RESEARCH ARTICLE

Improving visualization of colorectal metastatic cancer using laparoscopic fluorescence-guided surgery

Authors

Steinkamp P.J.[1#], van der Zant F.A.[1#], Hentzen J.E.K.R.[1,2], van Dam G.M.[3], Kruijff S.[1]

Affiliations

1. Department of Surgery, University Medical Center Groningen, University of Groningen, Groningen the Netherlands
2. Department of Surgery, Isala Clinics, Zwolle, the Netherlands
3. Department of Nuclear Medicine and Molecular Imaging / Medical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen the Netherlands

# These these authors contributed equally

ABSTRACT

Extensive surgical treatment for peritoneal metastases originating from colorectal cancer provokes high morbidity and perioperative mortality rates, therefore careful and objective patient selection prior to such a procedure is crucial. Tumor-specific fluorescence-guided laparoscopy is a potential imaging technique for the improvement of patient selection. The aim is to select the patient that benefits the most of extensive and valuable surgical treatment, in terms of disease-free survival, overall survival and quality of life. Cancer-upregulated proteins and tumor-specific biological processes with matching fluorescence imaging agents could guide the surgeon to improved identification of malignant tissue during diagnostic laparoscopy and for detection of non-visible small tumor lesions during cytoreductive surgery.

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed cancer types with a worldwide incidence of 1.8 million patients. Peritoneal metastases (PM) used to be a form of end-stage disease with an incidence differing between 4 to 40% worldwide. [1,2] PM is defined as the presence of metastatic tumor nodules spread over the peritoneal surface throughout the abdominal cavity and can arise from colorectal cancer, gynaecological cancers and peritoneal cancers. [3] Over the last two decades, performing cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) has improved overall survival (OS) shifting PM treatment from a palliative setting - using systemic chemotherapy - towards a potential curative treatment option depending on the preoperative condition of the patients and tumor load determined by the peritoneal carcinomatosis index (PCI). [4] In adequate selected patients, CRS combined with HIPEC to reduce microscopic malignant disease is performed. CRS combined with HIPEC improves OS from 21 to 63 months and increases the five-year survival rate up to 40% compared to palliative chemotherapy. [5-7] With a complete cytoreduction, mortality rate due to recurrence of disease decreases with 39% when compared to non-complete cytoreduction. [8] Despite promising initial results, this complex and extensive surgical treatment introduces a high risk of procedure-related mortality (0-8%), morbidity (12-68%) and extensive postoperative rehabilitation with prolonged hospitalization. [9] In 25% of all planned patients the initial surgical procedure is terminated prematurely due to excessive presence of irresectable tumor or high PCI, also referred to as an “open and close procedure (OC procedure)”. Moreover, 30% of patients develop recurrence of disease within one year. [10] These data illustrate the impactful consequences of this extensive treatment for our peritoneal metastasized patients and the need for technical improvement to identify the best patients for this extensive surgical treatment.

During the surgical treatment of PM originating from CRC there is a clear clinical need for introduction of innovative techniques to improve patient selection and eventually patient outcome. A more advanced, intra-operative imaging and diagnostic selection tool has the potential to create more tumor-specific and precise cancer surgery. The introduction of fluorescence-guided surgery (FGS) has demonstrated the feasibility to identify extra PM lesions during open surgery and could enable introduction of fluorescence-guided laparoscopy for more

adequate patient selection and improvement of the treatment regimen and outcomes. [11-13]

Spread of peritoneal deposits

PM pathogenesis can be explained by three different models: A) dissemination from the primary tumor, B) primary peritoneal tumor or C) peritoneal deposits with an independent origin. The most frequent form of dissemination is exfoliation of malignant cancer cells, when the tumor has expanded through the serosa. Subsequently, there are two different pathways for the attachment of the malignant cells to the peritoneum. In the first pathway, in absent or rounded (cuboidal) mesothelial cells, the integrins facilitate the attachment of the malignant cancer cells to the sub mesothelial connective tissue creating peritoneal deposits. In the second pathway, loose malignant cells adhere to mesothelium directly through adhesion molecules. [14]

Surgical Standard

The current surgical strategy in patients with colorectal PM is initiated by efforts to improve patient selection with a white light diagnostic laparoscopy (WL-DLS) to visualize the extent of disease by scoring the PCI. [15] A higher PCI indicates more tumor load and a lower 4-year survival rate. [16] WL-DLS is safe and reduces the amount of OC procedures with 20-40%. [17] During WL-DLS surgeons obviously depend on visual inspection alone without tactile information, and therefore adequate identification of malignant compared to benign tissue remains extremely challenging. The presence of benign scar tissue originating from neoadjuvant treatment and previous surgery makes adequate identification complex during WL-DLS and CRS. Consequently, small tumor lesions may be easily missed and clinically suspicious lesions could therefore be benign.

