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Intraoperative confocal laser endomicroscopy for real-time in vivo tissue characterization during surgical procedures

David Fuks[1] · Angelo Pierangelo[2] · Pierre Validire[3] · Marine Lefevre[3] · Abdelali Benali[3] · Guillaume Trebuchet[4] · Aline Criton[4] · Brice Gayet[1]

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Abstract

Introduction Probe-based confocal laser endomicroscopy (pCLE) is an innovative technique providing real-time, in vivo optical biopsies. A previous ex vivo phase of the study (PERSEE) allowed identifying accurate pCLE criteria for the diagnosis of hepatic and peritoneal surgical specimens. This study aimed at evaluating the pCLE role for in vivo intra-abdominal tissue characterization during digestive cancer surgical procedures.

Methods Between October 2014 and July 2015, consecutive patients diagnosed with digestive cancers and scheduled for a surgical resection or an exploratory laparoscopy were prospectively enrolled. Endomicroscopic images were acquired using a motorized Confocal Miniprobe™ with a bending distal tip providing easy access to abdominal organs. It was connected to an endomicroscopy system that allowed near-infrared illumination (at a wavelength of 785 nm) in conjunction with indocyanine green for contrast agent. A live audiovisual transmission was established between the surgeon and the pathologist for real-time interpretation of optical biopsies. Intraoperative pCLE performance for the diagnosis of suspicious nodules was assessed using corresponding surgical histopathology as reference standard.

Results 21 consecutive patients were successfully enrolled. Live audiovisual transmission between the surgeon and the pathologist was successfully established in all cases. 62 pCLE sequences were acquired from different tissues [peritoneum ($n=27$), liver ($n=21$), lymph node ($n=4$), diaphragm ($n=3$), colon ($n=3$), stomach ($n=2$), and adrenal gland ($n=2$)]. Malignant tissues were identified by fluorescently enhanced irregular cancerous tubes contrasting with dark glandular lumen and extracellular matrix. pCLE sensitivities and specificities were 67% and 100%, and 38% and 100% for peritoneal and hepatic carcinogenesis, respectively. One benign incident was reported during the trial with no patient consequence.

Conclusions Real-time intraoperative pCLE with near-infrared illumination is feasible and safe, provides additional information in terms of tissue characterization, and, in combination with telepathology, allows interactive collaboration between the surgeon and the pathologist during surgical procedures.

Trial registration clinicaltrials.gov Identifier: NCT02312167.

Keywords Probe-based confocal laser endomicroscopy · Cancer evaluation · Intraoperative collaboration · Diagnostic performance · Telepathology · Fluorescent-guided surgery · Diagnostic laparoscopy

Despite huge progresses in cross-sectional imaging techniques (such as contrast-enhanced multidetector CT-scan or magnetic resonance imaging), unexpected cancer extensions

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✉ David Fuks
david.fuks@imm.fr

[1] Department of Digestive Disease, Oncologic and Metabolic Surgery, Institut Mutualiste Montsouris, Université Paris-Descartes, 42 Boulevard Jourdan, 75014 Paris, France

[2] LPICM, Ecole Polytechnique, CNRS Engineering, 91120 Palaiseau, France

[3] Department of Pathology, Institut Mutualiste Montsouris, 42 Boulevard Jourdan, 75014 Paris, France

[4] Mauna Kea Technologies, 9 rue D'Enghien, 75010 Paris, France

are still encountered during surgery and lead physicians to change strategy [1, 2]. In this latter situation, accurate staging of local and regional extension of the cancer is crucial to determine the best therapeutic treatment and laparoscopic staging avoids unnecessary laparotomy reducing hospital stay and shortening administration of systematic therapy [3]. When a suspicious nodule is identified during laparoscopic staging, intraoperative "frozen section" analysis is routinely performed to distinguish malignant from non-malignant tissues [4]. However, this method is time consuming, expensive, and not systematically reproducible. Therefore, a limited number of frozen sections can be performed during surgical procedures [5]. It constrains the surgeon to select macroscopically samples that are suspicious or representative of the area. Additionally, some difficulty to access some specific organs, such as pancreas, may prevent suitable tissue sampling for microscopic analysis.

Probe-based confocal laser endomicroscopy (pCLE) is an emerging optical technology that enables real-time in vivo fluorescence imaging of tissues at the cellular level [6–12]. The ex vivo phase of the PERSEE study demonstrated that indocyanine green (ICG) endomicroscopic imaging with a 785-nm wavelength illumination can differentiate with high accuracy malignant from non-malignant tissues on ex vivo hepatic and peritoneal surgical specimens [13, 14].

The development of a dedicated pCLE device for surgery enabling intraoperative characterization of tissues in association with a real-time audiovisual connection between the surgeon and the pathologist would allow in vivo visualization and evaluation of tissue microstructures during surgery. This trial is the second phase of the PERSEE study which aimed at evaluating the feasibility and accuracy of ICG-aided pCLE for intraoperative characterization of suspicious nodules during surgery for digestive cancer.

Patients and methods

Patient selection

From October 2014 to July 2015, patients aged 18 years or older, scheduled for a surgical exploratory or resection of digestive cancer, were prospectively included in this study. Patients were excluded if they had allergy to any contrast agents (e.g., ICG), if they had previous life-threatening allergic reactions, if they were pregnant or breast feeding, if they had a history of cardio-pulmonary disease (including bronchial asthma), restricted renal function, or if they were under beta-blocker treatment. Written informed consent was obtained from all patients. The study was approved by the regional investigational review board and registered on clinicaltrial.gov (http://www.clinicaltrial.gov: NCT02312167, Feasibility Study for Robotic Endomicroscopy to Better Define Resection Strategies (PERSEE)).

