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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 performance, 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, focusing 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 transcutaneously 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 disappointing 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 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.

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}\text{Zr}]\text{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}\text{Zr}]\text{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 -fluorescence 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 heterogeneous patient population and with clear definitions for uncomplicated and complicated gallbladder disease.