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Confocal Laser Endomicroscopy vs Biopsies in the Assessment of Persistent or Recurrent Intestinal Metaplasia/Neoplasia after Endoscopic Treatment of Barrett's Esophagus related Neoplasia

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ABSTRACT

Background & Aims: Patients after endoscopic treatment of Barrett's esophagus (BE) related neoplasia (BORN) should enter endoscopic surveillance with biopsies to detect persistent or recurrent neoplasia or intestinal metaplasia (IM). Probe-based confocal laser endomicroscopy (pCLE) serves as a virtual biopsy and could replace standard biopsies. However, the role of pCLE in patients after endoscopic treatment of BORN has not been systematically assessed. The aim of this study was to compare pCLE with biopsies in detecting persistent/recurrent IM/neoplasia.

Methods: A single center, prospective and pathologist-blinded study was performed. Patients after endoscopic treatment of BORN (endoscopic resection or dissection, radiofrequency ablation) underwent surveillance endoscopy with pCLE followed by biopsies.

Results: A total of 56 patients were enrolled: initial diagnoses were low-grade dysplasia (LGD) in 24 patients (43%), high-grade dysplasia (HGD) in 12 patients (21%) and early adenocarcinoma (EAC) in 20 patients (36%). Only one patient (2%) experienced recurrent neoplasia (LGD), which was diagnosed by pCLE only. Twenty patients (35.7%) experienced persistent/recurrent IM, diagnosed by both pCLE and biopsies in 17 patients (17/30, 85%) and by pCLE only in 3 pts (3/30, 15%). Sensitivity, specificity, positive and negative predictive values to diagnose recurrent/persistent IM did not differ significantly between pCLE and biopsies; diagnostic accuracy was 100% (95%CI 93.6-100) for pCLE and 94.6 (95%CI 85.1-98.9%) for biopsies, p=0.25. In patients with IM detected by both tested methods, pCLE detected significantly more goblet cells (median 43 per patient) than biopsies (median 12 per patient), p=0.01.

Conclusion: pCLE is at least as effective as standard biopsies in the detection of persistent/recurrent IM after endoscopic treatment of BORN.

Key words: probe-based confocal laser endomicroscopy – Barrett's esophagus related neoplasia – radiofrequency ablation – endoscopic resection– neo-Z-line – intestinal metaplasia.

Abbreviations: BE: Barrett's esophagus; BORN: Barrett's esophagus related neoplasia; CLE: confocal laser endomicroscopy; CR-IM: complete remission of intestinal metaplasia; CR-N: complete remission of neoplasia; EAC: early adenocarcinoma; ER: endoscopic resection; ESD: endoscopic submucosal dissection; HD-WLE: high-definition white light endoscopy; HGD: high-grade dysplasia; IM: intestinal metaplasia; LGD: low-grade dysplasia; pCLE: probe-based confocal laser endomicroscopy; RFA: radiofrequency ablation.

INTRODUCTION

Barrett's esophagus (BE) is a premalignant condition with the risk of developing adenocarcinoma, which is gradually rising according to the presence of dysplasia. Patients with BE are undergoing regular surveillance endoscopies with biopsies to ensure a detection of dysplasia or early cancer at a stage when it may be treated endoscopically. Patients with detected low-grade dysplasia (LGD), high-grade dysplasia (HGD) and early cancer should undergo endoscopic treatment as it decreases the risk of progression. The aim of endoscopic treatment [endoscopic resection (ER), radiofrequency ablation (RFA)] is not only to remove neoplastic lesions, but also to eradicate the whole segment of BE to decrease the risk of metachronous malignancy. Histologically, the aims of endoscopic treatment are a complete remission of both neoplasia (CR-N) and of intestinal metaplasia (CR-IM). However, once BE is eliminated, patients still need endoscopic surveillance because recurrences of both neoplasia and IM

can occur [1-5]. On surveillance endoscopy, biopsies should be taken from the neo-Z-line (IM and/or neoplasia), from the previously treated area (to exclude buried glands) and from any macroscopically visible abnormality. Intervals between surveillance endoscopies vary and principally depend on the primary diagnosis (being the most frequent in patients with early cancer and the least frequent in patients with LGD) [6-7].

