of 2.5 mg total to 0.5 mg/kg [27-32]. The various moments at which ICG injection has been reported include 30-60 minutes before arrival in the operating room or the start of surgery, to just before induction of general anesthesia, and even following endotracheal intubation [31-36].

The dose used in the present study was 0.05 mg/kg. The first and third patient received ICG 1-2 hours prior to the start of surgery, based on the reported peak in concentration of ICG in bile 120 minutes after intravenous injection [24]. The second patient received ICG in the operating room only minutes before the start of surgery due to logistical delays. During NIR inspection in this patient, ICG was noted in the gallbladder, in the CBD, and in the CD within 20 minutes after intravenous administration. The remaining 28 patients received ICG after the start of general anesthesia.

To guarantee early visibility and to optimize pre-operative logistics, the authors suggest that intravenous administration should be carried out prior to the induction of general anesthesia.

Verbeek et al. [37]. studied the timing of administration and optimal dose of ICG during open hepatopancreatobiliary surgery and validated their results during elective conventional laparoscopic cholecystectomy in uncomplicated cholecystolithiasis. A prolonged interval (24 hours) between ICG administration and NIR cholangiography yielded the best results, with lower liver background fluorescence and optimal NIR-cholangiography [37]. . Although surgeons at our institution have not reported poor visibility due to high liver to background ratios, the most favorable interval and optimal timing of ICG application to best elucidate biliary structures remains unknown. However, it is clear that the prolonged interval between administration of ICG and NIR-cholangiography proposed by Verbeek and colleagues [37] would not be applicable in the clinical setting of acute cholecystectomy in complicated cholecystolithiasis.

In this study we have encountered one bile duct injury in 30 patients. Although ICG-NIR identified leakage of bile, the bile duct injury was not prevented. Further randomized studies will be required to demonstrate the possible benefit of ICG-NIR to decrease the occurrence of bile duct injuries during laparoscopic cholecystectomy.

Although others have reported good visibility of the CD (90-100%) and CBD (50-100%) using ICG, published data mainly include case reports or small patient series that lack substantial power [30-36;38]. For example, Schols et al. [30] performed a feasibility study in a series of 15 laparoscopic cholecystectomies in which use of NIR-ICG facilitated significantly earlier identification and a clear delineation of both CBD and CD - in agreement with the results of our study. Using ICG, Ishizawa et al. [32] reported identification of CD and CBD in a series of 52 patients undergoing laparoscopic cholecystectomy. Anatomic variants were seen in 8/52 patients during preoperative cholangiography and confirmed with NIR-ICG during the surgical procedure[32]. Both of these studies reported results from heterogeneous patient populations including patients with cholecystolithiasis, gallbladder polyps and (acute) cholecystitis [30;32].

In contrast, we validated ICG-NIR in a selective patient population consisting only of patients with uncomplicated cholecystolithiasis. The results presented in this study show early visibility of the extra hepatic bile ducts with ICG-NIR compared to CI in patients with uncomplicated symptomatic cholecystolithiasis. However, even in the setting of a cholecystectomy indicated by an uncomplicated case of cholecystolithiasis, ICG-NIR could be of great value to the surgeon, for example, when dissection turns out to be unexpectedly difficult due to anatomic

variation of the bile ducts. Secondly, we hypothesized that particularly for cases of complicated cholecystolithiasis such as cholecystitis, biliary pancreatitis, surgery after endoscopic retrograde cholangiopancreatography, or percutaneous gallbladder drainage with unclear anatomy, the ICG-NIR technique could be of tremendous value for safe dissection towards CVS. Recent years have seen the introduction of new, minimally invasive techniques including Single Incision Laparoscopic Surgery, Natural Orifice Transluminal Endoscopic Surgery (NOTES; American Society for Gastrointestinal Endoscopy [Oak Brook, IL] and Society of American Gastrointestinal and Endoscopic Surgeons [Los Angeles, CA]), and robot-assisted laparoscopic surgery.

The rate of bile duct injuries in single/incision laparoscopic surgery cholecystectomy is higher than laparoscopic cholecystectomy; 0,7% versus 0.26-0.6% respectively [8;39] This might be attributable to the different angle in which the surgical field is approximated or due to different positioning and introduction of a decreased number of trocars than in conventional laparoscopic cholecystectomy. Differences in visualization might impede or delay recognition of the extra-hepatic bile ducts and increase the risk of iatrogenic bile duct injury. Several studies suggest that ICG-NIR imaging could represent an important aid to surgeons performing cholecystectomies with single-incision laparoscopic surgery, NOTES or the robot [31;40-42]. Fluorescent cholangiography will allow better visualization of the bile ducts while it reduces the learning curve in implementing these new minimally invasive surgical techniques.
These new developments illustrate the potential value of image-guided surgery using novel and known techniques such as fluorescence with ICG or other fluorescent agents. In the future, imaging will become increasingly more important during surgical procedures and is expected to become an integral part of minimally-invasive surgery.

CONCLUSION

Use of ICG-NIR during elective laparoscopic cholecystectomy for uncomplicated cholecystolithiasis results in early and more frequent identification of the CD and CBD during surgery, without the need for bile duct cannulation required with IOC. Using ICG-NIR, unclear or abnormal biliary anatomy can be identified in uncomplicated cholecystolithiasis, and conversion can be prevented in case of unclear CVS. For patients with complicated cholecystolithiasis, we expect ICG-NIR to provide additional value for identification of the CD and CBD during laparoscopic cholecystectomy. We therefore recommend that further research be carried out to clarify whether ICG-NIR would allow elucidation of biliary anatomy in patients with a high risk of bile duct injury.

REFERENCES

1. Russell JC, Walsh SJ, Mattie AS, Lynch JT. Bile duct injuries, 1989-1993. A statewide experience. Connecticut Laparoscopic Cholecystectomy Registry. Arch Surg 1996 Apr;131(4):382-8.
2. Strasberg SM, Hertl M, Soper NJ. An analysis of the problem of biliary injury during laparoscopic cholecystectomy. J Am Coll Surg 1995 Jan;180(1):101-25.
3. Dolan JP, Diggs BS, Sheppard BC, Hunter JG. Ten-year trend in the national volume of bile duct injuries requiring operative repair. Surg Endosc 2005 Jul;19(7):967-73.
4. Giger U, Ouaissi M, Schmitz SF, Krahenbuhl S, Krahenbuhl L. Bile duct injury and use of cholangiography during laparoscopic cholecystectomy. Br J Surg 2011 Mar;98(3):391-6.
5. Khan OA, Balaji S, Branagan G, Bennett DH, Davies N. Randomized clinical trial of routine on-table cholangiography during laparoscopic cholecystectomy. Br J Surg 2011 Mar;98(3):362-7.
6. Nuzzo G, Giuliante F, Giovannini I, Ardito F, D'Acapito F, Vellone M, et al. Bile duct injury during laparoscopic cholecystectomy: results of an Italian national survey on 56 591 cholecystectomies. Arch Surg 2005 Oct;140(10):986-92.
7. Tantia O, Jain M, Khanna S, Sen B. Iatrogenic biliary injury: 13,305 cholecystectomies experienced by a single surgical team over more than 13 years. Surg Endosc 2008 Apr;22(4):1077-86.
8. Thurley PD, Dhingsa R. Laparoscopic cholecystectomy: postoperative imaging. AJR Am J Roentgenol 2008 Sep;191(3):794-801.
9. Moore DE, Feurer ID, Holzman MD, Wudel LJ, Strickland C, Gorden DL, et al. Long-term detrimental effect of bile duct injury on health-related quality of life. Arch Surg 2004 May;139(5):476-81.
10. Machado NO. Biliary complications postlaparoscopic cholecystectomy: mechanism, preventive measures, and approach to management: a review. Diagn Ther Endosc 2011;2011:967017.
11. Gigot J, Etienne J, Aerts R, Wibin E, Dallemagne B, Deweer F, et al. The dramatic reality of biliary tract injury during laparoscopic cholecystectomy. An anonymous multicenter Belgian survey of 65 patients. Surg Endosc 1997 Dec;11(12):1171-8.
12. Boerma D, Rauws EA, Keulemans YC, Bergman JJ, Obertop H, Huibregtse K, et al. Impaired quality of life 5 years after bile duct injury during laparoscopic cholecystectomy: a prospective analysis. Ann Surg 2001 Dec;234(6):750-7.
13. Melton GB, Lillemoe KD, Cameron JL, Sauter PA, Coleman J, Yeo CJ. Major bile duct injuries associated with laparoscopic cholecystectomy: effect of surgical repair on quality of life. Ann Surg 2002 Jun;235(6):888-95.
14. Livingston EH, Miller JA, Coan B, Rege RV. Costs and utilization of intraoperative cholangiography. J Gastrointest Surg 2007 Sep;11(9):1162-7.
15. El SG, Seow C, Sharp C, Mughrabi A, Nassar AH. Intraoperative cholangiography time in laparoscopic cholecystectomy: timing the radiographer. Surg Endosc 2005 Oct;19(10):1370-2.
16. Tabone LE, Sarker S, Fisichella PM, Conlon M, Fernando E, Yi S, et al. To 'gram or not'? Indications for intraoperative cholangiogram. Surgery 2011 Oct;150(4):810-9.
17. Podnos YD, Gelfand DV, Dulkanchainun TS, Wilson SE, Cao S, Ji P, et al. Is intraoperative cholangiography during laparoscopic cholecystectomy cost effective? Am J Surg 2001 Dec;182(6):663-9.
18. Nickkholgh A, Soltaniyekta S, Kalbasi H. Routine versus selective intraoperative cholangiography during laparoscopic cholecystectomy: a survey of 2,130 patients undergoing laparoscopic cholecystectomy. Surg Endosc 2006 Jun;20(6):868-74.

19. Hamad MA, Nada AA, Abdel-Atty MY, Kawashti AS. Major biliary complications in 2,714 cases of laparoscopic cholecystectomy without intraoperative cholangiography: a multicenter retrospective study. Surg Endosc 2011 Dec;25(12):3747-51.

20. Flum DR, Dellinger EP, Cheadle A, Chan L, Koepsell T. Intraoperative cholangiography and risk of common bile duct injury during cholecystectomy. JAMA 2003 Apr 2;289(13):1639-44.

21. Flum DR, Flowers C, Veenstra DL. A cost-effectiveness analysis of intraoperative cholangiography in the prevention of bile duct injury during laparoscopic cholecystectomy. J Am Coll Surg 2003 Mar;196(3):385-93.

22. Caratozzolo E, Massani M, Recordare A, Bonariol L, Antoniutti M, Jelmoni A, et al. Usefulness of both operative cholangiography and conversion to decrease major bile duct injuries during laparoscopic cholecystectomy. J Hepatobiliary Pancreat Surg 2004;11(3):171-5.

23. Buddingh KT, Weersma RK, Savenije RA, van Dam GM, Nieuwenhuijs VB. Lower rate of major bile duct injury and increased intraoperative management of common bile duct stones after implementation of routine intraoperative cholangiography. J Am Coll Surg 2011 Aug;213(2):267-74.

24. CHERRICK GR, STEIN SW, LEEVY CM. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 1960 Apr;39:592-600.

25. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Light-absorbing properties, stability, and spectral stabilization of indocyanine green. J Appl Physiol 1976 Apr;40(4):575-83.

26. Mordon S, Devoisselle JM, Soulie-Begu S, Desmettre T. Indocyanine green: physicochemical factors affecting its fluorescence in vivo. Microvasc Res 1998 Mar;55(2):146-52.

27. Kawaguchi Y, Ishizawa T, Masuda K, Sato S, Kaneko J, Aoki T, et al. Hepatobiliary surgery guided by a novel fluorescent imaging technique for visualizing hepatic arteries, bile ducts, and liver cancers on color images. J Am Coll Surg 2011 Jun;212(6):e33-e39.

28. Kaibori M, Ishizaki M, Matsui K, Kwon AH. Intraoperative indocyanine green fluorescent imaging for prevention of bile leakage after hepatic resection. Surgery 2011 Jul;150(1):91-8.

29. Harada N, Ishizawa T, Muraoka A, Ijichi M, Kusaka K, Shibasaki M, et al. Fluorescence navigation hepatectomy by visualization of localized cholestasis from bile duct tumor infiltration. J Am Coll Surg 2010 Jun;210(6):e2-e6.

30. Schols RM, Bouvy ND, Masclee AA, van Dam RM, Dejong CH, Stassen LP. Fluorescence cholangiography during laparoscopic cholecystectomy: a feasibility study on early biliary tract delineation. Surg Endosc 2013 May;27(5):1530-6.

31. Ishizawa T, Kaneko J, Inoue Y, Takemura N, Seyama Y, Aoki T, et al. Application of fluorescent cholangiography to single-incision laparoscopic cholecystectomy. Surg Endosc 2011 Aug;25(8):2631-6.

32. Ishizawa T, Bandai Y, Ijichi M, Kaneko J, Hasegawa K, Kokudo N. Fluorescent cholangiography illuminating the biliary tree during laparoscopic cholecystectomy. Br J Surg 2010 Sep;97(9):1369-77.

33. Ishizawa T, Tamura S, Masuda K, Aoki T, Hasegawa K, Imamura H, et al. Intraoperative fluorescent cholangiography using indocyanine green: a biliary road map for safe surgery. J Am Coll Surg 2009 Jan;208(1):e1-e4.

34. Mitsuhashi N, Kimura F, Shimizu H, Imamaki M, Yoshidome H, Ohtsuka M, et al. Usefulness of intraoperative fluorescence imaging to evaluate local anatomy in hepatobiliary surgery. J Hepatobiliary Pancreat Surg 2008;15(5):508-14.

35. Tagaya N, Shimoda M, Kato M, Nakagawa A, Abe A, Iwasaki Y, et al. Intraoperative exploration of biliary anatomy using fluorescence imaging of indocyanine green in experimental and clinical cholecystectomies. J Hepatobiliary Pancreat Sci 2010 Sep;17(5):595-600.

36. Buchs NC, Hagen ME, Pugin F, Volonte F, Bucher P, Schiffer E, et al. Intra-operative fluorescent cholangiography using indocyanin green during robotic single site cholecystectomy. Int J Med Robot 2012 Dec;8(4):436-40.

37. Verbeek FP, Schaafsma BE, Tummers QR, van der Vorst JR, van der Made WJ, Baeten CI, et al. Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. Surg Endosc 2013 Nov 14.

38. Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer 2009 Jun 1;115(11):2491-504.

39. Joseph M, Phillips MR, Farrell TM, Rupp CC. Single incision laparoscopic cholecystectomy is associated with a higher bile duct injury rate: a review and a word of caution. Ann Surg 2012 Jul;256(1):1-6.

40. Buchs NC, Pugin F, Azagury DE, Jung M, Volonte F, Hagen ME, et al. Real-time near-infrared fluorescent cholangiography could shorten operative time during robotic single-site cholecystectomy. Surg Endosc 2013 Oct;27(10):3897-901.

41. Spinoglio G, Priora F, Bianchi PP, Lucido FS, Licciardello A, Maglione V, et al. Real-time near-infrared (NIR) fluorescent cholangiography in single-site robotic cholecystectomy (SSRC): a single-institutional prospective study. Surg Endosc 2013 Jun;27(6):2156-62.

42. Daskalaki D, Fernandes E, Wang X, Bianco FM, Elli EF, Ayloo S, et al. Indocyanine Green (ICG) Fluorescent Cholangiography During Robotic Cholecystectomy: Results of 184 Consecutive Cases in a Single Institution. Surg Innov 2014 Mar 9.

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FLUORESCENT IMAGING WITH INDOCYANINE GREEN DURING LAPAROSCOPIC CHOLECYSTECTOMY IN PATIENTS AT INCREASED RISK OF BILE DUCT INJURY

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ABSTRACT

Background Although rare, injury to the common bile duct (CBD) during laparoscopic cholecystectomy (LC) can be reduced by better intraoperative visualization of the cystic duct (CD) and CBD. The aim of this study was to establish the efficacy of early visualization of the CD and the added value of CBD identification, using near-infrared (NIR) light and the fluorescent agent indocyanine green (ICG), in patients at increased risk of bile duct injury.

Materials and Methods Patients diagnosed with complicated cholecystitis and scheduled for LC were included. The CBD and CD were visualized with NIR light before and during dissection of the liver hilus and at critical view of safety (CVS).

Results Of the 20 patients originally included, 2 were later excluded due to conversion. In 6 of 18 patients, the CD was visualized early during dissection and prior to imaging with conventional white light. The CBD was additionally visualized with ICG-NIR in 7 of 18 patients. In 1 patient, conversion was prevented due to detection of the CD and CBD with ICG-NIR.

Conclusions Early visualization of the CD or additional identification of the CBD using ICG-NIR in patients with complicated cholecystolithiasis can be helpful in preventing CBD injury. Future studies should attempt to establish the optimal dosage and time frame for ICG administration and bile duct visualization with respect to different gallbladder pathologies.

INTRODUCTION

Laparoscopic cholecystectomy (LC) has become the standard surgical treatment for patients with symptomatic cholecystolithiasis [1-4] and today is one of the most frequently performed procedures in general surgery [5,6]. Although laparoscopy improves postoperative outcomes compared with open surgery, bile duct injury is a rare but serious and persistent complication. The classic injury occurs when the common bile duct (CBD) is mistaken for the cystic duct (CD), resulting in (partial) CBD resection [7]. Bile duct injury is associated with high morbidity rates, prolonged hospital stay, challenging and extensive surgery, and a significant negative impact on quality of life even 10 years after the event [8]. Therefore, it is now strongly advised that the critical view of safety (CVS) must be met prior to clipping of any tubular structure [9]. However, and despite the widespread implementation of CVS, recent literature suggests that bile duct injury still occurs in 0.26% to 0.7% of procedures [10-15]. In addition, conversion to an open procedure is indicated in cases where CVS cannot be reached, with reported conversion-to-open rates in LC presently around 2.6% to 10% [16-18]. Conversion is often due to unclear (biliary) anatomy associated with a wider range of conditions including acute cholecystitis, acute biliary pancreatitis, bleeding in Calot's triangle, fibrotic shrunken gallbladders due to previous infection, gallstones in Hartmann's pouch, a short cystic duct, Mirizzi's syndrome, or abnormal biliary anatomy [17-20].

Although the intraoperative cholangiogram (IOC) was introduced in open cholecystectomy for diagnostic and therapeutic reasons, use of the procedure in LC also helps prevent bile duct injury by allowing real-time imaging of the bile ducts. However, the routine use of IOC in LC is controversial due to a number of drawbacks including significantly increased operating time, added costs, requirement for cannulation of the bile duct with a concomitant increased risk of bile duct injury, radiation exposure, misinterpretation and the need for additional equipment and trained personnel [13,21-23]. IOC is therefore not part of the standard operative procedure in every country. More recently, extensive investigation of fluorescent image-guided surgery has shown that the technique can improve the identification of certain anatomic structures. A number of studies have demonstrated that real-time fluorescent imaging, using indocyanine green (ICG) (ICG-Pulsion, Pulsion medical Systems AG, Munich, Germany) in combination with a near-infrared (NIR) camera, is a viable approach to real-time fluorescent identification of the bile ducts in uncomplicated cholecystolithiasis [24-38], and without the disadvantages of IOC. Although real-time fluorescent imaging has clear potential in the enhanced visualization of the CBD in patients at risk for bile duct injury, the majority of the studies published to date actually excluded patients with acute cholecystitis, biliary pancreatitis, cholangitis, increased risk of

common bile duct stones, and conversion to an open procedure. These patients in particular would potentially derive the greatest benefit from intraoperative identification of the CD and CBD, and improved imaging would help in the move toward safe LC.

This study therefore sought to evaluate whether ICGNIR facilitates earlier identification of the CD compared with conventional white light imaging in patients with complicated cholecystolithiasis. Furthermore, we also attempted to determine if ICG-NIR contributes to the additional identification of the CBD compared with conventional imaging in these cases.

MATERIALS AND METHODS

Patients

All consecutive patients attending the outpatient clinic or the emergency room of the VU Medical Centre Amsterdam who met the inclusion criteria were asked to participate.

Inclusion criteria were the following: age 18 years and older, scheduled for LC after complicated gallstone disease as indicated by an acute or chronic cholecystitis, biliary pancreatitis, percutaneous gallbladder drainage, choledocholithiasis without signs of CBD stones at surgery or after endoscopic retrograde cholangiopancreaticography (ERCP).

Exclusion criteria were the following: isolated symptomatic cholelithiasis, iodine allergy, hyperthyroidism, hypothyroidism and use of listed medication interfering with hepatic ICG uptake as mentioned in the "Summary of Product Characteristics" (SPC) of ICG-Pulsion (anticonvulsive medication, cyclopropane, bisulphate connections, sodium bisulfite haloperidol, diamorphine, pethidine, morphine, nitrofurantoin, opium alkaloids, phenobarbital, phenylbutazon, probenicid, metamizole, rifamycin, methadone, or heroin). The study protocol was carried out in accordance with the Declaration of Helsinki, approved by the institutional ethics committee of the VU University Medical Centre Amsterdam and registered in the Dutch Trial Registration register (NTR 4680).

Indocyanine Green

Indocyanine green is a fluorescent contrast agent with a peak emission of 830 nm when assessed with NIR light. Following intravenous injection, ICG becomes proteinbound and is excreted exclusively by the liver into the bile without entering the enterohepatic circulation [39-41]. ICG is pharmacologically inactive, is not metabolized, and has been successfully and safely used in the assessment of liver function, arterial microcirculation, tissue perfusion, ophthalmic imaging, and imaging of the bile ducts during uncomplicated cholecystolithiasis.

