The role of endoscopic imaging for an improved diagnosis of colorectal neoplasia
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Citation for published version (APA):
van den Broek, F. J. C. (2010). The role of endoscopic imaging for an improved diagnosis of colorectal neoplasia

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Pilot study of probe-based confocal laser endomicroscopy during colonoscopic surveillance of patients with longstanding ulcerative colitis

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ABSTRACT

Background: Surveillance of patients with ulcerative colitis (UC) consists of taking targeted and random biopsies, which is time-consuming and its efficiency is doubtful. The use of probe-based confocal laser endomicroscopy (pCLE) may increase efficiency.

Objective: To evaluate the feasibility and diagnostic accuracy of pCLE in UC surveillance.

Design: Prospective pilot-study

Setting: Academic Medical Centre Amsterdam

Methods: In 22 UC-patients, 48 visible lesions and 87 random areas were initially evaluated by real-time narrow-band imaging (NBI) and high-definition endoscopy (HDE). Before taking biopsies, fluorescein-enhanced pCLE was performed. All pCLE videos were scored afterwards by 2 endoscopists who were blinded for histology and endoscopy.

Outcome measures: (1) Feasibility of pCLE, expressed as: required pCLE imaging time; percentage of imaging time with clear pCLE histology; and pCLE video quality rated by 2 endoscopists. (2) Diagnostic accuracy of pCLE.

Results: The median required pCLE imaging time was 98 sec for lesions vs. 66 sec for random areas (p=0.002). The median percentages of imaging time with clear pCLE histology were 61% vs. 81% respectively (p<0.001). The pCLE video quality was rated as good/excellent in 69%. Feasibility was significantly poorer for sessile and pedunculated mobile lesions. The sensitivity, specificity and accuracy of blinded pCLE were 65%, 82% and 81%, whereas these figures were 100%, 89% and 92% for real-time endoscopic diagnosis with NBI and HDE.

Limitations: Small sample size, blinded pCLE assessment

Conclusion: This study demonstrates that pCLE for UC surveillance is feasible with reasonable diagnostic accuracy. Future research should show whether increased experience with pCLE improves its ease-of-use and whether real-time pCLE diagnosis is associated with higher diagnostic accuracy.
Introduction

As patients with ulcerative colitis (UC) have an increased risk of developing neoplasia, guidelines recommend colonoscopic surveillance including targeted biopsies of suspicious lesions and multiple random biopsies. Taking many biopsies is time-consuming, has a low but non-negligible risk of secondary hemorrhage, and only has a moderate sensitivity for neoplasia detection. Furthermore, adenoma-like neoplasia may be treated inefficiently when first taking biopsies, whereas non-neoplastic lesions may be left in situ without biopsies at all. It is therefore desirable to replace the inefficient procedure of targeted and random biopsies by a more efficient method.

Several endoscopic techniques may facilitate differentiation of neoplasia from non-neoplastic mucosa, thereby increasing the efficiency of UC surveillance. Chromoendoscopy (CE) elucidates mucosal patterns that can be used for the prediction of histopathology in-vivo with an accuracy of 93%. Narrow-band imaging (NBI) reveals mucosal and vascular patterns and has a comparable diagnostic accuracy as CE with respect to differentiating neoplastic from non-neoplastic sporadic polyps. Whereas CE or NBI can be used for predicting histology, confocal laser endomicroscopy (CLE) is in fact in-vivo histology and has shown high agreement with true histopathology. Possible advantages of CLE during UC surveillance are that it may substitute random biopsies and may obviate targeted biopsies when CLE reveals non-neoplastic histology. If CLE suggests neoplasia, the endoscopist can decide whether to take biopsies or to perform endoscopic resection. To reach this goal however, CLE should be easy-to-use and have a high diagnostic accuracy when compared to true histopathology.

Until now, research on CLE in UC patients was limited to a system with the confocal technology integrated into the endoscope (Pentax inc., Tokyo, Japan). Recently, probe-based CLE has been launched (Mauna Kea Technologies, Paris, France) making use of probes that fit through the working channel of any standard colonoscope. The objective of our study was to evaluate the feasibility and diagnostic accuracy of probe-based CLE (pCLE) in UC surveillance.