Confocal endomicroscopy system

During laparoscopic assessment, real-time microscopic images were acquired with a Confocal Miniprobe™ (Mauna Kea Technologies, Paris, France) held by a specifically designed device called the Viziobot (Endocontrol, Grenoble, France). The Confocal Miniprobe™ prototype was based on the GastroFlex™ UHD Confocal Miniprobe™. It had a spatial resolution of 1 µm, a working distance (tissue imaging depth) of 40 µm, and a field of view (FOV) of 240 µm. The Viziobot is an instrument of 5 mm of diameter that allowed a motorized manipulation of the Confocal Miniprobe™ with a distal tip bending up to 80° in one direction, providing easy access to abdominal

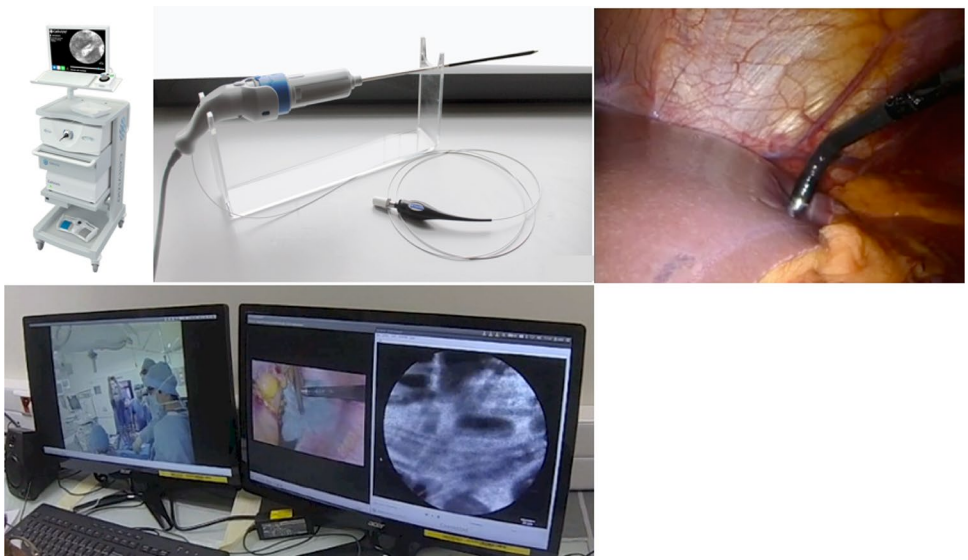

Fig. 1 Material **A** Cellvizio® endomicroscopy systems. **B** Motorized Confocal Miniprobe™ (combination of a Confocal Miniprobe™ and a Viziobot). **C** During the laparoscopic procedure, the Viziobot can bend up to 80° to provide easier access to lesions **D** Pathology station: operating room, laparoscopic view, and endomicroscopic view, from left to right

organs (Fig. 1). The motorized Confocal Miniprobe™ was connected to the Cellvizio® system (Mauna Kea Technologies, Paris, France) providing a 785-nm wavelength illumination.

Intraoperative confocal endomicroscopy during laparoscopic procedure

The procedure workflow is described in Fig. 2. The surgeon performed the acquisition of endomicroscopic images intraoperatively during the exploratory phase before surgical resection. The motorized Confocal Miniprobe™ was inserted through a port and the distal tip of the probe was positioned perpendicular to different tissues including peritoneal, hepatic, colonic, stomach and diaphragm tissues, lymph node, and adrenal gland. pCLE images of suspicious tissue areas were recorded. In case of open surgery, the motorized Confocal Miniprobe™ was held by the surgeon. As per standard of care, frozen sections were performed to assess suspicious lesions that could be considered as contra-indications to surgery.

Staining protocol

Prior to pCLE imaging, a contrast agent, indocyanine green (ICG, Infracyanine® SERB, Paris, France), was administered either intravenously or topically. Three different ICG concentrations were tested (0.25, 1.25, and 2.5 mg/ml). For topical application, a gauze soaked with the contrast agent solution was introduced through a trocar with forceps and rubbed on the area of interest, several times if needed.

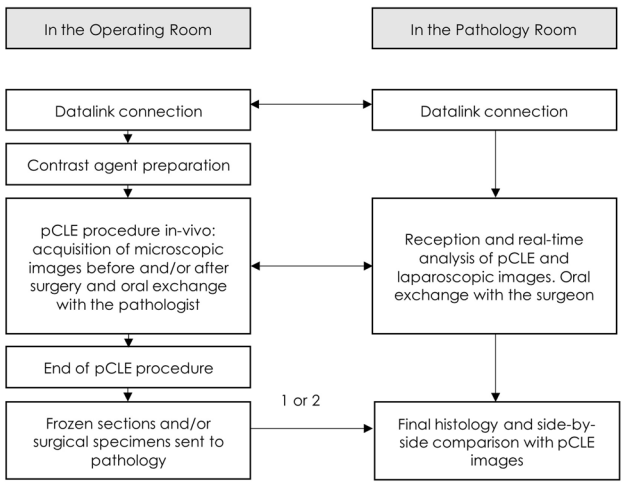

Fig. 2 General flowchart of the procedure

Audiovisual datalink between the operating room and the pathology lab

An audiovisual link was established between the operating room and the pathology laboratory. During the procedure, laparoscopic and endomicroscopic information as well as a webcam view of the operating room were simultaneously streamed to the pathology workstation (Fig. 1). All images received by the pathology workstation were recorded. Audio communication between the surgeon and the pathologist was achieved, thanks to a micro-headset.