Probe-based confocal laser endomicroscopy (pCLE) allows real-time microscopic imaging. Its use has been recommended as an add-on method to improve diagnosis of BE and to increase the detection of neoplasia while decreasing the number of biopsies needed [8-11]. On the other hand, pCLE has not been recommended yet for patients in the follow-up after endoscopic treatment of BE related neoplasia (BORN) given the lack of scientific evidence. Only one randomized study specifically addressed the role of pCLE in patients after RFA [12]. Wallace et al. [12] showed no evidence that the addition of pCLE to high-definition white light endoscopy (HD-WLE) imaging would improve the management (no-treatment in those without residual metaplasia/neoplasia or treatment in those with residual metaplasia/neoplasia). However, pCLE might also be a useful tool if it could replace standard biopsies in the detection of persistent/recurrent IM or neoplasia. Several studies have already shown that pCLE is equally effective as standard tissue sampling methods in specific indications [13-15].

So far, no study has compared the diagnostic yield of biopsies with that of pCLE in patients after endoscopic treatment of BORN. Therefore, we designed the present prospective, controlled and pathologist-blinded study to compare pCLE to biopsies in detecting recurrent neoplasia and/or persistent/recurrent IM in patients after endoscopic treatment of BORN.

METHODS

Study design

This was a single-center, prospective and pathologist-blinded study, which was approved by the local Ethical Committee in June 2015. The study was performed in agreement with the Declaration of Helsinki, including the changes accepted in Soul, South Korea, during the 59th WMA General Assembly. The study was registered at ClinicalTrials.gov as NCT02922049.

Patients' enrollment

All patients aged over 18 years who underwent a surveillance endoscopy after successful endoscopic treatment of BORN in our hospital between April 2016 and April 2019 were invited to participate. Before enrollment, all patients signed an informed consent form. Successful treatment of BORN was defined as a complete macroscopic and microscopic remission of neoplasia and macroscopic eradication of metaplastic mucosa (no visible segment of BE).

The endoscopic treatment consisted of endoscopic resection methods [ER or endoscopic submucosal resection (ESD)], an ablation method (radiofrequency ablation – RFA) or their combination. The exclusion criteria were no curative treatment of esophageal cancer (those patients were referred for surgery or adjuvant chemo-radiotherapy), no complete local remission of both neoplasia and/or BE, treatment with anticoagulants, esophageal varices, active esophagitis, allergy to fluorescein, polyvalent allergy or pregnancy. Prior to enrollment, all patients were administered long-term treatment with a proton pump inhibitor.

Endoscopy with pCLE

All patients underwent HD-WLE (Olympus 180 or 190, Olympus Medical Systems, Tokyo, Japan). Patients were given analgosedation with midazolam 2-5 mg intravenously, if necessary. After careful inspection of the area of interest in WLE, a pCLE examination was performed. Before pCLE, a 10% fluorescein was administered intravenously in dose of 2.5-5 ml (Fluorescite 100mg/ml inj sol, Alcon Pharmaceuticals, Czech Republic). Then the CLE probe (GastroFlex™, MP-009-HDG, Manua Kea Technologies, France) was introduced through the working channel of an endoscope and the target areas were then approached. Images were obtained and recorded from the neo-Z-line (the whole circumference), from visible tongues of metaplastic mucosa or any irregularities and from the neosquamous esophageal epithelium (treated area).

After pCLE examination had been completed, biopsies were obtained according to the study protocol (see below).

The recorded images were assessed by an experienced pathologist trained in CLE (M.K.) who was blinded with regard to the histology results of the standard biopsies.