Near-Infrared Laparoscopic Camera

The NIR camera used for this study was developed by Olympus (Olympus, Tokyo, Japan). A filter was designed to fit a rigid 0° laparoscope with a 10 mm diameter (Olympus, Tokyo, Japan). Using a lever, the image from the laparoscopic camera can be switched from conventional laparoscopic imaging to NIR imaging. The excitation barrier filter only admits light with a wavelength above 800 nm. Using the NIR camera, all fluorescent structures are displayed in green against a black (non-fluorescent) background. The NIR camera system in conjunction with ICG-enhanced fluorescence has already been used during a number of laparoscopic procedures including LC and sentinel lymph node imaging in colorectal cancer [28,42,43].

Surgical Procedures

Patients were scheduled and prepared according to the standard preoperative protocol for elective or acute LC, depending on the indication for surgery. In the operating room, directly after the time-out procedure and induction of general anesthesia, patients received an intravenous bolus of 0.2 mg/kg ICG diluted in water for intravenous injection. During the cholecystectomy, a conventional laparoscopic camera (EndoEye, Olympus, Tokyo, Japan) and a NIR camera (Olympus, Tokyo, Japan) were used and the image modes were switched during the surgical procedure. The NIR scope was used at set points during surgery (further referred to as "look") to visualize the fluorescent bile ducts, in particular the CD and the CBD. The first look performed in all patients, attempt to visualize the fluorescent bile ducts (first look) after incising the peritoneal fold in the hilus of the gallbladder and before dissection of Calot's triangle. If the CD could not be visualized at the start of dissection, a second look with the NIR scope followed at an early point during dissection but before skeletonizing of structures. In all patients, a third look with the NIR laparoscope was performed when CVS was reached with conventional imaging, to identify fluorescent bile ducts. Bile ducts, visualized by either NIR or conventional white-light imaging, were identified by the operating surgeon, the assisting surgeon and the attending investigator. The visualized structures and time of observation were noted on the case record form. The LC was performed in accordance with Dutch protocols, including achievement of CVS. An IOC was not performed since this is not part of standard LC in the majority of the hospitals in The Netherlands, including our hospital. After surgery, patients recovered on the surgical ward and were discharged according to standard postoperative procedures.

Statistical Analysis

Data were analyzed using SPSS software (SPSS version 20.0; IBM Corp, Armonk, NY). Continuous data are expressed as the median (minimum-maximum). The Mann-Whitney *U* test

was performed to determine the statistical significance between groups with and without bile duct visualization with ICG-NIR. Results were considered statistically significant when $P < 0.05$.

RESULTS

Following receipt of oral and written informed consent, 20 patients scheduled for LC were included in the study. The group included 13 males and 7 females, with a mean age of 65 (range 26-82) years and a mean body mass index (BMI) of 25.45 (range 16.8-38.0) kg/m^2. Two patients required early conversion to an open procedure due to dense adhesions as a result of acute cholecystitis. One of these patients had already undergone percutaneous gallbladder drainage and ERCP. Both patients were excluded from further analysis (Table 1).

Table 1. Patient Characteristics (N = 20).

Male:Female	13:7
Age, years, median (min-max)	65 (26-82)
Body mass index, kg/m^2, median (min-max)	25.45 (16.8-38.0)
ASA (American Society of Anesthesiologists)	
I	7
II	10
III	3
Conversion	2

Eighteen patients were included in the analysis (Table 2). Three patients suffered from biliary pancreatitis, one of whom had a complicated history of necrotizing biliary pancreatitis with renal failure and neuropathy, resulting in a prolonged stay at the intensive care unit before surgery (patient 1). Seven patients were diagnosed with acute cholecystitis. Seven patients were diagnosed with choledocholithiasis, of whom one had undergone a prior magnetic resonance cholangiopancreaticography (MRCP; patient 2), 4 patients a single ERCP (patients 3, 6, 7, 16), 1 patient 2 ERCPs (patient 10), and 1 patient 3 ERCPs (patient 14) before surgery.

The average time between injection of ICG and the first look with the NIR camera was 30 (range 20-72) minutes. At first look, the CD was observed in 3 of 18 patients using conventional white-light imaging and in 4 of 18 with ICG-NIR (Table 2). In 1 patient, the CD could be visualized with both conventional white light and ICGNIR imaging. The CBD was visible in 2 of 18 patients using ICG-NIR. These structures could not be visualized with white light at this time point (Table 2).

In the remaining 11 patients, no bile ducts could be visualized at first look. In the protocol a second look was planned for these patients early in dissection but before skeletonizing of structures. As CVS was reached quickly after the first look in 5 of the 11 patients, a second look was performed in just 6 patients. At second look, the CD could be visualized with both imaging modalities in 1 of 6 patients, and visualized only with conventional white light in a further 2 patients. In 2 patients, the CD and CBD could be visualized only with ICG-NIR. In the final patient (patient 14), anatomy was unclear with white light. Visualization of the CD and CBD with ICG-NIR during second look aided the surgeon and prevented conversion to an open procedure.

Overall, CVS was reached 51 (range 10-117) minutes after ICG injection. At CVS, the CD could be identified in all 18 patients using conventional white light, whereas the CBD could be identified in only 3 of 18 patients. Using ICG-NIR imaging, the CD was identified in 13 of 18 patients, and the CBD could be identified in 7 of 18 patients, all of whom also showed a fluorescent cystic duct (Table 3).

When comparing patients with a cystic duct of common bile duct identified by ICG-NIR at CVS to patients in whom no fluorescent bile ducts were identified at CVS, no differences were apparent with regard to age, BMI, or duration of symptoms before surgery (Table 4). Average total operating time was 82.5 (range 45-128) minutes, Time between ICG injection and first look and the and this did not differ between patients with and without time between injection and CVS were also comparable fluorescent bile duct imaging (Table 4 time between ICG injection and first look. Time between ICG injection and first look and the time between injection and CVS were also comparable between groups. This suggests that there were no significant differences in terms of difficulty of the surgical procedure between the patients with and without ICG-NIR bile duct imaging. No bile duct injuries occurred in the cohort and no other complications arose. In addition, no anatomical variations of the biliary tree were identified.

Table 2. Identification of the Cystic Duct (CD) and Common Bile Duct (CBD) by Conventional Imaging and ICG-NIR During Laparoscopic Cholecystectomy.

		First observation		Second observation		CVS	
No	**Diagnosis**	**Conventional imaging**	**ICG-NIR**	**Conventional imaging**	**ICG-NIR**	**Conventional Imaging**	**ICG-NIR**
1	Biliary pancreatitis	–	CD + CBD	×	×	CD	CD + CBD
2	Choledocholithiasis[a]	–	–	CD	–	CD	CD + CBD
3	Choledocholithiasis[b]	–	–	×	×	CD	CD + CBD
4	Acute cholecystitis	–	–	–	–	CD	–
5	Acute cholecystitis	–	CBD	×	×	CD	CD + CBD
6	Choledocholithiasis[b]	–	–	×	×	CD + CBD	CD + CBD
7	Choledocholithiasis[b]	CD	CD	×	×	CD	CD
8	Acute cholecystitis	–	CD	×	×	CD	CD
9	Acute cholecystitis	–	–	CD	–	CD	–
10	Choledocholithiasis[c]	–	–	CD	CD + CBD	CD + CBD	CD + CBD
11	Cholangitis	CD	–	×	×	CD	CD
12	Acute cholecystitis	–	–	×	×	CD	CD
13	Acute cholecystitis	–	–	×	×	CD	–
14	Choledocholithiasis[d]	–	–	–	CD+CBD	CD + CBD	CD + CBD
15	Biliary pancreatitis[b]	CD	–	×	×	CD	–
16	Choledocholithiasis[b]	–	–	–	–	CD	–
17	Acute cholecystitis	–	CD	×	×	CD	CD
18	Biliary pancreatitis	–	–	×	×	CD	CD

Abbreviations: CVS, critical view of safety; ICG, indocyanine green; NIR, near-infrared; –, not visible; ×, no second look performed.
[a] Magnetic resonance cholangiopancreaticography.
[b] Endoscopic retrograde cholangiopancreaticography.
[c] Two times endoscopic retrograde cholangiopancreaticography.
[d] Three times endoscopic retrograde cholangiopancreaticography.

Table 3. Bile Duct Imaging With Conventional and Indocyanine Green Near-Infrared (ICG-NIR) Imaging at Each "Look."

	Look 1[a]	Look 2[b]	Look 3[c]
No. of patients	18	6d	18
Time after ICG injection, minutes, median (min-max)	30 (20-72)	70 (28-91)	51 (10-117)
Bile duct imaging	7	4	18
Conventional imaging Cystic duct (CD)	3	3	18
Common bile duct (CBD)	0	0	3
No visualization of bile ducts	15	3	0
ICG-NIR imaging Cystic duct (CD)	4	2	13
Common bile duct (CBD)	2	2	7
No visualization of bile ducts	13	4	5

[a] After incising the peritoneal fold in the hilus of the gallbladder and before dissection of Calot's triangle.
[b] After the first look before skeletonizing of structures and only performed if the cystic duct could not be visualized at the first look.
[c] When critical view of safety was reached with conventional imaging.
[d] A second look was planned in 11 patients but performed in just 6 patients. In 5 patients, critical view of safety was reached quickly after the first look before a second look could have been performed.

Table 4. Characteristics of Patients With and Without ICG-NIR Imaging of the Bile Ducts at CVS.

	ICG-NIR Identification	No ICG-NIR Identification	*P*
No. of patients	13	5	
Age, years, median (min-max)	65 (39-82)	65 (26-73)	.964
BMI, kg/m^2, median (min-max)	25 (16.8-32.4)	25.6 (19.4-33.0)	.750
Diagnosis			
Cholecystitis acuta	4	3	
Biliary pancreatitis	2	1	
Choledocholithiasis	6	1	
Cholangitis	1	0	
Time symptoms to operation, days, median (min-max)	75 (2-714)	2 (2-238)	.213
First look, minutes, median (min-max)	30 (20-72)	42 (23-57)	.750
CVS, minutes, median (min-max)	49 (25-117)	60 (10-101)	.892
Operating time, minutes, median (min-max)	75 (45-128)	90 (50-120)	.892

Abbreviations: CVS, critical view of safety; ICG, indocyanine green; NIR, near-infrared.

DISCUSSION

This study was designed to assess the practicality and usefulness of ICG-NIR in early identification of the CD and the additional imaging of the CBD, compared with conventional white-light imaging, during LC in patients with complicated cholecystolithiasis. Fluorescent imaging of the CD was possible in the majority of the cases at CVS, and improved the identification of the CBD compared to conventional white-light imaging. In 1 patient, it was even possible to prevent conversion by detecting the CD and CBD with ICG-NIR before CVS was reached. However, we should emphasize that the number of patients included in the study was small and earlier CD identification by ICG-NIR was only possible in a few of these patients. Second, overall visualization rates of the CD and CBD with NIR fluorescence were much lower than the visualization rates described for uncomplicated cases of cholecystolithiasis [24-38].

The diminished visualization of the bile ducts with ICG-NIR in the patient population presented here may have been due to severe edema or dense adhesions as a result of an acute or past inflammation or an intervention such as ERCP [26,33,34]. It is important to bear in mind that the penetration depth of ICG is limited (±1.0 cm) and is probably insufficient for visualization of the bile ducts when covered by thickened tissue. This phenomenon is also suspected in obese patients (BMI >30 kg/m^2), in which case the bile ducts are covered by a fatty peritoneum. In agreement with current literature, we did not find significant differences in BMI in our patients in relation to successful or unsuccessful imaging of bile duct structures [44]. We hypothesize that other patient and surgical-related factors, including inflamed tissue, may interfere with and influence the success rate of fluorescence visualization of the bile ducts in complicated cases.

Important unresolved issues regarding the ICG-NIR technique are the large disparities in time intervals to imaging following injection of ICG and the wide variation in dosages. In current literature, the reported timing of ICG injection in uncomplicated cholecystolithiasis varies between 30 and 60 minutes prior to start of surgery until after endotracheal intubation. Whether a prolonged interval (up to 24 hours) improves visualization is not yet known. In terms of dosage, in the present study we used 0.2 mg/kg, which is in the range of a bolus of 2.5 mg in total up to 0.5 mg/kg used in described in current literature. Based on our earlier (satisfactory) results in uncomplicated cases and for logistical reasons, ICG was injected directly after induction of general anesthesia [28]. Our final look in all patients at CVS was reached at a median of 51 (10-117) minutes after ICG administration. Bearing in mind the low visualization rates of bile ducts in our study, particularly compared with uncomplicated cases, we presume that dosage and time

frame are decisive and crucial for the effectiveness of CD and CBD NIR fluorescence imaging in patients with complicated gallbladder pathologies.

With normal liver function, 95% of ICG is captured by hepatocytes within 15 minutes of injection and excreted into the bile [45]. In patients with decreased liver function, removal of ICG from blood to bile is delayed. This suggests that earlier injection of ICG could improve detection rates of the CD and CBD in complicated cases. Verbeek et al[46] have shown, in uncomplicated cases, that optimal visualization of the bile ducts is achieved by injection (10 mg ICG) 24 hours prior to surgery and is due to an increased contrast ratio of CBD versus liver background. These authors argued that administration of ICG should be performed as early as possible in all patients to allow optimal clearance of the contrast agent from the liver before undertaking NIR fluorescence assisted LC. However, this regime would be challenging in the case of elective LC and in patients suffering from acute cholecystitis requiring early surgery.

Four studies to date have described (small) patient cohorts in which ICG-NIR bile duct imaging was used in LC due to complicated cholecystitis [26,32,33,47]. The most complicated cases included in these studies were patients with acute cholecystitis. The CD and CBD visualization rates described in these studies were significantly higher than those found in our study (91.6% to 100% and 72% to 79.1% versus 72% and 38%, respectively). With the exception of patient selection, these studies show no great differences in timing and dosage compared to our study, thus suggesting that patient pathology influences the efficacy of ICG. It is presently unclear whether this phenomenon can be attributed to delayed clearance of ICG from blood to bile due to decreased liver clearance, as described above. Therefore, future studies should attempt to establish optimal dosages and timeframes for ICG administration and bile duct visualization, taking into account both the various gallbladder pathologies and feasibility in daily surgical practice.

CONCLUSIONS

NIR fluorescence-assisted LC has the potential to become a standard surgical procedure. Early visualization of the cystic duct and additional imaging of the CBD may increase safety in LC and might offer an alternative to the intraoperative cholangiogram in patients with an increased risk of CBD injury. In contrast to the ease and efficiency of CD and CBD detection by fluorescent imaging in uncomplicated cases, gallbladder pathology appears to create a much more challenging and complex situation. Further research is needed to optimize

techniques, dosage, timing, and patient selection in order to establish whether ICG-NIR can help prevent and manage bile duct injury, and whether there is a place for routine use of fluorescent imaging in those patients at increased risk of bile duct injury during LC.

REFERENCES

1. Soper NJ, Stockmann PT, Dunnegan DL, Ashley SW. Laparoscopic cholecystectomy. The new 'gold standard'? *Arch Surg*. 1992;127:917-921.
2. National Institutes of Health Consensus Development Conference Statement on Gallstones and Laparoscopic Cholecystectomy. *Am J Surg*. 1993;165:390-398.
3. Baird DR, Wilson JP, Mason EM, et al. An early review of 800 laparoscopic cholecystectomies at a universityaffiliated community teaching hospital. *Am Surg*. 1992;58: 206-210.
4. Dubois F, Berthelot G, Levard H. Laparoscopic chole-cystectomy: historic perspective and personal experience. *Surg Laparosc Endosc*. 1991;1:52-57.
5. Flum DR, Dellinger EP, Cheadle A, Chan L, Koepsell T. Intraoperative cholangiography and risk of common bile duct injury during cholecystectomy. *JAMA*. 2003;289:1639-1644.
6. Stewart L. Iatrogenic biliary injuries: identification, classification, and management. *Surg Clin North Am*. 2014;94:297-310.
7. Davidoff AM, Pappas TN, Murray EA, et al. Mechanisms of major biliary injury during laparoscopic cholecystectomy. *Ann Surg*. 1992;215:196-202.
8. Machi J, Johnson JO, Deziel DJ, et al. The routine use of laparoscopic ultrasound decreases bile duct injury: a multicenter study. *Surg Endosc*. 2009;23:384-388.
9. Avgerinos C, Kelgiorgi D, Touloumis Z, Baltatzi L, Dervenis C. One thousand laparoscopic cholecystectomies in a single surgical unit using the "critical view of safety" technique. *J Gastrointest Surg*. 2009;13:498-503.
10. Thurley PD, Dhingsa R. Laparoscopic cholecystectomy: postoperative imaging. *AJR Am J Roentgenol*. 2008;191:794-801.
11. Dolan JP, Diggs BS, Sheppard BC, Hunter JG. Ten-year trend in the national volume of bile duct injuries requiring operative repair. *Surg Endosc*. 2005;19:967-973.
12. Giger U, Ouaissi M, Schmitz SF, Krahenbuhl S, Krahenbuhl L. Bile duct injury and use of cholangiography during laparoscopic cholecystectomy. *Br J Surg*. 2011;98:391-396.
13. Khan OA, Balaji S, Branagan G, Bennett DH, Davies N. Randomized clinical trial of routine on-table cholangiography during laparoscopic cholecystectomy. *Br J Surg*. 2011;98:362-367.
14. Nuzzo G, Giuliante F, Giovannini I, et al. Bile duct injury during laparoscopic cholecystectomy: results of an Italian national survey on 56 591 cholecystectomies. *Arch Surg*. 2005;140:986-992.
15. Tantia O, Jain M, Khanna S, Sen B. Iatrogenic biliary injury: 13,305 cholecystectomies experienced by a single surgical team over more than 13 years. *Surg Endosc*. 2008;22:1077-1086.
16. Sakpal SV, Bindra SS, Chamberlain RS. Laparoscopic cholecystectomy conversion rates two decades later. *JSLS*. 2010;14:476-483.
17. Machado NO. Biliary complications postlaparoscopic cholecystectomy: mechanism, preventive measures, and approach to management: a review. *Diagn Ther Endosc*. 2011;2011:967017.
18. Trastulli S, Cirocchi R, Desiderio J, et al. Systematic review and meta-analysis of randomized clinical trials comparing single-incision versus conventional laparoscopic cholecystectomy. *Br J Surg*. 2013;100:191-208.
19. Machado NO. Laparoscopic cholecystectomy in cirrhotics. *JSLS*. 2012;16:392-400.
20. Zhang WJ, Li JM, Wu GZ, Luo KL, Dong ZT. Risk factors affecting conversion in patients undergoing laparoscopic cholecystectomy. *ANZ J Surg*. 2008;78:973-976.

21. Buddingh KT, Nieuwenhuijs VB. The critical view of safety and routine intraoperative cholangiography complement each other as safety measures during cholecystectomy. *J Gastrointest Surg.* 2011;15:1069-1070.

22. Massarweh NN, Devlin A, Elrod JA, Symons RG, Flum DR. Surgeon knowledge, behavior, and opinions regarding intraoperative cholangiography. *J Am Coll Surg.* 2008;207:821-830.

23. Tabone LE, Sarker S, Fisichella PM, et al. To 'gram or not'? Indications for intraoperative cholangiogram. *Surgery.* 2011;150:810-819.

24. Schols RM, Bouvy ND, Masclee AA, van Dam RM, Dejong CH, Stassen LP. Fluorescence cholangiography during laparoscopic cholecystectomy: a feasibility study on early biliary tract delineation. *Surg Endosc.* 2013;27:1530-1536.

25. Spinoglio G, Priora F, Bianchi PP, et al. Real-time nearinfrared (NIR) fluorescent cholangiography in single-site robotic cholecystectomy (SSRC): a single-institutional prospective study. *Surg Endosc.* 2013;27:2156-2162.

26. Daskalaki D, Fernandes E, Wang X, et al. Indocyanine green (ICG) fluorescent cholangiography during robotic cholecystectomy: results of 184 consecutive cases in a single institution. *Surg Innov.* 2014;21:615-621.

27. Osayi SN, Wendling MR, Drosdeck JM, et al. Near-infrared fluorescent cholangiography facilitates identification of biliary anatomy during laparoscopic cholecystectomy. *Surg Endosc.* 2015;29:368-375.

28. van Dam DA, Ankersmit M, van de Ven P, van Rijswijk AS, Tuynman JB, Meijerink WJ. Comparing near-infrared imaging with indocyanine green to conventional imaging during laparoscopic cholecystectomy: a prospective crossover study. *J Laparoendosc Adv Surg Tech A*. 2015;25:486-492.

29. Kaneko J, Ishizawa T, Masuda K, et al. Indocyanine green reinjection technique for use in fluorescent angiography concomitant with cholangiography during laparoscopic cholecystectomy. *Surg Laparosc Endosc Percutan Tech.* 2012;22:341-344.

30. Buchs NC, Hagen ME, Pugin F, et al. Intra-operative fluorescent cholangiography using indocyanine green during robotic single site cholecystectomy. *Int J Med Robot*. 2012;8:436-440.

31. Buchs NC, Pugin F, Azagury DE, et al. Real-time near infrared fluorescent cholangiography could shorten operative time during robotic single-site cholecystectomy. *Surg Endosc.* 2013;27:3897-3901.

32. Boni L, David G, Mangano A, et al. Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery. *Surg Endosc.* 2015;29:2046-2055.

33. Dip F, Roy M, Lo Menzo E, Simpfendorfer C, Szomstein S, Rosenthal RJ. Routine use of fluorescent incisionless cholangiography as a new imaging modality during laparoscopic cholecystectomy. *Surg Endosc*. 2015;29:1621-1626.

