Confocal Laser Endomicroscopy in GI Tract

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1.1 Introduction

Technologic advances in endoscopic imaging have improved the visualization of mucosal layer, allowing to distinguish neoplastic vs nonneoplastic tissue; however, the imaging is far from a perfect tool. Although histology is a highly accurate technique, it has few limitations: false-negative results in case of ulcers or inflammation, delayed final diagnosis and treatment, and increased costs in pathology in analysis with consequently repeated procedures. Moreover in some GI districts, the accuracy of cytopathology results is low like pancreatic cyst and bile duct due to the difficulties in acquiring tissue. Nevertheless histology is a postmortem analysis without informations about in vivo processes (blood flow, mucosal junction exchanges).

Confocal laser endomicroscopy (CLE), a recent advance of endoluminal imaging, allows an in vivo visualization of mucosal layer with a detailed visualization of tissue and subcellular structures with magnification up to 500–1000-folds. CLE has the potential to predict the final diagnosis (neoplastic vs nonneoplastic) and consequently to guide the next therapeutic procedure without the delay of a pathology response. Indeed, mucosa can be studied at a micron resolution providing an "optical biopsy". Forthcoming developments include the in vivo study of angiogenesis and inflammation in healthy and neoplastic tissues.

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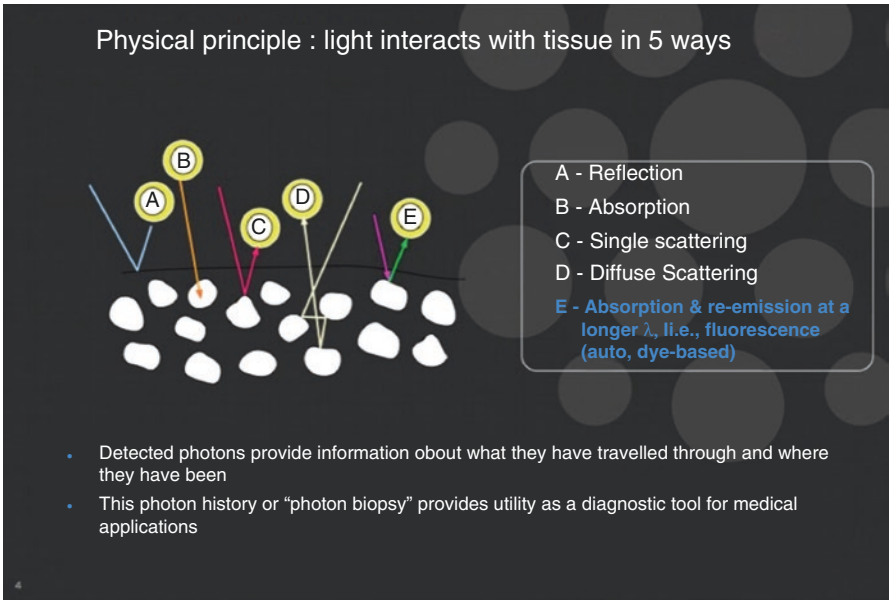

Fig. 1.1 (1) Reflection, (2) absorption, (3) single scattering, (4) diffuse scattering, (5) absorption and reemission at a different wavelength of fluorescence (Courtesy of Pr. Satish Singh, MD Department of Medicine & Biomedical Engineering, Boston University)

1.2 Physics

The physics of the CLE is based on tissue light interactions. Light interacts with tissue in five different ways (Fig. 1.1): (1) reflection, (2) absorption, (3) single scattering, (4) diffuse scattering, and (5) absorption and reemission at a different wavelength of fluorescence.

This last phenomenon can be an autofluorescence or a dye-based fluorescence. The light source is a blue laser beam with variable wavelength (488–660 nm) focused into the plane of interest, and the returned light is filtered by means of a small pinhole that rejects out-of-focus light. The illumination and detection systems are in the same focal plane and are termed "confocal." After passing the pinhole, the fluorescent light is detected by a photodetector device that stabilizes images from a system software transforming the light signal into an electrical one that is recorded by a computer. All detected signals from the illuminated spot are captured and measured. The gray-scale image created is an optical section representing one focal plane within the examined specimen. Because confocal images depend on fluorescence, a fluorescent dye (contrast agent) is required to make the objects visible. The contrast agents can be applied systemically (fluorescein) or topically (acriflavine and cresyl violet). Most studies in humans have been performed with intravenous administration of fluorescein sodium. As fluorescein distribution is outside the cell in intercellular space, it contrasts cellular and subcellular details, connective tissue, and vessel architecture at high resolution but does not stain the nuclei. The safety of

the fluorescein as contrast agent has been demonstrated in ophthalmology; it has been used for years for ophthalmological imaging of blood vessels. Wallace et al. [1] reported a cross-sectional survey study about the safety of fluorescein in CLE procedures. 2272 patients were enrolled and no serious adverse events were reported. Minor adverse events occurred in 1.4 % (transient hypotension, nausea, injection site erythema, mild epigastric pain), but none of them required additional intervention than observation. Acriflavine, another contrast agent, is applied topically and predominantly stains nuclei, but they are not allowed for human use, by FDA and EMEA.