Patients and Methods

Patients
Patients with UC, scheduled for colonoscopic surveillance at the Academic Medical Centre Amsterdam, were invited to participate when they met the following inclusion criteria: history of UC ≥ 8 yrs and inactive disease. Exclusion criteria were: contraindications to fluorescein, non-correctable coagulopathy, age ≤ 18 years, and inability to obtain informed consent. This study was approved by our institutional review board.

Endoscopic equipment
Colonoscopies were performed using the Lucera system (CV-260, Olympus, Tokyo, Japan), incorporating high-definition endoscopy (HDE), NBI, and optical magnification (100x). The accessory channel of the endoscopes (CF-H260Z, Olympus, Tokyo, Japan) was located at the right lower quadrant of the endoscopic view (Figure 1-2).
Confocal laser endomicroscopy was performed with the probe-based system (Cellvizio-GI, Mauna Kea Technologies, Paris, France). A laser scanning unit excites light with a wavelength of 488nm via a fiberoptic miniprobe (ColoFlex type UHD; Mauna Kea Technologies, Paris, France). The probe (diameter 2.5mm) can be inserted through the accessory channel of every standard colonoscope for contact with the mucosa (Figure 2). Only light of 500-650nm is collected by the probe to create optical slices with a field of view of 240µm, at a depth of 60µm below the mucosal surface (lateral resolution 1µm). The images are scanned with a rate of 12 frames per second, hence producing a real-time video on a second screen. For tissue contrast we used intravenous fluorescein (5mL, 10%) that has shown to be safe in ophthalmology and previous CLE studies.

**Colonoscopic procedure**

Patients underwent colonoscopy under conscious sedation using midazolam combined with fentanyl. Procedures were performed by 4 endoscopists with minimal experience in CLE. After cecal intubation, 20mg butyl-scopolamine was given to reduce colonic motility.

During withdrawal the colon was scrutinized for visible lesions (by either HDE or NBI at the discretion of the endoscopist). Detected lesions were assessed for size and location, and scored according to the revised Paris classification. Furthermore, real-time diagnoses (scored as either neoplasia or non-neoplastic) were made of all lesions based on mucosal and vascular patterns by NBI and HDE.

After HDE/NBI evaluation, each lesion was inspected with pCLE with a maximum of 7 lesions per patient (for reasons of time). While positioning the probe on the lesion, its location was noted with respect to the quadrant of the endoscopic view (left upper, left lower, right upper, right lower, central; Figure 1). This was noted since the position of the working channel relative to the lesion may affect the ease-of-use of pCLE. The recording of pCLE frames was started at positioning the probe against the lesion and stopped when removing the probe. The probe was removed when the endoscopist decided to have a video that clearly depicted the microscopic mucosal and vascular structures. No real-time pCLE diagnoses were made, but all pCLE videos were stored on a computer for assessment afterwards. Finally, biopsies were taken for histopathology.

After scrutinizing each colonic segment for suspicious lesions, four quadrant random biopsies were taken every 10 cm of colon, according to current guidelines. Of maximally 7 random areas a pCLE video was made which was also evaluated afterwards. Biopsy material that corresponded to the pCLE videos was sent in separate jars for histopathology.
Histopathology
Biopsies were evaluated by two pathologists who were blinded for endoscopic diagnosis. One of them was a gastrointestinal expert (SvE). Histopathology was classified according to the Vienna criteria.22

Evaluation of pCLE videos
The pCLE videos were stored as MKT-files (Cellvizio Viewer, Mauna Kea Technologies, Paris, France). Afterwards, only pCLE frames that demonstrated crypts/vessels were selected and converted into a new video in AVI-format. The total times of the original MKT-file and new AVI-file were noted.

The pCLE videos in AVI-format were subsequently scored by two endoscopists separately, blinded for histology and endoscopic information. Patient and time information was removed from the video, but no other post-processing was done. First, the videos were scored for quality (poor: no crypt/vessel visualization; moderate: visualization unsure and unclear; good: visualization sure but unclear; excellent: visualization sure and clear). Second, the pCLE histology was scored according to a recently developed classification scheme which categorizes crypt and vessel architecture (Table 1; Figure 3).23 In accordance with the Mainz criteria, crypt- or vessel-type 3 were expected to be neoplastic on histopathology.13

Figure 1: quadrants of the endoscopic view for defining the location of lesions while performing probe-based confocal laser endomicroscopy. LUQ left upper quadrant, LLQ left lower quadrant, RUQ right upper quadrant, RLQ right lower quadrant, C Center, P position of the working channel
Table 1: description of the recently developed pCLE classification in which crypts and vessels are scored separately on the pCLE videos

### Outcome measures

Primary outcome was the feasibility of pCLE, expressed as: (a) total time of pCLE imaging that was necessary to make a video of colonic mucosa; (b) proportion of total imaging time in which crypts/vessels were visible on the pCLE images; and (c) pCLE video quality rated by the two endoscopists.