34. Tagaya N, Shimoda M, Kato M, et al. Intraoperative exploration of biliary anatomy using fluorescence imaging of indocyanine green in experimental and clinical cholecystectomies. *J Hepatobiliary Pancreat Sci.* 2010;17:595-600.

35. Prevot F, Rebibo L, Cosse C, Browet F, Sabbagh C, Regimbeau JM. Effectiveness of intraoperative cholangiography using indocyanine green (versus contrast fluid) for the correct assessment of extrahepatic bile ducts during day-case laparoscopic cholecystectomy. *J Gastrointest Surg.* 2014;18:1462-1468.

36. Larsen SS, Schulze S, Bisgaard T. Non-radiographic intraoperative fluorescent cholangiography is feasible. *Dan Med J.* 2014;61:A4891.

37. Ishizawa T, Tamura S, Masuda K, et al. Intraoperative fluorescent cholangiography using indocyanine green: a biliary road map for safe surgery. *J Am Coll Surg.* 2009;208: e1-e4.

38. Schols RM, Bouvy ND, van Dam RM, Masclee AA, Dejong CH, Stassen LP. Combined vascular and biliary fluorescence imaging in laparoscopic cholecystectomy. *Surg Endosc.* 2013;27:4511-4517.

39. Landsman ML, Kwant G, Mook GA, Zijlstra WG. Lightabsorbing properties, stability, and spectral stabilization of indocyanine green. *J Appl Physiol*. 1976;40:575-583.

40. Mordon S, Devoisselle JM, Soulie-Begu S, Desmettre T. Indocyanine green: physicochemical factors affecting its fluorescence in vivo. *Microvasc Res*. 1998;55:146-152.

41. Cherrick GR, Stein SW, Leevy CM, Davidson CS. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. *J Clin Invest*. 1960;39:592-600.

42. van der Pas MH, Ankersmit M, Stockmann HB, et al. Laparoscopic sentinel lymph node identification in patients with colon carcinoma using a near-infrared dye: description of a new technique and feasibility study. *J Laparoendosc Adv Surg Tech A*. 2013;23:367-371.

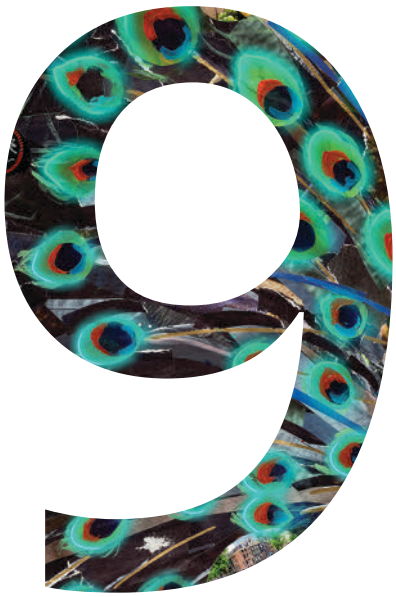
43. van der Pas MH, van Dongen GA, Cailler F, Pelegrin A, Meijerink WJ. Sentinel node procedure of the sigmoid using indocyanine green: feasibility study in a goat model. *Surg Endosc*. 2010;24:2182-2187.

44. Dip F, Nguyen D, Montorfano L, et al. Accuracy of near infrared-guided surgery in morbidly obese subjects undergoing laparoscopic cholecystectomy. *Obes Surg*. 2016;26:525-530.

45. Kawaguchi Y, Ishizawa T, Miyata Y, et al. Portal uptake function in veno-occlusive regions evaluated by real-time fluorescent imaging using indocyanine green. *J Hepatol*. 2013;58:247-253.

46. Verbeek FP, Schaafsma BE, Tummers QR, et al. Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. *Surg Endosc*. 2014;28:1076-1082.

47. Ishizawa T, Bandai Y, Ijichi M, Kaneko J, Hasegawa K, Kokudo N. Fluorescent cholangiography illuminating the biliary tree during laparoscopic cholecystectomy. *Br J Surg*. 2010;97:1369-1377.



BILIARY TRACT VISUALIZATION USING NEAR-INFRARED IMAGING WITH INDOCYANINE GREEN DURING LAPAROSCOPIC CHOLECYSTECTOMY: RESULTS OF A SYSTEMATIC REVIEW

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ABSTRACT

Background Near-infrared imaging with indocyanine green (ICG) has been extensively investigated during laparoscopic cholecystectomy (LC). However, methods vary between studies, especially regarding patient selection, dosage and timing. The aim of this systematic review was to evaluate the potential of the near-infrared imaging technique with ICG to identify biliary structures during LC.

Methods A comprehensive systematic literature search was performed. Prospective trials examining the use of ICG during LC were included. Primary outcome was biliary tract visualization. Risk of bias was assessed using ROBINS-I. Secondly, a meta-analysis was performed comparing ICG to intraoperative cholangiography (IOC) for identification of biliary structures. GRADE was used to assess the quality of the evidence.

Results Nineteen studies were included. Based upon the pooled data from 13 studies, cystic duct visualization was 86.5 (95%CI 71.2–96.6) prior to dissection of Calot's triangle with a 2.5-mg dosage of ICG and 96.5 (95%CI 93.9–98.4) after dissection. The results were not appreciably different when the dosage was based upon bodyweight. There is moderate quality evidence that the CD is more frequently visualized using ICG than IOC (RR 1.16; 95% CI 1.00–1.35); however, this difference was not statistically significant.

Conclusion This systematic review provides equal results for biliary tract visualization with near-infrared imaging with ICG during LC compared to IOC. Near-infrared imaging with ICG has the potential to replace IOC for biliary mapping. However, methods of near-infrared imaging with ICG vary. Future research is necessary for optimization and standardization of the near-infrared ICG technique.

INTRODUCTION

Laparoscopic cholecystectomy (LC) is one of the most frequently performed surgical procedures worldwide [2, 3]. The most serious complication is bile duct injury with an incidence 0.3–1.5% [4–6]. Bile duct injury has significant impact on quality of life and survival. Since the introduction of LC, prevention of complications has been a priority of surgeons [7]. To avoid complications such as bile duct injury, instruments have been developed to minimize risk. One of the strategies is the critical view of safety (CVS) during the dissection of the gallbladder according to Strasberg [8].

Despite introduction of CVS, the use of modern highresolution cameras and angled optics, the incidence of bile duct injury still remains 0.42% [9]. Most common causes of bile duct injury are poor identification of the biliary tract and inflammatory changes such as acute cholecystitis. To overcome risk of bile duct injury, extra intraoperative visualization techniques, such as intraoperative ultrasound and cholangiogram (IOC), have been introduced [10].

IOC is a technique that aids the surgeon to identify the biliary anatomy, possible common bile duct stones and anatomical variations during LC. The use of IOC is debateable. Advocates of the technique argue IOC should be used routinely during LC in order to avoid bile duct injury. Adversaries argue that the technique is not practically feasible, because additional trained staff is required during surgery [5]. Furthermore, incision of the cystic duct is required which could lead to possible bile duct injury. Also, IOC provides additional harmful radiation for the patient [5, 11, 12]. Another disadvantage is that partial dissection of Calot's triangle should be performed before IOC can be utilized and, therefore, bile duct injury can occur before IOC.

Recently, intraoperative visualization of the bile ducts using near-infrared light and the fluorescent dye indocyanine green (ICG) were introduced during cholecystectomy [13]. This technique provides real-time imaging of the biliary tract even before dissection of Calot's triangle.

ICG is an intravenously delivered agent, which when stimulated by near-infrared light (700–900 nm) provides fluorescent visualization of vascular and biliary structures. ICG is a water-soluble dye with peak spectral absorption at 800 nm. After intravenous injection, ICG is bound to plasma proteins and, therefore, remains intravascular. ICG is metabolized almost exclusively by hepatic parenchymal cells and secreted into the bile. Peak concentration in the bile occurs between 30 min and 2 h after injection, whereas peak concentration in the arterial system is reached within 1–2 min [14].

Near-infrared imaging with ICG during LC has only recently been introduced [15, 16]. Under near-infrared light, the biliary structures are fluorescently highlighted, potentially aiding anatomical identification and prevention of bile duct injury. Benefits of ICG include that it is less invasive and no incision of the cystic duct is required, nor is the patient exposed to radiation. Furthermore, ICG has the potential to identify the biliary tract before dissection of Calot's triangle, whereas IOC is generally performed after dissection of the CD.

Currently, near-infrared imaging with ICG is rapidly expanding to other surgical areas. The use of near-infrared imaging with ICG for LC is suggested to be safe and feasible [17–19]. However, between the published studies there is wide variation in technical and procedure-related factors, such as dosage, timing of ICG administration and patient pathology.

Primary question of this systematic review is to evaluate how the near-infrared imaging technique with ICG is used during LC in order to identify biliary structures. Secondary questions include: what is the influence of dosage and timing on biliary tract visualization before and after dissection of Calot's triangle? What is the influence of obesity and gallbladder disease aetiology on biliary tract visualization? And does near-infrared imaging with ICG provide more frequent biliary tract visualization compared to IOC?

METHODS

This systematic review was conducted in accordance with the preferred reporting items for systematic reviews and meta-analysis statement [20]. No approval of the medical ethics committee was required.

Criteria for considering studies for this review

Eligibility criteria

Articles were included if they fulfilled the following criteria: [1] the study describes the use of near-infrared imaging with ICG; [2] reports on laparoscopic cholecystectomies in humans for treatment of gallbladder disease (e.g. cholecystolithiasis and cholecystitis); and [3] used a prospective design (i.e. cohort study or randomized controlled trial). To avoid overlapping patient data in duplicate publications, the article with the largest sample size was included. No publication date or publication status restrictions were imposed. Only studies published in English, German or Dutch were included.

Types of outcome measures

Primary outcome of this review was extrahepatic biliary tract visualization of the cystic duct [1], common bile duct (CBD) and common hepatic duct (CHD) before and after dissection of Calot's triangle. Secondary outcomes were comparison of ICG to IOC, dosage and timing of administration of ICG and body mass index (BMI).

Search methods for selection of studies

Electronic searches

A comprehensive search was performed in the bibliographic databases PubMed, Embase.com, the Cochrane Library (via Wiley) and Web of Science from inception of the databases to the 8 February 2016, in collaboration with a medical librarian (LS). Search terms included controlled terms (Mesh in PubMed, Emtree in Embase) as well as free text terms. Free text terms were only used in the Cochrane Library and Web of Science. The following terms were used (including synonyms and closely related words) as index terms: 'biliary tract surgical procedures' or 'biliary tract' or 'biliary tract diseases' and 'cholangiography' or 'spectroscopy' or 'near-infrared' or 'surgery, computerassisted' and 'indocyanine green' or 'fluorescent dyes'. The full search strategies for all the databases are given in ''Supplementary Information S1''.

Searching other sources

Systematic reviews and narrative review articles that were identified during the search were checked for additional references.

Data collection and analysis

Selection of studies

Article selection was performed by three authors independent of one another (DvD, SV and MA). Titles and abstracts were first screened, and when unclear, the full text of an article was examined. In case of disagreement regarding inclusion or exclusion, a paper was discussed to establish consensus. To avoid overlapping patient data in duplicate publications, the article with the largest sample size was included. The process of inclusion is shown in a flow chart (Figure 1).

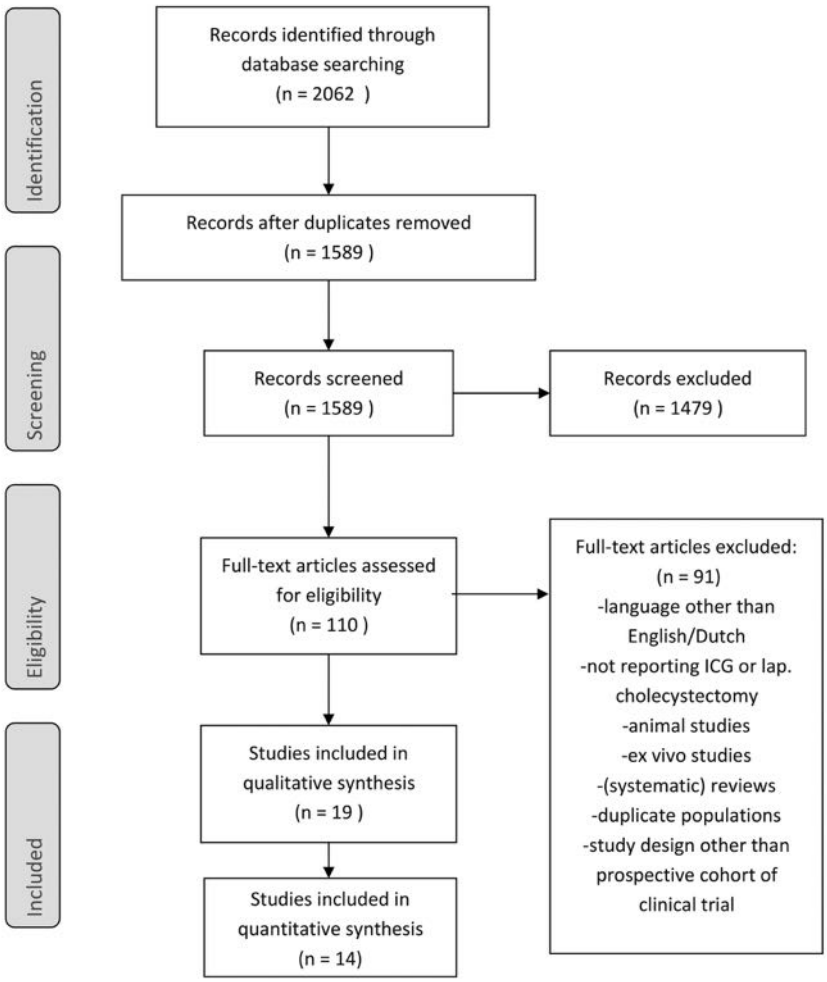


Figure 1. PRISMA flow diagram

Data extraction and management

Data were extracted by three authors (SV, DvD and MA) independent of one another using a data extraction form that included information on: publication details; dosage and timing of ICG administration; camera system used; general patient characteristics; general surgical procedure characteristics, including conversion and complication rate and operating time; LC indications and information on biliary tract visualization.

Risk of bias assessment

Two authors (SV and MA) assessed the risk of bias for studies that reported comparisons between ICG and IOC using the Cochrane's ROBINS-I tool (Risk Of Bias In Nonrandomized

Studies of Interventions) [21]. The following items were assessed: bias due to confounding; selection bias; bias in measurement of interventions; bias due to missing data; bias in measurement of outcomes; and bias in the selection of the reported result. Each domain was scored as low, moderate, serious or critical risk of bias. Overall risk of bias of the included study was scored serious if serious risk of bias was scored in at least one domain. If no serious risk of bias was scored in any domain, the study would receive an overall moderate risk of bias.

Measures of treatment effect

The primary outcome is dichotomous (i.e. identification biliary structure). Prevalence of biliary duct visualization for the CD, CBD, CHD and the cystic duct junction (CD-j) is given as proportions. Weighted mean biliary duct visualization was calculated through pooled analysis in a random effects model with 95% confidence intervals using MedCalc software (Oostende, Belgium).

ICG visualization was compared to IOC for the CD, CBD and CHD. Outcomes are weighted by inverse variance in a random effects model. Treatment effect is presented as relative risk with 95% confidence intervals. These analyses were conducted in RevMan5 [22]. Heterogeneity between studies was assessed through a number of manners: Eye-Ball, Q test and the I^2 statistic. Technical failure of IOC was not excluded from calculations, but analysed as not able to visualize the bile duct structures.

Data synthesis

The overall quality of the evidence and strength of recommendations was evaluated using GRADE [23]. At the start of GRADE assessment process, we assumed high quality for all studies and we downgraded the quality of the evidence for each comparison by one to two levels depending on the seriousness of the violations in each domain.

To assess the risk of bias for a comparison, the risk of bias tables for each study in that comparison was considered. For each comparison, the risk of bias was considered serious (-1) if a majority of the evidence in the studies included in the meta-analysis (in terms of number of participants) scored serious risk of bias. For consistency, we considered a majority of the following four items: an I^2 value of more than 50%, statistical significance of heterogeneity (p=0.05), large variation in size effect and no overlapping of confidence intervals for downgrading (-1). If half of the items were scored for inconsistency, the variation in size effect and confidence intervals were considered decisive. For imprecision of results, serious imprecision leading to downgrading (-1) was judged if a comparison included fewer than 22,000 participants or if wide

confidence intervals around the effect estimate were reported. Indirectness of the evidence was not an issue in this review, because the population, intervention, comparison and outcomes were directly correlated with the review question. Summary of Findings tables were generated for the primary analyses and for the primary outcome measures only. The quality of the evidence is described as:

- High: further research is very unlikely to change our confidence in the estimate of effect;
- Moderate: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate;
- Low: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
- Very low: the true effect is likely to be substantially different from the estimate of effect.

A 'Summary of Findings' table was created using GRADEpro software [24].

RESULTS

An overview of included studies is provided in Table 1. In total, 19 studies were included for qualitative evaluation. Of 19 studies [15, 16, 25–41], one non-randomized controlled trial was identified [27] and 18 prospective cohort studies. In total, 772 patients were reported, with a 0.5% (1/197 patients) conversion rate and 1.7% (10/578 patients) complication rate.

Of 19 studies, 13 reported near-infrared imaging with ICG during laparoscopic cholecystectomy [15, 16, 25, 28–30, 33–37, 40, 41] and six studies reported multiple laparoscopic techniques, including single incision and robotic surgery [26, 27, 31, 32, 38, 39]. Average time of first ICG administration varied between 74 min prior to surgery up to after tracheal intubation. Most studies administered ICG between 45 and 60 min prior to surgery. Timing of a second administration was reported on different moments during the surgical procedure in order to identify the cystic artery (CA). The CA was identified in 85.9% [SD 8.33] of the cases [32, 34, 37]. For near-infrared visualization, the use of six different camera systems has been reported.

In total, 14 studies included patients with only uncomplicated gallbladder disease (cholecystolithiasis, chronic cholecystitis or gallbladder polyp), encompassing 311 patients [15, 16, 26, 27, 30–32, 35, 37–41], and five studies included both complicated and uncomplicated gallbladder disease (acute cholecystitis, biliary pancreatitis, cholestasis and post-ERCP), encompassing 461 patients [25, 28, 29, 33, 34]. In total, 78 of 461 patients suffered complicated gallbladder disease.

Table 1. Study characteristics

References	Type of study	Visualisation techniques	Patients	F:M	BMI (range) [SD]	Age (range) [SD]	Included Indication	Conversion	Complicaties	Camera system	OT (range) [SD]
Aoki[15]	PCH	NIR-ICG, CI	14	6:8	-	61 (43-72)	SCL	-	0	HP	-
Ishizawa[16]	PCH	NIR-ICG, CI	52	23:29	23.2 (18.2-32.9)	59 (28-78)	SCL	-	0	HP	142 (91-366)
Ishizawa[31]	PCH	NIR-ICG, CI, SI	7	6:1	20.4 (16.6-27.4)	-	SCL	-	-	HP	-
Kaneko[32]	PCH	NIR-ICG, CI,SI (9)	28	18:10	20 (18-42)	51 (22-78)	SCL	0	0	HP	151 (98-343)
Buchs[26]	PCH	NIR-ICG, CI,SI, Rbt	12	8:4	28.5 (20-39)	47 (31-69)	SCL	0	0	DVI	85(57-125)
Buchs[27]	NRCT	NIR-ICG vs CI SI,rBT	44	31:13	27 [4.1]	48 [12]	SCL	0	3/23 (ICG) 1/21 (control)	DVI	85 [22]
Dip[30]	PCH	NIR-ICG, CI	65	35:30	20 (16-40)	42 (19-73)	SCL	-	-	KSE	120 (30-80)
Schols[37]	PCH	NIR-ICG, CI	30	19:11	26.7 (19.7-36.8)	53 (26-81)	SCL	1	0	KSE	-
Spinoglio[38]	PCH	NIR-ICG, CI,SI, Rbt	45	33:12	24.7 (19-43)	48 (23-76)	SCL	0	1	DVI	67 (35-110)
Tagaya[39]	PCH	NIR-ICG, CI,SI (4)	15	9:6	(19.5-27.3)	54 (37-69)	SCL	0	0	Os/HP	88 (68-118)
Boni[25]	PCH	NIR-ICG, CI	52	31:21	-	-	17 SCL 35 ACC	-	0	KSE	54 [13]
Dip[29]	PCH	NIR-ICG, CI	45	24:21	28.4 [6.5]	49 [14.76]	28 SCL 17 ACC	-	0	KSE	66 [19]
Osayi[35]	PCH	NIR-ICG, CI	82	64:18	31.5 [8.2]	43 [14]	SCL	-	0	SE	78 [30]
Prevot[36]	PCH	NIR-ICG, CI	23	21:2	-	45 (18-81)	SCL	0	2	KSE	72 (40-200)
Verbeek[41]	PCH	NIR-ICG, CI	14	-:-	25 (19-40)	61 (26-76)	SCL	-	-	mF	-
Larsen[34]	PCH	NIR-ICG, CI	35	26:9	28 (19-38)	48 (18-74)	33 SCL 2ACC	-	0	Os	43 (22-135)
Van Dam[40]	PCH	NIR-ICG, CI	30	21:9	27.5 [4.3]	50 [17]	SCL	-	3	Os	71 [20]
Kono[33]	PCH	NIR-ICG, CI	108	49:59	23.5 (15.6-42.2)	56 (19-92)	102 SCL 6 ACC	-	-	HP	-
Dip[28]	PCH	NIR-ICG, CI	71	42:29	53% > 30 47% <30	-	53 SCL 18 ACC	-	0	KSE	-

PCH, prospective cohort; RCH, retrospective cohort; NRCT, non-randomised controlled trial. NIR, near infrared; ICG, indocyanine green; CI, conventional image; SI, single incision; Rbt, Robot; F:M, female vs male ratio; BMI, body mass index (kg/ m2) with [SD] or (range); Age, in years with [SD] or (range); SCL, symptomatic biliary disease; ACC, acute cholecystitis; HP, Hamamatsu photonics; DVI, Da Vinci System/Intuitive; KSE, Karl Storz Endoskope; Os, Olympus; mF, mini-Flare; SE, Stryker Endoscopy.