Intestinal metaplasia in pCLE was defined by the presence of columnar epithelium with villiform-like pattern containing at least one regular cell with the dark ellipsoid vacuole with regular margin, with the size about 20 micrometers (goblet cell). The dysplastic BE was characterized by black cells with irregular borders and shapes, high dark contrast to the surrounding tissue, and irregular leaking capillaries in the mucosa (Miami classification) [13].

Biopsies

After completion of pCLE examination, at least 4-6 biopsies were taken from the neo-Z-line and at least 3 biopsies from the neo-squamous epithelium above the neo-Z-line. Biopsies were also taken from all visible abnormalities (tongues, islands). All biopsies were obtained with large-capacity forceps (Radial JawTM 4, Boston Scientific, Natick, MA, United States). The specimen was fixed in 10% neutral buffered formalin and processed for paraffin embedding. Five-micron tissue sections had been cut and stained with hematoxylin and eosin for histopathologic evaluation.

Definitive diagnosis of intestinal metaplasia and neoplasia

Definitive (final) diagnosis of IM and/or neoplasia was considered if both methods (biopsies and pCLE) reached the same diagnosis or either biopsies or pCLE confirmed IM or neoplasia. In such a case, histology slides or pCLE images had to be reviewed to confirm the definitive diagnosis.

Main outcomes

The primary endpoints were: 1) to determine the proportion of patients with recurrent neoplasia and with persistent/recurrent IM diagnosed by standard biopsies

and by pCLE; 2) to assess the sensitivity, specificity, positive predictive value and negative predictive value of pCLE in detecting persistent/recurrent IM and neoplasia with regard to a definitive diagnosis of neoplasia or IM; 3) to detect the number of glands and goblet cells detected on pCLE and on standard histology (pCLE could have a theoretical advantage of examining a larger area than biopsies).

Persistent IM was defined as the presence of IM in a patient after successful treatment of neoplasia and macroscopic eradication of BE. Recurrent metaplasia was defined as IM detected in a patient with previously confirmed CR-IM on at least 2 subsequent endoscopies.

Statistical analysis

Data are presented as counts and percentages, means with standard deviations or medians with range. Sensitivity, specificity, positive and negative predictive values with 95% confidence intervals (CI) for both pCLE and biopsies to confirm or exclude diagnosis of IM were calculated by using an artificial reference standard that combines true positives of both biopsies and pCLE [16]. Differences in proportions of true positives (sensitivity), true negatives (specificity) and true diagnoses (accuracy) were tested by McNemars test. An overall agreement between biopsies and pCLE in diagnosing IM was assessed by using a Cohens kappa statistic.

We were not able to calculate these parameters for neoplasia because recurrent neoplasia occurred only once. The Student's t-test was used to compare the mean number of glands/goblet cells assessed with either pCLE or biopsies and the value of less than 0.05 was considered significant. We planned to include at least 50 patients (cross over design, expected diagnostic accuracy of biopsies 80%, 95%CI: 65-90, p=0.05, 80% study power).

RESULTS

Patients' characteristics

A total of 56 patients (48 males, 8 females) were enrolled (Fig. 1) and baseline characteristics are shown in Table I. The endoscopic procedure with pCLE was well tolerated by all patients. One patient experienced a mild allergic reaction with rash caused by fluorescein, which was successfully treated with a single intravenous application of an antihistamine.

Probe-based CLE and biopsies in diagnosing recurrent neoplasia

Only 1 patient (1/56, 1.8%) experienced recurrent neoplasia - LGD, which was diagnosed by pCLE but not by histology (Fig. 2). This patient underwent successful re-RFA (HALO 60). As no other recurrences of BORN occurred, we were unable to compare the effectiveness of pCLE and of biopsies in detecting recurrent neoplasia as planned. However, our results show that biopsies and pCLE are comparable in excluding recurrent BORN, because in all the remaining patients no recurrent neoplasia was diagnosed with either biopsies or pCLE.