Study endpoints

The primary endpoint of this study was to evaluate the technical feasibility of performing endomicroscopic imaging during surgical procedures and transferring in real-time these images to a pathologist workstation. The secondary endpoint was to assess pCLE diagnosis provided in real-time by the pathologist, thanks to telepathology. pCLE diagnosis of malignancy relied on the observation of highly fluorescent cancerous tubes surrounded by a substantially darker extracellular matrix (ECM) as previously described ex vivo for ICG staining [13, 14]. Each endomicroscopic sequence acquired during the procedure was correlated side-by-side to the histological analysis from the corresponding specimen to highlight in vivo pCLE image criteria related to benign and malignant tissues. The last endpoint was to assess safety by recording incidents and adverse events related to the studied devices with their types and severities.

Statistical analysis

Baseline characteristics, including demographic data, indications, and pCLE outcomes, were described as percentages and ranges or means. The quality of endomicroscopic images was individually assessed by the pathologist and the surgeon, blinded to each other's rating, according to the following scale: poor, fair, good, and excellent video quality, respectively. pCLE imaging duration was also recorded as well as the duration of the surgical procedure. pCLE diagnostic performance was evaluated using surgical histology as the reference standard. pCLE Sensitivity (Se), Specificity (Spe), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated.

Results

Population studied

During the study period, 21 patients including 11 males and 10 females with a median age of 68 years (41–82) were

prospectively enrolled. The demographic characteristics are detailed in Table 1. Among these patients, 62 pCLE sequences were acquired from the peritoneum ($n=27$), liver ($n=21$), lymph node ($n=4$), diaphragm ($n=3$), colon ($n=3$), stomach ($n=2$), and adrenal gland ($n=2$) (Supplementary Table 1).

Table 1 Demographics and clinical data

Patients, *n*	21
Male, *n* (%)	11 (52)
Mean age, years old (min–max)	68 (41–82)
Primary cancer localization, *n* (%)	
Colon	12 (57)
Biliary	2 (10)
Rectum	3 (14)
Stomach	1 (4)
Anus	1 (4)
Cardia	1 (4)
Ovary	1 (4)
Presence of metastases, *n* (%)	18 (86)
Liver	9 (43)
Peritoneum/pleura/diaphragm	4 (19)
Other	5 (24)
Previous chemotherapy, *n* (%)	15 (71)
Indication, *n* (%)	
Surgical excision	18 (86)
Surgical exploration	3 (14)
Type of surgery, *n* (%)	
Hepatectomy	12 (57)
Colectomy	5 (24)
Gastrectomy	1 (5)
Hysterectomy	1 (5)
Laparoscopic assessment	2 (10)

Feasibility and technical results (Table 2)

Intravenous injection or topical application of the ICG contrast agent was assessed (Supplementary Table 1). Although enabling pCLE imaging of microvessels, ICG injected intravenously was not appropriate for the assessment of suspicious nodules as it was quickly washed out in all tissue types except in the liver, limiting to 3-min pCLE assessment. Unlike hepatic nodules, liver parenchyma remained stained with ICG 1 h after injection allowing the visualization of hepatocytes and their architecture. Topical application of ICG at 0.25 mg/ml was tested in one procedure and led to fair image quality. Concentrations of 1.25 or 2.5 mg/ml provided the best image quality. Live vocal transmissions and macroscopic video transmissions of the laparoscopic surgical field were successfully performed in all the cases. The overall quality of endomicroscopic images was judged by the pathologist and the surgeon as of excellent in 33% (7/21) and 33% (7/21), good in 62% (13/21) and 52% (11/21), and fair quality in 5% (1/21) and 14% (3/21) of the cases, respectively.

pCLE criteria description

Peritoneum (Fig. 3)

Topical applications of ICG revealed large black round and regular structures which were identified by the pathologist as adipocytes surrounded by a fluorescently enhanced fibrous ECM. Highly fluorescent thin fibers from connective network were visualized on the normal non-fatty peritoneum.

Table 2 Technical feasibility and safety

Mean procedure time, hours, and minutes (min–max)	
Standard surgical procedure	3 h and 44 min (55 min–6 h)
pCLE imaging	19 min (7–42 min)
Quality of endomicroscopic images evaluated by the pathologist, *n* (%)	
Excellent	7 (33)
Good	13 (62)
Fair	1 (5)
Poor	0 (0)
Quality of endomicroscopic images evaluated by the surgeon, *n* (%)	
Excellent	7 (33)
Good	11 (52)
Fair	3 (14)
Poor	0 (0)