Biliary tract visualization per dosage of ICG

Several dosage schemes were used: a fixed dosage of 2.5 mg ICG was used in nine studies [16, 26, 27, 31, 33, 35, 37–39], a dosage of 0.05 mg/kg bodyweight was used in six studies [28–30, 32, 34, 40], a dosage of 0.5 mg/kg bodyweight was used in two studies [25, 36] and two studies reported other dosage schemes [15, 41]. For analysis of biliary tract identification, the studies providing information on dosage scheme were stratified by fixed dosage (2.5 mg) and dosage per kilogram bodyweight (0.05 mg/kg) as given in Table 2.

Seven studies were stratified to the fixed dosage group encompassing 336 patients [16, 26, 31, 33, 35, 37, 38]. Six studies were stratified to the 0.05 mg/kg bodyweight group encompassing 274 patients [28–30, 2, 34, 40]. One study reported on visualization of the CD-j before dissection with positive identification in 35 of 35 cases [34]. No studies reported on the CHD and CD-j visualization after dissection. Two studies have used a dosage scheme of 0.5 mg/kg bodyweight, of which one has evaluated visualization of the biliary structures before and after dissection in 23 patients [36]. Two included studies with different dosage schemes [15, 41] did not report on biliary tract visualization.

In addition to the CD, CBD, CHD, other structures or aberrant anatomy were identified in 70/772 (9.1%) of the patients. Mostly, the confluence of the right and left hepatic duct was visualized [16, 30, 34]. Other identified structures include an extra CA [40], additional hepatic duct or aberrant course of the CD [16, 30, 34, 40] and a CBD cyst [30].

ICG versus IOC visualization

In total, four studies compared the use of ICG with IOC in 215 patients [29, 30, 35, 36]. Risk of bias was scored moderate for visualization of the CD and CBD and serious for visualization of the CHD (Table 3). Results of biliary structure visualization with ICG and IOC are presented in Figure 2A–C. There is moderate quality evidence that visualization of the cystic duct with ICG is better than using the IOC (RR 1.16; 95% CI 1.00–1.35), and, respectively, moderate and low quality evidence for the visualization of the CBD (RR 1.00; 95% CI 0.97–1.03) and CHD (RR 0.76; 95% CI 0.58–1.01)with ICG compared to visualization with IOC. None of the estimated effect sizes were statistically significant (Table 4). All three comparisons were downgraded for serious imprecision because few participants were examined. The comparison of the CHD also scored serious risk of bias due to possible selection bias.

Table 2. Biliary tract visualisation per dosage scheme

Study	*n*	adm. timing (mins)	CD before	CD after	CBD before	CBD after	CHD before	CHD after
Biliary tract visualisation with a 2,5mg fixed dosage of ICG *n (%)*								
Ishazawa [16]	52	30	52 (100)	52 (100)	-	-	50 (96.2)	52 (100)
Ishazawa [31]	7	15*	5 (71.4)	-	-	-	7 (100)	-
Buchs [26]	12	45	11 (91.7)	12 (100)	6 (50)	10 (83.3)	4 (33.3)	8 (66.7)
Schols [37]	30	15*	29 (96.7)	-	25 (83.3)	-	-	-
Spinoglio [38]	45	45	42 (93.3)	44 (97.8)	41 (91.1)	44 (97.8)	40 (88.9)	44 (97.8)
Osayi [35]	82	74	46 (56.1)	78 (95.1)	31 (37.8)	63 (76.8)	29 (35.4)	57 (69.5)
Kono [33]	108	-	88 (81.5)	103 (95.4)	-		94 (87.0)	100 (92.6)
Weighted mean % (95%C.I.)			**86.5 (71.2-96.6)**	**96.5 (93.9-98.4)**	**67.3 (35.5-92.1)**	**86.6 (67.1-98.0)**	**76.8 (51.2-94.7)**	**88.9 (73.5-98.2)**
Biliary tract visualisation with a 0,05mg per kg bodyweight dosage of ICG *n (%)*								
Kaneko [32]	28	15	26 (92.8)	-	-	-	27 (96.4)	-
Dip [30]	65	60	50 (76.9)	65 (100)	50 (76.9)	65 (100)	-	-
Dip [29]	45	60	44 (97.8)†	-	36 (80.0)†	-	27 (60.0)†	-
Larsen [34]	35	15*	-	-	-	-	-	-
van Dam [40]	30	15*	10 (33.3)	29 (96.7)	20 (66.7)	26 (86.7)	-	-
Dip [28]	71	60	71 (100)	-	62 (87.3)	-	50 (70.4)	-
Weighted mean % (95%C.I.)			**85.2 (60.2-98.9)**	**98.4 (92.4-99.9)**	**78.7 (70.3 - 86.0)**	**95.3 (73.4-99.0)**	**76.6 (54.5-92.9)**	

Number of biliary structure identifications n (% proportion) before and after dissection of Calot's triangle. Timing of ICG administration is equal (average 37.3 and 37.5 minutes before surgery) for both groups. ICG, indocyanine green; timing, timing of administration; mins, minutes; CD, cystic duct; CBD, common bile duct; CHD, common hepatic duct; * ICG administration after anaesthesia, † biliary structure identification before and during dissection

Table 3. Risk of bias , ROBINS-I

ROBINS-I	Dip[46]	Dip[46]	Osayi[35]	Prevot[36]
Participants	*65*	*45*	*82*	*23*
Domain				
Bias due to confounding	Low	Low	Low	Low
Bias in selection of participants into the study	Low	Low	Serious	Serious
Bias in measurement of interventions	Low	Low	Low	Low
Bias due to departures from intended interventions	Low	Low	Low	Low
Bias due to missing data	Low	Low	Low	Low
Bias in measurement of outcomes	Moderate	Moderate	Moderate	Moderate
Bias in selection of the reported result	Low	Low	Low	Low
Overall	**Moderate**	**Moderate**	**Serious**	**Serious**

Obesity and visualization accuracy

In addition to using BMI as a baseline characteristic, three studies reported on ICG visualization in different BMI groups [27,28,35]. Osayi et al. [35] reported similar visualization rates for the CD, CBD and CHD, but significantly increased visualization of the CD junction in the BMI ≤ 30 group ($p = 0.038$). Analysis of 20 patients with BMI > 35 revealed prevalence of visualization of the CD and CBD of 91 and 64%. Dip et al. [28] evaluated 71 patients of whom 53% had a BMI > 30. They reported no statistically significant differences between obese (BMI > 30) and non-obese (BMI ≤ 30) patients for biliary tract visualization. Obesity was mentioned as a cause of failure of ICG visualization of bile ducts in one study [15].

Buchs et al. [27] noted a significant faster dissection in the ICG group compared to the control group (conventional laparoscopy) in patients with a BMI < 25. Biliary tract visualization was not assessed.

Figure 2a. CD visualization

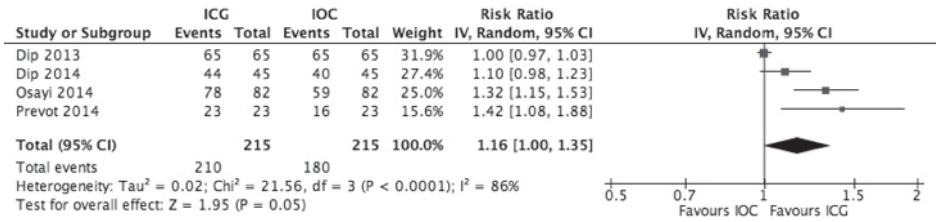


Figure 2b. CBD visualization

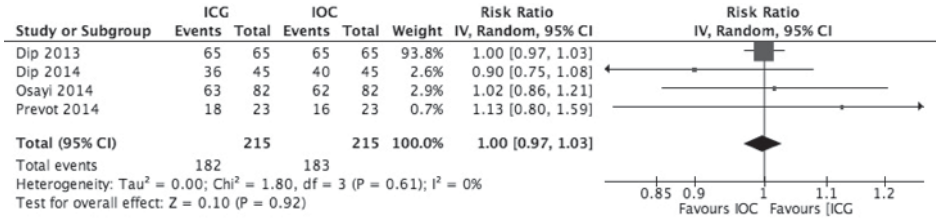


Figure 3b. CHD visualization

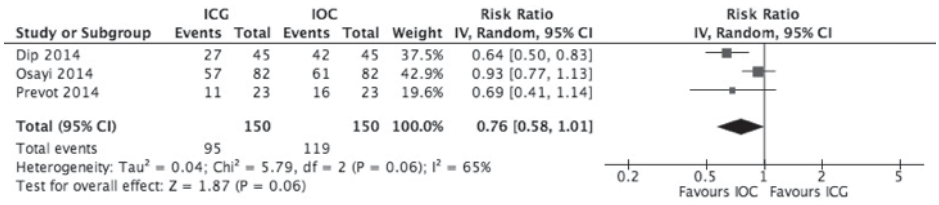


Table 4. GRADE Summary of evidence

ICG compared to IOC for Identification of the biliary ducts						
Patient or population: Identification of the biliary ducts **Setting**: VU University medical centre **Intervention**: ICG **Comparison**: IOC						
Outcomes	**Anticipated absolute effects*** (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (116)	Comments
	Risk with IOC	**Risk with ICG**				
Cystic duct	**Study population**		**RR 1.16** (1.00 to 1.35)	430 (4 observational studies)	Moderate [1,2,3,4,5,6]	Downgraded for imprecision
	837 per 1000	**971 per 1000** (837 to 1000)				
Common bile duct	**Study population**		**RR 1.00** (0.97 to 1.03)	430 (4 observational studies)	Moderate [5,6]	Downgraded for imprecision
	851 per 1000	**851 per 1000** (826 to 877)				
Common hepatic duct	**Study population**		**RR 0.76** (0.58 to 1.01)	300 (3 observational studies)	Low [2,5,6,7]	Downgraded for imprecision and serious risk of bias
	793 per 1000	**603 per 1000** (460 to 801)				
*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **RR:** Risk ratio						
GRADE Working Group grades of evidence **High quality:** We are very confident that the true effect lies close to that of the estimate of the effect **Moderate quality:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different **Low quality:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect						

DISCUSSION

This systematic review has examined the use of near-infrared imaging using ICG for visualization of the cystic duct and related structures. Results suggest that the use of the near-infrared imaging with ICG technique provides good overall visualization rates of the CD, CBD, CHD and CD junction prior to and following dissection of Calot's triangle. Although the number of studies is limited and, therefore, the strength of our conclusions is also limited, visualization rates of the biliary structures using near-infrared imaging techniques with ICG appear to be equally good for either 2.5 mg fixed dosage or 0.05 mg per kg dosage of ICG. Small variation of timing of ICG administration was seen, varying between over an hour before surgery until directly after anaesthesia. As of yet, studies have revealed little information regarding influence of obesity or gallbladder disease aetiology on biliary tract visualization. Also, biliary tract visualization was comparable for near-infrared imaging with ICG and conventional IOC.

Most studies used similar timing of ICG administration. Only Kono et al. [33] described in their cohort a difference in visualization of the CD-j after different administration times, with significantly more CD-j visualization after a longer preoperative administration of ICG (median 90 min prior to surgery (range 15–165) versus 47 min (range 21–205), p<0.01). Verbeek et al. [41] described that administration of ICG 24 h prior to surgery results in a significantly increased CBD-to-liver contrast. Longer preoperative administration of ICG could result in increased visualization rates of the biliary tract structures.

An important factor that could influence ICG imaging is the amount of intra-abdominal adipose tissue. Unfortunately, too few studies have examined this aspect and; therefore, strong conclusions cannot be derived. Interestingly, one study [35] reported improved visualization rates for the CD-j in patients with lower BMI, while another study reported no differences [28] with increasing BMI. Intra-abdominal adipose tissue has been known to be a risk factor in abdominal surgery, leading to increased number of post-operative complications [42]. More intra- abdominal adipose tissue results in a decreased penetration of near-infrared light and thus decreased visualization of biliary tract structures [33]. BMI has been used as a standard for obesity because of its simplicity; however, BMI does not distinguish between the type of adipose tissue or location of fat within the intra-abdominal cavity [43, 44]. In order to assess the effects of intra-abdominal adipose tissue on near-infrared imaging with ICG, the patients should be selected on basis of fat percentage assessed by either CT or MRI instead of BMI [45].

There is moderate quality evidence comparing near-infrared imaging using ICG to conventional IOC; therefore, future research is likely to have an important impact on confidence in the estimate of effect and may change the estimate. However, since IOC comes with higher costs, more difficult perioperative logistics, greater radiation exposure, greater use of radiographic contrast fluids, frequent technical failure and risk of bile duct injury due to cannulation of the CD, ICG might be considered to be the better option for visualization of the biliary tract [46], although further research is necessary to confirm this recommendation.

As IOC depends on at least partial dissection in order to cannulate the CD, ICG provides early imaging before start of dissection. Not only are early (prior to dissection) identification rates with ICG adequate, ICG can be used multiple times during dissection without increasing radiation or contrast load to the patient compared to IOC. Also, overall costs for ICG are 32.3 USD compared to 778.83 USD for IOC [46], although cost for the near-infrared imaging system is unclear.

Several studies have reported aberrant biliary anatomy with the use of ICG [16, 30, 34, 40]. Most commonly reported aberrant anatomy are variations in the hepatic duct. Failure to identify aberrant biliary anatomy could result in bile duct injury. In order to reduce major post-operative complications, ICG identification of aberrant anatomy might lead to a reduced rate of biliary tract leakages.

This systematic review is limited by the design of the included studies. Most included studies are prospective cohort studies and, therefore, highly subject to bias. To date, no randomized trials have been performed that investigate biliary tract visualization. The design of these prospective cohort studies is also limited because most studies did not compare intraoperative visualization with ICG to conventional white light laparoscopy. Therefore, no conclusions can be drawn whether the near-infrared with ICG technique provides advantages over conventional laparoscopy.

In addition, a rather heterogeneous population was examined. Some studies included only uncomplicated gallbladder disease, while others have included both complicated and uncomplicated disease. In the studies reporting both indications, biliary tract visualization results biliary structures during LC. Visualization rates were equal for both dosage schemes. When compared to IOC, ICG provides equal visualization of the bile ducts for both complicated and uncomplicated are pooled together. Furthermore, studies used different definitions for uncomplicated and complicated gallbladder disease. Therefore, no conclusions can be drawn on the use of biliary tract visualization for either complicated or uncomplicated gallbladder disease.

This systematic review and meta-analysis emphasize the need for further investigation. Randomized trials comparing near-infrared imaging with ICG to conventional white light laparoscopy are needed to assess the additive value of near-infrared imaging with ICG. Also, the effect of earlier preoperative ICG administration on biliary tract visualization should be further evaluated. To analyse the effect of uncomplicated and complicated gallbladder disease on ICG visualization results, further research should clearly define included patients groups.

CONCLUSIONS

This systematic review shows that near-infrared imaging with ICG provides good visualization of the biliary structures during LC. Visualization rates were equal for both dosage schemes. When comparing to IOC, ICG provides equal visualization of the bile ducts before dissection. Near-infrared imaging with ICG has the potential to replace IOC for biliary mapping. However, future research is necessary for optimization and standardization of the near-infrared ICG techniques.

REFERENCES

1. Lusch A, Bucur PL, Menhadji AD, Okhunov Z, Liss MA, PerezLanzac A, McDougall EM, Landman J (2014) Evaluation of the impact of three-dimensional vision on laparoscopic performance. J Endourol 28:261–266
2. Gollan J, Bulkley PL, Diehl A (1993) NIH Consensus conference.Gallstones and laparoscopic cholecystectomy. JAMA 269: 1018–1024
3. Perissat J (1993) Laparoscopic cholecystectomy: the Europeanexperience. Am J Surg 165:444–449
4. Deziel DJ, Millikan KW, Economou SG, Doolas A, Ko ST, AiranMC (1993) Complications of laparoscopic cholecystectomy: a national survey of 4,292 hospitals and an analysis of 77,604 cases. Am J Surg 165:9–14
5. Flum DR, Dellinger EP, Cheadle A, Chan L, Koepsell T (2003) Intraoperative cholangiography and risk of common bile duct injury during cholecystectomy. JAMA 289:1639–1644
6. Giger U, Ouaissi M, Schmitz SF, Krahenbuhl S, Krahenbuhl L(2011) Bile duct injury and use of cholangiography during laparoscopic cholecystectomy. Br J Surg 98:391–396
7. McMahon AJ, Fullarton G, Baxter JN, O'Dwyer PJ (1995) Bileduct injury and bile leakage in laparoscopic cholecystectomy. Br J Surg 82:307–313
8. Strasberg SM, Hertl M, Soper NJ (1995) An analysis of theproblem of biliary injury during laparoscopic cholecystectomy. J Am Coll Surg 180:101–125
9. Nuzzo G, Giuliante F, Giovannini I, Ardito F, D'Acapito F,Vellone M, Murazio M, Capelli G (2005) Bile duct injury during laparoscopic cholecystectomy: results of an Italian national survey on 56 591 cholecystectomies. Arch Surg 140:986–992
10. Aziz O, Ashrafian H, Jones C, Harling L, Kumar S, Garas G,Holme T, Darzi A, Zacharakis E, Athanasiou T (2014) Laparoscopic ultrasonography versus intra-operative cholangiogram for the detection of common bile duct stones during laparoscopic cholecystectomy: a meta-analysis of diagnostic accuracy. Int J Surg 12:712–719
11. Ausania F, Holmes LR, Ausania F, Iype S, Ricci P, White SA(2012) Intraoperative cholangiography in the laparoscopic cholecystectomy era: why are we still debating? Surg Endosc 26:1193–1200
12. Ford JA, Soop M, Du J, Loveday BP, Rodgers M (2012) Systematic review of intraoperative cholangiography in cholecystectomy. Br J Surg 99:160–167
13. Oddi A, Di Nicola V, Panzini A, Berni A, Lucci S, Greco L,Urciuoli P, Custureri F (1996) The intraoperative visualization of the bile ducts by the use of fluorescent substances. A feasibility study. G Chir 17:620–623
14. Alander JT, Kaartinen I, Laakso A, Patila T, Spillmann T, TuchinVV, Venermo M, Valisuo P (2012) A review of indocyanine green fluorescent imaging in surgery. Int J Biomed Imaging 2012:940585
15. Aoki T, Murakami M, Yasuda D, Shimizu Y, Kusano T, MatsudaK, Niiya T, Kato H, Murai N, Otsuka K, Kusano M, Kato T (2010) Intraoperative fluorescent imaging using indocyanine green for liver mapping and cholangiography. J Hepatobiliary Pancreat Sci 17(5):590–594
16. Ishizawa T, Bandai Y, Ijichi M, Kaneko J, Hasegawa K, KokudoN (2010) Fluorescent cholangiography illuminating the biliary tree during laparoscopic cholecystectomy. Br J Surg 97:1369–1377
17. Schnelldorfer T, Jenkins RL, Birkett DH, Georgakoudi I (2015) From shadow to light: visualization of extrahepatic bile ducts using image-enhanced laparoscopy. Surg Innov 22:194–200
18. Schols RM, Connell NJ, Stassen LP (2015) Near-infrared fluorescence imaging for real-time intraoperative anatomical guidance in minimally invasive surgery: a systematic review of the literature. World J Surg 39:1069–1079

19. Scroggie DL, Jones C (2014) Fluorescent imaging of the biliarytract during laparoscopic cholecystectomy. Ann Surg Innov Res 8:5

20. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 339:b2535

21. Thomson H, Sterne J, Higgins J (2015) ACROBAT-NRSi (Acochrane risk of bias assessment tool: for non-randomized studies of interventions) for non-clinical community based studies: a participatory workshop using a worked example from public health. Abstracts of the 23rd Cochrane Colloquium

22. Kishi K, Fujiwara Y, Yano M, Inoue M, Miyashiro I, Motoori M,Shingai T, Gotoh K, Takahashi H, Noura S, Yamada T, Ohue M, Ohigashi H, Ishikawa O (2012) Staging laparoscopy using ALAmediated photodynamic diagnosis improves the detection of peritoneal metastases in advanced gastric cancer. J Surg Oncol 106:294–298

23. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y,Alonso-Coello P, Schunemann HJ, Group GW (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336:924–926

24. Kondo Y, Murayama Y, Konishi H, Morimura R, Komatsu S,Shiozaki A, Kuriu Y, Ikoma H, Kubota T, Nakanishi M, Ichikawa D, Fujiwara H, Okamoto K, Sakakura C, Takahashi K, Inoue K, Nakajima M, Otsuji E (2014) Fluorescent detection of peritoneal metastasis in human colorectal cancer using 5-aminolevulinic acid. Int J Oncol 45:41–46

25. Boni L, David G, Mangano A, Dionigi G, Rausei S, Spampatti S,Cassinotti E, Fingerhut A (2015) Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery. Surg Endosc 29(7):2046–2055

26. Buchs NC, Hagen ME, Pugin F, Volonte F, Bucher P, Schiffer E,Morel P (2012) Intra-operative fluorescent cholangiography using indocyanin green during robotic single site cholecystectomy. Int J Med Robot 8:436–440