We also did not detect any patient with buried glands within neosquamous epithelium either with pCLE or with biopsies.

Probe-based CLE and biopsies in diagnosing persistent/recurrent IM

In a total of 36 patients (64.3%) IM was not detected by either pCLE or biopsies. Twenty patients experienced persistent or recurrent IM (35.7%). pCLE detected IM in all 20 patients (Fig. 3), biopsies detected IM in 17 patients (Fig. 4). Biopsies did not detect any patient with IM in whom pCLE was negative for IM. All persistent/recurrent IM occurred at the level of macroscopically normal neo-Z-line.

Diagnostic accuracy

Sensitivity, specificity, positive predictive value and negative predictive value of pCLE and biopsies did not differ significantly (Table II). Agreement between pCLE and histopathological findings in detecting IM was 94.6% (Table II). Cohens kappa statistic showed almost perfect agreement between the two tests (0.88, 95%CI 0.747 - 1.000).

Analysis of detected glands and goblet cells

A total of 639 biopsies were taken from all patients (8 -12 biopsies per patient) and 195 biopsies were taken from 17 patients with IM (detected in biopsies). In these IM positive patients, 169 biopsies (87%) were free of IM while 26 biopsies (13%) detected IM. Moreover, in these 17 patients, biopsies

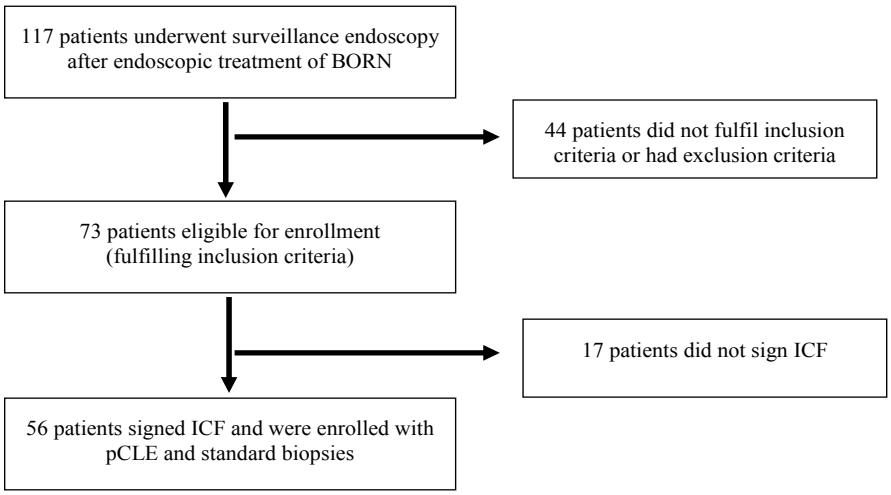

Fig. 1. Flow diagram of patient selection.

Table I. Baseline characteristics

	Adenocarcinoma	High-grade dysplasia	Low-grade dysplasia
No. of patients (%)	20 (36%)	12 (21%)	24 (43%)
Mean age - years (range)	68 (42-81)	68 (51-77)	62 (48-79)
Median length of BE - cm (range)	1 (1-7)	4 (1-12)	3 (1-10)
Modalities of BORN treatment:			
• ER or ESD only	6 (30%)	1 (8.3%)	2 (8.3%)
• ER/ESD with RFA	14 (70%)	7 (58.3%)	4 (16.7%)
• RFA only	0 (0%)	4 (33.3%)	18 (75%)
Median follow-up after therapy - months (range)	12 (4-66)	65 (4-97)	13 (4-65)

BE: Barrett's esophagus; BORN: Barrett's esophagus related neoplasia; ER: endoscopic resection; ESD: endoscopic submucosal dissection; RFA: radiofrequency ablation.

detected a total of 250 glands and 1,407 goblet cells while pCLE found 278 glands containing 1784 goblet cells (Table III). pCLE detected significantly more goblet cells (median 43 per patient, range 7-676) than biopsies (median 12 per patient, range 3-659), p=0.01.