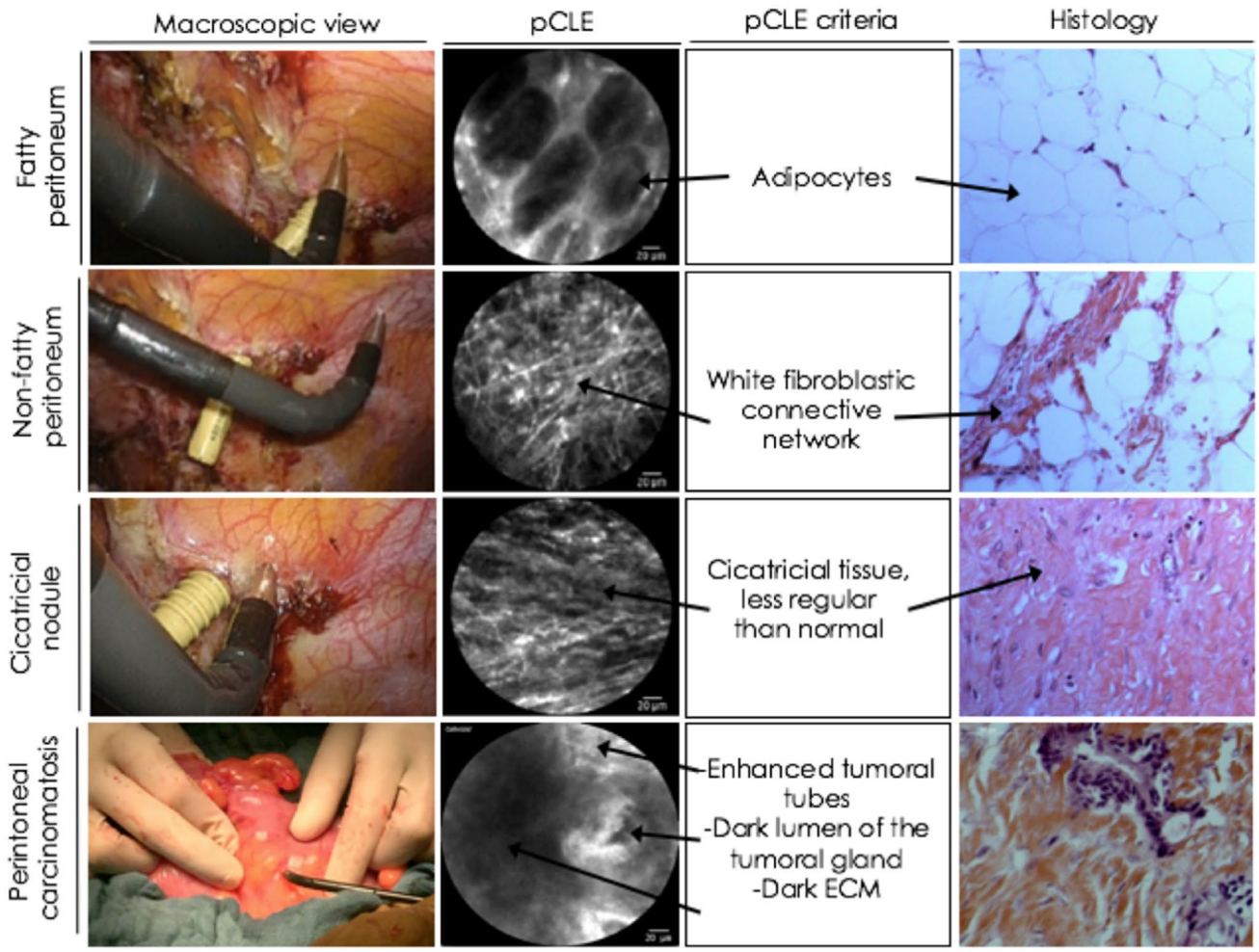

Fig. 3 pCLE images from peritoneum stained by topical application of ICG and side-by-side correlation with corresponding histology

On benign inflammatory nodule, white fibroblastic connective network appeared inhomogeneous with thickened fibers. On peritoneal carcinomatosis, strongly enhanced fluorescent irregular clusters or tubes of malignant cells were surrounded by a substantially darker ECM. The lumen of tumoral gland was visualized as substantially darker than the highly fluorescent cancerous tubes. Small black dots inside the bright malignant clusters were observed and were correlated to cancer cells' nuclei in histology.

Liver (Fig. 4)

Regular hepatocytes with non-fluorescent nuclei were clearly recognizable in normal liver, following either topical application or intravenous administration of ICG. ICG topically applied on the surface of normal liver revealed the liver capsule as a homogenous, dense, and bright network of fibers. Malignant nodules were identified as strongly fluorescent irregular cell cluster. The ECM and the lumen of tumoral glands were substantially less fluorescent than cancerous tubes. In nodules primarily treated with neoadjuvant chemotherapy, a very dense and strongly fluorescent fibrosis replaced tubular structures of cancerous cells (Fig. 4).

Other organs

pCLE imaging of other malignant tissues revealed enhanced and disorganized cancerous tubes surrounded by a substantially darker ECM suggesting that pCLE criteria for malignancy are similar between different organs. The surface of reactive lymph node was disorganized compared to normal lymph node. Benign and malignant criteria were not clearly visualized on the diaphragm. No pCLE criteria could be highlighted on stomach and adrenal gland.

