Near infrared fluorescence imaging in surgery

Summary

Misidentification of vital anatomical structures such as bile ducts, arteries and ureters may lead to iatrogenic injury. Therefore, detection of these structures is of utmost importance. An optical tool to enhance identification of structures might be helpful in preventing iatrogenic damage. One such optical tool is Near Infra-Red Fluorescence (NIRF) imaging. Preliminary studies, concerning experimental and limited human series, report encouraging results. However, at the start of the studies of this thesis, the technique was yet not standardly incorporated in daily practice. The overall aim of this thesis was to investigate and critically assess some of the possible applications of near infrared fluorescence imaging during surgery in order to prevent iatrogenic damage due to misrecognition of the anatomy.

The first part of the thesis concerns the use of NIRF cholangiography in laparoscopic cholecystectomy. In the second part, we investigated the possibilities of NIRF anatomical imaging in colorectal surgery. In the third part a pilot study is presented exploring the use of NIRF imaging to identify the parathyroid glands and to assess its remaining perfusion at the end of surgery.

PART I NEAR INFRARED FLUORESCENCE CHOLANGIOGRAPHY

Laparoscopic cholecystectomy is the most commonly performed laparoscopic procedure. The most feared complication in this type of surgery is bile duct injury. Although fortunately not occurring commonly (0.3-0.7% of cholecystectomies), bile duct injury results in major morbidity and in some cases even mortality.[1-9] Misidentification of the extrahepatic bile duct anatomy is the main cause for the occurrence of this complication.[10] The Critical View of Safety (CVS) technique is developed to minimize the chance for misidentification of the anatomy.[11] Pilot studies have shown that by the use of near infrared fluorescence (NIRF) cholangiography the visualization of the biliary anatomy can be enhanced, and the CVS can be achieved earlier. We assume that the CVS is thus easier to achieve using NIRF. In **Chapter 2**, the study protocol for an international multicenter randomized controlled trial is presented. This trial should prove whether the use of NIRF cholangiography does help the surgeon by obtaining earlier CVS or not.

When using NIRF cholangiography, great differences in obtained fluorescent signals in the images are found. These differences may originate form (differences in) background fluorescence from the liver and in the penetration depth of the dyes used, but more factors are of influence.[12] In **Chapter 3**, currently used techniques during NIRF cholangiography are compared. Factors of influence on the fluorescence intensity are explored in ex vivo experiments combined with knowledge presented in current literature. The factors found to be of influence are given dose, timing of ICG administration, route of administration, distance between the laparoscope and the area of interest, patient factors such as BMI and presence of cholecystitis, used system and used software to analyze the data. In this chapter, advise is given on how to use NIRF cholangiography. The concentration of ICG in the bile ducts should be between 0.00195 and 0.025 mg/ml, ICG should be given as early as logistically possible (up to 24h before surgery), the laparoscope should be held close to the tissue and the best available system should be used.

In **Chapter 4**, two new pre-clinical dyes are compared to a third, earlier tested preclinical dye. This third dye, IRDye® 800CW was very promising in previous experiments and possible even better than ICG.[13] However, this dye is expensive, and more expensive compared to the newly developed dyes IRDye® 800BK and IRDye® 800NOS. When comparing these three dyes in a pig model, the cystic artery was immediately visible using all three dyes. The cystic duct appeared later, but with the new dyes at least as good as with the use of IRDye®800CW. Thereby, IRDye® 800BK and IRDye® 800NOS seem to be good alternatives for IRDye® 800CW. Our conclusions are based on the subjective evaluation, but also based on the measured target to background ratio.

In **Chapter 5**, we compared the used methods in literature for objectification of the fluorescent signal. We found that the most commonly used software programs (Osirix and ImageJ) are compatible and comparison of results obtained with these programs seems legitimate. A third used software program (Photoshop) gives less comparable results and these are therefore not easily comparable with the results obtained with the other programs. When using the software programs both grey and blue-value (or green-value depending on the used system) can be measured to determine fluorescence intensity. However, in our images no clear difference between using the grey- and blue- value could be seen. Another aspect is that different formulas are used in literature to assess fluorescence intensity compared to the background. No clear advice can be given on the best formula to be used. Therefore, reporting of both target and background fluorescence intensity in manuscripts is recommended and not only the Target-to-background ratio. This will enable researchers and readers to make a more proper comparison of results between studies.

PART II NEAR INFRARED FLUORESCENCE IMAGING IN COLORECTAL SURGERY

During colorectal surgery there are two complications surgeons fear most. These two complications are anastomotic leakage in the patients in whom an anastomosis is made and ureter damage in pelvic surgery. Unfortunately, reported incidence of anastomotic leakage is high, namely 3-15% in colorectal surgery.[14-21] Anastomotic leakage is a very serious complication since it has a great impact on the patient by increasing morbidity, often requiring a second surgery, but also mortality rates are high.[16, 20-22] Several risk factors for anastomotic leakage have been identified, of which an important factor is perfusion of the bowel segments adjacent to the anastomosis.[23-25] A good perfusion is required for adequate anastomotic healing. Since the perfusion can be visualized using near infrared fluorescence imaging, it is thought that the use of near infrared fluorescence imaging can prevent part of the anastomotic leakages. In **Chapter 6**, we investigated this hypothesis in a systematic review and found that the use of NIRF angiography in anastomotic colorectal surgery seems easily applicable. After NIRF angiography, the resection margin was changed in 10.8% of cases and a significant lower anastomotic leak rate was found in published literature when using NIRF angiography. Especially an initially bright fluorescent signal seems to be predictive for good anastomotic healing.