1.3 Systems

In 2003, at the beginning of CLE research, two systems were available: one system inserted in the tip of the scope (eCLE, Pentax Corporation, Tokyo, Japan) and the other, a probe-based system, a separate device from the endoscope, able to be introduced in the working channel of any standard endoscope (pCLE, Cellvizio, Mauna Kea Tech, Paris, France). Currently, only the last one is commercially available and approved to perform CLE (Fig. 1.2).

1.4 Gastrointestinal Applications

In the following pages, we will describe all the current applications of CLE in gastrointestinal tract and literature results.

1.4.1 Barrett's Esophagus (BE)

Barrett's esophagus, defined as an abnormal change in squamous epithelium of the esophagus into an intestinal columnar epithelium (Fig. 1.3), is considered a premalignant condition and the most important risk factor for the development of esophageal adenocarcinoma.

The incidence of esophageal adenocarcinoma has been rapidly rising, increasing from threefold to sixfold since 1990 [2]. International guidelines suggest endoscopic surveillance with random four-quadrant biopsies every 1–2 cm through the extension of intestinal metaplasia for the detection of dysplasia (high grade/low grade) or early intraepithelial cancer (Seattle protocol) [3]. However, surveillance endoscopy has several limitations as dysplastic changes occurring in Barrett's esophagus are not easily identifiable by standard endoscopy. Moreover there is much controversy: first about the real efficacy of such an intense four-quadrant biopsy sampling protocol and second biopsies obtained using this technique are prone to sampling error, and interobserver agreement is low even between advanced operators and even among expert pathologists [4]. Nevertheless, the need for histology confirmation of neoplasia eliminates the ability to direct therapy during the

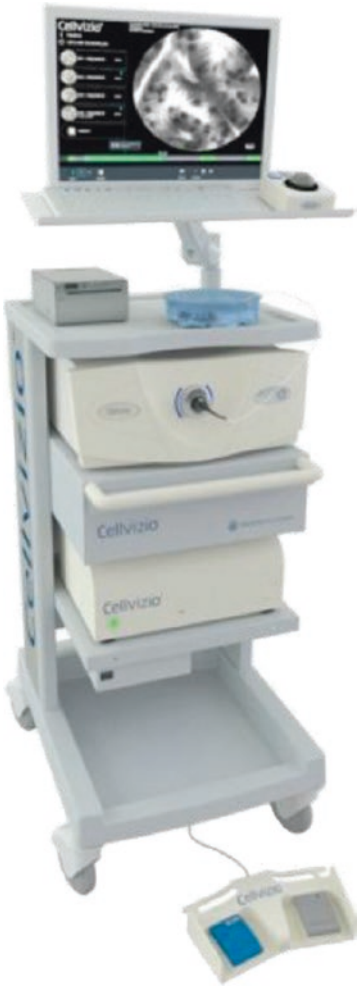

Fig. 1.2 pCLE, Cellvizio, Mauna Kea Tech, Paris, France

index endoscopy. Thus repeated procedures are needed, the first for the diagnosis and then for the therapy. A multiple biopsy protocol could also interfere with the next therapeutic steps.

EMR or ESD could be more difficult without adequate "lifting sign" due to scar tissue after repeated biopsies.

pCLE since its debut has demonstrated a really good accuracy in distinguishing visible neoplastic changes in epithelial cancers that occur at a cellular level. Randomized clinical studies have shown that eCLE or pCLE with white light endoscopy (WLE) can reduce up to 65 % of the number of biopsies needed to reach the same diagnostic yield of WLE alone [5, 6].

The interobserver agreement has been reported to be 86 % with a kappa estimate of 0.72 (CI 95 % 0.58–0.86) [7]. The observers in this study also rated individual