Secondary outcomes were sensitivity, specificity, and overall accuracy of the blinded pCLE classification and of the real-time endoscopic prediction of histology based on NBI and HDE. Histopathology was used as reference standard. Lastly, the interobserver agreement on the pCLE classification was calculated.

### Statistical analysis

Descriptive statistics were used to analyze the feasibility measures of pCLE. Feasibility measures were compared for different locations of lesions by using either Kruskal-Wallis (time comparisons) or Chi-square testing (video quality).

For calculating sensitivity, specificity and accuracy, a pCLE classification of 1-2 was considered non-neoplastic whereas a pCLE classification of 3 (either crypt-type or vessel-type) was considered neoplastic.

Finally, interobserver agreement of the pCLE classification was expressed as (1) percentage of full agreement between the two observers; and (2) kappa statistics (± 95%-confidence interval). Kappa-values were interpreted according to Landis and Koch.24 The manuscript was reported according to the STARD statements for diagnostic accuracy studies.25
Results

Patients
From October 2007-September 2008, 22 patients (mean age 54 yrs; 12 male) were included. Their median UC duration was 17 yrs (8-28); 18 had extensive colitis; and 4 had concomitant primary sclerosing cholangitis. Four endoscopists (ED, PF, PCFS, and CYP) performed 10, 9, 2 and 1 procedures each and no complications occurred.

Real-time endoscopy
Forty-eight lesions were examined by NBI, HDE and pCLE. Their median size was 10mm (3-100) and 37 (77%) were flat. Histopathology demonstrated low-grade intraepithelial neoplasia (LGIN) in 10; normal mucosa in 3; hyperplasia in 15; chronic inflammatory changes in 9; and active inflammation in 11 lesions.

The sensitivity, specificity and accuracy of the real-time endoscopic diagnosis of lesions, based on HDE and NBI, had a sensitivity, specificity and accuracy of 100% (95%-CI: 72-100), 89% (76-96), and 92% (80-97).

In addition, 87 random areas were examined by pCLE. Histology showed LGIN in 2; normal mucosa in 51; hyperplasia 11; chronic inflammatory changes in 16; and active inflammation in 7.

Probe-based confocal laser endomicroscopy
Feasibility
The median required time to obtain a reliable pCLE video was 98 seconds (interquartile range: 61-142) for lesions vs. 66 seconds (50-97) for random areas (p=0.002). The median percentage of the total pCLE imaging time that clearly demonstrated crypts/vessels was 61% (40-72) for lesions vs. 81% (60-93) for random areas (p=0.001). In 2 videos (1.5%) no histology was visualized at all, and hence these were excluded from assessment by the blinded observers.

The required time to make reliable videos did not differ between the quadrants of the endoscopic view (Kruskal-Wallis; p=0.975). However, the percentage of pCLE time in which crypts/vessels were visible, was significantly lower for areas located in the left lower quadrant (22 areas; median 42%) compared to all other quadrants (113 areas; medians 74-80%) (p=0.001). In addition, required pCLE time was shorter and the percentage of pCLE time with clear crypts/vessels was higher among flat than among protruded mobile lesions: medians 70 vs. 105 seconds (p=0.027), and 74% vs. 41% (p<0.001) respectively.

Of the 133 pCLE videos that were blindly assessed, 69% was rated as having good/excellent quality. Videos of areas in the left lower quadrant of the endoscopic view were less often rated as good/excellent (52% vs. 70-76%; p=0.014). The percentage of videos rated as good/excellent did not differ between lesions and random areas (64% vs. 72%; p=0.196).

Diagnostic accuracy
Of the 133 videos that were evaluated using the pCLE classification scheme, the two observers felt unable to score 1 and 6 videos respectively for vessel-type; and 0 and 6 videos for crypt-type (=inconclusive index-test results). Their scores are presented in Table 2. Ten out of 20 assess-
ments of neoplastic areas (50%) were correctly scored as crypt-type 3; and 11 out of 20 (55%) as vessel-type 3. Active inflammation and hyperplasia were associated with crypt-/vessel-type 3 in 18-22% of cases as well.