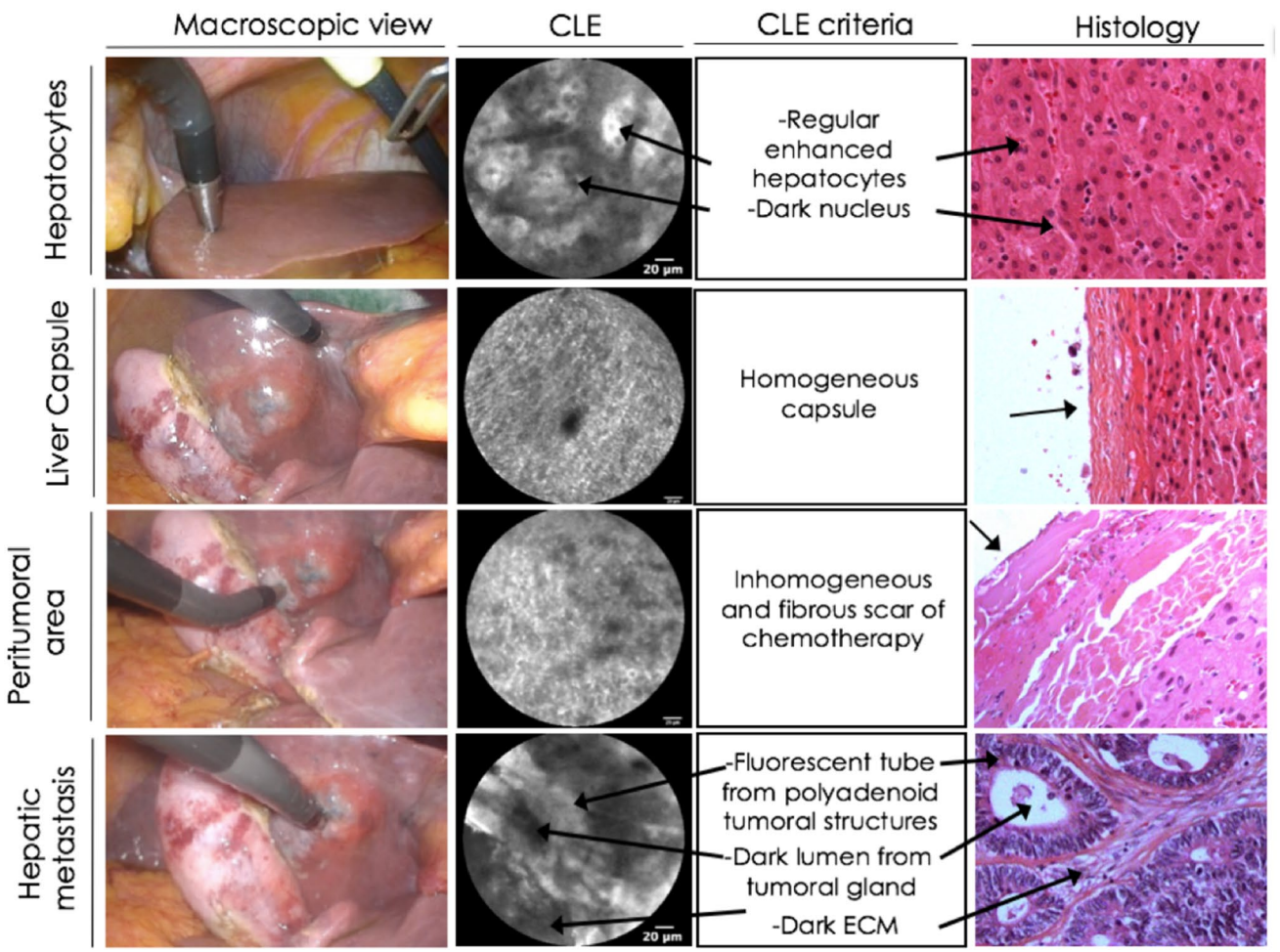

Fig. 4 pCLE images from liver stained by topical application of ICG and side-by-side correlation with corresponding histology

Assessment of pCLE performances through real-time telepathology

Peritoneum

The surfaces of six benign and six malignant peritoneal nodules were imaged by pCLE. The pathologist provided, six true-negative, four true-positive, and two false-negative diagnoses. In the two false-negative cases, a dense and inhomogeneous fluorescent scar capsular tissue was observed. pCLE Se, Spe, PPV, and NPV were 67%, 100%, 100%, and 75%, respectively.

Liver

The surfaces of eight malignant and one benign hepatic nodules were imaged by pCLE. Among the eight malignant nodules, highly fluorescent cancerous tubes surrounded by a dark ECM were observed in three cases leading to an overall Se of 38%. Among the 5 false-negative cases, pCLE images were uninterpretable due to a lack of stability in two cases, indeterminate structures were observed in one case and a very dense and strongly fluorescent capsule of fibrosis was visualized in two cases. The benign nodule was correctly diagnosed as such.

Safety evaluation (incidents and adverse events)

One incident related to investigational devices was reported during the trial: peeling of a motorized Confocal Miniprobe™ after multiple introductions and extractions in and out from the trocar. As soon as the damage was visualized, thanks to the laparoscopic display, the motorized Confocal Miniprobe™ and the trocar were immediately withdrawn ending the pCLE procedure. This incident did not lead to an adverse event.

Discussion

Although telepathology has been used since 1986 within the pathology community to support remote diagnostic consultation services [15], direct intraoperative communication between pathologists and surgeons is scarce. To our knowledge, this is the first time that malignant tissues have been assessed in vivo using confocal endomicroscopy during surgery by digitally transmitting real-time pCLE images to the pathologist for expert opinion. The pathologist was of a mandatory support to interpret endomicroscopic images and audio communication was established to discuss pCLE images in real-time [16–19]. Both the pathologist and the surgeon were satisfied with the data-link solution as it enhanced communication between them. The remote display of the laparoscopic surgical field brought an important piece of information to the pathologist as it provided the context of the endomicroscopic image and macroscopic tissue information. The pathologist could observe and apprehend the surgical context and was not anymore limited to the selection of samples provided by the surgeon. The pathologist could also provide the surgeon with some guidance with regard to what should be sampled for frozen section analysis. This setup enabled a real-time characterization of the tissues imaged by pCLE and allowed an interactive collaboration between surgeons and pathologists during the surgical procedure. During open surgery, only endomicroscopic images were sent yielding a more difficult contextualization for the pathologist.