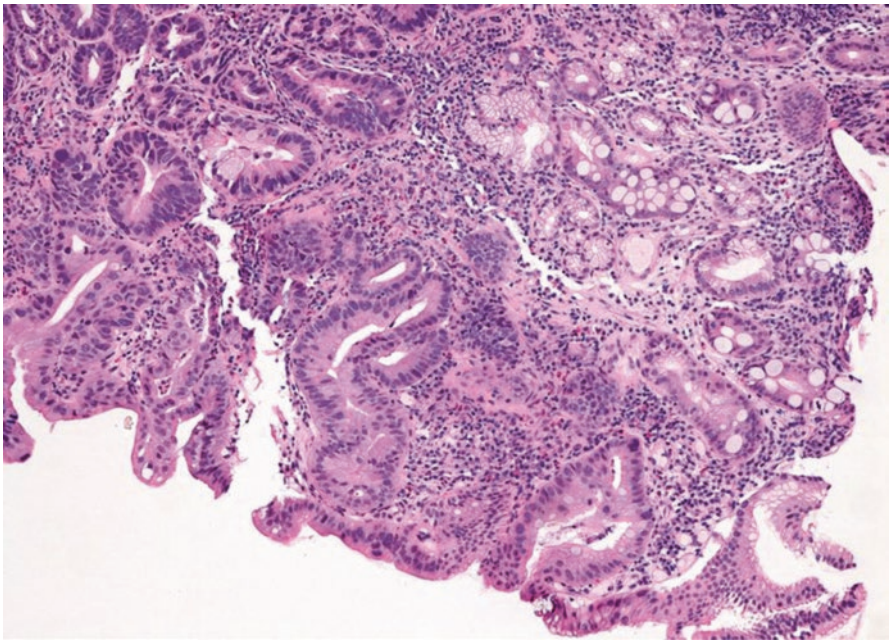

Fig. 1.3 Barrett's esophagus: intestinal metaplasia

features suggestive of neoplasia, such as irregular epithelial thickness, epithelial inhomogeneity, dark epithelial structures (lack of fluorescein uptake), crypt/villi fusion, and irregular vessels. These individual features had good specificity but lower sensitivity, and none of them appeared to compete with the overall diagnostic assessment.

In 2011 a classification has been proposed by a group of experts, the Miami classification, for real-time diagnosis of Barrett's neoplasia with pCLE, and later it has been widely accepted and validated in randomized controlled trials. BE pCLE criteria are uniform villiform architecture and columnar epithelial cells with dark goblet cells. In high-grade dysplasia (HGD), villiform structures have irregularly shaped crypts and dilated capillary vessels. In early adenocarcinoma (EAC), a complete loss of crypt and villiform architecture is observed with irregular and dilated capillaries [8] Fig. 1.4a, b.

A meta-analysis based on eight studies involving 709 patients and 4008 specimens showed a pooled sensitivity and specificity of CLE (in a per-patient analysis) for the detection of neoplasia of 89 % and 75 %, respectively [9].

Another recent application of confocal endomicroscopy is a role in guiding therapeutic endoscopic procedure (1) to localize and predict pathology, (2) to target biopsies and resections in surveillance and treatment, (3) to guide which therapy to use, and (4) to assess treatment adequacy and gauge need for further treatment [10].

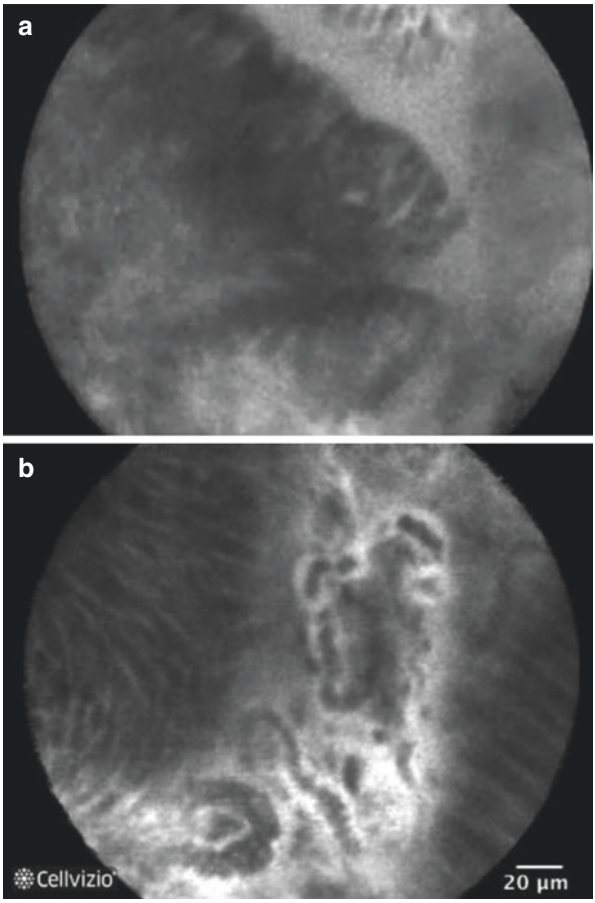

Fig. 1.4 (**a**) pCLE image of low-grade dysplasia with loss of crypt and villiform architecture. (**b**) pCLE image of low-grade dysplasia with irregular and dilated capillaries

1.4.2 Gastritis and Early Gastric Cancer

Gastric cancer remains the world's second leading cause of cancer-related deaths, with a mortality rate of 16.3 per 100,000 in men and 7.9 per 100,000 in women [11], and in eastern countries, the risk of gastric cancer is dramatically high. One of the strategies, to improve prognosis, essentially depends on the earlier detection of preneoplastic changes in mucosal layer because intraepithelial neoplasia and early gastric cancer have a dramatically better prognosis than the advanced one. Currently, the diagnosis of these lesions is based on pathologic assessment. Virtual chromoendoscopy and trimodal imaging endoscopy have demonstrated a significant value for the detection of early gastric neoplasia, whereas the detection of intraepithelial gastric neoplasia (GIN) has been less mentioned and investigated [12].