In our systematic review in **chapter 6**, we found that in most studies, the place of resection margin after NIRF angiography was based on the subjective view of the surgeon: whether the bowel was 'fluorescent enough'. However, a clear cut-off value of fluorescence intensity indicating sufficient perfusion is not available yet by would be desirable to base such decisions on rather than on a subjective view.

In **Chapter 7** therefore, we explored whether the fluorescent signal correlates to serum markers of anastomotic leakage and postoperative inflammation such as CRP, I-FABP and calprotectin. In the heterogenous pilot-group of 30 colorectal cancer-patients we included, no relation between the fluorescence intensity and the inflammatory markers studied could be found. However, a higher measured fluorescence intensity of the proximal bowel compared to the background both before and after surgery was found in the patients who did not develop anastomotic leakage. Also, a subjective scoring of the surgeon during surgery just before transection of the proximal bowel was higher in the patients who did not develop anastomotic leakage.

A second major complication after colorectal surgery is iatrogenic ureter damage. In this surgery, the ureter can be very close to the surgical field and may be hard to identify. Identification of the ureter is the most important step in avoiding ureter damage. By using a near infrared dye that is cleared by the kidneys, the ureters may be visualized during surgery and thereby lessen the chance of iatrogenic ureter damage. In **Chapter 8**, we investigated the feasibility of NIRF imaging with methylene blue to visualize the ureter in patients undergoing elective laparoscopic colorectal surgery. This dye is registered for clinical use and cheap: NIRF using this dye would be a very practical and promising technique. The only drawback was that a dedicated filter had to be developed, as the commercially available equipment didn't have filters for the appropriate wavelength. Ten patients were included, and different doses were tested. We could detect the ureter in five patients, who received the highest doses, namely between 0.75 and 1mg/kg. However, in all five cases the ureter was also visible in white light before detection in NIRF light. The results were disappointing. Therefore, the use of NIRF imaging with methylene blue in these cases was not considered of added value in clinical practice.

A potent dye with excitation and emission in the range of ICG would be preferable as the commercially available equipment can be used for obtaining the fluorescent signal. In **Chapters 9 and 10**, we explored the use of two new such dyes. These are still pre-clinical dyes, emitting fluorescent light at around 800nm instead of the 688nm at which methylene blue emits fluorescent light. The wavelength of 800nm is more in the near infrared range and thus has a higher penetration depth. Based on this theory we expected to see the ureters more clearly using these new dyes, IRDye® 800BK and IRDye® 800NOS. We compared these with another, earlier tested preclinical dye, IRdye® 800CW in a pig model. In these experiments three pigs were used, one of the three dyes each. These three dyes are cleared by the kidneys and showed a bright fluorescent signal, very clearly delineating the ureters in the pigs, especially during pulsatile movement of urine through the ureter. IRDye® 800BK seemed to give the best results of these two new dyes and is further explored in **Chapter 10.** Three different doses of the IRDye800BK were administered in three different pigs to search for the optimal dose. The used doses were 3mg, 6mg and 12 mg. The results obtained with these three doses were comparable. Also, the dye was used in explanted human and pig ureters to see if the penetration depth of the emitted light from the dye was high enough to penetrate through the human ureter. We found that this was possible, but that the presence of fat on the ureter is a limiting factor.

PART III NEAR INFRARED FLUORESCENCE IMAGING IN THYROID SURGERY

In thyroid surgery, iatrogenic injury of the parathyroid glands should be prevented. Here again, the key to prevention is identification of the anatomical structure, the parathyroid gland. However, in this type of surgery, only identifying is not enough to prevent injury. In some cases, the parathyroid glands are identified and kept in the patient, but due to manipulation of the thyroid, the blood supply towards the parathyroid is impaired, resulting in an insufficient remaining function of the parathyroid glands. In **Chapter 11** we evaluated the possibility of both identifying the parathyroid glands and assessing post-thyroidectomy perfusion of the parathyroid gland. In 30 (hemi) thyroidectomies, NIRF imaging was used. This technique was found safe and easily applicable. In 17 surgeries NIRF imaging was of added value, mainly due to providing more certainty to the operating surgeon about the location and remaining function of the parathyroid glands. In all patients, normal calcium levels were found 6 hours after surgery, however a temporary drop in calcium level the day after surgery occurred in three patients in whom the average TBR was lower compared to the measured TBR in the remaining patients. The calcium levels in these patients restored after two weeks.