Near-infrared image guidance in cancer surgery

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Chapter 8

Summary and Future perspectives

SUMMARY

Intraoperative imaging using near-infrared (NIR) fluorescence is a fast developing imaging modality as it provides real-time visual information during surgery **(Chapter 1)**. The ability to detect lymph nodes and tumours that need to be resected can assist the surgeon to improve surgery by reducing time of the procedure, reducing iatrogenic damage, and improve the number of radical resections. This thesis focuses on the introduction of NIR fluorescence imaging into the clinic. **Part 1** of this thesis describes the optimization of NIR fluorescence imaging for sentinel lymph node (SLN) biopsy using the clinically available NIR tracer Indocyanine green (ICG) in various cancer types. Moreover, these studies show both the limitations as the clinical benefit of NIR fluorescence for SLN biopsy. **Part 2** describes the use of NIR light for tumour detection. Tissue absorption and scattering in the NIR light spectrum was used for neoadjunvant treatment response monitoring in breast cancer patients. Moreover, NIR fluorescence imaging using NIR contrast agents was used for the intraoperative detection of otherwise difficult to localize liver metastases of colorectal cancer.

PART 1. Sentinel lymph node mapping

Sentinel lymph node mapping has been introduced for various types of cancer for either avoiding complete lymphadenectomy or improving nodal staging by selecting lymph nodes for ultrastaging. Up to now, radioactive colloids and blue dyes are most often used as standard of care for SLN biopsy in clinical practise. However, the use of radioactive colloids requires the involvement of a nuclear physician and localisation of the SLN can be difficult using a handheld gamma probe due the lack of visual information and interference of the injection site. Blue dyes cannot be seen easily through the skin and fatty tissue. Moreover, blue dye migrates quickly to 2nd-tier lymph nodes, which complicates differentiation between the SLN and 2nd-tier nodes. NIR fluorescence imaging allows non-ionizing detection of lymphatic tracers deeper into the tissue (up to 1 cm) and does not interfere with the surgical field. Moreover, as ICG binds to large proteins (such as albumin), this results in improved retention in the SLN. These favourable properties of NIR fluorescence imaging should therefore translate into improved detection of the SLN.

In previous preclinical studies, premixing of ICG with HSA showed clear advantages: it improved the retention of the dye in the SLN and increased the fluorescence brightness. **Chapter 2** aims to test this advantage in a clinical randomized setting, since lymph fluids consist of high protein levels, and ICG alone might rapidly bind to these endogenous proteins when draining in the lymphatic system. This could possible eliminate the need for premixing ICG with HSA. In vulvar cancer patients, no difference was found between groups injected with ICG with or without premixing with albumin in fluorescence contrast ($P = 0.65$) or number of fluorescent nodes detected

(P = 0.06). Similar results were found in patients with cervical cancer and described in **Chapter 3**. This indicates that premixing of ICG with HSA can be omitted. This simplifies the clinical procedure and can facilitate the introduction of this technique in clinical practice.

Though NIR fluorescence imaging provides some depth penetration, studies showed the need for radioactive colloids for the detection of deeper located SLNs. Therefore, in **Chapter 4**, ICG was covalently bound to radioactive Nanocolloid (99mTc-Nanocolloid) to form the hybrid radioactive and NIR fluorescence tracer ICG-99mTc-Nannocolloid tracer, which provides both preoperative imaging and intraoperative imaging. In breast cancer patients, this tracer permitted both fluorescence and radio-guidance of all SLNs. When the tracer is already injected for pre operative sentinel lymph node imaging, no additional injection prior to surgery is necessary and the intraoperative findings are comparable to those when using ICG alone. Moreover, NIR fluorescence staining outperformed blue dye staining as 100% of the SLNs were fluorescent and only 88% stained blue. Moreover, it was shown that increasing the particle density of ICG-Nanocolloid did not increase fluorescence brightness (P = 0.590), which indicates that a dose of 160 μM ICG-99mTc-Nanocolloid (in 200 μL) is optimal.