pCLE demonstrated a high accuracy for detecting gastric carcinomas compared with conventional histological biopsy, providing an excellent definition of the gastric pit pattern with high diagnostic accuracy on the detection of gastric atrophy and gastric intestinal metaplasia as well as *Helicobacter pylori* infection [13–15]. According to the study by Li, the sensitivity and specificity of gastric pit patterns and vessel architecture classification with pCLE for predicting atrophic gastritis were 88.51 % and 99.19 %, respectively. The sensitivity and specificity for predicting intestinal metaplasia were 92.34 % and 99.34 %, respectively. The overall sensitivity and specificity for predicting neoplasia were 89.89 % and 99.44 %, respectively. The use of CLE could possibly reduce the number of unnecessary biopsies and mistaken diagnoses before ESD [16–18]. The interobserver agreement was "substantial" (kappa = 0.70) for the differentiation of neoplasia versus non-neoplasia [19].

Another possible future application in the stomach is the "molecular CLE" that consists in the employment of fluorescein-labeled peptides that can be used for evaluating the expression of receptors in carcinomas in order to individualize the treatment regimens, but also for improving the diagnostic accuracy of endoscopic procedures by identifying otherwise invisible mucosal lesions. These novel applications need further evaluations about efficacy and safety because most of the studies have been conducted in animal facilities or in vitro, while only a limited number of trials have actually been carried out in vivo [20].

1.4.3 Celiac Disease

Many papers have been published about the role of CLE in the study of jejunal mucosa in celiac disease. Alterations of villa in terms of length, numbers, and distribution are easily recognized [21, 22].

1.4.4 Inflammatory Bowel Disease

The use of CLE in colon disease ranges from classifications of colorectal polyps to the study of inflammatory bowel disease (IBD). In particular patients affected by ulcerative colitis (UC) are at increased risk of developing colorectal cancer, so guidelines recommend surveillance including targeted biopsies of suspected lesions and multiple random biopsies. However, the sensitivity of this protocol for the detection of neoplasia is still low. Chromoendoscopy, virtual chromoendoscopy (NBI), and pCLE have been proposed to improve the detection of dysplastic lesions. Kiesslich et al. using the eCLE system reported a sensitivity of 97.4 %, specificity of 99.4 %, and accuracy of 99.2 % to predict the presence of neoplastic changes [23, 24]. Van den Broek et al. [25] reported similar data but lower sensitivity (65 %), specificity (82 %), and accuracy (81 %) due probably to a different system, learning curve in providing images and technical skills. Hurlstone et al. [26] assessed the clinical feasibility and predictive power of

CLE for in vivo differentiation between ALM and DALM in UC. The study evidenced high accuracy of the technique and consequently the possibility to differentiate patient eligible for endoscopic treatment from patients fit for surgery. Recently, De Palma et al. [27] reported the use of CLE applied in real-time inflammation activity assessment. The inflammation activity assessment includes polyps' architecture, cellular infiltration, and vessel architecture. These studies showed that images taken with CLE provide informations that were equivalent to conventional histology, differentiating between active and nonactive UC during ongoing colonoscopy. Recently the use of CLE has been applied also to functional studies in IBD, to evaluate epithelial gaps resulting from intestinal cell shedding rate higher than in healthy patients undergoing colonoscopy. Liu et al. [28] reported that patients with IBD had a significantly higher epithelial gap densities in the terminal ileum compared with controls without IBD. A novel and future application of CLE is the prediction of therapeutic response to TNF-α inhibitors. The utility and safeness of new contrast agent (fluorescent antibodies specific for TNF-alpha receptors) need to be confirmed in other studies [29–31].

1.4.5 Polyps

Colorectal cancer has been recognized as the second most common cause of cancer-related death in the United States [32]. Standard endoscopic inspection cannot by itself distinguish between neoplastic and nonneoplastic lesions; thus, all detected lesions need to be removed and then evaluated by a pathologist, and this approach still remains the gold standard. The first report of the potential role of CLE in predicting pathology of the colon polyps was by Kiesslich et al. [23]. They reported an accuracy in the prediction of intraepithelial neoplasia of 92 % (sensitivity of 97 % and specificity of 99 %). Hurlstone et al. [26] subsequently confirmed Kiesslich data, in particular confirmed the role of CLE in the visualization of high-quality cellular, subsurface vascular, and stromal imaging enabling prediction of intraepithelial neoplasia with accuracy of 99 %. Polglase et al. [33] also confirmed similar results. Recently Xie published that in polyps with diameter > 10 mm, the sensitivity of CLE was 97.1 % and specificity 100 % [34]. A study by Gomez et al. [7] reported also a moderate-to-good interobserver agreement between international collaborative colleagues for distinguishing neoplasia from nonneoplastic tissue. Buchner et al. reported a learning curve of the technique with accuracy of 82 % after 60 procedures [35]. In a meta-analysis that involved 15 studies and 719 patients, the pooled sensitivity of all studies was 0.94 [95 % confidence intervals (CI), 0.88–0.97], and pooled specificity was 0.95 (95 % CI, 0.89–0.97). Real-time CLE yielded higher sensitivity (0.96 vs 0.85, $P < 0.001$) and specificity (0.97 vs 0.82, $P < 0.001$) than blinded CLE. For real-time CLE, endoscopy-based systems had better sensitivity (0.96 vs 0.89, $P < 0.001$) and specificity (0.99 vs 0.82, $P < 0.0001$) than probe-based systems [36].