Fluorescence-guided tumor visualization

In the last decade, FGS using optical imaging and non-ionizing tumor-specific fluorescence imaging agents has emerged as an innovative real-time imaging technique to aid surgeons for the enhancement of intra-operative tumor visualization and increasing margin assessment in oncological surgery. [18-20] FGS as such serves as a ‘red-flag’ imaging technique to assist in visualization of small tumors, peritoneal deposits and leads to adequate differentiation between benign and malignant tissue. Various FGS studies showed an increase of real-time and adequate tumor-positive margin detection during surgery in a variety of tumors, potentially increasing surgical quality using FGS. [20-22]

Fluorescence-guided surgery uses optical properties of non-ionizing endo- and exogenous photons to visualize oncological phenotype and is relatively easy to implement during surgery. The near-infrared (NIR) spectrum (650-900 nm), invisible for the human eye, is used as NIR light can travel millimeters up to centimeters through tissue allowing for visualization of tumor-(non)specific fluorescence imaging agents. Different exogenous non-targeted or targeted contrast agents are described, where the targeted imaging agents contain a targeting moiety and a signal agent using fluorophores. Special imaging devices, like open fluorescence cameras, are used to visualize the imaging agent and therefore biological information of tumor tissue and benign tissue. Moreover, fluorescence imaging has become available in commercial laparoscopes, calibrated to adequately detect tumor non-specific indocyanine green (ICG, excitation 780 nm). [23] . However, for the detection of low-dose tumor-specific imaging agents, most commercially available imaging systems are not sensitive enough for adequate detection. Fluorescence imaging agents have relatively low fluorophore concentrations *in vivo* as conjugation with a tumor-specific component and human dose restrictions hinder increased fluorophore concentration. Moreover, imaging agent distribution varies in between tumor types, tumor size and patient characteristics.

Improving Peritoneal Metastases Visualization

Intra-operative imaging of colorectal PM using ICG has been performed, showing the potential of FGS for enhanced tumor visualization. [24] As ICG is not tumor-specific, a major drawback is the lack of sensitivity and specificity, which is extremely important during WL-DLS for adequate patient selection based on true-positive and true-negative imaging of suspected lesions.

A variety of studies investigated tumor-specific fluorescence imaging agents against cell surface receptors using intra-operative and *ex vivo* fluorescence imaging. Cancer-up-regulated proteins, with an enhanced expression on tumor cells, like Endothelial Growth Factor Receptor (EGFR), Vascular Endothelial Growth Factor (VEGF) and Carcinoma-Embryionic Antigen (CEA) have been widely used as molecular target for FGS. [12,20,22,25] For PM, a variety of potential targets were identified for intra-operative imaging, including VEGF-A and CEA. [26] VEGF-A, involved in tumor-induced angiogenesis, is upregulated in 93% of colorectal PM. [27] An improved surgical

outcome and survival using fluorescence-guided laparoscopy has been shown in mouse models of human pancreatic and colon cancer. [28] In our centre, the VEGF-A targeted Bevacizumab (Avastin®, Roche) conjugated to the NIR fluorescent agent IRDye-800CW (LI-COR Biosciences, Lincoln, NE, USA) is used in a variety of clinical trials. This fluorophore-labelled therapeutic monoclonal antibody showed adequate detection of intra-abdominal submillimetre malignant tissue in mouse models. [29] After IRB approval, Bevacizumab-800CW has shown to be safe in all dosing groups (4.5 to 50 milligrams) and is administered in patients intravenously two or three days prior to cancer surgery and showing promising results in the detection of breast cancer, sarcomas and oesophageal carcinoma. [30,31] In 2016, Harlaar *et al*. showed the clinical feasibility of Bevacizumab-800CW in seven patients with colorectal PM undergoing an explorative laparotomy as part of CRS+HIPEC. [11] Eighty peritoneal areas were imaged using an open fluorescence intra-operative camera system (SurgVision B.V., Groningen, The Netherlands). All 29 non-fluorescent resected areas proved to be benign on final histopathology, thus potentially indicating a sensitivity of 100%. On the other hand, in 27 out of 57 fluorescent resected areas (47%) from the fresh surgical specimen, tumor tissue was identified. These results show the potential for detecting peritoneal deposits using Bevacizumab-800CW also during WL-DLS, which could help to assess the true extent of peritoneal disease more accurately and to prevent performing CRS+HIPEC surgery in patients who will not benefit from this complex abdominal procedure in terms of survival and quality of life.