Table 2: Correspondence between the recently developed pCLE classification (scored by two endoscopists) and the final histopathology (i.e. reference standard). Data are represented as number of colonic random areas or lesions (%).

<table>
<thead>
<tr>
<th>Histopathology (reference standard)</th>
<th>Normal</th>
<th>Hyperplastic</th>
<th>Chronic inflammation</th>
<th>Active inflammation</th>
<th>Neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crypt-type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>65 (60)</td>
<td>14 (28)</td>
<td>12 (25)</td>
<td>1 (3.0)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>2a</td>
<td>16 (15)</td>
<td>7 (14)</td>
<td>11 (23)</td>
<td>6 (18)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>2b</td>
<td>6 (5.6)</td>
<td>6 (12)</td>
<td>8 (17)</td>
<td>5 (15)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>2c</td>
<td>2 (1.9)</td>
<td>3 (5.9)</td>
<td>5 (10)</td>
<td>4 (12)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>2d</td>
<td>2 (1.9)</td>
<td>2 (3.9)</td>
<td>5 (10)</td>
<td>2 (6.1)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>2e</td>
<td>12 (11)</td>
<td>8 (16)</td>
<td>7 (15)</td>
<td>9 (27)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>3</td>
<td>5 (4.6)</td>
<td>11 (22)</td>
<td>0 (0)</td>
<td>6 (18)</td>
<td>10 (50)</td>
</tr>
<tr>
<td><strong>Vessel-type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>58 (54)</td>
<td>23 (46)</td>
<td>30 (64)</td>
<td>13 (38)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>2</td>
<td>43 (40)</td>
<td>16 (32)</td>
<td>11 (23)</td>
<td>14 (41)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>3</td>
<td>7 (6.5)</td>
<td>11 (22)</td>
<td>6 (13)</td>
<td>7 (21)</td>
<td>11 (55)</td>
</tr>
</tbody>
</table>

For differentiating neoplastic from non-neoplastic mucosa, the pCLE classification had a sensitivity of 65% (43-82), specificity of 82% (77-86), and accuracy of 81% (76-85). The accuracy was 84% for videos rated as good/excellent vs. 73% for videos rated as poor/moderate (p=0.037).

**Interobserver agreement**

The pCLE classification had a *fair* interobserver agreement (kappa 0.33; 0.23-0.42) for crypt-type and *moderate* agreement (kappa 0.52; 0.39-0.64) for vessel-type. Their respective percentages of full agreement were 44% and 67% (p<0.001).

When simplifying the classification to neoplasia (crypt-/vessel-type 3) versus non-neoplastic (all remaining types), the agreement became *moderate* (kappa 0.47; 0.29-0.66) with a percentage full agreement of 83%.

**Discussion**

The main objective of this study was to assess the feasibility of pCLE during UC surveillance, expressed as (a) required imaging time; (b) proportion of imaging time that crypts/vessels were visible; and (c) percentage of pCLE videos that had good/excellent quality. These parameters reflect the effort that is necessary to obtain pCLE videos of sufficient quality.
The median time required to obtain a pCLE video of lesions was 98 seconds, whereas this was 66 seconds for random areas. This difference was caused by the difficulty of performing pCLE for mobile (sessile/pedunculated) lesions. We did however not yet use a small transparent cap on the tip of the endoscope which should help to stabilize the probe and thus the image. With the miniprobe inserted through the working channel of an endoscope it is difficult to apply same-time suction for stabilization. Kiesslich et al., who used the integrated CLE, showed that the imaging time per lesion was approximately 34 seconds (calculated from their results). The difference can be explained by their longer experience but also by their different system. The variable scanning depth of the integrated system (0-250mm) may contribute to an increased ability to visualize histology in a shorter time whereas pCLE has a fixed imaging plane at ~60mm. Moreover, the integrated system has a working channel opposite to the CLE-window, enabling same-time suctioning for stabilization.