27. Buchs NC, Pugin F, Azagury DE, Jung M, Volonte F, Hagen ME,Morel P (2013) Real-time near-infrared fluorescent cholangiography could shorten operative time during robotic single-site cholecystectomy. Surg Endosc Other Interv Tech 27(10):3897–3901

28. Dip F, Nguyen D, Montorfano L, Noste MES, Menzo EL,Simpfendorfer C, Szomstein S, Rosenthal R (2015) Accuracy of near infrared-guided surgery in morbidly obese subjects undergoing laparoscopic cholecystectomy. Obes Surg 26(3):525–530

29. Dip F, Roy M, Menzo EL, Simpfendorfer C, Szomstein S,Rosenthal RJ (2014) Routine use of fluorescent incisionless cholangiography as a new imaging modality during laparoscopic cholecystectomy. Surg Endosc 29(6):1621–1626

30. Dip FD, Nahmod M, Alle L, Sarotto L, Anzorena FS, Ferraina P(2013) Fluorescence cholangiography in laparoscopic cholecystectomy experience in Argentina. Front Gastr Res 31:80–85

31. Ishizawa T, Kaneko J, Inoue Y, Takemura N, Seyama Y, Aoki T,Beck Y, Sugawara Y, Hasegawa K, Harada N, Ijichi M, Kusaka K, Shibasaki M, Bandai Y, Kokudo N (2011) Application of fluorescent cholangiography to single-incision laparoscopic cholecystectomy. Surg Endosc 25:2631–2636

32. Kaneko J, Ishizawa T, Masuda K, Kawaguchi Y, Aoki T, Sakamoto Y, Hasegawa K, Sugawara Y, Kokudo N (2012) Indocyanine green reinjection technique for use in fluorescent angiography concomitant with cholangiography during laparoscopic cholecystectomy. Surg Laparosc Endosc Percutaneous Tech 22:341–344

33. Kono Y, Ishizawa T, Tani K, Harada N, Kaneko J, Saiura A,Bandai Y, Kokudo N (2015) Techniques of fluorescence cholangiography during laparoscopic cholecystectomy for better delineation of the bile duct anatomy. Medicine 94:1–9

34. Larsen SS, Schulze S, Bisgaard T (2014) Non-radiographicintraoperative fluorescent cholangiography is feasible. Dan Med J 61:A4891

35. Osayi SN, Wendling MR, Chaudhry UI, Drosdeck JM, Perry KA,Noria SF, Hazey JW, Muscarella P, Melvin WS, Narula VK (2014) Near infrared fluorescent cholangiography facilitates identification of biliary anatomy during laparoscopic cholecystectomy. Surg Endosc Other Interv Tech 28:267

36. Prevot F, Rebibo L, Cosse C, Browet F, Sabbagh C, RegimbeauJ-M (2014) Effectiveness of intraoperative cholangiography using indocyanine green (versus contrast fluid) for the correct assessment of extrahepatic bile ducts during day-case laparoscopic cholecystectomy. J Gastrointest Surg 18(8):1462–1468

37. Schols RM, Bouvy ND, van Dam RM, Masclee AA, De jong CH,StassenLP (2013) Combined vascular and biliary fluorescence imaging in laparoscopic cholecystectomy. Surg Endosc 27:4511–4517

38. Spinoglio G, Priora F, Bianchi PP, Lucido FS, Licciardello A,Maglione V, Grosso F, Quarati R, Ravazzoni F, Lenti LM (2013) Real-time near-infrared (NIR) fluorescent cholangiography in single-site robotic cholecystectomy (SSRC): a single-institutional prospective study. Surg Endosc 27:2156–2162

39. Tagaya N, Sugamata Y, Makino N, Saito K, Okuyama T,Koketsu S, Oya M (2013) Fluorescence cholangiography in laparoscopic cholecystectomy: experience in Japan. Front Gastr Res 31:73–79

40. van Dam DA, Ankersmit M, van de Ven P, van Rijswijk AS,Tuynman JB, Meijerink WJ (2015) Comparing near-infrared imaging with indocyanine green to conventional imaging during laparoscopic cholecystectomy: a prospective crossover study. J Laparoendosc Adv Surg Tech Part A 25(6):486–492

41. Verbeek FP, Schaafsma BE, Tummers QR, van der Vorst JR, vander Made WJ, Baeten CI, Bonsing BA, Frangioni JV, van de Velde CJ, Vahrmeijer AL, Swijnenburg RJ (2014) Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. Surg Endosc 28:1076–1082

42. Morris K, Tuorto S, Gonen M, Schwartz L, DeMatteo R,D'Angelica M, Jarnagin WR, Fong Y (2010) Simple measurement of intra-abdominal fat for abdominal surgery outcome prediction. Arch Surg 145:1069–1073

43. Deurenberg P, Andreoli A, Borg P, Kukkonen-Harjula K, deLorenzo A, van Marken Lichtenbelt WD, Testolin G, Vigano R, Vollaard N (2001) The validity of predicted body fat percentage from body mass index and from impedance in samples of five European populations. Eur J Clin Nutr 55:973–979

44. Rothman KJ (2008) BMI-related errors in the measurement ofobesity. Int J Obes (Lond) 32(Suppl 3):S56–S59

45. Yoshizumi T, Nakamura T, Yamane M, Islam AHMW, Menju M,Yamasaki K, Arai T, Kotani K, Funahashi T, Yamashita S, Matsuzawa Y (1999) Abdominal fat: standardized technique for measurement at CT. Radiology 211:283–286

46. Dip FD, Asbun D, Rosales-Velderrain A, Lo Menzo E, Simpfendorfer CH, Szomstein S, Rosenthal RJ (2014) Cost analysis and effectiveness comparing the routine use of intraoperative fluorescent cholangiography with fluoroscopic cholangiogram in patients undergoing laparoscopic cholecystectomy. Surg Endosc 28:1838–1843

SUPPLEMENTARY INFORMATION

1. Search strategy and search results:

Supplementary information S1

Search strategy for PubMed (February 8th 2016)

[Mesh] = Medical subject headings

[tiab] = words in title or abstract

Search	Query	Items found
#4	#1 AND #2 AND #3	244
#3	"Indocyanine Green"[Mesh] OR "Fluorescent Dyes"[Mesh] OR indocyanine green[tiab] OR wofaverdin[tiab] OR vophaverdin[tiab] OR icg[tiab] OR fluorescen*[tiab] OR fluorochrome*[tiab] OR fluorogenic*[tiab] OR cw800*[tiab] OR irdye*[tiab] OR fluorophore[tiab] OR fluorescein[tiab] OR indocyanine green[ot] OR wofaverdin[ot] OR vophaverdin[ot] OR icg[ot] OR fluorescen*[ot] OR fluorochrome*[ot] OR fluorogenic*[ot] OR cw800*[ot] OR irdye*[ot] OR fluorophore[ot] OR fluorescein[ot]	405300
#2	"Cholangiography"[Mesh] OR "Spectroscopy, Near-Infrared"[Mesh] OR "Surgery, Computer-Assisted"[Mesh] OR cholangiogra*[tiab] OR cholangiopancreatograph*[tiab] OR near-infrared[tiab] OR nir[tiab] OR image guided surger*[tiab] OR computer-aided surger*[tiab] OR computer assisted surger*[tiab] OR robot*[tiab] OR fireflight[tiab] OR biliary map*[tiab] OR angiogra*[tiab] OR ((real time[tiab] OR realtime[tiab] OR biliary[tiab] OR optical[tiab] OR intra-operative[tiab] OR intraoperative[tiab]) AND (imag*[tiab] OR visual*[tiab])) OR cholangiogra*[ot] OR cholangiopancreatograph*[ot] OR near-infrared[ot] OR nir[ot] OR image guided surger*[ot] OR computer-aided surger*[ot] OR computer assisted surger*[ot] OR robot*[ot] OR fireflight[ot] OR biliary map*[ot] OR angiogra*[ot] OR ((real time[ot] OR realtime[ot] OR biliary[ot] OR optical[ot] OR intra-operative[ot] OR intraoperative[ot]) AND (imag*[ot] OR visual*[ot]))	321028
#1	"Biliary Tract Surgical Procedures"[Mesh] OR "Biliary Tract"[Mesh] OR "Biliary Tract Diseases"[Mesh] OR Biliar*[tiab] OR gallbladder[tiab] OR bile duct*[tiab] OR ampulla of vater*[tiab] OR cystic duct*[tiab] OR hepatic duct*[tiab] OR cholecystectom*[tiab] OR choledochostom*[tiab] OR cholelithiasis[tiab] OR cholangitis[tiab] OR choledoch*[tiab] OR cholecyst*[tiab] OR gallstone*[tiab] OR hepatobiliary*[tiab] OR gall bladder[tiab] OR biliar*[ot] OR gallbladder[ot] OR bile duct*[ot] OR ampulla of vater*[ot] OR cystic duct*[ot] OR hepatic duct*[ot] OR cholecystectom*[ot] OR choledochostom*[ot] OR cholelithiasis[ot] OR cholangitis[ot] OR choledoch*[ot] OR cholecyst*[ot] OR gallstone*[ot] OR hepatobiliary*[ot] OR gall bladder[ot]	207453

2. Search strategy for Embase.com (February 8th 2016)

/exp = EMtree keyword with explosion

:ab,ti = words in title or abstract

NEAR/3= words near to each other, 3 places apart

NEXT/1= words next to each other, 1 place apart

Search	Query	Items found
#4	#1 AND #2 AND #3	1227
#3	'indocyanine green'/exp OR 'fluorescent dye'/exp OR 'indocyanine green':ab,ti OR wofaverdin:ab,ti OR vophaverdin:ab,ti OR icg:ab,ti OR fluorescen*:ab,ti OR fluorochrome*:ab,ti OR fluorogenic*:ab,ti OR cw800*:ab,ti OR irdye*:ab,ti OR fluorescein:ab,ti	505845
#2	'cholangiography'/exp OR 'near infrared spectroscopy'/exp OR 'computer assisted surgery'/exp OR cholangiogra*:ab,ti OR cholangiopancreatograph*:ab,ti OR 'near infrared':ab,ti OR nir:ab,ti OR 'image guided surgery':ab,ti OR 'image guided surgeries':ab,ti OR ('real time' NEAR/3 imag*):ab,ti OR (realtime NEAR/3 imag*):ab,ti OR (optical NEAR/3 imag*):ab,ti OR ('computer aided' NEAR/3 surger*):ab,ti OR ('computer assisted' NEAR/3 surger*):ab,ti OR robot*:ab,ti OR fireflight:ab,ti OR (biliary NEAR/3 map*):ab,ti OR visual*:ab,ti	643692
#1	'biliary tract surgery'/exp OR 'hepatobiliary system'/exp OR 'biliary tract disease'/exp OR biliar*:ab,ti OR gallbladder:ab,ti OR (bile NEXT/1 duct*):ab,ti OR 'ampulla of vater':ab,ti OR (cystic NEXT/1 duct*):ab,ti OR (hepatic NEXT/1 duct*):ab,ti OR cholecystectom*:ab,ti OR choledochostom*:ab,ti OR cholelithiasis:ab,ti OR cholangitis:ab,ti OR choledoch*:ab,ti OR cholecyst*:ab,ti OR gallstone*:ab,ti OR hepatobiliary*:ab,ti OR 'gall bladder':ab,ti	793399

3. Search strategy for Wiley/Cochrane Library (February 8th 2016)

ti,ab,kw = words in title, abstract or keyword

Search	Query	Items found
#1	biliar* or gallbladder or (bile and duct*) or (ampulla and vater*) or (cystic and duct*) or (hepatic and duct*) or cholecystectom* or choledochostom* or cholelithiasis or cholangitis or choledoch* or cholecyst* or gallstone* or hepatobiliary* or gall bladder	9099
#2	cholangiogra* or cholangiopancreatograph* or near-infrared or nir or (image guided and surger*) or (computer-aided and surger*) or (computer assisted and surger*) or robot* or fireflight or (biliary and map*) or angiogra* or ((real time or realtime or biliary or optical or intra-operative or intraoperative) and (imag* or visual*))	22320
#3	indocyanine green or wofaverdin or vophaverdin or icg or fluorescen* or fluorochrome* or fluorogenic* or cw800* or irdye* or fluorophore or fluorescein	4684
#4	#1 and #2 and #3	20

4. Search strategy for Web of Science (February 8th 2016)

TS = words in topic

TI = words in title

Search	Query	Items found
#4	#3 AND #2 AND #1	390
#3	TS=('indocyanine green' OR wofaverdin OR vophaverdin OR icg OR fluorescen* OR fluorochrome* OR fluorogenic* OR cw800* OR irdye* OR fluorophore OR fluorescein)	1,163,990
#2	TS=(cholangiogra* OR cholangiopancreatograph* OR 'near-infrared' OR 'near infrared' OR nir OR (image guided AND surger*) OR (computer-aided AND surger*) OR (computer assisted AND surger*) OR robot* OR fireflight OR (biliary AND map*) OR angiogra* OR ((real time OR realtime OR biliary OR optical OR 'intra-operative' OR intraoperative) AND (imag* OR visual*)))	943,839
#1	TS=(biliar* OR gallbladder OR (bile AND duct*) OR (ampulla AND vater*) OR (cystic AND duct*) OR (hepatic AND duct*) OR cholecystectom* OR choledochostom* OR cholelithiasis OR cholangitis OR choledoch* OR cholecyst* OR gallstone* OR hepatobiliary* OR 'gall bladder')	370,644

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

During surgical procedures the surgeon still relies mainly on inspection and palpation to identify structures. It is often very difficult to distinguish malignant from healthy tissue, or to distinguish between fibrotic and inflamed tissue while avoiding injury to structures that should be spared. The introduction of laparoscopic and robotic surgery, which both minimize intraoperative tactile feedback, has accelerated the need for additional perioperative imaging modalities. Molecular image-guided surgery, using radionuclides or optical tracers, has the potential to improve current surgical performance. After administration of radionuclides, the radioactive signal can be used for preoperative nuclear imaging and for intraoperative tumor localization by identification of an acoustic signal produced by a hand-held detection probe. Intraoperative guidance, using near-infrared (NIR) fluorescence imaging in particular, allows for real-time visualization of tissue and vital structures.

Depending on the specific pathology and the aim of tissue visualization, imaging modalities can be used either separately or in combination. Real-time NIR fluorescence imaging has found applications in the imaging of nerves, bile ducts, ureters and in the evaluation of adequate blood flow. Optical imaging, alone or combined with nuclear imaging (PET or SPECT), is preferred in oncological surgery as it can be utilized for sentinel lymph node (SLN) identification or to localize tumor tissue in order to determine disease extent and improve radical resection.

In this thesis, we illustrate the application and feasibility of molecular image-guided surgery in abdominal surgical procedures. Our research focused on 1) the identification of the SLN in colon cancer, and 2) bile duct imaging during laparoscopic cholecystectomy. The technique was suitable for both technical applications. To improve the diagnostic and clinical performance of molecular image-guided surgery, several technical hurdles first need to be tackled, and a better understanding is required of disease pathology and patient characteristics in order to improve perioperative visualization. In general, molecular image-guided surgery has the potential to dramatically improve the treatment and prognosis of the individual patient.

TECHNICAL CHALLENGES IN MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

Intraoperative real-time imaging

The use of high definition (HD) and 3-dimensional (3D) imaging systems has improved intraoperative imaging dramatically in the last few years [1, 2]. NIR fluorescence imaging has advantages compared to conventional white light imaging due to the emission of light in the

NIR range (700-100nm), resulting in deeper penetration depth and less autofluorescence from surrounding tissue. A fluorescent fluorophore is required to produce a NIR-fluorescent signal, and currently only Indocyanine Green (ICG) and methylene blue are approved for clinical use by the Food and Drug Administration and the European Medicines Agency.

ICG is the most widely used fluorophore in clinical studies. Due to its short serum half-time of only a few minutes, combined with exclusive clearance by the liver, intravenous administration of ICG allows visualization of micro-perfusion of the bowel and imaging of the bile duct. Peritumoral injection of ICG facilitates SLN mapping and is preferred over methylene blue due its favorable excitation peak of around 800 nm and its larger particle size [3].

In this thesis, we used ICG as a fluorescent agent for SLN mapping and bile duct imaging. NIR fluorescence imaging proved to be feasible for both applications. However, a major drawback is the limited penetration depth of 5-10 mm, meaning that fluorescent structures located beneath a thick layer of (fatty) tissue, severe edema or dense adhesions are difficult to identify. To improve

NIR fluorescence imaging, new dyes with better fluorescent properties might improve intraoperative imaging quality. The fluorescent dye IRDye800CW is currently the best NIR-fluorescent dye available and is close to FDA approval. Animal studies have shown an excellent contrast ratio between SLNs stained with IRDye800CW and surrounding tissue. Additionally, conjugation of IRDye800CW to a nanocolloid improved retention time in the node to up to 24 hrs. ZW800-I is another interesting new dye which is currently being clinically tested [4]. ZW800-I has low toxicity and is excreted through the kidneys, enabling visualization of the ureters and perfusion of anastomoses. It also has a favorable emission wavelength of 788 nm, which allows for visualization with current camera systems adjusted for ICG. Dyes that emit light in the NIR-II range from 1000 to 1700 nm rather than the NIR-I range (700-1000 nm) might also improve imaging [4]. These dyes have longer wavelengths and therefore less attenuation and scattering, which is especially favorable when imaging deeply located tissue. However, these favorable properties are accompanied by increased autofluorescence. Furthermore, these dyes have a lower quantum yield compared to NIR-dyes in current use, they show different pharmacokinetics, and they require visualization adjustment in current clinical imaging systems [5].

Another interesting new technique that might improve intraoperative imaging is optoacoustic imaging [6]. A major advantage of optoacoustic imaging is better spatial resolution in deep tissue, with less photo bleaching and autofluorescence compared to NIR-imaging. Optoacoustic imaging is a method based on the absorption of tissue when illuminated by NIR-light. The

absorbed optical energy is converted into heat, causing a transient thermoelastic expansion that generates ultrasound waves from the tissue that absorbs the NIR-light. These ultrasound waves can be detected, resulting in visualization of NIR-absorbing tissue. NIR-dyes (ICG and IRDye800CW) can be used as contrast agents with optoacoustic imaging to visualize tissues of interest. Optoacoustic imaging studies in mouse models have shown favorable results regarding the sensitivity of detection and the differentiation of fluorescent targets, suggesting that this technique could overcome the problem of restricted penetration depth of current NIR fluorescence imaging modalities [7].

The performance of intraoperative imaging may also be enhanced through improvement of imaging systems. First, the sensitivity of imaging systems can be improved by modification of software, emission filters and background corrections. Secondary to these camera system improvements, it also appears to be essential to keep the imaging probe perpendicular during inspection in order to obtain an optimal fluorescent signal and better distinguish the fluorescent target from normal tissue [8]. Although seemingly simple, due to the limited flexibility of the laparoscopic camera this can be quite challenging during surgery. A DROP-IN NIR camera, which can be inserted through a trocar port and picked up by the surgeon with laparoscopic devices, could overcome this problem. A prototype DROP-IN gamma probe has already been investigated for use in SLN imaging in prostate cancer. This study demonstrated an enlarged probe maneuverability to freely scan in every direction in the abdominal cavity and increased the autonomy of the operating surgeon [9].

Perioperative molecular nuclear imaging

Preoperative molecular nuclear imaging is used to plan the surgical strategy and define the extent of disease. The radioactive signal can subsequently be used for intraoperative localization of the tissue of interest using a handheld radionuclide probe.

For preoperative nuclear imaging, positron emission tomography (PET) combined with conventional computed tomography (CT) scans is currently the most sophisticated nuclear imaging modality. Compared to single photon emission computed tomography (SPECT), PET scanners have a higher sensitivity and much better temporal resolution, producing high-quality whole-body 3D images. PET characteristics are favorable regarding SLN identification in colon cancer, since lymphatic drainage patterns are unknown. However, it often appears that the majority of SLNs are located near the injection site, and the limited resolution of SPECT and a shine-through effect from the tracer depot may preclude accurate identification when a SLN is close to the tumor [10, 11]. PET/CT lymphoscintigraphy is able to detect SLNs near the

primary tumor and can provide a detailed localization of these nodes in oral cancer patients [12]. PET/CT imaging has the potential to improve the SLN procedure in colon cancer patients and has therefore been investigated in this thesis as a nuclear imaging technique for this application.

PET/CT is a relatively new technique in the molecular nuclear imaging field. Specific PET tracers are needed for PET imaging and over the last decade 89-Zirconium has garnered a great deal of attention as an emergent radionuclide for PET imaging. Its favorable half-life of 78.4 hrs facilitates quantitative analysis of tracer distribution over time. Secondly, it can be easily coupled to various compounds, in particular to a (nano)colloid resulting in the radiocolloid [^{89}Zr] Zr-Nanocoll. Nanocoll enlarges the radionuclide molecule and therefore improves retention of the radiocolloid in the SLN. Drawbacks of PET imaging are the high associated costs and the limited availability of PET/CT cameras. Additionally, a handheld PET probe for intraoperative localization of the radioactive signal would be desirable but is not yet available. The development of this type of PET-probe is challenging and expensive due to high-energy photons that need a large collimated and shielded detector, which is probably unsuitable for laparoscopic surgery [13, 14]. Meanwhile, improved hand-held gamma cameras and SPECT imaging systems have been developed. Additionally, new SPECT radionuclides such as indium-111, with a half-life of 67.32 days, are now available. However, it is unclear if these imaging probes and dyes will be able to overcome the limited temporal resolution of SPECT and associated shine-through, and the diminished visualization of adjacent tissues of interest.