Very limited studies have investigated the feasibility of endomicroscopy during laparoscopic surgery [20–22]. The first two studies assessed the use of endomicroscopy for hepatic fibrosis and steatosis staging while the third study evaluated the use of pCLE during robotic-assisted radical prostatectomy. Our pilot study demonstrated the feasibility and the safety of pCLE during a surgical procedure using a motorized Confocal Miniprobe™ specifically designed for laparoscopic procedures. ICG is used worldwide to evaluate liver function during surgery and its safety has already been validated in several studies [23]. Here, we demonstrated that the topical application of ICG is suitable for the intraoperative visualization and the assessment of tissue at cellular level, in different intra-abdominal organs including the peritoneum and the liver.

As non-specific peritoneal nodule characterization is challenging for surgeons, frozen sections of those nodules at the time of laparoscopic staging are routinely performed to detect the presence of invisible metastases. However, frozen sections are time consuming and their accuracy is limited [4, 5]. Therefore, having immediate and real-time access to the pathologist expertise for tissue characterization during laparoscopy could potentially provide valuable information. A previous study described specific pCLE criteria based on ex vivo benign and malignant peritoneal nodules exhibiting a very high pCLE diagnostic accuracy of 93% for both the surgeon and the pathologist [13]. Here, we showed that the criteria identified ex vivo are well reproducible in vivo with the topical application of ICG at 2.5 mg/ml. pCLE clearly allowed the differentiation of inflammatory from malignant tissues when the cancerous cells reached the peritoneal surface. The current in vivo sensitivity of 67% and specificity of 100% of endomicroscopic criteria raise a potential interest in clinical practice for ruling out malignant nodules thus redirecting patients toward chemotherapy.

Intraoperative examination of the liver in patients with colorectal liver metastases has been reported to identify at least one additional lesion in 10–25% of the cases [1, 24]. The preoperatively planned surgical strategy may change in 9 to 72% of cases, based on intraoperative findings [25–27]. The present series showed that topical application of ICG is optimal for endomicroscopic visualization of liver tissue microstructures in vivo. Out of 9 patients with liver colorectal metastases, 7 had a preoperative chemotherapy leading to tumoral transformation which created a scar and fibrotic tissue within the metastasis. In case of partial regression of subcapsular metastases, viable tumor cells were observed at the periphery. However, it was not possible to visualize malignant tissue microstructures on most of the hepatic metastases because they were located deeper than 40 µm below the capsule. An increased confocal depth of the Confocal Miniprobe™ would increase pCLE sensitivity for the diagnosis of malignancy in the liver. Several studies demonstrated the very high sensitivity and specificity of needle-based confocal endomicroscopy (nCLE) during pancreatic cyst puncture procedure using 19G needle preloaded with AQ-Flex™19 Confocal Miniprobe™ [11, 28]. The intraoperative use of nCLE during indeterminate liver masses biopsies could be used as an alternative for their diagnosis thus overcoming both limitations of the thickened lesion capsule and the Confocal Miniprobes™ confocal depth.

Interestingly, the intravenous administration of ICG allowed microscopic visualization of a contrast agent accumulation in hepatocyte cytoplasm. This was not observed in other organs confirming that ICG accumulates in the liver before being excreted [29]. ICG fluorescence imaging (ICG-FI) has been shown to be safe and useful for identifying small liver tumors that were not identified at surgical exploration under standard white light [30, 31]. The pattern of ICG staining could also allow to distinguish between well-differentiated and poorly differentiated hepatocellular carcinoma and liver metastases of colorectal carcinoma origin [23, 32]. In the present study, ICG was intravenously administrated during the procedure and did

not reveal liver tumors probably because of the reduced ability of liver tumors and surrounding hepatocytes to uptake ICG. However, in this study, the assessment of ICG fluorescence endomicroscopy for intraoperative suspicious hepatic mass characterization was not evaluated when ICG was administered intravenously 1–14 days before laparoscopic surgery [32]. This type of ICG administration remains to be studied.

Our study has several limitations: This pilot study aimed to evaluate the feasibility and the safety of endomicroscopic imaging during laparoscopic surgery and was limited to short-term outcomes. Morbidity and long-term oncological outcomes were not assessed. Another study design would be required to confirm diagnostic performance and to evaluate the impact of this technology on patient management. The optical properties of pCLE enabled imaging of a tissue slice only at 40 µm depth below the surface which limits malignant tissue detection if located several hundreds of microns below the nodule capsule surface. A deeper working distance would enable an increased pCLE sensitivity for malignancy. Then, pCLE image stabilization was the second main limitation. Due to a very narrow field of view, it was difficult for the surgeon to provide real-time stable pCLE images to the pathologist to allow for interpretation. This was enhanced when imaging was performed close to large beating vessels or close to ventilated lungs. While the anesthesiologists could control breathing induced motion, blood pressure could not be controlled during surgical procedure. Further development of the motorized Confocal Miniprobe™ should focus on image stabilization either through a hardware or through a software solution.