DISCUSSION

In our study, we have demonstrated that pCLE has a comparable effectiveness to standard biopsies in detecting persistent or recurrent IM in patients who have undergone endoscopic treatment of BORN. Furthermore, pCLE was equally effective as biopsies in excluding recurrent neoplasia and finally, pCLE detected significantly more goblet cells than biopsies in patients with IM.

Barrett's esophagus is a premalignant condition; patients with BE have an increased risk of developing esophageal adenocarcinoma with an annual incidence of 0.12-0.2% in patients without dysplasia, 10-15% in patients with LGD and 13-25% in patients with HGD [17-21]. For this reason, patients with nondysplastic BE should enter endoscopic surveillance with biopsies to detect dysplasia or early cancer and subsequently the majority of patients could be successfully managed endoscopically.

Patients with LGD, HGD or early adenocarcinoma are good candidates for endoscopic treatment, consisting of ER or ESD (any visible lesion or cancer) and/or of ablative method (most frequently RFA). Endoscopic resection combined with RFA is now considered as a gold standard for treatment of patients with early esophageal adenocarcinoma. Radiofrequency ablation alone is the first-line treatment for patients with flat BE with confirmed LGD or HGD [6, 22-24].

The aim of endoscopic treatment is to remove both neoplasia and the remaining segment of BE. Ideally, a patient after a successful endoscopic treatment should be free of both neoplasia and IM; CR-N and CR-IM are considered as the main outcome in patients undergoing endoscopic treatment of BORN. Several studies demonstrated that endoscopic treatment is effective and safe. The rates of CR-N and CR-IM are 80-93% and 70-88%, respectively [14, 25-27].

However, recurrences of both BE and neoplasia may occur and persistent IM detected after successful endoscopic treatment, (when BE macroscopically disappeared and CR-N but not CR-IM was achieved) carries, at least a theoretical risk of further progression. For example, Orman et al. [26] showed in a meta-analysis of 18 studies with 3,802 patients that CR-IM and CR-N were achieved in 78% and 91%, respectively. Recurrences of IM and neoplasia were detected in 13% and 1.6% of patients. Our recently published study found that among patients with BORN who had undergone endoscopic treatment, 98.5% of patients achieved CR-N and 77.9% patients achieved CR-IM [14]. Neoplasia and IM recurred in 4.5% and 15% of patients. Worth noting is that the majority of IM recurrences occurred without a recurrence of the macroscopically visible BE segment. Also other studies reported a recurrence of IM in 2-20% and recurrence of neoplasia in 2-7% [28, 29].

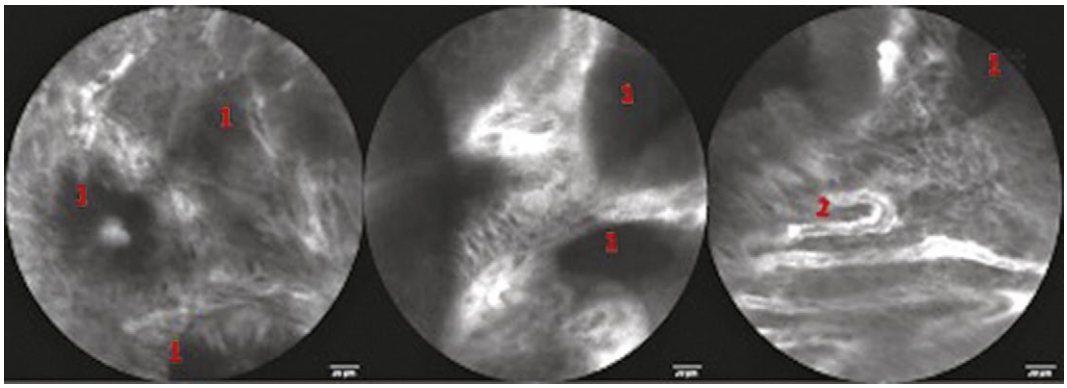

Fig. 2. The only patient with low grade dysplasia detected with pCLE (but not with histology). On pCLE image we can differentiate black epithelium with irregular cellular border (1) and irregular vessels (2).