In addition to the clinical available NIR tracer ICG, novel fluorophores are developed, which have improved fluorescence properties and can be easily bound to tumour specific ligands or large proteins. In **Chapter 5** the novel fluorophore IRDye 800CW was bound to human serum albumin (complex: HSA800) and evaluated for SLN mapping. The procedure was performed ex vivo after resection in patients with colon cancer, as IRDye 800CW has not yet been approved by the FDA and EMA. HSA800 allowed clear fluorescent identification of the SLNs and was of added value to conventional blue dye, as NIR fluorescence allowed detection of additional SLNs and allowed easy detection of SLNs located deeper into the mesenteric fat. Moreover, NIR fluorescence cannot be confused with blue nodal staining resulting from preoperative tattooing of the tumour.

PART 2. Tumour detection

Chapter 6 describes the use of NIR light for tumour detection using tissue absorption and scattering characteristics of tumour tissue. This is used for longitudinal response measurements of breast cancer to neoadjuvant chemotherapy. Using diffuse optical spectroscopy in the NIR light spectrum, the Softscan can assess metabolic parameters such as Hb, HbO_2, $\%H_2O$, and %lipid. As tumour tissue has high tissue density (high H_20 content), contains less breast tissue (high lipid content) and has high metabolic activity, which requires high blood supply and high oxygen usage, tumour tissue can be clearly differentiated from normal breast tissue. As early as after the first chemotherapy

cycle a significant difference between responders and non-responders was found using the Softscan (P= 0.023).

In addition to SLN mapping, the use of ICG is also explored for tumour imaging as ICG is cleared by the liver and has been shown to passively accumulate around colorectal liver metastases. **Chapter 7** focuses on the clinical optimization of ICG dose and timing of injection for the detection of colorectal liver metastases and assess patient benefit. No difference in signal-to-background were found between the different treatment groups (10mg 24h, 10mg 48h, 20mg 24h, 20mg 48; P = 0.70). Therefore an injection of 10mg ICG at 24 h before surgery is considered desirable from a safety and logistical point of view. Importantly, in 5 of 40 patients (12.5%, 95% CI: 5.0-26.6), additional small and superficially located lesions were detected using NIR fluorescence, and were otherwise undetectable by preoperative computed tomography (CT), intraoperative ultrasound (IOUS), visual inspection, and palpation.

FUTURE PERSPECTIVES

Pre-operative imaging modalities such as HD-US, CT, MRI, PET and SPECT are rapidly improving. However, the occurrence of non-radical tumour resections and iatrogenic injuries during surgery is not uncommon. Therefore, the need for improved intraoperative imaging tools is imperative. Image-guided surgery using NIR fluorescence is real-time, can provide depth penetration and is highly specific, without altering the surgical field. For these reasons, NIR fluorescence imaging is currently attracting major interest world wide as new intra-operative imaging modality.[1]

This thesis shows the use of NIR fluorescence imaging for identifying SLNs and tumour tissue and presents the advantages over other intra-operative imaging modalities using clinical available technologies. As the NIR fluorescence tracer ICG has already been used since the sixties with hardly any toxicity, safety is not an issue.[2] This has led to the exponential use of ICG in clinical trials for NIR fluorescence imaging. However, several hurdles will have to be conquered before the surgeon will use NIR fluorescence imaging in daily practice.

Cost-benefit analysis will have to show the additional benefit of NIR fluorescence imaging before it will be widely accepted as standard-of-care. This thesis and most current studies mainly focus on the feasibility and optimization of the technique. This is necessary before the start of large multi-center trials to provide the most efficient technique and allow for sample size calculations. Large multi-center studies are now in progress using already clinical available NIR probes, which will hopefully lead to wider implementation of NIR fluorescence imaging. Currently the costs of NIR fluorescence imaging mainly depend on the used imaging system, as costs of a vial of ICG are less than 100 Euro. Therefore, it is reasonable to suggest that with the benefit of NIR fluorescence imaging presented in the thesis, cost-benefit analysis will be favouring NIR fluorescence imaging in clinical practice.

Surgery is evolving to minimal invasive techniques. Both laparoscopic and robotic surgical techniques have shown to provide less morbidity and shorter hospital stays for multiple indications including large oncologic operations. Both techniques use camera systems to provide the surgeon with visual information of the operation field on a screen. Therefore, adding NIR fluorescence is relatively easy and only requires small adaptation of the camera sensor and light source. Already multiple commercial suppliers of laparoscopic and robotic surgical systems provide the option to use NIR fluorescence imaging. This will attribute to the fast introduction of intraoperative NIR fluorescence imaging.