Furthermore, 61%-81% (medians of lesions and random areas) of pCLE time led to actual visualization of crypts/vessels. After selecting frames that truly demonstrated crypts/vessels for assessing video quality, 69% was rated as good/excellent. These two feasibility measures were significantly inferior for lesions located in the left lower quadrant of the endoscopic view. As the miniprobe can be inserted through the accessory channel of any endoscope with variable locations of the working channel, this finding is likely to vary between different endoscopes. We used colonoscopes which have the opening of the working channel at the 5 o’clock position. By torquing the endoscope however, one can easily position the lesion in the most appropriate quadrant. Since integrated CLE has a fixed imaging window at the left lower quadrant, the area of interest should always be brought into this corner of the endoscopic view. To the contrary, pCLE has the possibility of targeting areas further away from the endoscopic tip, enabling endomicroscopy of the upper quadrants as well. Kiesslich et al. scored 84% of CLE images of lesions as having good quality. The difference with our results again may be attributed to their higher experience but may also be caused by interobserver variability in the assessment of video or image quality and by the slightly higher lateral resolution (0.7 vs. 1mm) of the integrated system. Because studies using pCLE in the colon are lacking, our feasibility results cannot be compared to other studies using this system.

From our feasibility results and while still in our learning curve, it can be deduced that using pCLE would lead to an increase in colonoscopy time of about 30-40 minutes, as visualizing one random area would take 66 seconds. Additional pCLE of detected lesions would account for more colonoscopy time. A question that remains to be answered is whether increased experience with pCLE would substantially reduce this time and would increase the proportion of images with clear histology and good quality. Direct comparison between the integrated and probe-based system would then be interesting.

Secondary aim of our study was to assess the accuracy of our pCLE classification, which was 81% (sensitivity 65%; specificity 82%). The disappointing specificity may be caused by the fact that hyperplasia and inflammation also frequently led to reduced goblet cells and increased striped/irregular epithelium, which are associated with neoplasia too. On the other hand, neoplasia was associated with regenerative epithelium (crypt-type 2a-2e) in several cases, contributing to the poor sensitivity (Figure 4). In this regard, the classification scheme we developed should probably be refined to account for neoplasia more accurately.
We have to stress however that our observers were blinded and scored pCLE videos afterwards. Kiesslich et al. used real-time CLE next to chromoendoscopy, showing an accuracy of 97.8%. In order to put their results in perspective, we calculated that our endoscopic diagnosis, based on NBI/HDE in real-time, led to a sensitivity of 100% and specificity of 89%. One can imagine that real-time endoscopy and pCLE would also lead to a higher accuracy, since the endoscopist is aware of the endoscopic nature of the lesion. Alternatively, one may argue that there is little possible gain for CLE as add-on test next to HDE/NBI, as these already had an accuracy of 92%. The clinical value of pCLE as add-on test in UC surveillance should therefore also be studied as a real-time increment to HDE, with and without NBI.

Meining et al. also used 2 blinded observers for pCLE evaluation with comparable accuracies of 74-82%. Subanalysis of high quality videos led to an accuracy of ~93% in that study. The accuracy of pCLE in our study was maximally 84% when excluding videos of poor/moderate quality. Meining et al. however included non-colonic lesions and used cresyl-violet as contrast agent instead of fluorescein, making comparison with our study difficult.

Lastly, we assessed the reproducibility of our pCLE classification that was created by an international collaboration of pCLE users. The classification included 7 crypt-types which had a poor agreement (kappa 0.33; full agreement 44%). When simplifying the classification to neoplasia versus non-neoplastic, the agreement increased to moderate (kappa 0.47; full agreement 83%) which is acceptable in view of our learning phase. Hurlstone et al. demonstrated a kappa value of 0.81 for integrated CLE, although they evaluated non-UC patients only. Inflammation may interfere with reproducibility as the distinction between neoplasia and inflammation is more difficult. Nevertheless, our interobserver results force us to refine and simplify our pCLE classification in order to increase its clinical value in UC surveillance.

In summary, this study showed that pCLE is feasible during UC surveillance with reasonable results. Probe-based CLE in our first experience increased colonoscopy time significantly. Technical enhancements are needed to provide images of sufficient quality and increased experience should reveal whether enhanced technical skills improve its ease-of-use. Secondly, the achieved diagnostic accuracy and reproducibility are justifiable in view of our learning phase and blinded assessments, but are currently falling short when compared to the accuracy achieved with real-time HDE and NBI. Future research should focus on whether real-time use of pCLE will improve the overall diagnostic accuracy.

>> For figures 2-4; see page 142
Reference List