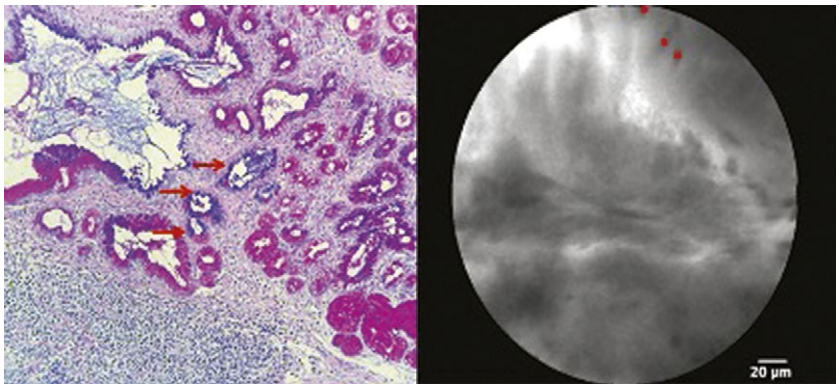

Fig. 3. Disagreement in detection persistent/recurrent intestinal metaplasia, histology without intestinal metaplasia, pCLE with intestinal metaplasia (left – H&E; right – pCLE), arrow – tall blue cells, without intestinal metaplasia, * intestinal metaplasia – goblet cells.

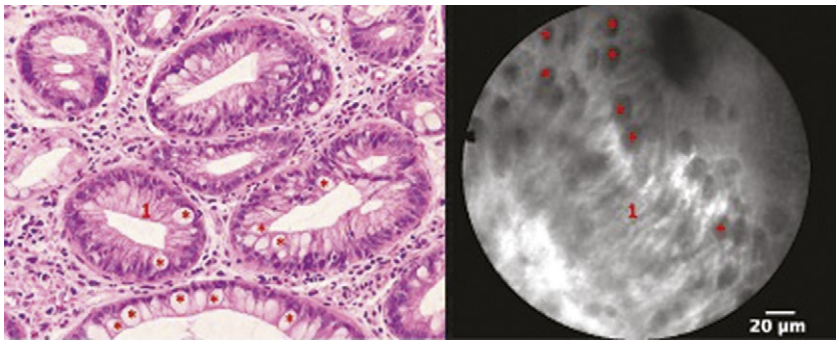

Fig. 4. Agreement in detection persistent/recurrent intestinal metaplasia (left – H&E; right – pCLE.), 1= epithelium, * intestinal metaplasia – goblet cells.

Table II. pCLE vs. biopsies in detecting IM after treatment of BORN

	pCLE	Biopsies	p
Sensitivity	100% (95%CI: 80–100%)	85% (95%CI: 62.1–96.8)	0.25
Specificity	100% (95%CI: 88–100)	100% (95%CI: 88–100)	1
Positive predictive value	100% (95%CI: 80–100)	100% (95%CI: 77.1-100)	1
Negative predictive value	100% (95%CI: 88-100)	92.3% (95%:CI: 78 – 98)	1
Accuracy	100% (95%CI: 93.6-100)	94.6% (95%CI: 85.1-98.9)	0.25
Agreement between pCLE and histopathological findings	94.6 %		0.88 (95%CI:0.747-1.000)*

* Cohen kappa statistic; pCLE: probe-based confocal laser endomicroscopy; IM: intestinal metaplasia

Table III. pCLE vs. biopsies in detection of glands and goblet cells (analysis of 17 patients with intestinal metaplasia in both biopsies and pCLE). pCLE detected significantly more goblet cells.