1.4.6 Pancreas

Pancreatic cystic lesions are relatively common findings in the general population due to the widespread use of cross-sectional imaging. They are a heterogeneous group of lesions as some show a benign behavior and others have a premalignant or malignant potential. A different management should be applied for each type: benign cysts are usually referred for follow-up (based on imaging), while premalignant or malignant lesions should be surgically resected. Endoscopic ultrasound (EUS) is used to evaluate pancreatic lesions and to identify its features as it offers a good visualization of the lesion and its relation with pancreatic main duct. When combined with fine-needle aspiration and cystic fluid analysis, the diagnosis potential is increased, although its accuracy for differentiating benign and malign tumors remains modest [37].

EUS-guided needle-based confocal laser endomicroscopy (nCLE) is a confocal procedure based on a confocal miniprobe (AQ-flex Cellvizio Technology, Mauna Kea Tech, France) thin enough to be passed through a 19-G FNA needle. The miniprobe (0.632 mm diameter) preloaded and screwed by a locking device in the EUS needle is guided endosonographically in the target lesion, and then the miniprobe is pushed under the EUS guidance in gentle contact with the cyst wall. It potentially provides in vivo images of the pancreas at a cellular level, offering the possibility to precisely define a lesion.

The first multicenter study was the INSPECT study [38] with the primary aim to develop descriptive image interpretation criteria and a classification of nCLE findings in pancreatic cysts through a review of prospectively obtained nCLE videos from proven malignant and benign cases. Secondary aims included assessing procedure-related adverse events, technical feasibility of nCLE, and developing a first atlas of nCLE images in pancreatic cysts. A total of 66 patients underwent nCLE imaging, and images were available for 65 patients, eight of whom were subsequently excluded due to insufficient information for consensus reference diagnosis. The presence of epithelial villous structures based on nCLE was associated with pancreatic cystic neoplasm [intraductal papillary mucinous neoplasm (IPMN)] ($P = 0.004$) and provided a sensitivity of 59 %, specificity of 100 %, positive predictive value (PPV) of 100 %, and negative predictive value (NPV) of 50 %. The overall complication rate was 9 % and included pancreatitis (one mild case, one moderate case), transient abdominal pain ($n = 1$), and intracystic bleeding not requiring any further measures ($n = 3$). These preliminary data suggested that nCLE has a high specificity in the detection of IPMN, but may be limited by a low sensitivity.

The second published multicenter study (CONTACT study) [39] aimed to define the criteria of serous cystadenoma (SCA) and to differentiate mucinous from serous pancreatic lesion using nCLE. A total of 31 patients with a solitary pancreatic cystic lesion of unknown diagnosis were prospectively included at three centers. The final diagnosis was based on either a stringent gold standard (surgical specimen and/or positive cytopathology) or a committee consensus. Six not-blinded investigators reviewed nCLE sequences from patients with the most stringent final diagnosis and

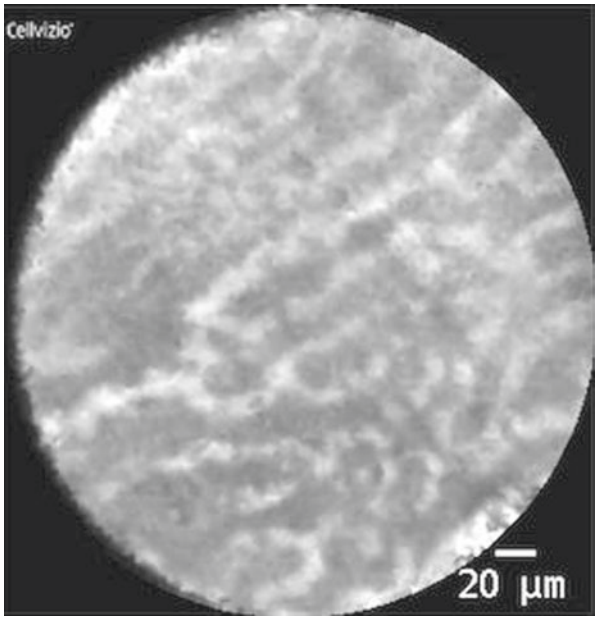

Fig. 1.5 A superficial vascular network pattern visualized on nCLE which corresponds to a dense and subepithelial capillary vascularization visible only in SCA

identified a single feature that was only present in SCA. The findings were correlated with the pathology of archived specimens. After a training session, four blinded independent observers reviewed, with a separate independent video set, and the yield and interobserver agreement for the criterion were assessed. A superficial vascular network pattern visualized on nCLE was identified as the criterion. It corresponded on pathological specimen to a dense and subepithelial capillary vascularization only seen in SCA (Fig. 1.5).