First results of a phase I study in renal cell carcinoma using indium-111 as a tracer, combined with preoperative SPECT imaging, are promising [8]. However, It must be emphasized that the study only included solitary tumors of at least 1.5 cm. Future studies must demonstrate whether indium-111, combined with SPECT imaging modalities, is capable of detecting signals from smaller, adjacent and more widely-spread tumor tissues. It seems likely that an optimal radionuclide should be chosen in light of the precise clinical question for each specific disease pathology [15].

FUTURE PERSPECTIVES OF IMAGE-GUIDED ABDOMINAL SURGERY

The sentinel lymph node procedure in colon cancer

As shown in **Chapter 3**, the sensitivity of the SLN procedure (SNP) in colon cancer is with 56% much lower compared to breast cancer and melanoma. It seems to be difficult to identify the correct SLN(s) in colon cancer probably due to technical and anatomical properties which are unique for colon cancer. This combined with the wide variation of the used SLN mapping

methods, patients selection and histopathological analysis of lymph nodes, we have to conclude that the performance of SNP in colon cancer is still insufficient. To improve the SNP, several patient, tumor and procedure-related factors should be optimized and standardized.

The first step is to select only those patients with stage I and eventually stage II disease, who have potentially the greatest benefit of the SLN procedure. More advanced tumor stages already meet criteria for adjuvant chemotherapy and their treatment will not be changed by a SLN procedure. It is difficult to distinguish early and more advanced tumor stages with current preoperative imaging techniques (CT scan). Therefore, future studies should integrate patient selection as part of the research protocol.

Subsequently, refinement of the preoperative and intraoperative SLN method should be further investigated with imaging techniques other than planar lymphoscintigraphy, gamma-counting and blue dye. In **Chapter 6** we demonstrated that preoperative PET/CT lymphoscintigraphy, combined with NIR fluorescence imaging, could guide the surgeon to the location of a number of SLNs in early-staged colon cancer, even when they were located near the injection site. However, physical movement of the bowel that changes the anatomical position of SLNs, combined with a minimized but not entirely absent 'shine-through effect' from the injection depot of both tracers, still impedes perioperative SLN identification. Additionally, the limited penetration depth of NIR fluorescence imaging and the fast migration of ICG to higher echelon nodes hampered intraoperative localization of the SLNs. To improve perioperative SLN identification, a handheld PET-probe would be desirable but as mentioned is not yet clinically feasible. However, the lack of a handheld PET-probe, combined with the limiting properties of NIR fluorescence imaging, means that an additional ex vivo PET/CT scan of the specimen is unavoidable. When the only aim of SLN mapping in colon cancer is improvement of lymph node staging, this technique should be simplified and investigated in future large volume studies. However, if the purpose of the SNP is to diminish the extent of surgery through SLN biopsy and local excision of the primary tumor, it is doubtful whether preoperative SLN identification using PET/CT lymphoscintigraphy combined with intraoperative NIR fluorescence imaging will be sufficient. Several studies, in multiple types of cancer, have shown promising results using the hybrid tracer ICG-^{99m}Tc-Nanocolloid, which allows preoperative SPECT/CT lymphoscintigraphy combined with intraoperative NIR-imaging and gamma-probe guided SLN detection [16, 17]. Since ^{99m}Tc-Nanocoll has exactly the same behavior in vivo as [^{89}Zr]Zr-Nanocoll, the applicability of ICG-^{99m}Tc-Nanocolloid as an SLN mapping technique should be reinvestigated in colon cancer using insights derived from our results. An additional advantage of ICG-^{99m}Tc-Nanocoll is the wide availability in several countries, whereas [^{89}Zr]Zr-Nanocoll is not yet FDA approved. As

mentioned earlier, it is uncertain whether SPECT actually contributes to improvement of the SNP, since it has an unfavorably low resolution compared to PET/CT.

We also expect that, in addition to the imaging technique used, procedure-related factors may play an important role in improving current SLN performance in colon cancer.
First, injection into the submucosal layer shows favorable results compared to the subserosal injection technique. This is probably the result of more accurate injection close to the tumor after submucosal injection, with consequent improvement of uptake by all tumor-draining lymphatic vessels. Secondly, we found that an ex vivo performed SNP showed better sensitivity. We hypothesized that this is probably explained by a more aggressive dissection of the mesocolon to identify SLNs. However, ex vivo SLN identification after extraction of the specimen disrupts natural lymphatic pathways and may fail to identify SLNs when these are located outside the resection area. Meanwhile, an in vivo executed SNP could identify aberrant lymph node drainage patterns, which may change the mesocolonic resection margins when SLNs are located outside the conventional surgical field [18]. Furthermore, the in vivo technique has the potential to perform SLN picking, combined with local excision of the primary tumor when no metastases are found in the SLN. We therefore advocate that the SNP should be performed in vivo using an endoscopic submucosal injection technique.

In addition to technical improvements of SLN biopsy in colon cancer, the procedure would be greatly improved by better knowledge concerning lymphatic drainage patterns and thus understanding of lymphatic metastatic spread. In contrast to breast cancer and melanoma, SLNs of the colon are not located at a prespecified intra-abdominal location. Moreover, aberrant lymphatic drainage outside the standard resection margins has been described in up to 22% of patients, accompanied by lymph node metastases in 10% of these cases [18]. Better exploration and determination of lymphatic drainage patterns would help guide the surgeon to the location and number of SLNs that need to be retrieved. This knowledge would also be very helpful when SLNs are difficult to assign due to retention of fluorescent dye in fat tissue or when radioactive stool is mistaken for a SLN following tracer leakage into the lumen of the colon.

The potential benefit of the SNP in colon cancer is to improve staging, patient management and survival. The widely accepted practice for lymph node assessment is H&E-staining of a single or a few sections cut from each lymph node block representing only a small volume of the lymph node in a single axis, potentially producing sampling error [19]. Serial sectioning increases detection of (macro)metastases and especially of the "occult tumour cells", ie. isolated tumour cells and micrometastases. As shown in **Chapter 3**, true prevalence of LNM as derived from

the high quality concept validation studies was 48% (after extensive histopathology) versus 36% after conventional H&E-staining. These missed LNM after initial surgical treatment could explain the disease recurrence in patients considered to have no lymph node metastases after primary surgery with standard histopathology. However. the prognostic relevance of occult tumour cells and treatment of these patients with adjuvant chemotherapy to improve survival is still unclear and must be established. It is suggested that only micrometastases are associated with a significant reduction in 5-year survival while their presence is much lower compared to isolated tumour cells [20-23]. The EnRoute study (NCT01097265) was designed to determine the benefits of adjuvant chemotherapy in those patients with SLNs revealing micrometastases [24]. Unfortunately, this study had to be terminated prematurely due to low patient numbers and slow inclusion. Saha et al. [25] recently showed an improved survival in patients treated with chemotherapy if only *one* lymph node with micrometastases was found compared to those who refused adjuvant treatment. In patients treated with chemotherapy, an average survival of 108 months was reported compared to 50 months in those not treated with chemotherapy. However, only 30 patients were included in the study and the authors did not stratify for tumor stage. Future studies should investigate the prognostic relevance of isolated tumour cells and micrometastases separately, as identified by SLN procedures. Future randomized trials should establish if adjuvant chemotherapy improves survival in these patients. When a reliable SNP has been developed and the clinical relevance of occult tumour cells is known, studies which investigate SLN-picking combined with local excision of the primary tumour when no metastases are found in the SLNs can be designed as final step. This treatment approach would dramatically change therapy options for patients with early staged tumours, and could potentially decrease surgery-related morbidity rates while improving patient survival [26, 27].

Intraoperative real-time optical molecular imaging

Since ICG is exclusively cleared by the liver and excreted into the bile, it can be used for imaging of the biliary tree during laparoscopic cholecystectomy. The main advantage of intraoperative NIR fluorescence imaging of the extrahepatic biliary structures is the potential to avoid bile duct injuries in patients. For patients with complicated cholecystolithiasis this application could be of particular value because identification of structures is often difficult due to adhesions or severe edema caused by an acute or post inflammatory state. Currently, only a few studies have included patients with complicated cholecystolithias in particular cholecystitis [28-30]. Moreover, ours are the only results on fluorescent bile duct imaging after biliary pancreatitis, percutaneous gallbladder drainage or after endoscopic retrograde cholangiopancreatography (ERCP). In contrast to patients with uncomplicated gallstone disease, fluorescent bile duct imaging seems to be difficult in patients with complicated gallstone disease, see **Chapter 8**.

The cause of this difference in performance between gallbladder pathologies is unclear. We hypothesized that a suboptimal dosage, concentration or inappropriate time interval between administration of ICG and surgery contributes to this diminished visualization. In patients with uncomplicated cholecystitis the optimal time-frame between ICG administration and imaging seem to be between 3 to 24 hrs [31,32]. In patients with complicated cholecystitis a decreased liver function could cause a delayed hepatic clearance of ICG. As a consequence this time-interval should be exceeded, although this is probably not feasible in daily practice since patients with complicated cholecystitis are often operated in an unplanned acute setting. Secondly, thickened (post) inflammatory tissue may also impede the fluorescent signal. Future studies must show whether these limitations can be overcome, as NIR fluorescence cholangiography has real potential in the prevention of bile duct injuries in patients with complicated cholecystolithiasis.

One strategy to improve extrahepatic bile duct visualization is injection of the fluorophore directly into the gallbladder. A major advantage of this technique is the absence of background fluorescence in the liver due to accumulation after intravenous injection. Current published results regarding visualization of the bile duct using this method are similar to those for intravenous injection [33]. An additional complex surgical procedure consisting of intraoperative needle puncture, preparation of a purse-string at the gallbladder fundus and injection of ICG into the purse-string requires advanced surgical skills and can be time-consuming. The major limitation of this technique is the optical contamination of the operative field at NIR visualization due to ICG leakage. The technique could be interesting for patients who already have a transhepatic drain or in difficult cases when additional imaging would increase the safety of the cholecystectomy. Future studies should seek to refine the technique and compare systematic versus intragallbladder injection in patients with complicated cholecystolithiasis.

Based on our results in this thesis, we believe that NIR-fluorescent cholangiography with ICG is a potentially promising technique for the avoidance of bile duct injuries or to replace IOC for biliary mapping. However, future research focusing on optimization of the technique and standardization of doses, concentration and timing of administration is necessary to gain wide clinical acceptance. Moreover, the low rate of bile duct injury in uncomplicated cholecystolithiasis indicates that a very large number of patients would need to be monitored. Practical clinical efficacy of the technique will probably not be found in the prevention of bile duct injuries but rather in the earlier establishment of CVS, resulting in cost reductions. The first multicenter randomized controlled study is currently underway with this alternative endpoint as its main objective [34]. Meanwhile, there could be a tremendous potential benefit of early visualization of the extrahepatic bile ducts in patients with complicated cholecystolithiasis using fluorescent

cholangiography. However, NIR fluorescence cholangiography in patients with more severe gallbladder pathologies appears to create a complex situation which influences the efficacy of the technique. The technique, dosage and timing in these patients should be investigated separately from patients with uncomplicated cholecystolithiasis.

Another interesting application of NIR fluorescence imaging in abdominal surgery not yet discussed in this thesis is the visualization of micro-perfusion of the bowel to prevent anastomotic leakage. Anastomotic insufficiency leading to anastomotic leakage is a serious complication during colorectal (cancer) surgery. Currently, the selection of an optimal site for anastomosis with adequate perfusion is determined by the intraoperative opinion of the surgeon. Several studies have suggested that fluorescence ICG-mediated angiography improves the outcome of laparoscopic anastomotic bowel surgery by changing the surgical plan [35, 36]. Although NIR fluorescence imaging in this application appears to be easy, safe and effective, there is still a lack of large, well-designed randomized trial evidence for its routine use in colorectal surgery. Furthermore, most studies have not used an objective quantification of the fluorescent signal to assess optimal tissue perfusion. Imaging software that measures fluorescence intensity is currently under investigation in several studies [36-38]. Tools that quantify bowel perfusion will help develop the technique as a reliable instrument and should be integrated in future studies.

Tumor-targeted molecular imaging

Tumor-targeted molecular imaging has only briefly been discussed in this thesis so far but will probably prove to be the most important and revolutionary application in abdominal surgery, especially in the treatment of malignancies.

Visualization of tumor boundaries and tumor spread is pivotal to complete tumor resection, while preventing excision of unnecessary tissue that may cause surgery-related complications. Tumor-targeted molecular imaging exploits tumor-specific biomarkers that are overexpressed by the tumor of interest and which can be targeted by molecules (e.g. monoclonal antibodies or small peptides) [39] easily conjugated to fluorophores, radioactive isotopes or both.

In abdominal surgery, tumor-targeted molecular imaging could improve surgical outcomes in patients with peritoneal metastases and might help achieve complete resection in organ-sparing surgery. Treatment of patients with peritoneal metastases consists of cytoreduction surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC). Survival of patients depends on the extent of disease and completeness of the cytoreduction. Therefore,

accurate patient selection and macroscopically complete cytoreduction are of the utmost importance. Current preoperative imaging modalities have limited value in the detection of peritoneal metastases since most lesions are too small to be identified with PET, CT or MRI [40]. Tumor-targeted molecular imaging would be desirable in patients with PM in terms of selecting those patients who will ultimately benefit from surgery and achieving complete cytoreduction. In pancreatic and rectal cancer the technique can be used to improve radical resection and diminish local recurrence or distant metastases. Additionally, it could help to evaluate response to chemoradiotherapy in rectal cancer patients, which in turn might improve selection of patients who could benefit from additional surgery. Several phase I and II studies with fluorophore-labeled tumor-specific antibodies are underway in patients with colon, rectal and pancreatic cancer (NTR5673; NCT02743975; NCT03384238; NCT02973672; NCT03659448; NCT01972373). Furthermore, a study using a combination of the SPECT/CT nuclide Indium-III, fluorescent tracer IRDye800CW and the carcinoembryonic antigen-specific antibody, Labetuzumab, is presently being conducted in patients with peritoneal metastases of colorectal cancer (NCT03699332). Tumor localization and resection will be defined by a combination of preoperative imaging with intraoperative targeted radio- and fluorescent-guided surgery. The results of this study will be very interesting with regard to the impact of tumor-targeted imaging on perioperative clinical decision making in these patients. Eventually, future phase III studies should focus on the impact of tumor-targeted imaging on clinical endpoints such as morbidity rates, quality of life and progression-free or overall survival rates.

General conclusion

In this thesis we described the application of image-guided molecular imaging techniques for SLN identification in colon cancer and during laparoscopic cholecystectomies. Intraoperative NIR fluorescence imaging proved feasible for SLN mapping in colon cancer and bile duct imaging, and in both applications has the potential to improve surgical outcomes, patient survival and safety. The development of new fluorescent tracers and clinical imaging systems that improve the penetration depth of the NIR-signal are important to overcoming current limits of visualization. To improve SLN mapping in colon cancer, a highly sensitive preoperative nuclear imaging technique would bring additional benefit by creating a road-map of the number and location of SLNs. Moreover, careful patient selection regarding disease pathology would help identify those who would potentially derive the most benefit from additional pre-and/or intraoperative molecular imaging. In the future, the characteristics of the patient and pathology should be matched to the specific properties of an imaging technique, thus optimizing molecular image-guided surgery for each indication. This should be followed by large, well-designed

randomized trials to assess the clinical implementation of these techniques in abdominal surgery.

REFERENCES

1. Hagiike M, Phillips EH, Berci G. Performance differences in laparoscopic surgical skills between true high-definition and three-chip CCD video systems. Surg Endosc. 2007;21(10):1849-54.
2. Wilhelm D, Reiser S, Kohn N, Witte M, Leiner U, Muhlbach L, et al. Comparative evaluation of HD 2D/3D laparoscopic monitors and benchmarking to a theoretically ideal 3D pseudodisplay: even well-experienced laparoscopists perform better with 3D. Surg Endosc. 2014;28(8):2387-97.
3. Hojo T, Nagao T, Kikuyama M, Akashi S, Kinoshita T. Evaluation of sentinel node biopsy by combined fluorescent and dye method and lymph flow for breast cancer. Breast. 2010;19(3):210-3.
4. Handgraaf HJM, Boonstra MC, Prevoo H, Kuil J, Bordo MW, Boogerd LSF, et al. Real-time near-infrared fluorescence imaging using cRGD-ZW800-1 for intraoperative visualization of multiple cancer types. Oncotarget. 2017;8(13):21054-66.
5. Hong G, Robinson JT, Zhang Y, Diao S, Antaris AL, Wang Q, et al. In vivo fluorescence imaging with Ag2S quantum dots in the second near-infrared region. Angew Chem Int Ed Engl. 2012;51(39):9818-21.
6. Stoffels I, Morscher S, Helfrich I, Hillen U, Leyh J, Burton NC, et al. Metastatic status of sentinel lymph nodes in melanoma determined noninvasively with multispectral optoacoustic imaging. Sci Transl Med. 2015;7(317):317ra199.
7. Chen Z, Dean-Ben XL, Gottschalk S, Razansky D. Performance of optoacoustic and fluorescence imaging in detecting deep-seated fluorescent agents. Biomed Opt Express. 2018;9(5):2229-39.
8. Hekman MC, Rijpkema M, Muselaers CH, Oosterwijk E, Hulsbergen-Van de Kaa CA, Boerman OC, et al. Tumor-targeted Dual-modality Imaging to Improve Intraoperative Visualization of Clear Cell Renal Cell Carcinoma: A First in Man Study. Theranostics. 2018;8(8):2161-70.
9. Meershoek P, van Oosterom MN, Simon H, Mengus L, Maurer T, van Leeuwen PJ, et al. Robot-assisted laparoscopic surgery using DROP-IN radioguidance: first-in-human translation. Eur J Nucl Med Mol Imaging. 2019;46(1):49-53.
10. de Haas RJ, Wicherts DA, Hobbelink MG, van Diest PJ, Vleggaar FP, Borel Rinkes IH, et al. Sentinel lymph node mapping in colon cancer using radiocolloid as a single tracer: a feasibility study. Nucl Med Commun. 2012;33(8):832-7.
11. Tiffet O, Kaczmarek D, Chambonniere ML, Guillan T, Baccot S, Prevot N, et al. Combining radioisotopic and blue-dye technique does not improve the false-negative rate in sentinel lymph node mapping for colorectal cancer. Dis Colon Rectum. 2007;50(7):962-70.
12. Heuveling DA, van Schie A, Vugts DJ, Hendrikse NH, Yaqub M, Hoekstra OS, et al. Pilot study on the feasibility of PET/CT lymphoscintigraphy with 89Zr-nanocolloidal albumin for sentinel node identification in oral cancer patients. J Nucl Med. 2013;54(4):585-9.
13. Heller S, Zanzonico P. Nuclear probes and intraoperative gamma cameras. Semin Nucl Med. 2011;41(3):166-81.
14. Heuveling DA, Karagozoglu KH, Van Lingen A, Hoekstra OS, Van Dongen G, De Bree R. Feasibility of intraoperative detection of sentinel lymph nodes with 89-zirconium-labelled nanocolloidal albumin PET-CT and a handheld high-energy gamma probe. EJNMMI Res. 2018;8(1):15.
15. Hekman MCH, Rijpkema M, Bos DL, Oosterwijk E, Goldenberg DM, Mulders PFA, et al. Detection of Micrometastases Using SPECT/Fluorescence Dual-Modality Imaging in a CEA-Expressing Tumor Model. J Nucl Med. 2017;58(5):706-10.
16. Van Den Berg NS, Buckle T, Kleinjan GI, Klop WM, Horenblas S, Van Der Poel HG, et al. Hybrid tracers for sentinel node biopsy. Q J Nucl Med Mol Imaging. 2014;58(2):193-206.

17. KleinJan GH, van Werkhoven E, van den Berg NS, Karakullukcu MB, Zijlmans H, van der Hage JA, 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.

18. Saha S, Johnston G, Korant A, Shaik M, Kanaan M, Johnston R, et al. Aberrant drainage of sentinel lymph nodes in colon cancer and its impact on staging and extent of operation. Am J Surg. 2013;205(3):302-5; discussion 5-6.

19. Verrill C, Carr NJ, Wilkinson-Smith E, Seel EH. Histopathological assessment of lymph nodes in colorectal carcinoma: does triple levelling detect significantly more metastases? J Clin Pathol. 2004;57(11):1165-7.

20. Sloothaak DA, Sahami S, van der Zaag-Loonen HJ, van der Zaag ES, Tanis PJ, Bemelman WA, et al. The prognostic value of micrometastases and isolated tumour cells in histologically negative lymph nodes of patients with colorectal cancer: a systematic review and meta-analysis. Eur J Surg Oncol. 2014;40(3):263-9.

21. Iddings D, Ahmad A, Elashoff D, Bilchik A. The prognostic effect of micrometastases in previously staged lymph node negative (N0) colorectal carcinoma: a meta-analysis. Ann Surg Oncol. 2006;13(11):1386-92.

22. Nicastri DG, Doucette JT, Godfrey TE, Hughes SJ. Is occult lymph node disease in colorectal cancer patients clinically significant? A review of the relevant literature. J Mol Diagn. 2007;9(5):563-71.

23. Sloothaak DAM, van der Linden RLA, van de Velde CJH, Bemelman WA, Lips DJ, van der Linden JC, et al. Prognostic implications of occult nodal tumour cells in stage I and II colon cancer: The correlation between micrometastasis and disease recurrence. Eur J Surg Oncol. 2017;43(8):1456-62.

24. Lips DJ, Koebrugge B, Liefers GJ, van de Linden JC, Smit VT, Pruijt HF, et al. The influence of micrometastases on prognosis and survival in stage I-II colon cancer patients: the Enroute plus sign in circle Study. BMC Surg. 2011;11:11.