Other colleagues from Leiden University showed the safety and feasibility of SGM-101, an anti-CEA monoclonal antibody, during open surgery in patients with colorectal PM. [12] Fluorescence-positive malignant lymph nodes, both superficially and deeper seated, which were missed during standard surgery were visualized changing the surgical strategy in one out of three patients. During a follow-up study, additional lesions were observed using SGM-101 during laparotomy mostly increasing fluorescence PCI. FGS improved macroscopic cytoreduction and thus potentially improves surgical quality, and in later stage overall and disease-free survival for patients. [13] Based on both clinical studies, we conclude that FGS using monoclonal antibodies could be used during WL-DLS for adequate patient selection for CRS+HIPEC and during CRS+HIPEC for adequate surgical

cytoreduction. A recent study showed the possibility of visualization of PM and colorectal cancer targeting tumor acidosis with a smart-activatable pH-activated nanoprobe, showing the potential for dual use of this generic imaging agent and the potential of using a variety of imaging agents and fluorophores for PM detection (table 1). [21]

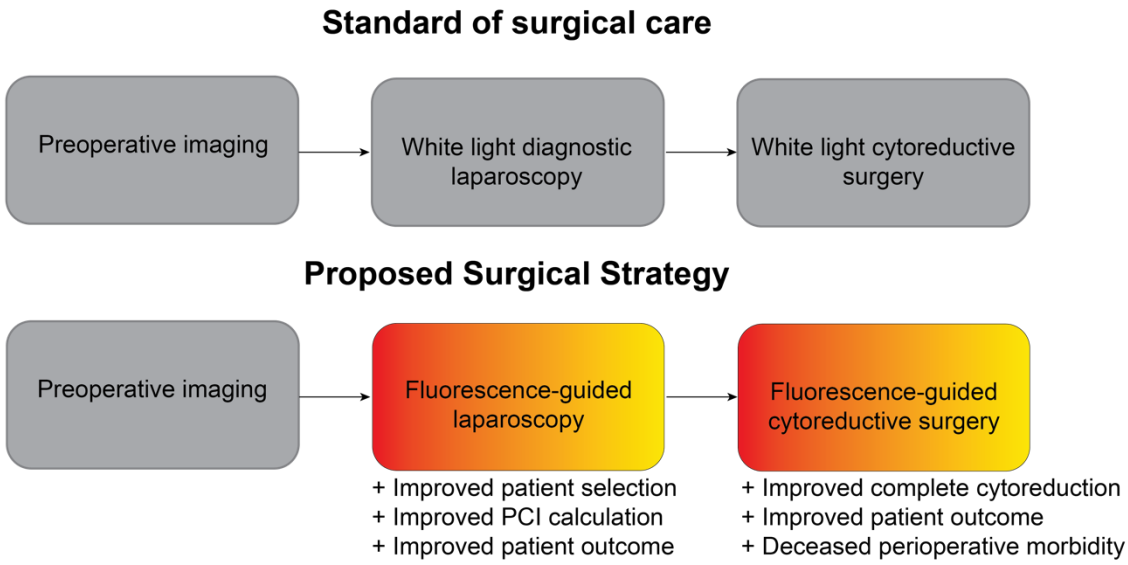
Table 1 | Clinical available fluorescence imaging agents for PM visualization

GMP available fluorescence imaging agent	Description	Excitation/emission wavelength
Bevacizumab-800CW [11]	VEGF targeting antibody with IRDye800. Administration 3 days prior to surgery	778 / 794 nm
SGM-101 [12,13]	Monoclonal antibody against CEA. Administration 4 days prior to surgery.	686 / 704 nm
ONM-101 [21]	pH-sensitive micelles with ICG. Administration one day prior to surgery.	780 / 820 nm

Optimizing Surgical Treatment

In order to optimize surgical strategy in this complex patient group, we aim to perform a feasibility WL/fluorescence-guided-DLS study using bevacizumab-800CW in patients with colorectal PM. The ability of laparoscopic fluorescence imaging to detect extra fluorescence-positive PM, as has been shown using bevacizumab-800CW and SGM-101 before, could potentially change clinical decision making (Fig. 1). The first aim is to identify the optimal dosage for laparoscopic imaging and to compare fluorescence PCI to clinical PCI during WL-DLS. In the future, a combination of fluorescence WL-DLS and fluorescence imaging during CRS+HIPEC could be performed to compare both fluorescence imaging methods and their effects in the same patient group.

Figure 1 | Proposed Surgical Workflow



Conclusion

Adequate selection of eligible patients with colorectal PM for CRS+HIPEC remains difficult. Improving patient selection and surgical quality before undergoing CRS+HIPEC is clinically relevant due to high morbidity and perioperative mortality rates of this intentionally curative treatment. FGS using tumor-specific imaging agents targeting cancer-upregulated proteins has proven its feasibility to delineate malignant tissue during laparotomy and thus has the potential to increase intra-operative detection of colorectal PM during laparoscopy. [11,13] However, those studies are performed in relatively small numbers of patients. Further expansion in phase II validation studies is needed to confirm those primary results during laparotomy and laparoscopy. We propose to perform a phase II clinical study targeting VEGF-A with Bevacizumab-800CW to improve intra-operative detection using fluorescence-guided laparoscopy.

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