The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of this sign for the diagnosis of SCA were 87 %, 69 %, 100 %, 100 %, and 82 %, respectively. Interobserver agreement was substantial ($k = 0.77$). This new nCLE criterion seems highly specific for the diagnosis of SCA.

Recently a single-center trial by Nakai et al. combined nCLE with an EUS-guided cystoscopy (DETECT study). The goal of this study was to assess the feasibility, safety, and diagnostic yield of the combination of cystoscopy and nCLE in the clinical diagnosis of pancreatic cystic lesion. Thirty patients were included. The procedure was technically successful with the exception of one probe exchange failure. In two patients (7 %), post-procedure pancreatitis developed. Specific features associated with the clinical diagnosis of mucinous cysts were identified: mucin on cystoscopy and papillary projections and dark rings on nCLE. The sensitivity of cystoscopy was 90 % (9/10) and that of nCLE was 80 % (8/10), and the combination was 100 % (10/10) in 18 high-certainty patients. The combination of dual through-the-needle imaging (cystoscopy and nCLE) of pancreatic cysts appears to have strong concordance with the clinical diagnosis of pancreatic cyst [40].

1.4.7 Biliary Tract

Despite the technological developments in the field of imaging as well as available options for endoscopic evaluation through endoscopic retrograde cholangiopancreatography (ERCP), the diagnostic yield in biliary and pancreatic duct strictures and preoperative diagnosis of undetermined biliary strictures are still suboptimal.

The probe usually used for confocal imaging of the pancreatobiliary system is the CholangioFlex miniprobe (Mauna Kea Technologies, Paris, France) that requires a working channel of at least 1.2 mm and has a working length of 4 m. The lateral resolution of the probe is 3.5 μm with a field of view of 325 μm.

The first study aimed to classify confocal patterns related to biliary strictures in the so-called Miami classification study. This study was an attempt to identify as well as standardize the interpretation of finding on pCLE of the biliary system in cases on indeterminate biliary strictures. The combination of thick white bands with dark clumps or epithelial structures provided a 94 % diagnostic sensitivity and 46 % diagnostic specificity. On the other hand, the combination of white bands with thick white bands or fluorescein leakage or dark clamps provided a 61 % diagnostic sensitivity and a 100 % diagnostic specificity [8].

When using a cholangioscope, pCLE had a sensitivity of 96 % (95 % CI, 84–100 %) and a specificity of 76 % (95 % CI, 53–91 %), while when using a catheter, the sensitivity was 100 % (95 % CI, 83–100 %) and the specificity was 62 % (95 % CI, 45–78 %), but there was no statistical difference in the accuracy between these delivery techniques, but the operator confidence about the diagnosis was much higher when using cholangioscopy when compared to a catheter-based approach for pCLE of biliary strictures (43.2 % vs 9.8 %, respectively) [41]. In a randomized trial for the comparison between catheter-guided (fluoroscopy only) pCLE and cholangioscopy-guided pCLE, the accuracy of cholangioscopy-guided pCLE was 82 % compared to 78 % for catheter-guided pCLE. Of note, the sample size of the study was small [42]. The addition of pCLE with ERCP in the evaluation of indeterminate pancreatobiliary strictures can increase the detection of [43] with a sensitivity of (98 % vs 45 %) and NPV (97 % vs 69 %), although it decreased the specificity (67 % vs 100 %) and the PPV (71 % vs 100 %) when compared to index pathology [44].

Although conventionally the use of pCLE for the evaluation of biliary strictures is through a side-viewing duodenoscope, a case series showed pCLE through direct peroral cholangioscopy in 22 out of 24 patients with biliary strictures [45]. In this case series, they classified patients based on the pre-pCLE evaluation for the probability of a malignant etiology for biliary stricture into a range from very unlikely to certainly based on the clinical evaluation as well as imaging, pCLE was found to be complementary to peroral cholangioscopy and ERCP in cases where a malignant etiology was suspected and did not affect the management decision, but it might be sufficient for the confirmation of a malignant etiology when tissue acquisition is not required. pCLE in hilar strictures has also been proven to be of use in a series of 19

patients with the correct identification of all cases with neoplasia, but one false-positive case was reported [46].