25. Saha S, Elgamal M, Cherry M, Buttar R, Pentapati S, Mukkamala S, et al. Challenging the conventional treatment of colon cancer by sentinel lymph node mapping and its role of detecting micrometastases for adjuvant chemotherapy. Clin Exp Metastasis. 2018;35(5-6):463-9.

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

27. Cahill RA, Bembenek A, Sirop S, Waterhouse DF, Schneider W, Leroy J, et al. Sentinel node biopsy for the individualization of surgical strategy for cure of early-stage colon cancer. Ann Surg Oncol. 2009;16(8):2170-80.

28. Daskalaki D, Fernandes E, Wang X, Bianco FM, Elli EF, Ayloo S, et al. Indocyanine green (ICG) fluorescent cholangiography during robotic cholecystectomy: results of 184 consecutive cases in a single institution. Surg Innov. 2014;21(6):615-21.

29. Dip F, Roy M, Lo Menzo E, Simpfendorfer C, Szomstein S, Rosenthal RJ. Routine use of fluorescent incisionless cholangiography as a new imaging modality during laparoscopic cholecystectomy. Surg Endosc. 2015;29(6):1621-6.

30. Tagaya N, Shimoda M, Kato M, Nakagawa A, Abe A, Iwasaki Y, et al. Intraoperative exploration of biliary anatomy using fluorescence imaging of indocyanine green in experimental and clinical cholecystectomies. J Hepatobiliary Pancreat Sci. 2010;17(5):595-600.

31. Boogerd LSF, Handgraaf HJM, Huurman VAL, Lam HD, Mieog JSD, van der Made WJ, et al. The Best Approach for Laparoscopic Fluorescence Cholangiography: Overview of the Literature and Optimization of Dose and Dosing Time. Surg Innov. 2017;24(4):386-96.

32. Verbeek FP, van der Vorst JR, Schaafsma BE, Hutteman M, Bonsing BA, van Leeuwen FW, et al. Image-guided hepatopancreatobiliary surgery using near-infrared fluorescent light. J Hepatobiliary Pancreat Sci. 2012;19(6):626-37.

33. Liu YY, Liao CH, Diana M, Wang SY, Kong SH, Yeh CN, et al. Near-infrared cholecystocholangiography with direct intragallbladder indocyanine green injection: preliminary clinical results. Surg Endosc. 2018;32(3):1506-14.

34. van den Bos J, Schols RM, Luyer MD, van Dam RM, Vahrmeijer AL, Meijerink WJ, et al. Near-infrared fluorescence cholangiography assisted laparoscopic cholecystectomy versus conventional laparoscopic cholecystectomy (FALCON trial): study protocol for a multicentre randomised controlled trial. BMJ Open. 2016;6(8):e011668.

35. Blanco-Colino R, Espin-Basany E. Intraoperative use of ICG fluorescence imaging to reduce the risk of anastomotic leakage in colorectal surgery: a systematic review and meta-analysis. Tech Coloproctol. 2018;22(1):15-23.

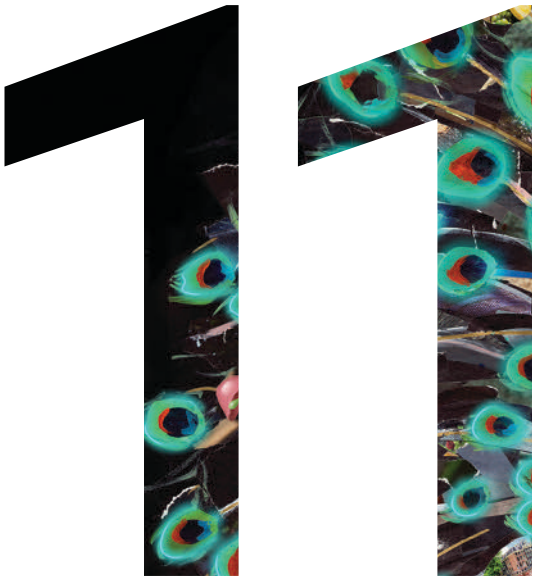
36. van den Bos J, Al-Taher M, Schols RM, van Kuijk S, Bouvy ND, Stassen LPS. Near-Infrared Fluorescence Imaging for Real-Time Intraoperative Guidance in Anastomotic Colorectal Surgery: A Systematic Review of Literature. J Laparoendosc Adv Surg Tech A. 2018;28(2):157-67.

37. Sherwinter DA, Gallagher J, Donkar T. Intra-operative transanal near infrared imaging of colorectal anastomotic perfusion: a feasibility study. Colorectal Dis. 2013;15(1):91-6.

38. Diana M, Agnus V, Halvax P, Liu YY, Dallemagne B, Schlagowski AI, et al. Intraoperative fluorescence-based enhanced reality laparoscopic real-time imaging to assess bowel perfusion at the anastomotic site in an experimental model. Br J Surg. 2015;102(2):e169-76.

39. Zhang RR, Schroeder AB, Grudzinski JJ, Rosenthal EL, Warram JM, Pinchuk AN, et al. Beyond the margins: real-time detection of cancer using targeted fluorophores. Nat Rev Clin Oncol. 2017;14(6):347-64.

40. Klumpp BD, Schwenzer N, Aschoff P, Miller S, Kramer U, Claussen CD, et al. Preoperative assessment of peritoneal carcinomatosis: intraindividual comparison of 18F-FDG PET/CT and MRI. Abdom Imaging. 2013;38(1):64-71.



SUMMARY

DUTCH SUMMARY

SUMMARY

SUMMARY

PART I MOLECULAR IMAGE-GUIDED SURGERY IN COLON CANCER

The introduction of nationwide screening programs, increasing life expectancy, healthy ageing and increasing awareness of symptoms, will lead to a dramatic increase of patients diagnosed with early staged tumours without lymph node metastases (stage I-II). Especially patients with stage I disease, can be potentially treated with local excision of the primary tumour that would prevent them from surgery-related complications and even mortality. However, up to 20% of patients with stage I-II disease, show disease recurrence and eventually die within five years after initial treatment despite complete surgical resection. This is probably the result of understaging due to missed occult tumour cells (micrometastases or isolated tumour cells) during routine histopathological examination. It is known that advanced histopathological techniques like serial sectioning combined with immunohistochemistry are highly sensitive for detection of occult tumour cells. However, the resulting workload of extensive histopathological examination to all lymph nodes is not compatible with daily practice. The Sentinel Lymph Node (SLN) is the first node in the orderly lymphatic drainage pattern from the primary tumour and has therefore the highest change of harboring metastases. Identification of the SLN allows the pathologist to perform detailed histopathological analysis to only the SLNs in addition to conventional H&E-staining. As a result, identification of the SLN could improve lymph node staging while diminishing the surgical damage in patients with early colon cancer without lymph node metastases.

In **Chapter 2** we describe the concept of the SLN procedure (SNP) and the current drawbacks in colon cancer. Major concernings towards the SNP are the wide variation of SLN identification techniques and lack of standardized methods regarding patient and tumour related factors. At that time the most recent literature showed a sensitivity of 86% and detection rate of 96% for the SNP in colon cancer. Results voor sensitivity are lower compared to other types of cancer in which the SLN procedure is the gold standard for surgical staging of the lymph nodes. However, it seems that identification of the true SLN is difficult in colon cancer. Technical limitations and disadvantageous characteristics of current used tracers seem to be the main causes of the unsatisfactory results. First, blue dyes have limited penetration depth and are therefore difficult to be visualized in fatty mesocolon. Secondly, the particle size of blue dye is relative small resulting in fast migration of dye to regional lymph nodes. The additional use of gamma tracers present the problem of the 'shine-through effect' of SLN close to the primary tumour which are hidden by the highly radioactive injection site. Near-infrared (NIR) fluorescence imaging

using Indocyanine Green (ICG) as contrast agent, is mentioned as solution for these technical problems. First the NIR-range (700-1000 nm) penetrates more deeply into the living tissue compared to conventional white light imaging or blue dye. Secondly, the fluorescent agent ICG exhibits strong excitation and fluorescence in the NIR-range (+/- 800 nm) and moreover it is clinically approved. The additional conjugation of humanized serum albumin (HSA) improves the fluorescent signal and enlarges the particle size, which facilitates dye retention in the SLN. In same chapter we also discuss some drawbacks of NIR fluorescence imaging based on our own experiences at that time. First spillage of dye caused by incorrect needle positioning during dye administration, results in high background fluorescence of peritoneum which makes SLN identification impossible. We also noticed that the penetration depth of NIR fluorescence imaging is better compared to blue dye, but still limited up to a maximum of 0.5-1.0 cm. We shortly discussed the promising new fluorophore IRDye800CW (LI-COR bioscience, Lincoln, NE) that has several advantages above ICG. First, IRDye800CW seems to have a stronger fluorescence signal compared to ICG. Additionally it can be covalently conjugated to several biomolecules, which makes it the dye of choice for tumour-targeted imaging. At the time, IRDye800CW was not FDA approved yet and therefore not clinically investigated.

As mentioned, no consensus exists on the validity of the SNP in colon cancer. Additionally to the tracer used, several tumour and procedure related factors are supposed to influence the performance of the SNP. In **Chapter 3** we present a systematic review and meta-analysis which provides an overview of the diagnostic performance of the SLN procedure in terms of sensitivity, negative predictive value and detection rate. To validate the hypothesis that SLNs are the most likely hosts for potential lymph node metastases and assess to what extent the SLNs are representative of the total lymph node yield, we selected high quality concept validation studies. These studies are characterized by their pathological assessment of all SLNs and regional lymph nodes with advanced histopathological techniques and therfore ensure an unbiased assessment. After a search in Embase and Pubmed we identified 47 eligible studies of which six were selected as high quality concept validation studies. Overall analysis showed a low sensitivity of 73% with a corresponding negative predictive value of 82%. Diagnostic outcome measurements decreased further to a disappointing sensitivity of 56% and negative predictive value of 69% in the high quality concept validation studies. Subanalysis of procedure related factors showed better results for sensitivity after an ex vivo procedure while in vivo harvestigs detected twice as many SLNs. The better sensitivity of the ex-vivo approach may result from better real-time visualization of lymph flow dynamics and a more specific dissection of the mesocolon to detect SLNs. On the other hand, node positive (S)LNs can be missed with the ex vivo technique when they are located outside the resection area.

Despite to overall disappointing SLN perfomance, we showed that the prevalence of lymph node metastases increased from 34% after conventional H&E-staining to 48% with advanced immunohistochemistry. These results underline the potential benefit of the SNP in colon cancer to improve staging, patient management and survival. However, it must be emphasized that the prognostic and predictive relevance of occult tumor cells is still unclear. Additionally it is suggested that only micrometastases are associated with a significant reduction in 5-year survival while their presence is much lower compared to isolated tumour cells. Future studies should investigate the prognostic relevance of isolated tumour cells and micrometastases, and establish if adjuvant chemotherapy improve survival of these patients. Moreover we conclude that the SLN performance is currently insufficient due to anatomical and technical difficulties combined with the wide variation of used SLN mapping methods, patient selection and histopathological analysis of lymph nodes. Therefore a standardized SNP must be developed, focussing on low invasive tumours and real-time imaging of lymph flow towards the SLN.

In **Chapter 4** and **Chapter 5** we continued our research towards an accurate SNP using NIR fluorescence imaging in colon cancer. In **Chapter 4** we present the result of the first 14 patients undergoing the SNP in vivo using a transcutanously subserosal injection technique. At least one SLN could be assigned in all patients of which none contained metastases. However, in four patients metastases were found in regional lymph nodes resulting in a sensitivity of 0%. We hypothesized that these very dissapoiting results could be caused by incorrect needle positioning not close enough to the tumor. This could have led to drainage of dye into adjacent lymph vessels instead of the vessels draining the SLN, resulting in high false negativity rates. Also dislocation of the needle during tracer administration occurred in several patients with extravasation of dye into the peritoneum as consequence. Therefore we switched to a submucosal injection technique by colonoscopy. In the subsequent 15 patients as described in **Chapter 5**. We experienced no spillage of dye using the submucosal injection technique and sensitivity rates increased to 80%. Additionally, a systematic review and meta-analysis was conducted to identify currently used methods and results for SLN mapping with NIR fluorescence imaging. This systematic review and meta-analysis included 8 studies describing 227 SLN procedures. A sensitivity of 63% was found accompanied by a negative predictive value of 81% and detection rate of 94%. Upstaging as a result of extended histopathological assessment was 15%. Stratified analysis showed no improvement of SLN performance regardless of used tracer, injection site, in vivo or ex vivo SLN performance, number of injections and timing between tracer administration and SLN identification. Overall we concluded that evidence regarding SLNM with NIR fluorescence imaging in colon cancer is still limited. However, the SNP in colon cancer seems to be more challenging compated to other types of cancer and considerable expertise will be required

before large patient-related studies can be undertaken to validate SLNM as part of the standard surgical treatment in colon cancer.

In **Chapter 6** we show the first clinical results of SLN identification using PET/CT lymphoscintigraphy combined with real-time NIR fluorescence imaging with ICG. We aimed to establish if preoperative [^{89}Zr]Zr-nanocoll PET/CT imaging is a useful technique to identify the number and location of SLNs and if the additional use of NIR fluorescence imaging allows for intraoperative identification of these SLNs. Three preoperative PET/CT lymphoscintigraphy and additional PET/CT scan of the surgical specimen were made. A lymph node visible at preoperative PET/CT and identified at PET/CT of the specimen was classified as SLN. In two out of ten patients injection of [^{89}Zr]Zr-nanocoll failed. In seven out of the eight remaining patients, perioperative SLN identification succeeded with a median number of three harvested SLNs. One SLN showed isolated tumour cells. All SLNs revealed radioactivity and fluorescence. Six SLN located near the primary tumour (< 2 cm) were not identified with NIR- fluorescence imaging. This result suggests that PET/CT lymphoscintigraphy has a better performance when SLNs are located close to the injection site. The preoperative PET/CT guided the surgeon towards the fluorescent SLN intraoperatively, whereas the postoperative imaging identified additional SLNs not seen during surgery. As long as optimization of the SNP is considered as the primary aim of the current research, we recommend to perform a preoperative lymphoscintigraphy 24 hrs after injection combined with postoperative scan of the specimen.

PART II MOLECULAR IMAGE GUIDED SURGERY DURING LAPAROSCOPIC CHOLECYSTECTOMY

In the second part of this thesis we focus on NIR fluorescence imaging for visualization of biliary structures during laparoscopic cholecystectomy. NIR fluorescence imaging could prevent bile duct injuries and may replace the current intraoperative cholangiography (IOC) as imaging technique.

In **Chapter 7** we investigated the value of NIR fluorescence imaging with ICG in addition to the Critical View of Safety (CVS) for early identification of the extrahepatic bile ducts during laparoscopic cholecystectomy in patients with uncomplicated cholecystolithiasis. Thirty patients were included. An intravenous injection of 0.05 mg/ kg ICG diluted in water was administrated prior to surgery. The Common Bile Duct (CBD) and Cystic Duct (CD) were both significant earlier identified with NIR fluorescence imaging compared to conventional white light imaging. Additionally, the CBD was identified significantly more frequently during dissection and at CVS with NIR fluorescence imaging.

Early visualization of the CD and additional identification of the CBD could be of great value when biliary anatomy is unclear or abnormal. Therefore the efficacy and early visualization of the CD and added value of CBD identification with NIR fluorescence imaging in patients with complicated gallbladder disease, was investigated in **Chapter 8**. Eighteen patients were included in this study. Patients received an intravenous bolus of 0.2 mg/kg ICG diluted in water directly after induction of general anesthesia. At the first look, which was set just before dissection of Calot's triangle, the CD was observed in three patients with conventional imaging and in four patients using NIR fluorescence imaging. At this time point, the CBD could be additionally visualized in only two patients using NIR fluorescence imaging. A second look was established early during dissection but before skeletonizing biliary structures. In five patient CVS was already reached before the second look could be obtained. The CD and CBD could be visualized in two patients using NIR fluorescence imaging at the second time-point, preventing conversion to an open procedure in one patient. Disappointingly, at CVS the CD was visualized in only 13 patients using NIR fluorescence imaging while conventional white light imaging identified the CD in all 18 patients. Additionally, the CBD could only be visualized in seven patients. We hypothesized that these inferior results of fluorescent bile duct imaging in complicated cases compared to uncomplicated gallbladder disease, could be caused by severe edema or dense adhesions as a result of acute or past inflammation or after ERCP combined with the limited penetration depth of NI- fluorescence imaging. Other patient and procedure related factors such as high Body Mass Index (BMI), optimal dosages and timeframes between ICG administration and bile duct visualization might also influence the success rate of the procedure, especially in complicated cases. Therefore we concluded that future research should focus on optimizing the technique, dosage, timing and patient selection in order to establish whether NIR -luorescence imaging can help to prevent bile duct injuries and if there is a place for routing use of the technique during laparoscopic cholecystectomy.

In **Chapter 9** we present a systematic review in which we evaluated visualization of the CD, CBD and Common Hepatic Duct (CHD) before and after dissection of Calot's triangle with NIR fluorescence imaging using ICG during laparoscopic cholecystectomy. We additionally compared biliary structure visualization between ICG and IOC in a meta-analysis. After a search in PubMed, Embase, the Cochrane Library and Web of Science, 19 eligible studies were identified, presenting 779 patients whereof only 78 patients suffered from complicated gallbladder disease. Results suggest that the use of NIR fluorescence imaging with ICG provides good overall visualization rates of the CD, CBD, CHD and CD junction prior to and following dissection of Calot's triangle. Visualization rates of the biliary structures appear to be equally good for either 2.5 mg fixed dosage or 0.05 mg/ kg dosage of ICG. Small variation of timing of

ICG administration was seen, varying between over an hour before surgery until directly after anesthesia. We found moderate quality evidence that visualization of the CD and CBD with ICG is better than IOC. This finding combined with the higher costs of IOC, more difficult perioperative logistics, radiation exposure and risk of bile duct injury, ICG might be considered to be a better option for visualization of the biliary tract. However, further research is necessary to confirm this recommendation. Overall, no conclusions could be drawn whether NIR fluorescence imaging with ICG provides advantages over conventional white light imaging during laparoscopic cholecystectomy or in the prevention of bile duct injuries. Future randomized trials must be enrolled, including a hetrogenous patient population and with clear definitions for uncomplicated and complicated gallbladder disease.

DUTCH SUMMARY

DUTCH SUMMARY

DEEL I MOLECULAIRE BEELDGELEIDENDE CHIRURGIE BIJ DIKKE DARM TUMOREN

Door de invoering van landelijke screeningsprogramma's, stijgende levensverwachting, toenemende aantal gezonde ouderen en bewustzijn van (vroege) alarmsymptomen, neemt aantal patiënten met dikke darmkanker in een vroeg stadium zonder tekenen van lymfklierstadiering (stadium I-II) snel toe. Potentieel kunnen deze tumoren behandeld worden met een lokale excisie van de tumor, met name wanneer het een T1 of T2 tumor betreft. Deze behandeling voorkomt blootstelling aan chirurgische gerelateerde complicaties inclusief mortaliteit van een uitgebreide en-block resectie. Het is echter ook bekend dat 20% van de patiënten na een radicale resectie van een stadium I-II tumor, zich later alsnog presenteert met metastasen op afstand waarvan een deel binnen 5 jaar na initiële behandeling overlijdt. Dit is mogelijk het gevolg van onderstadiëring door het missen van occulte tumor cellen in de lymfklieren (micrometastasen en geïsoleerde tumor cellen) tijdens conventionele histopathologische beoordeling. Het is bekend dat de detectie van occulte tumor cellen toeneemt door gebruik te maken van aanvullende uitgebreide histopathologische technieken, zoals het uitsnijden van meerdere opeenvolgende coupes gecombineerd met immuunhistochemie. Deze aanvullende technieken zijn echter duur en tijdrovend waardoor niet geschikt voor dagelijks gebruik. De schildwachtklier (SWK) is de eerste lymfklier waar de lymfebanen vanaf de primaire tumor naartoe draineren, en heeft daardoor de grootste kans op het bevatten van metastasen. Het identificeren van deze SWK biedt de mogelijkheid om juist op deze lymfklier uitgebreide en gedetailleerde histopathologische analyse te doen aanvullend op conventionele H&E-kleuring. De SWK-procedure kan enerzijds een lokale excisie van de primaire tumor en daarmee dus de kans op chirurgische complicaties verkleinen, wanneer deze negatief is voor metastasen. Anderzijds kan de SWK-procedure het detecteren van metastasen juist optimaliseren en daarmee patientoverleving verbeteren.