	Biopsies	pCLE	p
No of glands detected (total)	250	278	
No. of glands per patient (median, range)	3 (1-87)	8 (4-95)	0.45
No. of detected goblet cells (total)	1,407	1,784	
No. of goblet cells per patient (median, range)	12 (3-659)	43 (7-676)	0.01

Therefore, patients after successful endoscopic treatment of BORN still need endoscopic surveillance. In contrast to patients with naïve BE, the risk factors for recurrences of neoplasia or IM are not fully elucidated and, therefore, the appropriate intervals between endoscopies are still matter of discussion [30]. Nevertheless, it seems that the risk of recurrence is higher in patients with advanced initial histopathological diagnosis (e.g. cancer) compared to patients with less advanced stages (e.g. LGD) [7, 14]. Thus, surveillance endoscopies should be adhered to more frequently in patients with adenocarcinoma and less frequently in patients with an initial diagnosis of LGD. Surveillance endoscopy consists of careful inspection and of biopsies taken from any visible abnormality (lesion, new islands of tongues of metaplastic mucosa), from the neo-Z-line and from the esophageal neo-squamous epithelium (to rule out

buried glands). In cases of recurrence (visible segment of BE or neoplasia), another session of endoscopic treatment should be considered. In cases of persistent/recurrent IM within macroscopically normally appearing neo-Z-line, re-treatment is usually not indicated [14, 31, 32].

Confocal laser endomicroscopy is a relatively new method enabling real-time microscopic imaging. The currently available system for pCLE allows examination throughout the whole digestive system as the probe may be introduced through the working channel of a standard endoscope (esophagus, stomach, colon) or a cholangioscope (biliary tree, pancreatic duct) or through a needle for biopsy during endoscopic ultrasound. Confocal laser endomicroscopy may be useful either as a complementary tool to increase diagnostic yield of standard diagnostic methods or as a tool allowing replacement (full or partial) of current diagnostic methods.

Probe-based CLE reliably diagnoses IM in patients with BE [8-10] and is not inferior to biopsies in excluding BORN [33]. Two studies in patients with BE [9, 15] demonstrated that the use of pCLE may decrease the number of biopsies needed to diagnose neoplasia and that pCLE improved detection of HGD/early adenocarcinoma if added on HD-WLE. A combination of narrow band imaging, pCLE and HD-WLE resulted in an excellent sensitivity (100%) for diagnosis of HGD/adenocarcinoma [9]. Moreover, biopsies could be avoided in 60% of patients. Our recent study showed that pCLE is at least as effective as standard biopsies in terms of diagnostic accuracy in patients with esophageal/gastric macroscopically visible lesions [34]. Thus, the usefulness of pCLE in the assessment of patients with BE lies in improving the diagnostic yield for BORN and/or in reducing the number of biopsies (in patients with a visible esophageal lesion pCLE could even replace biopsies).

In this study we examined whether pCLE could also be useful in patients after successful endoscopic treatment of BORN. We demonstrated that pCLE may be useful in this indication because it was at least equally effective as biopsies in detecting persistent/recurrent IM and in excluding neoplasia. Among 20 patients with IM, pCLE diagnosed all of them while biopsies missed IM in 3 patients. So far, only one randomized trial assessed the role of pCLE in the assessment of residual BE during endoscopic treatment of BORN. The main outcome was the proportion of optimally managed patients (correctly indicated treatment of patients with residual IM/neoplasia and no treatment of patients without IM/neoplasia). The study concluded that complementary use of pCLE failed to increase the number of patients with optimal management [12]. However, there are several differences when interpreting results of these two studies: firstly we compared both pCLE and biopsies, but did not test whether pCLE could have improved the diagnostic yield of biopsies; secondly, we did not examine patients before achieving treatment success, but we included only patients who had successfully completed treatment of BORN; finally the image interpretation in our study was performed off-line (and not real-time), which could have influenced their "negative" result.