Two years later a refinement of the Miami classification named the Paris classification was published [47]. The aim of the Paris classification was to decrease the number of false-positive results when evaluating indeterminate strictures of the biliary system as in inflammatory strictures. Caillol et al. [48] identified four characteristics on biliary pCLE that were associated with benign inflammatory strictures: vascular congestion, dark granular patterns with scales, increased inter-glandular space, and thickened reticular structure. In this study the authors sought to explain the false-positive cases in 60 cases that were enrolled in a registry and found that pCLE diagnosis was either influenced by the ERCP impression or the presence of less than three malignant Miami classification criteria. In a validation study for the Paris classification, it was found to increase the specificity to 73 % compared to 67 % when using the Miami criteria [8]; a similar finding was obtained in a second study [49].

Giovannini et al. [50] evaluated the effect of biliary stenting in 54 patients with indeterminate biliary stenosis and found that biliary stenting decreased the accuracy of pCLE when using the Miami criteria, similar findings were replicated where a decrease in the sensitivity from 88 to 75 %, and specificity from 83 to 71 % was found in those who had cholangitis or a stent inserted prior to pCLE imaging [51]. Although this requires validation in other series, it might be prudent to perform pCLE prior to biliary stenting in cases with biliary strictures of unknown etiology. Also, of note, in the study by Caillol et al. [47], they noted that stricture dilation could induce fluorescein leakage, thus giving the impression of a malignant stricture, while it was subsequently found to be benign.

A recent consensus report by 16 physicians validated seven statements with regard to the use of pCLE in biliary strictures: (1) CLE can be used to evaluate biliary strictures, and the probe can be delivered via a catheter or a cholangioscope; (2) CLE is more accurate than ERCP with brush cytology and/or forceps biopsy in determining malignant or benign strictures, using established criteria; (3) The accuracy of CLE in indeterminate biliary strictures may be decreased by prior presence of plastic stent; (4) The NPV of CLE is very high; (5) The use of CLE can assist clinical decision-making such as excluding malignancy; (6) CLE should be cited as a valuable tool for an increased diagnostic yield in official guidelines; (7) The "black bands" that can be seen in pCLE images have been shown to be collagen fibrils that predictably increase in pathologic tissue [52]. A preliminary analysis of the multicenter FOCUS trial demonstrated that the clinical impression of physicians and pCLE during the workup of biliary strictures outperform tissue sampling where the combination of brush cytology and biopsy would have missed five malignant strictures out of 36 patients [53].

Adding histology/cytology to pCLE resulted in a marginal increase in sensitivity (from 89 to 93 %) but did not change specificity (79 %) compared to the addition of pCLE alone [54].

1.4.8 Primary Sclerosing Cholangitis (PSC)

In a series of 15 patients, 19 strictures, both extra and intrahepatic, were evaluated by ERCP and pCLE. Due to the inflammatory nature of PSC, the authors used a scoring system based on the Miami classification. When there were two of five malignant criteria, the lesion was classified as "suspicious." When there was one criterion, the lesion was classified as "reactive," and the finding of a reticular pattern was deemed as "benign." The findings on pCLE were compared to ERCP, cholangioscopy, histology/cytology, liver explants, fluorescence in situ hybridization (FISH), or 12 months of follow-up. Visualization was successful in 95 % of the procedures; pCLE was found to have a sensitivity of 100 % (95 % CI, 40–100 %), a specificity of 50 % (95 % CI, 9–90 %), a PPV of 67 % (95 % CI, 24.5–94 %), and a NPV of 100 % (95 % CI, 20–100 %). The authors suggested the high-negative predictive value of pCLE could guide in the interval of surveillance in patients with dominant biliary strictures [55, 56].

1.4.9 Solid Organs

With the availability of new probes, CLE allows virtual biopsies of solid organs and other intraperitoneal structures during EUS, laparoscopy, or natural-orifice transluminal endoscopic surgery (NOTES) procedures providing thus a pathological diagnosis based on the morphological features of the solid tissue.

The first report [57] about the use of a probe designed to be used like a handheld laparoscope (FIVE1, Optiscan, Notting Hill, Australia) was used in a liver of a healthy mice and in pathological tissue in human liver disease of a rodent model. Thus, chronic hepatitis, steatosis, and fibrosis were studied using different fluorescent-staining protocols, and images in rodents were collected after topical application or bolus injection of fluorescent agents. No toxic effects on the animals had been observed. Most images were deemed good to excellent quality, and the correlation with ex vivo histopathology was substantial. In the same study group, a handheld probe was used in 25 patients [58] to examine their liver diseases during mini laparoscopy under conscious sedation. Subsurface serial images allowed the visualization of hepatocytes, bile ducts, sinusoids, and collagen fibers in vivo. Typical appearances of liver diseases were identified. Confocal diagnosis of moderate-to-severe steatosis and pericellular fibrosis correlated well with histopathologic analysis of subsequent biopsies (83.3 % and 84.6 %, respectively).