Moreover, we are only at the start of this new imaging technology. Multiple research groups are developing new NIR fluorescent probes, which have improved properties for identification of tumour tissue or vital anatomical structures. Using intraoperative fluorescence imaging, cancer in the head and neck region can be clearly

visualized using cetuximab conjugated to a NIR fluorophore[3] and folate-FITC allows for intraoperative detection of ovarian and lung cancer.[4,5] The development of these new probes will further expand the use of NIR fluorescence image-guided surgery.

The development of these new tracers is not only focussed on the targeting part (e.g. HER2, EGFR) of the probe. Other aspects such as fluorescence brightness, particle size and particle charge are crucial for providing sufficient tumour-to-background signal.[6] For example, during imaging of colorectal liver metastases, the tracer needs to be cleared preferably by the kidneys as liver take-up can result in a significant increase of the background signal, which subsequently decreases tumour-to-background signal.

The development of new FDA en EMA approved drugs require extensive research and validation. With the increasing interest of commercial partners, partly also as result of the successful use of currently available probes such as ICG, the introduction of new FDA and EMA fluorescent agents is coming closer.

Though, still for some indications, in which more depth penetration will be required, other solutions will have to be found. Several possibilities are currently investigated, such as opto-acoustic imaging and multimodal imaging combining fluorescence imaging with PET or SPECT.[7] Combining fluorescence with PET or SPECT has the additional advantage of combining pre-operative imaging, which will further allow for improved surgical planning and could also provide a surgical roadmap for intraoperative navigation. This concept was recently demonstrated in patients with melanoma that underwent SLN biopsy.[8] Using the same tracer pre- and intra-operative allows for real-time navigation after feeding SPECT/CT images to the intra-operative navigation system, and when approaching the targeted tissue, fluorescence imaging provides the real-time visual confirmation.

If the future of NIR fluorescence imaging will continue to develop as it currently is, in the near future not one NIR tracer but a combination of NIR fluorescent probes will be used to delineate the tumour and vital structures both pre-operative as provide real-time intra-operative navigation. All with the goal to reduce unnecessary tissue damage, which will reduce morbidity and reduce time of hospitalization and to improve oncological outcome by increasing the number of patients in whom a complete resection of tumour tissue will be achieved. This will reduce the need for re-interventions and improve disease free survival.

In addition to the use of NIR imaging during surgery, the use of NIR imaging as a non-invasive imaging modality has shown great potential. In this thesis, NIR imaging was successfully used to identify breast cancer patients who did not benefit from neoadjuvant chemotherapy and would therefore possibly benefit from a different chemotherapy regime or from direct surgery. With the increasing use of neoadjuvant chemotherapy in cancer in general (e.g. breast cancer, oesophageal cancer and rectal

cancer), NIR imaging could be of great benefit for monitoring tumour response to chemotherapy, thereby providing patient tailored medicine.

Not only the use of neoadjuvant chemotherapy is increasing. More medicinal tumour targeted treatment options are available every year. Tumour targeted treatments have shown improvement in the survival of cancer in for example stage IV melanoma and stage IV breast cancer.[9,10] With the growing knowledge of pathways that are responsible for tumour progress, more treatment options will become available. This has already led to speculations that some cancers will not be the cause of death in patients with cancer, but will rather become a chronic illness.[11]

With the improving options to treat the advanced stages of cancer, we simultaneously try less aggressive treatment strategies of in situ carcinoma or early stage cancer. This, to reduce morbidity from non-beneficial treatment. In for example patients with rectal cancer, patients with a clinical complete response to chemoradiotherapy did no undergo surgery. Instead, patients received intensive follow-up: "wait-and-see", without effect on disease-free survival.[12] Moreover, in breast cancer patients it is debated to be less aggressive with surgery in ductal carcinoma in situ, which is currently evaluated in a clinical trial (EORTC 1401-BCG).

The field of oncology is evolving rapidly: earlier detection of small tumours, more advanced surgical techniques, and innovative medicinal treatment strategies. All of which are focused to reduce morbidity and improve survival. In all these developments clear visualisation of tumour is essential to correctly assess tumour extend and observe the effect of treatment. NIR imaging, as a novel imaging modality, will therefore make a significant contribution to the treatment of cancer.

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