Conclusion

Intraoperative pCLE with a dedicated motorized Confocal Miniprobe™ and a near infrared illumination is feasible and safe during laparoscopic procedures. These preliminary results suggest that by technical improvements, pCLE could provide valuable additional information intraoperatively for the characterization of peritoneal carcinomatosis and subcapsular liver nodules. Despite the current technical limitation for the characterization of subcapsular tissues, pCLE represents a promising tool for in vivo surgical margin assessment during procedures, potentially avoiding time consuming and expensive frozen sections. Furthermore, the implementation of intraoperative connection between the surgeon and the pathologist could lead to new avenues for remote collaboration.

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Compliance with ethical standards

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References

1. Hoch G, Croise-Laurent V, Germain A, Brunaud L, Bresler L, Ayav A (2015) Is intraoperative ultrasound still useful for the detection of colorectal cancer liver metastases? HPB 17:514–519. https://doi.org/10.1111/hpb.12393
2. Dromain C, Leboulleux S, Auperin A, Goere D, Malka D, Lumbroso J, Schumberger M, Sigal R, Elias D (2008) Staging of peritoneal carcinomatosis: enhanced CT vs. PET/CT. Abdom Imaging 33:87–93. https://doi.org/10.1007/s00261-007-9211-7
3. Jarnagin WR, Conlon K, Bodniewicz J, Dougherty E, DeMatteo RP, Blumgart LH, Fong Y (2001) A clinical scoring system predicts the yield of diagnostic laparoscopy in patients with potentially resectable hepatic colorectal metastases. Cancer 91:1121–1128
4. Grobmyer SR, Fong Y, D'Angelica M, Dematteo RP, Blumgart LH, Jarnagin WR (2004) Diagnostic laparoscopy prior to planned hepatic resection for colorectal metastases. Arch Surg 139:1326–1330. https://doi.org/10.1001/archsurg.139.12.1326
5. Oberschmid B, Dietrich A, Wittekind C (2012) Frozen sections diagnostics in visceral surgery. Stomach and intestines. Pathol 33:407–412. https://doi.org/10.1007/s00292-012-1601-0
6. Sharma P, Meining AR, Coron E, Lightdale CJ, Wolfsen HC, Bansal A, Bajbouj M, Galmiche J-P, Abrams JA, Rastogi A, Gupta N, Michalek JE, Lauwers GY, Wallace MB (2011) Real-time increased detection of neoplastic tissue in Barrett's esophagus with probe-based confocal laser endomicroscopy: final results of an international multicenter, prospective, randomized, controlled trial. Gastrointest Endosc 74:465–472. https://doi.org/10.1016/j.gie.2011.04.004
7. Gaddam S, Mathur SC, Singh M, Arora J, Wani SB, Gupta N, Overhiser A, Rastogi A, Singh V, Desai N, Hall SB, Bansal A, Sharma P (2011) Novel probe-based confocal laser endomicroscopy criteria and interobserver agreement for the detection of dysplasia in barrett's esophagus. Am J Gastroenterol 106:1961–1969. https://doi.org/10.1038/ajg.2011.294
8. Neumann H, Kiesslich R (2013) Endomicroscopy and endocytoscopy in IBD. Gastrointest Endosc Clin N Am 23:695–705. https://doi.org/10.1016/j.giec.2013.03.006
9. Chang TC, Liu J-J, Hsiao ST, Pan Y, Mach KE, Leppert JT, McKenney JK, Rouse RV, Liao JC (2013) Interobserver agreement of confocal laser endomicroscopy for bladder cancer. J Endourol 27:598–603. https://doi.org/10.1089/end.2012.0549
10. Yserbyt J, Dooms C, Ninane V, Decramer M, Verleden G (2013) Perspectives using probe-based confocal laser endomicroscopy of the respiratory tract. Swiss Med Wkly. https://doi.org/10.4414/smw.2013.13764
11. Napoléon B, Lemaistre A-I, Pujol B, Caillol F, Lucidarme D, Bourdariat R, Morellon-Mialhe B, Fumex F, Lefort C, Lepilliez V, Palazzo L, Monges G, Filoche B, Giovannini M (2014) A novel approach to the diagnosis of pancreatic serous cystadenoma: needle-based confocal laser endomicroscopy. Endoscopy 47:26–32. https://doi.org/10.1055/s-0034-1390693