In **hoofdstuk 2** beschrijven we het concept van de SWK-procedure en huidige tekortkomingen van de techniek bij dikke darmkanker. Destijds liet de literatuur een sensitiviteit van 86% en detectie van 96% zien, wat lager is ten opzichte van andere tumoren waarbij de SWK-procedure al standaard wordt toegepast. De grote variatie in gebruikte technieken en de afwezigheid van standaardisatie ten aanzien van patiënt- en tumor gerelateerde factoren, worden aangewezen als oorzaken van dit mindere resultaat. Ook technische tekortkomingen en eigenschappen van de gebruikte tracers lijken hieraan bij te dragen. Als eerste wordt de penetratiediepte van de meest gebruikte tracer ' blue dye' besproken. Deze is zeer beperkt en daardoor

moeilijk te visualiseren wanneer SWKs diep in het vettige mesocolon liggen. Tevens is de molecuulgrootte van blue dye relatief klein waardoor deze snel migreert naar opeenvolgende regionale lymfklieren. Het additioneel gebruik van gamma-tracers lijkt niet direct bij te dragen aan de verbetering van de SWK-procedure. Dit komt doordat SWKs nabij de tumor overstraald worden door het zeer radioactieve injectiedepot ter plaatse van de primaire tumor. Nabij-infrarode fluorescentiebeeldvorming (NIR fluorescent imaging) met Indocyanine Groen (ICG) als geleidende fluorescerende tracer in plaats van blue dye, wordt gezien als mogelijke oplossing voor deze technische problemen. De verhouding tussen het signaal en de achtergrond is het groots bij nabij-infrarode golflengtes (700-1000 nm) waardoor diepgelegen structuren beter gevisualiseerd kunnen worden ten opzichte van conventioneel wit licht en blue dye. Indocyanine Groen heeft een emissiegolflengte rond 800 nm waarmee het geschikt is voor NIR fluorescente beeldvorming en wordt al klinisch gebruikt. Het toevoegen van gehumaniseerd albumine (HSA) lijkt tevens het fluorescente signaal te versterken en het molecuul te vergroten wat retentie van ICG in de SWK bevorderd. In dit hoofdstuk bespreken we naar aanleiding van onze eigen ervaringen, ook enkele tekortkomingen van NIR fluorescente beeldvormende techniek als methode voor de SWK-procedure in dikke darmkanker. Ten eerste kan tijdens het injecteren ICG lekken in de intra-abdominale ruimte waardoor er veel achtergrond fluorescentie ontstaat, wat identificatie van de SWK onmogelijk maakt. Ook bleek dat diepgelegen lymfklieren nog steeds moeilijk te detecteren zijn met NIR fluorescente beeldvorming door de alsnog beperkte penetratiediepte van 0.5-1.0 cm. In dit hoofdstuk bespreken we kort een veelbelovende nieuwe fluorescerende tracer IRDye800CW (LI-COR bioscience, Lincoln, NE) welke enkele voordelen heeft ten opzichte van ICG. Ten eerste lijkt het een sterker fluorescent signaal te hebben dan ICG. Daarnaast is het mogelijk IRDye800CW covalent te binden met verschillende biomoleculen waardoor het een zeer geschikte is voor specifieke aankleuring van tumoren. Destijds was IRDye800CW nog niet goedgekeurd door de FDA en kon dus niet klinisch onderzocht worden.

In **hoofdstuk 3** gaan we dieper in op de validiteit van de SWK-procedure en beschrijven we in een systematic review met meta-analyse de huidige sensitiviteit, negatief voorspellende waarde en detectie van de SWK-procedure bij dikke darmkanker. Na een uitgebreide zoekopdracht in Pubmed en Embase, werden 47 studies geïncludeerd. Deze studies laten een sensitiviteit van 73% zien met een bijbehorende negatief voorspellende waarde van 82%. Om vast te stellen of de SWK een correct voorspellende waarde heeft voor lymfkliermetastasen, zouden alle SWKs en regionale lymfklieren geanalyseerd moeten worden met aanvullende histopathologische technieken naast conventionele H&E-kleuring. Daarom worden zes 'high-quality studies' geselecteerd die alle lymfklieren met deze aanvullende technieken onderzoeken. In deze high-quality studies daalt de sensitiviteit naar 56% en negatief voorspellende waarde naar 69%.

Subanalyses laten zien dat een ex-vivo uitgevoerde SWK-procedure een betere sensitiviteit geeft maar dat er bij een in-vivo procedure twee keer meer SWKs worden gevonden. Waarschijnlijk is de betere sensitiviteit van de ex-vivo procedure toe te schrijven aan de directe visualisatie van de lymfedrainage en meer specifieke dissectie van het mesocolon. Aan de andere kant kunnen positieve (schildwacht) klieren juist gemist worden met de ex-vivo techniek wanneer deze buiten het resectiegebied liggen. Ondanks de teleurstellende resultaten van de SWK-procedure beschreven in dit hoofdstuk, blijkt dat het aanvullend uitgebreid histopathologisch analyseren van de lymfklieren wel degelijk een toename geeft van het aantal lymfkliermetastasen van 34% naar 48%. Hierbij moet worden opgemerkt dat de prognostisch en voorspellende waarde van de veelal gevonden occulte tumor cellen nog steeds onduidelijk is. Daarbij wordt in de literatuur gesuggereerd dat alleen micrometastasen van invloed zijn op de vijfjaarsoverleving terwijl deze minder frequent voorkomen dan geïsoleerde tumorcellen. Toekomstige studies moeten dan ook onderzoeken wat de prognostische waarde is van micrometastasen danwel geïsoleerde tumorcellen, en of behandeling met adjuvante chemotherapie de overleving van deze patiënten beïnvloed. Bovendien blijkt uit deze studie dat de SWK-procedure door anatomische en technische uitdagingen gecombineerd met de grote variatie in de gebruikte methoden nog niet voldoende is ontwikkeld bij dikke darmkanker. Om de SWK-procedure te verbeteren is gestandaardiseerd onderzoek nodig, wat zich specifiek richt op vroegtumoren en gebruik maakt van optimale beeldvormende technieken.

In **hoofdstuk 4** en **hoofdstuk 5** beschrijven we onze resultaten van de SWK-procedure met NIR fluorescente beeldvorming. In **hoofdstuk 4** laten we de resultaten zien van de SWK-procedure na transcutane subserosale injectie met ICG. In alle 14 patiënten kon een SWK zonder metastasen worden aangewezen. Echter in vier patiënten werden wel uitzaaiingen gevonden in regionale lymfklieren wat resulteert in een sensitiviteit van 0%. Dit zeer teleurstellende resultaat is mogelijk veroorzaakt door het onvermogen om met deze injectietechniek, de tracer dichtbij de tumor te injecteren. Dit kan zorgen voor drainage van tracer in aangrenzende lymfebanen in plaats van de lymfebanen, die direct vanaf de tumor draineren. Daarnaast bleek het ook lastig te zijn de naald goed gepositioneerd te houden gedurende injectie, met dislocatie en extravasatie van dye in het peritoneum als gevolg. Derhalve werd in de daaropvolgende 15 patiënten een coloscopie verricht waarbij ICG nabij de tumor en in de submucosale laag werd geïnjecteerd, zie **hoofdstuk 5**. Dit bleek een technisch eenvoudige injectiemethode te zijn zonder lekkage van ICG in de peritoneale ruimte, wat resulteerde in een sensitiviteit van 80%. Aanvullend werd, in **hoofdstuk 5**, een systematic review en meta-analyse verricht om de huidige methoden en resultaten van de SWK-procedure met NIR fluorescente beeldvorming in kaart te brengen. Er werden 8 studies geïncludeerd waarin 227 SWK-procedures werden uitgevoerd. Een sensitiviteit

van 63% werd gevonden met een daarbij behorende negatief voorspellende waarde van 81% en detectie ratio van 94%. In 15% van de patiënten werden metastasen gevonden in de SWKs na aanvullende histopathologische analyse. Er werden geen verschillen gezien voor de SWK-procedure op basis van gebruikte tracer, injectie methode, tijdsinterval tussen injectie en SWK identificatie of wanneer deze in- of ex vivo werd uitgevoerd. Echter het aantal studies die de SWK-procedure met NIR fluorescente beeldvorming heeft onderzocht is nog beperkt. Wel concluderen we aan de hand van deze resultaten, dat de SWK-procedure bij dikke darmkanker ook met NIR fluorescente beeldvorming moeilijker is in vergelijking met andere vormen van kanker. Er is dan ook meer expertise nodig voordat grote klinische studies naar de validatie van de SWK-procedure als onderdeel van de standaard chirurgische behandeling van dikke darmkanker kunnen worden gestart.

In **hoofdstuk 6** beschrijven we de eerste resultaten van de SWK-procedure waarbij we preoperatieve PET/CT lymfscintigrafie combineren met intra-operatieve NIR fluorescente beeldvorming. We maken hierbij gebruik van de radioactieve stof [^{89}Zr]Zr-nanocoll en ICG. Het doel in deze studie is om vast te stellen of preoperatieve beeldvorming met [^{89}Zr]Zr-nanocoll PET/CT bijdraagt aan het lokaliseren van aantal en locatie SWKs, en of deze overeenkomen met de SWKs die tijdens de operatie geïdentificeerd worden met NIR fluorescente beeldvorming. Er worden drie preoperatieve [^{89}Zr]Zr-nanocoll PET/CT lymfscintigrafieën gemaakt om de kinetiek en biodistributie van de tracer vast te stellen. Tevens wordt een postoperatieve PET/CT van het preparaat gemaakt. Een lymfklier wordt beschouwd als SWK wanneer deze gezien wordt op preoperatieve PET/CT en op de scan van het preparaat. In twee van de tien patiënten was er sprake van inadequate injectie van [^{89}Zr]Zr-nanocoll. In zeven patiënten konden SWKs geïdentificeerd worden, welke allen naast radioactief ook fluorescent waren. In één SWK werden geïsoleerde tumorcellen aangetroffen. Zes SWKs nabij de primaire tumor (< 2 cm) werden niet gedetecteerd met NIR fluorescente beeldvorming. Dit suggereert dat PET/CT lymfscintigrafie beter in staat is om SWKs dichtbij de primaire tumor te detecteren ten opzichte van NIR fluorescente beeldvorming. Verder faciliteerde de preoperatieve PET/CT de chirurg bij het intra-operatief detecteren van de fluorescente SWKs en werden nog enkele aanvullende SWKs geïdentificeerd op de postoperatieve PET/CT-scan. In deze fase van het onderzoek waarin optimalisatie van de SWK-procedure nog het primaire doel is, adviseren wij dan ook een preoperatieve lymfscintigrafie scan 24 uur na tracer injectie te combineren met een postoperatieve scan van het preparaat.

DEEL II MOLECULAIRE BEELDGELEIDENDE CHIRURGIE TIJDENS DE LAPAROSCOPISCHE CHOLECYSTECTOMIE

In het tweede deel van dit proefschrift onderzoeken we de visualisatie van biliaire structuren tijdens de laparoscopische cholecystectomie met NIR fluorescente beeldvorming. Het doel van NIR fluorescente beeldvorming bij deze operatie is het voorkomen van galwegletsel en het vervangen van de intra-operatieve cholangiografie (IOC) als beeldvormende techniek.

In **hoofdstuk 7** onderzoek we of vroege identificatie van de extraheptische galwegen met behulp van NIR fluorescente beeldvorming van toegevoegde waarde is als aanvulling op de 'Critical View of Safety' (CVS) bij patiënten die een laparoscopische cholecystectomie ondergaan voor ongecompliceerd galsteenlijden. Bij 30 patiënten werd preoperatief een oplossing van 0.05 mg/kg ICG/water intraveneus geïnjecteerd. De ductus cysticus werd significant eerder gedetecteerd met NIR fluorescente beeldvorming ten opzichte van conventionele beeldvorming. Tevens werd de ductus choledochus met NIR fluorescente beeldvorming vaker geïdentificeerd tijdens het vrijmaken van de driehoek van Calot en ook ten tijde van CVS.

Aangezien vroege visualisatie van de ductus cysticus en aanvullende identificatie van de ductus choledochus juist van grote toegevoegde waarde kan zijn wanneer de anatomie onduidelijk of afwijkend is, wordt de techniek in **hoofdstuk 8** onderzocht in patiënten met gecompliceerd galsteenlijden. In 18 patiënten werd net na algehele anesthesie een oplossing van 0.02 mg/kg ICG/water intraveneus geïnjecteerd. Tijdens de operatie werd vlak voor het vrijmaken van de driehoek van Calot en vroeg tijdens de dissectie, getracht de biliaire structuren met NIR fluorescente beeldvorming te visualiseren. De ductus cysticus werd drie keer gevisualiseerd met conventionele beeldvorming en vier keer met NIR fluorescente beeldvorming vlak voor het vrijmaken van de driehoek van Calot. Daarnaast kon de ductus choledochus twee keer geïdentificeerd worden met NIR fluorescente beeldvorming. In vijf patiënten werd CVS bereikt nog voordat er een tweede keer met NIR laparoscoop gekeken kon worden. In twee patiënten werden zowel de ductus cysticus als ductus choledochus gevisualiseerd met NIR fluorescente beeldvorming, waardoor conversie naar een open procedure in één patiënt voorkomen kon worden. Helaas kon de ductus cysticus op CVS slechts in 13 patiënten gevisualiseerd worden met NIR fluorescente beeldvorming terwijl deze met conventionele beeldvorming wel in alle patiënten geïdentificeerd werd. Aanvullend werd de ductus choledochus met NIR fluorescente beeldvorming in zeven patiënten gedetecteerd. Het moeizaam zichtbaar maken van de galwegen in deze patiëntenpopulatie, is waarschijnlijk het gevolg van oedeem en adhesies rondom de hilus bij of na een acute cholecystitis of ERCP, gecombineerd met de beperkte penetratie van NIR

fluorescente beeldvorming. Andere patiënt- en procedure gebonden factoren zoals Body Mass Index (BMI), dosis en tijdsinterval tussen ICG toediening en beeldvorming, zijn mogelijk ook van invloed. Toekomstige studies moeten zich dan ook richten op deze factoren om zodoende de techniek te kunnen verbeteren en vast te stellen of galwegletsel voorkomen kan worden door het routinematig toepassen van NIR fluorescente beeldvorming tijdens de laparoscopische cholecystectomie.

In **hoofdstuk 9** onderzoeken we in een systematic review de toegevoegde waarde van NIR fluorescente beeldvorming in het voorkomen van galwegletsel tijdens de laparoscopische cholecystectomie. Visualisatie met NIR fluorescente beeldvorming van de ductus cysticus, ductus choledochus en ductus hepaticus communis zowel voor als na dissectie van de driehoek van Calot, wordt geëvalueerd. Aanvullend wordt een meta-analyse verricht waarbij visualisatie van de biliaire structuren tussen NIR fluorescente beeldvorming en IOC worden vergeleken. Negentien studies met 779 patiënten worden geïdentificeerd na een zoekopdracht in PubMed, Embase, the Cochrane Library en Web of Science. Slechts 78 geïncludeerde patiënten met gecompliceerd galsteenlijden werden geincludeerd. Resultaten van het review suggereren dat NIR fluorescente beeldvorming met ICG de ductus cysticus, ductus choledochus, ductus hepaticus communis en overgang tussen ductus cysticus en ductus hepaticus communis, zowel voor als tijdens het vrijmaken van de driehoek van Calot goed kan visualiseren. Een optimale dosis wordt gezien bij 2.5 mg ICG of bij een oplossing van 0.05 mg/kg. Het toedienen van ICG varieert tussen een uur preoperatief tot direct na algehele anesthesie. Er is mild bewijs dat visualisatie van de ductus cysticus en ductus choledochus beter is met NIR fluorescente beeldvorming ten opzichte van IOC. Echter, aangezien IOC gepaard gaat met hoge kosten, uitdagende perioperatieve logistieke planning, radioactieve straling en toenemend risico op galwegletsel, kan NIR fluorescente beeldvorming overwogen worden als betere optie voor het visualiseren van de biliaire structuren. Aanvullend onderzoek is nodig om dit daadwerkelijk vast te kunnen stellen. Aan de hand van dit review kunnen geen eenduidige conclusies worden getrokken aangaande het voordeel van NIR fluorescente beeldvorming ten opzichte van conventionele beeldvorming tijdens de laparoscopische cholecystectomie en in het voorkomen van galwegletsel. Hiervoor zullen eerst gerandomiseerde studies uitgevoerd moeten worden met een heterogene patiëntenpopulatie die tevens gebruik maken van eenduidige definities aangaande ongecompliceerd en gecompliceerd galsteenlijden.

LIST OF PUBLICATIONS

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CURRICULUM VITAE

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Ankersmit M, van der Pas MHGM, van Dam DA, Meijerink WJHJ. Near infrared fluorescence lymphatic laparoscopy of the colon and mesocolon. Colorectal Dis. 2011;7:70-73.

van der Pas MHGM, **Ankersmit M**, Stockmann HB, Silvis R, van Grieken NC, Bril H, Meijerink WJHJ. Laparoscopic sentinel lymph node identification in patients with colon carcinoma using a near-infrared dye: description of a new technique and feasibility study. J Laparoendosc Adv Surg Tech A. 2013;23:367-371.

Ankersmit M, van Dam DA, van de Ven P, van Rijswijk AS, Tuynman JB, Meijerink WJHJ. Comparing Near-Infrared Imaging with Indocyanine Green to Conventional Imaging During Laparoscopic Cholecystectomy: A Prospective Crossover Study.J Laparoendosc Adv Surg Tech A. 2015;25:486-492.

Luttikhold J, van Norren K, Buijs N, **Ankersmit M**, Heijboer AC, Gootjes J, Rijna H, van Leeuwen PA, van Loon LJ. Jejunal Casein Feeding Is Followed by More Rapid Protein Digestion and Amino Acid Absorption When Compared with Gastric Feeding in Healthy Young Men. J Nutr. 2015;145:2033-2038.

Luttikhold J, van Norren K, Rijna H, Buijs N, **Ankersmit M**, Heijboer AC, Gootjes J, Hartmann B, Holst JJ, van Loon LJ, van Leeuwen PA. Jejunal feeding is followed by a greater rise in plasma cholecystokinin, peptide YY, glucagon-like peptide 1, and glucagon-like peptide 2 concentrations compared with gastric feeding in vivo in humans: a randomized trial. Am J Clin Nutr. 2016;103:435-443.

Vlek SL, Lier MC, **Ankersmit M**, Ket JC, Dekker JJ, Mijatovic V, Tuynman JB. Laparoscopic Imaging Techniques in Endometriosis Therapy: A Systematic Review. J Minim Invasive Gynecol. 2016;23:886-892.

Gorter RR, Eker HH, Gorter-Stam MA, Abis GS, Acharya A, **Ankersmit M**, Antoniou SA, Arolfo S, Babic B, Boni L, Bruntink M, van Dam DA, Defoort B, Deijen CL, DeLacy FB, Go PM, Harmsen AM, van den Helder RS, Iordache F, Ket JC, Muysoms FE, Ozmen MM, Papoulas M, Rhodes M, Straatman J, Tenhagen M, Turrado V, Vereczkei A, Vilallonga R, Deelder JD, Bonjer J. Diagnosis and management of acute appendicitis. EAES consensus development conference 2015. Surg Endosc. 2016;30:4668-4690.

Vlek SL, van Dam DA, Rubinstein SM, de Lange-de Klerk ESM, Schoonmade LJ, Tuynman JB, Meijerink WJHJ, **Ankersmit M**. Biliary tract visualization using near-infrared imaging with indocyanine green during laparoscopic cholecystectomy: results of a systematic review. Surg Endosc. 2017;31:2731-2742.

Ankersmit M, van Dam DA, van Rijswijk AS, van den Heuvel B, Tuynman JB, Meijerink WJHJ. Fluorescent Imaging With Indocyanine Green During Laparoscopic Cholecystectomy in Patients at Increased Risk of Bile Duct Injury. Surg Innov. 2017;24:245-252.

Grewal S, Korthouwer R, Bögels M, Braster R, Heemskerk N, Budding AE, Pouw SM, van Horssen J, **Ankersmit M**, Meijerink J, van den Tol P, Oosterling S, Bonjer HJ, Gül N, van Egmond M. Spillage of bacterial products during colon surgery increases the risk of liver metastases development in a rat colon carcinoma model. Oncoimmunology. 2018;26:e1461302.

Ankersmit M, Hoekstra OS, van Lingen A, Bloemena E, Jacobs MAJM, Vugts DJ, Bonjer HJ, van Dongen GAMS, Meijerink WJHJ. Perioperative PET/CT lymphoscintigraphy and fluorescent real-time imaging for sentinel lymph node mapping in early staged colon cancer. Eur J Nucl Med Mol Imaging. 2019;46:1495-1505.

Lier MCI, Vlek SL, **Ankersmit M**, van de Ven PM, Dekker JJML, Bleeker MCG, Mijatovic V, Tuynman JB. Comparison of enhanced laparoscopic imaging techniques in endometriosis surgery: a diagnostic accuracy study.Surg Endosc. 2019; Epub ahead of print.

Ankersmit M, Bonjer HJ, Hannink G, Schoonmade LJ, van der Pas MHGM, Meijerink WJHJ. 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;23:1113-1126.

Ankersmit M. Heymans MW, Hoekstra OS, Vlek SL, Schoonmade LJ, van der Pas MHGM, de Vet HCW, Meijerink WJHJ and collaborating authors. Sentinel lymph node procedure in colon cancer; a systematic review and meta-analysis of individual patient data. Submitted.

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Pap, omdat niemand zo wijs, eerlijk en integer is.

CURRICULUM VITAE

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Marjolein Ankersmit was born on the 11th of November 1986 in Purmerend, the Netherlands. After graduating from the Jan van Egmond College in Purmerend, she started medical school in 2006 at the Vrije Universiteit Amsterdam.

During her medical school, Marjolein developed her interest in research and started a research project under supervision of Prof.dr. W.J.H.J. Meijerink in collaboration with the laboratory of Prof.dr. M. van Egmond. This was later turned into a PhD research project resulting in the present doctoral thesis. After having obtained the medical degree in 2013, she worked as a PhD student for four years at the Department of Surgery (Prof.dr. H.J. Bonjer and Prof.dr. W.J.H.J. Meijerink) and Department of Radiology and Nuclear medicine (Prof.dr. O.S.Hoekstra). Subsequently, she worked as a senior house officer at the Department of Surgery.

In January 2020, Marjolein started her surgical training at Ziekenhuis Gelderse Vallei Ede and Radboud UMC Nijmegen under the supervison of dr. A.M. Bosch and dr. B.H. Verhoeven.