Recently the AQ-flex probe was used through a 19-G needle in solid organs. Mennone and colleagues [59] evaluated, in in vivo feasibility study, the ability of nCLE to distinguish between normal and cirrhotic liver tissue in a non-survival rat model. In this study three healthy and four cirrhotic rats were examined under general anesthesia using three prototypes of confocal miniprobes with different working distances. During laparotomy features were acquired on the surface of the liver capsule and through a 19-gauge needle inserted into the liver parenchyma.

Real-time sequences were recorded after intravenous injection of fluorescein. Biopsy specimens were taken for standard histopathology. All the three miniprobes identified different features like cords of hepatocytes radiating toward central venules in normal livers and distorted hepatic architecture in cirrhotic livers.

Another feasibility study of nCLE in a porcine model by Becker [60] was applied in various abdominal organs such as the pancreas, lymph node, spleen, and liver. At three academic centers, ten pigs were examined in a non-survival experiment with the animals under general anesthesia. Either EUS-guided organ puncture or NOTES procedure was technically feasible allowing real-time in vivo images at histologic resolutions when compared to standard histopathology.

Subsequent human clinical trials were focused on the evaluation of the pancreas and of its pathological features. The first multicenter pilot study [38] evaluated the feasibility of nCLE in sixteen pancreatic cysts and two pancreatic masses. No complications occurred after the puncture of pancreatic solid mass. The final diagnosis of the solid lesions was established after surgical resection in one case (pancreatic endocrine tumor) and by cytology in the other case (adenocarcinoma). Of the two solid masses, image quality was respectively deemed good (NET) and moderate (adenocarcinoma). Karstensen et al. [61] published a feasibility study in 25 patients with pancreatic masses studied with nCLE preloaded into the needle at the same location of EUS-FNA. No adverse advents were registered, but the diagnostic value was considered limited. In a second paper [62], the same group evaluated prospectively 20 patients with pancreatic masses selected for EUS-FNA. Also for these patients, the FNA was performed at the same location studied with nCLE. Features like dark aggregates and pseudoglandular structures were observed in all pancreatic adenocarcinomas.

An interesting field of application of pCLE consists in the use of fluorescein-labeled antibodies that have shown the feasibility of in vivo immunohistological staining. Moreover, the fluorescein-labeled antibodies direct to a specific target could evaluate the expression of cellular receptors. The detection of these receptors in solid neoplasia might potentially be correlated to the efficacy of treatment regimens (tailored therapy).

Nakai and colleagues [63] evaluated whether this method was feasible using needle-based confocal laser endomicroscopy (nCLE) for extraluminal investigation of the pancreas in conjunction with topical administration of antihuman EGFR-fluorescein-conjugated monoclonal antibodies and antihuman surviving-fluorescein-conjugated monoclonal antibodies. In pancreatic cancer the expression of EGFR and of anti-apoptosis protein surviving is significantly upregulated. Although the number of pigs was limited, the technique was feasible. However, the resolution of the pictures obtained was low. Other problems were the immunogenic nature of antibodies, long half-life in serum, and slow penetration into tissues due to their high molecular weight. Furthermore, antibodies are expensive to produce in high amounts.

Another experimental study [64] showed a precise identification of perigastric lymph nodal metastasis using CLE systems to detect fluorescein-labeled hepatic cells in original noncancer animal model. Various tumor cell lines coupled with dye substances can be injected in the submucosa of the GI tract to migrate to regional

lymph nodes and allow for testing node navigation technologies or advanced optical imaging systems. They choose hepatic cells as they are easy to be collected in a large amount and for their ability to be differentiated from the lymphoid tissue by IHC. This model enables the potential of cancer-specific fluorescent antibodies of recognizing cancer cells in real time. This model is reproducible and simulated metastatic spread of gastric cancer.

CLE technology was used also to make several important observations on functional and molecular features of apoptosis. Goetz and colleagues [65] reported hepatocyte apoptosis studied with confocal endomicroscopy: different features were seen in living rodents following distinct morphological, functional, and molecular features of apoptosis in intact liver in vivo and at high resolution. In another study [66], the injection of fluorescent apoptosis marker was used to study the effect of high-linear energy transfer radiation on the HCC tumor model orthotopically transplanted.

Conclusion

In conclusion CLE may be a useful virtual biopsy of GI organs. Real-time confocal laser endomicroscopy has the potential to improve sampling error and potentially reduce the number of procedure needed for a diagnosis in more difficult organs to access such as the bile duct and pancreas. In situations in which there is no on-site cytopathologist available, endomicroscopy could facilitate cytology acquisition. Therefore, safety issues still need further evaluations. However, a limited number of trials have actually been carried especially in solid organs. However, this finding has to be confirmed in larger studies. Further studies are needed to assess the diagnostic accuracy and if nCLE in focal masses is clinically relevant in selected cases.

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