12. Slivka A, Gan I, Jamidar P, Costamagna G, Cesaro P, Giovannini M, Caillol F, Kahaleh M (2015) Validation of the diagnostic accuracy of probe-based confocal laser endomicroscopy for the characterization of indeterminate biliary strictures: results of a prospective multicenter international study. Gastrointest Endosc 81:282–290. https://doi.org/10.1016/j.gie.2014.10.009
13. Pierangelo A, Fuks D, Benali A, Validire P, Gayet B (2017) Diagnostic accuracy of confocal laser endomicroscopy for the ex vivo characterization of peritoneal nodules during laparoscopic surgery. Surg Endosc 31:1974–1981. https://doi.org/10.1007/s00464-016-5172-7
14. Pierangelo A, Fuks D, Validire P, Benali A, Gayet B (2017) Diagnostic accuracy of confocal laser endomicroscopy for the characterization of liver nodules. Eur J Gastroenterol Hepatol 29:42–47. https://doi.org/10.1097/MEG.0000000000000741
15. Weinstein RS, Bloom KJ, Rozek LS (1987) Telepathology and the networking of pathology diagnostic services. Arch Pathol Lab Med 111:646–652
16. Kayser K, Drlicek M, Rahn W (1993) Aids of telepathology in intra-operative histomorphological tumor diagnosis and classification. Vivo Athens Greece 7:395–398
17. Kayser K, Fritz P, Drlicek M (1995) Aspects of telepathology in routinary diagnostic work with specific emphasis on ISDN. Arch Anat Cytol Pathol 43:216–218
18. Nordrum I, Engum B, Rinde E, Finseth A, Ericsson H, Kearney M, Stalsberg H, Eide TJ (1991) Remote frozen section service: a telepathology project in northern Norway. Hum Pathol 22:514–518
19. Vitkovski T, Bhuiya T, Esposito M (2015) Utility of telepathology as a consultation tool between an off-site surgical pathology suite and affiliated hospitals in the frozen section diagnosis of lung neoplasms. J Pathol Inform 6:55. https://doi.org/10.4103/2153-3539.168515
20. Goetz M, Kiesslich R, Dienes H-P, Drebber U, Murr E, Hoffman A, Kanzler S, Galle P, Delaney P, Neurath M (2008) In vivo confocal laser endomicroscopy of the human liver: a novel method for assessing liver microarchitecture in real time. Endoscopy 40:554–562. https://doi.org/10.1055/s-2008-1077296
21. Goetz M, Deris I, Vieth M, Murr E, Hoffman A, Delaney P, Galle PR, Neurath MF, Kiesslich R (2010) Near-infrared confocal imaging during mini-laparoscopy: a novel rigid endomicroscope with increased imaging plane depth. J Hepatol 53:84–90. https://doi.org/10.1016/j.jhep.2010.01.039
22. Lopez A, Zlatev DV, Mach KE, Bui D, Liu J-J, Rouse RV, Harris T, Leppert JT, Liao JC (2016) Intraoperative optical biopsy during robotic assisted radical prostatectomy using confocal endomicroscopy. J Urol 195:1110–1117. https://doi.org/10.1016/j.juro.2015.10.182
23. Ishizawa T, Fukushima N, Shibahara J, Masuda K, Tamura S, Aoki T, Hasegawa K, Beck Y, Fukayama M, Kokudo N (2009) Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer 115:2491–2504. https://doi.org/10.1002/cncr.24291
24. Ferrero A, Langella S, Giuliante F, Viganò L, Vellone M, Zimmitti G, Ardito F, Nuzzo G, Capussotti L (2013) Intraoperative liver ultrasound still affects surgical strategy for patients with colorectal metastases in the modern era. World J Surg 37:2655–2663. https://doi.org/10.1007/s00268-013-2183-6
25. Tamandl D, Herberger B, Gruenberger B, Schoppmann SF, Puhalla H, Schindl M, Schima W, Jakesz R, Gruenberger T (2008) Adequate preoperative staging rarely leads to a change of intraoperative strategy in patients undergoing surgery for colorectal cancer liver metastases. Surgery 143:648–657. https://doi.org/10.1016/j.surg.2007.11.020
26. Niekel MC, Bipat S, Stoker J (2010) Diagnostic imaging of colorectal liver metastases with CT, MR imaging, FDG PET, and/or FDG PET/CT: a meta-analysis of prospective studies including patients who have not previously undergone treatment. Radiology 257:674–684. https://doi.org/10.1148/radiol.10100729
27. Wagnetz U, Atri M, Massey C, Wei AC, Metser U (2011) Intraoperative ultrasound of the liver in primary and secondary hepatic malignancies: comparison with preoperative 1.5-T MRI and 64-MDCT. AJR Am J Roentgenol 196:562–568. https://doi.org/10.2214/AJR.10.4729
28. Napoleon B, Lemaistre A-I, Pujol B, Caillol F, Lucidarme D, Bourdariat R, Morellon-Mialhe B, Fumex F, Lefort C, Lepilliez V, Palazzo L, Monges G, Poizat F, Giovannini M (2016) In vivo characterization of pancreatic cystic lesions by needle-based confocal laser endomicroscopy (nCLE): proposition of a comprehensive nCLE classification confirmed by an external retrospective evaluation. Surg Endosc 30:2603–2612. https://doi.org/10.1007/s00464-015-4510-5
29. Cherrick GR, Stein SW, Leevy CM, Davidson CS (1960) Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 39:592–600. https://doi.org/10.1172/JCI104072
30. Gotoh K, Yamada T, Ishikawa O, Takahashi H, Eguchi H, Yano M, Ohigashi H, Tomita Y, Miyamoto Y, Imaoka S (2009) A novel image-guided surgery of hepatocellular carcinoma by indocyanine green fluorescence imaging navigation. J Surg Oncol 100:75–79. https://doi.org/10.1002/jso.21272
31. Liberale G, Bourgeois P, Larsimont D, Moreau M, Donckier V, Ishizawa T (2017) Indocyanine green fluorescence-guided surgery after IV injection in metastatic colorectal cancer: a systematic review. Eur J Surg Oncol 43:1656–1667. https://doi.org/10.1016/j.ejso.2017.04.015
32. Shimada S, Ohtsubo S, Ogasawara K, Kusano M (2015) Macro- and microscopic findings of ICG fluorescence in liver tumors. World J Surg Oncol 13:198. https://doi.org/10.1186/s12957-015-0615-5