Unfortunately, due to a very low number of patients with recurrent neoplasia, we were unable to compare pCLE with biopsies in detecting recurrent neoplasia. This would have been the most relevant endpoint from a clinical point of view. Nevertheless, in view of a very low rate of recurrent neoplasia after ER/RFA (2-7%), a study comparing pCLE and biopsies in detecting recurrent neoplasia is probably unrealistic, because it would require inclusion of a high number of patients (e.g. with 2% neoplastic recurrence rate, such a study would need to include 1,000 patients to find 20 with recurrent neoplasia). However, as pCLE reliably diagnoses BORN in patients with naïve BE, we may anticipate that CLE could also reliably diagnose recurrent neoplasia. Of note, the diagnosis (and subsequent re-RFA) of the only patient with recurrent LGD in our study was solely based on pCLE as biopsies were negative (Fig. 2).

Our findings may raise questions about their clinical significance because the real clinical impact of persistent/recurrent IM within a normal neo-Z-line remains unclear (in contrast to recurrent neoplasia or recurrent visible segment of BE with IM). Nevertheless, biopsies from normal neo-Z-line are recommended during surveillance endoscopies to detect IM, because eradication of IM is considered as an important target of endoscopic treatment of BORN [23, 27].

On the other hand, our study shows that pCLE is reliable in excluding recurrent neoplasia as well as buried glands beneath the neosquamous epithelium because pCLE did not show any false-positive result.

Our study also assessed how many glands and goblet cells were examined by pCLE compared to biopsies in patients with IM. Probe-based CLE detected significantly more goblet cells compared to biopsies. This result is not very surprising taking into account that biopsies were taken from 3-6 specific sites within the neo-Z-line but pCLE images were recorded from around the whole circumference of the neo-Z-line. We may therefore speculate, that pCLE could have a higher likelihood of detecting recurrent neoplasia.

The major drawback of pCLE is the high cost, which is significantly higher compared to histopathology (500 USD for pCLE vs. 150-200 USD for 5 biopsies) and unfortunately, pCLE is generally not reimbursed.

Our study has some limitations. First only a limited number of patients were included and consequently only one patient with recurrent neoplasia which did not permit the assessment of pCLE in diagnosing recurrent neoplasia. The limited number of patients with persistent/recurrent IM may also be responsible for surprisingly excellent results of pCLE (sensitivity and specificity 100%); with an increased number of patients, the results would have been probably more realistic. However, Kiesslich et al. [10] previously reported "close to perfect" sensitivity (98.1%) in diagnosing IM in patients with BE. Off-line assessment of pCLE images may also be considered as a limitation as the possibility of real-time diagnosis belongs to the principal advantage of pCLE.

CONCLUSIONS

Our study shows that pCLE may be useful in patients undergoing endoscopic surveillance after endoscopic treatment of BORN because it is at least as effective as biopsies in detecting persistent/recurrent IM and in excluding recurrent neoplasia and buried glands. Due to the low number of

patients with recurrent neoplasia we were unable to compare pCLE with biopsies in diagnosing recurrent neoplasia. Nevertheless, we believe that this clinical setting might be another good indication for pCLE among already tested and proven indications.

Conflicts of interest: None to declare.

Authors' contributions: J.K.: data analysis, follow-up endoscopies with pCLE; M.K.: pathology–assessment of all pCLE, critical review of the manuscript; J. Maluskova: pathology–assessment of all histopathological slides, critical review of the manuscript; M.J: statistics, data analysis, critical review of the manuscript; Z.V: performing follow-up endoscopies with pCLE, critical review of the manuscript; J.S: critical review of the manuscript; J. Martinek: study design, performing follow-up endoscopies with pCLE, manuscript.

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